Effects of empagliflozin on fluid overload, weight and blood pressure in chronic kidney disease

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ABSTRACT

BACKGROUND

Chronic kidney disease (CKD) is common worldwide and associated with considerable morbidity and mortality, largely due to cardiovascular disease. As CKD progresses, fluid overload is a common manifestation which has both clinical and prognostic implications. Bioimpedance spectroscopy is commonly used in the dialysis setting to assess fluid status but less so in earlier stages of CKD.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors have recently been shown to slow the progression of kidney disease and additionally have cardiovascular benefits. The underlying mechanisms of SGLT2 inhibitors remain incompletely understood but include diuretic effects and so they may reduce fluid overload. Trial reports demonstrate consistent treatment effects across a range of patient subgroups however these don't address the real-life scenario in which the highest risk patients (such as those with frailty) exhibit several characteristics in combination. Clinicians may hesitate to prescribe SGLT2 inhibitors in patients with frailty (which is common in CKD) due to perceived altered risk-benefit profile.

The hypothesis of this work is that the beneficial effects of empagliflozin (an SGLT2 inhibitor) on kidney and cardiovascular outcomes in CKD may be in part explained by fluid reduction which can be assessed using bioimpedance spectroscopy. This work will explore potential contributory mechanisms (through effects on fluid overload and blood pressure) and whether there is heterogeneity of treatment effect (focusing on frailty).

METHODS

A systematic review was conducted to inform the analysis strategy for analyses of the effects of empagliflozin on fluid overload. The full methods of the EMPA-KIDNEY trial have been reported elsewhere. In brief, 6609 patients with CKD at risk of progression with an estimated glomerular filtration rate (eGFR) $\geq$20 and <90 mL/min/1.73m^2 were randomised to either empagliflozin or matching placebo. The primary outcome was the first occurrence of kidney disease progression or death from cardiovascular causes. An EMPA-KIDNEY bioimpedance substudy obtained bioimpedance measurements in

addition to routine trial procedures at the randomisation, 2- and 18-month follow-up visits in 660 participants. Weight and blood pressure were measured at all visits. “Risk of hospitalisation during follow-up” at baseline was used as the primary frailty indicator with supplementary analyses by multimorbidity, polypharmacy and health-related quality of life at baseline.

Effects of empagliflozin on fluid status, blood pressure and weight were assessed using a mixed model repeated measures (MMRM) approach; and effects on binary endpoints were assessed using Cox regression models; with adjustment for age, sex, prior diabetes, eGFR, and urinary albumin-to-creatinine ratio (uACR) in the categories used in the minimised randomisation algorithm. Pre-specified subgroups were categories of sex, diabetes, eGFR and N-terminal pro B-type natriuretic peptide (NT-proBNP) and subgroup-specific treatment effects were estimated by fitting interaction terms in the MMRM or Cox models and calculating heterogeneity or trend statistics. Frailty (predicted risk of hospitalisation) was determined by logistic regression models with recorded hospitalisation (first event) as the response variable. All variables which were significantly associated ($P<0.01$) with hospitalisation in univariable models proceeded to inclusion in multivariable model building using forward stepwise selection and likelihood ratio tests with significance threshold $P<0.01$. Frailty subgroups were defined according to predicted risk of hospitalisation and relative and estimated absolute effects on the trial’s primary outcome and all-cause hospitalisation were assessed.

RESULTS

In 620 substudy participants included in the primary assessment, compared to placebo, the study-average absolute difference in absolute “Fluid Overload” was -0.24 L (95% CI -0.38, -0.11). Effects were similar at 2- and 18-months (-0.23 [-0.37, -0.08] and -0.26 [-0.46, -0.06] L, respectively; P value for interaction with time = 0.11) and across the pre-specified subgroups. There were no significant between-group differences in bioimpedance-derived fat or lean tissue parameters. In the bioimpedance substudy cohort, the study-average between-group difference in total body weight in kg was -0.7 kg (95% CI -1.3, -0.1), consistent with the -0.9 kg (95% CI -1.2, -0.6) difference observed in the full trial cohort. In the full trial, the effects on weight persisted over time (P for interaction = 0.47) with consistent effects across subgroups. The study-average between-group differences in

systolic and diastolic blood pressure in the full trial cohort were -2.6 mmHg (95% CI -3.3, -1.9) and -0.5 mmHg (95% CI -0.9, -0.1), respectively with similar results in the substudy cohort. There was no evidence of heterogeneity of the blood pressure lowering effect of empagliflozin when subdivided by sex, baseline eGFR or NT-proBNP but there was some evidence to suggest greater systolic blood pressure lowering in patients with diabetes (-4.1 [0.3] versus -1.9 [0.3] mmHg; heterogeneity P value = 0.001).

Overall, compared to placebo, empagliflozin reduced the risk of the primary composite outcome of kidney disease progression or cardiovascular death by 28% (HR 0.72, 95% CI 0.64-0.82), with no significant difference in relative effects by baseline frailty (P for heterogeneity all >0.05). In absolute terms, there was evidence of larger estimated benefits on the primary outcome in participants in the highest category of frailty (based on risk of hospitalisation) compared to those with lesser degrees of frailty: per 1000 participants treated, empagliflozin was estimated to result in 38 fewer primary outcomes among those in the highest frailty category compared to 14 primary outcomes avoided annually in the lowest third of frailty. Safety outcomes such as dehydration and bone fractures were more common in participants with indicators of increased frailty, but there was no significant effect of study treatment on any of these outcomes overall or among those who were most frail.

CONCLUSION

The findings from this thesis demonstrate that the benefits of SGLT2 inhibitor treatment extend beyond the main effects on slowing kidney disease progression and provide some mechanistic insights into how these benefits are mediated. Empagliflozin resulted in sustained reductions in “Fluid Overload”, weight and blood pressure in patients with CKD with and without diabetes with no demonstrable effect on fat mass. This effect was evident even in patients with low levels of kidney function. In the studied population, the absolute benefits of empagliflozin on the primary outcome (kidney disease progression or cardiovascular death) were greater in patients with the highest levels of frailty and outweighed the absolute risks of adverse events.

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PEER-REVIEWED PUBLICATIONS DIRECTLY ARISING FROM THIS THESIS

1. **Mayne KJ**, Sardell RJ, Staplin N, Judge PK, Zhu D, Sammons E et al. Frailty, multimorbidity and polypharmacy: exploratory analyses of the effects of empagliflozin from the EMPA-KIDNEY trial. *Clin J Am Soc Nephrol*. 2024 Sep;19(9):1119–29. doi: 10.2215/CJN.0000000000000498. PMID: 38949880; PMCID: PMC11390031.
2. **Mayne KJ**, Staplin N, Keane DF, Wanner C, Brenner S, Cejka V et al. Effects of Empagliflozin on Fluid Overload, Weight and Blood Pressure in Chronic Kidney Disease. *J Am Soc Nephrol.* 2024 Feb 1;35(2):202-215. doi: 10.1681/ASN.0000000000000271. PMID: 38082486; PMCID: PMC7615589.
3. **Mayne KJ**, Shemilt R, Keane DF, Lees JS, Mark PB, Herrington WG. Bioimpedance indices of fluid overload and cardiorenal outcomes in heart failure and chronic kidney disease: a systematic review. *J Card Fail*. 2022;28(11):1628-1641. doi: 10.1016/j.cardfail.2022.08.005. PMID: 36038013; PMCID: PMC7613800.
4. **Mayne KJ**, Lees JS, Herrington WG. Bioimpedance in CKD: an untapped resource? *Nephrol Dial Transplant*. 2022;38(3):583-585. doi: 10.1093/ndt/gfac275. PMID: 36260361; PMCID: PMC9976732.

SUPPLEMENTARY PEER-REVIEWED PUBLICATIONS

1. **Mayne KJ**, Sardell RJ, Staplin N, Judge PK, Zhu D, Sammons E et al. Empagliflozin lowers serum uric acid in chronic kidney disease: exploratory analyses from the EMPA-KIDNEY trial. *Nephrol Dial Transplant*. 2024 Sep 14:gfae203. doi: 10.1093/ndt/gfae203. Epub ahead of print. PMID: 39277784; PMCID: PMC7616479.
2. Fletcher RA, Herrington WG, Agarwal R, **Mayne KJ,** Arnott C, Jardine MJ et al. Effects of SGLT2 inhibitors on cause-specific cardiovascular death in patients with chronic kidney disease: a meta-analysis of CKD progression trials. *Clin J Am Soc Nephrol*. 2024 Sep. doi: 10.2215/CJN.0000000000000470. PMID: 38622766; PMCID: PMC11390023.
3. Nangaku M… **Mayne KJ**… et al. Effects of empagliflozin in patients with chronic kidney disease from Japan: exploratory analyses from EMPA-KIDNEY. *Clin Exp Nephrol*. 2024 Jun;28(6):588-595. doi: 10.1007/s10157-024-02489-4. PMID: 38643286; PMCID: PMC11116192.

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7. Staplin N, Haynes R, **Mayne KJ**, Roddick AJ, Neuen BL, Hauske SJ et al. Impact of diabetes on the effects of sodium glucose co-transporter-2 inhibitors on kidney outcomes: collaborative meta-analysis of large placebo-controlled trials. *Lancet*. 2022;400(10365):1788-1801. doi: 10.1016/S0140-6736(22)02074-8. PMID: 36351458; PMCID: PMC7613836.
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9. EMPA-KIDNEY Collaborative Group including **Mayne KJ**. Design, recruitment, and baseline characteristics of the EMPA-KIDNEY trial. *Nephrol Dial Transplant.* 2022; 37(7):1317-1329. doi: 10.1093/ndt/gfac040. PMID: 35238940; PMCID: PMC9217655.

SCIENTIFIC PRESENTATIONS AND ABSTRACTS

1. Oral presentation ("Free Communication" – highest scoring abstracts) at the European Renal Association Congress, May 2024
   Title: 'The impact of multimorbidity on the effects of empagliflozin in chronic kidney disease (CKD): exploratory analyses from the EMPA-KIDNEY trial'
2. Poster presentation at the International Society of Nephrology World Congress of Nephrology, Apr 2024
   Title: 'The impact of frailty on the effects of empagliflozin: *post-hoc* analyses from the EMPA-KIDNEY trial'
3. Poster presentation at the American Society of Nephrology Kidney Week, Nov 2023

Title: ‘Effects of empagliflozin on weight & blood pressure in chronic kidney disease: analyses from the EMPA-KIDNEY trial’

4. Oral presentation (“Free Communication” – highest scoring abstracts) at the European Renal Association Congress, Jun 2023
   Title: ‘Effects of empagliflozin on fluid overload in chronic kidney disease: an EMPA-KIDNEY bioimpedance substudy’
5. Poster presentation at Oxford Population Health 10th Anniversary Symposium, Jun 2023
   Title: ‘Effects of empagliflozin on fluid overload in chronic kidney disease: an EMPA-KIDNEY bioimpedance substudy’
6. Co-author oral presentation at the American Society of Nephrology (ASN) Kidney Week, Nov 2022
   Title: ‘Sodium glucose co-transporter-2 (SGLT2) inhibitors among patients with and without diabetes: collaborative meta-analysis of large placebo-controlled trials’
   Authors: Natalie Staplin, Kaitlin J. Mayne, Richard Haynes, William G. Herrington

INVITED PRESENTATIONS

1. Invited speaker, Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU) Joint Annual Collaborator Meeting, May 2024 & Annual Symposium June 2024, Nuffield Department of Population Health, University of Oxford
   Title: ‘Too frail for flozins?’
2. Invited speaker, Oxford Kidney Unit Academic Meeting, Dec 2023 & May 2024
   Title: ‘Results from the EMPA-KIDNEY bioimpedance substudy’ &
3. Invited speaker, South West Renal Meeting, Jul 2023
   Title: ‘Results from the EMPA-KIDNEY trial’
4. Invited plenary speaker, Scottish Renal Association Annual Conference, Nov 2022
   Title: ‘Results from the EMPA-KIDNEY trial’

PREFACE

THESIS CONTEXT

EMPA-KIDNEY was a multicentre randomised double-blind placebo-controlled clinical trial of the sodium-glucose cotransporter 2 inhibitor, empagliflozin versus placebo to assess cardiovascular and kidney outcomes in patients with chronic kidney disease at risk of progression. A formal interim analysis in March 2022 found that the trial had met the pre-specified criteria for stopping early for efficacy. The trial therefore closed in July 2022 and the main results were reported in November 2022 in the *New England Journal of Medicine* (EMPA-KIDNEY Collaborative Group, 2023). The doctoral research project is based on data collected from the EMPA-KIDNEY trial which I had direct access to as a Clinical Research Fellow, working with the Renal Studies Group at the central coordinating office (Nuffield Department of Population Health, University of Oxford) full-time for three years (2021-2024).

EMPA-KIDNEY was initiated, designed, conducted and analysed by the Renal Studies Group. My role in the group included development and implementation of trial procedures; monitoring the safety of trial participants day-to-day and providing advice for clinicians caring for trial participants around the world, and adjudication of trial outcomes. I therefore had a key role in data collection and co-authoring key manuscripts from the trial (as a member of the trial's Steering Committee). The main EMPA-KIDNEY results are briefly discussed in Chapter 1 and do not form part of this thesis.

A 660-participant subset of the trial's 6609 participants were enrolled in an optional bioimpedance substudy which forms the basis of this doctoral research project and is reported herein. Results presented within this thesis are only those I have personally analysed (unless otherwise stated). The bioimpedance substudy had been fully recruited at the beginning of this doctoral project but I then took over responsibility for the day-to-day running and delivery of the substudy in October 2021 under the supervision of the trial's Principal Investigators. I coordinated substudy operations, drafted the data analysis plan (with supervision from the Chief Investigator and trial statistician) and conducted all of the data cleaning and statistical analyses of the substudy.

Once the main results of the trial were published, I was afforded access to the main trial dataset and, in addition to analyses of the bioimpedance substudy population, was then able to conduct related analyses in the complete trial population to explore effects on anthropometry, blood pressure and the impact of frailty (as well multimorbidity, polypharmacy and health-related quality of life) as reported herein.

My doctoral project was completed as part of a unique collaboration between the University of Oxford and the University of Glasgow. Data were generated by the University of Oxford which necessitated residing in Oxford for the duration of this doctoral research programme and studying under University of Glasgow "furth of Glasgow" regulations. This was possible due to existing links with the University of Glasgow where I held Honorary Clinical Lecturer status in association with a West of Scotland National Training Number in Renal and General Internal Medicine. A highly effective supervisory arrangement developed via approximately monthly virtual meetings with supervisors from both institutions supplemented with in-person meetings several times per year often at conferences. Lead University of Glasgow supervisor Professor Patrick Mark signed a confidentiality disclosure agreement to allow sharing of confidential results necessary for the supervision of my project without direct access to data which could be accessed by myself and Professor Will Herrington. Funding for the EMPA-KIDNEY trial was from Boehringer Ingelheim and Eli Lilly (along with core departmental funding to the University of Oxford from the UK Medical Research Council, the British Heart Foundation, the National Institute for Health and Care Research Biomedical Research Council, and Health Data Research UK) paid my salary and tuition fees as a result of competitive application to the post of Clinical Research Fellow.

BACKGROUND TO THE PROJECT

Chronic kidney disease (CKD) is common worldwide and associated with considerable morbidity and mortality, largely through associated cardiovascular disease burden. CKD has numerous causes with diabetes and hypertension among the most common. CKD is a progressive condition which may eventually necessitate kidney replacement therapy by dialysis or kidney transplantation. Treatments to slow the progression of CKD have, for many decades, been very limited. In recent decades, disease-modifying treatment has focused on renin-angiotensin system (RAS) inhibitors which were shown, in large randomised controlled trials of patients with diabetes, to reduce proteinuria and progression of kidney disease as well as modestly reducing blood pressure in CKD. Additional treatments are desperately needed to further reduce the burden of progressive CKD.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors which were originally developed to lower blood glucose in the treatment of type 2 diabetes were incidentally found to have beneficial effects on cardiovascular and renal outcomes in safety trials (Zinman et al., 2015, Neal et al., 2017). This led to the dedicated testing of these agents in heart failure populations in which exploratory analyses suggested the drugs may also have kidney-protective effects. This hypothesis was confirmed in the CREDENCE trial assessing kidney disease outcomes in a population with type 2 diabetes (Perkovic et al., 2019). Hypotheses emerged that SGLT2 inhibitors may also be efficacious in patients without diabetes, hypotheses which were again confirmed in further trials in heart failure populations without diabetes (Packer et al., 2020, Anker et al., 2021, Solomon et al., 2022, McMurray et al., 2019). There was therefore a need to test these drugs in a dedicated CKD trial in patients with and without diabetes which led to the design of EMPA-KIDNEY. A similar trial, the DAPA-CKD trial, of the SGLT2 inhibitor dapagliflozin ran in parallel and reported in late 2020. The results of DAPA-CKD showed that dapagliflozin reduced the risk of a composite kidney disease progression or cardiovascular death outcome by 29% (hazard ratio 0.71, 95% confidence interval 0.55-0.92) (Heerspink et al., 2020). DAPA-CKD studied 4304 participants with CKD with an estimated glomerular filtration rate (eGFR) between 25 and 75 mL/min/1.73m^2 and a urinary albumin-to-creatinine ratio (uACR) of 200-5000 mg/g; two-thirds of whom had type 2 diabetes. The EMPA-KIDNEY trial would extend these findings having recruited a larger population of 6609 participants and address remaining uncertainty in certain patient groups namely those with CKD

without diabetes (69% had a non-diabetic cause of kidney disease) and those with lower levels of eGFR and low levels of or no detectable albuminuria.

Although the benefits of SGLT2 inhibitors in diabetes, heart failure and CKD have been increasingly recognised and are now well-established, the underlying mechanisms remain incompletely understood. Benefits were originally largely attributed to glycosuric effects however the persistence of effect in individuals without diabetes challenged this hypothesis. Amongst other mechanisms, the diuretic and natriuretic properties of SGLT2 inhibitors are thought to play a role however this has not been properly evaluated, particularly in CKD. Large randomised trials, in addition to testing key hypotheses, offer opportunities to conduct additional analyses seeking to better understand the mechanisms underlying the observed effects on primary efficacy outcomes. The EMPA-KIDNEY trial therefore included a substudy in an approximate 10% subset of the recruited population incorporating additional assessments using bioimpedance measurements at randomisation and twice during scheduled follow-up. The substudy was conceived with the aim of better understanding the effects of empagliflozin on fluid status and body composition to contribute to mechanistic understanding. Once the main results of the trial and substudy were known, additional exploratory analyses were possible to complement pre-specified analyses where these were deemed scientifically and clinically important and approved by the trial's Steering Committee. To contextualise results of the bioimpedance substudy, expanded analyses were conducted of the effects of empagliflozin on anthropometry, blood pressure and related laboratory parameters in the whole trial population. The results of the EMPA-KIDNEY trial informed clinical guideline updates, extending the use of SGLT2 inhibitors in patients with CKD. The United Kingdom Kidney Association's updated guidelines, published in 2023, acknowledged the need to take account of frailty and multimorbidity and consider the balance of disease and treatment burden when prescribing SGLT2 inhibition in CKD. In order to provide randomised evidence to specifically support such clinical decisions, the impact of frailty (and related metrics) were analysed and related to effects on body water and composition as discussed in this thesis.

The following pages outline the research questions addressed in this doctoral research project and provide an outline of thesis chapters.

RESEARCH QUESTIONS

HYPOTHESIS

The hypothesis of this thesis is that empagliflozin (and the sodium-glucose cotransporter 2 inhibitor class of drugs) has beneficial effects on kidney and cardiovascular outcomes in patients with CKD which may be in part explained by effects on fluid status which can be assessed using bioimpedance spectroscopy. I will explore potential contributory mechanisms (through effects on fluid overload, body composition and blood pressure) and whether there is heterogeneity of treatment effect (focusing on indicators of patients who may be vulnerable to diuresis and blood pressure lowering).

These research questions and associated objectives outlined below are expanded upon in 1.7 Aims of the thesis.

RESEARCH QUESTION 1

Is bioimpedance spectroscopy a valuable tool in clinical and research settings in non-dialysis chronic kidney disease - what are the associations between bioimpedance-derived fluid overload and clinical outcomes?

RESEARCH QUESTION 2

What are the effects of empagliflozin on fluid status estimated by bioimpedance spectroscopy?

RESEARCH QUESTION 3

What are the effects of empagliflozin on body composition and is weight lost due to fluid or reduced adiposity?

RESEARCH QUESTION 4

What are the effects of empagliflozin on blood pressure and how do these relate to effects on fluid status?

RESEARCH QUESTION 5

What is the impact of frailty, multimorbidity, polypharmacy and health-related quality of life on the effects of empagliflozin on clinical outcomes and physical measurements?

OUTLINE OF THESIS CHAPTERS

Chapter 1 provides background of the existing literature on chronic kidney disease, its treatment, fluid overload as a manifestation of chronic kidney disease and the use of bioimpedance techniques. The aims of this thesis are summarised at the end of this chapter.

Chapter 2 details the methods for all aspects of the thesis: systematic review, EMPA-KIDNEY trial and bioimpedance substudy, frailty analyses and statistical methods.

Chapter 3 reports the findings of a systematic review of bioimpedance-derived fluid overload and associations with clinical outcomes in CKD and heart failure (research question 1).

Chapter 4 reports results on the effects of empagliflozin on bioimpedance-derived fluid overload (research question 2).

Chapter 5 reports results on the effects of empagliflozin on body composition (research question 3).

Chapter 6 reports results on the effects of empagliflozin on blood pressure (research question 4).

Chapter 7 reports results on the impact of frailty, multimorbidity, polypharmacy and health-related quality of life on the effects of empagliflozin on clinical outcomes and physical measurements (research question 5).

Chapter 8 summarises the key findings from chapters 3-7 in the context of existing literature and discusses implications and future directions.

ACKNOWLEDGEMENT

I am indebted to my inspiring supervisory team of Professor William Herrington, Professor Patrick Mark and Dr Jennifer Lees. As lead University of Glasgow supervisor, I'm grateful to Professor Mark for his enthusiasm and creativity in pursuing "furth of Glasgow" doctoral study and taking me on in slightly unconventional circumstances which importantly allowed me to keep a connection with Glasgow and foster collaboration. I sincerely thank Dr Lees for her role not only as supervisor but mentor, role model and friend. It has been my privilege to be her first PhD student and having a third supervisor at an earlier career stage has added enormous value to my experience. I thank Professor Herrington for braving the Courts of Glasgow to facilitate my PhD and trusting me with analysis of EMPA-KIDNEY data. I thank him especially for exceptionally timeous and attentive review of my work amidst many other priorities.

None of this would have been possible without Professor Richard Haynes and I sincerely thank him for his unwavering support. I'm grateful, not least, to Professor Haynes (along with Professor Herrington) for employing me and paying my salary and tuition fees to the University of Glasgow with grant funding for the trial coming from Boehringer Ingelheim and Eli Lilly. It has been an incredible privilege to work on a trial such as EMPA-KIDNEY which has formed the basis of my research and I am grateful for the invaluable experience and indoctrination in large randomised trial methodology. I'm also particularly grateful for the opportunities Professors Haynes & Herrington provided for me to travel internationally to present work which has contributed importantly to my professional and personal development and my enjoyment.

This work also would not have been possible without those who supported me to conduct the analyses. Trial statistician Associate Professor Natalie Staplin provided consistent support with statistical analyses, demonstrating incredible patience and always making herself available to me. Though not officially a supervisor, these analyses would not have been possible without her support and my data programming and statistical analysis skills have flourished under her guidance. I also thank Dr Rebecca Sardell for her time and patience in verifying analyses reported in Chapter 7. I owe most of what I know about the technical aspects of bioimpedance to Dr David Keane and sincerely thank him for donating his expertise, patiently educating me on Cole-Cole plots and always making time to discuss

out of genuine interest and enthusiasm. I also owe thanks to Daniele Trinca for his essential contribution in the initial processing of bioimpedance data.

I must thank friends, peers and colleagues at the Clinical Trial Service Unit and Epidemiological Studies Unit, Oxford for their support. Dr Doreen Zhu and Dr Sarah Ng welcomed me so warmly when I joined and have become cherished friends and cheerleaders, enriching my life as a Research Fellow both in and outside of work. My thanks for their support and friendship also to Associate Professor Kirsty Reith, Dr Waseem Karsan, Dr Nikita Agarwal and Dr Ryoki Arimoto. I must also thank many others at CTSU who delivered the EMPA-KIDNEY trial: Professor David Preiss, Professor Jonathan Emberson and Dr Parminder Judge all added value in reviewing manuscripts and generally supporting; and colleagues who were particularly key to the successful conduct of the bioimpedance substudy include: Rejive Dayanandan, Dr Ryonfa Lee, John Nolan, Akiko Omata, Mo Gray and based in Wurzburg, Germany: Dr Marcela Fajardo-Moser.

I thank my brother in arms and fellow University of Glasgow PhD student Dr Benjamin Elyan for his friendship. Our approximately monthly VIRMs and in-person catch-ups when in Glasgow or at conferences have provided much light relief and fuel for the road. I am incredibly grateful to others in Glasgow who provided research opportunities for me at an early stage and motivated me to pursue this path: Dr Kenneth Mangion, Dr Alastair Rankin and Dr Keith Gillis. Without such experience and their mentorship, the University of Oxford might not have employed me and none of this would have been possible. I am also incredibly thankful for my Glasgow-based non-renal support network of friends and family. My family tolerated 300-mile physical distance during difficult times while I pursued this experience in Oxford and always encourage me. They have supported me in many ways despite physical distance and found creative ways to overcome this.

Lastly, it has been a real honour to be able to study and analyse data from EMPA-KIDNEY, a project which was the work of hundreds of investigators worldwide. I express my sincere gratitude to all individuals involved (local site staff, regional and central coordinating centre staff, and all members of EMPA-KIDNEY committees) and most importantly, the patients who bravely volunteered to participate and made this work possible.

AUTHOR'S DECLARATION

The work presented in this thesis was conducted by myself under the supervision of the supervisors. I acknowledge support from statisticians Associate Professor Natalie Staplin and Dr Rebecca Sardell (Nuffield Department of Population Health, University of Oxford) in advising on statistical methods and replicating analyses for necessary verification purposes required by the Renal Studies Group prior to publication. All analyses reported herein were conducted by me personally (and verified by departmental statisticians) with the exception of (i) analysis of effects on waist:hip ratio requiring multiple imputation (see 5.2.3) and (ii) analyses of first and recurrent hospitalisations (see 7.2.5) due to the need for trial statisticians to be able to exactly replicate my results later if requested (e.g. by regulators) and the complexity of producing these particular analyses in two different statistical programmes (R which I used versus SAS used by trial statisticians).

I also acknowledge the contribution of Dr David Keane and Daniele Trinca in processing the raw bioimpedance data to derive parameters suitable for analysis using proprietary coefficients provided to them by the device manufacturer. I also acknowledge support from Dr Richard Shemilt (University of Glasgow) in performing dual screening and data extraction for the systematic review. Most importantly, I acknowledge the critical contribution of hundreds of investigators from the 241 contributing sites worldwide who conducted the EMPA-KIDNEY trial, the associated coordinating centres and committees who collaborated between 2018 and 2022, and the 6609 participants.

This thesis, including all statistical analysis (unless otherwise stated above) and presentation of results, is my own work. It has not previously been submitted for a higher degree.

Kaitlin J Mayne

October 2024

DEFINITIONS/ABBREVIATIONS

Abbreviation	Definition
ACEi	Angiotensin converting enzyme inhibitor
ACR	Albumin-to-creatinine ratio
AF	Atrial fibrillation
AHF	Acute heart failure
AIC	Akaike information criterion
AKI	Acute kidney injury
ANOVA	Analysis of variance
ARB	Angiotensin receptor blocker
ATM	Adipose tissue mass
AUC	Area under the curve
AUROC curve	Area under the receiver operating characteristic curve
BCM	Body composition monitor
BIA	Bioimpedance analysis
BIS	Bioimpedance spectroscopy
BIVA	Bioimpedance vector analysis
BMI	Body mass index
BNP	Brain natriuretic peptide
BP	Blood pressure
CA125	Cancer antigen 125
CHF	Chronic heart failure
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKD-MBD	Chronic kidney disease mineral and bone disorder
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DEXA	Dual energy x-ray absorptiometry
DPP-4	Dipeptidyl peptidase-4
ECW	Extracellular water
EDMS	Electronic document management system
ESKD	End-stage kidney disease

ESRD	End-stage renal disease
eGFR	Estimated glomerular filtration rate
FTI	Fat tissue index
GFR	Glomerular filtration rate
GLP-1	Glucagon-like peptide-1
HbA1c	Glycated haemoglobin
HD	Haemodialysis
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HHF	Hospitalisation for heart failure
HI	Hydration index
HR	Hazard ratio
HRQoL	Health-related quality of life
ICW	Intracellular water
IQR	Interquartile range
KDIGO	Kidney Disease Improving Global Outcomes
KF	Kidney failure
KRT	Kidney replacement therapy
LCC	Local Clinical Centre
LDL	Low density lipoprotein
LL	Lower limit
LRC	LCC Research Coordinator
LRT	Likelihood ratio test
LTI	Lean tissue index
LTM	Lean tissue mass
LVEF	Left ventricular ejection fraction
MACE	Major adverse cardiovascular events
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
MMRM	Mixed model repeated measures
MRI	Magnetic resonance imaging
MV	Multivariable
MVSA	Multivariable survival analysis
NGAL	Neutrophil gelatinase-associated lipocalin

NT-proBNP	N-terminal pro B-type natriuretic peptide
NYHA	New York Heart Association
OH	Overhydration
OR	Odds ratio
PCI	Percutaneous coronary intervention
PD	Peritoneal dialysis
PVD	Peripheral vascular disease
RAS	Renin-angiotensin system
RCT	Randomised controlled trial
ROB	Risk of bias
ROC	Receiver operating characteristic
RR	Relative risk
RRT	Renal replacement therapy
SD	Standard deviation
SE	Standard error
SGLT2	Sodium-glucose cotransporter 2
SOP	Standard operating procedure
TBW	Total body water
uACR	Urinary albumin-to-creatinine ratio
UL	Upper limit
VAD	Ventricular assist device
WCC	White cell count

CHAPTER 1 – INTRODUCTION

This chapter sets out the background to this thesis. It discusses chronic kidney disease (CKD), its treatment, fluid overload as a manifestation of CKD and the use of bioimpedance techniques to assess fluid status. The aims of this thesis are summarised at the end of this chapter.

1.1 BACKGROUND – CHRONIC KIDNEY DISEASE

1.1.1 CHRONIC KIDNEY DISEASE DEFINITION AND EPIDEMIOLOGY

CKD is defined as abnormal kidney structure or function which persists for at least three months (KDIGO, 2024). This includes biochemical evidence of kidney dysfunction as well as imaging or histopathological findings. The heterogeneous term includes kidney disease of any aetiology and encompasses a wide range of pathophysiology. CKD is now thought to affect around 10% of the global population and risk factors include advancing age, female sex, non-White race, diabetes and hypertension (Kovesdy, 2022, GBD Chronic Kidney Disease Collaboration, 2020). Temporal trends in incidence and prevalence are difficult to ascertain with discrepant patterns observed between countries. However, CKD is an important cause of death worldwide and projected to be the fifth leading cause of death worldwide by 2040 (Foreman et al., 2018). CKD-related mortality has remained stubbornly high while mortality from other cardiovascular diseases like stroke and myocardial infarction has fallen in recent decades (Hockham et al., 2022).

Diabetes and hypertension cause the majority of CKD worldwide though the potential causes of CKD are manifold. Other causes can be largely grouped into glomerulonephritis, multisystem disorders, genetic conditions and other/unknown disorders. Glomerulonephritis is an umbrella term for conditions, usually immunological in nature, which damage the glomerulus of the kidney. Multisystem diseases most commonly implicated in CKD include systemic vasculitis, connective tissue disorders such as systemic lupus erythematosus, and haematological conditions such as multiple myeloma. Genetic or inherited causes of CKD such as adult polycystic kidney disease and Alport's syndrome are responsible for ~10-15% of CKD in adults and a much greater proportion of early-onset CKD (Torra et al., 2021). Acute kidney injury (AKI) due to any cause is also an important risk factor for CKD – both due to non-recovery following the initial insult causing persisting kidney disease as well as increased risk of CKD in later life in those who have recovered from AKI. Though the list of potential causes of CKD is long,

diabetes and high blood pressure are considered to be by far the largest contributors to CKD burden. CKD attributed to diabetes is associated with much greater morbidity and mortality (measured in disability-adjusted life years, DALYs) than CKD due to glomerulonephritis or other/unspecified causes (GBD Chronic Kidney Disease Collaboration, 2020).

1.1.2 STAGING AND PROGRESSION OF CHRONIC KIDNEY DISEASE

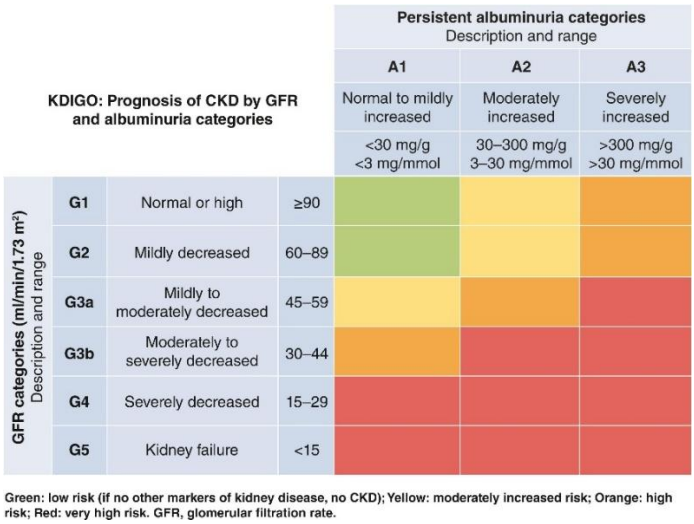
The staging of CKD is determined by laboratory measures: estimated glomerular filtration rate (GFR, eGFR) and albuminuria (or proteinuria). Kidney function can be more accurately determined by directly measured GFR by the clearance of exogenous filtration markers which are purely renally excreted (such as iohexol, chromium 51-ethylenediamine tetraacetic acid) however this requires repeated blood collection over several hours which is costly and impractical hence the needed for indirect estimations of GFR using endogenous filtration markers (serum creatinine or cystatin C). Estimated GFR is derived using equations of which several exist, the most commonly used in current European practice being the race-free 2009 CKD-EPI equation (Levey et al., 2009) which uses serum creatinine, age and sex. The inclusion of race is controversial since its use often reflects ethnicity rather than the biological construct of race and so a 2021 update to the CKD-EPI equation was designed not to include a race coefficient at all. The 2021 equation was found to underestimate GFR in Black populations but overestimate GFR in non-Black populations (Inker et al., 2021) therefore the 2009 equation (without the race coefficient) has remained the preferred method in European nephrology (largely White populations) (Gansevoort et al., 2023). Attempts to improve the estimation of GFR have seen the development of equations using the biomarker cystatin C which overcomes some of the limitations of non-GFR determinants of serum creatinine which can bias estimates of GFR especially in those with extremes of muscle mass. Equations using both serum creatinine and cystatin C perform better in estimation of GFR than either analyte in isolation (Inker et al., 2021). Nevertheless, cystatin C too has limitations (its own non-GFR determinants and systematic differences between manufacturers at present) meaning there is still a need for further work to improve the estimation of GFR.

Albuminuria describes the presence of abnormal levels of albumin (protein) in the urine which is pathognomonic of kidney damage. Since albumin’s small molecules are not

normally filtered across the semi-permeable membrane of the glomerulus in health, albuminuria is an early indicator of glomerular damage and often precedes reduction in GFR. Albuminuria is preferred to the less specific assessment of proteinuria. Proteinuria is a more general term reflecting abnormal levels of protein in the urine which can have several causes; not just the glomerular damage resulting in albuminuria. Other causes of proteinuria include "tubular proteinuria" due to failure of normal tubular reabsorption of filtered proteins; as well as increased protein production, for example due to haematological disorders producing excess immunoglobulin light chains. Guidelines recommend the quantification of albuminuria rather than proteinuria in the assessment of CKD since albumin is the main component of proteinuria in most CKD and has also been shown to be independently associated with kidney and cardiovascular outcomes (KDIGO, 2024).

CKD is broadly categorised as stages 1-5 based on eGFR however a more detailed classification also takes account of albuminuria in which these GFR-based stages 1-5 are reported as G1-G5 with an additional categorisation applied reflecting levels of albuminuria A1-A3 in mg/g or mg/mmol (varies geographically). CKD stage is therefore reported, in its more detailed form, for example, as CKD stage G3bA2. The staging of CKD as per the Kidney Disease Improving Global Outcomes (KDIGO) guidelines is depicted in Figure 1-1 in which heat map colouring reflects the associated combined risks of CKD progression, cardiovascular outcomes and mortality for each stage (KDIGO, 2024).

Figure 1-1: KDIGO staging of CKD (KDIGO, 2024)



The clinical manifestations of CKD are hugely variable and somewhat affected by cause and individual disease course. Though often an incidental finding and asymptomatic until the later stages of disease, the diagnosis of CKD has important health implications which can be broadly categorised as (1) progressive decline in kidney function; (2) complications of reduced glomerular filtration; and (3) cardiovascular disease.

CKD is typically a progressive condition and the eventual outcome is kidney failure necessitating kidney replacement therapy with either dialysis or kidney transplantation. The need for kidney replacement therapy occurs in a minority of people with CKD but is a very costly treatment which is associated with markedly reduced life expectancy.

A more sophisticated estimation of risk of CKD progression has recently been developed in the Kidney Failure Risk Equation (KFRE) which incorporates age, sex, eGFR and albuminuria in the 4-variable equation (Tangri et al., 2011). An eight-variable equation was also developed which additionally incorporates serum albumin, bicarbonate, calcium and phosphate. The 4-variable KFRE has been validated in UK (and multinational) cohorts for the prediction of 2- and 5-year risk of kidney failure (Major et al., 2019) and adopted to guide referrals from primary to secondary care in the UK.

1.1.3 COMPLICATIONS OF REDUCED GLOMERULAR FILTRATION

Complications of CKD occur increasingly at more advanced stages of disease and commonly include hypertension, anaemia, mineral bone disorder and other metabolic disturbance. These objective and measurable complications are also often accompanied by less well-defined symptoms such as nausea, itch, anorexia, cachexia, sexual dysfunction, fatigue, poor sleep and overall reduced quality of life.

High blood pressure is both a cause and consequence of CKD and is a particularly concerning complication since it is thought to further accelerate the progression of declining glomerular filtration as well as being independently associated with mortality and cardiovascular disease (Bello et al., 2017). Hypertension occurs as a complication of CKD by several mechanisms including disordered salt and water homeostasis and activation of the sympathetic nervous and renin-angiotensin systems. CKD causes anaemia

since red blood cell production relies on renal production of the hormone erythropoietin as well as functional iron deficiency. Anaemia may cause fatigue and shortness of breath and is treated with erythropoiesis-stimulating agents and iron supplementation in CKD. Mineral bone-related complications of CKD are defined as a syndrome referred to as CKD-mineral and bone disorder (CKD-MBD). CKD-MBD encompasses a range of manifestations of hyperphosphataemia, vitamin D deficiency and secondary hyperparathyroidism as direct consequences of CKD; all of which have specific pharmacological treatments. Salt and water retention, electrolyte disturbance and metabolic acidosis are all further sequelae of advanced CKD which contribute to the morbidity of CKD. All of the aforementioned complications generally rely on monitoring and clinician identification since they are either asymptomatic or manifest as non-specific symptoms.

The increased burden of cardiovascular disease (CVD) is an important and modifiable consequence of CKD since it is the commonest cause of death in people with CKD and more common than reaching kidney failure (requiring initiation of kidney replacement) (Thompson et al., 2015). Both reduced eGFR and albuminuria are independently associated with the increased risk of CVD in CKD (Matsushita et al., 2010, van der Velde et al., 2011). Recent figures from the US Renal Data System (USRDS) 2022 report that 65% of those with CKD have CVD, increasing to 80% in those aged 85 years and over. Cardiovascular disease overall is 2-3 times more common in individuals with CKD than those without, with the largest gaps seen for heart failure and myocardial infarction specifically, both of which are around 4 times more common in individuals with CKD. The burden of CVD in CKD also increases with advancing CKD stage, particularly for heart failure which was 1.5 times more common in CKD stages 4-5 versus stage 3 in the 2022 United States data (United States Renal Data System, 2022).

The associations between CKD and CVD are in part due to shared risk factors such as diabetes, blood pressure and obesity; but also pathophysiological changes associated with advancing CKD (as discussed in the previous section) which affect the cardiovascular system such as uraemic toxicity, anaemia, inflammation, fluid overload and activation of the sympathetic and renin-angiotensin systems (Matsushita et al., 2022). Heart failure, arrhythmia and related sudden cardiac death are more prominent pathologies in CKD than atherosclerotic cardiovascular disease presentations. Treatment of CKD must importantly

be aimed at addressing associated cardiovascular comorbidity as well as slowing kidney disease progression.

1.2 BACKGROUND – TREATMENT OF CHRONIC KIDNEY DISEASE

For about two decades, pharmacotherapy to slow the progression of CKD was largely limited to the use of renin-angiotensin system (RAS) inhibitors from ~2001-2019. RAS inhibitors were shown, in large randomised controlled trials, to reduce proteinuria and progression of kidney disease as well as modestly reducing blood pressure in CKD. The REIN trial, reported in 1997, assessed the effect of the angiotensin-converting enzyme inhibitor ramipril on GFR decline in 352 patients with non-diabetic causes of proteinuric kidney disease. Ramipril significantly slowed GFR decline and reduced proteinuria versus placebo though blood pressure was similar in both groups (Porter, 1997). The RENAAL trial, reported in 2001, tested the angiotensin receptor blocker losartan in 1513 patients with type 2 diabetes and nephropathy and reported risk reductions of around one quarter in kidney disease progression outcomes relative to placebo, after adjustment for blood pressure, though no effect was observed on mortality. Losartan use was also associated with a 35% reduction in proteinuria (Brenner et al., 2001). The discovery of RAS inhibitors had an important impact on the treatment of CKD however considerable excess risk of adverse outcomes remained, driving the need for discovery of additional therapies.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are the first class of drugs to show promise in slowing the progression of CKD since the advent of RAS inhibition. SGLT2 inhibitors were originally developed to lower blood glucose in the treatment of type 2 diabetes and were incidentally found to have beneficial effects on cardiovascular outcomes in safety trials. This led to the dedicated testing of these agents in heart failure populations in which exploratory analyses suggested the drugs may also have kidney-protective effects. This hypothesis was confirmed in the CREDENCE trial assessing kidney disease outcomes in a population with type 2 diabetes (Perkovic et al., 2019). Hypotheses emerged that SGLT2 inhibitors may also be efficacious in patients without diabetes, hypotheses which were again confirmed in further trials in heart failure populations without diabetes. There was therefore a need to test these drugs in a dedicated CKD trial in patients with and without diabetes which led to the EMPA-KIDNEY trial. A similar trial, the DAPA-CKD trial, of the SGLT2 inhibitor dapagliflozin ran in parallel and reported its results in late

2020. The results of DAPA-CKD reported that dapagliflozin reduced the risk of a composite kidney disease progression or cardiovascular death outcome by 29% (hazard ratio 0.71, 95% confidence interval 0.55-0.92) (Heerspink et al., 2020). DAPA-CKD studied 4304 participants with CKD with an eGFR between 25 and 75 mL/min/1.73m^2 and a urinary albumin-to-creatinine ratio (uACR) of 200-5000 mg/g; two-thirds of whom had type 2 diabetes. The EMPA-KIDNEY trial would extend these findings having recruited a larger population of 6609 participants and address remaining uncertainty in certain patient groups namely those with CKD without diabetes and those with low levels of or no detectable albuminuria.

EMPA-KIDNEY was also stopped early for benefit having met its pre-specified criteria for stopping early for efficacy in March 2022. The results of the trial are reported elsewhere but in brief, empagliflozin 10 mg once daily reduced the risk of kidney disease progression or cardiovascular death by 28% (95% CI 18-36%) in 6609 patients with CKD at risk of progression (EMPA-KIDNEY Collaborative Group, 2023). Meta-analyses combining results from EMPA-KIDNEY with twelve other trials in diabetes, heart failure and CKD populations conclusively demonstrated the benefits of SGLT2 inhibitors in reducing adverse kidney and cardiovascular outcomes irrespective of diabetes status or kidney function. Furthermore, although EMPA-KIDNEY alone was unable to quantify effects on cardiovascular outcomes and acute kidney injury owing to low numbers of events, meta-analyses of data from four CKD trials demonstrate that SGLT2 inhibitors reduce the risk of cardiovascular death or hospitalisation for heart failure by 23% (relative risk [RR] 0.77, 95% confidence interval [CI] 0.74-0.81) and reduce the risk of acute kidney injury by 23% (RR 0.77, 95% CI 0.70-0.84) (Nuffield Department of Population Health Renal Studies Group and SGLT2 inhibitor Meta-Analysis Cardio-Renal Trialists' Consortium, 2022).

SGLT2 inhibitor trials have also consistently demonstrated modest blood pressure lowering effects and weight loss in the region of 1-2 kg with reduction in waist circumference (Zinman et al., 2015). However, the mechanisms underlying the beneficial effects on clinical outcomes are incompletely understood. Moreover, whether weight loss reflects fat loss versus reduction in fluid volume or even muscle mass is uncertain. Several purported mechanisms have been proposed to explain the kidney and cardioprotective effects observed. Haemodynamic effects represent a primary mechanism and of particular

relevance to fluid overload and the focus of this thesis, is the diuretic and natriuretic effect of SGLT2 inhibitors (Herrington et al., 2021). This results in a reduction in interstitial fluid and blood volume (Griffin et al., 2020, Hallow et al., 2018) as well as blood pressure which, in combination, reduce cardiac preload and afterload (Herrington et al., 2021). SGLT2 inhibitors also exert metabolic effects which are also highly relevant to body composition. Previous studies in people with diabetes and without kidney disease using dual energy X-ray absorptiometry have found weight loss with SGLT2 inhibitors to be largely attributable to reduced adiposity (Ridderstråle et al., 2014). However, other randomised evidence suggests these weight loss benefits are maintained at lower levels of kidney function despite the reduced glycosuric effect of these drugs with reduced kidney function (Petrykiv et al., 2017, Kohan et al., 2014, Cherney et al., 2018) which supports the hypothesis of reduction in body water as a major determinant of weight loss. These studies also highlight important differences between people at different stages of the CKD trajectory who, as well as behaving differently with respect to glycosuria, have differing degrees of disturbed salt and water homeostasis contributing to fluid overload.

1.3 BACKGROUND – RELEVANCE OF FRAILTY, MULTIMORBIDITY, POLYPHARMACY AND HEALTH-RELATED QUALITY OF LIFE IN THE TREATMENT OF CHRONIC KIDNEY DISEASE

The effects of empagliflozin on the primary outcome of progression of kidney disease or cardiovascular death in the EMPA-KIDNEY trial were broadly consistent across the subgroups studied. Key subgroups were by diabetes status, eGFR and uACR with additional subgroups including age, sex, region, prior disease status (cardiovascular disease, heart failure, peripheral arterial disease and cause of kidney disease separately), medication use and other clinical and laboratory parameters measured at recruitment. In reality, patients often have several of these subgroup characteristics in combination, particularly patients who are older in age and/or who have frailty. In clinical practice, uncertainty exists surrounding the benefit-risk profile of disease-modifying drugs like SGLT2 inhibitors in older patients with frailty. Clinical guidelines recommend an individualised approach to prescription of SGLT2 inhibition in CKD taking account of frailty and multimorbidity; and the balance of disease and treatment burden. Since frailty is associated with increased burden of long-term conditions and associated polypharmacy, there may be clinician and/or patient reluctance to prescribe drugs like SGLT2 inhibitors due to perceived altered benefit-risk ratio. Conversely, frail patients may be at high

absolute risk of adverse outcomes and consequently may particularly benefit from the effects of SGLT2 inhibition. Reliable evidence is therefore needed to enable practical implementation of current SGLT2 inhibitor guidelines since without randomised evidence, perceived altered benefit-risk profile may lead to under-treatment of frail patients.

Frailty can be defined as the state of increased vulnerability due to age-related decline in physiological reserve. The relationship between frailty and CKD is bidirectional and frailty occurs more commonly in CKD relative to the general population (Hurst et al., 2022), particularly as CKD progresses (Wilkinson et al., 2022). Frailty confers poor prognosis with greater risks of death, hospitalisation and progression to kidney failure compared to non-frail adults with CKD (Mei et al., 2021, Zhang et al., 2020, Wilkinson et al., 2022). Multimorbidity and polypharmacy are closely related but distinct concepts to frailty. Multimorbidity is typically defined as the presence of two or more long term conditions (Ho et al., 2022), and polypharmacy is generally defined as the regular prescription of five or more drugs (Masnoon et al., 2017). Various instruments are also used to assess health-related quality of life, one such tool is the EuroQol EQ-5D-5L questionnaire (Herdman et al., 2011). Importantly, health-related quality of life is a patient-reported measure reflecting symptom burden and may therefore add valuable information to the assessment of frailty beyond clinician assessments. There are several different tools applied to quantify frailty in clinical practice and research. “Validated” approaches in general populations include the Fried frailty phenotype (Fried et al., 2001), the Clinical Frailty Scale (CFS)(Rockwood et al., 2005) and the Rockwood Frailty Index (Searle et al., 2008). Common components of frailty assessments include comorbid medical conditions, quality of life, cognitive assessments, physical measurements and laboratory parameters; all of which reflect deficits in health which accumulate with aging. The Rockwood Frailty Index was developed in community-dwelling adults aged over 70 years in the United States of America and applies weights to each comorbidity (Searle et al., 2008) which may not be generalisable across diseased populations. A bespoke approach was therefore used in analyses of the EMPA-KIDNEY trial population as outlined in sections 2.6 and 2.7 of the Methods Chapter.

1.4 BACKGROUND – FLUID OVERLOAD IN CHRONIC KIDNEY DISEASE

Though CKD is often asymptomatic, fluid overload is a common manifestation as the disease progresses and has both clinical and prognostic implications (prognostic implications are discussed in section 1.6.1.3 and Chapter 3). Disturbed water and sodium homeostasis causes expansion of the extracellular water compartment (Liu et al., 2021) and as CKD progresses, removal of excess fluid is impaired which manifests as symptomatic fluid overload, ultimately requiring kidney replacement therapy. An important factor which contributes to fluid overload in CKD is concomitant heart failure. The conditions commonly coexist but are often considered separately in research and clinical practice. The burden of heart failure increases with advancing CKD with estimated prevalence of clinical heart failure of around 40% in patients requiring dialysis (House et al., 2019, Foley, 2003, Mark et al., 2022). Structural heart disease on echocardiography is perhaps twice as common, with heart failure with preserved ejection fraction (HFpEF) the more frequent phenotype in CKD than heart failure with reduced ejection fraction (HFrEF) (Foley, 2003, Mark et al., 2022). This interrelationship may be explained in part by shared risk factors but also by bidirectional aetiological mechanisms. Heart failure increases the risk of CKD due to impaired perfusion of the kidneys and neurohormonal activation (Tuegel and Bansal, 2017, Fonarow and Heywood, 2006), and there are a number of pathophysiological changes associated with advancing CKD which contribute to heart failure. These include chronic hypertension and fluid overload as well as the possibility for direct uraemia-related cardiotoxicity (Tuegel and Bansal, 2017). The mechanisms underlying the development of fluid overload in heart failure specifically are less well understood than the development of fluid overload due to impaired kidney function however its consequences are clear – increased diastolic filling pressures and impaired cardiac function (Miller, 2016). Though there may be distinct mechanisms at play, CKD and heart failure share evidence-based treatments, first in RAS inhibitors and more recently in SGLT2 inhibitors and mineralocorticoid receptor antagonists.

SGLT2 inhibitors are now established treatments for both CKD and heart failure and though their osmotic and diuretic mechanisms are undoubted, their effects on fluid status have not been well-demonstrated in previous trials. *Post-hoc* analyses of the earliest cardiovascular outcome SGLT2 inhibitor trial, EMPA-REG OUTCOME which studied 7020 people with type 2 diabetes and established cardiovascular disease explored effects on haematocrit, a surrogate of plasma volume (Inzucchi et al., 2018). Analyses suggested

significant increases in haematocrit in those receiving empagliflozin versus placebo and that this mediated 52% of the treatment effect on cardiovascular death. However, a key limitation of haematocrit as a fluid status surrogate in this setting is that SGLT2 inhibitors promote erythropoiesis and so increases in haematocrit are likely multifactorial (Inzucchi et al., 2018). In the EMPEROR-Reduced trial assessing the effects of empagliflozin in 3730 people with heart failure with reduced ejection fraction, a crude clinical assessment of fluid status was made at baseline but there were no repeated measures of fluid status and therefore no assessment of treatment effect on fluid status (Packer et al., 2021). Randomised evidence relating to fluid status is even more limited in CKD-specific populations with no randomised data available prior to the work described in this thesis. Small observational studies largely in cohorts with diabetes and/or heart failure but with preserved kidney function do provide some evidence of transient reductions in body fluid (both plasma volume and interstitial fluid) with SGLT2 inhibitor administration using a variety of fluid status assessment methods, as summarised in Table 1-1.

Fluid overload is traditionally assessed non-quantitatively by clinical examination (Miller, 2016) taking account of factors such as perceptible oedema, chest auscultation and jugular venous pressure combined with objective assessments of body weight and blood pressure. Serological markers can also give information on volume status such as sodium, haematocrit and natriuretic peptides. Each of these methods have limitations therefore bedside medical devices which may have the potential to automate and standardise clinical assessment have been developed using ultrasound and bioimpedance technology. Both methods can be employed with relatively little training and allow rapid clinic-based measurements. Ultrasound modalities include lung ultrasonography (Rastogi et al., 2022, Ekinci et al., 2018, Zoccali et al., 2021) and less commonly, vascular ultrasound of the inferior vena cava and internal jugular veins (Ekinci et al., 2018, Pellicori et al., 2021). Bioimpedance methods have been demonstrated to be highly reproducible and have been validated against gold standard techniques (Wabel et al., 2009) more extensively than ultrasound approaches. Ultrasound methods require specially-trained operators however bioimpedance devices can be employed with little training required, making this an attractive approach. Bioimpedance is widely employed in clinical management of CKD in the dialysis setting but not routinely in earlier CKD nor at all in the management of heart failure.

Table 1-1: Existing literature on the effects of SGLT2 inhibition on fluid status

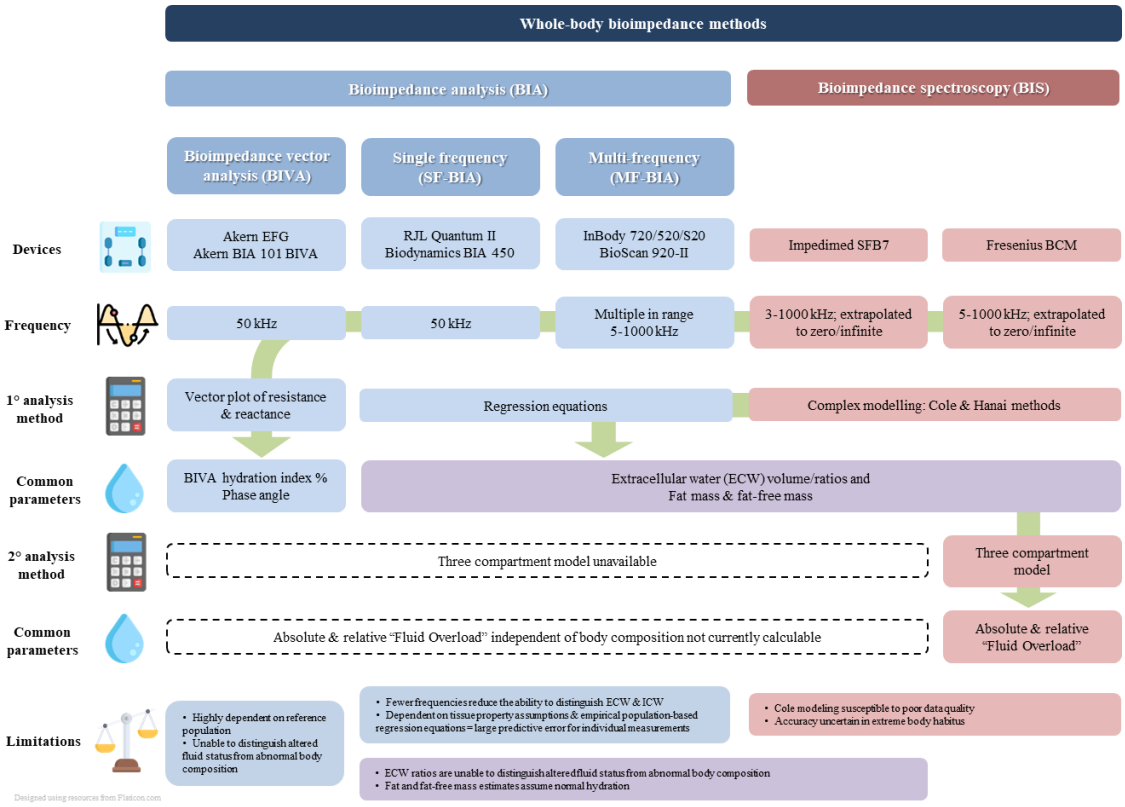
Study	Design	Population	Intervention/control	Outcomes	Fluid assessment method	Findings
Van Ruisen, 2022 Cardiovasc Diabetology DECREASE trial	Randomised controlled trial (RCT) 16 weeks	n=66 Type 2 diabetes without CKD	Dapagliflozin, exenatide, dapagliflozin plus exenatide, placebo	Estimated plasma volume Haemodynamic parameters	Estimated plasma volume using haematocrit ImpediMed bioimpedance spectroscopy device	Dapagliflozin compared with placebo decreased extracellular fluid and estimated plasma volume after 10 days, but not after 16 weeks
Sen, 2022 Diab Obes Metab DAPASALT & DIAMOND studies	Single arm interventional study (DAPASALT) & crossover RCT (DIAMOND)	DAPASALT n=6 with CKD (stratum 3) (mean eGFR 39) DIAMOND n=53 with CKD (mean eGFR 59)	Dapagliflozin	24-hour sodium excretion, blood pressure Bioimpedance-derived extracellular and intracellular water	ImpediMed bioimpedance spectroscopy device	Transient reduction in extracellular (but not intracellular) water at day 4, recovered by day 14 (Effects on sodium excretion, blood pressure reported from both studies in same paper, appears bioimpedance data from DAPASALT only, not DIAMOND)
Scholtes, 2021 Diabetes Care DAPASALT study*	Single arm interventional study 18 days	n=14 Type 2 diabetes, preserved eGFR (stratum 2)	Dapagliflozin	24-hour sodium excretion Bioimpedance-derived extracellular and intracellular water	ImpediMed bioimpedance spectroscopy device	Transient reduction in extracellular (but not intracellular) water at day 4, recovered by day 14
Espriella, 2021 Int J of Cardio	Retrospective cohort Median 1.8 years	n=60 Heart failure with type 2 diabetes	Empagliflozin	CA125, NT-proBNP (as surrogates of fluid overload)	CA125, NT-proBNP	Decrease in log CA125 but not NT-proBNP
Jensen, 2021 Lancet Diabetes Endocrinol Empire HF Renal trial	RCT 12 weeks	n=120 Heart failure (HFrEF)	Empagliflozin versus placebo	Estimated plasma and extracellular volumes Measured GFR	Estimated plasma volume using haematocrit Estimated extracellular volume using 51Cr-EDTA distribution volume and the body surface area	Empagliflozin reduced estimated plasma and extracellular volumes versus placebo at 12 weeks
Schork, 2019 Cardiovasc Diabetology	Longitudinal observational study 6 months	n=27 Type 2 diabetes eGFR>60	Empagliflozin or dapagliflozin initiated routinely	HbA1c, weight, body mass index BCM-derived overhydration, extracellular water, adipose, fat and lean tissue mass Plasma renin	Fresenius BCM	Transient reductions in OH, ECW at day 3, recovered by 3- and 6 months Significant decrease in adipose and fat tissue mass Concluded weight loss is due to fat loss
Schwaiger, 2019 Am J Transplant EMPTRA-DM study	Pilot study 1 year	n=8 Kidney transplant recipients with post-transplant diabetes	Empagliflozin non-inferiority to insulin	Glycaemic markers eGFR, bioimpedance-derived extracellular and total body water	Fresenius BCM	Transient reductions in extracellular and total body water at 4 weeks then recovered
Heerspink, 2013 Diab Obes Metab	RCT 12 weeks	n=75 Type 2 diabetes without CKD	Dapagliflozin versus hydrochlorothiazide versus placebo	Plasma volume NT-proBNP	Plasma volume using radioisotope techniques with 51Cr-labelled erythrocytes and 125I-labelled human serum albumin	Plasma volume fell in dapagliflozin group NT-proBNP increased slightly in dapagliflozin group
Santos-Gallego [ongoing study] ERTU-SODIUM trial NCT05152940	RCT (crossover)	n=28 Heart failure (HFrEF)	Ertugliflozin versus placebo	Skin water content Secondary outcomes include interstitial fluid volume (extracellular minus plasma volume)	Method not reported on registration	Ongoing study (estimated completion December 2024)

* The DAPASALT study had three strata: patients with type 2 diabetes and impaired kidney function (stratum 1), type 2 diabetes and preserved kidney function (stratum 2), and no diabetes but impaired kidney function (stratum 3), with a total sample size of 51 patients (17 patients per stratum); results are reported in separate papers as above for strata 2 and 3.

1.5 BACKGROUND – BIOIMPEDANCE TECHNIQUES AND DEVICES

Bioimpedance is a non-invasive measure of resistance and reactance of body tissues quantified by application of an electrical current via electrodes attached to the skin from which fluid compartment volumes and body composition can be estimated. Bioimpedance methods include both bioimpedance analysis (BIA) (single-, multi-frequency and bioimpedance vector analysis [BIVA]) and bioimpedance spectroscopy (BIS). Although often incorrectly used interchangeably, bioimpedance analysis and bioimpedance spectroscopy are not synonymous terms. These four main bioimpedance approaches are summarised in Figure 1-2 including their analysis methods, commonly reported parameters and key advantages/disadvantages. BIA traditionally used only a single frequency before multi-frequency BIA devices were developed which measure impedance at 50-200 discrete frequencies between 3-1000 kHz. BIS extends this range by extrapolation to zero and infinity kHz. Greater frequency range improves discrimination of extracellular (ECW) from intracellular water.

Figure 1-2: Summary of whole-body bioimpedance methods



References: Khalil et al. Sensors (Basel). 2014; Piccoli et al. Kidney Int. 2005; Jaffrin et al. Med Eng Phys. 2008; Keane. University of Leeds (thesis). 2016. Chamney et al. Am J Clin Nutr. 2007.

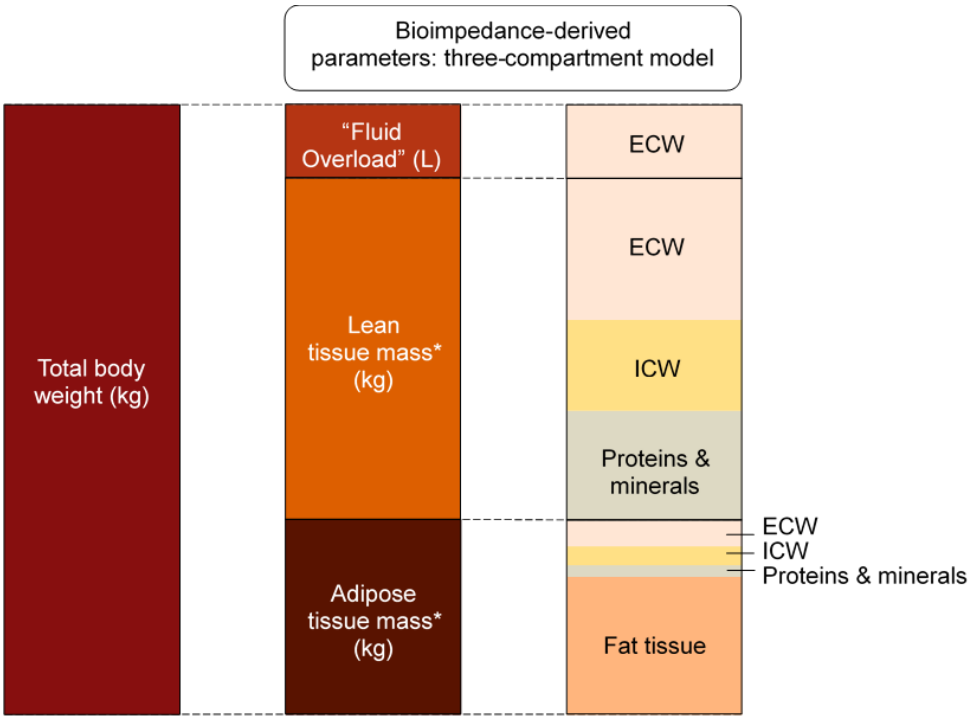
Both single and multiple frequency BIA methods are dependent upon empirical regression equations to translate impedance data into fluid overload parameters (Jaffrin and Morel, 2008). BIS uses a more complex method involving extrapolation of impedance to zero and infinity frequencies to estimate extracellular and total body water volumes (Jaffrin and Morel, 2008). Bioimpedance vector analysis (BIVA) is an alternative technique which is less reliant on assumptions of tissue properties than BIA/BIS methods and directly plots raw reactance and resistance as vectors on a graph for which reference ranges have been established however BIVA is a single-frequency technique (Keane, 2016).

While both BIA and BIS have been applied in heart failure and CKD, the Fresenius Medical Care (FMC) Body Composition Monitor (BCM) which uses BIS is the most widely employed in patients with kidney disease and was selected for use in the EMPA-KIDNEY bioimpedance substudy. A key advantage of this device is that it employs secondary calculations taking account of estimates of lean and adipose tissue mass (by applying a three compartment model (Chamney et al., 2007)) thereby providing more specific estimates of fluid excess, independent of body composition – the only commercially-available device to do this. As indicated by the green arrows in Figure 1-2, vector plots, BIVA hydration index and phase angle can be derived by all devices; ECW ratios, fat and fat-free mass can only be derived from BIA & BIS devices; and only the BCM device produces absolute and relative “Fluid Overload” independent of body composition.

The three-compartment model (Figure 1-3) was described by Chamney et al. in 2007 (Chamney et al., 2007) and builds upon methodology published in the earlier paper by the same group (Moissl et al., 2006). The group first present equations for determination of extracellular and intracellular water with correction for body mass index. The subsequent paper then expanded the methodology to allow derivation of excess fluid volume which accumulates in pathological states, distinct from total body (extracellular and intracellular) water. This led to the three-compartment model separately estimating normally-hydrated lean tissue mass, normally-hydrated adipose tissue mass and the volume of excess extracellular fluid (termed “overhydration” and more recently “absolute Fluid Overload”) which can reside in either adipose or lean tissue. This assumes that in a state of health, lean and adipose tissue are considered “normally-hydrated” and the excess extracellular fluid

volume accumulates in disease states. Excess fluid volume can be estimated with precision of ±0.5 kg based on the assumption of fixed tissue hydration parameters (i.e. any given mass of tissue has a fixed proportion of extra- and intracellular water, irrespective of body composition (Chamney et al., 2007)). The ratio of extra- to intracellular water in both lean and adipose tissue is therefore considered constant. The methodology was developed in adults and has not been directly applied in children since the water content of adipose tissue changes during childhood. Normally-hydrated lean tissue comprises largely water (extra- and intracellular), plus protein, minerals (osseous and non-osseous) and essential lipids while normally-hydrated adipose tissue is composed largely of fat tissue (stored lipids), with some water, protein and non-osseous minerals to a lesser extent (see Figure 1-3). Lean and fat tissue masses are indexed to height squared and expressed as lean and fat tissue indices (LTI and FTI) in kg/m^2, these indexed parameters are also reported directly by the device and preferred for analysis purposes. Of note, relating these indices to the three-compartment model, LTI is calculated directly from lean tissue mass (LTM) indexed to height squared whereas FTI comes from the fat tissue component of the adipose tissue mass compartment (not the entire compartment mass), indexed to height squared.

Figure 1-3: Relationship of the derived "Fluid Overload" parameter to body weight and tissue mass



Based upon the three-compartment model described by Chamney et al.(Chamney et al., 2007) * Refers to normally-hydrated lean and adipose tissue mass. ECW = extracellular water; ICW = intracellular water. The figure is not to scale since compartment proportions vary between individuals and "Fluid Overload" is usually smaller than depicted (and can be a negative value in fluid depletion). The mean baseline values in the EMPA-KIDNEY substudy were: total body weight 88.8 kg; "Fluid Overload" 0.4 L; lean tissue mass 38.8 kg; and adipose tissue mass 49.6 kg. In the EMPA-KIDNEY substudy, mean total ECW at baseline was 18.7 L and ICW 20.4 L.

Importantly, the three-compartment model methodology employed by the BCM device does not delineate intravascular or plasma volume. Since plasma contains both lipids and proteins and since excess fluid or "overhydration" accumulates both intravascularly and in interstitial tissue, plasma volume likely resides within all three compartments of the three-compartment model.

The BCM device has been validated for fluid status assessment in kidney failure populations against gold standard techniques (Wabel et al., 2009) and shown to have acceptable reproducibility (Hannan et al., 1995, Wabel P, 2007). Differing reference methods are used for difference bioimpedance-derived parameters: bromide dilution for extracellular water; total body potassium for intracellular water; and deuterium dilution for total body water (Wabel et al., 2009). The "Fluid Overload" parameter is described by the device manufacturer Fresenius as validated "by expert clinical assessment" – no specific method exists for this comparison however "Fluid Overload" is derived from extracellular water which is validated with an accepted reference method. Lean and adipose tissue mass are compared to dual energy x-ray absorptiometry (DEXA) assessments with additional techniques employed to compare adipose tissue mass (air displacement plethysmography and under water weighing).

1.6 BACKGROUND – APPLICATIONS OF BIOIMPEDANCE SPECTROSCOPY IN CKD & HEART FAILURE

Bioimpedance spectroscopy has many potential applications in CKD but is presently almost exclusively used in clinical practice to assess fluid status in patients with kidney failure requiring haemodialysis (and much less so in the setting of peritoneal dialysis). Similarly, research using bioimpedance spectroscopy in CKD is largely restricted to analysis of fluid parameters in kidney failure populations. These clinical and research applications are discussed further in sections 1.6.1.1 and 1.6.1.2 which follow. The adiposity parameters derived by the BCM device (lean and fat tissue indices, LTI and FTI) are much less studied and much less used in clinical practice despite theoretical benefits to nutritional assessment.

1.6.1 APPLICATIONS IN DIALYSIS CONTEXT

1.6.1.1 CLINICAL APPLICATIONS IN DIALYSIS

Clinically, accurate volume assessment is an essential component of dialysis prescription. Although routine clinical assessments may often be sufficient to avoid extremes of hydration status, adjunctive BCM assessments have theoretical advantages in kidney failure requiring replacement therapy. Tracking fluid status using a BCM can be achieved with minimal training and should provide objective measures with less potential for between-observer differences than clinical assessments. Measurements are typically made pre-dialysis (rather than after fluid removal achieved by ultrafiltration during haemodialysis treatment) and observational associations with mortality are more consistent when fluid status is assessed pre- rather than post-haemodialysis (Tangvoraphonkchai and Davenport, 2016, Dekker et al., 2017, Hecking et al., 2018).

The use of the BCM in routine clinical practice is much less common in patients receiving peritoneal dialysis compared with haemodialysis. It is generally considered that BCM measurements are not affected by presence or absence of peritoneal dialysate (Van Biesen et al., 2011) although measurements are generally obtained with dialysate *in situ* (O'Lone et al., 2014, Jotterand Drepper et al., 2016) and there is some uncertainty (Arroyo et al., 2015). The presence or absence of indwelling dialysate is likely inconsequential in practice if serial measurements follow a consistent approach for the individual patient.

1.6.1.2 RESEARCH APPLICATIONS IN DIALYSIS

The BCM device has also been widely used in clinical research in dialysis populations. The most common research application is the study of associations between BCM parameters and clinical outcomes (as discussed in section 1.6.1.3) and other related parameters in observational study designs. However the BCM device has also been employed in randomised controlled trials in haemodialysis populations, both as an intervention itself (Table 1-2) and as a mechanistic assessment of another intervention (Table 1-3).

Bioimpedance-based assessment of fluid status versus standard clinical assessment has been shown in randomised controlled trials to improve parameters such as blood pressure,

left ventricular mass and arterial stiffness (Scotland et al., 2018, Hur et al., 2013, Onofriescu et al., 2014, Huan-Sheng et al., 2016). Reduction in intradialytic hypotension was also a theorised benefit of bioimpedance however this has not been conclusively demonstrated in randomised trials (Beaubien-Souligny et al., 2019). The observed clinical benefits are, however, yet to be shown to impact upon risk of hard clinical outcomes: randomised trials comparing bioimpedance added to standard care versus standard of care alone have not demonstrated meaningful impact on hospitalisations (Huan-Sheng et al., 2016, Siriopol et al., 2017a), preservation of residual kidney function (Davies et al., 2023, Yoon et al., 2019, Oh et al., 2018), cardiovascular outcomes or death (Siriopol et al., 2017a, Tian et al., 2020, Onofriescu et al., 2014, Huan-Sheng et al., 2016), but numbers of outcomes in completed trials are generally small. The most recent of these trials was the BISTRO trial, reported in 2023, which randomised 439 UK haemodialysis patients with residual kidney function (more than 500 ml urine production per day or eGFR greater than 3 mL/min/1.73m^2) to either bioimpedance-supplemented fluid assessment or a standardised clinical assessment proforma (without bioimpedance) for up to 2 years. The trial found no significant between-group differences in the primary outcome of time to anuria nor were there any differences in rate of residual kidney function decline, blood pressure or patient-reported outcomes. There were only 32 deaths during follow-up, 15 in the intervention group versus 17 in the control group. The trial was unfortunately limited by under-recruitment during the COVID-19 pandemic and lower-than-expected decline in residual kidney function limiting power for this assessment. The investigators concluded that the standardised protocol (used in the control group) was associated with excellent clinical management and preservation of residual kidney function which could not be improved by the addition of bioimpedance assessments (Davies et al., 2023).

In trials of other interventions, the BCM has been used to assess eligibility and outcomes and guide interventions. In the BVM-Reg trial of different techniques to monitor ultrafiltration (Antlanger et al., 2017), BCM-assessed severe “Fluid Overload” ≥15% was an inclusion criterion. The SOLiD trial (Marshall et al., 2020) found that although allocation to a lower dialysate sodium of 135 mmol/L versus 140 mmol/L did not lead to any significant effect on the primary outcome of left ventricular mass index, the intervention did reduce BCM-measured ECW by about 0.6 L over the 12-month trial. Bioimpedance is also being employed in the ongoing RESOLVE trial (ClinicalTrials.gov Identifier: NCT02823821) evaluating dialysate sodium seeking to provide further

randomised evidence in this area. The BCM was also used as an outcome assessment in a small crossover trial of salt-restricted diet in people with stage 3-4 CKD and hypertension in which the intervention was shown to reduce extracellular water volume (McMahon et al., 2013). Though not the BCM device, a multifrequency bioimpedance analysis device (InBody 720) was used in a trial in a slightly different manner to inform the intervention protocol: the BELIEVE pilot trial tested intravenous bicarbonate for prevention of contrast-induced acute kidney injury after coronary angiography and used bioimpedance measurements to dictate the volume of intravenous bicarbonate to be prescribed (Kananuraks et al., 2020). The pilot study found no significant effect of the intervention but nevertheless demonstrates another potential application of the technology in clinical trials.

Table 1-2: Trials of bioimpedance as an intervention

Trial	Design	Population	Intervention/control	Outcome	Results
Haemodialysis					
Davies, 2023 Kidney International BISTRO trial	RCT 2 year	n=439 Haemodialysis UK (34 centres)	1-to-3-monthly BCM-guided dry weight assessment Control: BCM performed but not available to treating clinicians	Residual kidney function	No significant difference
Sommerer, 2021 HD International	RCT <6 months	n=132 Haemodialysis Germany (single centre)	BCM at first & last visit & according to clinical need Control: BCM at first & last visit but not available to clinicians	NT-proBNP	No significant difference More hypovolaemia adverse events in intervention group (41 vs 24, p=0.002)
Liu, 2020 BMC Nephrology BOCOMO study	RCT 1 year	n=445 Haemodialysis China (11 centres)	2-monthly BCM-guided dry weight assessment Control: BCM performed but not available to treating clinicians	Composite of death, MI, stroke, peripheral vascular disease	No significant difference
Patel, 2019 Indian J Nephrol	RCT 6 months	n=50 Haemodialysis India (2 centres)	2-weekly BCM-guided dry weight assessment Control: BCM performed but not available to treating clinicians	Blood pressure, intradialytic complications	Blood pressure similar but with reduced pill burden in intervention group Less intradialytic hypotension in intervention group
Siriopol, 2017 Int Urol Nephrol BUST study	RCT 2 year	n=250 Haemodialysis Romania (2 centres)	Weekly/monthly lung ultrasound + BCM if clinical hypovolaemia Control: routine care	Composite all-cause mortality and CVE - death, stroke, MI	No significant difference Less pre-dialysis dyspnoea, but more intradialytic cramps in intervention
Huan-Sheng, 2016 Int Urol Nephrol ABISAD-III	RCT 1 year	n=298 Haemodialysis Taiwan (6 centres)	Monthly BCM-guided dry weight assessment Control: Monthly BCM performed but not available to treating clinicians	All-cause hospitalisation Acute fluid overload or CV-related event, hypertension, intra-dialysis morbidity	No significant difference
Onofriescu, 2014 Am J Kid Dis	RCT 2.5 year	n=131 Haemodialysis Romania (single centre)	3-monthly BCM-guided dry weight assessment Control: 3-monthly BCM performed but not available to treating clinicians	All-cause mortality Arterial stiffness, fluid overload, blood pressure	Only 1 versus 8 deaths – underpowered (adjusted HR 0.11, 95% CI 0.01-0.92, p=0.04) Significantly lower arterial stiffness but not blood pressure, lower fluid overload achieved
Ponce, 2014 Port J Nephrol Hypert	RCT 1 year	n=189 Online haemodiafiltration Portugal (23 centres)	Monthly BCM-guided dry weight assessment Control: Monthly BCM performed but not available to treating clinicians	Fluid overload, intradialytic hypotension, blood pressure, hospitalisation, mortality	Fluid overload reduced No significant difference hospitalisations/mortality
Hur, 2013 Am J Kid Dis	RCT 1 year	n=156 Haemodialysis Turkey (2 centres)	Twice monthly BCM-guided dry weight assessment Control: 3-monthly BCM but not available to treating clinicians	Left ventricular mass index Blood pressure, left atrial volume, arterial stiffness by pulse-wave velocity	Significant regression of LVMI, mean difference between groups: -10.2 g/m^2 (95% CI, -19.2, -1.17 g/m^2; p=0.04). Other parameters also decreased in the intervention group, but not control.
Onofriescu, 2012 Int Urol Nephrol	RCT 1 year	n=135 Haemodialysis Romania (single centre)	3-monthly BCM-guided dry weight assessment Control: 3-monthly BCM performed but not available to treating clinicians	Blood pressure, arterial stiffness, NT-proBNP	No significant difference (Blood pressure & NT-proBNP fell in both groups; arterial stiffness improved intervention)
Peritoneal dialysis					
Brimble, 2022 Am J Kid Dis FLUID trial	2 by 2 factorial RCT 1 year	n=65 Peritoneal dialysis Canada (6 centres)	(1) Multifrequency BIA using Quadscan 4000 (Bodystat) versus routine care (2) Colecalciferol vs placebo	Left ventricular mass index	No significant difference
Tian, 2020 CJASN	RCT 1 year	n=240 Peritoneal dialysis China (single centre)	1-to-3-monthly BCM-guided management Control: routine care	All-cause mortality, cardiovascular mortality, technique survival	No significant difference
Yoon, 2019 Nephrology (APSN)	RCT 1 year	n=198 Peritoneal dialysis Korea (5 centres)	1-to-3-monthly BCM-guided dry weight assessment Control: BCM performed 0, 6 & 12 months but not available to treating clinicians	Residual kidney function, all-cause mortality, cardiovascular events, peritonitis, hospitalisations	No significant difference
Oh, 2018 PD International COMPASS study	RCT 1 year	n=137 Peritoneal dialysis Korea (5 centres)	2-monthly BCM-guided dry weight assessment Control: BCM performed at start & end but not available to treating clinicians	Residual kidney function	No significant difference
Luo, 2011 Blood Purif	RCT 12 weeks	n=160 Peritoneal dialysis China (single centre)	~6-weekly BCM-guided dry weight assessment Control: BCM performed but not available to treating clinicians	Fluid overload Blood pressure	Significant reduction in fluid overload & blood pressure in intervention versus control groups

Table 1-3: Ongoing/published trials using bioimpedance for eligibility/supplementary assessment

Trial	Design	Population	Intervention/control	Outcome	Application of bioimpedance
Marshall, 2020 CJASN SOLiD trial	RCT 1 year New Zealand (11 centres)	n=99 Haemodialysis	Low dialysate sodium versus conventional	Left ventricular mass index on cardiac MRI Secondary outcomes included BCM-derived extracellular fluid volume	Device: Fresenius BCM **Application: outcome assessment** No significant effect on primary outcome but intervention led to significant reduction in extracellular fluid volume
Kananuraks, 2020 KI Reports BELIEVE trial	Pilot RCT Thailand (single centre)	n=61 At risk of AKI	BIA-guided volume expansion with intravenous bicarbonate for prevention of contrast-induced AKI after coronary angiography	Contrast-induced AKI	Device: MF-BIA (InBody 720) **Application: guided intervention** (intervention prescribed according to ECW/TBW) No significant effect
Antlanger, 2017 BMC Nephrol BVM-Reg trial	RCT 4 weeks Austria (multi-centre)	n=50 Haemodialysis	Fluid removal techniques to improve dry weight reduction: ultrafiltration and dialysate conductivity (UCR) and/or regulation of ultrafiltration and temperature (UTR) versus conventional dialysis	Dry weight reduction, blood pressure, ultrafiltration rates, complications	Device: Fresenius BCM **Application: eligibility** - inclusion required BCM "Fluid Overload" >15%
Beduschi, 2013 Renal Failure	RCT 16 weeks Brazil (single centre)	n=52 Haemodialysis	Dialysate sodium reduction from 138 to 135 mEq/L versus no reduction	Biomarker (TNF-α, IL-6) reduction, blood pressure, interdialytic weight gain, dialysis adequacy, complications	Device: SF-BIA (Biodynamics analyser 450) **Application: outcome assessment** Reduction in biomarkers but no significant change in extracellular water (BIA)
McMahon, 2013 JASN LowSALT study	RCT (crossover) 6 weeks (first phase) UK (single centre)	n=20 Stage 3-4 CKD with hypertension	Salt restricted diet versus control	Blood pressure, extracellular fluid volume, albuminuria	Device: Fresenius BCM **Application: outcome assessment** Reductions in blood pressure, extracellular fluid volume and albuminuria
Jagodzinska, 2011 J Ren Nutri	Trial (not randomised/controlled)	n=38 Haemodialysis	Chewing gum to reduce thirst, dry mouth and fluid overload	Symptom questionnaires, monthly bioimpedance assessment	Device: not stated **Application: outcome assessment** Chewing gum did not improve fluid status
DAPA-HD NCT05179668 [ongoing trial]	RCT 6 months	n=108 Haemodialysis	Dapagliflozin versus placebo	Left ventricular mass index on cardiac MRI BCM fluid assessment at baseline, 3 months and end of study	Device: Fresenius BCM **Application: additional monitoring assessment/outcome** (if hypervolaemia, dialysis prescription sdjusted) Estimated completion September 2025
DAPA-advKD NCT05196347 [trial completed, results not yet published at thesis submission]	RCT 1 year Taiwan (3 centres)	n=225 CKD with GFR 10-30	Dapagliflozin versus routine care	eGFR slope	Device: Fresenius BCM **Application: additional monitoring assessment/outcome** (used to control "Fluid Overload" between 0-1 Litres)
RESOLVE NCT02823821 [ongoing trial]	Cluster RCT Event-driven (~5 years) International	n~50000 Maintenance haemodialysis patients	Dialysate sodium 140mmol/l versus 137mmol/l	Major cardiovascular events and all-cause death	Estimated completion December 2024

1.6.1.3 ASSOCIATIONS WITH ADVERSE OUTCOMES

Widespread measurement of BCM-determined "Fluid Overload" across the Nephrocare-FMC 26-country dialysis centre network has facilitated large-scale observational studies, which have demonstrated strong positive associations with all-cause mortality in patients requiring dialysis, independent of blood pressure (Zoccali et al., 2017, Barra et al., 2022, Siriopol et al., 2019). These studies used relative "Fluid Overload" derived by indexing absolute excess fluid volume to the volume of the extracellular water (ECW) compartment as the exposure, thereby allowing for comparisons between individuals (Wizemann et al., 2009).

In the Wizemann et al. cohort of 269 patients on haemodialysis, 86 died during 3.5 years of follow-up and the adjusted hazard ratio (HR) for all-cause mortality associated with pre-dialysis relative "Fluid Overload" >15% was 2.1 (95% confidence interval [CI] 1.4-3.2) compared to ≤15% (Wizemann et al., 2009). Pre-dialysis relative "Fluid Overload" >15% was more strongly associated with death than age or systolic blood pressure (Wizemann et al., 2009). The >15% threshold, equivalent to approximately +2.5 L absolute "Fluid Overload" (Wizemann et al., 2009), has subsequently been widely used in confirmatory studies in haemodialysis populations (Zoccali et al., 2017, Dekker et al., 2017, Kim et al., 2015, Onofriescu et al., 2015, Siriopol et al., 2019).

Other studies have also used a lower threshold of >7% relative "Fluid Overload", which in a healthy reference population is equivalent to approximately +1.1 L absolute "Fluid Overload" and to the 90th percentile, which has also been associated with poorer survival (Siriopol et al., 2016, Siriopol et al., 2017b, Van Biesen et al., 2011, Siriopol et al., 2019, Dekker et al., 2017). Dekker et al. reported that, compared with patients considered to be euvolaemic pre-dialysis (defined as "Fluid Overload" -1.1 L to +1.1 L), those with pre-dialysis values of >+1.1, ≤+2.5 L (equivalent to 7-15% relative "Fluid Overload") and >+2.5, ≤+5.0 L (equivalent to >15% relative "Fluid Overload"), hazards of death were increased by 1.6- and 2.7-times, respectively (HR 1.6 [95% CI 1.4-2.0] & 2.7 [95% CI 2.3-3.4]). Siriopol et al. report similar findings comparing haemodialysis patients with moderate (>+1.1 L, <+2.5 L) and severe (>+2.5 L) "Fluid Overload" pre-dialysis to those

considered normovolaemic (-1.1 L to +1.1 L) (HR 1.5 [95% CI 1.2–1.9] & 2.0 [95% CI 1.6–2.6]; respectively) (Siriopol et al., 2019).

Studies more commonly report "Fluid Overload" assessed prior to a haemodialysis treatment session (pre-HD) than after treatment (post-HD). A few studies have sought to compare pre- and post-HD "Fluid Overload" and associations with mortality and largely reported similar-sized hazard ratios pre- and post-HD (Dekker et al., 2017, Hecking et al., 2018). However, Tangvoraphonkchai et al. found increased hazards of mortality to be associated with pre-HD "Fluid Overload" but not post-HD (Tangvoraphonkchai and Davenport, 2016) and another study found neither pre- nor post-HD "Fluid Overload" to be associated with mortality (Siriopol et al., 2013) however this small study represents an outlier compared to the body of evidence supporting the mortality risks of "Fluid Overload". In summary, pre-HD "Fluid Overload" measurements seem to be more consistently associated with clinical outcomes and should be favoured since this aligns with clinical practice.

In peritoneal dialysis (PD) populations, a study by Jotterand Drepper et al. applied the same 15% threshold to a cohort of 54 PD patients and demonstrated a significant association with hazards of death (HR for each for 1-SD [11%] increase in relative "Fluid Overload" 7.8 [95% CI 1.1–29.1]) (Jotterand Drepper et al., 2016). In the IPOD-PD study, van Biesen et al. studied the association between serial measurements of relative "Fluid Overload" and mortality in a cohort of 1054 incident PD patients in which moderate relative "Fluid Overload" was defined as >7% and severe >17.3% (Van Biesen et al., 2019). The severe threshold value of >17.3% is derived from the 75th percentile of their population at 1 month since commencing PD. It was associated with 59% increased hazards of all-cause mortality (HR 1.6 [95% CI 1.1-2.3], compared with relative "Fluid Overload" ≤17.3%). In a subgroup analysis of the cohort who developed PD technique failure (composite of death or transfer to haemodialysis), they used the same principle of a cut-off value based on the 75th percentile of the study population (who developed technique failure) and therefore a value of >14.4% was used. This was associated with a significantly higher risk of PD technique failure (HR 2.7 ([95% CI 1.8-4.3) (Vrtovsnik et al., 2021). O'Lone et al. applied another alternative threshold value of ≥10% (representing the top 30% of the studied cohort), and reported that relative "Fluid Overload" of ≥10%

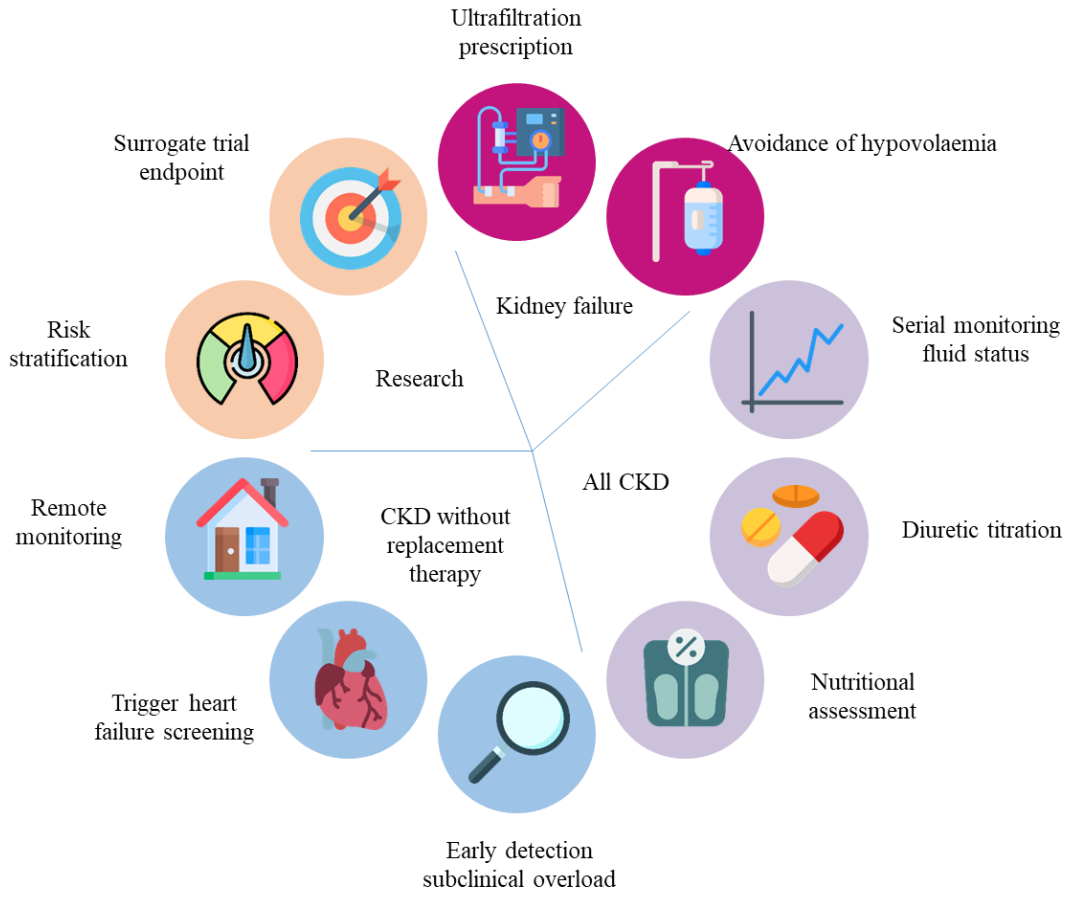
was strongly associated with risk of death in 529 patients on PD (HR 2.1 [95% CI 1.4-3.2]) compared to those with <10% “Fluid Overload” (O'Lone et al., 2014). Other cohorts have not assessed the same 17.3% or 10% threshold used in these studies.

1.6.2 APPLICATIONS IN NON-DIALYSIS CKD

Use of bioimpedance is relatively unexplored in CKD without kidney replacement therapy (KRT), but observational studies are emerging. These have focused on moderate “Fluid Overload” (>+1.1L or >+7%) as an exposure since severe “Fluid Overload” is relatively uncommon before kidney failure develops. A study by Tsai et al. demonstrated that in patients with CKD stages G4-5 not requiring dialysis, relative “Fluid Overload” ≥7% was associated with about a doubling of the hazards of the composite outcome of death or cardiovascular event (incident myocardial infarction, stroke, peripheral artery disease, or hospitalisation for heart failure or arrhythmia) compared to <7% “Fluid Overload” (HR 1.9 [95% CI 1.0-3.7]) (Tsai et al., 2015). Associations were similar in a study by Hung et al. conducted in patients with CKD stages G3-5 (not requiring dialysis): relative “Fluid Overload” ≥7% was associated with significantly increased risk of a composite outcome of myocardial infarction, hospitalisation for congestive heart failure or unstable angina, or death from cardiovascular causes (HR 2.7 [95% CI 1.1-6.5]) (Hung et al., 2015). These and other studies have also reported associations between relative “Fluid Overload” >7% and kidney disease progression (Liu et al., 2021, Schork et al., 2020) but these may simply reflect “Fluid Overload” as a marker of risk rather than be directly responsible for CKD progression.

Beyond the fluid status assessment applications discussed, bioimpedance spectroscopy has many other potential applications in CKD, though not currently employed in clinical practice or well-researched, as outlined in Figure 1-4.

Figure 1-4: Potential roles for bioimpedance spectroscopy in CKD



Despite the wealth of observational research on BCM-derived "Fluid Overload" parameters in both dialysis populations and more recently, in non-dialysis CKD; there is relatively little reported on BCM-derived lean and adipose/fat tissue parameters and studies report inconsistent results. Several studies have reported protective effects of greater lean tissue mass generally demonstrating associations between higher lean tissue index (LTI) and lower all-cause mortality in CKD populations on haemodialysis (Castellano et al., 2016), peritoneal dialysis (Parthasarathy et al., 2019, Kim et al., 2021) and in earlier CKD – stages 3-5 not on dialysis (Lin et al., 2018) though associations were lost in fully-adjusted multivariable models in some cases. The relationship between fat tissue index (FTI) and clinical outcomes is far less clear and studies report inconsistent results. Longitudinal increase in fat tissue index was associated with increased hazards of all-cause mortality in one peritoneal dialysis cohort though the association was no longer significant once C-reactive protein was added as a covariate (Kim et al., 2021). Conversely, another peritoneal dialysis study reported that each standard deviation increase in FTI was associated with lower hazards of mortality though upper confidence interval limits approach 1.00 throughout (Parthasarathy et al., 2019). Data in haemodialysis

populations is limited, one small study similarly reported protective effects of increased FTI and lower hazards of all-cause mortality though categorising FTI as high or low based on its median value in the cohort studied. In earlier stages of CKD, Lin et al. found no association between FTI and all-cause mortality or cardiovascular outcomes when FTI was again dichotomised based on the median value (Lin et al., 2018). These inconsistent associations may in part be explained by the established "obesity paradox" in CKD (Park et al., 2014) such that obesity is associated with poorer survival in earlier CKD but actually confers survival advantage in advanced CKD therefore making it difficult to clearly demonstrate associations with FTI in small studies in the absence of a linear relationship. It remains unclear whether lean mass or adiposity is the more important factor in determining clinical outcomes and typically small studies using bioimpedance spectroscopy are unable to satisfactorily answer this considering the limitations of the BCM device methodology since both lean and fat tissue mass are originally derived from the same parameters of extracellular and intracellular resistance.

1.6.3 APPLICATIONS IN HEART FAILURE

Bioimpedance spectroscopy is in routine use in patients with advanced CKD as discussed in the previous section however it has not yet found a place in heart failure management. Fluid overload is a hallmark of decompensated heart failure (Gheorghiade et al., 2010, Njoroge and Teerlink, 2021) and therefore BCM-derived "Fluid Overload" could be a surrogate for decompensated heart failure though not well-tested in randomised trials in heart failure populations. Bioimpedance devices have been shown to detect subclinical fluid overload (Albert, 2006, Miller, 2016) which, in people with heart failure, is associated with increased risk of death or need for cardiac transplant (Androne et al., 2004). Bioimpedance may therefore support clinical decisions on when to intensify diuretic therapy to modify risk. Bioimpedance devices are generally portable and could be utilised in outpatient heart failure and CKD clinic assessments, and even in patients' homes. This strategy is being assessed in a small feasibility study of at-home BIA in heart failure (NCT05177081). Other registered trials using whole-body bioimpedance devices were not completed (NCT02662439, NCT00843245).

Specific to the heart failure setting, localised impedance methods can also be employed via intra- and transthoracic measures of lung impedance available via implanted cardiac

devices and wearable vests. There is some evidence that fluid overload indicated by thoracic impedance predicts hospitalisation and has clinical potential to monitor diuresis (Smeets et al., 2020, Yu et al., 2005, Shochat et al., 2015). However despite some evidence supporting their use in predicting hospitalisation and association with clinical outcomes, these methods have not shown any demonstrable impact on clinical management (Smeets et al., 2020, Yu et al., 2005, Shochat et al., 2015). Currently, tools to aid in clinical management of heart failure are limited. N-terminal pro B-type natriuretic peptide (NT-proBNP) has been studied as a potential serological marker and although established as a prognostic tool, it is not specific to heart failure and its serial measurement in monitoring/response to treatment is not supported by current guidelines (McDonagh et al., 2021). Other approaches such as pulmonary artery pressure monitoring are being studied (McDonagh et al., 2021) however whole-body bioimpedance spectroscopy has the advantage of not requiring an indwelling device. Perhaps the utility of whole-body bioimpedance spectroscopy in heart failure has been limited by the theoretical concern that whole-body bioimpedance devices may inhibit unipolar pacing in patients dependent on pacemakers, but the majority of pacemakers are now bipolar and overall risk is considered low.

Observational studies in both dialysis and non-dialysis CKD have demonstrated that bioimpedance-derived fluid overload is strongly associated with mortality (as discussed in the previous section) but fewer data exist in heart failure. Data in heart failure are typically limited to small cohorts and use bioimpedance analysis (BIA, a less precise technique than bioimpedance spectroscopy employed by the BCM). Associations of these parameters with outcomes are reported in Chapter 3.

1.7 AIMS OF THE THESIS

The aims of this thesis focus on testing the key hypothesis that empagliflozin has beneficial effects on kidney and cardiovascular outcomes in patients with chronic kidney disease which may be in part explained by effects on fluid status which can be assessed using bioimpedance spectroscopy. The thesis will explore potential contributory mechanisms (focusing on fluid status, body composition and blood pressure) and heterogeneity of treatment effect (focusing on indicators of patients who may be vulnerable to diuresis and blood pressure lowering) by addressing the following five research questions.

Research question 1: Is bioimpedance spectroscopy a valuable tool in clinical and research settings in non-dialysis chronic kidney disease - what are the associations between bioimpedance-derived fluid overload and clinical outcomes?

Objectives:

- Review the use of bioimpedance in chronic kidney disease in existing literature
- Conduct a systematic review (± meta-analysis if data allow) of associations between bioimpedance-derived fluid parameters and clinical outcomes
- Summarise commonly used definitions and analysis approaches to bioimpedance-derived fluid parameters to inform own analyses

Research question 2: What are the effects of empagliflozin on fluid status estimated by bioimpedance spectroscopy?

Objectives:

- Establish whether empagliflozin reduces bioimpedance estimates of fluid excess
- If so, establish:
    - Whether effects are transient or sustained (or differ over time)
    - Whether effects differ depending on other patient characteristics
    - To what extent reduction in fluid excess contributes to weight loss

Research question 3: What are the effects of empagliflozin on body composition and is weight lost due to fluid or reduced adiposity?

Objectives:

- Establish whether empagliflozin reduces adipose and/or lean tissue mass as estimated by bioimpedance spectroscopy
- If so, establish:
    - Whether effects are transient or sustained (or differ over time)
    - To what extent reduction in tissue mass contributes to weight loss
- Further characterise the effects of empagliflozin on anthropometric measurements (weight, body mass index and waist-to-hip ratio) in EMPA-KIDNEY
    - Assess whether effects on weight:
        - Are transient or sustained (or differ over time)
        - Differ depending on other patient characteristics
- Assess the effect of empagliflozin on glycated haemoglobin and haematocrit in relation to effects on body composition and fluid status

Research question 4: What are the effects of empagliflozin on blood pressure and how do these relate to effects on fluid status?

Objectives:

- Further characterise the effects of empagliflozin on blood pressure in EMPA-KIDNEY
    - Assess whether effects on blood pressure:
        - Are transient or sustained (or differ over time)
        - Differ depending on other patient characteristics
- Relate the effects of empagliflozin on fluid status to effects on blood pressure

Research question 5: What is the impact of frailty, multimorbidity, polypharmacy and health-related quality of life on the effects of empagliflozin on clinical outcomes and physical measurements?

Objectives:

- Derive a frailty indicator using EMPA-KIDNEY trial data
- Relate the frailty indicator to the related concepts of multimorbidity, polypharmacy and health-related quality of life and characterise these in EMPA-KIDNEY

- Use the frailty indicator(s) to categorise participants into levels of frailty to assess whether there is heterogeneity in the effect of empagliflozin on clinical (efficacy and safety) outcomes according to frailty
- Characterise the benefit-risk profile of empagliflozin across levels of frailty by estimating absolute benefits and harms of treatment

CHAPTER 2 – METHODS

The first section of this methods chapter describes methods of the systematic review (reported in Chapter 3). The second section pertains to the design and conduct of the EMPA-KIDNEY trial: the main trial methods are discussed in brief since these are not the focus of, but provide the context for, this thesis. Methods of clinical and laboratory assessments in the main trial are expanded upon since these were required for tertiary analyses related to the bioimpedance substudy (reported in Chapters 5 & 6). The majority of the methods chapter then focuses on the methods of the EMPA-KIDNEY bioimpedance substudy, the core of this thesis (reported in Chapters 4 & 5), separately considering (i) design and conduct; (ii) data quality assessment; and (iii) analysis including outcomes. The methodology underpinning the bioimpedance spectroscopy technique is discussed in Chapter 1 (section 1.5). The final sections of this methods chapter describe methods specific to analyses of the impact of frailty (and related metrics) on the effects of empagliflozin (reported in Chapter 7) and statistical analysis methods.

2.1 SYSTEMATIC REVIEW OF BIOIMPEDANCE-DERIVED FLUID OVERLOAD AND ASSOCIATIONS WITH CLINICAL OUTCOMES IN CKD AND HEART FAILURE

2.1.1 REVIEW CONCEPTION AND PROTOCOL REGISTRATION

Associations between bioimpedance-derived fluid status and clinical outcomes in patients receiving dialysis had previously been well-documented in systematic reviews and meta-analyses as summarised in Table 2-1 (Tabinor et al., 2018, Wang and Gu, 2021, Shu et al., 2018, Scotland et al., 2018). These associations had not previously been summarised in patients with earlier stages of chronic kidney disease (CKD) and much less was known in heart failure populations. This review therefore aimed to systematically review and meta-analyse the available data in populations with CKD (not requiring dialysis) and/or heart failure. This approach also appropriately aligned with the study population of the EMPA-KIDNEY trial and its bioimpedance substudy which forms the core of this thesis. It was anticipated that the majority of identified reports would be observational studies though interventional studies were also included in the review.

Table 2-1: Existing systematic review and meta-analyses summarising associations between bioimpedance-derived fluid excess and clinical outcomes in CKD/heart failure populations

	Intervention(s) reviewed	**Population(s)**	**Outcome(s)**	**Size**	**Findings**
Wang, 2021 J Int Med Res	BIS/BIA – OH, ECW, ICW, TBW	Dialysis (any)	Mortality Cardiovascular events	55 studies, n=104,758	OH/ECW >15% predicts mortality (RR 2.72, 95% CI 2.01–3.44) ECW/TBW >0.4 predicts mortality (RR 5.92, 95% CI 2.02–17.34) and cardiovascular events (RR 2.68, 95% CI 1.35–5.34)
Tabinor, 2018 Nature Sci Reports	BIS/BIA – OH, phase angle/BI vector, ECW:ICW	Dialysis (95% HD, 5% PD)	Mortality Hospitalisation, Cardiovascular events	42 cohorts, n=60,790	OH predicts mortality independent of comorbidity OH >15% (RR 2.28, 95% CI 1.56–3.34)
Shu, 2018 Blood Purif	BIS/BIA – OH (ECW:TBW)	PD	Mortality Technique failure	8 studies, n=1989	ECW/TBW "might" predict all-cause mortality (RR 1.08, 95% CI 0.96, 3.36)
Scotland, 2018 Health Technol Assess	Fresenius BCM – absolute and relative OH	Dialysis	Clinical effectiveness versus standard clinical assessment Cost-effectiveness	5 RCTs, n=904, 6 non-randomised studies, n=4915	OH lower with BCM vs standard clinical care; Non-significant effect on blood pressure, arterial stiffness, mortality (mortality RR 0.69, 95% CI 0.23-2.08)
Covic, 2017 Int Urol Nephrol	BIA	Dialysis	All-cause, Cardiovascular mortality Dry weight assessment, SBP, volume control, arterial stiffness	7 RCTs, n=1312	BIA did not reduce mortality (RR 0.87, 95% CI 0.54–1.39); improved blood pressure and reduced overhydration
Da Silva, 2018 Clinical Nutrition ESPEN	BIA	Variety of medical conditions (excluded healthy) – critical illness, heart failure, CKD	Mortality	12 studies, n=8617	Hyperhydration by BIA associated with mortality (OR 4.38, 95% CI 2.76-6.94)
Rodriguez, 2019 Kidney Int Reports	Various non-pharmacological approaches including bioimpedance	Any renal replacement therapy	Arterial stiffness by carotid-femoral pulse wave velocity (cf-PWV)	33 studies, n=2166	Control of OH by BIA reduced arterial stiffness
Beaubien-Souligny, 2019 Kidney Int Reports	Technological adjuncts for fluid management: BIS/BIA	Dialysis (any)	Mortality Cardiovascular events, hospitalisation, intradialytic hypotension, blood pressure, left ventricular mass index	10 studies, n=2111	Adjunct technologies did not reduce mortality (RR 0.71 95% CI 0.43-1.17)

The initial title/abstract screening was performed in two stages: firstly screening all studies and then secondly removing all retained studies which clearly studied only dialysis (kidney failure) populations. Records of these studies were retained for review of fluid overload definitions and threshold values in existing studies across all CKD (non-dialysis and dialysis) and heart failure populations.

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines were followed and the review was prospectively registered on PROSPERO international prospective register of systematic reviews on 16 March 2022 (Appendix 1, PROSPERO identifier CRD42022316312).

2.1.2 PI(E)COS FRAMEWORK

PI(E)COS (Population, Intervention/Exposure, Comparison, Outcome, Setting) criteria for study inclusion are outlined in Table 2-2.

Table 2-2: PI(E)COS framework

CRITERIA	DEFINITION
Population	Adult populations (aged over 18 years or as defined by the study) with heart failure and/or CKD
Intervention/(Exposure)	Fluid status assessed by whole-body bioimpedance analysis or spectroscopy
Comparison	Largely not applicable for observational studies. Studies may include, as a comparator, standard clinical assessment of fluid status used in routine care
Outcome(s)	All-cause mortality Cardiovascular event or composite outcomes using study-specific definitions Kidney disease progression using study-specific definitions
Study design	Observational and interventional studies

2.1.3 EXCLUSION CRITERIA

CKD was defined according to the KDIGO staging system and included CKD stages 1-5, not yet requiring kidney replacement therapy (dialysis or transplant). Studies of people with functioning kidney transplants or on maintenance dialysis were excluded. Studies exclusively of acute kidney injury and other acute disease states were also excluded (e.g., sepsis, critical illness and perioperative studies), with the exception of acute decompensated heart failure. Studies of other chronic diseases in which fluid overload may manifest (e.g., liver disease) were also excluded.

2.1.4 EXPOSURES AND COMPARISONS

All whole-body bioimpedance indices of fluid overload were considered relevant, including absolute and relative “Fluid Overload” (or overhydration), ratios of body water compartments, phase angle, vector length, and bioimpedance vector analysis (BIVA) hydration index; whether reported as continuous or categorical exposures. Results of both absolute “Fluid Overload” in litres and the related relative “Fluid Overload” parameter (indexed to measured extracellular water volume, expressed as a percentage) were tabulated where both were reported and analyses of variables as both continuous and categorised exposures were summarised. Fluid status assessments at any time point and studies reporting both single and serial measurements were included. Where serial measurements were reported, baseline measurements were favoured since serial measurements may have been affected by intervention.

Studies using only segmental/localised (as opposed to whole-body) bioimpedance such as intra/transthoracic or calf measurements were excluded. Where eligibility was unclear, authors were contacted by email.

2.1.5 OUTCOMES

The primary outcome of interest was mortality (as the more specific outcome of cardiovascular mortality was not widely reported). Studies reporting cardiovascular and kidney disease progression outcomes were also reviewed. Definitions of these outcomes were expected to differ (Table 2-3), the protocol specified that all outcome definitions would be included as reported by the study authors and heterogeneity of definitions reviewed to consider whether statistical aggregation would be appropriate. For heart failure populations, composite outcomes comprising all-cause death and hospitalisation were included as a cardiovascular outcome on the presumption that a large proportion of deaths in these composite outcomes reflect cardiovascular disease in the included populations (and particularly in heart failure populations).

Table 2-3: Systematic review outcome definitions

Outcome	Possible components/definitions
All-cause mortality	Death from any cause
Cardiovascular outcomes	Fatal and nonfatal incident cardiovascular disease requiring hospitalisation, including: • Myocardial infarction • Stroke • Cardiovascular death • Coronary heart disease including unstable angina and revascularisation procedures • Hospitalisation for heart failure
Progression of chronic kidney disease	Incident end-stage kidney disease requiring initiation of renal replacement therapy (RRT) (haemodialysis, peritoneal dialysis or renal transplantation including pre-emptive transplantation) • By percentage decline in glomerular filtration rate (GFR) or eGFR (definitions vary e.g. ≥25, ≥30%, ≥40% or ≥50%) • By decline in GFR or eGFR per year (definitions vary e.g. ≥3 mL/min/1.73m^2 per year) • Doubling of serum creatinine from baseline • Onset of self-reported persistent anuria (definitions vary e.g. urine volume <100 mL/24 hours)

It was expected that different nomenclature would be found to describe kidney failure. A Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference in 2019 reviewed the nomenclature used to describe kidney disease, aiming to achieve greater uniformity. The recommendations were published in 2020 (Levey et al., 2020) and it is expected the suggested nomenclature will, in time, replace the current commonly used terminology. Results are discussed used the newly proposed terms (for example, kidney failure, KF) in summarising findings however existing literature was likely to instead feature the terms end-stage kidney disease (ESKD) or end-stage renal disease (ESRD). Table 2-4 outlines how these terms were handled in this review.

Table 2-4: Kidney disease outcome nomenclature

KDIGO 2020 nomenclature	Definition	Related terms
Kidney failure (KF)	GFR <15 mL/min/1.73 m^2 or treatment by dialysis For ≥3 months	End-stage kidney disease (ESKD) End-stage renal disease (ESRD) End-stage kidney failure (ESKF) End-stage renal failure (ESRF)
Kidney replacement therapy (KRT)	Includes dialysis and transplantation	Renal replacement therapy (RRT)
Kidney failure with replacement therapy (KFRT)	CKD G5 treated by dialysis or CKD G1-G5 after transplantation; for epidemiologic studies, both should be included	ESKD/ESRD/ESKF/ESRF requiring dialysis/transplantation
Kidney failure without replacement therapy (CKD G5 without KRT)	CKD G5 where KRT is not chosen or not available	ESKD/ESRD/ESKF/ESRF not on dialysis/transplanted
Chronic kidney disease without KRT (CKD without KRT)	CKD G1–G5, A1–A3 of any cause, not receiving dialysis or transplantation	Non-dialysis CKD

2.1.6 SEARCH STRATEGY

The systematic search was conducted within MEDLINE (Ovid), Embase (Ovid) and Cochrane Central Register of Controlled Trials (CENTRAL) from inception to 14th March 2022 (see appendix 2 for search strategy). The database search was not restricted by language however all studies were available in English. Conference abstracts were

excluded. Studies were only included once and the approach to selection of the report used for extraction was based upon the following factors: maximal outcome data, maximal follow-up time and largest population. Where different fluid overload parameters were used, this was considered alongside the aforementioned factors and parameters most synonymous with other studies were favoured. Search results were exported using Endnote software (EndNote X9, Clarivate, Philadelphia, USA, 2013) and imported into Covidence software (Covidence, Veritas Health Innovation, Melbourne, Australia [no version number/date]) where duplicates were removed. Two reviewers (Kaitlin Mayne & Richard Shemilt) independently screened all unique studies first by title/abstract followed by a review of full texts for those studies which appeared potentially relevant with disagreement resolved by consensus discussion.

2.1.7 DATA EXTRACTION AND REPORTING

A bespoke Covidence electronic data extraction form was created for independent data extraction (Kaitlin Mayne & Richard Shemilt), and included data fields for study design, funding, population characteristics, measures of kidney function and cardiac status, blood pressure and other laboratory parameters relevant to fluid overload at recruitment, as well as bioimpedance-outcome associations. To simplify presentation, for studies reporting multiple fluid exposures, tabulations preferentially included the parameter most commonly reported across all studies, unless in reviewers' opinions, there were important differences in findings with less frequently used exposures. Results from multivariable confounder-adjusted models were emphasised, wherever possible. Results from models which also included potential mediators of associations were extracted and are presented for comparison. Studies were grouped according to study population (CKD or heart failure) and by outcomes reported and results tabulated.

2.1.8 RISK OF BIAS IN INDIVIDUAL STUDIES

Risk of bias was independently assessed by both reviewers using the Quality In Prognosis Studies (QUIPS) tool for observational prognostic studies (see appendix 3) (Hayden et al., 2013) which scores the following six domains as either low, moderate or high risk of bias: study participation; study attrition; prognostic factor measurement; outcome(s) measurement; study confounding; and finally, statistical analysis and reporting. Differences were resolved by discussion to reach consensus.

2.1.9 SUMMARY MEASURES

The preferred effect estimates extracted for all outcomes were hazard ratios and 95% confidence intervals with odds ratios (and 95% confidence intervals) also accepted.

2.1.10 SYNTHESIS OF RESULTS

Meta-analysis was planned by study population and clinical outcome however considerable heterogeneity in exposure measurement and outcome reporting precluded robust aggregation. Studies were grouped according to study population (CKD or heart failure) and by outcomes reported and results tabulated.

2.2 METHODS OF THE EMPA-KIDNEY TRIAL

The full methods of the EMPA-KIDNEY trial and the main results have been reported elsewhere (EMPA-KIDNEY Collaborative Group, 2023) and the Trial Protocol is publicly available for download: https://www.empakidney.org/downloads. In brief, patients with CKD at risk of progression were identified based on historical and screening local laboratory measurements of an estimated glomerular filtration rate (eGFR) ≥20 but <45 mL/min/1.73m^2, or an eGFR ≥45 but <90 mL/min/1.73m^2 with a urinary albumin-to-creatinine ratio (uACR) ≥200 mg/g. The primary outcome was the first occurrence of progression of kidney disease or death from cardiovascular causes. Progression of kidney disease was defined as end-stage kidney disease (ESKD; the initiation of maintenance dialysis or receipt of a kidney transplant), a sustained decrease in the eGFR to less than 10 mL/min/1.73m^2, a sustained decrease from baseline in the eGFR of at least 40%, or death from kidney failure. The pre-specified key secondary outcomes were a composite of hospitalisation for heart failure or death from cardiovascular causes, hospitalisation for any cause (including the first and any subsequent hospitalisations), and death from any cause. The effects of empagliflozin versus placebo on all hospitalisations were analysed according to key pre-specified subgroups by diabetes status, baseline eGFR and uACR. Other secondary outcomes were progression of kidney disease, death from cardiovascular causes, and a composite of ESKD or death from cardiovascular causes. Safety outcomes were serious occurrences of urinary tract infection, genital infection, hyperkalaemia, acute kidney injury, hypoglycaemia; as well as liver injury, ketoacidosis, lower limb amputation, bone fracture; and serious and symptomatic dehydration, separately. Other pre-specified

safety assessments also included analyses of the effects of empagliflozin versus placebo on weight (kg) and blood pressure (systolic and diastolic; mmHg).

2.2.1 CLINICAL ASSESSMENTS IN THE FULL TRIAL COHORT

Analyses include effects on weight, body mass index, waist-to-hip ratio and blood pressure (systolic and diastolic blood pressure, pulse pressure and mean arterial pressure) in the full EMPA-KIDNEY trial cohort and exclude participants with missing baseline values of the outcome variable of interest in each analysis. These measurements were made at routine trial visits using Local Clinical Centre (LCC) equipment as would be typical in usual clinical practice. Weight (kg) and blood pressure (mmHg) were measured at the randomisation visit and all subsequent scheduled visits. Pulse pressure (*post-hoc*) was calculated by subtracting diastolic from systolic blood pressure for each measurement. Mean arterial pressure (*post-hoc*) was calculated as diastolic blood pressure + 1/3(systolic – diastolic blood pressure). Height (metres) was measured at randomisation and used to calculate body mass index (BMI) as weight divided by height squared for each study visit. Waist (i.e. the smallest part of the trunk or the level of the umbilicus if natural indent not apparent) and hip (the widest area around the hips) circumferences were measured in centimetres at randomisation, 18 months and the final visit only. Weight, height and waist/hip circumferences were required to be measured in the specified units – no conversion from imperial units was permitted. Trained LCC Research Co-ordinators (LRC) were advised to obtain measurements without footwear, outer clothing and with items removed from pockets. Guidance was provided to measure waist circumference in the standing position during exhalation, with arms folded. Blood pressure was measured using an automated digital sphygmomanometer or manual device if more appropriate (e.g. if the participant had an irregular heart beat) using an appropriately sized cuff. LRCs were advised the participant should sit comfortably for five minutes prior; to apply the cuff to the exposed upper arm at the level of the heart; neither the LRC nor the participant should speak during measurement and the participant should be advised to remain still. Only one reading was required, in accordance with streamlined trial principles.

2.2.2 LABORATORY ASSESSMENTS IN THE FULL TRIAL COHORT

Analyses include effects on glycated haemoglobin (HbA1c) and haematocrit in the full EMPA-KIDNEY trial cohort. HbA1c was measured in the central laboratory at

randomisation, 2 and 18 months and the final follow-up visit (varies by participant); measurements at 2-, 18- 24- and 30-month time points were included in analyses of the full trial cohort. Kit boxes were provided by the Central Co-ordinating Office (CCO) to be used to collect and store the samples required for central analysis. Guidance was provided on centrifugation and storage prior to transfer to the central laboratory. HbA1c determination used the high-performance liquid chromatographic (HPLC) method using ethylenediaminetetraacetic acid (EDTA) blood on an Arkray HA8180 analyser and reagents with a calibrator supplied by Menarini Diagnostics UK traceable to International Federation of Clinical Chemistry (IFCC) reference standards. Haematocrit was assessed in an approximately 20% subset of the full trial cohort using local laboratory measurements at randomisation and 18 months only. Sample collection bottles/tubes for local laboratory testing were not supplied by the study, so used the bottles which are sourced locally for routine clinical use. LRCs were instructed to enter all test results from the local laboratory into the electronic care report form within 48 hours of collection and were requested to keep a paper copy of any tests results provided by the local laboratory specifically for the study within the participant's study records for monitoring purposes.

2.3 BIOIMPEDANCE SUBSTUDY: DESIGN AND CONDUCT

2.3.1 SUBSTUDY DESIGN

The EMPA-KIDNEY bioimpedance substudy was an optional substudy conducted in a subset of sites in the United Kingdom (UK) and Germany. All participants in the subset of UK and Germany participating sites were eligible for invitation. Consenting participants underwent additional bioimpedance measurements at routine trial visits, in addition to the trial's main protocol-specified procedures. These additional measurements occurred at the randomisation visit and twice during follow-up: at the 2- and 18-month visits. Local Clinical Centres used/obtained their own approved device (Fresenius Body Composition Monitor) with instructions provided on regular calibration.

2.3.2 SUBSTUDY TRAINING

All substudy bioimpedance measurements were performed by trained LRCs during routine trial visits. A training video was produced by trial investigators at the Central Co-ordinating Office, Oxford and sent to LRCs to view independently. The slides used within

the training video are included in appendix 4. LRCs were also asked to review documentation relating to the substudy (Substudy Protocol Supplement [appendix 5]; Substudy Information Leaflet and Consent Form [appendix 6]; and Substudy Instruction ("kit") Leaflet [appendix 7]) and signed a Training Signature Form [appendix 8] confirming completion of substudy training.

2.3.3 DATA CAPTURE

To obtain measurements, four disposable adherent electrodes were attached to the participant's hand, wrist, foot and ankle on either side of the body (Figure 2-1) with the participant in the supine position. Local Research Coordinators were advised to request removal of wrist and/or ankle jewellery at the measurement site and instructed to clean the skin if required (for example if moisturising cream had recently been applied which may affect electrode adhesion). Electrodes were then connected to the Fresenius BCM device by red and black wires with the red wires always attached distally (nearest fingers/toes) to the black wires (Figure 2-1 & Figure 2-2). The BCM device then passes low level painless electrical current at frequencies of 5-1000 kHz (with results extrapolated from zero to infinity kHz) through body tissue. Once prepared, the measurement takes around 20 seconds and results are available within minutes.

Figure 2-1: BCM electrode placement

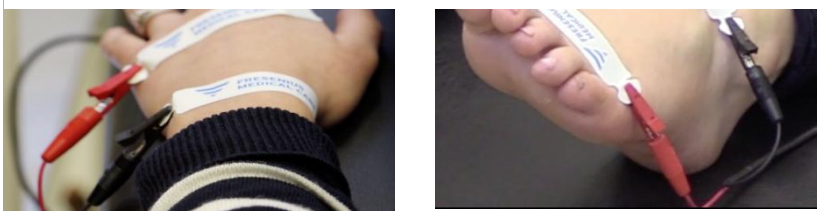
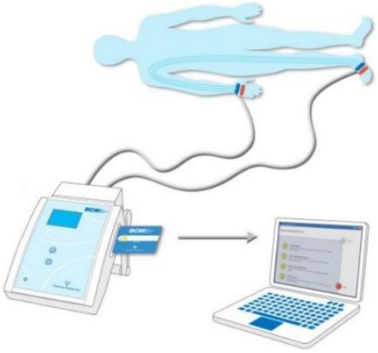


Figure 2-2: BCM device set-up and data card



2.3.4 INITIAL DATA QUALITY ASSESSMENT

When the measurement has been obtained, the BCM device displays an assessment of measurement quality in the form of a Q value and Cole-Cole plot. The Q values ranges from 0 to 100 and a value of ≥80 is generally accepted to reflect acceptable quality. LRCs were trained to repeat bioimpedance measurements when the Q value was <80 after checking electrode and participant positioning. LRCs were not required to interpret the Cole-Cole plot; these were used later by expert reviewers as part of data quality assessments as outlined in section 2.4.

2.3.5 DATA STORAGE

Once the measurement had been made, bioimpedance data were then stored on a dedicated BCM data card (Figure 2-2) unique to the patient but pseudonymised. The process of linking the BCM data to the participant was achieved by LRCs entering the unique BCM data card identifier (a ten digit number beginning 1001-) into the electronic case record form. This then allowed the BCM data card to later be linked to the participant and visit data via the trial database. LRCs were advised to use the same BCM data card if repeat measurement was required (i.e. each participant should have one data card for each visit). BCM data cards were stored securely at the research site until courier transfers to the trial's Central Co-ordinating Office in Oxford were arranged at certain points during the substudy.

2.3.6 ACCOMPANYING DATA

Derivation of analysis parameters also required recording of participant age, sex, height and weight at the time of the BCM measurement. In practice, BCM users enter these data into the BCM device however this function was overridden by pre-programming the data storage cards with dummy data for these variables so that analysis parameters could be derived manually. Sex and date of birth were recorded at randomisation and entered into the electronic case report form by the LRC. Date of birth was used to calculate age at the time of each BCM measurement within the trial database. Height measured at randomisation was entered into the electronic case report form and assumed to remain constant throughout the trial (the streamlined trial design sought to minimise unnecessary assessments). Weight was measured at every study visit according to the main trial

protocol and also entered into the electronic case report form. These variables (age, sex, height and weight) were extracted from the main trial database and provided in Excel spreadsheet form along with bioimpedance data at the point of data transfer for outcome derivation.

2.3.7 DATA EXTRACTION AND TRANSFER

Courier transfer of data cards was arranged by the Central Co-ordinating Office approximately six-monthly according to a pre-specified Internal Operating Procedure (appendix 9). Once data cards were received, trial administrators downloaded the data using a dedicated encrypted substudy laptop with Fluid Management Tool software installed (designed by Fresenius Medical Care specifically for use with the BCM device). All data were downloaded (including multiple measurements recorded on the same card). Q values were also reviewed as measurements were downloaded and administrators noted where measurements from certain research sites had recurrently low Q values (<85%) and these sites were contacted and asked to review the training video before obtaining further BCM measurements. Downloaded data were then stored in PAT file format with a back-up created and securely electronically transferred in ZIP file format to collaborators based at Leeds Teaching Hospitals NHS Trust, formalised in a Service Agreement and outlined in a pre-specified Internal Operating Procedure (appendix 10). The PAT file data transferred in ZIP format was also accompanied by an encrypted Excel spreadsheet containing additional data extracted from the main trial database which would be required for derivation of analysis parameters (BCM data card identifier and corresponding age, sex, height, weight). Once derivation of analysis parameters was complete (see next section 2.3.8), an Excel spreadsheet was then returned using the same secure electronic approach to the Central Co-ordinating Office containing the derived parameters suitable for analysis. Following completion of analyses at the end of the trial, BCM data cards were returned to local research sites for archiving.

2.3.8 DERIVATION OF ANALYSIS PARAMETERS

Derivation of analysis parameters was done manually by expert collaborators rather than employing the device-automated functions to allow for application of updated optimised methodology. The substudy collaborators had access to more recent optimised tissue hydration parameters provided by the device manufacturer to be used in the modelling

approach deriving analysis parameters. These optimised tissue hydration parameters are unpublished and commercially sensitive and could not be made available for inclusion in this thesis. Although this data processing step necessarily had to be performed by collaborators with access to these optimised tissue hydration parameters, I maintained overall oversight of the data throughput.

The steps taken to derive the analysis parameters were as follows:

1. Extracellular and intracellular resistance (Re and Ri, respectively) measurements extracted from the PAT files using an Excel macro
2. The BCM data card ID was used to match Re and Ri to the corresponding participant data (age, sex, height and weight)
3. Extracellular and intracellular water volumes (ECW and ICW, respectively) are then calculated using formulae by Moissl et al (Figure 2-3) (Moissl et al., 2006)
4. OH (overhydration, termed "Fluid Overload") is then derived from ECW, ICW and weight based on methodology developed by Chamney et al (Chamney et al., 2007) but using updated optimised tissue hydration coefficients
5. Lean tissue mass (LTM) is derived from OH, ICW and weight using the same method and indexed to height and expressed as lean tissue index (LTI) in kg/m^2
6. Adipose tissue mass (ATM) is then derived from OH, LTM and weight using the same methodology and tissue hydration coefficients. Adipose tissue mass consists of fat tissue mass plus proteins, minerals and fluid. Fat tissue mass (FTM) is computed separately to allow calculation of the fat tissue index (FTI): fat tissue mass indexed to height squared and expressed in kg/m^2
7. A final age adjustment factor is applied to OH to derive the adjusted OH parameter which corresponds with the parameter reported by the BCM device

Figure 2-3: Excerpt from Moissl et al paper illustrating the formulae (formulae 9 to 12) used to calculate extracellular and intracellular water volumes

$$\mathrm{ECW_{BCS}} = k_{\mathrm{ECW}} \left(\frac{H^2 \cdot \sqrt{W}}{R_0} \right)^{2/3} \quad (9)$$

$$\mathrm{ICW_{BCS}} = k_{\mathrm{ICW}} \left(\frac{H^2 \cdot \sqrt{W}}{R_I} \right)^{2/3} \quad (10)$$

where R_I is the intracellular resistance and k_{ECW} and k_{ICW} are functions of BMI:

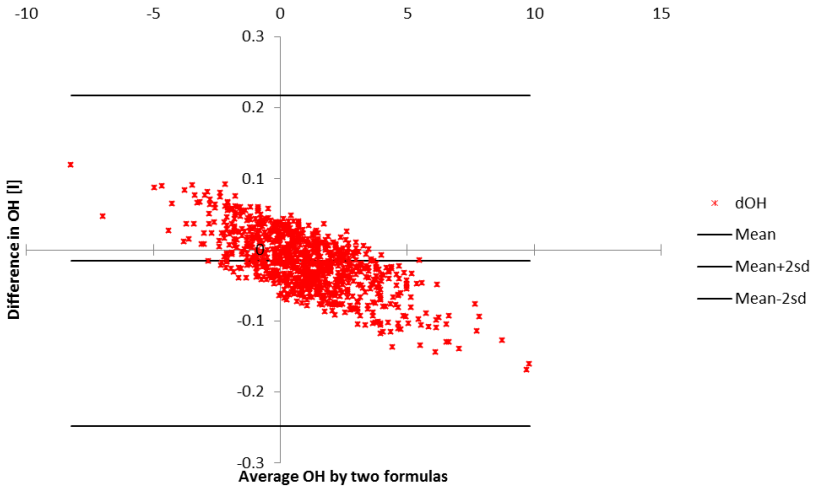
$$k_{\mathrm{ECW}} = \frac{a}{\mathrm{BMI}} + b, \qquad k_{\mathrm{ICW}} = \frac{c}{\mathrm{BMI}} + d. \quad (11)$$

These functions were found empirically by regressing BMI against the true values of $k_{ECW,ref}$ and $k_{ICW,ref}$ which can be found by solving equations (9) and (10) for the unknown parameters and applying volumes from the references (compare figure 5):

$$k_{\mathrm{ECW,ref}} = \frac{\mathrm{ECW_{ref}}}{\left(\frac{H^2 \cdot \sqrt{W}}{R_0}\right)^{2/3}}; \qquad k_{\mathrm{ICW,ref}} = \frac{\mathrm{ICW_{ref}}}{\left(\frac{H^2 \cdot \sqrt{W}}{R_I}\right)^{2/3}}. \quad (12)$$

The expert collaborators previously validated their approach using 892 measurements from 141 patients (in a clinical practice setting) and demonstrated an appropriate level of agreement (Figure 2-4) with bias (average of OH from manual derivation – BCM-computed OH) calculated to be 0.02 Litres.

Figure 2-4: Bland-Altman plot showing agreement of manually-derived OH with BCM-computer OH to validate the methods used for substudy analyses (figure provided by collaborators)



2.3.9 DATA CLEANING

Data cleaning was then required to address occurrences of multiple measurements; missing or untraceable bioimpedance data and discrepant data owing to infrequent data errors at the time of measurement at research sites; in accordance with the principles pre-specified in the Data Analysis Plan (appendix 11) and described in the next section. Handling of the

data is also documented in the pre-specified University of Glasgow Data Management Plan (appendix 12).

2.4 BIOIMPEDANCE SUBSTUDY: DATA QUALITY ASSESSMENT

Data quality assessment was devised, piloted and revised blind to treatment allocation using a preliminary dataset in April 2022. The final data quality assessment process was then applied to the complete dataset in November 2022, while reviewers still remained blinded to individual participants' treatment allocation. The main results of the EMPA-KIDNEY trial were published on 4th November 2022 therefore reviewers were inevitably unblinded to the main trial results.

Data quality assessment was based upon review of Cole-Cole plots (see next section 2.4.1) and was completed independently by two reviewers (myself and a clinician scientist expert reviewer; following training by the expert reviewer), with differences resolved by discussion to determine inclusion in the primary analysis. Two levels of assessment were devised; the first as a "screening" step to detect measurements likely to be of poor quality (A) and then secondly, criteria (B) to be applied to further assess measurements identified in step (A):

> (A) Criteria to be applied to all bioimpedance readings to identify readings which may be of poor quality and should be further assessed by visual inspection of Cole-Cole plots
>
> (B) Criteria to be applied when visually inspecting Cole-Cole plots for readings identified in step (A) to determine inclusion in the primary analysis

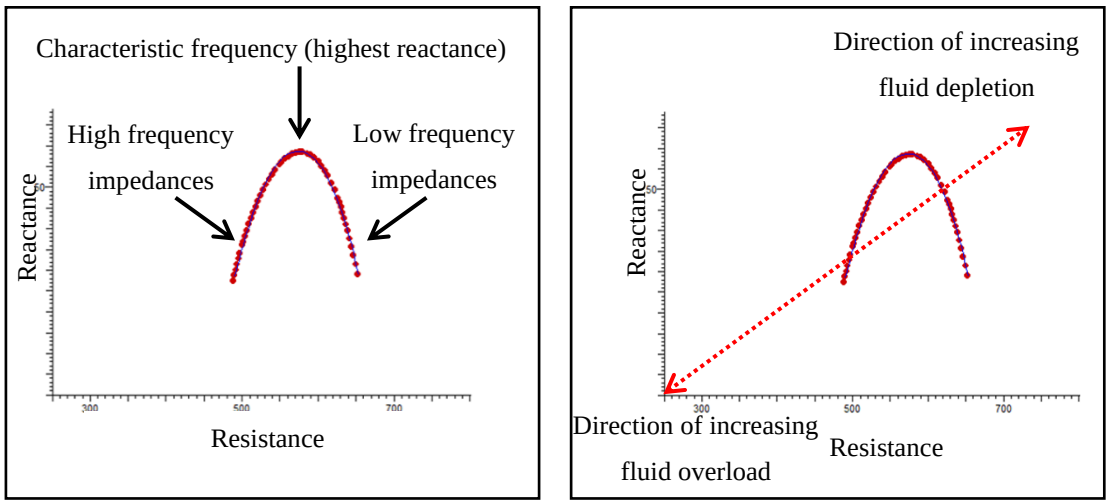
A pilot data quality assessment process was then completed and both criteria (A) and (B) were revised accordingly to determine final data quality assessment procedures to be applied to the complete dataset to determine inclusion in the primary outcome assessment.

2.4.1 COLE-COLE PLOTS

The Cole-Cole plot generated by the BCM device fits a curve to the measured impedance data and defines the extracellular and intracellular resistances upon which all body composition data are based (Ward et al., 2006). Visual inspection of Cole-Cole plots

identifies artefact within the impedance data. Figure 2-5 illustrates the basic interpretation of the Cole-Cole plot.

Figure 2-5: Interpretation of the Cole-Cole plot



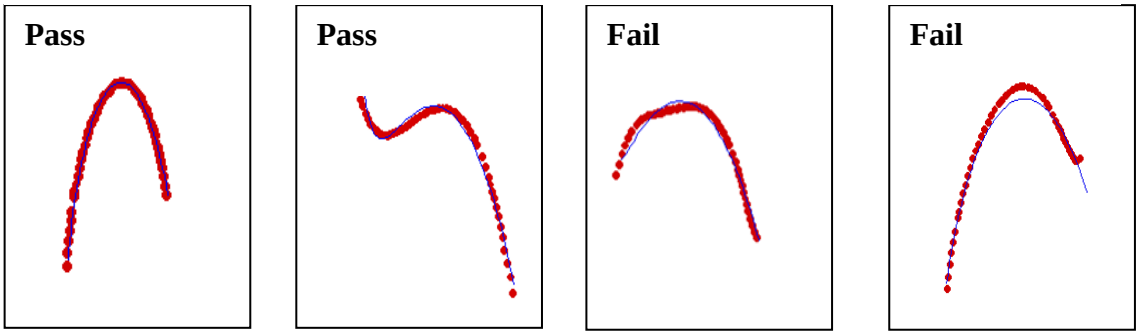
There are no published guidelines for assessment of the Cole-Cole plot therefore these were devised based upon expert knowledge of a collaborator and revised in an iterative process throughout three rounds of Cole-Cole plot review. Discrepancies were discussed, further education delivered and rules (or criteria) revised.

Following testing of various potential selection criteria using descriptions of elements of the Cole-Cole plot, reviewers 1 (Kaitlin Mayne) and 2 (expert reviewer David Keane) jointly agreed upon the following rule to definitively classify measurements as having poor data quality:

> In the opinion of the observer blind to treatment allocation, a good quality Cole-Cole plot should have the basic structure of a parabola, ignoring any artefacts at the high and low frequency end, and the plotted blue curve should closely fit the raw data red.

Iterations during the pilot process attempted to specifically characterise “artefacts at the high and low frequency end” however this was found to lead to overly harsh review by reviewer 1 (Kaitlin Mayne) and therefore unnecessary exclusion of data of acceptable quality. Examples are shown in Figure 2-6.

Figure 2-6: Examples of good ("pass") and poor ("fail") quality bioimpedance data (according to the principles in the box above)



Parabola with good fit of plotted curve against raw data	Ignoring artefact at high frequency, acceptable parabola	Raw data is not parabolic in shape & consequently poor fit	Unacceptable fit of plotted curve against raw data

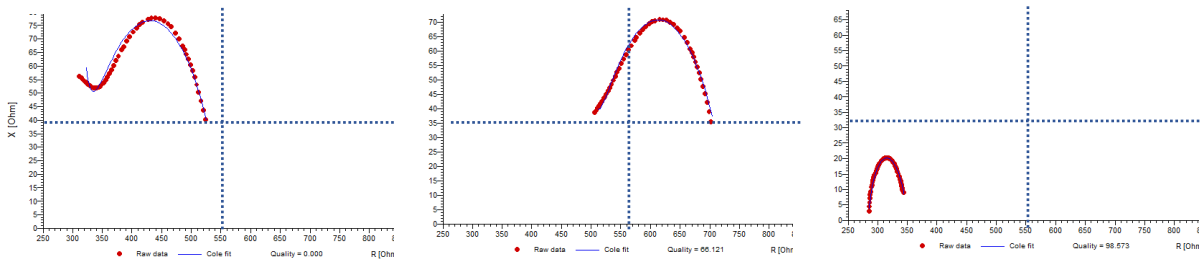
Note: review of the Cole-Cole plot is not affected by the height or width of the plot, length of either end of a parabola, nor its position in the plot region.

The position of the Cole-Cole plot in relation to the reactance/resistance axes can also be assessed in the context of known patient characteristics however these factors were not applied in blinded assessment of data quality in the EMPA-KIDNEY bioimpedance substudy.

Expected associations based upon quadrants of the reactance/resistance plot are shown in Figure 2-7:

- Top left: extreme lean body composition
- Top right: lean/petite individual with dehydration
- Bottom left: extreme fluid overload/obesity
- Bottom right: diseased state (not pictured)

Figure 2-7: Expected associations based on the Cole-Cole plot



2.4.2 ORIGINAL PROPOSED DATA QUALITY ASSESSMENT CRITERIA

In the first released version of the substudy Data Analysis Plan (appendix 11), the following data quality assessment criteria were specified:

Validity of BCM measurements will be assessed, prior to unblinding. If any of the following is true of a BCM measurement, the Cole-Cole plot will be visually inspected to assess data quality and determine inclusion in analyses:

- A Q value of <80 (staff were trained to repeat BCM measurements if the Q value was <80)
- Absolute "Fluid Overload" value considered an extreme outlier (defined as >2 standard deviations from the mean)
- Multiple measurements exist on the same datacard and the difference between the highest and lowest values for absolute "Fluid Overload" is >0.5 litres

Revised data quality assessment criteria following the pilot process (next section 2.4.3) are outlined in section 2.4.4.

2.4.3 PILOT DATA QUALITY ASSESSMENT

In April 2022, a preliminary dataset was available which included 1495 BCM cards containing BCM data. This dataset contained BCM data (combination of randomisation, 2-month and 18-month data) downloaded up until 15th December 2021. At this time, the EMPA-KIDNEY trial had not yet completed the on-treatment phase of the trial and investigators remained blinded to treatment allocation. This was used as a preliminary dataset to pilot the data cleaning process and data quality assessments and to determine criteria (whilst still blinded to minimise bias) which would later be applied to the final analysis dataset (containing data up until 4th July 2022, the end of the on-treatment phase of the trial). Of these 1495 readings, 172 readings were identified as meeting at least one of the criteria (section 2.4.2) triggering visual inspection of the Cole-Cole plot (measurements may satisfy more than one criteria).

2.4.4 REVISED DATA QUALITY ASSESSMENT CRITERIA

The final data quality assessment criteria to be applied to determine inclusion in the primary outcome assessment, as informed by the pilot data quality process, are outlined here:

- Absolute "Fluid Overload" values more negative than -5 L excluded due to implausibility
    - Otherwise outliers included
- Multiple measurements on the same card (for the same participant on the same date) handled by favouring the reading with the highest Q score and excluding other measurements
- After applying these exclusions, all readings with Q<80 will be reviewed by two reviewers to determine inclusion with discrepancies resolved by discussion
- A random subset of readings with Q scores >80 will be assessed by two reviewers with discrepancies resolved by discussion to ensure Q >80 is largely an acceptable measure of quality

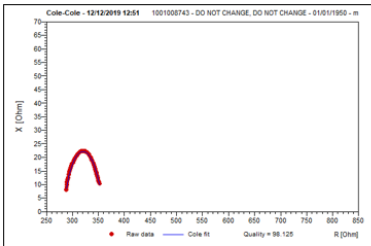
The justification for revising the data quality assessment criteria for application to the final dataset to determine inclusion in the primary analysis was that the original criteria meant discarding data unnecessarily which, when further assessed, was found to be of acceptable quality.

2.4.4.1 EXTREME OUTLIER CRITERION

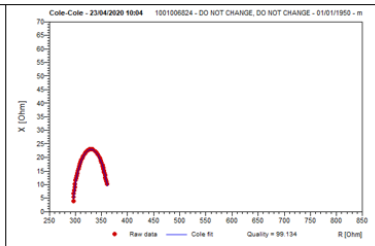
The original "extreme outlier" criteria were found to be too broad. This was originally defined as >2 SD from the mean. Review of all extreme outliers identified using this criterion were reviewed in the pilot data quality assessment process and it was found that measurements with extremely positive values (of "Fluid Overload"/overhydration) had consistently acceptable Cole-Cole plots (Figure 2-8) whereas the opposite was true of extreme negative measurements (Figure 2-9). The cut-off of -5 L was selected based on biological plausibility in combination with the distribution of data within the pilot dataset. The mean value of "Fluid Overload"/overhydration in the pilot dataset was 0.5 L with a standard deviation of 1.58 L meaning that -5 L is ~3.5 standard deviations below the mean (Figure 2-10). Thresholds 1 L higher and lower than -5 L were also assessed by examining the Cole-Cole plots of measurements falling between -5 and -6 L; and between -5 and -4 L. Applying a cut-off of -6 L would mean inclusion of two measurements which were of

clearly unacceptable quality based on the Cole-Cole plot (Figure 2-11). Conversely applying a cut-off of -4 L would exclude an additional five measurements from the pilot dataset (relative to the -5 L threshold), two of which are of clearly acceptable quality based on Cole-Cole plots (Figure 2-12). Therefore, -5 L was confirmed as an appropriate cut-off below which measurements should be deemed of poor data quality and excluded from the primary analysis, without need for further review of the Cole-Cole plot.

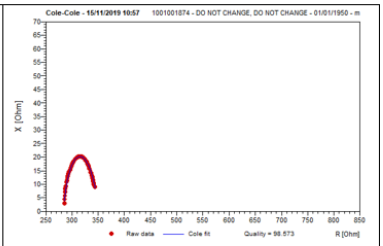
Figure 2-8: Extreme positive outliers



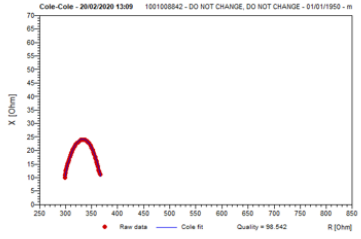
BCM ID	1001008743
OH (L)	6.4
Q score	98.1
Plot	Clear pass



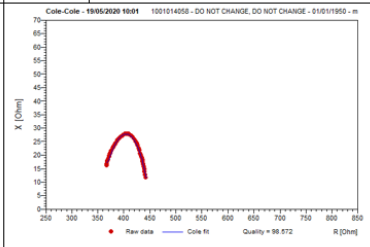
BCM ID	1001006824
OH (L)	6.4
Q score	99.1
Plot	Clear pass



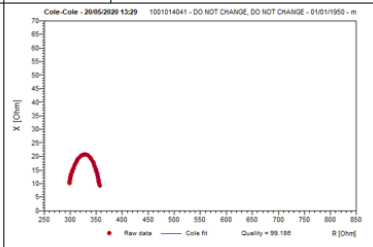
BCM ID	1001001874
OH (L)	6.1
Q score	98.6
Plot	Clear pass



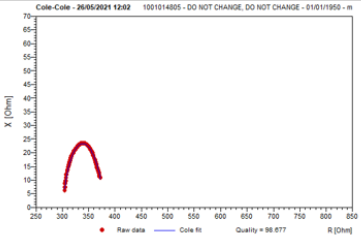
BCM ID	1001008842
OH (L)	6.0
Q score	98.5
Plot	Clear pass



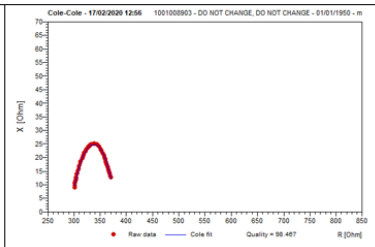
BCM ID	1001014058
OH (L)	6.0
Q score	98.6
Plot	Clear pass



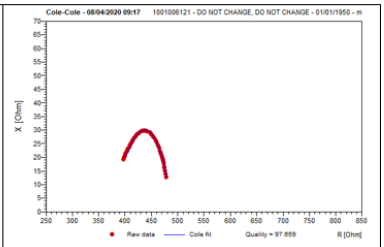
BCM ID	1001014041
OH (L)	5.9
Q score	99.1
Plot	Clear pass



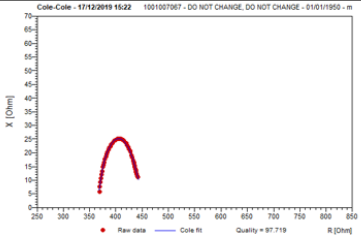
BCM ID	1001014805
OH (L)	5.8
Q score	98.7
Plot	Clear pass



BCM ID	1001008903
OH (L)	5.6
Q score	98.5
Plot	Clear pass

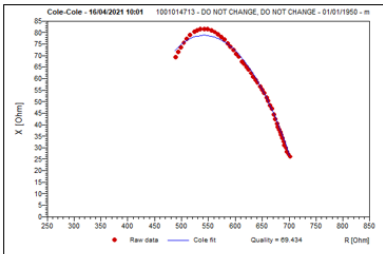


BCM ID	1001006121
OH (L)	5.1
Q score	97.7
Plot	Clear pass

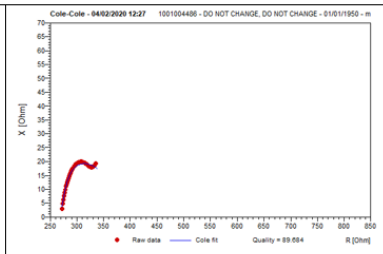


BCM ID	1001007067
OH (L)	5.0
Q score	97.7
Plot	Clear pass

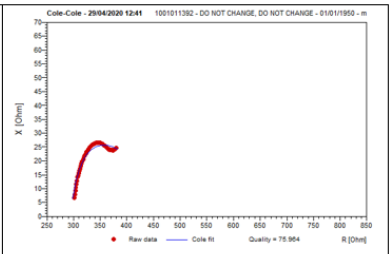
Figure 2-9: Extreme negative outliers



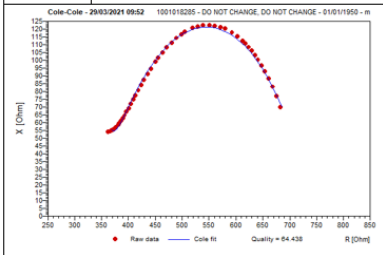
BCM ID	1001014713
OH (L)	-10.8
Q score	69.4
Plot	Clear fail



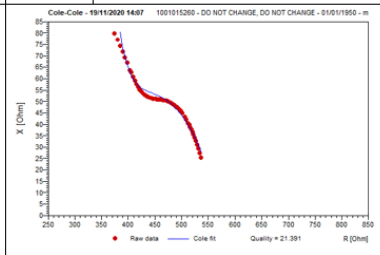
BCM ID	1001004486
OH (L)	-9.5
Q score	89.7
Plot	Clear fail



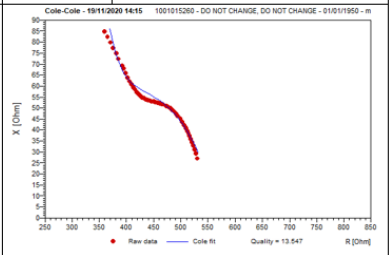
BCM ID	1001011392
OH (L)	-8.8
Q score	76.0
Plot	Clear fail



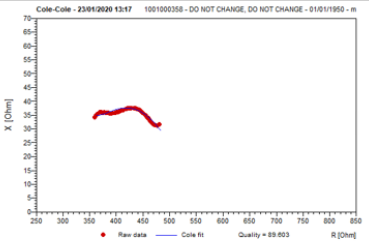
BCM ID	1001018285
OH (L)	-8.5
Q score	64.4
Plot	?Pass



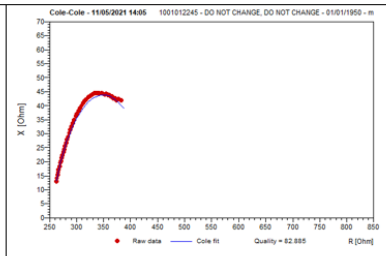
BCM ID	1001015260_1
OH (L)	-6.4
Q score	21.4
Plot	Clear fail



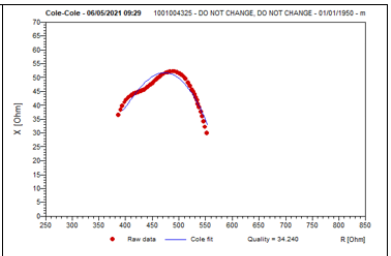
BCM ID	1001015260_2
OH (L)	-8.2
Q score	13.5
Plot	Clear fail



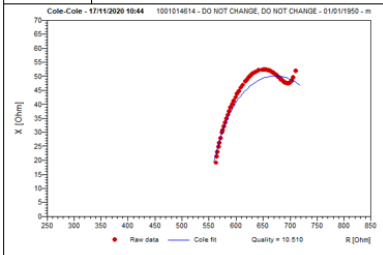
BCM ID	1001000358
OH (L)	-7.8
Q score	89.6
Plot	Clear fail



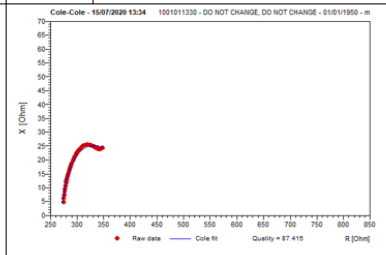
BCM ID	1001012245
OH (L)	-7.9
Q score	82.9
Plot	Clear fail



BCM ID	1001004325_1
OH (L)	-6.3
Q score	34.2
Plot	Clear fail



BCM ID	1001014614
OH (L)	-5.7
Q score	10.5
Plot	Clear fail



BCM ID	1001011330
OH (L)	-5.3
Q score	87.4
Plot	Clear fail

Figure 2-10: Identification of the -5 L extreme outlier cut-off

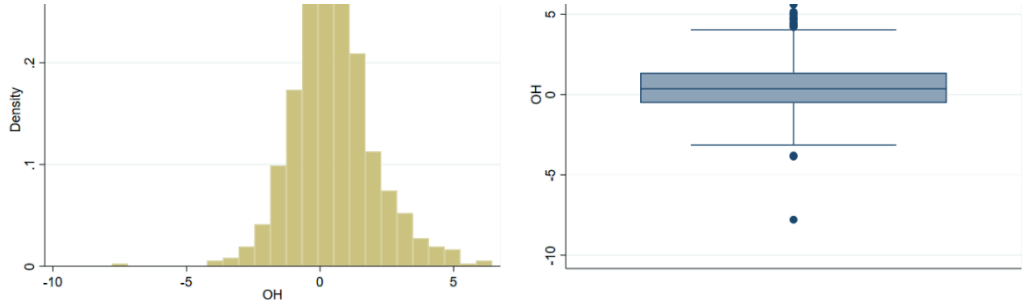


Figure 2-11: Application of a -6 L cut-off and inappropriate inclusion of poor quality measurements

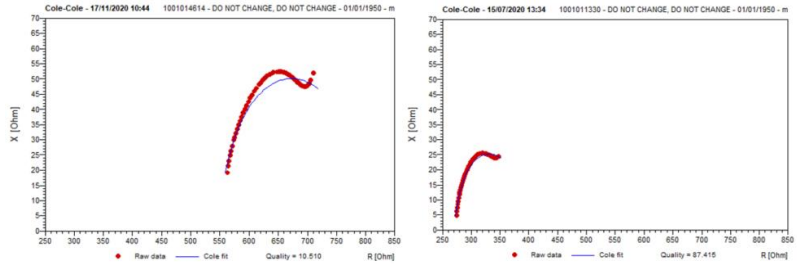
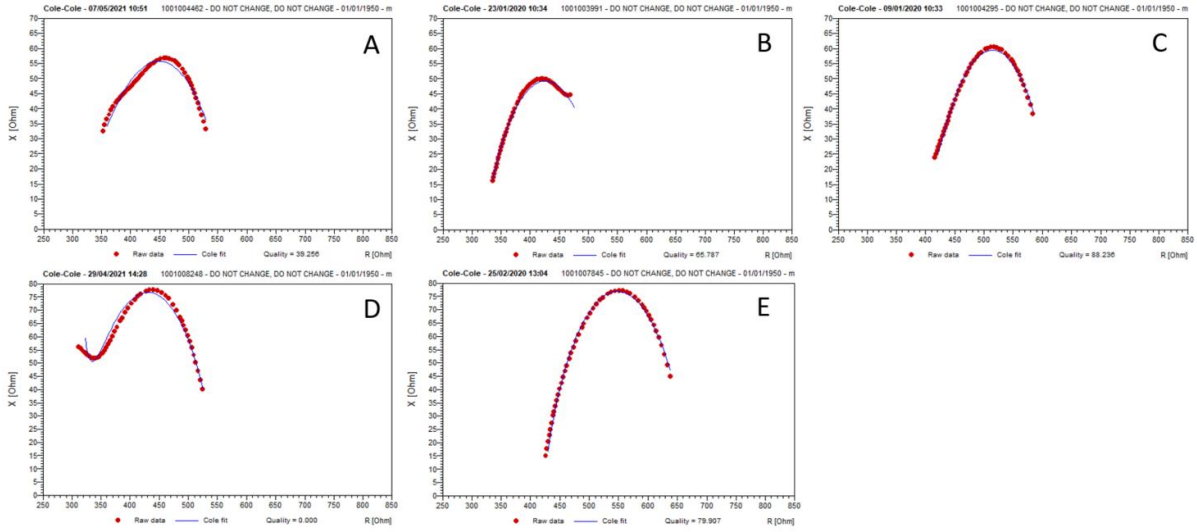


Figure 2-12: Application of a -4 L cut-off and inappropriate exclusion of two measurements of acceptable data quality

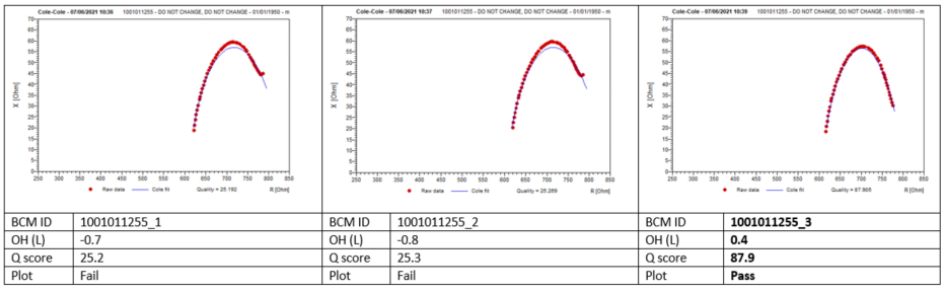


Measurements C and E are of good quality. Plots A, B and D are of poor quality but it should be noted these measurements have Q values <80 therefore although these measurements would not be excluded based on the extreme outlier criteria, they would be identified for further review based on the Q value and excluded after review of the Cole-Cole plot therefore supporting application of the -5 L cut-off.

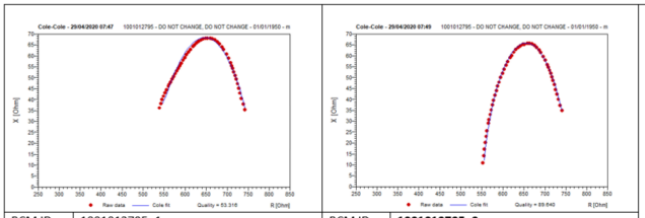
2.4.4.2 MULTIPLE MEASUREMENTS ON THE SAME CARD CRITERION

The pilot data quality assessment demonstrated that it would be unnecessary to review the Cole-Cole plots of all measurements where multiple were recorded on the same card. All instances of this were examined in the pilot data quality process demonstrating that in every case, the measurement with the highest Q value also had the most favourable Cole-Cole plot (Figure 2-13). This therefore confirmed that an approach of retaining the measurement with the highest Q value was appropriate in cases where multiple measurements exist on the same card, without need for additional review of Cole-Cole plots.

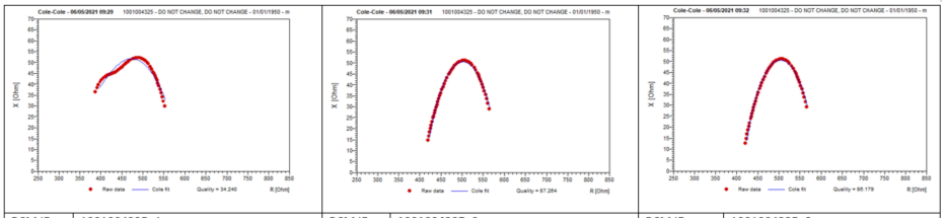
Figure 2-13: Cole-Cole plots for measurements with multiple measurements on the same card



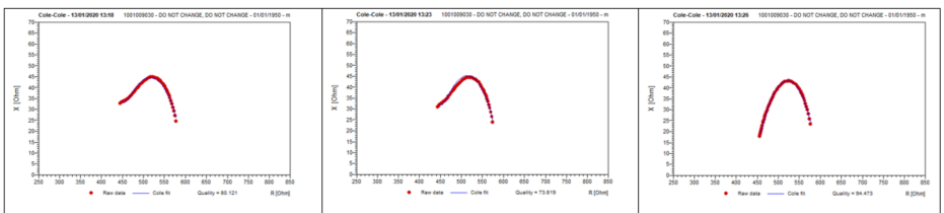
BCM ID	1001011255_1	BCM ID	1001011255_2	BCM ID	**1001011255_3**
OH (L)	-0.7	OH (L)	-0.8	OH (L)	**0.4**
Q score	25.2	Q score	25.3	Q score	**87.9**
Plot	Fail	Plot	Fail	Plot	**Pass**



BCM ID	1001012795_1	BCM ID	**1001012795_2**
OH (L)	-2.2	OH (L)	**-1.6**
Q score	53.3	Q score	**89.6**
Plot	Fail/borderline	Plot	**Pass**



BCM ID	1001004325_1	BCM ID	**1001004325_2**	BCM ID	1001004325_3
OH (L)	-6.3	OH (L)	**-2.6**	OH (L)	-2.6
Q score	34.2	Q score	**87.3**	Q score	86.2
Plot	Fail	Plot	**Pass**	Plot	Pass



BCM ID	1001009030_1	BCM ID	1001009030_2	BCM ID	**1001009030_3**
OH (L)	-0.1	OH (L)	0.4	OH (L)	**0.9**
Q score	80.1	Q score	73.8	Q score	**94.5**
Plot	Pass/borderline	Plot	Fail/borderline	Plot	**Pass**

Each row represents a different participant and data card. In each case, the most favourable Cole-Cole plot corresponds with the highest Q value (bold highlight).

2.4.4.3 QUALITY VALUE CRITERIA

Based on assessments of extreme outliers and multiple measurements on the same card, it was therefore concluded that; after removal of extreme outliers more negative than -5 L; screening for poor quality measurements should be focused on the BCM's automated quality score (Q value). All measurements in the complete dataset with a Q value <80 would be identified for Cole-Cole plot review. To ensure robustness of this approach, a random subset of measurements with a Q value ≥80 would also be identified from the complete dataset and undergo Cole-Cole plot review seeking to confirm that Q values ≥80 are reliable indicators of acceptable data quality.

The revised data quality assessment procedure (Table 2-5) would necessitate reviewing fewer Cole-Cole plots (46 versus 172 in the pilot dataset) overall thereby allowing double review (i.e. by two reviewers independently) of all identified measurements with discrepancies resolved by discussion. This may not have been feasible if larger numbers of measurements were identified for Cole-Cole plot review in which case I would review all plots with only a subset double-reviewed by the expert reviewer to ensure inter-observer reliability, a less favourable approach. It is also possible than unnecessary review of large numbers of Cole-Cole plots could potentially risk introducing bias which must be avoided.

Table 2-5: Summary of original and revised data quality criteria identifying measurements for Cole-Cole plot review

Original criterion	Revised criterion	Justification
Exclusion* if Q value <80	Q value <80 triggered manual review of Cole-Cole plot in each case	All Cole-Cole plots with a Q value <80 were reviewed and >50% deemed of acceptable quality based on Cole-Cole plot review therefore this revised criterion is necessary to avoid automatic exclusion of valid readings based on Q<80 alone. A random subset of 50 measurements with a Q score ≥80 were also selected for Cole-Cole plot review to test this criterion. Q scores above this threshold were confirmed to be a reliable indicator of good data quality.
Exclusion* if absolute "Fluid Overload" extreme outlier: >2 standard deviations (SD) from the mean	Absolute "Fluid Overload" more negative than -5 L automatically excluded. All other outliers retained and do not warrant Cole-Cole plot review.	Cole-Cole plots for all measurements lying >2 SD from the mean were reviewed. It was found that outlying positive values of "Fluid Overload" were reliably associated with satisfactory Cole-Cole plots whereas outlying negative values were consistently associated with poor Cole-Cole plots. The -5 L cut-off was selected based on review of each individual plot combined with clinical reasoning based upon plausibility.
Exclusion* if multiple measurements at the same time with more than 0.5L between the highest and lowest absolute "Fluid Overload" values	If more than one valid bioimpedance measurement is available at a single Follow-up visit (i.e. date), the measurement with the highest Q value will be used and additional measurements ignored.	All duplicate measurements were reviewed in the pilot dataset and the measurement with the highest Q score also had the most favourable Cole-Cole plot in every case. MMRM analysis requires a single reading for each participant at each time point.

* exclusion from primary outcome assessment

2.5 BIOIMPEDANCE SUBSTUDY: ANALYSIS

2.5.1 BASELINE CHARACTERISTICS

In order to assess balance of baseline characteristics between randomised arms of the BCM substudy, the following variables were recorded at randomisation (or at screening) and presented for the empagliflozin and placebo groups. Categories were specified to be consistent with those from the main trial publications or subgroup analyses:

a. History of prior disease:
    i. Diabetes mellitus (presence *vs* absence);
    ii. Self-reported heart failure (presence *vs* absence);
    iii. Primary renal diagnosis (diabetic kidney disease, hypertensive/renovascular disease, glomerular disease, other or unknown [1])
b. Patient characteristics;
    i. Age (continuous and categorised: <60; ≥60 <70; ≥70 years);
    ii. Sex (male *vs* female);
    iii. Race (White, Black/African American, South Asian, Southeast Asian, Mixed or Other);
    iv. Smoking status (ever smoked regularly at randomisation, yes *vs* no);
    v. Weight in kg*;
    vi. Body mass index (BMI) (continuous and categorised: <25; ≥25 <30; ≥30 kg/m^2);
    vii. Waist-to-hip ratio*;
    viii. Extracelllular water (ECW) in litres*;
    ix. Intracellular water (ICW) in litres*;
    x. Absolute "Fluid Overload" in litres*;
    xi. Relative "Fluid Overload" (%)*;
    xii. Clinically significant "Fluid Overload" (%, presence *vs* absence)*;
        - Moderate (>7%, ≤15% relative "Fluid Overload")
        - Severe (>15% relative "Fluid Overload")
    xiii. Lean tissue index (LTI) (lean tissue mass [LTM] indexed to height)*;
    xiv. Fat tissue index (FTI) (fat tissue mass [ATM] indexed to height)*;

[1] Other includes tubulointerstitial disease, familial/hereditary nephropathies, other systemic disorders and miscellaneous renal disorders. Glomerular disease is subcategorised as follows: focal segmental glomerulosclerosis, Immunoglobulin A (IgA) nephropathy, membranous nephropathy, minimal change disease and other glomerular disease.

xv. Systolic blood pressure (continuous and categorised: <130; ≥130 <145; ≥145 mmHg);

xvi. Diastolic blood pressure (continuous and categorised: <75; ≥75 <85; ≥85 mmHg);

c. Laboratory values at randomisation:
    a. CKD-EPI eGFR (continuous and categorised: <30, ≥30 <45, ≥45 mL/min/1.73m^2 estimated from central enzymatic creatinine [or local creatinine where central value unavailable])
    b. UACR: (continuous and categorised: <30, ≥30 ≤300, >300 mg/g)
    c. Glycated haemoglobin (HbA1c) (continuous and categorised: <39 [normoglycaemia], ≥39<48 [pre-diabetes], ≥48<75 [well-controlled diabetes], ≥75 [poor glycaemic control] mmol/mol, or missing
    d. N-terminal pro B-type natriuretic peptide (NT-proBNP) (continuous and categorised: <110, ≥110 <330, ≥330 ng/L)
    e. Haematocrit (continuous and categorised: <37%; ≥37% <41%; ≥41%)

d. Medication use at randomisation:
    i. RAS inhibition (yes *vs* no);
    ii. Diuretics (yes *vs* no, and analyses by type [loop *vs* thiazide *vs* mineralocorticoid receptor antagonist *vs* other potassium-sparing].
    iii. Antidiabetic medications (yes *vs* no, and analyses by type [biguanide *vs* sulphonylurea *vs* insulin *vs* DPP-4 inhibitor *vs* GLP-1 agonist *vs* other]

* continuous and categorised into approximate thirds of the distribution.

2.5.2 BIOIMPEDANCE-DERIVED "FLUID OVERLOAD" TERMINOLOGY

There is no standard nomenclature for bioimpedance-derived fluid overload parameters in existing literature, with a range of terminology and threshold values to infer clinical significance employed. Following a systematic review of all existing literature in non-dialysis CKD and a supplementary scoping review of literature in dialysis populations, the approach outlined in Table 2-6 was adopted for reporting of the EMPA-KIDNEY bioimpedance substudy.

Table 2-6: Description of "Fluid Overload" parameters applied in the EMPA-KIDNEY bioimpedance substudy

EMPA-KIDNEY terminology	Definition	Equivalent terminology used in other literature
Absolute "Fluid Overload"	Overhydration in litres, computed as the difference between expected (based upon weight and body composition) versus measured extracellular water (ECW) volume, with positive values representing excess fluid "Fluid Overload" = $ECW_{measured}$ - $ECW_{expected}$	Overhydration (Wang and Gu, 2021) Hydration status (Wabel et al., 2008) Absolute tissue hydration (Van Biesen et al., 2011)
Relative "Fluid Overload"	Overhydration index relative to measured ECW volume, expressed as a percentage Relative "Fluid Overload" = "Fluid Overload" ÷ $ECW_{measured}$	Overhydration index (Wang and Gu, 2021, Tabinor et al., 2018) Relative hydration status (Wizemann et al., 2009, Tsai et al., 2015) Relative tissue hydration (Van Biesen et al., 2011)
Clinically Significant "Fluid Overload"		
Moderate "Fluid Overload"	Relative "Fluid Overload" >7%, ≤15% where 7% reflects the 90th percentile in a healthy reference population and is approximately equivalent to absolute "Fluid Overload" of +1.1L	-
Severe "Fluid Overload"	Relative "Fluid Overload" >15% which represents the highest quartile in a haemodialysis reference population; approximately equivalent to absolute "Fluid Overload" of +2.5L	Hyperhydration (Wizemann et al., 2009)

Definitions in the above table originate from work by authors Wabel, Wizemann, Van Biesen, Zoccali, Dekker and colleagues (Wabel et al., 2008, Wizemann et al., 2009, Van Biesen et al., 2011, Dekker et al., 2017, Zoccali et al., 2017).

Table 2-6 also summarises alternative terminology employed in existing research using the BCM device, illustrating the lack of standard nomenclature. Some scientific literature has used the term "overhydration index" to refer to both absolute "Fluid Overload" in litres and relative "Fluid Overload" (Tabinor et al., 2018, O'Lone et al., 2014) however use of this term would seem more appropriate for relative "Fluid Overload" which is indexed to ECW volume. Hydration status expressed as ΔHS has also been used to refer to both absolute "Fluid Overload" in litres (Wabel et al., 2008) and relative "Fluid Overload" (Wizemann et al., 2009, Tsai et al., 2015). Absolute and relative "Fluid Overload" were adopted for use in EMPA-KIDNEY and throughout this thesis and related publications, "Fluid Overload" (including "" and capitalisation of each word) is used to refer to the bioimpedance-derived parameter with terms fluid status and fluid excess used as descriptions of physiological state wherever possible.

The substudy primary assessment uses the absolute "Fluid Overload" parameter in litres in preference to relative "Fluid Overload" (expressed as a percentage) since estimation in litres is likely to be more readily interpretable in a clinical context. Relative "Fluid Overload" is commonly reported in observational research since indexing to ECW is thought to allow for more appropriate comparison between individuals (Wizemann et al., 2009). The reference range for absolute "Fluid Overload" based on general population data

is -1.1 L to +1.1 L (Wabel et al., 2008). An absolute value of +1.1 L approximately corresponds to relative "Fluid Overload" of 7%, both representing the 90th centile in a healthy reference population (Van Biesen et al., 2011).

The EMPA-KIDNEY definitions of moderate and severe clinically significant "Fluid Overload" are based upon existing literature which largely represents populations with advanced CKD requiring dialysis (because fluid excess is less common in earlier CKD). Various thresholds have been used in previous studies as summarised in Table 2-7. Wizemann et al. established a 15% threshold value of relative "Fluid Overload" (referred to as relative hydration status) based upon the highest quartile of a reference haemodialysis population (Wizemann et al., 2009). This threshold is approximately equivalent to >+2.5 L absolute "Fluid Overload" in patients on haemodialysis (Wizemann et al., 2009, Wabel et al., 2008, Wieskotten et al., 2008), and is strongly associated with mortality (Zoccali et al., 2017, Dekker et al., 2017, Tabinor et al., 2018, Caetano et al., 2016, Chazot et al., 2012, Kim et al., 2015, Onofriescu et al., 2015, Siriopol et al., 2019, Wizemann et al., 2009). In EMPA-KIDNEY, the threshold of >15% relative "Fluid Overload" is referred to as "severe" as the study population can be expected to exhibit lower levels of fluid excess than dialysis populations. The moderate "Fluid Overload" threshold of >7% has also been associated with risk of death in dialysis cohorts (Dekker et al., 2017, Siriopol et al., 2019, Siriopol et al., 2017b, Siriopol et al., 2016) and more recently, in those with earlier stages of CKD (Tsai et al., 2015, Hung et al., 2015).

An important distinction between the Fresenius BCM device and other devices is that the BCM device derives an estimation of "Fluid Overload" which is corrected for body composition. The device also estimates fat and lean tissue mass though these values are age- and sex-specific and should be interpreted accordingly.

Table 2-7: "Fluid Overload" thresholds used in previous cohort studies employing the BCM

Study	Population	Absolute "Fluid Overload" threshold(s) analysed	Relative "Fluid Overload" threshold(s) analysed
Haemodialysis			
Lesquevas Barra, 2022, BMC Nephrol	n=5081 (EuCliD/Nephrocare)	-	>13% women >15% men
Keber, 2021, Clinical Nephrol	n=92	>1.1 L, >2.5 L = severe	-
Schwermer, 2021, Polish J Int Med	n=511	Normal <1 L; mild 1-2L; moderate 2-3 L; severe >3 L	-
Song, 2020, BMC Nephrol	n=88	-	>15%
Mizuri, 2020, HD International	n=215	-	Continuous
Siriopol, 2019, NDT	n=4114 (EuCliD/Nephrocare)	>1.1 L, >2.5 L = severe	-
Valente, 2019, Nephrology	n=3696 (EuCliD/Nephrocare)	-	>15%
Zhang, 2019, Renal Failure	n=123	Continuous	-
Arrigo, 2018, NDT	n=144	-	>15%
Huang, 2018, Kidney Blood Press Res	n=178	-	≥7%
Zoccali, 2017, JASN	n=39,566 (EuCliD/Nephrocare)	-	≥13% women, ≥15% men (note ≥)
Dekker, 2017, Kidney International	n=8883 (MONDO initiative)	FO >2.5 L moderate, >2.5 L severe, >5 L extreme	-
Garagarza, 2017, Int Urol Nephrol	n=3552	-	>15%
Siriopol, 2017, Arch Med Sci	n=285	-	>6.9% (median)
Siriopol, 2016, Int J Cardiovasc Imaging	n=173	-	>6.88%
Tangvoraphonkchai, 2016, Int J Artif Organs	n=362	-	Continuous
Caeatano, 2016, J Ren Nutr	n=697	-	>15%
Schwermer, 2015, Pol Arch Med Wewn	n=321	-	≥4% (not justified)
Onofriescu, 2015, PLoS One	n=221	-	>15% & >17.4%
Kim, 2015, Kidney Res Clin Pract	n=344	-	>15%
Hoppe, 2015, Blood Purif	n=241	-	Continuous
Mathew, 2014, Renal Failure	n=99 (HD & PD)	>3.2 vs ≤3.2 L (median)	-
Siriopol, 2013, NDT	n=96	-	>15%
Chazot, 2012, NDT	n=208	-	>15%
Wizemann, 2009, NDT	n=269	-	>15%
Peritoneal dialysis			
Vrtovsnik, 2021, Clin Kidney J IPOD-PD study	n=719	-	>14.4% (75th percentile at 6 months)
van Biesen, 2019, CJASN IPOD-PD study	n=1054	-	Moderate >7% Severe >17.3% (75th percentile at 1 month)
Kim, 2019, PD International	n=297	>2.5 L	Not analysed
Kim, 2018, Am J Nephrol	n=284	-	>15%
Ng, 2018, PLoS One	n=311	Continuous	Continuous
Jotterand Drepper, 2016, PLoS One	n=54	-	>15%
Hassan, 2015, Int Heart J	n=38	1.7 L (cut-off found to be associated with presence of left ventriculsr hypertrophy)	-
O'Lone, 2014, NDT	n=529	Per L increment	Per % increment & ≥10% (based upon highest 30%)
van Biesen, 2011, PLoS One EuroBCM study	n=639	-	>7% "fluid overload" & >15% "severe FO"
Luo, 2011, Blood Purif	n=137	>2.0 L	-
Non-dialysis CKD			
Liu, 2021, Diabetes Res Clin Pract	n=1065	Tertiles (L)	>7% vs ≤7%
Schork, 2020, Kidney Blood Press Res	n=179	Per L increment; >1 L vs ≤1 L	
Tsai, 2014, 2017 & 2018, Am J Kid Dis & PLoS One	n=472	Tertiles (L) 2014 >1.1 vs ≤1.1 L 2017	Per % increment
Vega, 2018, Clin Kidney J	n=356	Per L increment	Per % increment
Esmeray, 2018, Med Princ Pract	n=100	>0 L vs ≤0 L >0.5 L vs ≤0.5 L	-
Khan, 2016, PLoS One	n=312	Per L increment	-
Hung, 2015, J Am Heart Assoc	n=338	Per L increment	≥7% vs <7%
Heart failure			
Siriopol, 2021, Int J Cardiovasc Imaging	n=151	Per 1 L increment	Per % increment
Koell, 2017, Int J Cardiol	n=162	-	≥7% vs <7%

2.5.3 TIME WINDOWS

BCM measurements were specified to be performed at randomisation, 2 and 18 month follow-up visits. The COVID-19 pandemic caused a substantial proportion of face-to-face follow-up visits to be delayed, however BCM measurements were permitted at later attended follow-up visit appointments and grouped into two follow-up periods/windows around the 2 and 18 month visit time points, as outlined in Table 2-8. Flexibility was also incorporated into the Data Analysis Plan (appendix 11) to allow for inclusion of final follow-up measurements from participants who had not reached 18 months since randomisation at the time of their final follow-up visit due to early stopping of the trial in the 18-month time "window" for analyses.

Table 2-8: Scheduled follow-up visits relative to the randomisation visit date

Trial visit number	Follow-up month	Ideal Follow-up day	Follow-up period (or "window")
1	2	60 days	≥30, <400 days
4	18	540 days	≥400, <680 days*

* Measurements obtained ≥680 days were not included

Where analyses are reported for the full trial cohort alongside the substudy cohort (e.g. weight and blood pressure), analyses of the full trial cohort include data from all available time points (up to 36 months for some participants). For comparison with analyses from the substudy, sensitivity analyses were conducted in the full trial cohort applying the same time windows (2 and 18 months) as were used in the bioimpedance substudy.

2.5.4 WEIGHTING OF MEASUREMENTS

Values obtained corresponding to the 2- and 18-month follow-up visits were weighted according to the relative duration of each follow-up time period ("window"). The ideal time point for a 2-month measurement was 60 days post-randomisation but measurements taken on or after day 30 but before day 400 were mapped to the 2-month visit (or "window"). The 18-month visit ideally occurred on day 540 post-randomisation and readings on or after day 400 and before day 680 could be analysed in reference to this time point (or "window"). The first follow-up window is therefore 370 days in duration (≥30 to <400 days) and the second spanning 280 days (≥400 to <680 days) therefore analyses were pre-specified to weight information from the first time period at approximately 55%

compared to 45% for the second time period (weighting factors of 0.569 and 0.431). This was considered appropriate based on a hypothesised larger effect on "Fluid Overload" at the early 2-month time period relative to 18 months based on known haemodynamic mechanisms of the intervention. It was specified in a minor revision to the revised Data Analysis Plan (dated 17th November 2022, appendix 11) that statistical comparisons by treatment would be presented for the distribution of time-to-measurements from randomisation for each follow-up window.

2.5.5 SUBSTUDY OUTCOMES

2.5.5.1 PRIMARY AND SECONDARY OUTCOMES: "FLUID OVERLOAD" AS A CONTINUOUS OUTCOME

The substudy's pre-specified primary outcome was the effect of empagliflozin versus placebo on mean absolute "Fluid Overload" averaged over time (with weights proportional to the length of time between visits, see section 2.5.4), with effects on relative "Fluid Overload" provided for comparison. It was calculated that at least 382 participants would be required to have 90% power (2-sided p value=0.05) to detect at least a 0.3 L difference in absolute "Fluid Overload" between treatment groups (the substudy was not powered to detect expected differences in adiposity parameters). The effect on "Fluid Overload" at the different time points was analysed as a secondary outcome.

2.5.5.2 KEY SECONDARY COMPOSITE OUTCOME

The key secondary outcome was effects of empagliflozin versus placebo on time to the first event of a composite defined as the death from heart failure, heart failure hospitalisation, or development of new moderate or severe relative "Fluid Overload" (in participants without this level of "Fluid Overload" at baseline). Important data on fluid overload captured by BCM measurements was missed when remote follow-up visits were necessary (e.g. as a result of the COVID-19 pandemic) or after death, so the composite outcome served to capture all recorded data on fluid excess and its clinical consequences (whether measured by BCM or reflected in reported adverse events). This outcome was based upon the relative "Fluid Overload" parameter which is commonly reported in existing literature and favoured in observational research because normalisation to extracellular water facilitates comparison between individuals.

The moderate and severe "Fluid Overload" components are defined as follows:

- Development of moderate "Fluid Overload" defined as >7% to ≤15% relative "Fluid Overload" among those without this outcome at baseline;
- Development of severe "Fluid Overload" defined as >15% relative "Fluid Overload" among those without this outcome at baseline

2.5.5.3 TERTIARY OUTCOMES

Tertiary analyses included:

(i) Effects of empagliflozin versus placebo on the primary outcome by pre-specified subgroups by sex, diabetes status, NT-proBNP and eGFR at recruitment. *Post-hoc* exploratory subgroup analyses by race and baseline hydration status were also performed with dehydration; normohydration; moderate; and severe "Fluid Overload" defined based on as ≤ -7%; > -7%, ≤ +7%; > +7%, ≤ +15%; and > +15% relative "Fluid Overload" respectively. The established normohydration category (representing 60% of the substudy population at baseline) was further split into low- (> -7% ≤ 0%) and high-normohydration (>0% ≤ +7%) to further assess for evidence of trend.

(ii) Tertiary analyses also included effects of empagliflozin versus placebo on:

a. Extracellular water (ECW)
b. Intracellular water (ICW)
c. Lean tissue index (LTI) (lean tissue mass [LTM] indexed to height)
d. Fat tissue index (FTI) (fat tissue mass [FTM] indexed to height)
e. Body weight
f. BMI
g. Waist circumference
h. Hip circumference
i. Waist-to-hip ratio

(iii) Further tertiary analyses assessed effects of empagliflozin on each of the four components of the key secondary outcome.

(iv) The final tertiary assessment was an analysis of time to first outcome of regression of "Fluid Overload" (i.e. regression of moderate or severe "Fluid Overload" at randomisation to any lower hydration category).

2.6 FRAILTY ANALYSIS

The relevance of exploring heterogeneity of treatment effect according to indicators of frailty, multimorbidity, polypharmacy and health-related quality of life is discussed in section 1.3 of the introductory chapter of this thesis. In brief, these analyses focus on characteristics of patients who may be vulnerable to the diuretic and blood pressure lowering effects which are the focus of this thesis. There are several approaches to assessing and defining frailty in clinical practice and research settings (discussed in section 1.3). To address some of the limitations of these approaches and in the absence of a gold standard method, a bespoke method of assessing "frailty" in EMPA-KIDNEY was devised. This approach used "predicted risk of hospitalisation during follow-up" at baseline as the primary frailty indicator based on its established association with clinical frailty and event numbers in the EMPA-KIDNEY trial relative to mortality outcomes thereby maximising statistical power. All potential predictor variables based on data availability and biological plausibility were assessed in logistic regression models with first observed hospitalisation occurring during follow-up as the response variable (see Statistical methods section 2.7.4). Potential predictor variables included self-reported comorbidities recorded at randomisation, baseline EQ-5D-5L (quality of life) domains, physical measurements and laboratory parameters which reflect deficits in health. All potential predictor variables captured in the trial dataset were assessed, the full list is presented in Table 2-9. This list includes combinations of variables, for example any history of myocardial infarction and/or angina was collapsed into a single variable reflecting ischaemic heart disease for inclusion in model building. All variables were assessed in univariable model but only those most strongly associated with hospitalisation were retained in multivariable model building (i.e. combination variables and their component variables reflecting the same conditions were not included together in multivariable models). From the final multivariable model, predicted risk of hospitalisation during follow-up was derived for each participant and used as the primary frailty indicator.

Table 2-9: Variables assessed as potential predictors of hospitalisation

COMORBIDITIES		
Cardiovascular comorbidities assessed in isolation:	**Alternative cardiovascular disease variables combining individual variables:**	
Heart failure		Pre-specified trial definition of prior cardiovascular disease (myocardial infarction, heart failure, stroke, transient ischemic attack, or peripheral arterial disease)
Myocardial infarction	Ischaemic heart disease (myocardial infarction and/or angina)	
Angina		
Stroke	Cerebrovascular disease (stroke and/or TIA)	
Transient ischaemic attack (TIA)		
Peripheral arterial disease		
Atrial fibrillation		
Diabetes variables:	**Alternative diabetes variable combining individual variables:**	
Pre-specified trial definition of diabetes (patient-reported history of diabetes of any type, use of glucose-lowering medication, or HbA1c ≥48 mmol/mol (6.5%) at randomisation)	Diabetes with retinopathy (diabetes with retinopathy, diabetes without retinopathy, no diabetes)	
Diabetic retinopathy (yes/no)		
Other comorbidities recorded at randomisation:		
Peripheral neuropathy		
Self-reported swollen ankles		
Gout		
CLINICAL MEASUREMENTS	**LABORATORY MEASUREMENTS**	
Body mass index, kg/m^2	eGFR, mL/min/1.73 m^2	
Waist:hip ratio	uACR, mg/g	
Systolic blood pressure, mmHg	NT-proBNP, ng/L	
Diastolic blood pressure, mmHg	Haemoglobin, mg/dL	
Pulse pressure, mmHg		
HEALTH-RELATED QUALITY OF LIFE	**DEMOGRAPHICS**	
Mobility (EQ-5D-5L)	Age, years	
Self-care (EQ-5D-5L)	Sex	
Usual activities (EQ-5D-5L)	Region	
Pain/discomfort (EQ-5D-5L)		
Anxiety/depression (EQ-5D-5L)		
Self-rated health (EQ-5D-5L visual analogue scale)		
Exercise tolerance (NYHA)		

Abbreviations: eGFR = estimated glomerular filtration rate; uACR = urinary albumin-to-creatinine ratio; NT-proBNP = N-terminal pro B-type natriuretic peptide.

Additional related indicators assessed included multimorbidity, polypharmacy and health-related quality of life at baseline. Multimorbidity was defined according to the presence or absence of eight self-reported comorbid medical conditions at baseline: diabetes, heart failure, ischaemic heart disease (any history of myocardial infarction or angina), cerebrovascular disease (any history of stroke or transient ischaemic attack), peripheral arterial disease, atrial fibrillation, peripheral neuropathy and gout (Table 2-10); in addition to CKD which determined eligibility.

Table 2-10: Composition of multimorbidity subgroups

	No. of conditions (excluding CKD)			
	None or one (N=3864)	**Two (N=1369)**	**Three or more (N=1376)**	**Overall (N=6609)**
Diabetes*	923 (23.9)	982 (71.7)	1135 (82.5)	3040 (46.0)
Heart failure	20 (0.5)	98 (7.2)	540 (39.2)	658 (10.0)
Ischaemic heart disease	108 (2.8)	276 (20.2)	711 (51.7)	1095 (16.6)
Cerebrovascular disease	94 (2.4)	183 (13.4)	386 (28.1)	663 (10.0)
Atrial fibrillation	88 (2.3)	187 (13.7)	510 (37.1)	785 (11.9)
Peripheral arterial disease	31 (0.8)	100 (7.3)	339 (24.6)	470 (7.1)
Peripheral neuropathy	102 (2.6)	459 (33.5)	755 (54.9)	1316 (19.9)
Gout	564 (14.6)	453 (33.1)	690 (50.1)	1707 (25.8)

Data are n (%) of participants in each category of "No. of conditions (excluding CKD)" with that condition. * Defined as patient-reported history of diabetes of any type, use of glucose-lowering medication, or a glycated haemoglobin level of at least 48 mmol/mol (6.5%) at the randomisation visit.

Polypharmacy was derived from the number of concomitant medications recorded on the randomisation visit form for each participant. Health-related quality of life at randomisation was assessed using the EuroQoL EQ-5D-5L tool which asks participants to rate on an ordinal scale five domains (mobility, self-care, usual activities, pain or discomfort and anxiety or depression) and additionally to rate their overall health on that day between zero and 100 reflecting the worst and best health imaginable, respectively, using the visual analogue scale. The five individual domain scores for each participant were mapped to an index value using a Microsoft Excel macro which can be downloaded from: https://www.sheffield.ac.uk/nice-dsu/methods-development/mapping-eq-5d-5l-3l .

The same pre-specified efficacy and safety outcomes which have previously been reported for the overall trial population were assessed in analyses testing the impact of frailty on the treatment effects of empagliflozin versus placebo (see section 2.2).

2.7 STATISTICAL METHODS

Baseline characteristics were summarised using mean (standard deviation; SD) for normally distributed variables and median (interquartile range; IQR) and geometric mean (95% confidence interval; CI) if non-normally distributed. Normality was assessed by visual inspection of histograms and quantile-quantile plots. Numbers and percentages were used to summarise categorical variables.

All analyses followed the intention-to-treat principle (i.e. all participants included irrespective of whether they take none, some or all of their allocated treatment) (Peto and Peto, 1972, Peto et al., 1976, Peto et al., 1977). The substudy primary outcome was pre-specified to be assessed using a mixed model repeated measures (MMRM) approach adjusted for age, sex, prior diabetes, eGFR, and uACR in the categories used in the minimised randomisation algorithm. The MMRM model also included fixed categorical effects of time (to avoid assuming a linear association between treatment allocation and the outcome variable over time), treatment allocation, treatment-by-time interaction, and continuous effects of baseline (randomisation) measurements, and baseline-by-time interaction. The within-person error correlations were assumed to be unstructured. Analyses of the full trial cohort were additionally adjusted for region. Effects at each follow-up time point were estimated and used to derive study-average effects (with weights proportional to the amount of time between visits). All between-group differences were reported as empagliflozin minus placebo. To assess effect modification, subgroup-specific treatment effects were estimated by fitting interaction terms in the MMRM models. The null hypothesis was that the treatment effect is the same across all subgroups. This was tested by calculating a heterogeneity or trend statistic from subgroup-specific means and standard errors, without correction for multiplicity of testing.

The key secondary outcome and its components were analysed using an adjusted Cox proportional hazards regression using the same covariates in the minimisation algorithm (age, sex, prior diabetes, eGFR and uACR) and included the complete substudy population of 660 participants (i.e. it included participants without a valid follow-up bioimpedance measurement who were excluded from MMRM analyses but were at risk of clinical outcomes). Tertiary analyses used the same MMRM approach as described for the primary outcome and assessed effects on ECW, ICW, LTI, FTI, body weight and BMI. Waist and

hip circumference measurements were obtained at a single follow-up time point (18 months) and were therefore analysed by analysis of covariance (ANCOVA), adjusted for the baseline value and minimisation variables. Missing baseline measurements were handled by mean imputation and missing follow-up measurements were handled by multiple imputation. The same approach was followed for the substudy and full trial population for effects on waist-to-hip ratio. All 6609 participants were included and the imputed dataset was then subset to derive an imputed dataset for analysis of effects in substudy participants specifically. The imputation model included non-missing values of baseline and follow-up measurements of waist, hip and waist-to-hip ratio as appropriate in separate imputation models for each of these parameters; as well as weight. Treatment allocation and the minimisation algorithm variables (age, sex, eGFR, uACR and region) were also included as covariates in the imputation model. Associations between other variables and missingness of waist-to-hip ratio were assessed in univariable logistic regression to determine inclusion of additional covariates which identified NT-proBNP as an additional relevant covariate. Multiple imputation produced 20 imputed datasets which were each analysed by ANCOVA and treatment-specific estimated marginal means and standard errors were then pooled using the method of Rubin. All multiple imputation analyses were implemented using the multiple imputation procedure in SAS version 9.4 (SAS Institute, Cary NC), using the expectation-maximisation algorithm (which assumes a multivariate normal distribution) to impute values. Multiple imputation was conducted by trial statistician Natalie Staplin since it would not have been possible to exactly replicate an imputed dataset using this approach in R and SAS programmes.

P values for hypothesis testing for outcomes were limited to the primary outcome. P values for testing for any evidence of effect modification between subgroups, and between treatment effect and effects by time are presented. For all statistical tests (other than tests for heterogeneity or trend), the null hypothesis was that the effect of allocation to empagliflozin on the parameter of interest (e.g. “Fluid Overload”) in the target population is the same as the effect of allocation to placebo (and hence the alternative hypothesis will be that the effect of allocation to empagliflozin is not the same as the effect of allocation to placebo).

All bioimpedance substudy analyses were pre-specified before the main results of the trial were known and while investigators remained blind to study treatment allocation.

Additional analyses to provide context for the bioimpedance substudy results (and for the purposes of this doctoral research project) were planned following publication of the main trial results (November 2022). Analyses were performed using R Studio version 4.2.2 (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA).

2.7.1 HANDLING OF MISSING AND DUPLICATE DATA

Missing baseline data was handled differently in analyses of the bioimpedance substudy and full trial cohort. This is because substudy analyses were pre-specified with the aim of maximising inclusion (imputation rather than exclusion of missing baseline data). Related analyses of the full trial cohort were conducted later and it was necessary to follow procedures used in the pre-specified main trial analyses (as published and according to the main trial Data Analysis Plan) and since the analysis population for the full trial is ten-times larger than the substudy, exclusion of participants with missing baseline data was justifiable. This approach is outlined in more detail for the substudy and full trial cohort analyses separately in the following sections.

2.7.1.1 MISSING DATA IN THE BIOIMPEDANCE SUBSTUDY

In bioimpedance substudy analyses, participants with a missing baseline bioimpedance measurement could still be included if subsequent bioimpedance measurements were obtained within the 2- and/or 18-month follow-up windows. Missing baseline data were imputed with the mean observed value across both treatment groups combined. Participants with missing baseline values relevant to categorical groupings and/or subgroup analyses were included in the category containing the mean value (or the most frequent category for a binary variable). Analyses of continuous outcome variables using an MMRM approach required a consenting participant to have at least one valid measurement during follow-up and otherwise participants were excluded from the analysis of that outcome variable (but could be included in other analyses). The MMRM approach handles missing follow-up measurements implicitly where participants had at least one follow-up measurement available but missing data at other follow-up time points.

2.7.1.2 DUPLICATE DATA IN THE BIOIMPEDANCE SUBSTUDY

In all bioimpedance substudy analyses, if more than one valid bioimpedance measurement was available at a single follow-up visit (i.e. date), the measurement with the highest Q value was used and additional measurements ignored.

In all bioimpedance substudy analyses, if valid bioimpedance measurements were made on more than one day within a follow-up period, then the valid bioimpedance measurement made on the day nearest the ideal follow-up day was used. In the situation where >1 valid BCM measurement was obtained within a follow-up window on dates which were equidistant from the ideal follow-up date, a mean value was calculated and used in analyses. This was considered a more scientifically robust approach in this unique situation due to the hypothesised interaction of time in the association between treatment allocation and "Fluid Overload" which means that selecting one or other equidistant measurement on the basis of Q values could introduce bias.

Where data for two separate visits were recorded on a single BCM data card, valid BCM results were derived for the separate visits (and in some cases, separate participants due to site error).

2.7.1.3 MISSING DATA IN ANALYSES OF THE FULL TRIAL COHORT

In analyses of the full trial cohort, participants missing baseline measurements for weight, blood pressure, HbA1c or haematocrit were excluded from the analysis of that outcome variable (but could be included in other analyses). The MMRM approach handles missing follow-up measurements implicitly where participants had at least one follow-up measurement available but missing data at other follow-up time points.

Analyses of waist-to-hip ratio required to be handled differently. Since waist-to-hip ratio was only measured at a single follow-up time point (unlike bioimpedance parameters, body weight, blood pressure and laboratory parameters presented), analysis used linear regression and required multiple imputation of missing data (rather than the MMRM

approach). Since all published analyses required to be validated by the trial statistician but multiply imputed datasets generated by myself in R would not produce exactly the same imputations as by another operator using SAS; for simplicity this analysis was conducted only by the trial statistician (reported in section 5.2.3.2).

2.7.2 SENSITIVITY ANALYSES IN THE BIOIMPEDANCE SUBSTUDY

The effect of quality control steps was assessed in three sensitivity analyses for the primary outcome in the bioimpedance substudy. The first included all bioimpedance measurements irrespective of quality, imputing only missing baseline measurements; the second included all measurements with an automated quality value ≥80, imputing missing measurements and those with quality value <80; and the third was limited to participants with complete valid baseline BCM data only (no imputation).

It was specified in a minor revision to the Data Analysis Plan (dated 17th November 2022, appendix 11) that statistical comparisons by treatment would be presented for the distribution of Q values for measurements included in the main comparison and sensitivity analyses.

2.7.3 METHODS OF ADDITIONAL SUPPLEMENTARY ANALYSES IN THE FULL TRIAL COHORT

In order for inferences from the bioimpedance substudy assessments of fluid and adiposity to be put in the context of findings from all the available data, additional analyses from the full EMPA-KIDNEY trial cohort were also conducted. These included assessments of the effects of empagliflozin versus placebo on total weight, body mass index, waist-to-hip ratio, systolic and diastolic blood pressure, glycated haemoglobin and haematocrit. These analyses emphasised results of study average effects (with effects at 2 and 18 months also presented). These analyses were also intended to allow for exploration of subgroup effects on weight with greater reliability than would be possible in the smaller substudy population. Effects on weight and systolic blood pressure were explored according to sex, diabetes status, eGFR and NT-proBNP at baseline (the same subgroups as were pre-specified in the bioimpedance substudy). Additional subgroup analyses were conducted for all variables stratified by self-reported participant race to assess for any evidence of

differential effects by race in the full trial cohort since the substudy took place in the UK and Germany only.

Since outcome variables were only assessed at two time points in the bioimpedance substudy (2- and 18-month time windows) and data from the full trial cohort were available from all routine trial visits (at 2-, 6-, 12-, 18-, 24-, 30- and 36-month follow-up visits), handling of time required careful consideration for these additional analyses. In analyses of the full trial cohort, study averages include all available measurements to maximise statistical power and robustness of treatment effect estimates. In the bioimpedance substudy results publication, full trial results at the 2- and 18-month visits were emphasised and results from 6-, 12-, 24-, 30- and 36-month visits were not shown for simplicity of presentation. Substudy results were compared to results from the full cohort using standard statistical tests of heterogeneity.

A sensitivity analysis was conducted to assess an alternative approach of applying the two time windows which were pre-specified in the bioimpedance substudy to the full trial cohort analyses to allow for comparison between these assessments and effects on fluid overload in participants with measurements available for both outcomes within the bioimpedance substudy. Where multiple measurements were available within either time window (2- or 18-months as specified in the substudy Data Analysis Plan – appendix 11), the measurement which was nearest measurement to the "ideal" follow-up day in each window (60 and 540 days, respectively) was retained. This sensitivity analysis was conducted for effects on weight and systolic blood pressure.

2.7.4 PREDICTED RISK OF HOSPITALISATION

Logistic regression models adjusted for age, sex and region assessed the individual association of all potential predictor variables with recorded hospitalisation (first event). Models were developed first in the complete EMPA-KIDNEY population irrespective of treatment allocation to maximise power. The approach was then repeated restricted to the placebo group since allocation to empagliflozin can be expected to modify risk of hospitalisation (and may therefore confound associations between other variables and the outcome). Missing data in predictor variables was handled by mean (or median for non-

normal data) imputation (or the most common category for categorical variables) since missingness was infrequent and not associated with first hospitalisation on logistic regression. For continuous variables, nonlinearity was assessed by comparing models with and without quadratic terms in addition to the linear terms using likelihood ratio tests. If the inclusion of the quadratic term improved model fit (likelihood ratio test P <0.01), both terms were included in multivariable model building. All variables which were significantly associated (P<0.01) with hospitalisation in univariable (with age, sex and region forced to remain) models proceeded to inclusion in multivariable model building using forward stepwise selection, adding variables in order of best-fitting univariable (adjusted for age, sex and region) models (determined by lowest Akaike information criterion [AIC] values). The impact of stepwise additions was assessed using likelihood ratio tests with significance threshold P<0.01. Model performance was assessed using calibration plots and the area under the receiver operating characteristic curve (AUROC).

For each frailty indicator (predicted risk of hospitalisation, multimorbidity, polypharmacy and health-related quality of life), participants were categorised into approximate thirds of the variable's distribution to define subgroups. The top third was further dichotomised for the emphasised assessments by predicted risk of hospitalisation to provide greater discrimination among those at highest risk. The effects of allocation to empagliflozin versus placebo on the trial's pre-specified efficacy and safety outcomes were assessed using Cox regression models adjusted for age, sex, region, eGFR, uACR and diabetes status in the categories used in the minimised randomisation algorithm. Effects on weight and blood pressure were analysed using a mixed model repeated measures (MMRM) approach adjusted for age, sex, region, eGFR, uACR and diabetes status in the same categories. The MMRM model also included fixed categorical effects of time (to avoid assuming a linear association between treatment allocation and weight or blood pressure over time), treatment allocation, treatment-by-time interaction, and continuous effects of baseline (randomisation) measurements, and baseline-by-time interaction. The within-person error correlations were assumed to be unstructured. Effects at each follow-up time point were estimated and used to derive study-average effects (with weights proportional to the amount of time between visits). All between-group differences are reported as empagliflozin minus placebo. Analyses of total hospitalisations were performed using joint frailty models. Tests for trend across subgroups were used to assess for any evidence of treatment effect modification without correction for multiplicity of testing.

2.7.5 ESTIMATED ABSOLUTE EFFECTS

Estimated absolute effects in subgroups were calculated by applying hazard ratios to the observed event rate in the placebo arm of the subgroup. Which hazard ratio to use was determined by whether there was statistically significant evidence of heterogeneity in relative effects of treatment according to levels of the subgroup variable. If there was significant heterogeneity ($P<0.01$), the subgroup-specific hazard ratio was applied otherwise the overall hazard ratio was applied to all subgroup levels.

These estimated absolute effects were presented as absolute events avoided per 1000 patients treated with empagliflozin per year (and corresponding standard error) for each subgroup level; calculated as the difference between the absolute event rate in the placebo group and the estimated absolute event rate in the treatment group for each subgroup level.

Steps of these calculations were as follows:

art = arc x hr

arr = arc - art

se_arr = arc x hr x se_hr

where *arc* = observed absolute event rate in the control (placebo) group

art = estimated absolute event rate in the treatment (empagliflozin) group

hr = hazard ratio (overall/subgroup-specific dictated by heterogeneity tests above)

arr = absolute risk reduction; the estimated number of events avoided (or caused)

se = standard error

Estimated absolute effects were generally presented per 1000 patients treated for one year (based on event rates per 1000 patient-years) with the exception of analyses of all-cause hospitalisations which were presented per 100 patients treated for one year (based on event rates per 100 patient-years for consistency with analyses published in the trial's main results publication in November 2022).

CHAPTER 3 – SYSTEMATIC REVIEW OF BIOIMPEDANCE-DERIVED FLUID OVERLOAD AND ASSOCIATIONS WITH CLINICAL OUTCOMES IN CKD AND HEART FAILURE

3.1 INTRODUCTION

The previous chapters provide the introduction (sections 1.4-1.6) and methods (section 2.1) for this chapter reporting findings of a systematic review of the use of bioimpedance techniques in non-dialysis chronic kidney disease (CKD) and heart failure. This was intended to provide background on the use of bioimpedance in the disease population studied in the EMPA-KIDNEY bioimpedance substudy (with non-dialysis CKD, in whom heart failure is common) to inform the Data Analysis Plan for the bioimpedance substudy.

Bioimpedance has been widely used to assess fluid status in dialysis populations and is summarised in existing systematic reviews but not for non-dialysis CKD. This work sought to assess whether similar positive associations exist between fluid excess and adverse cardiorenal outcomes in earlier stages of CKD, where fluid excess may be less marked than in dialysis cohorts, but may still represent a key modifiable cause of morbidity and mortality. Much overlap exists between CKD and heart failure and fluid excess is a hallmark of heart failure however studies of bioimpedance parameters in heart failure populations are few therefore both populations were included in the review. Concerning terminology, as explained in section 2.5.2, throughout this thesis, “Fluid Overload” (including “” and capitalisation of each word) is used to refer to the bioimpedance-derived parameter with non-capitalised terms used as descriptions of physiological state wherever possible (fluid excess is used in preference to fluid overload in an attempt to distinguish from references to the bioimpedance parameter “Fluid Overload”).

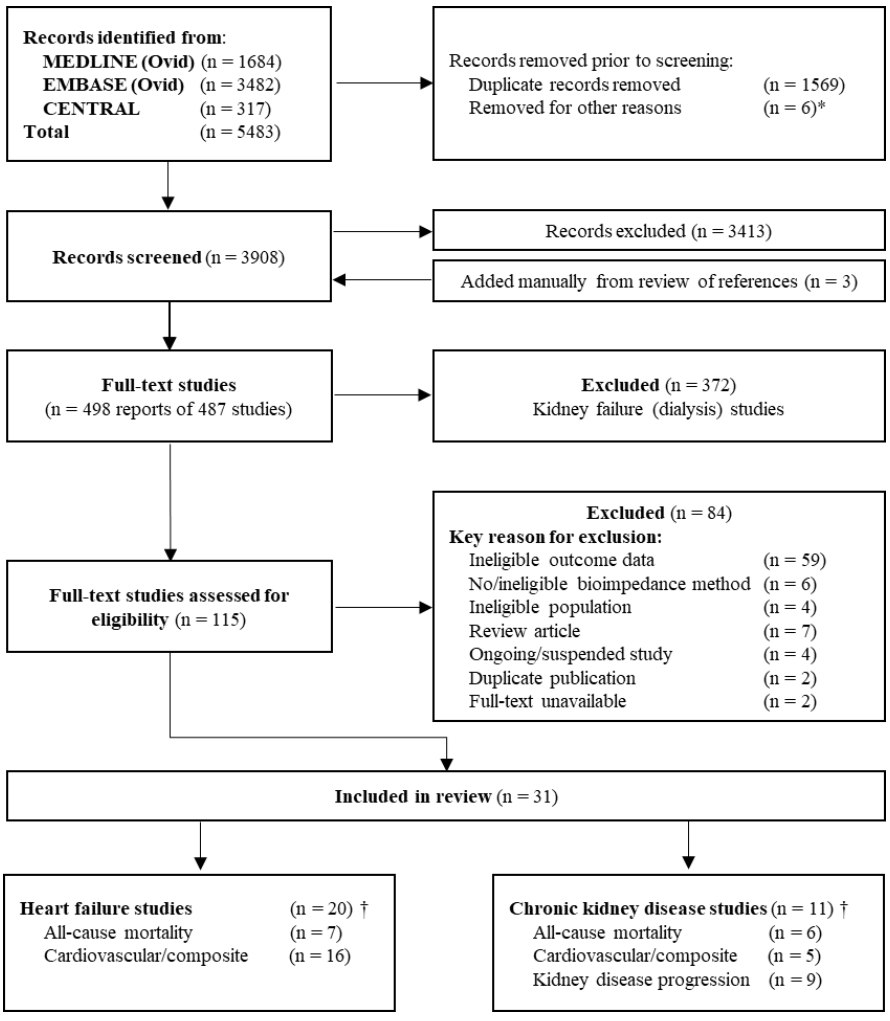
A secondary aim of the review was to summarise definitions and identify thresholds of “Fluid Overload” which are associated with adverse cardiorenal outcomes to inform pre-specified analyses of the EMPA-KIDNEY bioimpedance substudy, as summarised in section 2.5.2. The review was restricted to assessment of fluid parameters and did not seek to summarise bioimpedance-derived adiposity parameters based on a preliminary literature search which found inconsistent results reported by existing studies. Furthermore, the numbers of studies reporting these outcomes were small and fluid and adiposity parameters were infrequently analysed in the same study.

3.2 RESULTS

3.2.1 SEARCH RESULTS

Figure 3-1 presents search results, reasons for exclusion and included studies. The final number of included studies was 31, of which 20 studied heart failure populations, 10 studied CKD populations and one study included patients with type 2 diabetes with and without CKD. Seven studies were identified as having multiple reports, these are summarised in Table 3-1 with the selected report from which data were extracted highlighted in bold. The selection approach considered which report presented maximal outcome data and favoured analyses with the longest follow-up time and largest population. Where different fluid parameters were used, this was considered alongside the aforementioned factors and parameters most synonymous with other studies were favoured.

Figure 3-1: PRISMA flow diagram



*CENTRAL results produced 311 trials and 6 Cochrane reviews - 6 reviews removed as ineligible. † 6 CKD studies & 3 heart failure studies reported more than one relevant outcome.

Table 3-1: Studies with multiple reports identified

	Author	Year	Journal	Title	N	Recruitment dates	Follow-up (yrs)	Fluid overload parameter	Outcomes
A	Tsai	2013	PLoS One	Is Fluid Overload More Important than Diabetes in Renal Progression in Late Chronic Kidney Disease?	472	Jan-Dec 2011	1.4	Relative "Fluid Overload", %	KRT initiation; Δ eGFR
A	Tsai	2014	Am J Kidney Dis	Association of Fluid Overload With Kidney Disease Progression in Advanced CKD: A Prospective Cohort Study	472	Jan-Dec 2011	1.4	Absolute "Fluid Overload", L	KRT initiation; Δ eGFR
A	Tsai	2015	CJASN	Association of Fluid Overload with Cardiovascular Morbidity and All-Cause Mortality in Stages 4 and 5 CKD	478	Jan-Dec 2011	1.9	Relative "Fluid Overload", %	All-cause mortality; MACE
A	Tsai	2017	PLoS One	The interaction between fluid status and angiopoietin-2 in adverse renal outcomes of chronic kidney disease	290	Jan-Dec 2011	3.2	Absolute "Fluid Overload", L	KRT initiation; Δ eGFR
A	**Tsai**	**2018**	**PLoS One**	**The interaction between N-terminal pro-brain natriuretic peptide and fluid status in adverse clinical outcomes of late stages of chronic kidney disease**	**239**	**Jan-Dec 2011**	**3.3**	**Relative "Fluid Overload", %**	**All-cause mortality; MACE; KRT initiation; Δ eGFR**
B	**Hung**	**2015**	**J Am Heart Assoc**	**Volume Overload and Adverse Outcomes in Chronic Kidney Disease: Clinical Observational and Animal Studies**	**338**	**Sep 2011-Dec 2012**	**2.1**	**Relative "Fluid Overload", %**	**Composite Δ eGFR/KRT; composite CV morbidity/ mortality**
B	Hung	2015	J Am Heart Assoc	Association of Fluid Retention With Anemia and Clinical Outcomes Among Patients With Chronic Kidney Disease	326		2.2	Relative "Fluid Overload", %	
C	Khan	2016	PLoS One	Chronic Kidney Disease, Fluid Overload and Diuretics: A Complicated Triangle	312	NA	1.0	Absolute "Fluid Overload", L	Δ eGFR; KRT initiation [1]
C	**Khan**	**2017**	**Clin Exper Nephrol**	**Diuretics prescribing in chronic kidney disease patients: physician assessment versus bioimpedence spectroscopy**	**312**	**NA**	**1.0**	ECW, L (absolute "Fluid Overload", L)	Δ eGFR; KRT initiation
D	Orea-Tejeda	2010	Cardiology	Prognostic value of cardiac troponin T elevation is independent of renal function and clinical findings in heart failure patients	152	2002-2011	3.5	BIVA hydration index plot	All-cause mortality
D	**Colin-Ramirez**	**2012**	**Nutrition**	**Bioelectrical impedance phase angle as a prognostic marker in chronic heart failure**	**389**	**2002-2011 [2]**	**3.0**	**Phase angle, °**	**All-cause mortality**
D	Castillo-Martinez	2016	Nutrición hospitalaria	Body composition changes assessed by bioelectrical impedance and their associations with functional class deterioration in stable heart failure patients	275	2002-2011	0.5	Resistance/height, Ω/m	Change in NYHA class
E	Vega	2017	Clin Kidney J	Low lean tissue mass is an independent risk factor for mortality in patients with stages 4 and 5 nondialysis chronic kidney disease	356	NA	1.8	Absolute "Fluid Overload", L	All-cause mortality (CV events, KRT initiation) [3]
E	**Vega**	**2018**	**Clin Kidney J**	**Any grade of relative overhydration is associated with long-term mortality in patients with Stages 4 and 5 nondialysis chronic kidney disease**	**356**	**From Jan 2011**	**4.2**	**Relative "Fluid Overload", %**	**All-cause mortality**
F	Low	2021	J Diabetes Complicat	Higher extracellular water to total body water ratio was associated with chronic kidney disease progression in type 2 diabetes	1079	March 2011-March 2014	8.6	ECW:TBW ratio	Δ eGFR & worsening KDIGO category composite
F	**Liu**	**2021**	**Diab Res & Clin Practice**	**Association of overhydration and serum pigment epithelium-derived factor with CKD progression in diabetic kidney disease: A prospective cohort study**	**1065**	**March 2011-March 2014**	**8.6**	**Absolute (L) & (%) "Fluid Overload"**	**Δ eGFR & worsening KDIGO category composite**
G	Tai	2014	BMC Nephrol	Association between ratio of measured extracellular volume to expected body fluid volume and renal outcomes in patients with chronic kidney disease: a retrospective single-center cohort study	149	2005-2009	4.9	ECW:TBW	Composite Δ eGFR/KRT
G	**Ohashi**	**2015**	**J Nutr Health Aging**	**The associations of malnutrition and aging with fluid volume imbalance between intra- and extracellular water in patients with chronic kidney disease**	**149**	**2005-2009**	**4.9**	**ECW:ICW**	**All-cause mortality; CV events; Composite Δ eGFR/KRT**

The selected report from which data were extracted is highlighted in bold. [1] Multivariable survival analysis not reported by fluid overload (diuretic status only), author confirmed by email therefore 2017 report favoured. [2] Author confirmed all studies represent the same cohort and recruitment dates 2002-2011 provided by email; 2012 study favoured based upon population size and fluid overload measurement more synonymous with other studies. [3] 2017 paper states no association between fluid overload and KRT or CV events however not quantified. Several other included studies share authors but report upon distinct cohorts.

3.2.2 STUDY CHARACTERISTICS

Characteristics of the included studies are summarised overall in Table 3-2 and also presented separately for each included study, grouped by CKD (Table 3-3) and heart failure study populations (Table 3-4). Two studies included more than 1000 participants, but the majority of included studies were small. CKD studies were generally larger (range 100-3751 participants) than those in heart failure populations (51-706 participants) with longer durations of follow-up (range 1.0-8.6 years for CKD versus 0.02-3.0 years for heart failure cohorts). Heart failure studies more commonly studied participants with acute decompensated heart failure compared with stable chronic disease and heart failure subtypes (HFpEF vs HFrEF) were not frequently distinguished.

Table 3-2: Summary of characteristics of identified cohorts

	CKD cohorts (n=11)		Heart failure cohorts (n=20)	
Year of publication, n (%)				
2017 – 2021	8	(73)	11	(55)
2012 – 2016	2	(18)	8	(40)
Before 2012	1	(9)	1	(5)
Region, n (%)*				
Europe	4	(36)	12	(60)*
Asia	6	(55)	3	(15)
Russia	0	-	1	(5)
N. America	1	(9)	3	(15)*
S. America	0	-	3	(15)*
Median number of total participants (IQR)	236 (177-347)		175 (104-362)	
Median follow-up (IQR), years	3.3 (1.4-5.4)		0.9 (0.5-1.6)	
Median % male (IQR)	55 (50-61)		55 (49-63)	
Median % diabetes mellitus (IQR)	45 (36-49)		37 (35-44)	
Median % hypertension (IQR)	86 (82-87)		78 (71-79)	
Bioimpedance method, n (%)				
Bioimpedance analysis (BIA)	4	(36)	7	(35)
Bioimpedance vector analysis (BIVA)	0	-	11	(55)
Bioimpedance spectroscopy (BIS)	7	(64)	2	(10)
Bioimpedance device, n (%)				
Fresenius Body Composition Monitor	7	(64)	2	(10)
(BCM)	2	(18)	2	(10)
InBody S20/520/720	0	-	7	(35)
EFG	1	(9)	1	(5)
Quantum II/X	1	(9)	8	(40)
Other/not reported				
Fluid status parameter, n (%)				
Absolute & relative “Fluid Overload”	3	(27)	1	(5)
Absolute “Fluid Overload”	2	(18)	0	-
Relative “Fluid Overload”	1	(9)	1	(5)
Phase angle	2	(18)	3	(15)
BIVA hydration index/other BIVA	0	-	11	(55)
Extracellular water/ratio	3	(27)	4	(20)

For studies reporting more than one fluid status parameter, only those analysed in association with clinical outcomes are presented in the table (except for the related parameters absolute & relative “Fluid Overload”).
*Two studies included participants in two geographical regions

Table 3-3: Study characteristics for all included CKD cohorts

Author	Year	Region	Study design	N	Follow-up (yrs)	CKD inclusion criteria	Heart failure exclusion/reporting	Method	Device	Outcomes reported		
										All-cause mortality	CV	Kidney
Bansal	2018	North America	Prospective cohort	3751	7	eGFR 20-70	Excluded	BIA	Quantum II (RJL)	Y	Y	Y
Liu	2021	Asia	Prospective cohort	1065	8.6	CKD 1-4 & non-CKD with type 2 diabetes	Not excluded/reported	BIA	InBody S20	N	N	Y
Vega	2018	Europe	Prospective cohort	356	4.2	CKD 4-5	Included; reported baseline heart failure 27%	BIS	Fresenius BCM	Y	Y	N*
Hung	2015	Asia	Prospective cohort	338	2.1	CKD 3-5	Included in CVD outcome, baseline reported as CVD	BIS	Fresenius BCM	N	Y	Y
Khan	2017	Asia	Prospective cohort	312	1	CKD 3-5	Excluded	BIS	Fresenius BCM	N	N	Y
Tsai	2018	Asia	Prospective cohort	236	3.3	CKD 4-5	Included in outcome, baseline reported as CVD	BIS	Fresenius BCM	Y	Y	Y
Kohatsu	2021	Asia	Retrospective cohort	194	1.4	CKD 3-5	Not excluded/reported	BIA	BioScan 920-II	N	N	Y
Schork	2020	Europe	Retrospective cohort	179†	5.9	CKD 1-5	Not excluded/reported	BIS	Fresenius BCM	N	N	Y
Caravaca	2011	Europe	Prospective cohort	175	1.3	eGFR <40	Excluded	BIS	Fresenius BCM	Y	N	N
Ohashi	2015	Asia	Retrospective cohort	149	4.9	Not specified	Included in CVD outcome, baseline not reported	BIA	InBody S20	Y	Y	Y
Esmeray	2018	Europe	Non-randomised experimental	100	1	CKD 3-4	Excluded	BIS	Fresenius BCM	Y	N	Y

* Event numbers reported for progression to dialysis however no analysis reported. †179 included, 177 with outcome data for kidney outcomes.

Table 3-4: Study characteristics for all included heart failure cohorts

Author	Year	Region	Study design	N	Follow-up (years)	Heart failure inclusion criteria	CKD exclusion criteria/reporting	Method	Device	Outcomes reported		
										All-cause mortality	CV	Kidney
Massari	2019	Europe	Retrospective cohort	706	0.02	AHF; LVEF <40%, 40-49%, ≥50%	Included CKD	BIVA	CardioEFG (Akern)	N	Y	N
Massari	2020	Europe	Retrospective cohort	436	1.3	AHF & CHF; LVEF <40%, 40-49%, ≥50%	Included CKD	BIVA	CardioEFG (Akern)	Y	N	N
Colin-Ramirez	2012	North America	Retrospective cohort	389	3	CHF; HFrEF & HFpEF	Included CKD	BIA	Quantum X (RJL)	Y	N	N
Di Somma	2014	Europe	Prospective cohort	381[1]	0.1	AHF	Excluded eGFR <30	BIVA	Tetrapolar 50 kHz (Akern Srl)	N	Y	N
Nunez	2016	Europe	Prospective cohort	369	1	AHF	Included CKD	BIVA	CardioEFG (Akern)	Y	Y	N
Lyons	2017	North America	Prospective cohort	359	2.1	CHF; HFrEF & HFpEF	Not reported	BIA	InBody 520	N	Y	N
Santarelli, EHJ Acute CV Care	2017	Europe	Prospective cohort	336[2]	0.3	AHF	Included CKD	BIVA	EFG (Akern)	Y	Y	N
Santarelli, Intern Emerg Med	2017	Europe & South America	Prospective cohort	292	0.3	AHF	Included CKD	BIVA	EFG (Akern)	N	Y	N
De Berardinis	2014	Europe & North America	Prospective cohort	194	1.5	AHF	Excluded KRT; reported baseline CKD 31%	BIVA	Tetrapolar 50 kHz (Akern Srl)	Y	Y	N
Sakaguchi	2015	Asia	Prospective cohort	190[3]	0.5	AHF	Excluded sCr >3 mg/dL	BIA	BioScan 920-2 (Maltron Intl)	N	Y	N
Liu	2012	Asia	Randomised controlled trial	159[4]	0.5	AHF; LVEF <40%	Excluded sCr >5 mg/dL / nephritic; reported baseline CKD 38%	BIA	InBody 720	N	Y	N
Siriopol	2021	Europe	Prospective cohort	151	1.7	CHF; LVEF <45%	Excluded ESKD	BIS	Fresenius BCM	Y	N	N
Koell	2017	Europe	Prospective cohort	150	2	CHF; HFpEF (LVEF>50%)	Included CKD	BIS	Fresenius BCM	N	Y	N
Soloveva	2019	Russia	Prospective cohort	149	0.8	AHF	Excluded ESKD; reported baseline CKD 23%	BIVA	ABC-01 (Medass)	N	Y	N
Trejo-Velasco	2016	Europe	Prospective cohort	105	0.9	AHF	Included CKD	BIVA	CardioEFG (Akern)	N	Y	N
Sakaguchi	2020	Asia	Prospective cohort	100[5]	0.5	AHF	Included CKD	BIA	Bioscan 920-2 (Maltron Intl)	N	Y	N
Curbelo	2019	Europe	Prospective cohort	99	1	CHF	Excluded dialysis	BIA	Bodygram (Akern)	N	Y	N
Villacorta	2021	South America	Prospective cohort	80	0.6	AHF	Excluded dialysis (or imminent)	BIVA	EFG (Akern)	N	Y	N
Alves	2016	South America	Prospective cohort	71	2	AHF; LVEF ≤45%	Excluded serum creatinine >2.5 mg/dL or dialysis	BIA	Biodynamics 450	Y	N	N
Di Somma	2010	Europe	Prospective cohort	51[6]	0.3	AHF	Excluded eGFR <60	BIVA	NA	N	Y	N

[1] 270/381 with AHF; 111 controls. [2] 221/336 with AHF. [3] 130 with AHF + 60 hospitalized controls; controls used to determine predicted values ECW only, analysis is of AHF patients (not compared to controls). [4] 53 in case management with BIA group; 53 in case management without BIA; 53 controls (routine care). [5] 100 with central venous catheter and therefore included in survival analysis reporting fluid overload. [6] 25 AHF + 26 controls.

Table 3-5: Baseline participant characteristics from CKD cohorts

Author	Year	Age, years	Male %	eGFR, mL/min/1.73m^2	Urine protein	BMI, kg/m^2	Systolic BP, mm/Hg	Primary renal diagnosis, %	Diabetes %	Hypertension %	Smoking %	Diuretic %	RASi %	NT-proBNP/ BNP pg/ml	Haemoglobin g/dL	Serum albumin g/dL	Serum sodium mmol/L or mEq/L
Liu	2021	59 (9)	51	92 (69-103)*	uACR 21.6 (7.3-80.0) mg/g*	28 (5)	139 (18)	-	100	-	-	-	63	-	-	-	-
Vega	2018	67 (13)	64	16 (6)	Urine protein 0.5 (0.2-1.5) g/24h*	28 (5)	-	GN 23%; DM 19%; Vasc 28%; Interstitial 13%; PKD 10%; Other 7%	36	87	-	-	-	840 (370-1810)*	-	4.1 (0.4)	-
Hung	2015	66 (14)	69	29 (15)	uPCR 0.9 (0.3-2.5) g/g*	26 (4)	138 (17)	-	45	46	21	33	59	242 (78-771)*	-	3.6 (0.4)	136 (4)
Khan	2017	65 (6)	57	21 (9)	Urine protein >1 52%	24 (5)	140 (21)	-	64	86	31	-	57	-	-	4.2 (0.4)	139 (3)
Tsai	2018	65 (12)	53	16 (8)	uPCR 1.9 (2.1) mg/mg	24 (4)	138 (19)	-	39	81	18	22	53	262 (125-742)*	10.5 (1.8)	4.1 (0.4)	-
Schork	2020	60 (48-71)*	55	47 (30-71)*	uACR 43 (5-198) mg/g*	28 (26-32)*	134 (125-149)*	GN 38%; DM/ HTN 33%; Interstitial 2%; PKD 5%; Other 23%	21	81	-	61	78	182 (68-613)*	-	-	-
Kohatsu	2021	71 (12)	76	24 (11)	Urine protein 0.9 (0.2-2.2) g/24h*	25 (5)	133 (16)	GN 12%; DM 32%; Vasc 28%; Other 28%	47	94	-	32	68	-	11.4 (1.8)	3.9 (0.5)	-
Caravaca	2011	66 (14)	56	16 (6)	uACR 1.8 (2.1) mg/g	30 (5)	159 (24)	-	35	11†	-	52	-	-	12.0 (1.4)	4.1 (0.3)	-

Limited to studies reporting characteristics for the full cohort. Baseline characteristics for entire cohort not available for Bansal, Ohashi & Esmeray studies. None of the CKD studies reported left ventricular ejection fraction or NYHA class. Data are presented as mean (SD) unless denoted by * which indicates median (IQR). † Reported uncontrolled hypertension only.

Table 3-6: Baseline participant characteristics from heart failure cohorts

Author	Year	Age, years	Male %	eGFR, mL/min/ 1.73m^2	Serum creatinine mg/dL	BMI, kg/m^2	Systolic BP, mm/Hg	LVEF, %	NYHA class III-IV %	Diabetes %	Hypertension %	Smoking %	Diuretic %	RASi %	NT-proBNP/ BNP pg/ml	Haemo-globin g/dL	Serum albumin g/dL	Serum sodium mmol/L or mEq/L
Massari	2019	78 (10)	52	CrCl 46 (23)	1.6 (1.1)	28 (5)	-	44 (14)	-	24	-	-	Loop 97%	ACEi 36%; ARB 12%	830 (479-1810)*	12.0 (2.0)	3.2 (0.5)	139 (4)
Massari	2020	75 (11)	52	-	1.2 (0.8)	28 (5)	130 (25)	Preserved = 48% Mid-range = 10% Reduced = 42%	56	24	-	-	Loop 69%; MRA 69%	ACEi 39%; ARB 21%	503 (197-1000)*	13.0 (2.0)	3.3 (0.6)	139 (4)
Di Somma†	2014	77 (11)	53	57 (29)	-	27 (6)	140 (29)	-	-	37	79	-	-	-	717 (786)	-	-	-
Nunez	2014	73 (11)	50	-	1.2 (0.5)	-	148 (35)	49 (16)	27	46	78	11	Loop 97%; MRA 36%	ACEi 35%; ARB 30%	4041 (5921)	12.1 (1.9)	-	138 (4)
Lyons	2017	56 (14)	72	-	1.3 (1.1)	28 (6)	115 (19)	36 (16)	34	24	-	-	MRA 52%	84	334 (500)	-	4.7 (4.4)	-
Santarelli, EHJ Acute CV Care	2017	79 (8)	41	55 (26)	-	-	140 (27)	-	-	-	-	-	-	-	859 (985)	-	-	138 (5)
De Berardinis	2014	76 (11)	56	55 (29)	1.6 (1.1)	29 (14)	150 (34)	-	-	45	78	-	Loop 65%; MRA 13%	ACEi 28%; ARB 14%	873 (1024)	11.9 (0.0)	-	137 (64)
Sakaguchi	2015	74 (11)	55	49 (24)	1.2 (0.6)	-	137 (33)	45 (19)	-	37	71	-	Loop 67%; MRA 31%	56	653 (586)	12.0 (2.5)	3.7 (0.4)	141 (4)
Siriopol	2021	67(12)	70	67 (25)	1.1 (0.9-1.4) *	29 (5)	125 (18)	33 (10)	48	35	62	40	-	-	800 (400-1500)*	13.4 (2.1)	-	137 (5)
Soloveva	2019	69 (12)	70	54 (44-69)*	1.2 (1.0-1.5)	-	141 (28)	40 (14)	95	41	93	-	-	-	4046 (1956-5456)*	-	-	-
Trejo-Velasco	2016	69 (13)	56	-	1.1 (0.4)	-	127 (20)	39 (16)	-	44	78	49	Loop 98%; MRA 40%	76	4629 (3768)	12.4 (1.8)	-	136 (4)
Sakaguchi	2020	71 (12)	64	-	1.3 (0.8)	-	137 (34)	43 (18)	-	38	70	-	-	63	759 (599)	-	-	-
Curbelo	2019	84 (7)	41	55 (22)	1.2 (0.5)	27 (6)	126 (20)	58 (15)	26	34	93	-	Loop 84%; MRA 53%; Thiazide 18%	ACEi 35%; ARB 32%	1637 (2289)	12.7 (1.6)	-	140 (3)
Alves	2016	61 (12)	63	-	1.3 (0.4)	-	-	26 (8)	100	-	-	-	-	-	921 (545-1932)*	12.4 (2.0)	3.7 (0.5)	139 (4)

Limited to studies reporting characteristics for the full cohort. Baseline characteristics for entire cohort not available for Colin-Ramirez, Di Somma 2010, Koell, Liu, Santarelli & Villacorta studies. None of the heart failure studies reported urine protein. Data are presented as mean (SD) unless denoted by * which indicates median (IQR). † Baseline characteristics are for 270 with AHF who were studied in MV regression analysis.

Baseline characteristics were reported for the entire cohort in 71% (22/31) of studies and are summarised in Table 3-2 and presented for each study in Table 3-5 and Table 3-6. Average age ranged from 56 to 84 years; average proportion of male participants was 55% in both CKD and heart failure cohorts; and diabetes and hypertension were more common in CKD cohorts than heart failure cohorts (diabetes: 45 vs 37%; and hypertension: 86 vs 78%, respectively). Ethnicity was not widely reported although studies represent wide geographical coverage. Confounding variables associated with CKD (such as albuminuria, CKD stage and measures of kidney function) were not widely reported in heart failure studies; and vice versa: baseline heart failure, left ventricular ejection fraction, New York Heart Association class and N-terminal pro B-type natriuretic peptide (NT-proBNP) were not widely reported in CKD studies.

3.2.3 MEASUREMENT OF FLUID STATUS

Fluid status was assessed using 10 different bioimpedance parameters (6 parameters in CKD and 8 in heart failure) which are described in Table 3-7. There were no observable clear temporal trends in the parameter used by year of study publication as was reported in a review of largely dialysis populations (Tabinor et al., 2018) however clear differences exist between practice in CKD and heart failure populations. The commonest parameters applied in CKD studies were absolute and relative “Fluid Overload” (also termed overhydration) measured by the Fresenius Body Composition Monitor (BCM) device. This device was only used in 2 (10%) heart failure cohorts. Bioimpedance analysis (BIA) and bioimpedance vector analysis (BIVA) devices were more commonly used in heart failure studies in which BIVA hydration index was the most commonly reported parameter.

The majority of studies (21 studies [68%]) reported single baseline measurements as opposed to serial measurements. Serial measurements were slightly more common in heart failure (7 studies [35%]) than in CKD studies (3 studies [27%]), with serial measurements commonly recorded over short timeframes during heart failure admissions. Reports tended to select preferentially a single exposure time point for observational analyses relating fluid status to future risk of outcomes, rather than considering time-updated exposures or applying adjustment for regression dilution bias.

Table 3-7: Summary of bioimpedance indices of fluid status employed in included studies

	Units	Method	Represents	Reference ranges
Absolute "Fluid Overload"	Litres	BIS using Fresenius Body Composition Monitor (requires 3-compartment model)	Absolute measure of excess fluid volume, independent of body composition.	Manufacturer normal range: -1.1 L to +1.1 L. Authors have suggested additional threshold representing severe "Fluid Overload" >+2.5 L.
Relative "Fluid Overload"	%	BIS using Fresenius Body Composition Monitor (requires 3-compartment model)	Excess fluid volume indexed to measured ECW volume allowing for comparison between individuals.	Not provided by manufacturer. Suggested by multiple authors: normal -7% to +7%, 7-15% mild and >15% severe "Fluid Overload". Approximately equal to above absolute thresholds.
Reactance Xc/H	Ohms/metre (Ω/M)	Theoretically can be derived from all BIA & BIS devices	Raw impedance parameter measuring obstruction to the flow of electrical current where resistance relates to extra- and intracellular resistance & reactance relates to the cell membrane. Indexed to height in metres.	Not applicable.
Resistance R/H	Ohms/metre (Ω/M)	Theoretically can be derived from all BIA & BIS devices		
Phase angle	Degrees (°)	Theoretically can be derived from all BIA & BIS devices	Derived from a vector plot of reactance against resistance which describes the relationship between water resistance & cell resistance. Phase angle indicates the direction of the vector.	No existing reference ranges. Lower phase angle reflects higher degrees of fluid overload. Phase angle increases with increasing numbers of intact cells in the body and therefore also reflects other factors such as nutrition.
BIVA hydration index	%	Theoretically can be derived from all BIA & BIS devices	Derived from a vector plot of reactance against resistance presented as nomogram. Standard deviation ellipses are used to define normal ranges.	Dehydration <72.7%; normohydration 72.7-74.3%; fluid overload >74.3%.
Extracellular water (ECW)	Litres	Multifrequency BIA & BIS devices	Volume of the extracellular water compartment.	Dependent upon age and sex. Approximately one third of total body water volume.
ECW:total body water ratio (or ECW:intracellular water ratio)	NA (ratio)	Multifrequency BIA & BIS devices	Ratio of ECW volume to other body fluid compartments: either as a fraction of the total body water volume or a ratio compared to the intracellular compartment.	0.36-0.39 considered normal by InBody device manufacturers.

3.2.4 ALL-CAUSE MORTALITY

Associations between fluid status and specific causes of death were not widely reported, limiting the review to all-cause mortality. Associations between fluid status and death were presented in 13 studies, 10 of which reported multivariable-adjusted estimates. Significant between-study differences in exposures and model approaches precluded meta-analysis. Considering CKD cohorts first (Table 3-8), the largest study by Bansal et al. (3751 participants) demonstrated that phase angle <5.59° (where lower phase angle represents higher degrees of fluid overload) vs ≥6.4° was associated with double the hazards of all-cause mortality (hazard ratio [HR] 2.02, 95% confidence interval [CI] 1.67-2.43 [776 deaths]) after adjustment for age, sex, ethnicity and clinical site (Bansal et al., 2018). Studies by Tsai et al. (236 participants in the included analysis) and Vega et al. (356 participants) using BCM-derived parameters were much smaller and, perhaps as a consequence, were unable to confirm statistically significant associations consistently (Tsai et al.: adjusted HR per % relative "Fluid Overload" 1.07, 95% CI 0.99-1.14 [23 deaths]; Vega et al.: adjusted HR per L absolute "Fluid Overload" 1.10, 95% CI 0.99-1.20 & HR per % relative "Fluid Overload" 3.18, 95% CI 2.09-4.97 [113 deaths]) (Tsai et al., 2018, Vega et al., 2018).

In heart failure cohorts, the largest studies demonstrated significant positive associations between bioimpedance indices of fluid excess and all-cause mortality (Table 3-9). Massari et al. reported on 436 individuals finding BIVA hydration index >73.8% was associated with twice the risk of all-cause mortality compared to those with less fluid excess (adjusted HR 2.00, 95% CI 1.20-3.20 [92 deaths]) (Massari et al., 2020). Of note, Massari et al. included heart failure status (acute vs chronic), brain natriuretic peptide (BNP) and estimated plasma volume status (derived from haemoglobin and haematocrit, surrogate measures of intravascular volume status) in the multivariable model alongside BIVA hydration index, meaning models were estimating the relevance of total body fluid excess for a given level of intravascular status. The associations therefore estimate the relevance of excess extravascular fluid - rather than total body fluid excess - with risk. Similarly-sized cohorts studying stable chronic heart failure (Colín-Ramírez et al., 2012) and acute heart failure (Núñez et al., 2016) populations studied markers of total body fluid excess and found strong positive associations with risk of all-cause mortality, whether estimated by phase angle (Colín-Ramírez et al., 2012) or BIVA hydration index (Núñez et al., 2016).

Table 3-8: Associations between fluid excess and risk of all-cause mortality in CKD cohorts

Author	Population	N	Follow-up (yrs)	Fluid overload definition	Baseline fluid overload Mean (SD)/ median (IQR)	n deaths	% deaths	Deaths/100 person yrs[1]	Analysis	Covariates						HR	95% CI LL	95% CI UL
										Age	Sex	Diabetes	CVD	eGFR[2]	Other			
Bansal	CKD	3751	7.0	Phase angle (◦) Quartile 1 vs 3 & 4 combined (<5.59 vs ≥6.4)	6.6 (1.8) ◦	776	21	3.0	Cox MVSA	**X**	**X**				**Ethnicity, clinical site**	**2.02**	**1.67**	**2.43**
									Cox MVSA	X	X	X	X	X	uACR, blood pressure, serum albumin, clinical site, ethnicity, smoking	1.31	1.09	1.58
Vega	CKD	356	4.2	Absolute "Fluid Overload" (L) Per 1 L increment	0.6 (-0.4 to 1.5) L	113	32	7.6	Cox MVSA	X	-	X	X	-	Serum albumin, Charlson comorbidity, prealbumin, CRP	1.10	0.99	1.20
				Relative "Fluid Overload" (%) Per % increment	2.3 (0.8) %	113	32	7.6	Cox MVSA	X	-	X	X	-		3.18	2.09	4.97
Tsai	CKD	236	3.3	Relative "Fluid Overload" (%) Per % increment	7.8 (8.6) %	23	10	3.0	Cox MVSA	X	X	X	X	X	uACR, medication (ACEi, ARB, diuretic, statin), LDL	1.07	0.99	1.14
Caravaca	CKD	175	1.3	Phase angle (◦) Per ◦ increment	5.4 (1.0) ◦	16	9	7.0	Cox MVSA	Not reported						0.49	0.26	0.92
Ohashi	CKD	149	4.9	ECW:ICW; assumed per increment (not specified)	NA	25	17	3.4	Cox MVSA	X	-	X	-	X	-	1.29	1.11	1.50
Esmeray	CKD	100	1.0	Absolute "Fluid Overload" (L) >0 L vs ≤0 L	NA	10	10	10.0	Univariable only: Kaplan-Meier. Cumulative survival significantly greater in ≤0L vs >0L, p=0.003 (not quantified)									

Lower phase angle indicates higher degrees of fluid overload. BIVA hydration index (%) ranges are based on standardised plots: hyperhydration >74.3%, normohydration 72.7-74.3%, dehydration <72.7%. Where more than one multivariable model is presented with different levels of adjustment, the preferred model is highlighted in bold. [1] Event rate calculated for all studies from N, n and follow-up in years. [2] eGFR or other measure of kidney function.

Table 3-9: Associations between fluid excess and risk of all-cause mortality in heart failure cohorts

Author	Population	N	Follow-up (yrs)	Fluid overload definition	Baseline fluid overload Mean (SD)/ median (IQR)	n deaths	% deaths	Deaths/100 person yrs[1]	Analysis	Covariates						HR	95% CI LL	95% CI UL
										Age	Sex	Diabetes	CVD	eGFR[2]	Other			
Massari, 2020	HF	436	1.3	BIVA hydration index (%) categories; >73.8%	73.7 (73.1-76.8) %	92	21	16.2	Cox MVSA	-	-	-	-	X	Acute vs chronic heart failure, BNP, estimated plasma volume status (based upon haematocrit/ haemoglobin)	2.00	1.20	3.20
Colin-Ramirez	HF	389	3.0	Phase angle (◦) Quartile 1 vs 4 (<4.2 vs ≥5.7)	5.0 (NA) ◦ [3]	66	17	5.7	Cox MVSA	X	-	X	-	-	Haemoglobin	3.08	1.06	8.99
Nunez	HF	369	1.0	BIVA hydration index (%) >74.3% vs 72.7-74.3%	73.6 (73.0-76.2) %	80	22	21.7	Cox MVSA	Not reported in main publication						2.08	1.21	3.58
Santarelli, EHJ Acute CV Care	HF	336	0.3	R/H, Xc/H Ω/m & BIVA hydration index (%); dR/H = change admission-discharge (median dR/H 11 Ω/m)	Xc/H: 36 (14) Ω/m	33[4]	15	49.8	Cox MVSA[5]	"dR/H was associated with better prognosis (hydration index 0.417, p<0.01)"[5]								
De Berardinis	HF	194	1.5	BIVA phase angle (◦) Per ◦ increment	4.4 (1.7) ◦	47	24	16.2	Univariable only: ROC. AUC for death at 30 days: 0.64 (p=0.01) & 18 months: 0.86 (p<0.001), cut-off not given									
Siriopol	HF	151	1.7	Absolute "Fluid Overload" (L) Per 1L increment	1.1 (2.8) L	53	35	20.7	Univariable only: Cox	-	-	-	-	-	-	1.11	1.02	1.19
				Relative "Fluid Overload" (%) Per % increment	4.8 (13.5) %	53	35	20.7	Univariable only: Cox	-	-	-	-	-	-	1.02	1.01	1.04
Alves	HF	71	2.0	Phase angle (◦) <4.8 vs >4.8	5.6 (2.1) ◦	29	41	20.4	Cox MVSA	X	-	-	-	X	Left ventricular ejection fraction	2.67	1.21	5.89

[1] Event rate calculated for all studies from N, n and follow-up in years. [2] eGFR or other measure of kidney function. [3] 50th percentile = 5.0 °; IQR not reported. [4] 33 deaths in 221 with AHF out of total 336 cohort (115 controls). [5] Cox MVSA results are not presented in tabular form; dR/H is the difference between R/H at admission & discharge however these results cannot be meaningfully interpreted.

3.2.5 CARDIOVASCULAR OUTCOMES

Of the 5 CKD studies reporting relevant cardiovascular/composite outcomes, the largest study reported 48% (HR 1.48, 95% CI 1.15-1.91) increased hazards of atherosclerotic cardiovascular disease (420 events, defined as incident myocardial infarction, ischaemic stroke or peripheral arterial disease) and 80% (HR 1.80, 95% CI 1.46-2.23) increased hazards of heart failure events (581 events, not dependent on hospitalisation; see Table 3-10 footnote for definition) for participants with phase angle <5.59° (indicating higher level of fluid overload) vs ≥6.4° and after adjustment for age, sex, ethnicity and clinical site (Bansal et al., 2018). Notably, when additional variables such as albuminuria, blood pressure and serum albumin were added to the models – all factors which may mediate any causal effect between fluid overload and adverse outcomes – the associations were substantially attenuated suggesting these factors have key mediating contributions. Studies by Hung et al. and Tsai et al. also reported significantly increased risk of composite cardiovascular morbidity and mortality outcomes associated with "Fluid Overload" measured by the Fresenius BCM device, but were based on relatively small numbers of events (47 and 48 events, respectively) (Tsai et al., 2018, Hung et al., 2015). Vega et al. reported only univariable analyses (Vega et al., 2018) and the final study by Ohashi et al. found a significant association between fluid excess (using ECW:ICW) and risk of all-cause hospitalisations (83 events) but not for the smaller number of cardiovascular outcomes (18 outcomes) (Ohashi et al., 2015).

Associations with composite cardiovascular outcomes were reported in 16 heart failure studies, 7 of which reported multivariable Cox regression analyses, a further 4 reported other multivariable regression analyses and 5 reported only univariable associations (Table 3-11). Composite cardiovascular outcomes in both CKD and heart failure cohorts commonly included death (all-cause, cardiovascular or cardiac) and heart failure hospitalisation. CKD studies also often reported nonfatal myocardial infarction and stroke in cardiovascular composites. Substantial between-study differences in exposure definitions, modelling, +/- outcome definitions again precluded statistical aggregation of study results. Considering individual heart failure studies, 6 of the 7 studies which reported multivariable Cox models included hospitalisation for heart failure in their composite cardiovascular outcome. Despite less than 100 of such outcomes in each study, all 6 reported statistically significant positive associations between increased baseline fluid overload assessed by a variety of parameters (BIVA hydration index in 3 studies; ECW

volume/ratio in 2 studies; and relative “Fluid Overload” in 1 study) and risk of these cardiovascular outcomes. The seventh study (Lyons et al., 2017) reported on a composite of death, urgent transplant or ventricular assist device implantation and found no significant association between the ratio of ECW-to-total body water (TBW) >0.39 vs ≤0.39 (adjusted HR 1.21, 95% CI 0.51-2.90, 56 outcomes). Adjustment for BNP and heart failure symptoms in this and other studies may result in models underestimating any causal relevance of associations, and for the majority of studies, it was not possible to find less adjusted models which are more relevant to the aetiological scientific focus of this systematic review.

3.2.6 PROGRESSION OF CKD

Progression to kidney replacement therapy initiation was reported in 4 studies with a further 4 incorporating this into a composite outcome using percentage eGFR decline (Table 3-12). Two studies also included eGFR slope analyses (Tsai et al., 2018, Hung et al., 2015). The largest studies consistently report increased risk of composite kidney outcomes associated with fluid overload defined by absolute/relative “Fluid Overload” or phase angle (Table 3-12).

Table 3-10: Associations between fluid excess and risk of cardiovascular outcomes in CKD cohorts

Author	N	Follow-up (yrs)	Fluid overload definition	Baseline fluid overload Mean (SD)/ median (IQR)	Outcome definition	n outcomes	% outcomes	Outcomes/100 person yrs[1]	Events included in CV event/MACE definition									Analysis	Covariates						HR	95% CI LL	95% CI UL
									Death	Nonfatal MI	Nonfatal stroke	Angina	PCI	Heart failure	Arrhythmia	PVD	Other		Age	Sex	Diabetes	CVD	eGFR[2]	Other			
Bansal	3751	7.0	Phase angle (◦); quartile 1 vs quartiles 3 & 4 combined (<5.59 vs ≥6.4)	6.6 (1.8) ◦	(1) Atherosclerotic CV disease	420	11	1.6	-	X	X	-	-	-	-	X	-	Cox MVSA	**X**	**X**				**Ethnicity, site**	**1.48**	**1.15**	**1.91**
																		Cox MVSA	X	X	X	X	X	uACR, BP, albumin, site, ethnicity, smoking	1.12	0.86	1.45
					(2) Heart failure events[3]	581	15	2.2	-	-	-	-	-	X[3]	-	-	-	Cox MVSA	**X**	**X**				**Ethnicity, site**	**1.80**	**1.46**	**2.23**
																		Cox MVSA	X	X	X	X	X	uACR, BP, albumin, site, ethnicity, smoking	1.03	0.82	1.29
Vega	356	4.2	Absolute (L) & relative "Fluid Overload" (%)[4]	0.6 (-0.4-1.5) L 2.3 (0.8) %	CV events	150[5]	42	10.0	-	X	X	-	-	X[6]	-	X	-	Univariable logistic regression; not reported in table, text suggests no significant association with "Fluid Overload" and assume therefore not included in MVSA									
Hung	338	2.1	Absolute "Fluid Overload" (L); per 1L increment	NA	Composite CV morbidity & mortality	47	14	6.6	X	X	-	X	-	X[7]	-	-	-	Cox MVSA	**X**	**X**					**1.42**	**1.25**	**1.62**
																		Cox MVSA	X	X	X	X		BP, medication (ACEi, ARB)	1.28	1.09	1.50
																		Cox MVSA	X	X	X	X	X	uACR, BP, medication (ACEi/ARB/diuretic)	1.25	1.04	1.51
			Relative "Fluid Overload" (%); ≥7% vs <7%	8.3 (8.6) %	Composite CV morbidity & mortality	47	14	6.6	X	X	-	X	-	X[7]	-	-	-	Cox MVSA	**X**	**X**					**6.22**	**2.78**	**13.92**
																		Cox MVSA	X	X	X	X		BP, medication (ACEi, ARB)	3.84	1.68	8.76
																		Cox MVSA	X	X	X	X	X	uACR, BP, medication (ACEi, ARB)	2.71	1.14	6.48
Tsai	236	3.3	Relative "Fluid Overload" (%); per % increment	7.8 (8.6) %	(1) CV events (MACE)	31	13	4.0	-	X	X	X	-	X[7]	X	-	-	Cox MVSA	X	X	X	X	X	uACR, medication (ACEi, ARB, statin diuretic), LDL	1.07	1.02	1.13
					(2) Composite MACE & all-cause mortality	48	20	6.2	X	X	X	X	-	X[7]	X	-	-	Cox MVSA	X	X	X	X	X	uACR, medication (ACEi, ARB, statin diuretic), LDL	1.08	1.03	1.13
Ohashi	149	4.9	ECW:ICW; assumed per increment (not specified)	NA	(1) CV events	18	12	2.5	-	X	X	-	X	X[7]	-	-	-	Cox MVSA	X	-	-	-	X	uACR, BP	1.12	0.93	1.31
					(2) Hospitalisation (all-cause)	83	56	11.4	-	-	-	-	-	-	-	-	X	Cox MVSA	X	-	-	-	X	uACR, BP	1.18	1.08	1.28

Lower phase angle indicates higher degrees of fluid overload. Where more than one multivariable model is presented with different levels of adjustment, the preferred model is highlighted in bold. [1] Event rate calculated for all studies from N, n and follow-up in years. [2] eGFR or other measure of kidney function. [3] "Heart failure events were determined based on clinical symptoms, radiographic evidence of pulmonary edema, physical examination of the heart and lungs, central venous hemodynamic monitoring data, and echocardiographic imaging in hospitalized patients based on the Framingham35 and ALLHAT36 criteria". [4] Unclear which was used in CV event analysis, both are analysed as continuous variables in all-cause mortality analysis. [5] 150 participants experienced an event – total number of events not reported. [6] Heart failure defined as "presence of acute pulmonary oedema and an echocardiogram with ventricular systolic dysfunction and left ventricular ejection fraction <45" – does not specify hospitalisation required. [7] Hospitalisation for heart failure.

Table 3-11: Associations between fluid excess and risk of cardiovascular outcomes in heart failure cohorts

Author	N	Follow-up (yrs)	Fluid overload definition	Baseline fluid overload Mean (SD)/ median (IQR)	Outcome definition	n outcomes	% outcomes	Outcomes/100 person yrs[1]	Outcome summary			Analysis	Covariates						HR/ OR	95% CI LL	95% CI UL
									Death	Heart failure	Other		Age	Sex	Diabetes	CVD	eGFR[2]	Other			
Massari, 2019	706	0.02	BIVA HI (%), assumed per increment – not stated	77.7 (5.8) %	Length of stay Median 7.5 (7.4-8.1) days	NA	NA	NA	-	-	X	MV linear regression	-	-	-	-	X	BNP, NYHA, haemoglobin, oedema	β 0.183 (p<0.001)		
Di Somma, 2014	381 [3]	0.1	BIVA HI (%) >74.3% vs ≤74.3%	81.2 (6.7) %	CV death[4] or hospitalisation	97	36	359.3	X	-	X	MV logistic regression	X	-	-	-	X	BP	1.96	1.05	3.66
Nunez	369	1.0	BIVA HI (%) Per increment	73.6 (73.0-76.2) %	HHF	93	25	25.2	-	X	-	Cox MVSA; Fine & Gray[5]	Not reported						1.06	1.03	1.10
Lyons	359	2.1	ECW:TBW >0.39 vs ≤0.39[6]	NA	Death, urgent transplant, or VAD	56	16	7.4	X	-	X	Cox MVSA	X	X	X	-	X	BMI, HF aetiology, NYHA, BNP	1.21	0.51	2.90
Santarelli, EHJ Acute CV Care	336[7]	0.3	R/H, Xc/H Ω/m & BIVA HI (%); per increment	Xc/H: 36 (14) Ω/m	Death or hospitalisation (presumed all-cause)	74	33	111.6	X	-	X	Univariable only· Methods state Cox MVSA - not reported for death/rehospitalisation outcome; univariable analyses cannot be clearly interpreted (ORs & ROC analysis reported stating Xc predicts events with an AUC 0.56, p=0.04 however cut-off value not given).									
Santarelli, Intern Emerg Med	292	0.3	BIVA HI (%), assumed per increment (not stated)	NA	CV death[4]	36	12	41.1	X	-	-	MV regression[8]	X	-	-	-		BNP, R, Xc, rales	1.10	0.97	1.25
De Berardinis	194	1.5	Phase angle (◦) Per ◦ increment	4.4 (1.7) ◦	(1) Death or rehospitalisation at 30 days[9]	40[9]	21	13.7	X	-	X	MV regression[9]	X	-	-	-	X	Beta blocker use, WCC, galectin-3	β -1.462 (p<0.03)		
			BIVA HI (%) Per increment	79.4 (6.6) %	(2) Death or rehospitalisation at 30 days[9]	40[9]	21	13.7	X	-	X	MV regression[9]	X	-	-	-	X	Beta blocker use, WCC, galectin-3	β 0.103 (p≥0.05)		
Sakaguchi, 2015	190[10]	0.5	ECW (L) measured/ predicted at discharge; per 0.1 unit	15.0 (5.5) L	Cardiac death[11] or HHF	37	28	56.9	X	X	-	Cox MVSA	Not reported						1.48	1.20	1.83
Liu	159[12]	0.5	ECW:TBW pre-discharge[12;] per 0.001 increment	0.39 (0.01)	HF events (death or hospitalisation)	10[12]	9	18.9	X	X	-	Cox MVSA	X	-	-	-	X	Allocation, haemoglobin, uric acid, sodium, NYHA, ACEi/ARB	1.06	1.02	1.10
Koell	150	2.0	Relative "Fluid Overload" (%); ≥7% vs <7%	NA	Cardiac death or HHF	51	34	17.0	X	X	-	Cox MVSA	X	X	X	-	-	BMI, 6-minute walking distance, NT-proBNP, AF	3.09	1.68	5.68
Soloveva	149	0.8	BIVA "congestion status at discharge per 1 rank" (hydration index)	79.5 (6.5) %	(1) All-cause death or heart transplant	29	19	24.3	X	-	X	Univariable only: Cox							1.73	1.23	2.45
					(2) All-cause death, heart transplant, HHF	60	40	50.3	X	X	X	Univariable only: Cox							1.40	1.10	1.79
Trejo-Velasco	105	0.9	BIVA HI (%); HI (<72.7 & >74.3%) vs 72.7-74.3%[13]	NA	All-cause death or HHF	37	35	39.2	X	X	-	Cox MVSA	X	-	-	-	X	AF	2.60	1.05	6.44
Sakaguchi, 2020	100[14]	0.5	ECW (L), assumed per increment (not stated)	15.3 (6.9) L	Cardiac death[11] or HF readmission	27	27	54.0	X	X	-	Univariable only: Cox							0.96	0.89	1.04
Curbelo	99	1.0	Phase angle (◦)	3.8 (1.5) ◦	HF events (death or hospitalisation)	36	36	36.4	X	X	-	Univariable only: ROC. AUC 57.0 95% CI 43.2-70.8 [15]									
Villacorta	80	0.6	BIVA HI (%) categories >74.3% at discharge	NA	Cardiac death[11] or HF hospitalisation	27	34	56.3	X	X	-	Cox MVSA	X	X	-	-	X	BNP, NGAL	1.39	1.25	1.54
Di Somma, 2010	51[16]	0.3	BIVA HI (%); cut-off >80.5%	79.0 (6.0) %	Death or rehospitalisation for cardiogenic event	NA	NA	NA	X	-	X	Univariable only: ROC. Sensitivity 22%, specificity 94%, p=0.04 (no AUC)									

Lower phase angle indicates higher degrees of fluid overload. BIVA hydration index (%) ranges are based on standardised plots: hyperhydration >74.3%, normohydration 72.7-74.3%, dehydration <72.7%. [1] Event rate calculated for all studies from N, n and follow-up in years. [2] or other measure of kidney function. [3] 270/381 with AHF; 111 controls. [4] Not defined. [5] Unclear if HR from Cox or Fine & Gray analysis. [6] Manufacturer reference. [7] 221/336 with AHF. [8] Unclear analysis method. [9] 10 deaths + 30 rehospitalisations at 30 days; death and rehospitalisation are assumed to be all-cause; unclear analysis methods. [10] 130 with AHF + 60 hospitalised controls used to determine predicted values ECW only (not comparison). [11] Death from HF, MI, sudden cardiac death. [12] 53 with BIA; 53 case management without BIA; 53 controls (routine care). [13] Dehydrated and hyperhydrated groups combined in MVSA; HR not reported for hyperhydrated alone. [14] 100 with central venous catheter and therefore included in survival analysis reporting fluid overload. [15] Cut-off value not given. [16] 25 AHF + 26 controls.

Table 3-12: Associations between fluid excess and risk of kidney disease progression

Author	N	Follow-up (yrs)	Fluid overload definition	Baseline fluid overload Mean (SD)/ median (IQR)	Outcome definition	n outcomes	% outcomes	Outcomes/100 person yrs[1]	Outcome summary: Composite	Outcome summary: KRT/ESKD	Outcome summary: Δ eGFR	Analysis	Covariates: Age	Sex	Diabetes	CVD	eGFR[2]	uACR	BP	Other	HR/ OR	95% CI LL	95% CI UL
Bansal	3751	7.0	Phase angle (◦) Quartile 1 vs quartiles 3 & 4 combined (<5.59 vs ≥6.4)	6.6 (1.8) ◦	>30% eGFR decline or ESKD (KRT)	1597	43	6.1	X	-	-	Cox MVSA	**X**	**X**						**Ethnicity, clinical site**	**1.78**	**1.56**	**2.04**
												Cox MVSA	X	X	X	X	X	X	X	Serum albumin; ethnicity, clinical site, smoking	0.99	0.86	1.14
Liu	1065	8.6	Absolute "Fluid Overload" (L); Tertiles: tertile 3 vs tertile 1	1.3 (0.6-1.9) L	Worsening KDIGO CKD category (eGFR) and ≥25% eGFR decline	465	44	5.1	X	-	-	Cox MVSA	**X**	**X**	-	-	-	-	-	**Ethnicity**	**1.94**	**1.54**	**2.46**
												Cox MVSA	X	X	X		X	X	X	HbA1c, BMI, medication (RASi)	1.45	1.14	1.85
			Relative "Fluid Overload" (%); >7% vs ≤7%	10.2 (4.8-14.4) %	Worsening KDIGO CKD category (eGFR) and ≥25% eGFR decline	465	44	5.1	X	-	-	Cox MVSA	**X**	**X**	-	-	-	-	-	**Ethnicity**	**1.59**	**1.30**	**1.95**
												Cox MVSA	X	X	X		X	X	X	HbA1c, BMI, medication (RASi)	1.29	1.05	1.59
Hung	338	2.1	Absolute "Fluid Overload" (L) Per 1L increment	NA	(1) ≥50% eGFR decline or ESKD requiring chronic dialysis	100	30	14.1	X	-	-	Cox MVSA	**X**	**X**							**1.34**	**1.23**	**1.45**
												Cox MVSA	X	X	X	X			X	Medication (ACEi, ARB)	1.26	1.14	1.39
												Cox MVSA	X	X	X	X	X	X	X	Medication (ACEi, ARB)	1.25	1.11	1.41
			Relative "Fluid Overload" (%); ≥7% vs <7%	8.3 (8.6) %	(1) ≥50% eGFR decline or ESKD requiring chronic dialysis	100	30	14.1	X	-	-	Cox MVSA	**X**	**X**							**4.56**	**2.83**	**7.36**
												Cox MVSA	X	X	X	X			X	Medication (ACEi, ARB)	3.63	2.20	5.99
												Cox MVSA	X	X	X	X	X	X	X	Medication (ACEi, ARB)	2.44	1.44	4.13
					(2) eGFR slope analysis	NA	NA	NA	-	-	X	NA	Significantly greater eGFR decline in ≥7% vs <7%[3]										
Khan	312	1.0	ECW (L); assume per increment (not reported)	16.7 (3.7) L	KRT initiation	36	12	11.5	-	X	-	MV logistic regression	X	X	X	-	X	X	X	Medication (ACEi/ARB/ diuretic)	3.25	1.42	1.18[4]
Tsai	236	3.3	Relative "Fluid Overload" (%); >7% vs ≤7%	7.8 (8.6) %	(1) Dialysis initiation	129	55	16.6	-	X	-	Cox MVSA	X	X	X	X	X	X	-	Medication (ACEi, ARB, diuretic, statin), LDL	1.53	1.02	2.28
					(2) Rapid eGFR decline >3ml/min/1.73m^2/year	88	37	11.3	-	-	X	MV logistic regression	X	X	X	X	X	X	-	Medication (ACEi, ARB, diuretic, statin), LDL	2.89	1.51	4.45
					(3) eGFR slope analysis	NA	NA	NA	-	-	X	NA. Significantly greater eGFR decline in >7% vs ≤7%[5]											
Kohatsu	194	1.4	ECW:TBW > median (0.48)	0.48 (0.04)	≥30% eGFR decline or ESKD (KRT or death)	107	55	39.4	X	-	-	Cox MVSA (figure only, HR not reported). "No significant difference" high vs low ECW:TBW groups											
Schork	177	5.9	Absolute "Fluid Overload" (L); Per 1L increment	0.2 (-0.5 to 1.2) L	Progression to ESKD with KRT initiation	33	19	3.2	-	X	-	Cox MVSA	-	-	X	-	X	X	X	NT-proBNP	1.24	0.83	1.90
			Absolute "Fluid Overload" (L); >1L vs ≤1L		Progression to ESKD with KRT initiation	33	19	3.2	-	X	-	Cox MVSA	-	-	X	-	X	X	X	NT-proBNP	3.32	1.26	8.76
Ohashi	149	4.9	ECW:ICW; assumed per increment (not specified)	NA	≥50% eGFR decline or KRT initiation	52	35	7.1	X	-	-	Cox MVSA	X	-	X	-	X	X	X		1.15	1.03	1.26
Esmeray	100	1.0	Absolute "Fluid Overload" (L) >0.5L vs ≤0.5L	NA	ESKD requiring chronic dialysis	14	14	14.0	-	X	-	MV logistic regression	Not reported								1.76	1.20	2.57

Lower phase angle indicates higher degrees of fluid overload. Where more than one multivariable model is presented with different levels of adjustment, the preferred model is highlighted in bold. [1] Event rate calculated for all studies from N, n and years follow-up. [2] or other kidney function measure. [3] eGFR slope ≥7% vs <7%: -4.3 [-12.6, 1.2] vs -1.7 [-7.8, 2.7] mL/min/1.73m^2/year; p<0.05. [4] Assume error in the UL reported. [5] eGFR slope presented overall & in 4 groups based upon "Fluid Overload" ≤7%/>7% & median NT-proBNP: entire cohort -2.3(-4.1,-1.1); "Fluid Overload" ≤7% NT-proBNP ≤ median -1.8(-3.2,-0.9); "Fluid Overload" ≤7% NT-proBNP > median -1.6(-2.9,-0.9); "Fluid Overload" >7% NT-proBNP ≤ median -2.6(-5.4,-1.0); "Fluid Overload" >7% NT-proBNP >median -3.1(-6.1,-1.4) mL/min/1.73m^2/year; p<0.001.

3.2.7 RISK OF BIAS

Methodological quality varied across studies however no studies were excluded due to high risk of bias (Table 3-13). See section 2.1.8 and appendix 3 for risk of bias methods.

Table 3-13: Risk of bias assessment for all included studies

Study		1. Study Participation	2. Study Attrition	3. Prognostic Factor Measurement	4. Outcome(s) Measurement	5. Study Confounding	6. Statistical Analysis & Reporting
Alves	2016	L	M	L	L	M	L
Bansal	2018	L	L	L	L	L	L
Caravaca	2011	L	L	L	L	M	M
Colin-Ramirez	2012	L	L	L	L	M	M
Curbelo	2018	L	L	L	L	M	M
De Berardinis	2014	L	L	L	M	M	M
Di Somma	2010	L	L	L	L	M	M
Di Somma	2014	L	L	L	L	M	L
Esmeray	2018	L	L	L	M	M	M
Hung	2015	L	L	L	L	L	L
Khan	2017	L	L	L	L	L	M
Koell	2017	L	L	L	L	M	L
Kohatsu	2021	L	L	L	L	L	L
Liu	2012	L	L	L	L	L	L
Liu	2021	L	L	L	L	L	L
Lyons	2017	M	L	L	L	L	L
Massari	2019	L	L	L	L	M	M
Massari	2020	L	L	L	L	M	M
Nunez	2014	L	L	L	L	M	M
Ohashi	2015	M	L	L	L	L	M
Sakaguchi	2015	L	L	L	L	M	M
Sakaguchi	2020	L	L	L	L	M	M
Santarelli, EHJ Acute CV Care	2017	L	L	L	L	M	M
Santarelli, Intern Emerg Med	2017	L	L	L	M	M	M
Schork	2020	M	L	L	L	M	M
Siriopol	2021	L	L	L	L	M	M
Soloveva	2019	L	L	L	L	M	M
Trejo-Velasco	2016	L	L	L	L	M	M
Tsai	2018	L	L	L	L	L	L
Vega	2018	L	L	L	L	M	M
Villacorta	2021	L	L	L	L	M	M

L = low risk of bias; M = moderate risk of bias; H = high risk of bias. **Study participation**: All considered low ROB unless inclusion/exclusion criteria not adequately described. **Study attrition**: Retrospective studies are not subject to loss to follow-up therefore rated low ROB. Loss to follow-up rarely reported in included studies therefore assumed minimal if not reported and rated low ROB. Alves et al. table 2 reports 29 deaths & 30 survivors in overall cohort of N=71, outcome not reported for remaining 12. **Prognostic factor (fluid overload) measurement**: ROB depends on measurement being different for different levels of the outcome – all studies used the same measurement for all participants therefore rated low ROB. Some studies used fluid parameters which are subject to bias (ECW:ICW, ECW:TBW or inappropriate thresholds such as 0 L absolute "Fluid Overload") however because the same measurement was used for all participants, this does not introduce significant bias within the study. **Outcome measurement**: ROB assessment based upon whether outcome measurement is different related to baseline level of fluid overload therefore despite suboptimal methods in some studies, if these were not deemed likely to differ according to baseline fluid status, studies were rated low ROB. **Study confounding & Statistical analysis & reporting**: confounding and statistical analysis commonly overlap and were therefore considered together. Observational studies are very likely to be subject to unmeasured confounding and studies generally adjusted for some, but not all, relevant factors therefore commonly rated moderate ROB. Where issues were identified with confounding variables/covariates, this is reflected in ROB assessment for domains 5 & 6.

3.3 DISCUSSION

Whole-body bioimpedance is frequently used and well-studied in dialysis populations. In order to address the potential role of bioimpedance in non-dialysis CKD and heart failure populations, a systematic review was conducted to summarise existing evidence and determine threshold values of clinically significant “Fluid Overload” for consistent research application. In total, 31 eligible studies (11 CKD and 20 heart failure cohorts) were identified which used 10 different fluid parameters derived from bioimpedance analysis or spectroscopy to assess associations with cardiorenal outcomes. Studies also varied greatly in size, duration, approaches to model construction, and outcome definitions which precluded statistical aggregation of results by meta-analysis. Nevertheless, there was convincing evidence from individual studies that bioimpedance indices of fluid excess were associated with increased hazards of death in both populations with CKD and/or heart failure. Similarly, significant positive associations were observed with study-defined cardiovascular outcomes across a majority of studies. These associations appeared clearest for heart failure hospitalisation outcomes, whereas evidence of a link with ischaemic events were limited to CKD cohorts.

Associations between fluid excess and mortality and cardiovascular disease are supported by robust mechanistic principles due to diastolic dysfunction and myocardial fibrosis (Miller, 2016) however the purported associations with progression of kidney disease lack established rationale. Progressive CKD in fluid overloaded individuals is likely to occur as a result of the haemodynamic effects of volume overload on cardiovascular structure and function (Liu et al., 2021) however an alternative mechanism of kidney interstitial congestion and fibrosis is postulated (Liu et al., 2021, Kuriyama, 2019, Low et al., 2021). Interstitial congestion causes increased interstitial pressure which alters the trans-glomerular pressure gradient as well as compressing the tubules thereby increasing luminal pressure as well as causing local hypoxia (Kuriyama, 2019). These mechanisms, when coupled with dysregulated tubuloglomerular feedback and renin-angiotensin-aldosterone system activation amongst other mechanisms, may contribute to declining kidney function (Kuriyama, 2019). The interstitial space constitutes the majority of the extracellular fluid compartment (Low et al., 2021), the source of bioimpedance-derived measures of fluid excess, which may therefore support the interstitial congestion hypothesis and a potential mechanism by which fluid excess may increase risk of CKD progression. Despite these hypotheses, it seems most likely that the observed association between fluid excess and

CKD progression reflects generally poor prognosis and cardiovascular comorbidity rather than a direct relationship with progression of intrinsic kidney disease per se.

The findings from this systematic review are qualitatively consistent with the much larger body of evidence from dialysis populations. Such data are based largely on the Fresenius BCM device used in 7/11 [64%] CKD cohorts and 2/20 [10%] heart failure cohorts in this review. Dialysis studies have assessed a variety of threshold values of BCM-derived "Fluid Overload". Wizemann et al. first established a >15% threshold value of relative "Fluid Overload" based upon the highest quartile of a reference haemodialysis population (measured pre-dialysis) (Wizemann et al., 2009), which was followed by studies of a >7% threshold, derived from the 90th percentile of a healthy reference population (Van Biesen et al., 2011). Both thresholds (or equivalents in litres) have been consistently linked to poorer survival. In our review, no studies in non-dialysis CKD or heart failure reported associations with the 15% threshold value, perhaps because this degree of fluid excess is uncommon in earlier stages of CKD and heart failure compared with the extreme phenotype of fluid excess which manifests in kidney failure requiring kidney replacement therapy. The 7% relative "Fluid Overload" threshold was applied in two CKD cohorts and one heart failure cohort and was positively associated with cardiorenal outcomes. Based on this, the thresholds of relative "Fluid Overload" >7% and >15% (described as "moderate" and "severe", respectively) were adopted as outcome definitions for use in the EMPA-KIDNEY bioimpedance substudy. These terms were consistent with descriptors used by other authors (Dekker et al., 2017, Van Biesen et al., 2019, Siriopol et al., 2019, Keber et al., 2021).

A key advantage of the BCM over all other commercially-available bioimpedance devices is the ability to quantify fluid status independent of body composition (i.e. lean and adipose tissue mass) by application of a three compartment model (Chamney et al., 2007). It is not possible to equate BCM-derived "Fluid Overload" to other bioimpedance parameters, such as phase angle or BIVA hydration index, which were more commonly employed in heart failure cohorts identified in the review. Established BIVA hydration index reference ranges (fluid overload defined as hydration index >74.3%)(Valle et al., 2011) were applied in the identified heart failure studies but, like phase angle and ECW ratios, this parameter may reflect differences in fluid volume, body composition or a combination of both.

Multivariable analysis adjusted for body composition and nutritional factors may not completely address this limitation and is not practical for clinical application. It therefore seems that the Fresenius BCM device is the optimum currently-available method to assess fluid excess for patients with CKD and/or heart failure.

The systematic review has a number of limitations largely dictated by the nature of existing studies. Firstly, the studies identified in this review were almost exclusively observational in nature: there is very limited randomised data from non-dialysis CKD and heart failure populations which therefore precludes causal inferences and there remains the possibility of residual confounding. Fluid excess is associated with inflammation, endothelial dysfunction and cardiac dysfunction, therefore purported associations between fluid excess and adverse outcomes may therefore at least in part be explained by other factors. Reporting and analysis of key factors pertaining to fluid excess such as albumin and proteinuria were not widely reported. Dietary sodium intake, another key factor influencing fluid status, was generally not assessed in included studies. Secondly, as described in the results, significant between-study differences in the fluid parameters and definitions of clinical outcomes precluded quantitative aggregation of results by meta-analysis. Furthermore, the wide range of different reported models each considered a different set of covariates often adjusting for combinations of potential confounders and mediators of associations simultaneously. This means models often addressed somewhat different research questions. Consequently, this review is limited to qualitative conclusions. Availability of individual participant data from included studies could address some of these limitations, but does not address the different approaches to fluid status assessment or relatively small size of completed studies. Thirdly, studies commonly reported only a single baseline bioimpedance measurement, which does not account for fluctuation in fluid status, resulting in regression-dilution bias and reported associations underestimating the full importance of fluid excess in relation to outcomes. Fourthly, studies rarely characterised both baseline and follow-up cardiac and CKD phenotypes precluding the joint consideration of these overlapping populations. Lastly, the review assessed associations with fluid parameters only and not the adiposity measures reported by bioimpedance devices since data are lacking and inconsistent; the significance of bioimpedance-derived adiposity measures in relation to clinical outcomes warrants further study.

In summary, whole-body bioimpedance indices of fluid excess appear to be consistently and positively associated with risk of death and adverse cardiovascular outcomes in non-dialysis CKD and heart failure populations, but there are limitations to the currently available evidence. Bioimpedance has several potential roles in clinical management and in clinical research in non-dialysis CKD and heart failure. The considerable heterogeneity in bioimpedance parameters used to measure fluid status in both CKD and heart failure populations can be considered a key finding of this review which has important implications for interpretation and clinical application. Further development for these populations would benefit from consensus on the optimum device and standardisation of analytical methods for such patients. The review also highlights under-appreciation of the interplay between CKD and heart failure in patients with fluid excess. Large studies recording serial measurements and more detailed baseline and follow-up characterisation of both cardiac and renal phenotypes in a range of patients with CKD and heart failure are also needed.

CHAPTER 4 – EFFECTS OF EMPAGLIFLOZIN ON BIOIMPEDANCE-DERIVED FLUID OVERLOAD

4.1 INTRODUCTION

In the previous chapter, results from a systematic review demonstrated that bioimpedance-derived "Fluid Overload" is significantly associated with adverse cardiorenal outcomes in patients with chronic kidney disease (CKD). The review highlighted heterogeneity in devices, parameters and methods of analysis but nevertheless confirmed that bioimpedance is a valuable tool in assessing fluid status in CKD and heart failure. In this chapter, the primary and secondary assessments of the EMPA-KIDNEY bioimpedance substudy are reported, which describe the effects of empagliflozin on bioimpedance-derived fluid status. The bioimpedance substudy methods and Data Analysis Plan are outlined in Chapter 2 and were informed by the systematic review reported in Chapter 3.

As discussed in Chapter 1, fluid excess is a common manifestation in CKD with clinical and prognostic implications. The diuretic and natriuretic effects of sodium-glucose cotransporter 2 (SGLT2) inhibitors are hypothesised to reduce fluid excess however data to support this hypothesis were previously lacking. Furthermore, fluid excess can be assessed in numerous ways and surrogates of fluid excess used in previous analyses have limitations (discussed in 4.3). SGLT2 inhibitors have been hypothesised to reduce interstitial more than plasma volume (Hallow et al., 2018). While whole body bioimpedance spectroscopy has its own limitations, it is a useful method of quantifying interstitial (extracellular) fluid. Furthermore, the Fresenius Body Composition Monitor (BCM) bioimpedance spectroscopy device has the attractive property of uniquely estimating extracellular water excess (overhydration or "Fluid Overload") independently of body composition. This device was therefore an obvious choice for use in the EMPA-KIDNEY bioimpedance substudy seeking to understand effects of empagliflozin on fluid status and body composition separately and the contribution of these factors to weight loss.

The substudy primary assessment uses the absolute "Fluid Overload" parameter in litres in preference to relative "Fluid Overload" (expressed as a percentage) since estimation in litres is likely to be more readily interpretable in a clinical context. These parameters and their references ranges are discussed in more detail in section 2.5.2. The EMPA-KIDNEY

definitions of moderate and severe clinically significant "Fluid Overload" are based upon existing literature using the relative "Fluid Overload" parameter (Table 2-7).

4.2 RESULTS

4.2.1 SUBSTUDY PARTICIPANTS, COMPLETENESS, DATA QUALITY AND ADHERENCE

A total of 668 participants consented to participate in the bioimpedance substudy at their randomisation visit between 22nd May 2019 and 14th April 2021. Eight consenting participants were excluded: one due to a metal knee implant which precludes use of the BCM device; and a further seven due to issues with the baseline (randomisation) measurement[2]. The analysis population therefore comprised 660 participants. Out of a possible 1980 BCM measurements for 660 individuals (three measurements per participant at randomisation, 2- and 18-month follow-up visits), a total of 1728 measurements were available for analysis, of which 1682 were deemed valid and 46 deemed invalid according to principles outlined in section 2.4.4. The proportion of valid measurements was therefore 84.9% overall (out of all expected measurements) and similar between treatment groups (Table 4-1 & Figure 4-1).

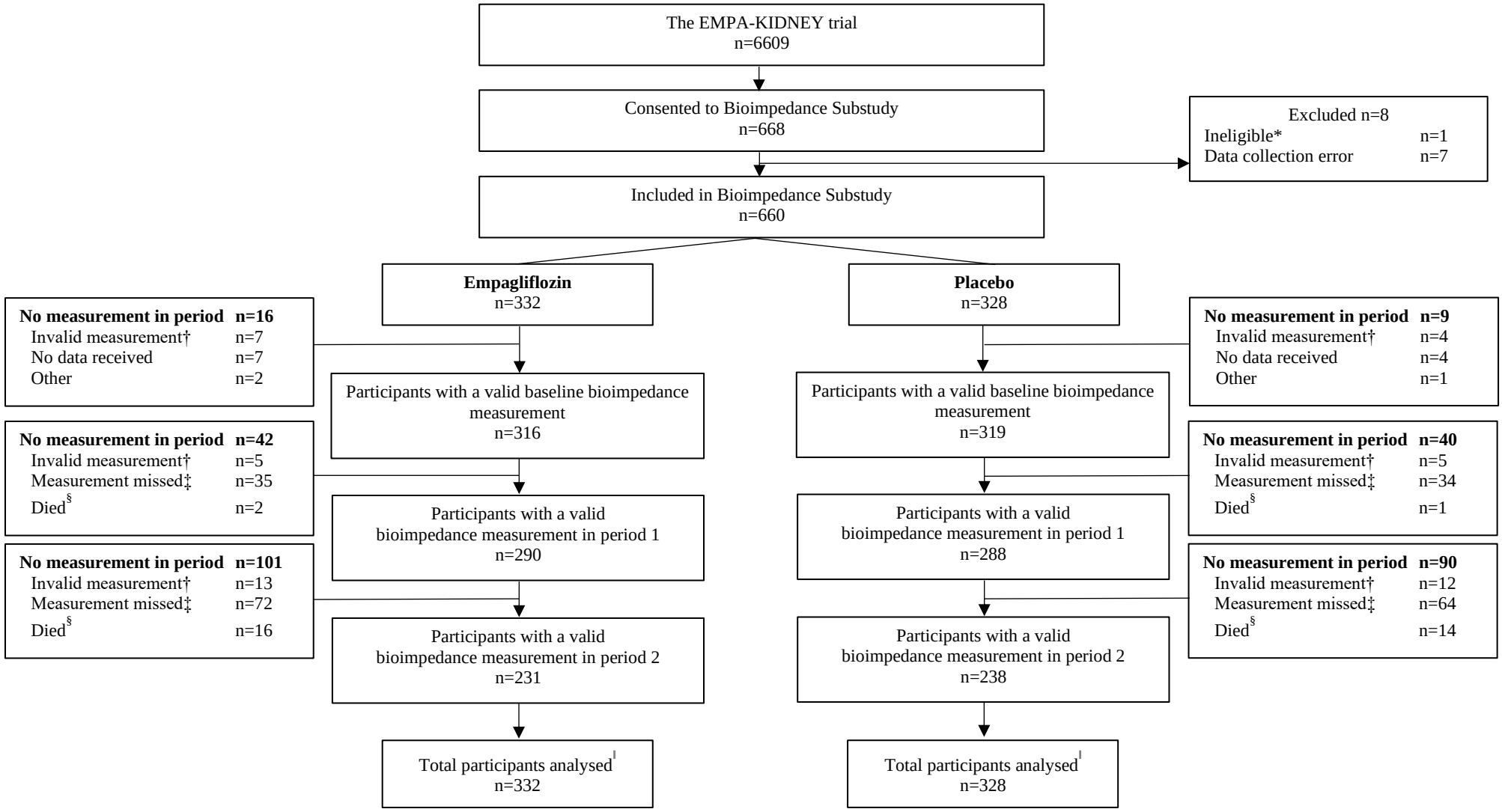
Table 4-1: Summary of valid, invalid and missing BCM measurements

	Valid			**Invalid**			**Missing**		
	Empagliflozin	Placebo	**Overall**	Empagliflozin	Placebo	**Overall**	Empagliflozin	Placebo	**Overall**
Baseline	316	319	**635**	7	4	**11**	9	5	**14**
2-month follow-up*	290	288	**578**	5	5	**10**	37	35	**72**†
18-month follow-up*	231	238	**469**	13	12	**25**	88	78	**166**‡
Total	**837**	**845**	**1682**	**25**	**21**	**46**	**134**	**118**	**252**

*2-month follow-up = ≥30 <400 days; 18-month follow-up ≥400 <680 days.
†3/72 due to participant death, 69/72 missed due to other reasons; ‡30/166 due to participant death, 136/166 missing due to other reasons; Missing measurements were largely due to missed in-person follow-up visits due to COVID-19 restrictions.

[2] Data cards lost at one research site for five participants; readings were only recorded on paper for one participant (protocol violation); and one participant had the baseline BCM measurement obtained on a different day to randomisation weight measurement (paired weight required; protocol violation).

Figure 4-1: Bioimpedance substudy cohort CONSORT flowchart



* Metal knee implants. † Invalid reasons: inadequate data quality, implausible outlying values or missing accompanying weight measurement (see Data Analysis Plan – Supplemental Material). ‡ Reasons for missed measurements were not recorded for all participants but include telephone follow-up (due to COVID-19), missed follow-up or rarely patient refusal/technical failure. § Died within time window of missing bioimpedance measurement; does not include deaths after valid measurement obtained; total deaths until end of follow-up = 35 (empagliflozin=18; placebo=17). ‖ Number (proportion) of participants who reported taking "most" of their study treatment at 12 months: empagliflozin 282 (89%); placebo 292 (91%). All 660 participants were included in secondary analyses. The analysis population for MMRM analyses excluded 40 participants who did not have a single valid follow-up measurement.

4.2.1.1 DATA QUALITY ASSESSMENT

Data quality was assessed for all measurements in November 2022, blind to treatment allocation, as detailed in section 2.4. The reasons for deeming a measurement invalid are also described in detail in section 2.4.4 but, in brief, were either due to (i) absolute "Fluid Overload" measurements more negative than -5 L which were deemed implausible; and/or (ii) visual inspection of the Cole-Cole plot identified poor data quality; and/or (iii) missing accompanying weight measurement (needed for derivation of analysis parameters). In total only 46 measurements (46/1728 = 2.7%) were deemed invalid overall across all three measurement time points (Table 4-2).

Table 4-2: Reasons for measurements being deemed invalid

	Empagliflozin	**Placebo**	**Overall**
Implausible negative value	11	6	**17**
Cole-Cole plot	13	14	**27**
Missing accompanying weight	1	1	**2**
Total	**25**	**21**	**46**

Greater numbers of measurements were deemed invalid in the empagliflozin group compared to placebo and in particular, due to absolute "Fluid Overload" measurements more negative than -5 L which were deemed implausible. This could theoretically be explained by a pronounced treatment effect causing profound dehydration in some participants receiving empagliflozin however this was considered unlikely and more likely explained as a chance finding.

Of the 660-participant analysis population, 600 (90.9%) survived until the final follow-up visit; 35 (5.3%) died and 25 (3.8%) were lost to follow-up. Valid measurements were available for 413/660 (62.6%) participants at all time points; numbers of participants with valid measurements at each time point are summarised in Table 4-3.

Table 4-3: Number of participants with valid measurements by visit

	Empagliflozin	Placebo	Overall
Valid measurements at all time points	203	210	**413**
Valid measurements at 2/3 time points			
Randomisation & 2-month follow-up	73	71	**144**
Randomisation & 18-month follow-up	19	19	**38**
2- & 18-month follow-up	7	7	**14**
Single valid measurement only			
Randomisation only	21	19	**40**
2-month follow-up only	7	0	**7**
18-month follow-up only	2	2	**4**
Total	**332**	**328**	**660**

The quality score (Q value) produced by the BCM device was used to identify BCM measurements requiring further assessment of data quality by visual inspection of the Cole-Cole plot (process described in detail in section 2.4.1). It was pre-specified in the Data Analysis Plan (appendix 11) that statistical comparisons by treatment allocation would be presented for the distribution of Q values for measurements included in the main comparison and sensitivity analyses. In all analyses, Q values were marginally lower in the empagliflozin group compared with placebo (Table 4-4); however, overall, Q values were very high (median across all measurements 94.2, IQR 90.4-96.7).

Table 4-4: Distribution of BCM quality scores (Q values)

	Empagliflozin	Placebo	P*
All measurements	94.0 (90.3-96.6)	94.3 (90.5-96.7)	0.14
Primary analysis	94.0 (90.2-96.4)	94.5 (91.2-96.7)	0.05
Sensitivity analysis 1	93.8 (89.7-96.3)	94.4 (90.7-96.7)	0.05
Sensitivity analysis 2	94.1 (90.6-96.5)	94.6 (91.6-96.7)	0.03
Sensitivity analysis 3	94.0 (90.2-96.4)	94.4 (91.1-96.7)	0.09

*Wilcoxon rank sum test P value

4.2.1.2 ADHERENCE TO STUDY TREATMENT

At 12 months of follow-up (the approximate midpoint of the trial), of substudy participants who remained alive, 282/318 (89%) in the empagliflozin group and 292/320 (91%) in the placebo group reported taking at least 80% of their allocated study treatment. These figures were similar to the full trial cohort as a whole: empagliflozin 2909/3245 (90%) versus placebo 2924/3239 (90%).

4.2.2 BASELINE CHARACTERISTICS

Baseline characteristics of substudy participants were reasonably well balanced between treatment groups (Table 4-5 & Table 4-6), with perhaps the exception of weight which was slightly higher among patients allocated to empagliflozin though not a statistically significant difference (P = 0.21). In the substudy overall, mean age was 65 (15) years, 205 (31%) participants were female and 256 (39%) participants had diabetes at baseline. Mean body weight was 88.8 (19.8) kg, mean body mass index (BMI) was 30.3 (6.2) kg/m^2 and mean systolic blood pressure was 137.3 (18.9) mmHg. Mean (SD) estimated glomerular filtration rate (eGFR) was 36.0 (12.4) $mL/min/1.73m^2$ and median (IQR) urinary albumin-to-creatinine ratio (uACR) was 203 (26-936) mg/g. Known heart failure was reported by 136 (21%) of participants at baseline, median N-terminal pro B-type natriuretic peptide (NT-proBNP) was 211 (93-581) ng/L and a similar proportion of participants reported taking any diuretic therapy at baseline in both groups (empagliflozin 180/332, 54% vs placebo 173/328, 53%). Mean absolute “Fluid Overload” at baseline was 0.4 (1.7) L, 126 (19%) with moderate “Fluid Overload” and 30 (5%) with severe “Fluid Overload”.

Although the substudy was only conducted in a subset from the UK and Germany, substudy participants were generally representative of all participants in their regions (UK and Germany combined) and demographically similar to the full trial population as a whole (all regions including Asia and North America) with the exception of race (Table 4-7). The proportion of participants with diabetes in the substudy was slightly lower and the proportion with heart failure slightly higher, relative to the full trial population.

Table 4-5: Key baseline characteristics of the bioimpedance substudy population

	Empagliflozin (N=332)	Placebo (N=328)
DEMOGRAPHICS		
Age (years)	65.2 (14.2)	64.1 (14.9)
Female sex	102 (30.7)	103 (31.4)
White race	321 (96.7)	315 (96.0)
PRIOR DISEASE		
Diabetes	135 (40.7)	121 (36.9)
Heart failure	62 (18.7)	74 (22.6)
CLINICAL MEASUREMENTS		
Weight (kg)	89.8 (20.2)	87.9 (19.3)
Body mass index (kg/m^2)	30.5 (6.2)	30.1 (6.3)
Waist-to-hip ratio	1.0 (0.1)	1.0 (0.1)
Systolic blood pressure (mmHg)	137.0 (18.8)	137.5 (18.9)
Diastolic blood pressure (mmHg)	77.8 (12.2)	78.6 (11.9)
BIOIMPEDANCE MEASUREMENTS*		
Absolute "Fluid Overload" (L)	0.45 (1.68)	0.32 (1.68)
Relative "Fluid Overload" (%)		
Mean (SD)	1.9 (8.7)	1.3 (8.3)
Moderate "Fluid Overload"	70 (21.1)	56 (17.1)
Severe "Fluid Overload"	14 (4.2)	16 (4.9)
Extracellular water (L)	19.0 (3.8)	18.4 (3.7)
Intracellular water (L)	20.7 (4.5)	20.1 (4.6)
Lean tissue index (kg/m^2)	13.3 (3.1)	12.9 (3.0)
Fat tissue index (kg/m^2)	12.6 (5.4)	12.5 (5.1)
LABORATORY MEASUREMENTS		
Estimated GFR ($mL/min/1.73m^2$)		
Mean (SD)	36.1 (13.4)	35.8 (11.4)
Distribution		
<30	123 (37.0)	118 (36.0)
≥30 <45	148 (44.6)	154 (47.0)
≥45	61 (18.4)	56 (17.1)
Urinary albumin-to-creatinine ratio (mg/g)	203 (26-958)	205 (29-865)
HbA1c (mmol/mol)	43.9 (11.3)	43.5 (10.9)
NT-proBNP (ng/L)	197 (90-596)	225 (95-550)
MEDICATIONS		
RAS inhibitor	304 (91.6)	288 (87.8)
Any diuretic therapy	180 (54.2)	173 (52.7)

Data are presented as mean (SD) or median (Q1-Q3) for continuous variables and n (%) for categorical variables.
Abbreviations: GFR = glomerular filtration rate; HbA1c = glycated haemoglobin; NT-proBNP = N-terminal pro B-type natriuretic peptide; RAS = renin-angiotensin system. *Bioimpedance measurements for all participants with non-missing bioimpedance measurements at baseline (n=644; n=16 missing).

Table 4-6: Additional baseline characteristics of the bioimpedance substudy population

	Empagliflozin (N=332)	**Placebo (N=328)**	**Overall (N=660)**
DEMOGRAPHICS			
Age (years)			
<60	103 (31.0)	107 (32.6)	210 (31.8)
≥60 <70	81 (24.4)	83 (25.3)	164 (24.8)
≥70	148 (44.6)	138 (42.1)	286 (43.3)
CAUSE OF KIDNEY DISEASE			
Diabetic kidney disease	70 (21.1)	52 (15.9)	122 (18.5)
Hypertension/renovascular	64 (19.3)	75 (22.9)	139 (21.1)
Glomerular	87 (26.2)	87 (26.5)	174 (26.4)
Other	47 (14.2)	61 (18.6)	108 (16.4)
Unknown	64 (19.3)	53 (16.2)	117 (17.7)
HISTORY OF SMOKING	178 (53.6)	161 (49.1)	122 (18.5)
CLINICAL MEASUREMENTS			
Weight (kg)			
<80	107 (32.2)	121 (36.9)	228 (34.5)
≥80 <95	113 (34.0)	100 (30.5)	213 (32.3)
≥95	112 (33.7)	106 (32.3)	218 (33.0)
Missing	0 (0.0)	1 (0.3)	1 (0.2)
Waist-to-hip ratio			
<0.9	39 (11.7)	40 (12.2)	79 (12.0)
≥0.9 <1.0	101 (30.4)	96 (29.3)	197 (29.8)
≥1.0	192 (57.8)	192 (58.5)	384 (58.2)
Missing	0 (0)	0 (0)	0 (0)
Body mass index (kg/m^2)			
<25	54 (16.3)	62 (18.9)	116 (17.6)
≥25 <30	133 (40.1)	133 (40.5)	266 (40.3)
≥30	145 (43.7)	132 (40.2)	277 (42.0)
Missing	0 (0)	1 (0.3)	1 (0.2)
Systolic blood pressure (mmHg)			
<130	111 (33.4)	114 (34.8)	225 (34.1)
≥130 <145	112 (33.7)	106 (32.3)	218 (33.0)
≥145	109 (32.8)	108 (32.9)	217 (32.9)
Missing	0 (0)	0 (0)	0 (0)
Diastolic blood pressure (mmHg)			
<75	140 (42.2)	126 (38.4)	266 (40.3)
≥75 <85	86 (25.9)	102 (31.1)	188 (28.5)
≥85	106 (31.9)	100 (30.5)	206 (31.2)
Missing	0 (0)	0 (0)	0 (0)
BIOIMPEDANCE-DERIVED PARAMETERS*			
Absolute "Fluid Overload" (L)			
Mean (SD)	0.45 (1.68)	0.32 (1.68)	0.39 (1.68)
Distribution			
<-1	32 (9.6)	34 (10.4)	66 (10.0)
≥-1 <+1	136 (41.0)	155 (47.3)	291 (44.1)
≥+1	154 (46.4)	133 (40.5)	287 (43.5)
Relative "Fluid Overload" (%)			
Mean (SD)	1.9 (8.7)	1.3 (8.3)	1.6 (8.5)
Distribution			
<-3	75 (22.6)	90 (27.4)	165 (25.0)
≥-3 <+5	117 (35.2)	129 (39.3)	246 (37.3)
≥+5	130 (39.2)	103 (31.4)	233 (35.3)
Extracellular water (L)			
Mean (SD)	19.0 (3.8)	18.4 (3.7)	18.7 (3.8)
Distribution			
<16	61 (18.4)	72 (22.0)	133 (20.2)
≥16 <20	122 (36.7)	133 (40.5)	255 (38.6)
≥20	139 (41.9)	117 (35.7)	256 (38.8)
Intracellular water (L)			
Mean (SD)	20.7 (4.5)	20.1 (4.6)	20.4 (4.6)
Distribution			
<17	61 (18.4)	68 (20.7)	129 (19.5)

≥17 <22	135 (40.7)	138 (42.1)	273 (41.4)
≥22	126 (38.0)	116 (35.4)	242 (36.7)
Lean tissue index (kg/m^2)			
Mean (SD)	13.3 (3.1)	12.9 (3.0)	13.1 (3.1)
Distribution			
<11	57 (17.2)	69 (21.0)	126 (19.1)
≥11 <14	126 (38.0)	128 (39.0)	254 (38.5)
≥14	139 (41.9)	125 (38.1)	264 (40.0)
Fat tissue index (kg/m^2)			
Mean (SD)	12.6 (5.4)	12.5 (5.1)	12.5 (5.3)
Distribution			
<10	105 (31.6)	91 (27.7)	196 (29.7)
≥10 <14	92 (27.7)	123 (37.5)	215 (32.6)
≥14	125 (37.7)	108 (32.9)	233 (35.3)
LABORATORY MEASUREMENTS			
Urinary albumin-to-creatinine ratio (mg/g)			
<30	91 (27.4)	84 (25.6)	175 (26.5)
≥30 ≤300	90 (27.1)	101 (30.8)	191 (28.9)
>300	151 (45.5)	143 (43.6)	294 (44.5)
HbA1c (mmol/mol)			
<39	129 (38.9)	141 (43.0)	270 (40.9)
≥39 <48	107 (32.2)	96 (29.3)	203 (30.8)
≥48 <75	86 (25.9)	78 (23.8)	164 (24.8)
≥75	4 (1.2)	6 (1.8)	10 (1.5)
Missing	6 (1.8)	7 (2.1)	13 (2.0)
NT-proBNP (ng/L)			
<110	100 (30.1)	90 (27.4)	190 (28.8)
≥110 <330	110 (33.1)	114 (34.8)	224 (33.9)
≥330	117 (35.2)	117 (35.7)	234 (35.5)
Missing	5 (1.5)	7 (2.1)	12 (1.8)
Haematocrit (%)			
Mean (SD)	39.2 (4.8)	39.3 (4.9)	39.3 (4.8)
Distribution			
<37	84 (25.3)	98 (29.9)	182 (27.6)
≥37 <41	107 (32.2)	89 (27.1)	196 (29.7)
≥41	124 (37.3)	131 (39.9)	255 (38.6)
Missing	17 (5.1)	10 (3.0)	27 (4.1)
MEDICATIONS			
Mineralocorticoid receptor antagonist	42 (12.7)	42 (12.8)	84 (12.7)
Loop diuretic	120 (36.1)	123 (37.5)	243 (36.8)
Thiazide diuretic	64 (19.3)	57 (17.4)	121 (18.3)
Potassium-sparing diuretic	5 (1.5)	0 (0)	5 (0.8)
Any diabetes therapy	119 (35.8)	102 (31.1)	221 (33.5)
Insulin	77 (23.2)	62 (18.9)	139 (21.1)
Sulfonylurea	19 (5.7)	14 (4.3)	33 (5.0)
Metformin	41 (12.3)	35 (10.7)	76 (11.5)
GLP-1 agonist	19 (5.7)	12 (3.7)	31 (4.7)
DPP-4 inhibitor	38 (11.4)	40 (12.2)	78 (11.8)
Other diabetes drug	4 (1.2)	1 (0.3)	5 (0.8)

Data are presented as mean (SD) or median (Q1-Q3) for continuous variables and n (%) for categorical variables. History of smoking = "ever smoked tobacco regularly" as determined by the participant. Weight, waist-to-hip ratio and all bioimpedance-derived parameters are presented as approximate tertiles, all other categorisations use pre-specified groupings as per the Data Analysis Plan. Bioimpedance-derived parameters were missing at baseline for 10 participants in the empagliflozin group (3.0%) and 6 participants in the placebo group (1.8%). Abbreviations: NT-proBNP = N-terminal pro B-type natriuretic peptide; GLP-1 = glucagon-like peptide-1 receptor; DPP-4 = dipeptidyl-peptidase 4. *Bioimpedance measurements for all participants with non-missing bioimpedance measurements at baseline (n=644; n=16 missing).

Table 4-7: Baseline characteristics of the substudy population relative to trial regions

	Bioimpedance Substudy Cohort (N=660)	**Bioimpedance Substudy Countries** (i.e. all UK & Germany participants) **(N=2402)**	**Full Trial Cohort (N=6609)**
DEMOGRAPHICS			
Age (years)	64.6 (14.5)	65.5 (13.9)	63.8 (13.9)
Female sex	205 (31.1)	710 (29.6)	2192 (33.2)
Race			
White	636 (96.4)	2256 (93.9)	3859 (58.4)
Black/African American	3 (0.5)	74 (3.1)	262 (4.0)
Asian	12 (1.8)	38 (1.6)	2393 (36.2)
Mixed/Other	9 (1.4)	34 (1.4)	95 (1.4)
PRIOR DISEASE			
Diabetes	256 (38.8)	966 (40.2)	3040 (46.0)
Heart failure	136 (20.6)	380 (15.8)	658 (10.0)
CLINICAL MEASUREMENTS			
Weight (kg)	88.8 (19.8)	89.9 (19.9)	84.1 (21.4)
Body mass index (kg/m^2)	30.3 (6.2)	30.7 (6.3)	29.7 (6.8)
Waist-to-hip ratio	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)
Systolic blood pressure (mmHg)	137.3 (18.9)	137.0 (18.6)	136.5 (18.3)
Diastolic blood pressure (mmHg)	78.2 (12.0)	78.2 (11.6)	78.1 (11.8)
LABORATORY MEASUREMENTS			
Estimated GFR ($mL/min/1.73m^2$)			
Mean (SD)	36.0 (12.4)	35.0 (12.0)	37.3 (14.4)
Distribution, n (%)			
<30	241 (36.5)	933 (38.8)	2282 (34.5)
≥30 <45	302 (45.8)	1098 (45.7)	2928 (44.3)
≥45	117 (17.7)	371 (15.4)	1399 (21.2)
Urinary albumin-to-creatinine ratio (mg/g)	203 (26-936)	220 (29-909)	329 (49-1069)
HbA1c (mmol/mol)	43.7 (11.1)	44.5 (12.7)	45.0 (13.6)
NT-proBNP (ng/L)	211 (93-581)	209 (92-571)	160 (69-419)
MEDICATIONS			
RAS inhibitor	592 (89.7)	2092 (87.1)	5628 (85.2)
Any diuretic therapy	353 (53.5)	1270 (52.9)	2815 (42.6)

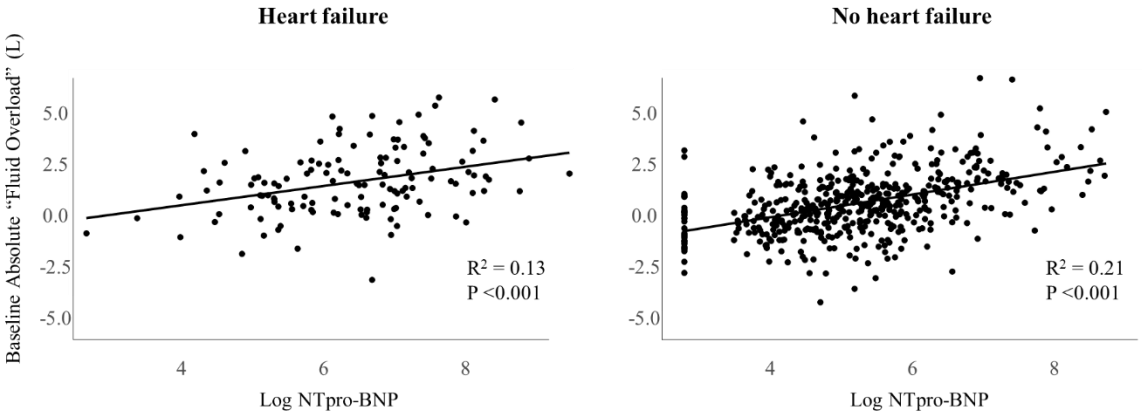
Abbreviations: GFR = glomerular filtration rate; HbA1c = glycated haemoglobin; NT-proBNP = N-terminal pro B-type natriuretic peptide; RAS = renin-angiotensin system.

4.2.3 CORRELATION BETWEEN BIOIMPEDANCE-DERIVED FLUID OVERLOAD AND OTHER CHARACTERISTICS AT BASELINE

There were some differences in baseline characteristics when the substudy cohort was stratified according to bioimpedance-derived fluid status at baseline (Table 4-8). Participants with moderate or severe "Fluid Overload" were more typically male, older in age and more likely to have diabetes and heart failure. Diuretic use was also more common among the categories with moderate or severe "Fluid Overload". Correlations were assessed between bioimpedance-derived "Fluid Overload" as a continuous variable (in litres) and relevant laboratory parameters; as well as with systolic blood pressure; in

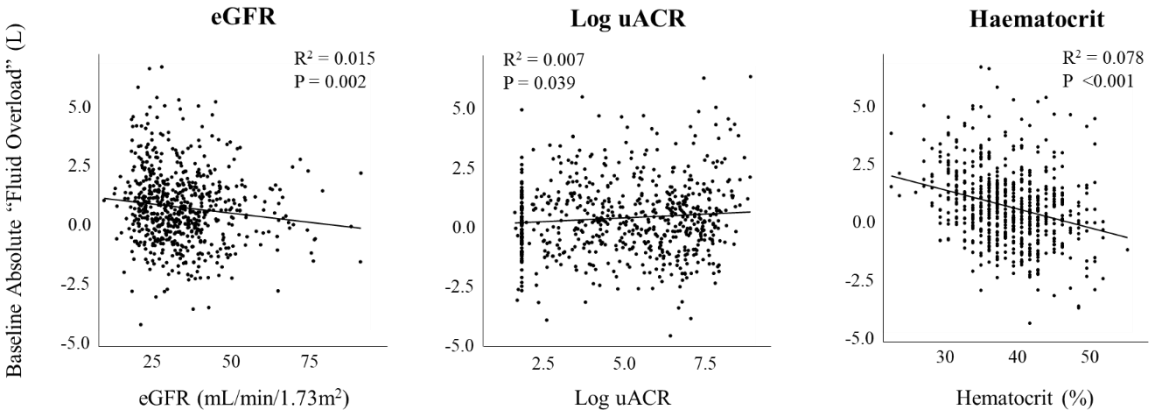
hypothesis tests using Pearson's product moment correlation coefficient. NT-proBNP (log-transformed) was the variable most strongly correlated with absolute "Fluid Overload" and positive correlations were observed both in participants with and without heart failure (R^2 = 0.13 and = 0.21, respectively; P <0.001; Figure 4-2).

Figure 4-2: Correlation between "Fluid Overload" and NT-proBNP



Absolute "Fluid Overload" was weakly negatively associated with eGFR and weakly positively associated with log uACR. Higher "Fluid Overload" also correlated with lower haematocrit.

Figure 4-3: Correlations between "Fluid Overload" and other laboratory parameters



Increased “Fluid Overload” was also weakly correlated with elevated systolic and inversely with diastolic blood pressure (R^2 = 0.02 and = 0.03, respectively; P <0.001; Figure 4-4).

Figure 4-4: Correlation between “Fluid Overload” and blood pressure

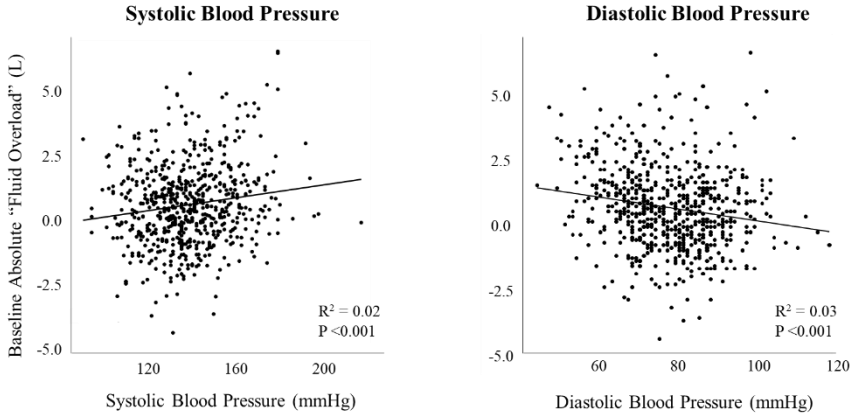


Table 4-8: Key baseline characteristics according to baseline fluid status

	Fluid-deplete (N=89)	Normohydrated (N=399)	Moderate "Fluid Overload" (N=126)	Severe "Fluid Overload" (N=30)
DEMOGRAPHICS				
Age (years)	58.9 (14.6)	63.9 (14.9)	68.7 (12.8)	71.0 (9.1)
Female sex	47 (52.8)	115 (28.8)	37 (29.4)	3 (10.0)
Race				
White	85 (95.5)	383 (96.0)	122 (96.8)	30 (100.0)
Black/African American	0 (0.0)	3 (0.8)	0 (0.0)	0 (0.0)
Asian	4 (4.5)	7 (1.8)	1 (0.8)	0 (0.0)
Mixed/Other	0 (0.0)	6 (1.6)	3 (2.4)	0 (0.0)
PRIOR DISEASE				
Diabetes	26 (29.2)	143 (35.8)	58 (46.0)	21 (70.0)
Heart failure	10 (11.2)	78 (19.5)	34 (27.0)	13 (43.3)
CLINICAL MEASUREMENTS				
Weight (kg)	97.0 (22.9)	88.2 (18.9)	84.8 (20.1)	89.2 (15.6)
BMI (kg/m^2)	34.1 (7.2)	29.9 (5.9)	28.9 (6.0)	29.2 (4.6)
Waist-to-hip ratio	0.9 (0.1)	1.0 (0.1)	0.9 (0.1)	1.0 (0.2)
Systolic BP (mmHg)	132.4 (15.4)	137.5 (18.9)	137.0 (20.1)	148.8 (19.4)
Diastolic BP (mmHg)	80.3 (10.7)	79.2 (11.7)	74.4 (12.7)	76.4 (13.7)
BIOIMPEDANCE-DERIVED PARAMETERS				
Absolute "Fluid Overload" (L)	-2.1 (1.4)	0.1 (0.7)	2.1 (0.7)	4.1 (1.0)
Relative "Fluid Overload" (%)	-12.0 (7.2)	0.5 (3.8)	10.6 (2.4)	18.2 (2.9)
Extracellular water (L)	17.4 (3.6)	18.4 (3.5)	19.5 (4.1)	22.4 (3.2)
Intracellular water (L)	21.3 (5.9)	20.6 (4.4)	19.3 (4.3)	20.2 (2.9)
Lean tissue index (kg/m^2)	13.4 (4.3)	13.2 (2.9)	12.5 (2.6)	12.8 (2.2)
Fat tissue index (kg/m^2)	15.8 (6.7)	12.3 (5.0)	11.5 (4.4)	11.0 (3.7)
LABORATORY MEASUREMENTS				
Estimated GFR ($mL/min/1.73m^2$)				
Mean (SD)	39.5 (12.9)	36.0 (12.2)	34.5 (12.7)	33.0 (11.0)
Distribution				
<30	17 (19.1)	141 (35.3)	61 (48.4)	15 (50.0)
≥30 <45	48 (53.9)	192 (48.1)	43 (34.1)	11 (36.7)
≥45	24 (27.0)	66 (16.5)	22 (17.5)	4 (13.3)
Urinary albumin-to-creatinine ratio (mg/g)	231 (21-852)	207 (22-938)	166 (29-982)	311 (47-1466)
HbA1c (mmol/mol)	43.3 (12.3)	43.3 (10.9)	44.5 (10.8)	46.7 (11.6)
NT-proBNP (ng/L)	100 (48-223)	187 (88-399)	549 (172-1250)	953 (463-2168)
MEDICATIONS				
RAS inhibitor	84 (94.4)	357 (89.5)	111 (88.1)	26 (86.7)
Any diuretic therapy	49 (55.1)	191 (47.9)	86 (68.3)	21 (70.0)

Data are not presented for 16/660 (2%) participants with missing bioimpedance data at baseline.

4.2.4 EFFECTS ON "FLUID OVERLOAD" AS A CONTINUOUS VARIABLE

Of the 660-participant analysis population, 40 participants who had no valid BCM measurements during the follow-up period (randomisation measurements only) were excluded from the primary assessment and all analyses of continuous bioimpedance-derived parameters, since the mixed model repeated measures (MMRM) approach requires participants to have at least one available follow-up measurement. These 40 excluded participants were approximately evenly distributed between treatment groups (empagliflozin versus placebo: 21 versus 19 respectively) and the reasons for not having at least one valid follow-up measurement were: death before first follow-up measurement (n=3); inadequate data quality (n=9); and no follow-up measurement performed (e.g. due to Covid-19 precluding visits, n=28). The 620 participants included in MMRM analyses had, in total, 1047 valid follow-up measurements available for inclusion in analyses.

4.2.4.1 PRIMARY AND SECONDARY ASSESSMENTS

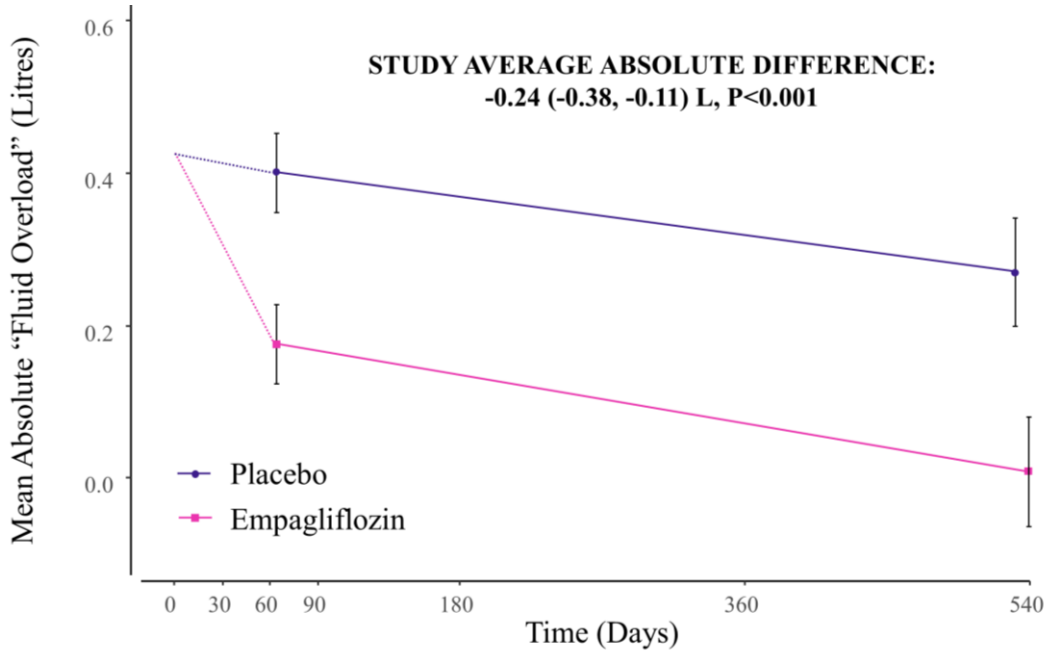
Compared to placebo, the study average absolute difference in absolute "Fluid Overload" was -0.24 L (95% confidence interval [CI] -0.38, -0.11; Table 4-9). Effects were similar at 2- and 18-months (-0.23 [-0.37, -0.08] and -0.26 [-0.46, -0.06] L, respectively; P value for interaction with time = 0.11; Table 4-9 & Figure 4-5). Analysis of effects on the related parameter relative "Fluid Overload" were also consistent: the study average absolute difference was -1.19 (-1.90, -0.48) % with similar effects at 2- and 18-months (-1.12 [-1.88, -0.37] and -1.28 [-2.32, -0.23] %, respectively; P value for interaction with time = 0.39).

Table 4-9: Effects of empagliflozin on bioimpedance-derived parameters

	Empagliflozin (N=311)		**Placebo (N=309)**				
	Adjusted* Mean	SE	Adjusted* Mean	SE	Absolute Difference	95% CI	P
Absolute "Fluid Overload", L							
Randomisation	0.50	0.09	0.35	0.09			
2-month follow-up	0.18	0.05	0.40	0.05	-0.23	(-0.37, -0.08)	
18-month follow-up	0.01	0.07	0.27	0.07	-0.26	(-0.46, -0.06)	
Study average	**0.10**	**0.05**	**0.34**	**0.05**	**-0.24**	**(-0.38, -0.11)**	**<0.001**
Relative "Fluid Overload", %							
Randomisation	2.24	0.47	1.39	0.45			
2-month follow-up	0.52	0.27	1.65	0.27	-1.12	(-1.88, -0.37)	
18-month follow-up	-0.36	0.38	0.92	0.37	-1.28	(-2.32, -0.23)	
Study average	**0.14**	**0.25**	**1.33**	**0.25**	**-1.19**	**(-1.90, -0.48)**	**0.001**

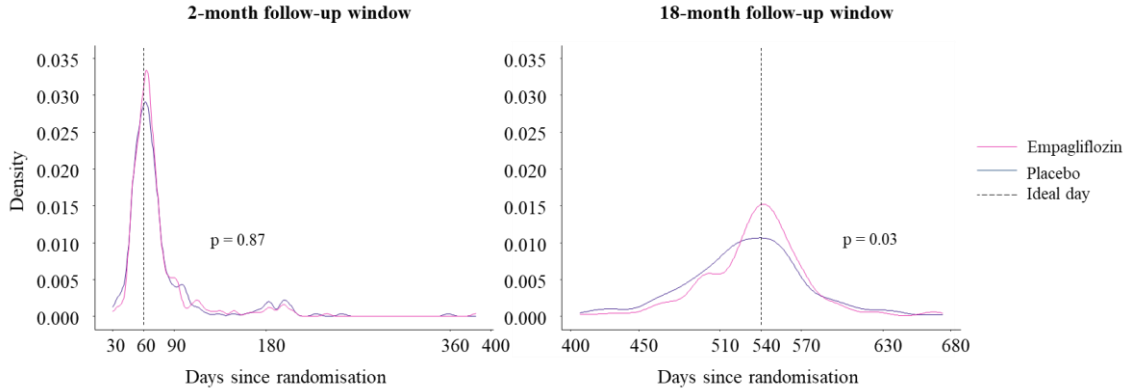
*Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) between treatment groups with study averages weighted in proportion to the amount of time between follow-up visits.

Figure 4-5: Effects of empagliflozin on "Fluid Overload" by time



The distribution of the timing of measurements (in days since randomisation) was assessed. Timing of the 2-month measurement adhered closely to the substudy protocol with the median follow-up day being very close to the ideal day 60 in both groups (median [IQR] 64 [57-74] days in the empagliflozin group versus 64 [57-75] days in the placebo group; P = 0.87; Figure 4-6). There was greater spread around the 18-month visit but the median day of measurement was again very close to the ideal day 540 although visits occurred slightly earlier in the placebo group (540 [519-555] days in the empagliflozin group versus 532 [505-554] days in the placebo group; P = 0.03; Figure 4-6).

Figure 4-6: Distribution of time to bioimpedance measurement



4.2.4.2 SENSITIVITY ANALYSES FOR THE PRIMARY ASSESSMENT

Sensitivity analyses assessing the impact of different procedures to determine validity of measurements demonstrated findings consistent with the primary assessment (Table 4-10).

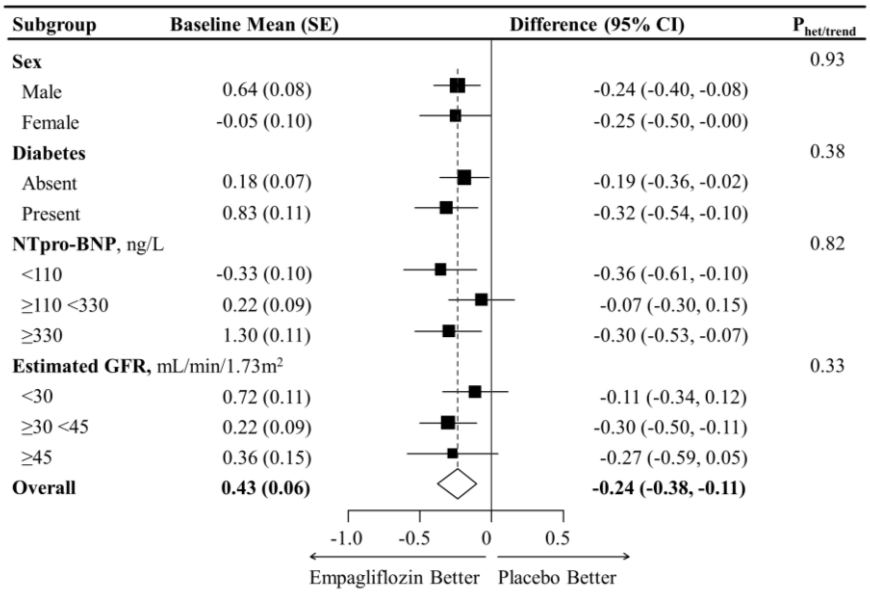
Table 4-10: Sensitivity analyses

	Analysis includes:		**Empagliflozin**		**Placebo**			
	Follow-up measurements	N	Mean	SE	Mean	SE	Absolute Difference	95% CI
PRIMARY ASSESSMENT	1047	620	0.10	0.05	0.34	0.05	-0.24	(-0.38, -0.11)
Sensitivity analysis 1								
Maximal inclusion: irrespective of data quality /implausible values	1082	629	-0.07	0.07	0.25	0.07	-0.32	(-0.52, -0.12)
Sensitivity analysis 2								
Limited to measurements with Q value ≥80	1029	614	0.07	0.06	0.37	0.06	-0.30	(-0.46, -0.14)
Sensitivity analysis 3								
Cohort with complete baseline bioimpedance data	1008	595	0.09	0.05	0.33	0.05	-0.24	(-0.38, -0.11)
All results are study averages (and absolute difference between treatment groups) for absolute "Fluid Overload" in L with adjustment as before.								

4.2.4.3 SUBGROUP ANALYSIS (TERTIARY AND POST-HOC ASSESSMENTS)

Effects on "Fluid Overload" were similar in men and women; in people with and without diabetes; and across the spectrum of eGFR and NT-proBNP studied (Figure 4-7).

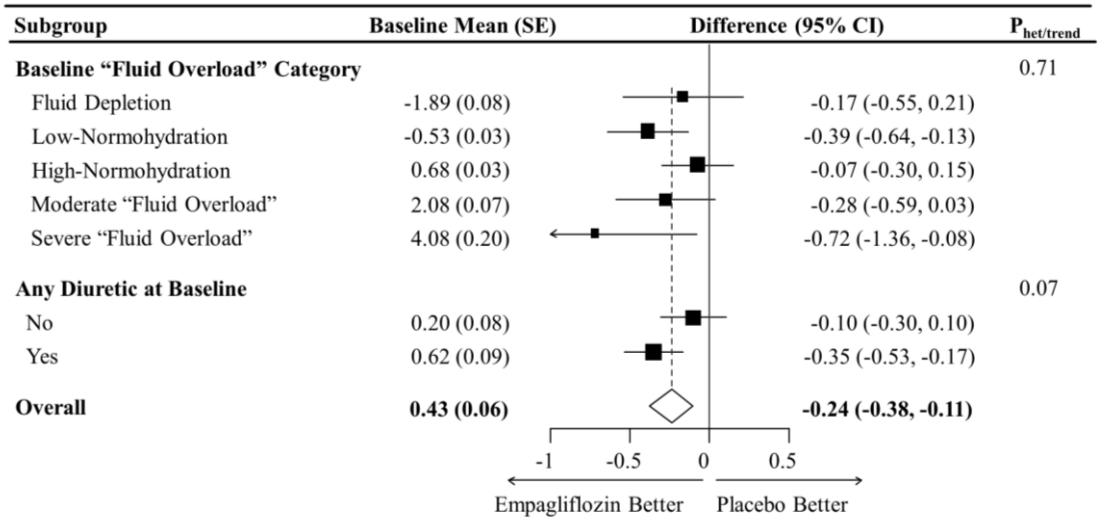
Figure 4-7: Effects on absolute "Fluid Overload" by pre-specified subgroups



Study-average differences are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) between treatment groups and weighted in proportion to the amount of time between follow-up visits.

Post-hoc exploratory subgroup analyses stratified by baseline fluid status and reported use of any diuretic agent at baseline also did not find any statistical evidence for effect modification (Figure 4-8). Baseline "Fluid Overload" was categorised using relative "Fluid Overload": fluid depletion = ≤ -7%, low-normohydration = > -7% ≤ 0%, high-normohydration = >0% ≤ +7%, moderate "Fluid Overload" = > +7% ≤ +15%, severe "Fluid Overload" = > +15%; participants without a valid baseline bioimpedance measurement were included in the high-normohydration category based upon the imputed mean value.

Figure 4-8: Effects on absolute "Fluid Overload" by post-hoc subgroups

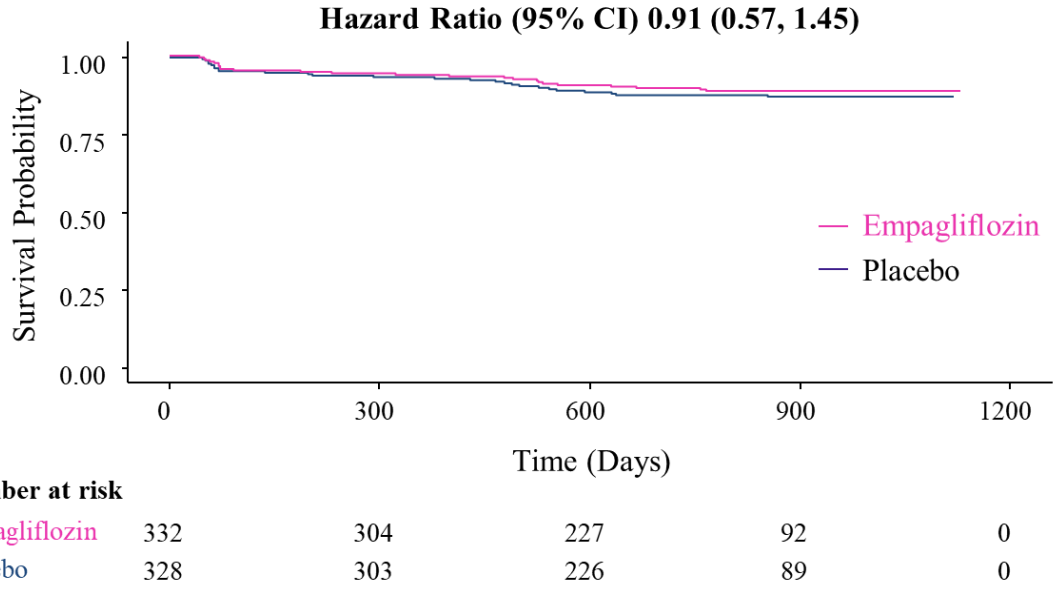


Study-average differences are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) between treatment groups and weighted in proportion to the amount of time between follow-up visits.

4.2.5. EFFECTS ON THE COMPOSITE OF CATEGORICAL FLUID OVERLOAD OUTCOMES AND HEART FAILURE

The number of key secondary outcomes was low, and there was no significant difference in the hazards of the composite outcome between treatment groups (35/332 [10%] versus 38/328 [12%]; hazard ratio (HR) 0.91, 95% CI 0.57, 1.45; P = 0.69, Figure 4-9 & Table 4-11) with consistent effects for its components (Table 4-11). There were no events in either treatment group for the death from heart failure component of this composite outcome and only 27 first hospitalisations for heart failure (11/332 [3%] in the empagliflozin group versus 16/328 [5%] in the placebo group; HR 0.67, 95% CI 0.31-1.46. This result was consistent with findings from the full trial cohort in which there were there were 88 (2.7%) first hospitalisations for heart failure in the empagliflozin group versus 107 (3.2%) in the placebo group (HR 0.80, 95% CI 0.60-1.06).

Figure 4-9: Effects on the composite cardiovascular secondary outcome



In participants who had either moderate or severe absolute "Fluid Overload" at baseline, regression to a lower fluid status category was no more common in the empagliflozin or placebo groups (46/84 [55%] for empagliflozin versus 35/72 [49%] for placebo; Table 4-11).

Table 4-11: Effects of empagliflozin on cardiovascular composite outcome (bioimpedance substudy cohort)

	Empagliflozin		**Placebo**				
	n/N	%	n/N	%	Hazard Ratio	95% CI	P
KEY SECONDARY ASSESSMENT **Death from heart failure, hospitalisation for heart failure, development of new moderate or severe "Fluid Overload"**	35/332	11	38/328	12	0.91	(0.57-1.45)	0.69
Death from heart failure	0/332	0	0/328	0	-	-	
Hospitalisation for heart failure	11/332	3	16/328	5	0.67	(0.31-1.46)	
Development of new moderate "Fluid Overload"*	18/232	8	25/247	10	0.68	(0.37-1.26)	
Development of new severe "Fluid Overload"†	8/302	3	4/303	1	1.96	(0.57-6.71)	
TERTIARY ASSESSMENT							
Regression of "Fluid Overload"‡	46/84	55	35/72	49	1.33	(0.82-2.18)	

Cox proportional hazards models include adjustment for the covariates used in the minimisation algorithm: age, sex, diabetes status, eGFR and uACR. * Requires randomisation value of relative "Fluid Overload" ≤7% and follow-up value >7%, ≤15%. † Requires randomisation value of relative "Fluid Overload" ≤15% and follow-up value >15%. ‡ Requires randomisation value consistent with moderate or severe relative "Fluid Overload" and regression to any lower hydration category at any follow-up (limited to first event).

4.2.6 EFFECTS ON EXTRACELLULAR AND INTRACELLULAR WATER

The study-average absolute differences in bioimpedance-estimated extracellular (ECW) and intracellular water (ICW) were -0.49 L (95% CI -0.69, -0.30) and -0.30 L (95% CI -0.57, -0.03), respectively (Table 4-12). Effects on total body water (TBW) were explored on a *post-hoc* exploratory basis and provide confirmatory results (Table 4-12).

Table 4-12: Effects of empagliflozin on tertiary bioimpedance-derived parameters by time

	Empagliflozin (N=311)		**Placebo (N=309)**			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
EXTRACELLULAR WATER, L						
Randomisation	18.96	0.22	18.40	0.21		
2-month follow-up	18.19	0.07	18.70	0.07	-0.52	(-0.72, -0.32)
18-month follow-up	18.13	0.10	18.59	0.10	-0.46	(-0.74, -0.19)
Study average	**18.16**	**0.07**	**18.66**	**0.07**	**-0.49**	**(-0.69, -0.30)**
INTRACELLULAR WATER, L						
Randomisation	20.63	0.27	20.12	0.25		
2-month follow-up	20.02	0.10	20.37	0.10	-0.35	(-0.64, -0.07)
18-month follow-up	20.20	0.14	20.44	0.13	-0.24	(-0.61, 0.14)
Study average	**20.10**	**0.10**	**20.40**	**0.10**	**-0.30**	**(-0.57, -0.03)**
TOTAL BODY WATER, L						
Randomisation	39.59	0.46	38.51	0.44		
2-month follow-up	38.19	0.16	39.09	0.16	-0.89	(-1.33, -0.45)
18-month follow-up	38.32	0.21	39.04	0.21	-0.73	(-1.30, -0.15)
Study average	**38.25**	**0.15**	**39.07**	**0.15**	**-0.82**	**(-1.24, -0.40)**

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) between treatment groups and weighted in proportion to the amount of follow-up time represented.

4.2.7 EFFECTS ON DEHYDRATION ADVERSE EVENTS AND DIURETIC USE

The effects of empagliflozin on bioimpedance-derived estimates of "Fluid Overload" can be supplemented with additional information which was routinely collected as part of the main trial protocol in all 6609 participants and is of particular relevance to fluid status.

(i) Adverse event reports of dehydration

Serious dehydration was defined as any event of dehydration requiring inpatient hospitalisation, resulting in death or considered life-threatening or otherwise medically important in the opinion of a local investigator. Symptomatic dehydration was defined as symptoms attributed by participants to dehydration, such as feeling faint or fainting. In the full trial cohort, there were 54 reports of serious dehydration (empagliflozin 30/3304 vs

placebo 24/3305; HR 1.25, 95% CI 0.73–2.14) and 159 reports of symptomatic dehydration (empagliflozin 83/3304 vs placebo 76/3305; HR 1.10, 95% CI 0.81–1.51). Numbers of events in the smaller substudy population are reported in Table 4-13; stratified according to baseline bioimpedance-derived fluid status.

Table 4-13: Effects of empagliflozin on dehydration

	Empagliflozin		**Placebo**	
	n/N	%	n/N	%
SERIOUS DEHYDRATION				
Fluid-deplete	0/41	0.0	0/45	0.0
Normohydrated	1/207	0.5	2/211	0.9
Moderate "Fluid Overload"	3/70	4.3	2/56	3.6
Severe "Fluid Overload"	0/14	0.0	0/16	0.0
Bioimpedance substudy overall	**4/332**	**1.2**	**4/328**	**1.2**
Full trial cohort	**30/3304**	**0.9**	**24/3305**	**0.7**
SYMPTOMATIC DEHYDRATION				
Fluid-deplete	1/42	2.4	0/45	0.0
Normohydrated	3/207	1.4	3/211	1.4
Moderate "Fluid Overload"	6/70	8.6	2/56	3.6
Severe "Fluid Overload"	0/14	0.0	0/16	0.0
Bioimpedance substudy overall	**10/332**	**3.0**	**5/328**	**1.5**
Full trial cohort	**83/3304**	**2.5**	**76/3305**	**2.3**

Baseline "Fluid Overload" is categorised using relative "Fluid Overload": fluid depletion = ≤ -7%, normohydration = > -7% ≤ +7%, moderate "Fluid Overload" = > +7% ≤ +15%, severe "Fluid Overload" = > +15%; participants without a valid baseline bioimpedance measurement are included in the normohydrated category based upon the imputed mean value.

(ii) Initiation of new loop diuretic therapy

In the full trial cohort, loop diuretic therapy was initiated during follow-up when not recorded at randomisation in 159/2453 (6.5%) participants allocated empagliflozin versus 212/2409 (8.8%) allocated placebo; representing a 26% lower risk of requiring to start loop diuretics during follow-up among participants allocated empagliflozin versus placebo (risk ratio 0.74, 95% CI 0.60-0.90).

4.3 DISCUSSION

In patients with CKD, empagliflozin reduced bioimpedance-measured "Fluid Overload" across the broad range of participants studied and effects were sustained for at least 18 months. Averaged across the follow-up period, "Fluid Overload" was 0.24 L (95% CI 0.11-0.38) lower in the empagliflozin group compared with participants allocated to placebo. When considering total body water (total ECW and ICW), participants allocated to empagliflozin had approximately 0.8 L less total body water of which approximately 0.5

L was ECW (which includes the 0.24 L excess ECW referred to as “Fluid Overload”) and approximately 0.3 L ICW. These diuretic effects are considered potentially important contributing mechanisms to the cardiovascular benefits of SGLT2 inhibitors and had not previously been quantified using bioimpedance in randomised trials in CKD. Previously, the 16-week DECREASE trial provided the only peer reviewed published randomised evidence on the effects of SGLT2 inhibitors on bioimpedance parameters. The DECREASE trial found that, in 66 participants with type 2 diabetes—CKD status not reported—dapagliflozin reduced extracellular fluid by approximately 1 L and systolic blood pressure by approximately 4 mmHg at 10 days versus placebo. These results from the EMPA-KIDNEY bioimpedance substudy now substantially extend these previous findings by studying longer term effects (over 18 months) in a much larger number of participants in a placebo-controlled trial and in a CKD population specifically.

Mean baseline levels of “Fluid Overload” in EMPA-KIDNEY bioimpedance substudy participants were approximately consistent with observational studies (which have largely studied patients with comparatively lower levels of kidney function) (Tsai et al., 2018, Vega et al., 2018, Schork et al., 2020) and fall within the normal range based on a healthy reference population (absolute “Fluid Overload” -1.1 L to +1.1 L) (Wabel et al., 2008). Despite average baseline fluid status being within the normal range and the average difference in excess fluid volume being numerically small (-0.24 L); this effect is likely to be prognostically important. Observational analyses in a CKD stage 3-5 population have demonstrated significant increased hazards of cardiovascular morbidity and mortality per 1 L increment of increasing absolute “Fluid Overload” (adjusted HR 1.42, 95% CI 1.25-1.62) (Hung et al., 2015). In the same study, stratification of the cohort into fluid overloaded (relative “Fluid Overload” ≥7%) versus not, found hazards of the same composite outcome increased more than six-fold in association with moderate-severe levels of “Fluid Overload” (adjusted HR 6.22, 95% CI 2.78-13.92) (Hung et al., 2015). Severely fluid overloaded patients may conceivably exhibit larger diuresis with SGLT2 inhibition and therefore obtain particular benefit however *post-hoc* exploratory subgroup analysis did not demonstrate significant differences in treatment effect by baseline hydration status although few substudy participants had moderate or severe “Fluid Overload” at baseline, limiting statistical power.

Highly significant effects on the primary outcome assessment of absolute “Fluid Overload” in the EMPA-KIDNEY bioimpedance substudy did not translate into significant effects on the composite secondary outcome; likely due to lack of statistical power due to low event rates and loss of information by categorisation of the continuous “Fluid Overload” variable. Regardless, the clear effect demonstrated in the primary outcome assessment and associated established prognostic benefit can be expected to translate into important reductions in clinical heart failure outcomes.

Before the results of this substudy, attenuation of diuretic effects at low levels of kidney function was considered plausible as SGLT2 inhibitors have little effect on glycaemia at lower eGFR due to attenuated levels of glycosuria. Despite this, there were consistent effects on “Fluid Overload” across the eGFR-based subgroups. Similarly, effects did not vary by baseline fluid status, diuretic use, or albuminuria. These findings are analogous to results from large randomised trials in heart failure populations that included a large proportion of patients with CKD and low eGFR and demonstrated consistent effects of SGLT2 inhibitors on cardiovascular death or hospitalisation for heart failure irrespective of sex, diabetes, eGFR, or NT-proBNP at baseline.

These analyses convincingly demonstrate the diuretic effects of SGLT2 inhibitors in CKD and address uncertainty generated by previous conflicting reports. Authors of a *post-hoc* analysis of the EMPEROR-Reduced trial of empagliflozin in heart failure with reduced ejection fraction concluded that diuresis was not a dominant mechanism in the clinical benefits of empagliflozin in heart failure based on discordant effects on weight, haematocrit and natriuretic peptides and the observation that the magnitude of effect on major heart failure outcomes did not differ depending on whether participants had evidence of pre-existing fluid excess in the 4 weeks prior to enrolment or not (Packer et al., 2021). In light of bioimpedance data from EMPA-KIDNEY, these earlier conclusions can be considered relatively weak and unreliable especially since the definition of pre-existing fluid excess was unspecified and based on subjective investigator impression only. Contrasting reports from the EMPA-REG OUTCOME trial concluded that changes in markers of plasma volume (based on haematocrit) were the greatest mediators of the cardiovascular benefit of empagliflozin (Inzucchi et al., 2018). However, the use of haematocrit to reflect fluid status is inherently flawed since SGLT2 inhibitors are known to

promote erythropoiesis (Packer, 2023). There was also historic uncertainty on whether the diuretic effects of SGLT2 inhibition caused both intravascular and interstitial volume reduction (Scholtes et al., 2021). Robust assessments of the effects of SGLT2 inhibition on measured plasma volume are limited to isolated small studies (Lambers Heerspink et al., 2013) and more commonly rely on estimated plasma volume (van Ruiten et al., 2022, Jensen et al., 2021) a formula which is reliant on haematocrit. It is also relevant that the effect of empagliflozin on fluid loss in EMPA-KIDNEY was achieved safely. Although estimates of ECW reduction reflected loss of ECW that is not considered to be in excess by the three-compartment model, there was no increased risk of participant reports of symptomatic dehydration in the full trial or substudy cohorts nor any increased risk of acute kidney injury (EMPA-KIDNEY Collaborative Group, 2023).

The substudy has certain limitations. Firstly, analysed bioimpedance parameters are derived (from extracellular and intracellular resistance values) and are not direct measurements therefore it is not possible to precisely quantify differences in fluid and body composition. Furthermore, derivation is based on formulae normalised to healthy reference populations, not kidney disease populations, however the device has been extensively validated for fluid assessment in kidney failure cohorts. Secondly, some scheduled bioimpedance measurements were missed largely due to the impact of the COVID-19 pandemic necessitating remote follow-up. Thirdly, the substudy was only conducted in the UK and Germany and is therefore largely restricted to white participants. Fourthly, data on urinary and plasma sodium was not collected as part of EMPA-KIDNEY trial procedures precluding any assessment of natriuretic effects of empagliflozin alongside the diuretic effects on interstitial fluid volume.

In summary, in patients with CKD, empagliflozin reduced bioimpedance-measured “Fluid Overload” across the broad range of participants studied and effects were sustained for at least 18 months. These diuretic effects may contribute to the benefits of SGLT2 inhibitors particularly in reducing hospitalisations for heart failure. The following chapter will report the effects of empagliflozin on body weight and bioimpedance estimates of fat and lean tissue mass. These results will then allow for contextualisation of the effects on “Fluid Overload” in the final discussion chapter (Chapter 8).

CHAPTER 5 – EFFECTS OF EMPAGLIFLOZIN ON BODY COMPOSITION

5.1 INTRODUCTION

In the previous chapter, results from the EMPA-KIDNEY bioimpedance substudy were reported, demonstrating that empagliflozin brings about sustained reduction in levels of excess fluid in patients with chronic kidney disease (CKD). In this chapter, tertiary assessments of the bioimpedance substudy are reported which describe the effects of empagliflozin on body composition as assessed by bioimpedance-derived estimates of lean and fat tissue; as well as body weight, body mass index (BMI) and waist-to-hip ratio. This allows the effects on "Fluid Overload" reported in Chapter 4 to be put into context and assimilated in the final discussion in Chapter 8. This chapter concludes by briefly additionally reporting effects on laboratory parameters of relevance to effects on body composition and fluid status: glycated haemoglobin (HbA1c) and haematocrit.

There are established links between both general and central adiposity and eGFR decline, kidney failure and death in those with and without CKD (Zhu et al., 2021, Herrington et al., 2017, Chang et al., 2019, Elsayed et al., 2008, Vivante et al., 2012, Hsu et al., 2006). These associations are largely mediated by diabetes and hypertension though obesity is independently associated with CKD and Mendelian randomisation analyses support a causal role (Zhu et al., 2021). BMI is routinely used to reflect adiposity however it does not discriminate fat and muscle or lean tissue mass and changes in body composition in disease states such as CKD (Chintam and Chang, 2021). Furthermore, BMI is a measure of general adiposity however it is visceral or central adiposity which more strongly predict adverse outcomes (Postorino et al., 2009, Kramer et al., 2011, Zhu et al., 2021, Elsayed et al., 2008, Chintam and Chang, 2021). Alternative methods for assessing adiposity have been studied specifically in CKD such as waist circumference (Kramer et al., 2011) or waist-to-hip ratio (Elsayed et al., 2008, Zhu et al., 2021) and more recently, parameters derived from bioimpedance analysis or spectroscopy as applied in the EMPA-KIDNEY bioimpedance substudy. The methods and data analysis plan for the bioimpedance substudy are outlined in Chapter 2.

It was previously demonstrated in several trials that sodium-glucose cotransporter 2 (SGLT2) inhibitors reduce body weight although these effects appeared somewhat

attenuated in patients with CKD relative to heart failure and diabetes populations. Furthermore, it was not fully understood whether weight loss was synonymous with fat loss or may reflect loss of fluid volume or even skeletal muscle degradation. The analyses reported in this chapter therefore sought to address these uncertainties.

5.2 RESULTS

5.2.1 EFFECTS OF EMPAGLIFLOZIN ON BIOIMPEDANCE-DERIVED ADIPOSITY PARAMETERS

There were no significant between-group differences in bioimpedance-derived fat or lean tissue index and *post-hoc* analyses assessing effects on adipose, fat and lean tissue mass (from which the indices were derived) demonstrated consistent results (Table 5-1).

5.2.2 POST-HOC ANALYSES OF THE EFFECTS OF EMPAGLIFLOZIN ON EXTRACELLULAR AND INTRACELLULAR RESISTANCE

In *post-hoc* analyses of the effects of empagliflozin on the "raw" bioimpedance measurements of extracellular and intracellular resistance (from which all fluid and adiposity parameters are ultimately derived) there was a highly statistically significant difference in extracellular resistance of 15.65 (95% confidence interval [CI] 8.34, 22.96) ohms but not intracellular resistance (19.90 [95% CI -7.47, 47.26] ohms), averaged across the substudy (Table 5-2).

Table 5-1: Effects of empagliflozin on bioimpedance-derived adiposity parameters

	Empagliflozin (N=311)		Placebo (N=309)			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
LEAN TISSUE INDEX, kg/m^2						
Randomisation	13.22	0.18	12.92	0.16		
2-month follow-up	12.81	0.09	13.00	0.09	-0.20	(-0.46, 0.06)
18-month follow-up	13.02	0.13	13.10	0.13	-0.08	(-0.43, 0.28)
Study average	**12.90**	**0.09**	**13.05**	**0.09**	**-0.14**	**(-0.39, 0.10)**
FAT TISSUE INDEX, kg/m^2						
Randomisation	12.43	0.30	12.48	0.30		
2-month follow-up	12.44	0.10	12.50	0.10	-0.06	(-0.45, 0.30)
18-month follow-up	12.21	0.14	12.30	0.14	-0.10	(-0.66, 0.40)
Study average	**12.34**	**0.10**	**12.42**	**0.10**	**-0.07**	**(-0.48, 0.28)**
LEAN TISSUE MASS, kg						
Randomisation	39.40	0.66	38.24	0.61		
2-month follow-up	38.01	0.29	38.73	0.29	-0.72	(-1.52, 0.09)
18-month follow-up	38.65	0.39	39.05	0.38	-0.40	(-1.47, 0.66)
Study average	**38.28**	**0.27**	**38.87**	**0.27**	**-0.58**	**(-1.34, 0.18)**
FAT TISSUE MASS, kg						
Randomisation	36.30	0.88	36.11	0.81		
2-month follow-up	36.19	0.30	36.38	0.30	-0.18	(-1.01, 0.64)
18-month follow-up	35.52	0.42	35.76	0.41	-0.24	(-1.39, 0.91)
Study average	**35.90**	**0.30**	**36.11**	**0.30**	**-0.21**	**(-1.04, 0.62)**
ADIPOSE TISSUE MASS, kg						
Randomisation	49.39	1.19	49.13	1.10		
2-month follow-up	49.24	0.40	49.49	0.40	-0.25	(-1.37, 0.87)
18-month follow-up	48.33	0.57	48.66	0.56	-0.32	(-1.89, 1.24)
Study average	**48.85**	**0.41**	**49.13**	**0.41**	**-0.28**	**(-1.41, 0.85)**

The pre-specified analysis parameters lean tissue index and fat tissue index in kg/m^2 are calculated from lean tissue mass and adipose tissue mass in kg indexed to height squared. Adipose tissue mass consists of the fat tissue mass plus proteins, minerals and fluid. Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) between treatment groups and weighted in proportion to the amount of follow-up time represented.

Table 5-2: Effects of empagliflozin on extracellular and intracellular resistance

	Empagliflozin (N=311)		Placebo (N=309)			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
EXTRACELLULAR RESISTANCE, Ω						
Randomisation	571.78	5.60	590.08	5.70		
2-month follow-up	599.29	2.78	583.12	2.79	16.17	(8.40, 23.94)
18-month follow-up	601.49	3.87	586.52	3.82	14.97	(4.27, 25.67)
Study average	**600.24**	**2.62**	**584.59**	**2.62**	**15.65**	**(8.34, 22.96)**
INTRACELLULAR RESISTANCE, Ω						
Randomisation	1489.80	21.19	1530.51	21.40		
2-month follow-up	1547.51	10.35	1521.23	10.40	26.28	(-2.64, 55.20)
18-month follow-up	1531.34	14.39	1519.87	14.18	11.46	(-28.26, 51.19)
Study average	**1540.54**	**9.83**	**1520.64**	**9.80**	**19.90**	**(-7.47, 47.26)**

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR and uACR) and weighted in proportion to the amount of follow-up time represented.

5.2.3 EFFECTS OF EMPAGLIFLOZIN ON WEIGHT, BODY MASS INDEX AND WAIST-TO-HIP RATIO IN THE FULL TRIAL COHORT AND BIOIMPEDANCE SUBSTUDY

5.2.3.1 EFFECTS ON WEIGHT

In the bioimpedance substudy cohort, who had a higher baseline weight relative to the full trial cohort, the study average between-group difference in total body weight was -0.7 kg (95% CI -1.3, -0.1; Table 5-3). This finding was consistent with results from the larger full trial cohort: overall mean study average weight was 0.9 kg lower in the empagliflozin group (absolute difference -0.9 kg, 95% CI -1.2, -0.6; heterogeneity P value comparing substudy and full trial populations = 0.60). Furthermore, the effects on weight persisted over time: there was no significant interaction between time and treatment effect on weight (P for interaction with time in the substudy cohort = 0.44; full trial cohort = 0.47).

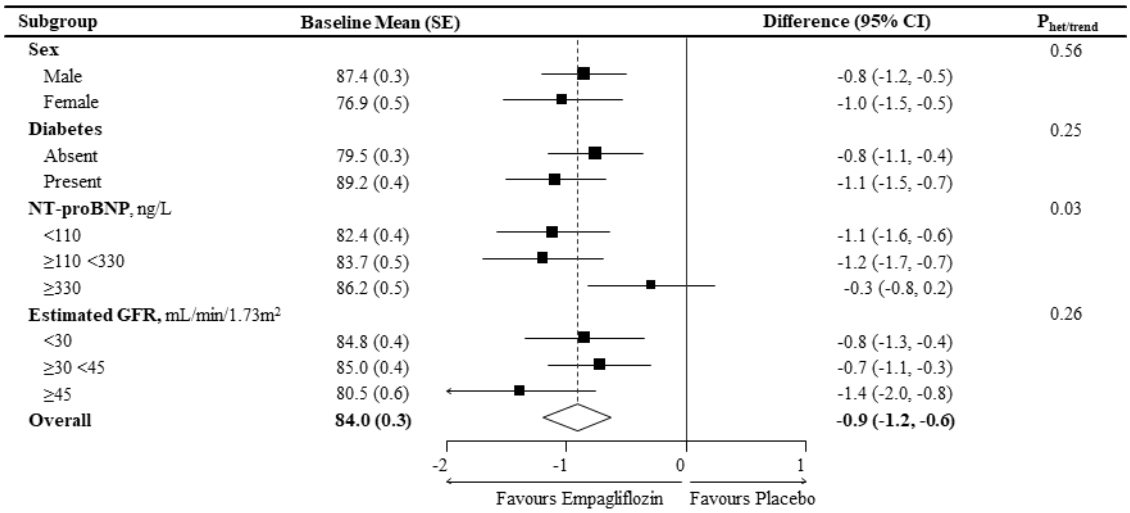
Table 5-3: Effects of empagliflozin on weight (kg)

	Empagliflozin		Placebo			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
Bioimpedance substudy cohort*						
Randomisation	89.0	1.1	88.2	1.1		
2-month follow-up	87.8	0.2	88.7	0.2	-0.9	(-1.4, -0.3)
18-month follow-up	87.4	0.3	88.0	0.3	-0.6	(-1.5, 0.4)
Study average	**87.6**	**0.2**	**88.4**	**0.2**	**-0.7**	**(-1.3, -0.1)**
Full trial cohort†						
Randomisation	84.0	0.4	83.9	0.4		
2-month follow-up	**83.3**	**0.1**	**84.1**	**0.1**	**-0.7**	**(-1.0, -0.5)**
6-month follow-up	82.7	0.1	83.7	0.1	-1.0	(-1.2, -0.7)
12-month follow-up	82.7	0.1	83.6	0.1	-0.9	(-1.2, -0.7)
18-month follow-up	**82.4**	**0.1**	**83.3**	**0.1**	**-0.9**	**(-1.2, -0.5)**
24-month follow-up	82.2	0.1	83.0	0.1	-0.8	(-1.2, -0.4)
30-month follow-up	81.9	0.2	82.9	0.2	-1.0	(-1.5, -0.4)
36-month follow-up	81.4	0.3	82.4	0.3	-1.0	(-1.7, -0.3)
Study average	**82.3**	**0.1**	**83.2**	**0.1**	**-0.9**	**(-1.2, -0.6)**

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups and weighted in proportion to the amount of follow-up time represented. *Analyses in the bioimpedance substudy cohort use the 2 and 18 month time windows pre-specified in the substudy Data Analysis Plan and analyse the 620 individuals included in the MMRM analyses of bioimpedance parameters. †Analyses in the full trial cohort use all available measurements.

Exploration of subgroup effects in the substudy cohort suggested a larger effect on weight in participants with diabetes and at higher eGFR however no strong evidence of heterogeneity was found between participants with or without diabetes in the more highly powered full trial cohort; nor for any other subgroup (Figure 5-1).

Figure 5-1: Effects of empagliflozin on weight (kg) in the full trial cohort by key bioimpedance substudy pre-specified subgroups



Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups and weighted in proportion to the amount of follow-up time represented.

Since study average results from substudy participants used two time windows centred on the 2 and 18 month study visits yet analyses of the full trial cohort used data from all available time points (up to 36 months for some participants), a sensitivity analysis was conducted in the full trial cohort applying the same time windows (2 and 18 months) as were used in the bioimpedance substudy (Figure 5-2). The study average between-group difference in weight in this analysis (-0.8, 95% CI -1.0, -0.5 kg) was very consistent with both the more reliable estimate using all available data in the full trial cohort (-0.9, 95% CI -1.2, -0.6 kg); and with the substudy average (-0.7, 95% CI -1.3, -0.1 kg) confirming that the analysis approach to handling of measurement time did not impact interpretation.

This sensitivity analysis exploring the impact of applying the two time window approach in the full trial cohort was also conducted for the previously selected subgroups (Figure 5-3). This demonstrated broadly consistent effects with greater certainty in the estimates from the full trial cohort (compared with the smaller substudy population); and there was no significant heterogeneity in the effect of empagliflozin on weight in any of these subgroups, consistent with the most reliable analysis using all available data in the full trial cohort as shown in the earlier Figure 5-1.

Figure 5-2: Effects of empagliflozin on weight over time in (i) the substudy population; (ii) the full trial cohort applying two time windows (as per substudy approach); and (iii) the full trial cohort using all available data

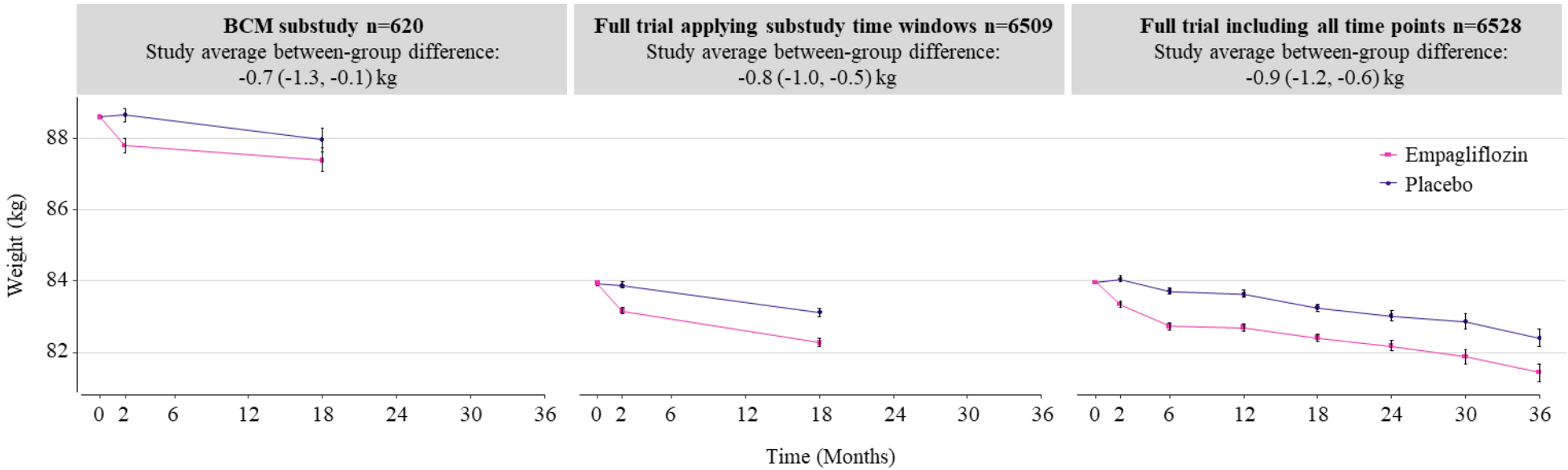
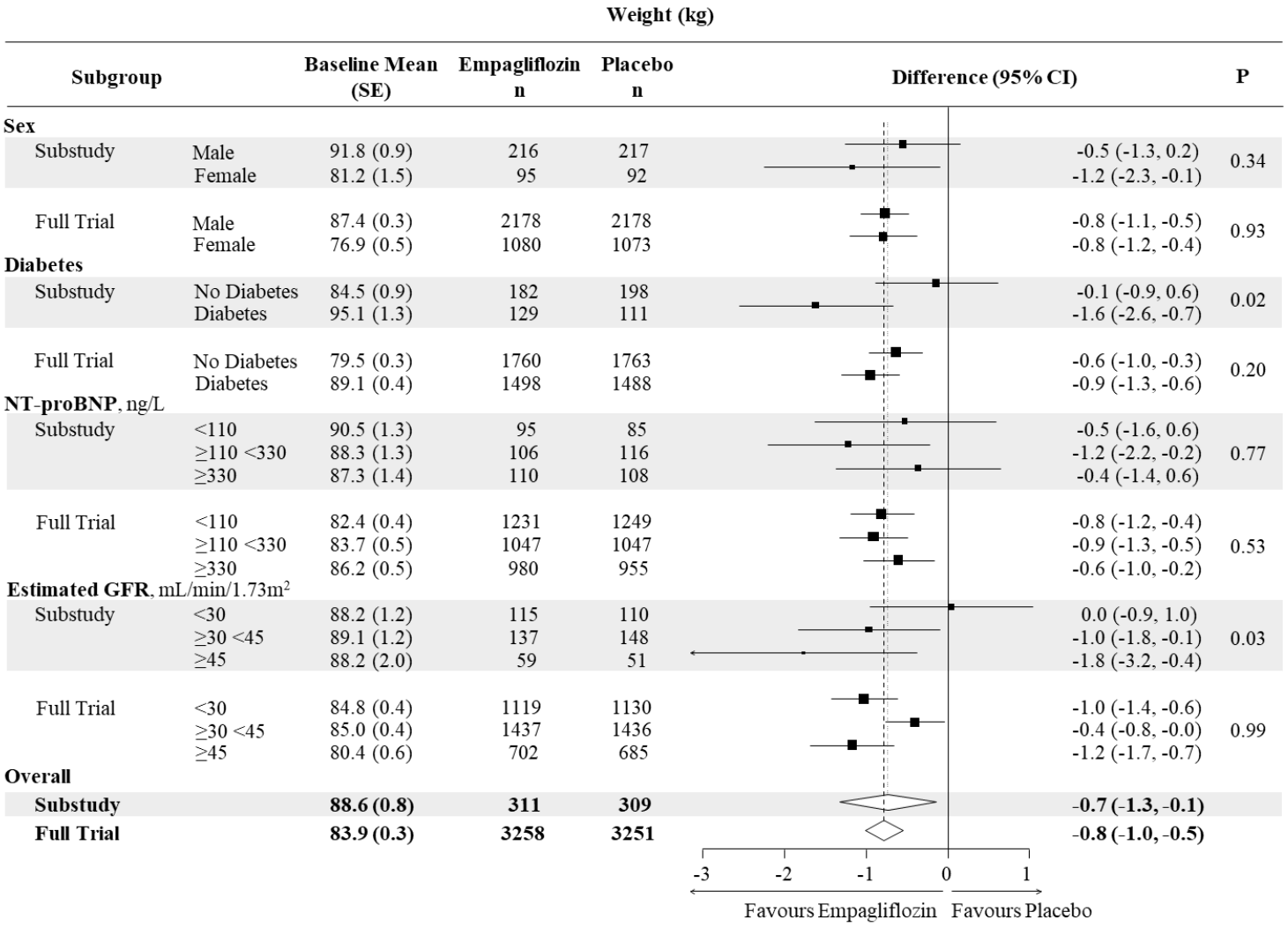


Figure 5-3: Sensitivity analysis applying two time window approach in subgroup analyses of effects on weight in the (i) substudy and (ii) full trial cohorts



5.2.3.2 EFFECTS ON BODY MASS INDEX AND WAIST-TO-HIP RATIO

Weight loss was also reflected in minor differences in BMI between treatment groups (Table 5-4). Waist-to-hip ratio at 18 months was not significantly different in those who received empagliflozin versus placebo in either the substudy or full trial cohort (Table 5-5).

Table 5-4: Effects of empagliflozin on body mass index (kg/m^2)

	Empagliflozin		Placebo			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
Bioimpedance substudy cohort						
Randomisation	30.2	0.3	30.1	0.4		
2-month follow-up	29.9	0.1	30.1	0.1	-0.3	(-0.5, -0.1)
18-month follow-up	29.7	0.1	29.9	0.1	-0.2	(-0.5, 0.1)
Study average	**29.8**	**0.1**	**30.0**	**0.1**	**-0.3**	**(-0.5, -0.1)**
Full trial cohort						
Randomisation	29.7	0.1	29.8	0.1		
2-month follow-up	**29.5**	**0.0**	**29.8**	**0.0**	**-0.3**	**(-0.4, -0.2)**
6-month follow-up	29.3	0.0	29.7	0.0	-0.4	(-0.5, -0.3)
12-month follow-up	29.3	0.0	29.6	0.0	-0.3	(-0.4, -0.2)
18-month follow-up	**29.2**	**0.0**	**29.5**	**0.0**	**-0.3**	**(-0.4, -0.2)**
24-month follow-up	29.1	0.1	29.4	0.1	-0.3	(-0.5, -0.2)
30-month follow-up	29.0	0.1	29.4	0.1	-0.4	(-0.6, -0.2)
36-month follow-up	28.8	0.1	29.2	0.1	-0.4	(-0.6, -0.1)
Study average	**29.1**	**0.0**	**29.5**	**0.0**	**-0.3**	**(-0.4, -0.2)**

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups and weighted in proportion to the amount of follow-up time represented.

Table 5-5: Effects of empagliflozin on waist-to-hip ratio

	Empagliflozin		Placebo			
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**
WAIST CIRCUMFERENCE, cm						
Bioimpedance substudy cohort						
Randomisation	105.2	(0.8)	105.5	(0.8)		
18-month follow-up	106.1	(0.5)	105.5	(0.4)	0.6	(-0.7, 1.8)
Full trial cohort						
Randomisation	102.8	(0.2)	102.7	(0.2)		
18-month follow-up	102.2	(0.2)	102.9	(0.2)	-0.8	(-1.2, -0.3)
HIP CIRCUMFERENCE, cm						
Bioimpedance substudy cohort						
Randomisation	109.3	(0.7)	109.4	(0.7)		
18-month follow-up	109.6	(0.4)	109.1	(0.4)	0.4	(-0.6, 1.5)
Full trial cohort						
Randomisation	107.3	(0.2)	107.2	(0.2)		
18-month follow-up	106.4	(0.2)	107.0	(0.2)	-0.6	(-1.1, -0.1)
WAIST-TO-HIP RATIO						
Bioimpedance substudy cohort						
Randomisation	0.96	(0.01)	0.96	(0.01)		
18-month follow-up	0.97	(0.00)	0.97	(0.01)	0.00	(-0.02, 0.02)
Full trial cohort						
Randomisation	0.96	(0.00)	0.96	(0.00)		
18-month follow-up	0.96	(0.00)	0.97	(0.00)	-0.00	(-0.01, 0.00)

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region). Waist, hip and the associated ratio measures were analysed at a single follow-up time point and are therefore analysed by analysis of covariance (ANCOVA). Full trial cohort analyses include all 6609 participants; missing measurements were handled by mean imputation for baseline and multiple imputation for follow-up measurements. Substudy cohort analyses include the 620 individuals included in the MMRM analyses of bioimpedance parameters, all of whom had a baseline waist-to-hip measurement; missing follow-up measurements were imputed following the same procedure for the full trial cohort.

5.2.4 EFFECTS OF EMPAGLIFLOZIN ON RELATED BIOCHEMICAL PARAMETERS IN THE FULL TRIAL COHORT AND BIOIMPEDANCE SUBSTUDY

5.2.4.1 EFFECTS OF EMPAGLIFLOZIN ON GLYCATED HAEMOGLOBIN

Since analyses of effects of empagliflozin on anthropometry demonstrated the ability to produce more reliable estimates using larger participant numbers in the full trial cohort, the same approach was followed for related laboratory parameters and results from the full trial cohort are emphasised first and substudy results are then related to these. The study-average difference in HbA1c in the full trial cohort was -0.4 mmol/mol (95% CI -0.8, -0.0; Table 5-6). There was some evidence that effects of empagliflozin appeared to differ according to whether or not participants had diabetes at baseline (heterogeneity P = 0.03); the between-group difference in HbA1c was -0.9 mmol/mol (95% CI -1.6, -0.1) in participants with diabetes at randomisation with no HbA1c difference between allocated treatment groups among participants without diabetes at baseline (0.0 mmol/mol, 95% CI -0.2, 0.2; Table 5-6). Such small effects on HbA1c were not detectable in the smaller substudy population (Table 5-6).

Table 5-6: Effects of empagliflozin on HbA1c (mmol/mol)

		Empagliflozin		**Placebo**				
	Participants analysed	**Mean**	**SE**	**Mean**	**SE**	**Absolute Difference (95% CI)**		**P_{het}**
Full trial cohort								
Prior diabetes	2914	53.4	0.3	54.3	0.3	-0.9	(-1.6, -0.1)	0.03
No prior diabetes	3444	36.9	0.1	36.9	0.1	0.0	(-0.2, 0.2)	
All participants	**6358**	**44.5**	**0.1**	**44.9**	**0.1**	**-0.4**	**(-0.8, -0.0)**	
Bioimpedance substudy cohort								
Prior diabetes	240	53.8	0.6	53.8	0.6	0.0	(-1.6, 1.6)	0.72
No prior diabetes	379	37.1	0.2	36.8	0.1	0.3	(-0.2, 0.7)	
All participants	**619**	**43.5**	**0.2**	**43.3**	**0.2**	**0.2**	**(-0.5, 0.9)**	

Study averages are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups. Analyses use central laboratory samples from randomisation, 2-, 18-, 24- and 30-month follow-up visits, weighted in proportion to the amount of follow-up time represented.

5.2.4.2 EFFECTS OF EMPAGLIFLOZIN ON HAEMATOCRIT

An increase in haematocrit was observed in the empagliflozin group relative to placebo: the full trial cohort average between-group difference in haematocrit at 18 months post-randomisation was 2.3% (95% CI 1.9, 2.7) and results in the substudy population were consistent (Table 5-7).

In the empagliflozin arm, change in haematocrit (randomisation to 18 months) in the substudy cohort correlated with change in absolute "Fluid Overload" in the 67 participants with such measurements (Spearman's correlation -0.3).

Table 5-7: Effects of empagliflozin on haematocrit (%)

		Empagliflozin		Placebo			
	Participants analysed	**Mean**	**SE**	**Mean**	**SE**	**Absolute Difference (95% CI)**	
Full trial cohort	1368	40.7	0.1	38.4	0.1	2.3	(1.9, 2.7)
Bioimpedance substudy cohort	196	41.7	0.3	39.1	0.3	2.5	(1.7, 3.4)

Study averages are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups. Haematocrit was only assessed in a ~20% subset of the full trial cohort using local laboratory measurements at randomisation and 18 months using analysis of covariance (ANCOVA) and excludes those with missing baseline measurements. Haematocrit is analysed in the 196 of the 620 bioimpedance substudy cohort with an 18-month measurement with mean imputation of missing baseline measurements for consistency with the substudy analysis approach.

5.3 DISCUSSION

Empagliflozin had no significant effect on adiposity in the substudy population. This is in contrast to other small trial substudies of SGLT2 inhibitors which used dual energy x-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI). These results should be interpreted in the context of population characteristics – existing body composition substudies are limited to participants with type 2 diabetes with largely normal kidney function (Bolinder et al., 2014, Ridderstråle et al., 2014, Sasaki et al., 2019). Although not previously used in SGLT2 inhibitor trials, bioimpedance has been used to assess body composition changes associated with SGLT2 inhibitor treatment in small observational studies, again only in participants with diabetes (Kurinami et al., 2018, Schork et al., 2019, Ohara et al., 2020) and fat loss was greater in participants with higher baseline HbA1c (Kurinami et al., 2018). In EMPA-KIDNEY, 37% of bioimpedance substudy participants had diabetes with a mean baseline glycated haemoglobin of 43.7 mmol/mol. Coupled with reduced levels of glomerular filtration (substudy mean baseline eGFR 36 mL/min/1.73m^2), filtered glucose load can be expected to be low, therefore limiting caloric loss associated with SGLT2 inhibitor treatment in this population.

Empagliflozin lowered body weight by ~1 kg which was reflected in a minor difference between the groups in BMI but no meaningful effect on waist-to-hip ratio. Effects on weight were consistent across subgroups analysed and did not importantly differ when different approaches to handling measurement time were applied in analyses. Effects on weight in EMPA-KIDNEY are remarkably consistent with the CREDENCE trial in a CKD population with diabetes (Ye et al., 2021) but somewhat smaller than pooled estimates of effects on weight from heart failure trials (Li et al., 2022). The differences observed in HbA1c between the empagliflozin and placebo groups in EMPA-KIDNEY were small (and not significant in participants without diabetes) and are insufficient to explain the observed ~1 kg weight loss caused by SGLT2 inhibitors. Furthermore, since there was no significant observable effect on lean or fat tissue parameters in this EMPA-KIDNEY bioimpedance substudy and an approximate 0.8 L between-group difference in total body water, this suggests that weight loss was almost entirely the result of fluid loss (and not fat loss) in the EMPA-KIDNEY substudy.

To further explore these findings in EMPA-KIDNEY, *post-hoc* analyses were conducted firstly assessing effects on lean, adipose and fat tissue mass (from which the indices are derived) and secondly on the "raw" bioimpedance measurements of extracellular and intracellular resistance (from which all fluid and adiposity parameters are ultimately derived). This analysis sought to test for a "true" between-group difference in the measured (rather than derived) parameters. A statistically significant effect was observed on extracellular but not intracellular resistance, in keeping with effects on the derived parameters and also with the derivation methods outlined in section 2.3.8 since bioimpedance-derived "Fluid Overload" reflects excess extracellular water and fat/lean tissue is intracellular. It is relevant that the effect of empagliflozin on intracellular water (reported in section 4.2.6) was only nominally significant and reductions in body water were largely extracellular. These *post-hoc* analyses therefore provide reassurance that the analysis parameters are reliable.

Supplementary analyses of the available laboratory parameters HbA1c and haematocrit in EMPA-KIDNEY further corroborate bioimpedance analyses. Empagliflozin had a negligible effect on HbA1c, consistent with the lack of effect on fat mass. Haematocrit was

significantly higher in participants receiving empagliflozin, compared with placebo. This observation is directionally consistent with the diuretic phenomenon however is likely to more closely reflect plasma volume rather than interstitial fluid dynamics which SGLT2 inhibitors are known to alter to a greater extent (Hallow et al., 2018). The more likely conclusion is that a rise in haematocrit reflects the erythropoiesis-stimulating effects of SGLT2 inhibitors and is not exclusively related to diuretic effects (Inzucchi et al., 2018, Zannad et al., 2022).

The substudy has certain limitations. Firstly, analysed bioimpedance parameters are derived (from extracellular and intracellular resistance values) and are not direct measurements therefore it is not possible to precisely quantify differences in body composition however *post-hoc* analyses of extracellular and intracellular resistance corroborate findings. Furthermore, derivation is based on formulae normalised to healthy reference populations, not kidney disease populations, however the device has been extensively validated (for fluid assessment) in kidney failure cohorts. Bioimpedance devices (and particularly the trial's selected device) are primarily used for fluid assessment and much less commonly to quantify body composition in people with kidney disease and the substudy was not powered for adiposity assessments. Secondly, some scheduled bioimpedance measurements were missed largely due to the impact of the COVID-19 pandemic necessitating remote follow-up. Thirdly, the substudy was only conducted in the UK and Germany and is therefore largely restricted to white individuals. Lastly, the substudy was not primarily powered to assess modest effects on adiposity, and so an effect on adiposity cannot be ruled out.

In summary, empagliflozin lowered body weight (by around 1 kg) but had no significant effect on fat mass or lean tissue mass in the EMPA-KIDNEY trial population with CKD, suggesting that weight loss is largely accounted for by fluid loss in CKD as reported in Chapter 4. The relationship between findings reported in Chapter 4 (effects on bioimpedance-derived "Fluid Overload") and Chapter 5 (effects of empagliflozin on weight and bioimpedance fat and lean tissue parameters) are further consolidated in the final discussion in Chapter 8.

CHAPTER 6 – EFFECTS OF EMPAGLIFLOZIN ON BLOOD PRESSURE

6.1 INTRODUCTION

High blood pressure is both a cause and consequence of chronic kidney disease (CKD) and is associated with increased cardiovascular morbidity and mortality. Sodium-glucose cotransporter 2 (SGLT2) inhibitors have consistently been shown, in large randomised controlled trials, to have modest blood pressure lowering effects in all studied populations. Reductions in blood pressure are in the order of around 4 mmHg for systolic and 1.5 mmHg for diastolic blood pressure in populations with type 2 diabetes (Mazidi et al., 2017) with reductions similar in magnitude in both heart failure and CKD trials (Beal et al., 2023, Heerspink et al., 2024). Moreover, the effect of SGLT2 inhibitors on blood pressure also appears to be sustained (Heerspink et al., 2024).

In the previous chapters, the effects of empagliflozin on bioimpedance-derived estimates of body water and body fat were reported in the context of the effects of empagliflozin on total body weight. Subgroup analyses assessed for heterogeneity of treatment effects on "Fluid Overload" and weight according to the same participant characteristics (sex, diabetes, estimated glomerular filtration rate [eGFR] and N-terminal pro B-type natriuretic peptide [NT-proBNP]) to allow comparison. In this chapter, the effects of empagliflozin on blood pressure are reported, since the antihypertensive effects of SGLT2 inhibitors are thought to be at least in part mediated by diuretic mechanisms. Furthermore, one of the consequences of fluid overload is worsening hypertension which is a key priority in the management of CKD. Blood pressure analyses were conducted in the full EMPA-KIDNEY trial population to contextualise analyses of the effects of empagliflozin on fluid status (Chapter 4) in larger participant numbers.

6.2 RESULTS

6.2.1 EFFECTS OF EMPAGLIFLOZIN ON SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

Results from the full trial cohort are emphasised (rather than the substudy population) since these are considered more reliable treatment estimates owing to larger numbers of participants and therefore greater statistical power. Results are presented separately for the

bioimpedance substudy cohort to allow interpretation in context of the effects on bioimpedance-derived fluid parameters. The study-average between-group differences in systolic and diastolic blood pressure in the full trial cohort were -2.6 mmHg (95% CI -3.3, -1.9) and -0.5 mmHg (95% CI -0.9, -0.1), respectively (Table 6-1). The effects of study treatment on blood pressure were similar in substudy versus non-substudy participants (systolic blood pressure heterogeneity P value = 0.52; Table 6-1).

Table 6-1: Effects of empagliflozin on systolic and diastolic blood pressure

	Empagliflozin		**Placebo**				
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**	**Interaction with time P**
SYSTOLIC BLOOD PRESSURE, mmHg							
Full trial cohort							<0.001
Randomisation	136.4	0.3	136.7	0.3			
2-month follow-up	**131.8**	**0.3**	**135.8**	**0.3**	**-4.0**	**(-4.7, -3.2)**	
6-month follow-up	131.7	0.3	134.7	0.3	-3.0	(-3.8, -2.2)	
12-month follow-up	133.3	0.3	136.3	0.3	-3.0	(-3.8, -2.2)	
18-month follow-up	**133.0**	**0.3**	**135.3**	**0.3**	**-2.3**	**(-3.2, -1.4)**	
24-month follow-up	133.8	0.4	135.3	0.4	-1.6	(-2.6, -0.5)	
30-month follow-up	132.5	0.5	135.4	0.5	-2.9	(-4.1, -1.6)	
36-month follow-up	132.7	0.9	134.5	0.9	-1.8	(-4.3, 0.6)	
Study average	**132.7**	**0.2**	**135.3**	**0.2**	**-2.6**	**(-3.3, -1.9)**	
Bioimpedance substudy cohort							0.029
Randomisation	137.0	1.1	137.3	1.1			
2-month follow-up	132.0	0.9	136.3	0.9	-4.3	(-6.7, -1.9)	
18-month follow-up	132.2	1.1	134.2	1.1	-2.0	(-4.9, 0.9)	
Study average	**132.1**	**0.8**	**135.4**	**0.8**	**-3.3**	**(-5.5, -1.2)**	
DIASTOLIC BLOOD PRESSURE, mmHg							
Full trial cohort							0.004
Randomisation	78.1	0.2	78.1	0.2			
2-month follow-up	**76.3**	**0.2**	**77.4**	**0.2**	**-1.1**	**(-1.6, -0.7)**	
6-month follow-up	76.4	0.2	77.2	0.2	-0.8	(-1.3, -0.3)	
12-month follow-up	76.8	0.2	77.3	0.2	-0.5	(-1.0, -0.1)	
18-month follow-up	**76.6**	**0.2**	**77.0**	**0.2**	**-0.4**	**(-0.9, 0.1)**	
24-month follow-up	76.8	0.2	76.3	0.2	0.4	(-0.2, 1.1)	
30-month follow-up	76.1	0.3	76.5	0.3	-0.3	(-1.1, 0.4)	
36-month follow-up	75.5	0.5	76.4	0.5	-0.9	(-2.3, 0.5)	
Study average	**76.3**	**0.1**	**76.8**	**0.1**	**-0.5**	**(-0.9, -0.1)**	
Bioimpedance substudy cohort							0.14
Randomisation	77.9	0.7	78.8	0.7			
2-month follow-up	77.3	0.5	77.8	0.5	-0.5	(-1.9, 0.9)	
18-month follow-up	77.7	0.6	77.5	0.6	0.2	(-1.5, 1.9)	
Study average	**77.5**	**0.4**	**77.7**	**0.4**	**-0.2**	**(-1.4, 1.0)**	

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups and weighted in proportion to the amount of follow-up time represented. The P values for the interaction with time are extracted from likelihood ratio tests comparing models with and without an interaction term testing for significant interaction between treatment allocation and time (using all available time points).

In the full trial cohort, there was no evidence of heterogeneity of the effect of empagliflozin on systolic blood pressure when subdivided by sex, baseline eGFR, NT-proBNP (Figure 6-1) but there was some evidence to suggest greater antihypertensive effects in patients with diabetes (-3.8 [-4.7, -2.8] versus -1.5 [-2.5, -0.6] mmHg; heterogeneity P value = 0.001; Figure 6-1).

Effects on systolic blood pressure did not differ according to baseline albuminuria category, blood pressure at baseline nor baseline body weight however there was some evidence to suggest that antihypertensive effects of empagliflozin appear larger in combination with RAS inhibitor therapy (heterogeneity P value = 0.02; Figure 6-2).

Figure 6-1: Effects of empagliflozin on systolic blood pressure in the full trial cohort by key bioimpedance substudy pre-specified subgroups

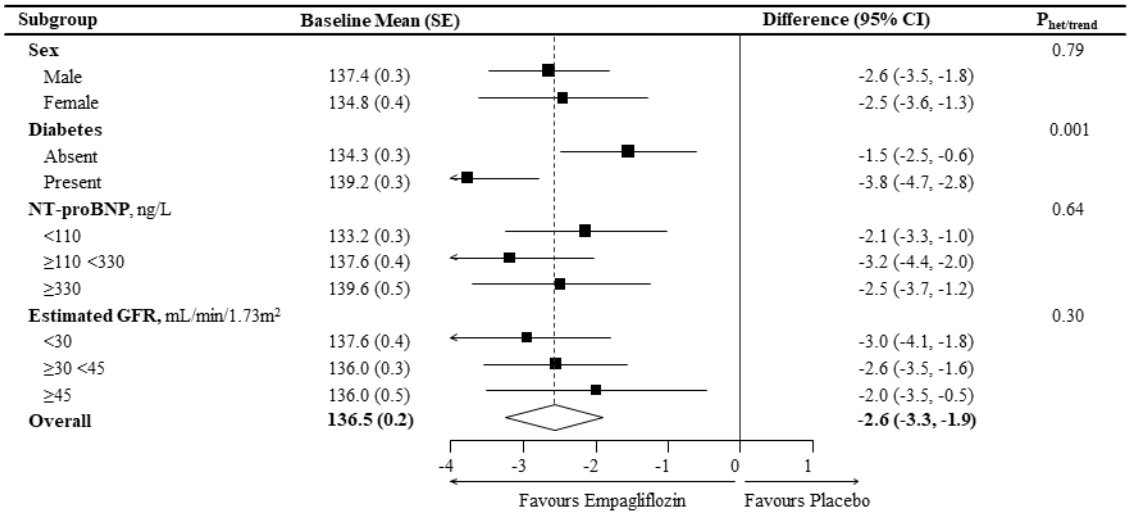
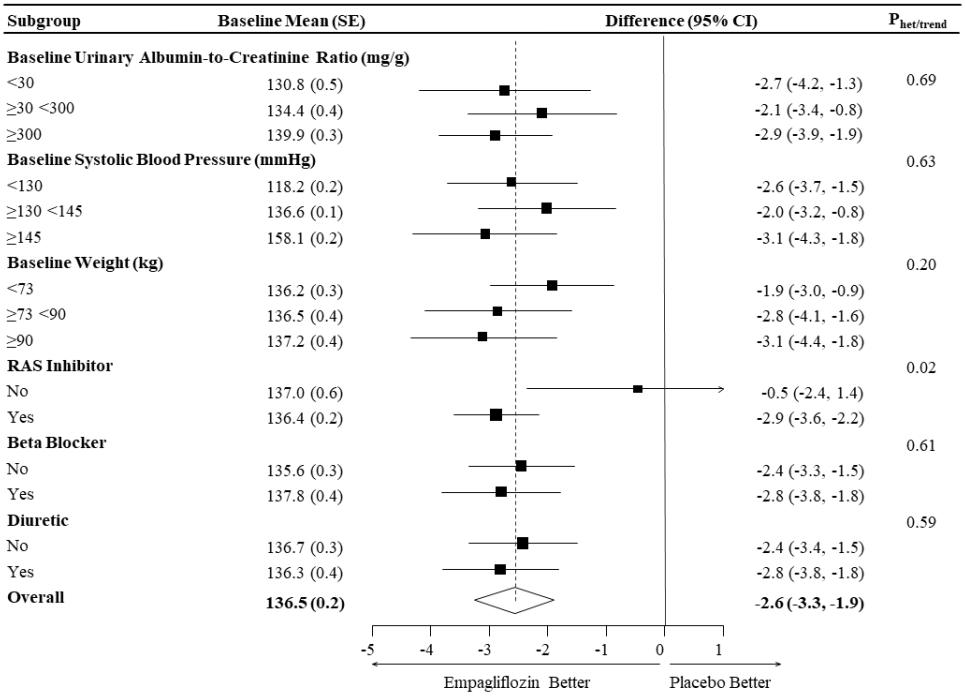


Figure 6-2: Effects of empagliflozin on systolic blood pressure in the full trial cohort by additional post-hoc subgroups



Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and, in the full trial cohort, region) between treatment groups and weighted in proportion to the amount of follow-up time represented. Participants with missing baseline weight are included in the median category.

Since study average results from substudy participants used two time windows centred on the 2 and 18 month study visits yet analyses of the full trial cohort used data from all available time points (up to 36 months for some participants), a sensitivity analysis was conducted assessing the effect of empagliflozin on systolic blood pressure in the full trial cohort applying the same time windows (2 and 18 months) as were used in the bioimpedance substudy. The study average between-group difference in systolic blood pressure in this analysis (-3.2, 95% CI -3.9, -2.6 mmHg) was very consistent with both the more reliable estimate using all available data in the full trial cohort (-2.6, 95% CI -3.3, -1.9 mmHg); and with the substudy average (-3.3, 95% CI -5.5, -1.2 mmHg) confirming that the analysis approach to handling of measurement time did not impact interpretation.

This sensitivity analysis exploring the impact of applying the two time window approach in the full trial cohort was also conducted for the previously selected subgroups. This demonstrated broadly consistent effects with greater certainty in the estimates from the full trial cohort (compared with the smaller substudy population); and there was no significant heterogeneity in the effect of empagliflozin on systolic blood pressure in any of these subgroups with the exception of the diabetes subgroup, consistent with the most reliable analysis using all available data in the full trial cohort as shown in the earlier Figure 6-1. This analysis is presented for systolic blood pressure only for simplicity of presentation though patterns were consistent in analysis of diastolic blood pressure.

The effects of empagliflozin on blood pressure differed across time with more pronounced antihypertensive effects evident at the early 2-month follow-up time point (P value for interaction between treatment effect and time in the full trial cohort <0.001 for systolic and = 0.004 for diastolic blood pressure; Table 6-1 & Figure 6-3).

Figure 6-3: Effects of empagliflozin on systolic and diastolic blood pressure over time in (i) the substudy population; (ii) the full trial cohort applying two time windows (as per substudy approach); and (iii) the full trial cohort using all available data

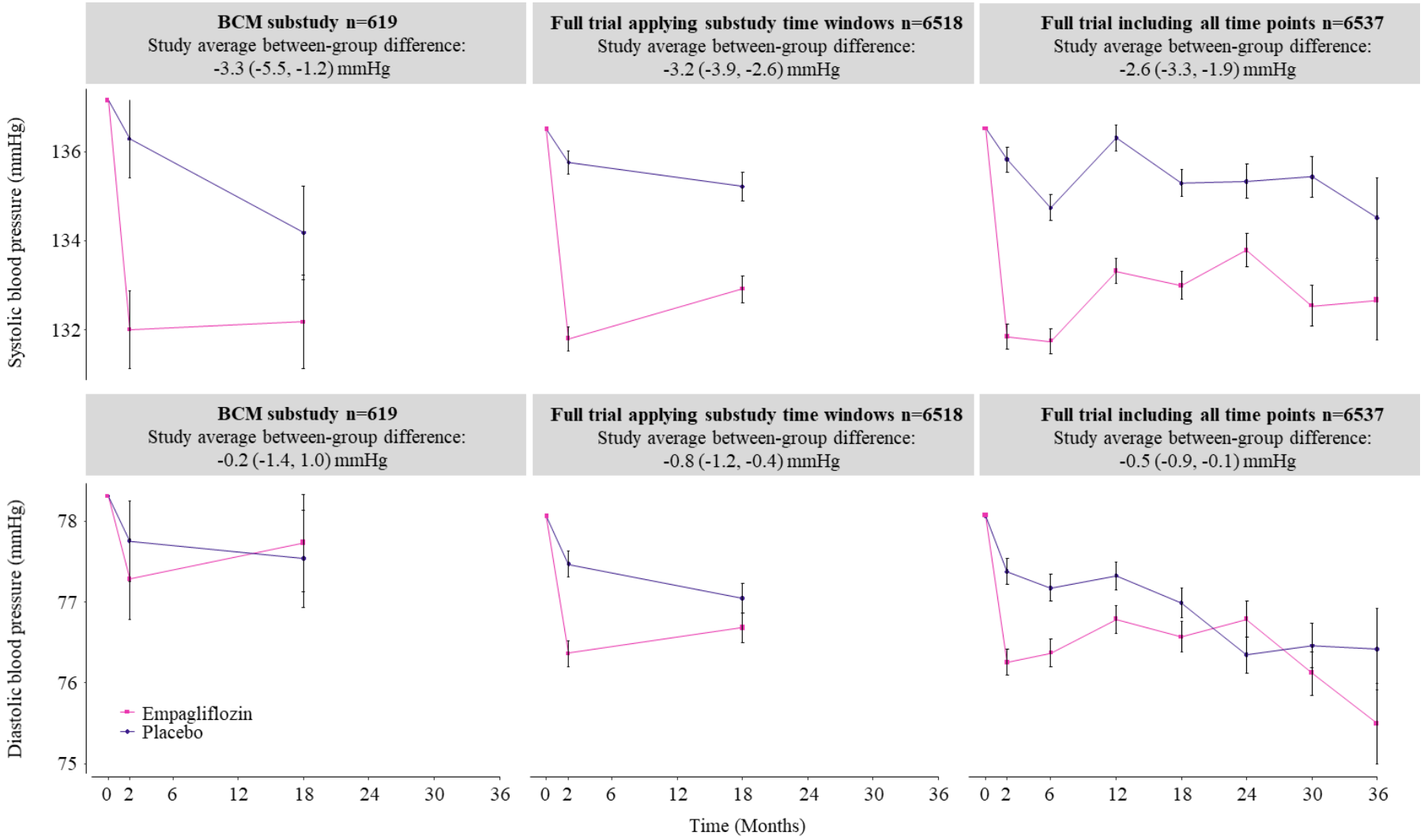
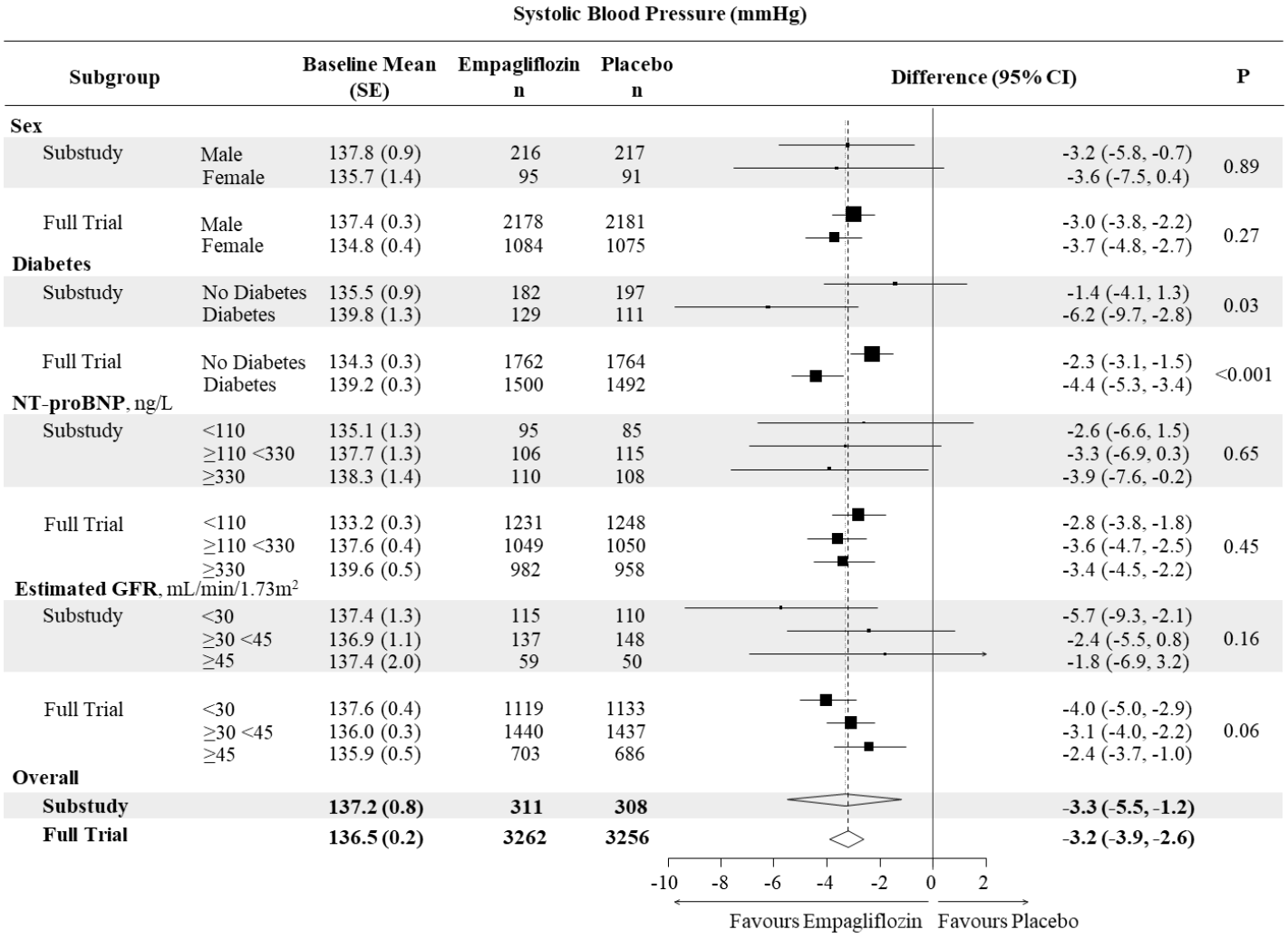


Figure 6-4: Sensitivity analysis applying two time window approach in subgroup analyses of effects on systolic blood pressure in the (i) substudy and (ii) full trial cohorts



Shown for systolic blood pressure only (and not diastolic) for simplicity of presentation; findings were consistent.

6.2.2 EFFECTS OF EMPAGLIFLOZIN ON PULSE PRESSURE AND MEAN ARTERIAL PRESSURE

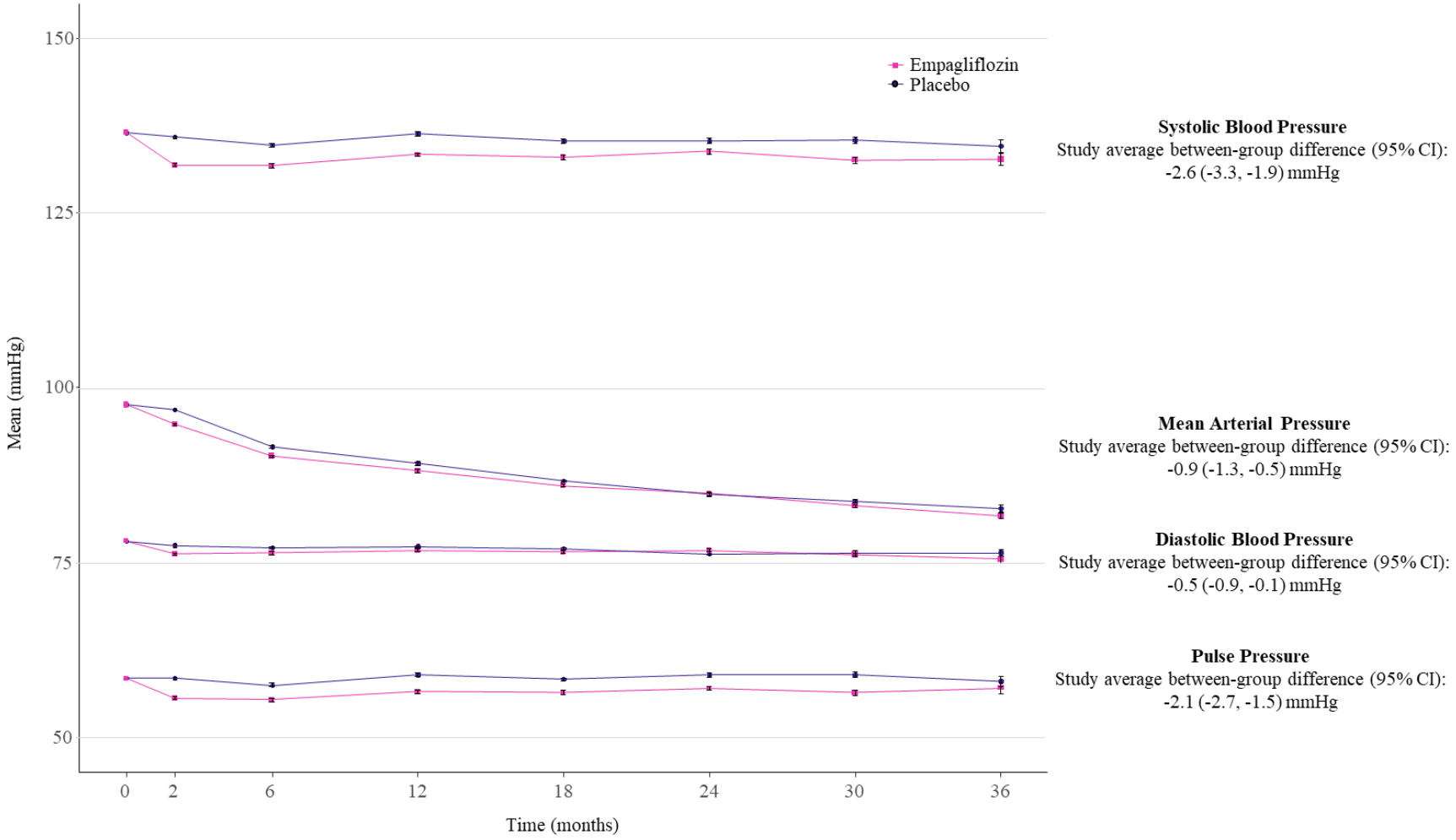
The effects of empagliflozin on blood pressure were also expressed in terms of effects on pulse pressure and mean arterial pressure (*post-hoc*), as well as systolic and diastolic blood pressure, for the full trial cohort. These were not reported for the smaller substudy cohort since the overall effect on diastolic blood pressure in the substudy was not statistically significant. Consistent with the effective reductions in systolic and diastolic blood pressure, both pulse pressure and mean arterial pressure were significantly lower in participants in the empagliflozin group versus placebo averaged across the study period. The study average between-group difference (95% CI) was -2.1 (-2.7, -1.5) mmHg for pulse pressure and -0.9 (-1.3, -0.5) mmHg for mean arterial pressure in the full trial cohort with available blood pressure measurements (Table 6-2).

Table 6-2: Effects of empagliflozin on pulse pressure and mean arterial pressure in the full trial cohort

	Empagliflozin		**Placebo**				
	Mean	**SE**	**Mean**	**SE**	**Absolute Difference**	**95% CI**	**Interaction with time P**
PULSE PRESSURE, mmHg							0.10
Randomisation	58.3	0.3	58.6	0.3			
2-month follow-up	55.6	0.2	58.4	0.2	-2.9	(-3.5, -2.2)	
6-month follow-up	55.4	0.2	57.6	0.2	-2.2	(-2.8, -1.5)	
12-month follow-up	56.5	0.2	59.0	0.2	-2.5	(-3.1, -1.8)	
18-month follow-up	56.4	0.3	58.3	0.3	-1.9	(-2.6, -1.2)	
24-month follow-up	57.0	0.3	59.0	0.3	-2.0	(-2.8, -1.1)	
30-month follow-up	56.4	0.4	59.0	0.4	-2.6	(-3.6, -1.5)	
36-month follow-up	57.1	0.8	58.1	0.8	-1.0	(-3.1, 1.1)	
Study average	**56.4**	**0.2**	**58.5**	**0.2**	**-2.1**	**(-2.7, -1.5)**	
MEAN ARTERIAL PRESSURE, mmHg							<0.001
Randomisation	97.5	0.2	97.6	0.2			
2-month follow-up	94.8	0.2	96.9	0.2	-2.1	(-2.6, -1.6)	
6-month follow-up	90.2	0.2	91.6	0.2	-1.3	(-1.8, -0.8)	
12-month follow-up	88.1	0.2	89.1	0.2	-1.0	(-1.5, -0.5)	
18-month follow-up	86.0	0.2	86.7	0.2	-0.7	(-1.3, -0.2)	
24-month follow-up	84.9	0.2	84.7	0.2	0.2	(-0.5, 0.8)	
30-month follow-up	83.1	0.3	83.7	0.3	-0.6	(-1.4, 0.1)	
36-month follow-up	81.7	0.5	82.8	0.5	-1.0	(-2.5, 0.4)	
Study average	**86.5**	**0.1**	**87.4**	**0.1**	**-0.9**	**(-1.3, -0.5)**	

Mean effects are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, eGFR, uACR and region) between treatment groups and weighted in proportion to the amount of follow-up time represented. The P values for the interaction with time are extracted from likelihood ratio tests comparing models with and without an interaction term testing for significant interaction between treatment allocation and time (using all available time points).

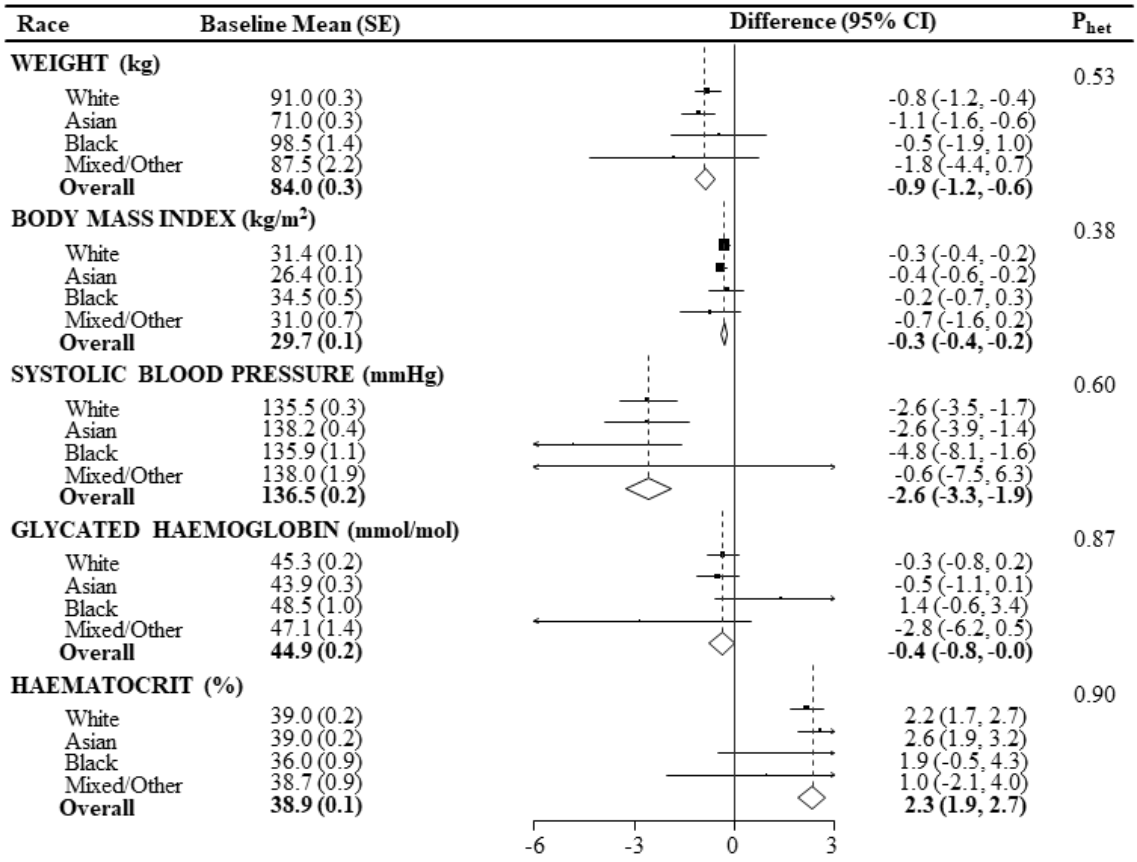
Figure 6-5: Effects of empagliflozin over time on systolic blood pressure, pulse pressure, diastolic blood pressure and mean arterial pressure in the full trial cohort



6.2.3 EFFECTS OF EMPAGLIFLOZIN ON ANTHROPOMETRY, BLOOD PRESSURE AND LABORATORY PARAMETERS BY RACE

This analysis was conducted in an attempt to address the limitation that the substudy population was restricted to largely white participants since it was conducted in the UK and Germany only. Effects on non-bioimpedance parameters were stratified by race in the full trial cohort to assess for any evidence of heterogeneity. Treatment effects on weight, body mass index (BMI), systolic blood pressure, glycated haemoglobin (HbA1c) and haematocrit were all consistent irrespective of race (heterogeneity P >0.3 for all analyses; Figure 6-6).

Figure 6-6: Effects of empagliflozin in the full trial cohort by race



6.3 DISCUSSION

In EMPA-KIDNEY, empagliflozin modestly lowered blood pressure (by 2.6 mmHg systolic and 0.5 mmHg diastolic) irrespective of baseline kidney function but with larger effects on blood pressure in patients with diabetes. Consistent effects were observed on pulse pressure and mean arterial pressure as could be expected. The overall blood pressure-lowering effects of empagliflozin observed are consistent with other large SGLT2 inhibitor trials demonstrating statistically significant modest effects on systolic blood pressure but negligible effects on diastolic blood pressure (Li et al., 2022, Heerspink et al., 2024). The antihypertensive mechanisms of SGLT2 inhibitors are not fully understood and are thought to include diuretic and natriuretic effects; weight loss and sympathetic nervous system inhibition amongst other mechanisms (van Ruiten et al., 2022, Wilcox, 2020).

In EMPA-KIDNEY, the antihypertensive effects of empagliflozin were maintained at lower levels of kidney function, consistent with existing literature (Cherney et al., 2018, Ye et al., 2021) however differential effects were evident according to diabetes status. This finding has not been reported previously and investigators of the DAPA-CKD and CREDENCE trials concluded that blood pressure-lowering effects were consistent irrespective of glycaemic status in their trial populations (Heerspink et al., 2024, Ye et al., 2021). EMPA-KIDNEY analyses do not adjust for multiple testing and chance findings cannot be excluded; a true difference might be explained by the inclusion of greater numbers of participants without diabetes in EMPA-KIDNEY and as such greater sensitivity to assess such effects. Furthermore, blood pressure-lowering effects were consistent across follow-up in the DAPA-CKD and CREDENCE (Ye et al., 2021) trials whereas in EMPA-KIDNEY analyses, the between-group difference in systolic blood pressure was almost twice as large at the 2-month versus 18-month time point. Taken together, the differential effects by diabetes status and variation over time in antihypertensive effects – patterns which were not observed in analyses of effects on "Fluid Overload" – suggest that antihypertensive actions of SGLT2 inhibitors are somewhat independent of their diuretic mechanisms. These distinct antihypertensive effects could be explained by effects on vascular stiffness or endothelial function (Lytvyn et al., 2017, Lytvyn et al., 2022, Cherney et al., 2014).

In EMPA-KIDNEY, baseline blood pressure did not appear to modify the antihypertensive effects of empagliflozin (when systolic blood pressure was categorised as <130, ≥130 <145 and ≥145 mmHg; Figure 6-2), consistent with analyses of the CREDENCE trial (Ye et al., 2021). There was some evidence suggesting larger reductions in systolic blood pressure were observed in participants prescribed RAS inhibitors at baseline (Figure 6-2). Numbers of participants not receiving these agents were small, limiting power however these assessments were not possible at all in previous SGLT2 inhibitor trials in CKD which mandated RAS inhibitor use. The magnitude of blood pressure-lowering effects of SGLT2 inhibitors is smaller than that of RAS inhibitors (Heran et al., 2008) but since hypertension in CKD is driven by both RAS stimulation and extracellular volume excess, SGLT2 inhibitors may aid management of hypertension in CKD in addition to their kidney protective effects. Antihypertensive effects of SGLT2 inhibitors are also more modest than those of diuretic agents (Chen et al., 2009) however potential synergistic effects (Wilcox, 2020) may benefit patients with treatment-resistant volume-driven hypertension in CKD. These findings have important clinical implications and might inform how clinicians initiate these drugs in combination.

The mechanisms underlying the antihypertensive effects of SGLT2 inhibitors are not fully understood, likely to be multifactorial and likely to differ in people with and without CKD and diabetes (Wilcox, 2020). Of particular relevance to this thesis, the diuretic and natriuretic mechanisms of SGLT2 inhibitors (and the associated reduction in fluid excess demonstrated in Chapter 4) are generally considered one of the most important contributing factors in blood pressure lowering hence exploring effects on blood pressure as supplementary analyses in this thesis. Furthermore, reduction in body weight itself (Chapter 5) is known to bring about blood pressure reduction, perhaps particularly in patients with diabetes in whom fat loss achieved by glycosuria is thought to account for up to 40% of antihypertensive effects (Cefalu et al., 2015). Amongst other purported antihypertensive mechanisms are direct effects on endothelial function and vascular stiffness (Lytvyn et al., 2017, Lytvyn et al., 2022, Cherney et al., 2014). SGLT2 inhibitors also reduce sympathetic nervous activity and so blood pressure reduction is not accompanied by increased heart rate (Wilcox, 2020).

Although clinically important, these antihypertensive effects appear to have only a minor mediating role in end-organ protection. Further analyses from the EMPA-KIDNEY trial estimated that, using the landmark method (adjusting the Cox regression model for 2-month biomarker values), reductions in systolic blood pressure after 2 months' treatment with empagliflozin explained 10% of the treatment effect of empagliflozin on the primary outcome (composite of cardiovascular death or progression of kidney disease). Reductions in diastolic blood pressure explained 4% of the treatment effect. In these analyses, urinary albumin-to-creatinine ratio (uACR) had the greatest mediating effect, explaining 40% of the treatment effect and when systolic and diastolic blood pressure and HbA1c were considered in combination with uACR, they added little with the combined proportion of treatment effect explained being 41%. Similar patterns were seen in assessments of the proportional treatment effect on chronic eGFR slope though only 26% of the treatment effect could be explained by these factors (Staplin et al., 2023). Furthermore, the end-organ protection afforded by SGLT2 inhibitors might itself contribute to long-term blood pressure control in CKD since preserved glomerular filtration results in lower blood pressure (Yu et al., 2020).

Nevertheless, when SGLT2 inhibitors are prescribed for their kidney-protective effects, the additional antihypertensive effect in addition to existing therapy is of clinical benefit. Treatment-resistant hypertension is problematic in CKD, occurring twice as commonly than in the general population with an estimated prevalence of around 40% in CKD with increasing frequency as eGFR declines (Rossignol et al., 2015, Thomas et al., 2016). The associated reduced need to commence additional agents to treat blood pressure (Ye et al., 2021) can be expected to be well-received by patients since polypharmacy adversely impacts quality of life.

These analyses have some limitations. Standardised measurement of blood pressure as recommended in clinical guidelines was not mandated in accordance with the streamlined design and procedures of EMPA-KIDNEY. Some guidance was provided on measurement procedure and research coordinators could use locally available devices whether automatic or manual sphygmomanometers therefore measurement error can be expected however randomisation eliminates the risk of this biasing treatment effect estimates. Secondly, additional *post-hoc* subgroups can be considered hypothesis-generating only especially

since small numbers of participants not taking RAS inhibitors at baseline limits assessment of these exploratory subgroups defined by baseline concomitant medications.

In summary, empagliflozin modestly lowers blood pressure in CKD even at low eGFR but with larger effects in patients with diabetes. The potential reason for this is unknown. The mechanisms by which SGLT2 inhibitors lower blood pressure are multifactorial and the diuretic effects of empagliflozin (reported in Chapter 4) are likely to contribute though cannot entirely be responsible. Blood pressure lowering may be an additional advantage in the treatment of patients with CKD (in whom hypertension is common) though it does not appear to explain the kidney-protective effects of SGLT2 inhibition.

CHAPTER 7 – IMPACT OF FRAILTY, MULTIMORBIDITY, POLYPHARMACY AND HEALTH-RELATED QUALITY OF LIFE ON THE EFFECTS OF EMPAGLIFLOZIN

7.1 INTRODUCTION

The rationale for exploring frailty (and related metrics) in relation to the effects of sodium-glucose cotransporter 2 (SGLT2) inhibitors in chronic kidney disease (CKD) is described in section 1.3. Briefly, uncertainty exists in clinical practice surrounding the benefit-risk profile of disease-modifying drugs in older patients with frailty in whom multimorbidity and polypharmacy are common. Such patients are at increased risk of adverse effects of treatment and as such there may be clinician and/or patient reluctance to prescribe drugs like SGLT2 inhibitors due to perceived altered benefit-risk ratio. Conversely, frail patients may be at high absolute risk of adverse outcomes and consequently may particularly benefit from the effects of SGLT2 inhibition therefore there is a need for reliable evidence to guide treatment decisions. This rationale is supported by evidence in populations with heart failure in whom the absolute benefits of both sacubitril/valsartan (Butt et al., 2022a) and SGLT2 inhibition (Butt et al., 2022b, Butt et al., 2022c) were greatest in those with the highest frailty indices yet patients with frailty are less likely to receive optimal guideline-directed medical therapy for heart failure (Khan et al., 2022).

The definition of frailty and available methods to assess frailty are introduced in section 1.3. The methods of the approach used in this work are reported in full in sections 2.6 and 2.7.

The aim of the analyses reported in this chapter was to use frailty indicators derived in the EMPA-KIDNEY population to assess the benefits and any harms of treatment with empagliflozin in CKD in patients with evidence (or risk) of frailty and by differing levels of multimorbidity, polypharmacy and health-related quality of life (HRQoL) in EMPA-KIDNEY. These analyses are needed to enable practical implementation of current SGLT2 inhibitor guidelines. Additional analyses will also further interrogate the effects of empagliflozin on fluid status, body composition and blood pressure reported in Chapters 4-6 in a specific group of participants who may be particularly vulnerable to diuretic and blood pressure lowering effects.

7.2 RESULTS

7.2.1 DERIVATION OF FRAILTY

Median (Q1-Q3) follow-up was 2.0 (1.5-2.4) years, during which time 1995 participants were hospitalised at least once (960 in the empagliflozin group and 1035 in the placebo group). Median predicted risk of hospitalisation was 27% (Q1-Q3: 18-40%). The strongest predictors of hospitalisation were N-terminal pro B-type natriuretic peptide (NT-proBNP) (baseline median [Q1-Q3] 160 ng/L [69-419], Table 7-1); poor mobility (based on EQ-5D-5L) and the presence of diabetes (Table 7-2). Restricting model development to the placebo group only yielded a very similar final model to the model developed in the full trial population therefore the latter was favoured for use in analyses due to greater statistical power (larger participant and event numbers).

Table 7-1: Univariable associations with hospitalisation: continuous

	Linear terms only					**Addition of quadratic terms**			
	Improvement in fit					**Improvement in fit**			
	ΔAIC[‡]	**LRT statistic**	**LRT P**	**Direct-ion**	**OR (95% CI)[†]**	**ΔAIC[§]**	**LRT statistic**	**LRT P**	**Direct-ion**
Ln NT-proBNP	-230.6	232.6	<0.001	+	1.40 (1.34-1.46)	-1.9	3.9	0.047	+
EQ-5D visual analogue scale score	-87.6	89.6	<0.001	-	0.98 (0.98-0.99)	1.2	0.8	0.360	-
Haemoglobin	-77.5	79.5	<0.001	-	0.98 (0.98-0.99)	-9.9	11.9	0.001	+
eGFR	-51.8	53.8	<0.001	-	0.98 (0.98-0.99)	-15.6	17.6	<0.001	+
Body mass index	-28.2	30.2	<0.001	+	1.02 (1.02-1.03)	2.0	0.0	0.923	-
Pulse pressure	-23.3	25.3	<0.001	+	1.01 (1.01-1.01)	1.8	0.2	0.669	+
Waist:hip ratio	-20.3	22.3	<0.001	+	3.25 (1.99-5.29)	-4.1	6.1	0.013	-
Ln uACR	-16.6	18.6	<0.001	+	1.07 (1.04-1.10)	-15.1	17.1	<0.001	+

[†] Adjusted for age, sex and region; for continuous variables the effect estimate is per one unit increment.[‡] Change in AIC for model including linear term for that predictor versus model fitting age, sex and region only. [§] Change in AIC for model with addition of quadratic term for that predictor versus model containing only linear term (adjusted for age, sex and region. Ln = natural logarithm; AIC = Akaike information criterion; LRT = likelihood ratio test.

Table 7-2: Univariable associations with hospitalisation: categorical & binary variables

	ΔAIC*	LRT statistic	LRT P	OR (95% CI)†
Mobility	-164.1	172.1	<0.001	
No problems				Ref
Slight problems				1.76 (1.52-2.04)
Moderate problems				2.41 (2.03-2.86)
Severe problems				3.09 (2.40-3.99)
Unable to walk about				4.45 (1.97-10.02)
Diabetes	-106.0	110.0	<0.001	
No diabetes				Ref
Without retinopathy				1.54 (1.36-1.73)
With retinopathy				2.40 (2.01-2.86)
Peripheral neuropathy	-83.0	85.0	<0.001	1.86 (1.63-2.12)
Heart failure	-74.9	76.9	<0.001	2.14 (1.81-2.53)
Ischaemic heart disease	-62.5	64.5	<0.001	1.77 (1.54-2.04)
Self-reported ankle swelling	-58.3	60.3	<0.001	1.64 (1.45-1.86)
Peripheral arterial disease	-41.5	43.5	<0.001	1.94 (1.59-2.35)
Atrial fibrillation	-29.6	31.6	<0.001	1.58 (1.35-1.86)
Cerebrovascular disease	-28.2	30.2	<0.001	1.61 (1.36-1.90)
Gout	0.6	1.4	0.240	1.08 (0.95-1.22)

* Improvement relative to model fitting age, sex and region only. † Adjusted for age, sex and region. AIC = Akaike information criterion; LRT = likelihood ratio test.

Variables which were significantly associated with hospitalisation in univariable models were included in multivariable model building and the final multivariable model contained 8 variables (in addition to age, sex and region) as presented in Table 7-3.

Table 7-3: Incremental impact of each variable in the final multivariable model

Model	AIC	ΔAIC	LRT statistic	LRT P value
Age, sex & region only	7870.0	NA	NA	NA
plus Ln NT-proBNP	7639.4	-230.6	232.6	<0.001
plus Mobility	7530.0	-109.3	117.3	<0.001
plus Diabetes	7479.8	-50.2	54.2	<0.001
plus Peripheral neuropathy	7462.9	-17.0	19.0	<0.001
plus Heart failure	7453.3	-9.5	11.5	<0.001
plus eGFR*	7434.3	-19.0	23.0	<0.001
plus Ischaemic heart disease	7425.6	-8.8	10.8	0.001
plus Self-reported ankle swelling	7419.9	-5.7	7.7	0.006

* Includes linear and quadratic eGFR terms. AIC = Akaike information criterion; LRT = likelihood ratio test; Ln = natural logarithm.

The change in Akaike information criterion (ΔAIC) with the addition of each variable relative to the model in the previous step with one fewer variable reflects the impact of the additional variable on model fit. Effect estimates (odds ratio and corresponding 95% confidence interval) for the final multivariable model are shown in Table 7-4.

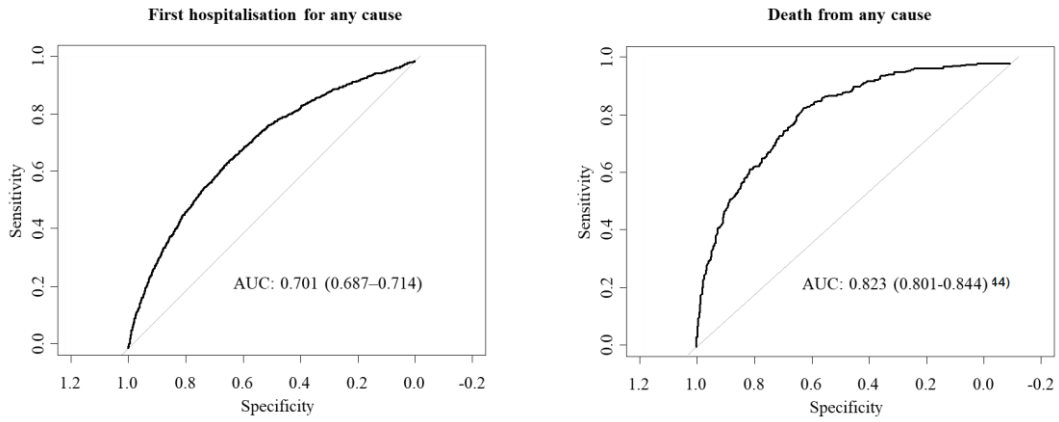
Table 7-4: Final multivariable model used to predict risk of hospitalisation

	Participants n (%)*	Hospitalised during follow-up n (%)*	OR (95% CI)	P†
Age, per 10 year increase	-	-	1.09 (1.04-1.15)	0.001
Female sex	2192 (33.2)	615 (28.1)	0.84 (0.74-0.95)	0.004
Region				<0.001
Europe	2648 (40.0)	909 (34.3)	Ref	
North America	1717 (26.0)	492 (28.7)	0.67 (0.58-0.77)	
China & Malaysia	1632 (24.7)	424 (26.0)	1.14 (0.97-1.33)	
Japan	612 (9.3)	170 (27.8)	1.20 (0.97-1.48)	
Ln NT-proBNP, per unit increase	-	-	1.26 (1.20-1.33)	<0.001
Mobility				<0.001
No problems	4411 (66.7)	1052 (23.8)	Ref	
Slight problems	1141 (17.3)	435 (38.1)	1.41 (1.21-1.64)	
Moderate problems	750 (11.3)	344 (45.9)	1.69 (1.41-2.02)	
Severe problems	282 (4.3)	149 (52.8)	1.93 (1.48-2.53)	
Unable to walk about	25 (0.4)	15 (60.0)	2.59 (1.11-6.07)	
Diabetes				<0.001
No diabetes	3569 (54.0)	851 (23.8)	Ref	
Diabetes without retinopathy	2375 (35.9)	853 (35.9)	1.27 (1.12-1.44)	
Diabetes with retinopathy	665 (10.1)	291 (43.8)	1.62 (1.34-1.96)	
Peripheral neuropathy‡	1316 (19.9)	557 (42.3)	1.34 (1.16-1.55)	<0.001
Heart failure‡	658 (10.0)	333 (50.6)	1.30 (1.08-1.57)	0.006
Estimated GFR, per 10 mL/min/1.73m^2 increase§	-	-	0.71 (0.60-0.84)	<0.001
Ischaemic heart disease‡	1095 (16.6)	494 (45.1)	1.30 (1.12-1.51)	0.001
Self-reported ankle swelling‡	1516 (22.9)	611 (40.3)	1.21 (1.06-1.38)	0.005

* Relevant for categorical variables only. † Wald test P value for continuous and binary outcomes; P value from likelihood ratio test comparing full model with and without the additional variable for categorical variables. ‡ Effect estimate for presence versus absence of. § Effect estimate for linear eGFR term, quadratic term also included in model due to non-linearity. Ln = natural logarithm.

Model performance was assessed using calibration plots and the area under the receiver operating characteristic curve (AUC) and found to adequately predict risk of hospitalisation (AUC [95% CI] 0.70 [0.69-0.71]; Figure 7-1). The model developed with all-cause hospitalisation as the response variable was also separately assessed using the AUC with death from any cause as the response variable demonstrating that the identified predictors (of risk of hospitalisation as an indicator of frailty) also had reasonable discrimination for death (which also has established associations with clinical frailty; AUC 0.82 [0.80-0.84]; Figure 7-1).

Figure 7-1: Performance of the final multivariable logistic regression model



7.2.2 FRAILTY INDICATORS AND BASELINE CHARACTERISTICS

Characteristics of participants at the highest risk of hospitalisation were generally as would be expected based upon the variables used in the prediction model: older age, history of diabetes and cardiovascular disease and lower eGFR (all P<0.001, Table 7-5). Elevated body mass index (BMI) was also associated with higher predicted risk of hospitalisation; there were few participants with low BMI in EMPA-KIDNEY (overall mean±SD 29.7±6.8 kg/m^2). Diabetic kidney disease was the commonest aetiology of kidney disease in those with the highest levels of frailty (based on risk of hospitalisation) whereas glomerular disease predominated in those in the lowest frailty category (Table 7-5). Related to primary kidney disease aetiology, participants with higher levels of frailty had lower levels of albuminuria (P<0.001) but greater 5-year risk of kidney failure (P<0.001) owing to older age and lower eGFR. Five-year risk of kidney failure (based on the 4-variable Kidney Failure Risk Equation) was 14% (95% CI 5-37) versus 6% (95% CI 2-19) in the groups at highest (>45%) versus lowest (≤20%) predicted risk of hospitalisation during follow-up. Predicted risk of hospitalisation varied geographically with the highest risk group largely being constituted of participants recruited from Europe (Germany, in particular) and comparatively very small numbers from China and Japan (Table 7-5).

Table 7-5: Characteristics of participants at recruitment by predicted risk of hospitalisation

	Predicted risk of hospitalisation during follow-up (median 2 years)				
	≤20% (N=1988)	**>20% ≤35% (N=2504)**	**>35% ≤45% (N=968)**	**>45% (N=1149)**	**P**
DEMOGRAPHICS					
Age at randomisation (years)					
Mean (SD)	52.8 (13.8)	65.6 (11.5)	71.4 (9.0)	72.6 (8.8)	<0.001
Category					<0.001
<60	1355 (68.2)	698 (27.9)	100 (10.3)	99 (8.6)	
≥60 <70	381 (19.2)	765 (30.6)	272 (28.1)	302 (26.3)	
≥70	252 (12.7)	1041 (41.6)	596 (61.6)	748 (65.1)	
Female sex	745 (37.5)	860 (34.3)	294 (30.4)	293 (25.5)	<0.001
Race (all regions)					<0.001
White	930 (46.8)	1435 (57.3)	624 (64.5)	870 (75.7)	
Black	99 (5.0)	96 (3.8)	36 (3.7)	31 (2.7)	
Asian	920 (46.3)	942 (37.6)	295 (30.5)	236 (20.5)	
Mixed	9 (0.5)	7 (0.3)	2 (0.2)	3 (0.3)	
Other	30 (1.5)	24 (1.0)	11 (1.1)	9 (0.8)	
Country					<0.001
UK	311 (15.6)	429 (17.1)	159 (16.4)	234 (20.4)	
Germany	210 (10.6)	415 (16.6)	220 (22.7)	424 (36.9)	
Italy	72 (3.6)	95 (3.8)	40 (4.1)	39 (3.4)	
USA	384 (19.3)	486 (19.4)	201 (20.8)	158 (13.8)	
Canada	149 (7.5)	195 (7.8)	71 (7.3)	73 (6.4)	
Malaysia	167 (8.4)	261 (10.4)	120 (12.4)	98 (8.5)	
China	503 (25.3)	346 (13.8)	81 (8.4)	56 (4.9)	
Japan	192 (9.7)	277 (11.1)	76 (7.9)	67 (5.8)	
PRIOR DISEASE					
Prior diabetes*					<0.001
Diabetes without retinopathy	308 (15.5)	980 (39.1)	478 (49.4)	609 (53.0)	
Diabetes with retinopathy	21 (1.1)	188 (7.5)	167 (17.3)	289 (25.2)	
Cause of kidney disease					<0.001
Diabetic kidney disease	203 (10.2)	773 (30.9)	460 (47.5)	621 (54.0)	
Hypertension/renovascular	348 (17.5)	627 (25.0)	233 (24.1)	237 (20.6)	
Glomerular	987 (49.6)	545 (21.8)	81 (8.4)	56 (4.9)	
Other/unknown	450 (22.6)	559 (22.3)	194 (20.0)	235 (20.5)	
Cardiovascular disease[†]	101 (5.1)	503 (20.1)	395 (40.8)	766 (66.7)	<0.001
Heart failure	9 (0.5)	103 (4.1)	130 (13.4)	416 (36.2)	<0.001
Ischaemic heart disease[*]	39 (2.0)	276 (11.0)	254 (26.2)	526 (45.8)	<0.001
Peripheral arterial disease	23 (1.2)	121 (4.8)	91 (9.4)	235 (20.5)	<0.001
Peripheral neuropathy	73 (3.7)	403 (16.1)	299 (30.9)	541 (47.1)	<0.001
Self-reported ankle swelling	146 (7.3)	476 (19.0)	335 (34.6)	559 (48.7)	<0.001
Count of conditions (other than CKD) at randomisation, median (Q1-Q3)	0 (0-1)	1 (0-2)	2 (1-3)	3 (2-4)	<0.001
CLINICAL MEASUREMENTS					
Systolic blood pressure (mmHg)					
Mean (SD)	132 (15)	138 (18)	140 (19)	138 (20)	<0.001
Category					<0.001
<130	925 (46.5)	802 (32.0)	278 (28.7)	393 (34.2)	
≥130 <145	678 (34.1)	836 (33.4)	301 (31.1)	374 (32.6)	
≥145	385 (19.4)	866 (34.6)	389 (40.2)	382 (33.2)	
Diastolic blood pressure (mmHg)					
Mean (SD)	82 (11)	79 (12)	75 (11)	73 (12)	<0.001
Category					<0.001
<75	503 (25.3)	913 (36.5)	499 (51.5)	665 (57.9)	
≥75 <85	678 (34.1)	820 (32.7)	265 (27.4)	289 (25.2)	
≥85	807 (40.6)	771 (30.8)	204 (21.1)	195 (17.0)	
Pulse pressure (mmHg)	49.8 (12.8)	59.6 (16.1)	65.5 (16.8)	65.1 (18.4)	<0.001
Body mass index (kg/m^2)					
Mean (SD)	28.3 (6.3)	29.4 (6.5)	30.8 (7.0)	32.1 (7.1)	<0.001
Category					<0.001
<25	620 (31.2)	662 (26.4)	172 (17.8)	165 (14.4)	
≥25 <30	746 (37.5)	877 (35.0)	340 (35.1)	334 (29.1)	
≥30	621 (31.2)	961 (38.4)	453 (46.8)	642 (55.9)	
Missing	1 (0.1)	4 (0.2)	3 (0.3)	8 (0.7)	
Waist-to-hip ratio	0.9 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	<0.001

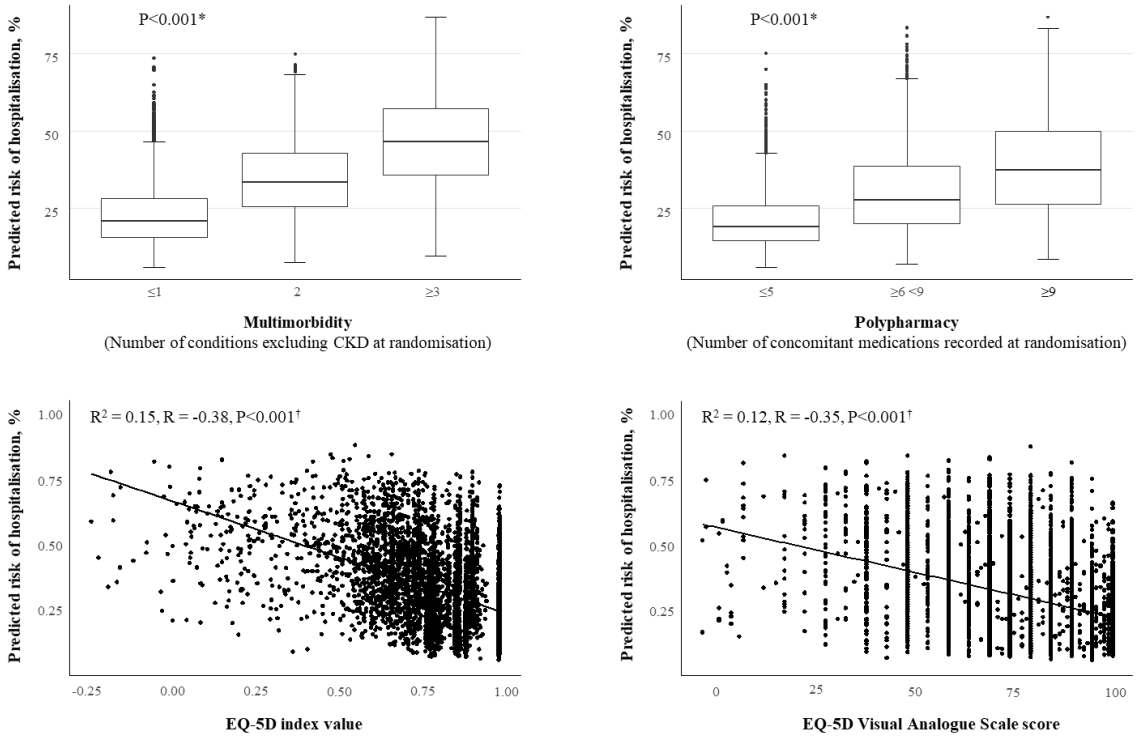
LABORATORY MEASUREMENTS					
Estimated GFR (mL/min/1.73m^2)					
Mean (SD)	45.1 (15.6)	36.2 (13.7)	33.1 (11.2)	30.0 (9.3)	<0.001
Category					<0.001
<30	275 (13.8)	898 (35.9)	444 (45.9)	665 (57.9)	
≥30 <45	915 (46.0)	1178 (47.0)	413 (42.7)	422 (36.7)	
≥45	798 (40.1)	428 (17.1)	111 (11.5)	62 (5.4)	
Urinary albumin-to-creatinine ratio (mg/g)					<0.001
Geometric mean (95% CI)	299 (277-323)	210 (194-227)	177 (154-202)	183 (162-206)	
Median (Q1-Q3)	440 (133-1056)	314 (43-1062)	220 (29-1060)	193 (34-1118)	
Category					<0.001
<30	279 (14.0)	543 (21.7)	245 (25.3)	261 (22.7)	
≥30 ≤300	507 (25.5)	692 (27.6)	281 (29.0)	384 (33.4)	
>300	1202 (60.5)	1269 (50.7)	442 (45.7)	504 (43.9)	
Glycated haemoglobin (mmol/mol)					
Mean (SD)	39.1 (10.3)	45.4 (13.6)	48.8 (13.7)	51.1 (14.6)	<0.001
Category					<0.001
<39	1284 (64.6)	953 (38.1)	240 (24.8)	205 (17.8)	
≥39 <48	463 (23.3)	729 (29.1)	291 (30.1)	354 (30.8)	
≥48 <75	168 (8.5)	688 (27.5)	366 (37.8)	502 (43.7)	
≥75	36 (1.8)	89 (3.6)	47 (4.9)	80 (7.0)	
Missing	37 (1.9)	45 (1.8)	24 (2.5)	8 (0.7)	
NT-proBNP (ng/L)					<0.001
Geometric mean (95% CI)	48 (46-50)	164 (158-171)	369 (346-393)	846 (792-903)	
Median (Q1-Q3)	52 (15-98)	160 (90-302)	356 (181-714)	851 (369-1865)	
Category					<0.001
<110	1552 (78.1)	821 (32.8)	104 (10.7)	33 (2.9)	
≥110 <330	382 (19.2)	1109 (44.3)	348 (36.0)	222 (19.3)	
≥330	37 (1.9)	542 (21.6)	507 (52.4)	890 (77.5)	
Missing	17 (0.9)	32 (1.3)	9 (0.9)	4 (0.3)	
Haematocrit (%)					
Mean (SD)	40.8 (4.9)	39.0 (4.9)	37.8 (4.9)	37.4 (5.3)	<0.001
Category					<0.001
<37%	331 (16.6)	688 (27.5)	332 (34.3)	467 (40.6)	
≥37 <41%	538 (27.1)	735 (29.4)	301 (31.1)	317 (27.6)	
≥41%	948 (47.7)	817 (32.6)	232 (24.0)	254 (22.1)	
Missing	171 (8.6)	264 (10.5)	103 (10.6)	111 (9.7)	
KDIGO risk category					<0.001
Low, moderate or high	678 (34.1)	640 (25.6)	197 (20.4)	157 (13.7)	
Very high	1310 (65.9)	1864 (74.4)	771 (79.6)	992 (86.3)	
5-YEAR RISK OF KIDNEY FAILURE (KFRE, %), median (Q1-Q3)	6 (2-19)	10 (3-32)	11 (4-34)	14 (5-37)	<0.001
CONCOMITANT MEDICATION USE					
Any diuretic	507 (25.5)	925 (36.9)	548 (56.6)	835 (72.7)	<0.001
Loop diuretic	164 (8.2)	474 (18.9)	385 (39.8)	724 (63.0)	<0.001
Thiazide diuretic	287 (14.4)	434 (17.3)	202 (20.9)	199 (17.3)	<0.001
Mineralocorticoid receptor antagonist	105 (5.3)	132 (5.3)	86 (8.9)	152 (13.2)	<0.001
Potassium sparing & other	12 (0.6)	13 (0.5)	9 (0.9)	4 (0.3)	0.343
Beta blocker	404 (20.3)	976 (39.0)	573 (59.2)	808 (70.3)	<0.001
Anticoagulant	20 (1.0)	81 (3.2)	69 (7.1)	146 (12.7)	<0.001
Antiplatelet therapy	308 (15.5)	809 (32.3)	476 (49.2)	646 (56.2)	<0.001
Diabetes treatment	292 (14.7)	1037 (41.4)	561 (58.0)	805 (70.1)	<0.001
Biguanide (e.g. metformin)	103 (5.2)	308 (12.3)	125 (12.9)	133 (11.6)	<0.001
Sulfonylurea	85 (4.3)	252 (10.1)	123 (12.7)	125 (10.9)	<0.001
Insulin	145 (7.3)	590 (23.6)	357 (36.9)	571 (49.7)	<0.001
DPP-4 inhibitor	74 (3.7)	345 (13.8)	186 (19.2)	277 (24.1)	<0.001
GLP-1 agonist	44 (2.2)	142 (5.7)	71 (7.3)	80 (7.0)	<0.001
Other antidiabetic agent	34 (1.7)	127 (5.1)	80 (8.3)	73 (6.4)	<0.001
Count of concomitant medications at randomisation, median (Q1-Q3)	5 (3-7)	7 (5-9)	9 (6-11)	10 (8-13)	<0.001

Figures are n (%) or mean (SD) or median (Q1-Q3). * Self-reported history of myocardial infarction or angina. P values are from Chi squared tests for categorical variables; one-way analysis of variance (ANOVA) for normally distributed and Kruskal-Wallis tests for non-normally distributed continuous variables, respectively. DPP-4 = dipeptidyl peptidase-4; GLP-1 = glucagon-like peptide-1.

The median (Q1-Q3) number of comorbid conditions (excluding CKD) prior to randomisation was 1 (0-2); range 0-7 and 71% of participants (4675/6609) had at least one condition in addition to CKD (i.e. multimorbidity). The median (Q1-Q3) number of concomitant medications recorded at randomisation was 7 (5-10), range 0-36 and 76% of participants (5044/6609) were prescribed five or more concomitant medications (i.e. polypharmacy). Median (Q1-Q3) indexed EQ-5D value was 0.891 (0.773-0.987) and median (Q1-Q3) self-rated health score on the visual analogue scale was 80 (70-90) with scores ranging from 0 to 100.

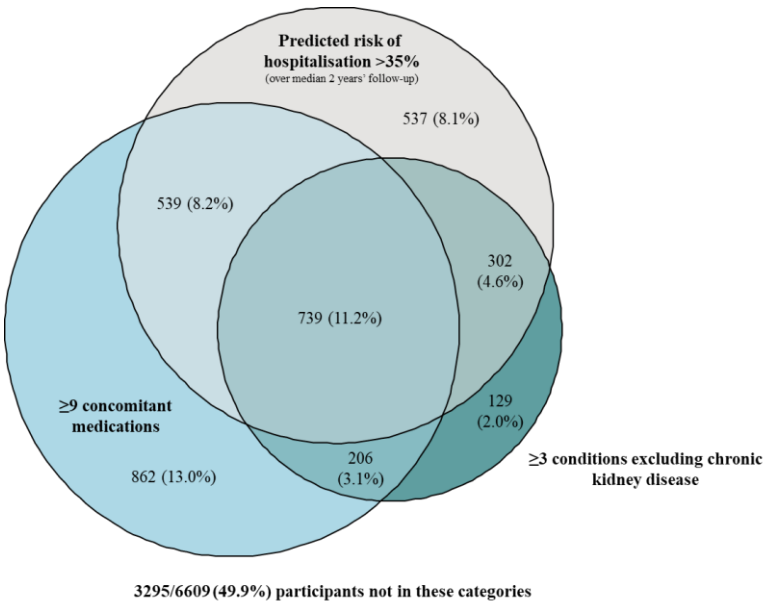
There was considerable overlap between the frailty indicator subgroups. Risk of hospitalisation was positively associated with multimorbidity, polypharmacy and inversely correlated with HRQoL (Figure 7-2). Of 5635 participants who fulfilled definitions of either polypharmacy or multimorbidity, 72% (4084/5635) were included in both groups (Figure 7-3).

Figure 7-2: Associations between predicted risk of hospitalisation and multimorbidity; polypharmacy; and health-related quality of life



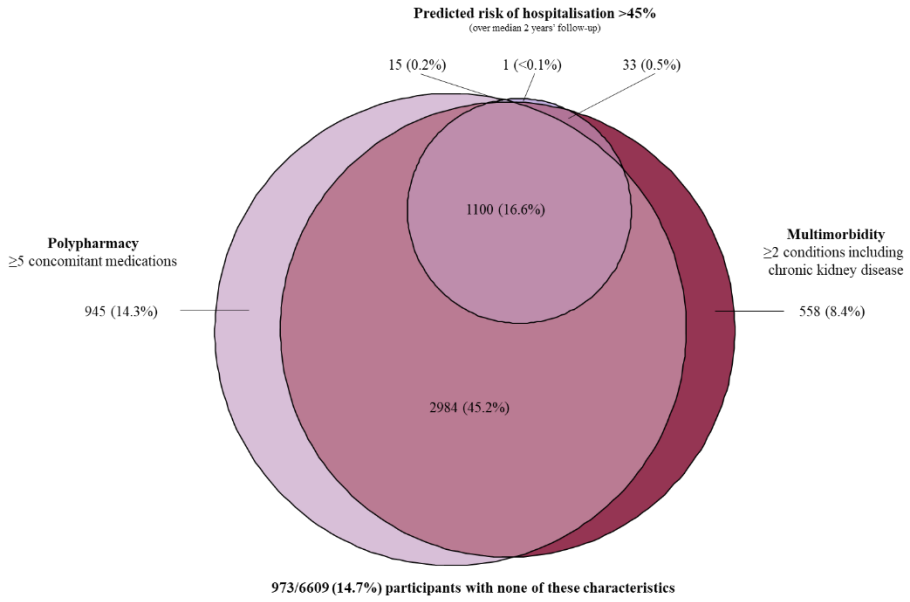
* P value = analysis of variance (ANOVA). † Spearman's rank-order correlation.

Figure 7-3: Number of participants in the top thirds of predicted risk of hospitalisation (>35%), multimorbidity (≥3 conditions excluding chronic kidney disease) and polypharmacy (≥9 concomitant medications) showing degrees of overlap



An alternative presentation showing overlap between the highest level of frailty defined in EMPA-KIDNEY (predicted risk of hospitalisation >45%) and conventional definitions of multimorbidity (≥2 conditions) and polypharmacy (≥5 medications) is shown in Figure 7-4.

Figure 7-4: Number of participants in the highest level of frailty (defined as predicted risk of hospitalisation >45%) in EMPA-KIDNEY showing overlap with conventional definitions of multimorbidity and polypharmacy



7.2.3 ADHERENCE TO STUDY TREATMENT

Adherence to study treatment was negatively associated with risk of hospitalisation. At 12 months of follow-up (the approximate midpoint of the trial), the proportion of participants reportedly taking most (>80%) of their study treatment was highest in patients in the lowest frailty category (≤20% predicted risk of hospitalisation during follow-up) at 1830/1982 (92.3%) and lowest in those with the highest level of frailty (>45% predicted risk of hospitalisation during follow-up) at 938/1090 (86.1%). Participants with greater degrees of frailty were more likely to discontinue study treatment in both empagliflozin and placebo groups thought cited reasons were uncommonly attributed to serious adverse events (Table 7-6).

Table 7-6: Reasons for discontinuing randomised treatment

	Predicted risk of hospitalisation					
	≤20%	**>20% ≤35%**	**>35% ≤45%**	**>45%**	**Total**	**P***
EMPAGLIFLOZIN						
Any reason	**139 (14.1%)**	**198 (15.9%)**	**93 (19.1%)**	**127 (21.6%)**	**557 (16.9%)**	<0.001
SAE	11	22	8	18	**59**	
NSAE	14	20	8	16	**58**	
Other	54	74	41	57	**226**	
Unknown	60	82	36	36	**214**	
PLACEBO						
Any reason	**147 (14.6%)**	**237 (18.8%)**	**99 (20.6%)**	**157 (28.0%)**	**640 (19.4%)**	<0.001
SAE	10	30	11	24	**75**	
NSAE	7	18	7	10	**42**	
Other	58	97	48	65	**268**	
Unknown	72	92	33	58	**255**	

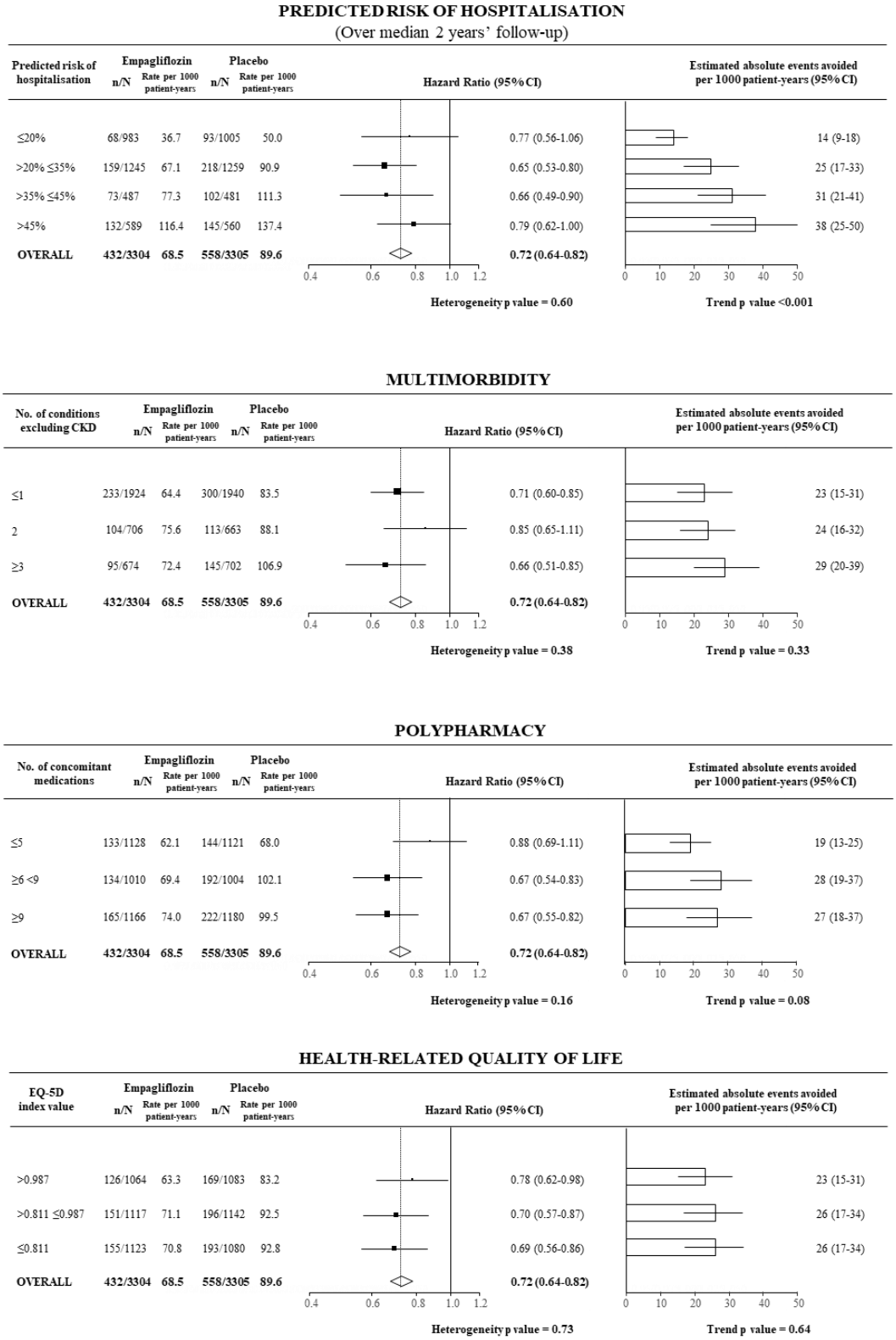
*P value from Chi squared test comparing proportion discontinuing treatment for any reason for across risk of hospitalization categories, separately for the empagliflozin and placebo groups. Abbreviations: SAE = serious adverse event; NSAE = non-serious adverse event. Other reasons for discontinuation are listed in a previous publication (EMPA-KIDNEY Collaborative Group, 2023).

7.2.4 EFFECTS OF EMPAGLIFLOZIN ON THE PRIMARY OUTCOME BY FRAILTY INDICATORS

7.2.4.1 RELATIVE EFFECTS ON THE PRIMARY OUTCOME

Overall, compared to placebo, empagliflozin reduced the risk of the primary composite outcome of kidney disease progression or cardiovascular death by 28% (hazard ratio [HR] 0.72, 95% confidence interval [CI] 0.64-0.82), with no significant difference in relative effects by baseline level of frailty, multimorbidity, polypharmacy or HRQoL (P for heterogeneity all >0.05, Figure 7-5). The majority of the 990 primary outcome events were due to kidney disease progression (888 events) and overall, empagliflozin reduced the risk of this secondary outcome by 29% (HR 0.71, 95% CI 0.62-0.81) with no strong evidence of differing relative effects across all four indicators of frailty (Table 7-7 to Table 7-10).

Figure 7-5: Effects of empagliflozin on the primary outcome of kidney disease progression or cardiovascular death by frailty indicators



Predicted risk of hospitalisation during follow-up (median 2 years) was derived from multivariable logistic regression models (first event). Multimorbidity was determined based on the presence/absence of 8 patient-reported comorbidities at randomisation excluding chronic kidney disease. The EQ-5D index value is a weighted index of the 5 EQ-5D domain scores (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) derived using established methodology; lower values indicate poorer quality of life. Due to absence of any evidence of effect modification by the presented characteristics, absolute events avoided per 1000 patients treated with empagliflozin per 1 year (95% CI) were estimated by applying the overall hazard ratio to the event rate per 1000 patient-years in the placebo group.

7.2.4.2 ABSOLUTE EFFECTS ON THE PRIMARY OUTCOME

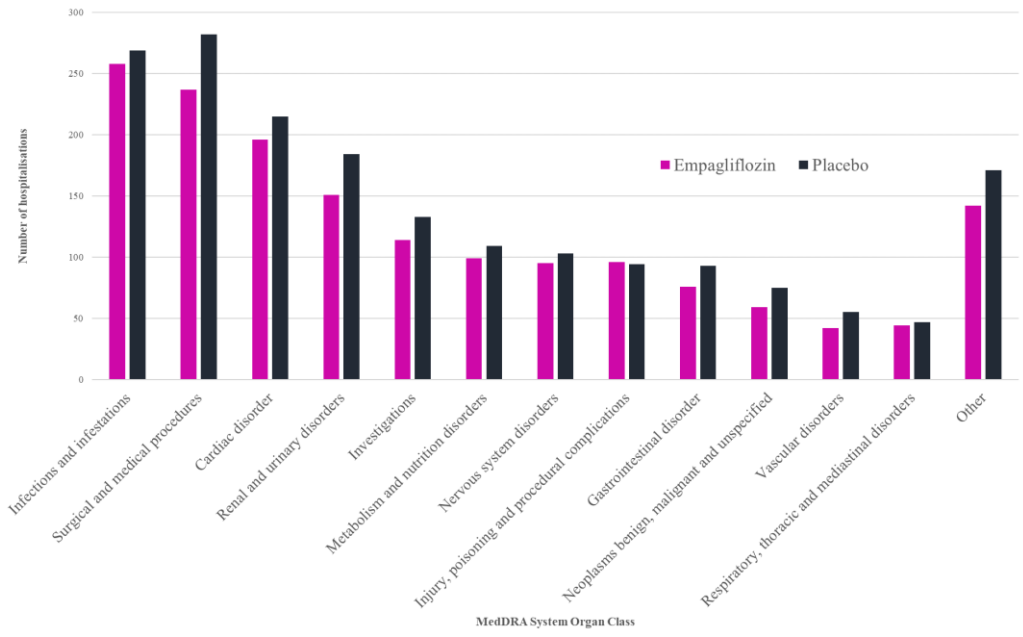
Although the proportional effects of empagliflozin were similar across levels of frailty indicators, there was evidence of larger estimated absolute benefits on the primary outcome of kidney disease progression or cardiovascular death in participants in the highest category of frailty (based on risk of hospitalisation) compared to those with lesser degrees of frailty. Per 1000 participants treated, empagliflozin was estimated to result in 38 fewer participants with a first occurrence of kidney disease progression or cardiovascular death (i.e. primary outcomes) among those in the highest frailty category compared to 14 primary outcomes avoided annually, per 1000 treated participants in the lowest third of frailty (Figure 7-5). A similar pattern was observed across levels of multimorbidity, polypharmacy and HRQoL though with less clear statistical evidence of trend (Figure 7-5).

7.2.5 EFFECTS OF EMPAGLIFLOZIN ON KEY SECONDARY OUTCOMES BY FRAILTY INDICATORS

7.2.5.1 RELATIVE EFFECTS ON KEY SECONDARY OUTCOMES

In total, 1611 hospitalisations occurred among 960 patients in the empagliflozin group, and 1895 among 1035 patients in the placebo group during follow-up. Overall, empagliflozin reduced total all-cause hospitalisations by 14% versus placebo (HR 0.86, 95% CI 0.78-0.95) though this was not clearly driven by a single cause of hospitalisation (by Medical Dictionary for Regulatory Activities [MedDRA] System Organ Class; Figure 7-6).

Figure 7-6: All hospitalisations grouped by cause



On a relative scale, analyses by baseline measures of frailty show no evidence of effect modification by baseline levels of risk of hospitalisation, multimorbidity, polypharmacy or baseline health-related quality of life (Figure 7-7).

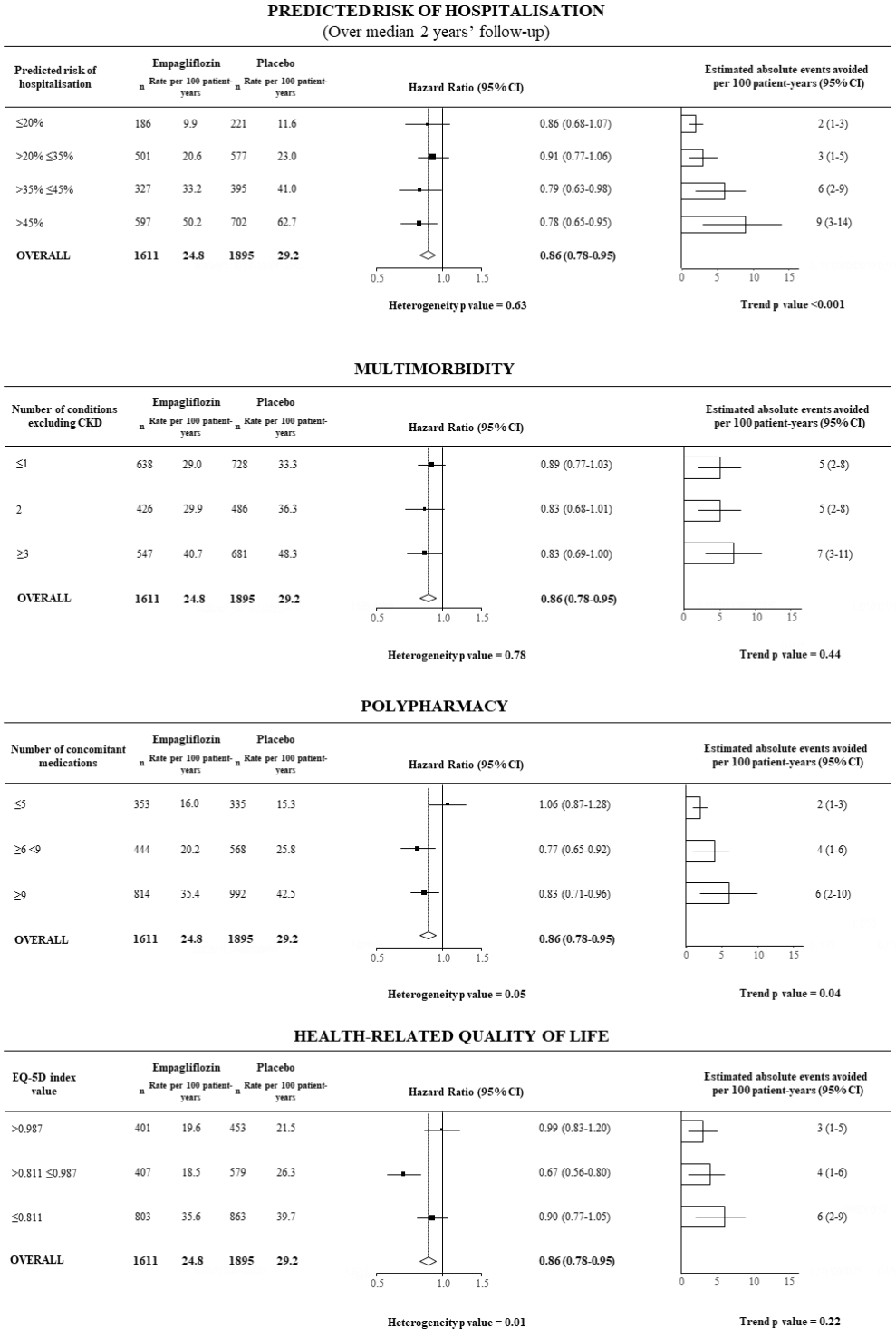
No significant effect was observed overall on the composite key secondary outcome of hospitalisation for heart failure or death from cardiovascular causes (HR 0.84, 95% CI 0.67-1.07); or death from any cause (HR 0.87, 95% CI 0.70-1.08), with consistent findings across frailty indicator subgroups for both of these outcomes (Table 7-7 to Table 7-10).

7.2.5.2 ABSOLUTE EFFECTS ON KEY SECONDARY OUTCOMES

Although the proportional effects of empagliflozin were similar across levels of frailty indicators, there was evidence of larger estimated absolute benefits on recurrent all-cause hospitalisations in participants in the top category of frailty (based on risk of hospitalisation) compared to those with lesser degrees of frailty. Per 100 participants treated, empagliflozin was estimated to result in 9 fewer total hospitalisations each year among those in the category with the greatest degree of frailty compared to 2 hospitalisations avoided annually, per 100 treated participants in the lowest third of frailty (Figure 7-7). A similar pattern was observed across levels of multimorbidity, polypharmacy and HRQoL though with less clear evidence of trend than for predicted risk of hospitalisation (Figure 7-7).

Uncertainty exists around the estimates of the effect of empagliflozin on the other key secondary outcomes of the composite of first hospitalisation for heart failure or cardiovascular death; and death from any cause. However, on an absolute scale, there were numerically more events avoided by empagliflozin for each of these outcomes in participants in the highest (versus lowest) categories of frailty, multimorbidity and polypharmacy and in those with poorest (versus greatest) HRQoL although there was no strong statistical evidence of trend (Table 7-7 to Table 7-10).

Figure 7-7: Effects of empagliflozin on recurrent all-cause hospitalisations by frailty indicators



The analysis of hospitalisations for any cause included the first and all subsequent events, n shown = total events; 1611 total hospitalisations occurred among 960 patients in the empagliflozin group, and 1895 total hospitalisations occurred among 1035 patients in the placebo group. Rates are presented per 100 patient-years to match previous reports. Predicted risk of hospitalisation during follow-up (median 2 years) was derived from multivariable logistic regression models adjusted for age, sex and region assessing the association of all potential predictor variables with recorded hospitalisation (first event). Multimorbidity was determined based on the presence/absence of 8 patient-reported comorbidities at randomisation in addition to chronic kidney disease. The EQ-5D index value is a weighted index of the 5 EQ-5D domain scores (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) derived using established methodology; lower values indicate poorer quality of life. Due to absence of any strong evidence of heterogeneity the presented characteristics, absolute events avoided per 100 patients treated with empagliflozin per 1 year (95% CI) were estimated by applying the overall hazard ratio (or 95% CI) to the event rate per 100 patient-years in the placebo group. If subgroup-specific hazard ratios (or CIs) were used to estimate absolute effects by health-related quality of life, based on P for heterogeneity = 0.01; estimated absolute events avoided (95% CI) would be 0.1 (-4, 4), 9 (6, 12) and 4 (-2, 9) rather than 3 (1, 5), 4 (1, 6) and 6 (2, 9).

Table 7-7: Primary and secondary outcomes by predicted risk of hospitalisation

Predicted risk of hospitalisation (%)	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events avoided per 1000 patient-years (SE)	P_{trend}
PRIMARY OUTCOME AND ITS COMPONENTS								
Primary outcome: progression of kidney disease or death from cardiovascular causes						0.60		<0.001
≤20%	68/983	36.7	93/1005	50.0	0.77 (0.56-1.06)		13.8 (2.3)	
>20% ≤35%	159/1245	67.1	218/1259	90.9	0.65 (0.53-0.80)		25.1 (4.2)	
>35% ≤45%	73/487	77.3	102/481	111.3	0.66 (0.49-0.90)		30.7 (5.2)	
>45%	132/589	116.4	145/560	137.4	0.79 (0.62-1.00)		37.9 (6.4)	
Overall	**432/3304**	**68.5**	**558/3305**	**89.6**	**0.72 (0.64-0.82)**			
KEY SECONDARY OUTCOMES								
Hospitalisation for heart failure or death from cardiovascular causes						0.27§		0.01
≤20%	3/983	1.6	1/1005	0.5	‡		0.1 (0.1)	
>20% ≤35%	16/1245	6.6	31/1259	12.4	0.53 (0.29-0.96)		1.9 (1.3)	
>35% ≤45%	22/487	22.6	22/481	23.1	1.02 (0.56-1.84)		3.6 (2.3)	
>45%	90/589	78.9	98/560	92.5	0.85 (0.64-1.13)		14.5 (9.3)	
Overall	**131/3304**	**20.4**	**152/3305**	**23.7**	**0.84 (0.67-1.07)**			
Hospitalisation for any cause (first and all subsequent events)						0.63		<0.001
≤20%	186	99	221	116	0.86 (0.68-1.07)		16.4 (5.1)	
>20% ≤35%	501	206	577	230	0.91 (0.77-1.06)		32.3 (10.0)	
>35% ≤45%	327	332	395	410	0.79 (0.63-0.98)		57.6 (17.8)	
>45%	597	502	702	627	0.78 (0.65-0.95)		88.1 (27.3)	
Overall	**1611**	**248**	**1895**	**292**	**0.86 (0.78-0.95)**			
Death from any cause						0.48§		0.03
≤20%	4/983	2.1	3/1005	1.6	‡		0.2 (0.2)	
>20% ≤35%	35/1245	14.4	41/1259	16.4	0.87 (0.56-1.37)		2.1 (1.6)	
>35% ≤45%	23/487	23.3	37/481	38.4	0.61 (0.36-1.02)		5.0 (3.8)	
>45%	86/589	72.3	86/560	76.8	0.95 (0.71-1.29)		10.1 (7.5)	
Overall	**148/3304**	**22.8**	**167/3305**	**25.8**	**0.87 (0.70-1.08)**			
OTHER SECONDARY OUTCOMES								
Any kidney disease progression						0.76		<0.001
≤20%	67/983	36.1	93/1005	50.0	0.77 (0.56-1.06)		14.6 (2.4)	
>20% ≤35%	150/1245	63.3	204/1259	85.1	0.65 (0.52-0.80)		24.8 (4.1)	
>35% ≤45%	66/487	69.9	90/481	98.2	0.66 (0.48-0.91)		28.6 (4.7)	
>45%	101/589	89.1	117/560	110.8	0.74 (0.57-0.97)		32.3 (5.3)	
Overall	**384/3304**	**60.9**	**504/3305**	**80.9**	**0.71 (0.62-0.81)**			
Death from cardiovascular causes						0.63§		-
≤20%	1/983	0.5	0/1005	0.0	‡		0.0 (0.0)	
>20% ≤35%	9/1245	3.7	15/1259	6.0	0.62 (0.27-1.41)		0.9 (0.9)	
>35% ≤45%	9/487	9.1	13/481	13.5	0.68 (0.29-1.59)		2.1 (2.0)	
>45%	40/589	33.6	41/560	36.6	0.92 (0.60-1.43)		5.7 (5.5)	
Overall	**59/3304**	**9.1**	**69/3305**	**10.6**	**0.84 (0.60-1.19)**			
ESKD or death from cardiovascular causes †						0.09		<0.001
≤20%	17/983	9.1	15/1005	7.9	1.23 (0.61-2.46)		2.2 (0.6)	
>20% ≤35%	46/1245	19.1	79/1259	32.1	0.55 (0.38-0.79)		8.7 (2.4)	
>35% ≤45%	25/487	25.7	42/481	44.5	0.58 (0.35-0.95)		12.1 (3.4)	
>45%	75/589	64.7	81/560	74.5	0.86 (0.62-1.17)		20.2 (5.6)	
Overall	**163/3304**	**25.4**	**217/3305**	**34.0**	**0.73 (0.59-0.89)**			

The p values shown are the p values for trend across categories of predicted risk of hospitalisation for the relative and estimated absolute effects; respectively. * Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. † ESKD: End-Stage Kidney Disease, defined as start of maintenance dialysis or receipt of a kidney transplant. ‡ Hazard ratios are not presented for outcomes with fewer than 10 events. § Heterogeneity test compares >20% ≤35%, >35% ≤45% and >45% since event numbers precluded reliable hazard ratio estimation for ≤20%; all other P_{het} refer to comparisons across all 4 levels of predicted risk of hospitalisation.

Table 7-8: Primary and secondary outcomes by multimorbidity

No. of conditions (excluding CKD)	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events avoided per 1000 patient-years (SE)	P_{trend}
PRIMARY OUTCOME AND ITS COMPONENTS								
Primary outcome: progression of kidney disease or death from cardiovascular causes						0.38		0.33
≤1	233/1924	64.4	300/1940	83.5	0.71 (0.60-0.85)		23.1 (3.9)	
2	104/706	75.6	113/663	88.1	0.85 (0.65-1.11)		24.3 (4.1)	
≥3	95/674	72.4	145/702	106.9	0.66 (0.51-0.85)		29.5 (5.0)	
Overall	**432/3304**	**68.5**	**558/3305**	**89.6**	**0.72 (0.64-0.82)**			
KEY SECONDARY OUTCOMES								
Hospitalisation for heart failure or death from cardiovascular causes						0.70		0.09
≤1	21/1924	5.6	29/1940	7.8	0.71 (0.41-1.25)		1.2 (0.8)	
2	37/706	26.3	36/663	27.4	0.98 (0.62-1.55)		4.3 (2.8)	
≥3	73/674	56.1	87/702	63.9	0.86 (0.63-1.17)		10.0 (6.4)	
Overall	**131/3304**	**20.4**	**152/3305**	**23.7**	**0.84 (0.67-1.07)**			
Hospitalisation for any cause (first and all subsequent events)						0.78		0.44
≤1	638	290	728	333	0.89 (0.77-1.03)		46.8 (14.5)	
2	426	299	486	363	0.83 (0.68-1.01)		51.0 (15.8)	
≥3	547	407	681	483	0.83 (0.69-1.00)		67.8 (21.0)	
Overall	**1611**	**248**	**1895**	**292**	**0.86 (0.78-0.95)**			
Death from any cause						0.86		
≤1	41/1924	11.0	48/1940	12.8	0.84 (0.55-1.27)		1.7 (1.3)	
2	40/706	28.1	38/663	28.4	0.98 (0.63-1.53)		3.7 (2.8)	
≥3	67/674	49.9	81/702	57.4	0.86 (0.62-1.19)		7.5 (5.6)	
Overall	**148/3304**	**22.8**	**167/3305**	**25.8**	**0.87 (0.70-1.08)**			
OTHER SECONDARY OUTCOMES								
Any kidney disease progression						0.59		0.92
≤1	223/1924	61.7	287/1940	79.9	0.71 (0.59-0.84)		23.3 (3.8)	
2	91/706	66.2	106/663	82.7	0.79 (0.60-1.04)		24.1 (4.0)	
≥3	70/674	53.4	111/702	81.8	0.63 (0.47-0.86)		23.8 (3.9)	
Overall	**384/3304**	**60.9**	**504/3305**	**80.9**	**0.71 (0.62-0.81)**			
Death from cardiovascular causes						0.44		0.36
≤1	12/1924	3.2	13/1940	3.5	0.91 (0.41-1.99)		0.5 (0.5)	
2	15/706	10.5	11/663	8.2	1.31 (0.60-2.85)		1.3 (1.2)	
≥3	32/674	23.8	45/702	31.9	0.73 (0.46-1.15)		5.0 (4.8)	
Overall	**59/3304**	**9.1**	**69/3305**	**10.6**	**0.84 (0.60-1.19)**			
ESKD or death from cardiovascular causes[†]						0.49		0.08
≤1	74/1924	20.1	94/1940	25.5	0.74 (0.54-1.00)		6.9 (1.9)	
2	41/706	29.1	45/663	34.3	0.90 (0.59-1.38)		9.3 (2.6)	
≥3	48/674	36.1	78/702	56.2	0.64 (0.45-0.92)		15.2 (4.3)	
Overall	**163/3304**	**25.4**	**217/3305**	**34.0**	**0.73 (0.59-0.89)**			

The p values shown are standard chi-square tests for trend across categories of predicted risk of hospitalisation for the relative and estimated absolute effects; respectively. Hazard ratios are not presented for outcomes with fewer than 10 events. * Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. [†] ESKD: End-Stage Kidney Disease, defined as start of maintenance dialysis or receipt of a kidney transplant.

Table 7-9: Primary and secondary outcomes by concomitant medication count

No. of concomitant medications	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events avoided per 1000 patient-years (SE)	P_{trend}
PRIMARY OUTCOME AND ITS COMPONENTS								
Primary outcome: progression of kidney disease or death from cardiovascular causes						0.16		0.08
≤5	133/1128	62.1	144/1121	68.0	0.88 (0.69-1.11)		18.8 (3.2)	
≥6 <9	134/1010	69.4	192/1004	102.1	0.67 (0.54-0.83)		28.2 (4.7)	
≥9	165/1166	74.0	222/1180	99.5	0.67 (0.55-0.82)		27.5 (4.6)	
Overall	**432/3304**	**68.5**	**558/3305**	**89.6**	**0.72 (0.64-0.82)**			
KEY SECONDARY OUTCOMES								
Hospitalisation for heart failure or death from cardiovascular causes						0.26		0.13
≤5	7/1128	3.2	14/1121	6.4	0.48 (0.19-1.19)		1.0 (0.6)	
≥6 <9	38/1010	19.3	35/1004	17.9	1.08 (0.68-1.72)		2.8 (1.8)	
≥9	86/1166	38.0	103/1180	45.3	0.82 (0.62-1.09)		7.1 (4.6)	
Overall	**131/3304**	**20.4**	**152/3305**	**23.7**	**0.84 (0.67-1.07)**			
Hospitalisation for any cause (first and all subsequent events)						0.05		0.04
≤5	353	160	335	153	1.06 (0.87-1.28)		21.5 (6.6)	
≥6 <9	444	202	568	258	0.77 (0.65-0.92)		36.2 (11.2)	
≥9	814	354	992	425	0.83 (0.71-0.96)		59.6 (18.5)	
Overall	**1611**	**248**	**1895**	**292**	**0.86 (0.78-0.95)**			
Death from any cause						0.75		
≤5	19/1128	8.6	19/1121	8.7	0.96 (0.51-1.81)		1.1 (0.9)	
≥6 <9	43/1010	21.6	43/1004	21.9	0.98 (0.64-1.49)		2.9 (2.1)	
≥9	86/1166	37.4	105/1180	45.0	0.82 (0.61-1.09)		5.9 (4.4)	
Overall	**148/3304**	**22.8**	**167/3305**	**25.8**	**0.87 (0.70-1.08)**			
OTHER SECONDARY OUTCOMES								
Any kidney disease progression						0.08		0.22
≤5	128/1128	59.7	138/1121	65.2	0.89 (0.70-1.13)		19.0 (3.1)	
≥6 <9	122/1010	63.2	178/1004	94.6	0.66 (0.52-0.83)		27.6 (4.6)	
≥9	134/1166	60.1	188/1180	84.3	0.63 (0.50-0.78)		24.5 (4.1)	
Overall	**384/3304**	**60.9**	**504/3305**	**80.9**	**0.71 (0.62-0.81)**			
Death from cardiovascular causes						0.81		0.33
≤5	5/1128	2.3	8/1121	3.7	0.61 (0.20-1.86)		0.6 (0.5)	
≥6 <9	16/1010	8.0	19/1004	9.7	0.82 (0.42-1.60)		1.5 (1.4)	
≥9	38/1166	16.5	42/1180	18.0	0.90 (0.58-1.40)		2.8 (2.7)	
Overall	**59/3304**	**9.1**	**69/3305**	**10.6**	**0.84 (0.60-1.19)**			
ESKD or death from cardiovascular causes[†]						0.53		0.02
≤5	37/1128	16.9	40/1121	18.5	0.87 (0.56-1.37)		5.0 (1.4)	
≥6 <9	46/1010	23.4	74/1004	38.3	0.63 (0.44-0.91)		10.4 (2.9)	
≥9	80/1166	35.3	103/1180	45.0	0.75 (0.56-1.00)		12.2 (3.4)	
Overall	**163/3304**	**25.4**	**217/3305**	**34.0**	**0.73 (0.59-0.89)**			

The p values shown are standard chi-square tests for trend across categories of predicted risk of hospitalisation for the relative and estimated absolute effects; respectively. Hazard ratios are not presented for outcomes with fewer than 10 events. * Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. [†] ESKD: End-Stage Kidney Disease, defined as start of maintenance dialysis or receipt of a kidney transplant.

Table 7-10: Primary and secondary outcomes by health-related quality of life

EQ-5D index value	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events avoided per 1000 patient-years (SE)	P_{trend}
PRIMARY OUTCOME AND ITS COMPONENTS								
Primary outcome: progression of kidney disease or death from cardiovascular causes						0.73		0.64
>0.987	126/1064	63.3	169/1083	83.2	0.78 (0.62-0.98)		23.0 (3.9)	
>0.811 ≤0.987	151/1117	71.1	196/1142	92.5	0.70 (0.57-0.87)		25.5 (4.3)	
≤0.811	155/1123	70.8	193/1080	92.8	0.69 (0.56-0.86)		25.6 (4.3)	
Overall	**432/3304**	**68.5**	**558/3305**	**89.6**	**0.72 (0.64-0.82)**			
KEY SECONDARY OUTCOMES								
Hospitalisation for heart failure or death from cardiovascular causes						0.21		0.45
>0.987	21/1064	10.3	30/1083	14.4	0.76 (0.43-1.32)		2.3 (1.4)	
>0.811 ≤0.987	25/1117	11.4	38/1142	17.4	0.59 (0.36-0.98)		2.7 (1.8)	
≤0.811	85/1123	38.5	84/1080	39.5	0.99 (0.73-1.33)		6.2 (4.0)	
Overall	**131/3304**	**20.4**	**152/3305**	**23.7**	**0.84 (0.67-1.07)**			
Hospitalisation for any cause (first and all subsequent events)						0.01		0.22
>0.987	401	196	453	215	0.99 (0.83-1.20)		30.2 (9.4)	
>0.811 ≤0.987	407	185	579	263	0.67 (0.56-0.80)		36.9 (11.4)	
≤0.811	803	356	863	397	0.90 (0.77-1.05)		55.7 (17.2)	
Overall	**1611**	**248**	**1895**	**292**	**0.86 (0.78-0.95)**			
Death from any cause						0.08		0.34
>0.987	22/1064	10.8	26/1083	12.4	0.91 (0.52-1.61)		1.6 (1.2)	
>0.811 ≤0.987	28/1117	12.7	47/1142	21.3	0.54 (0.34-0.86)		2.8 (2.1)	
≤0.811	98/1123	43.5	94/1080	43.2	1.02 (0.76-1.35)		5.7 (4.2)	
Overall	**148/3304**	**22.8**	**167/3305**	**25.8**	**0.87 (0.70-1.08)**			
OTHER SECONDARY OUTCOMES								
Any kidney disease progression						0.70		0.89
>0.987	118/1064	59.3	161/1083	79.3	0.77 (0.61-0.98)		23.1 (3.8)	
>0.811 ≤0.987	137/1117	64.5	183/1142	86.4	0.68 (0.54-0.85)		25.2 (4.2)	
≤0.811	129/1123	58.9	160/1080	76.9	0.68 (0.54-0.86)		22.4 (3.7)	
Overall	**384/3304**	**60.9**	**504/3305**	**80.9**	**0.71 (0.62-0.81)**			
Death from cardiovascular causes						0.89		0.56
>0.987	8/1064	3.9	10/1083	4.8	0.85 (0.34-2.16)		0.7 (0.7)	
>0.811 ≤0.987	15/1117	6.8	14/1142	6.3	0.98 (0.47-2.02)		1.0 (1.0)	
≤0.811	36/1123	16.0	45/1080	20.7	0.79 (0.51-1.23)		3.2 (3.1)	
Overall	**59/3304**	**9.1**	**69/3305**	**10.6**	**0.84 (0.60-1.19)**			
ESKD or death from cardiovascular causes‡						0.88		0.13
>0.987	34/1064	16.8	50/1083	24.0	0.72 (0.46-1.11)		6.5 (1.8)	
>0.811 ≤0.987	57/1117	26.3	68/1142	31.3	0.78 (0.55-1.11)		8.5 (2.4)	
≤0.811	72/1123	32.3	99/1080	46.4	0.69 (0.51-0.94)		12.6 (3.5)	
Overall	**163/3304**	**25.4**	**217/3305**	**34.0**	**0.73 (0.59-0.89)**			

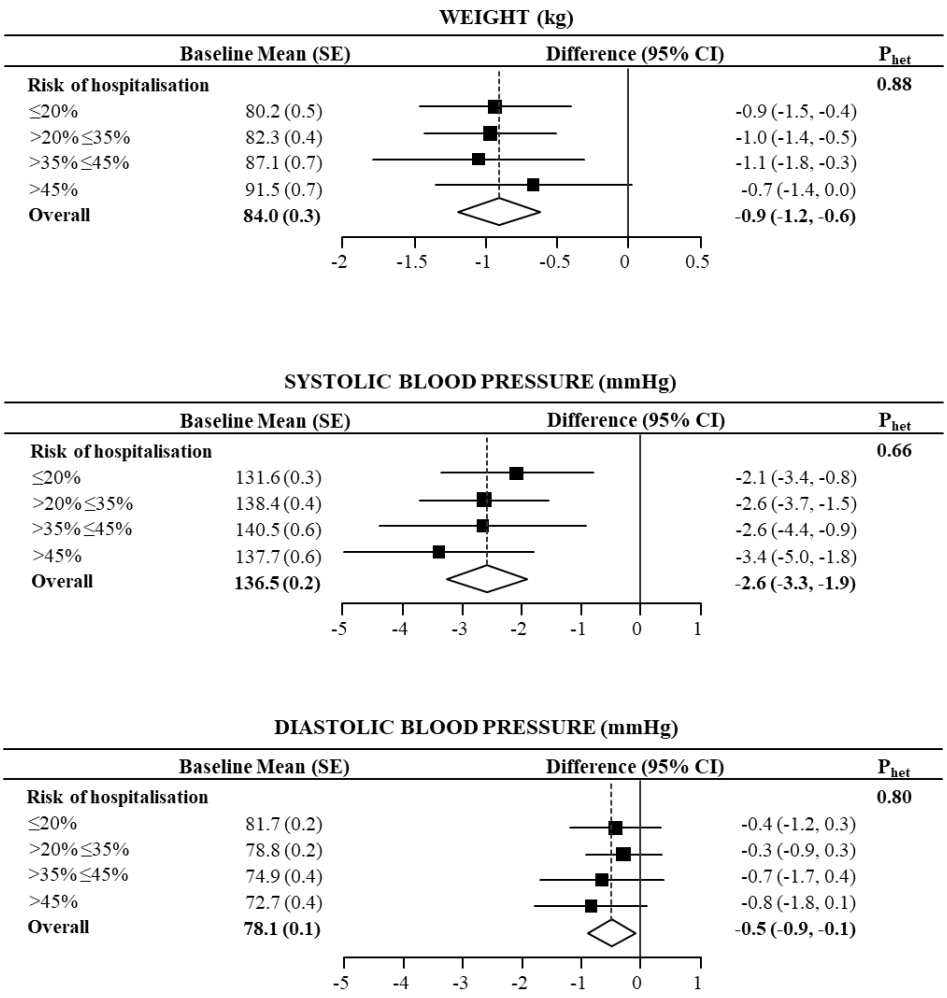
The p values shown are standard chi-square tests for trend across categories of predicted risk of hospitalisation for the relative and estimated absolute effects; respectively. Hazard ratios are not presented for outcomes with fewer than 10 events. * Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. † If subgroup-specific hazard ratios (or CIs) were used to estimate absolute effects on all-cause hospitalization by health-related quality of life, based on P for heterogeneity = 0.01 for relative effects; estimated absolute events avoided (SE) would be 1.3 (20.1), 87.0 (15.9) and 38.8 (28.4) rather than 30.2 (9.4), 36.9 (11.4) and 55.7 (17.2). ‡ ESKD: End-Stage Kidney Disease, defined as start of maintenance dialysis or receipt of a kidney transplant.

7.2.6 EFFECTS OF EMPAGLIFLOZIN ON SAFETY OUTCOMES BY FRAILTY INDICATORS

7.2.6.1 RELATIVE EFFECTS ON SAFETY OUTCOMES

Safety outcomes were more common in participants with indicators of increased frailty, but their incidence was not affected by study treatment. In particular, allocation to empagliflozin relative to placebo did not result in any excess of ketoacidosis, symptomatic dehydration or fractures (Table 7-11 to Table 7-14). Averaged across the follow-up period, total body weight was ~1 kg lower in the empagliflozin versus placebo group (-0.9 [-1.2, -0.6] kg) with similar between-group differences in all frailty (by predicted risk of hospitalisation) subgroups (P for heterogeneity = 0.88; Figure 7-8). Similarly, there was no evidence of heterogeneity of treatment effect on blood pressure (P for heterogeneity = 0.66 and 0.80 for systolic and diastolic blood pressure, respectively; Figure 7-8).

Figure 7-8: Effects of empagliflozin versus placebo on weight and blood pressure by frailty



Study-average differences are adjusted for baseline values of the dependent variable (in continuous form) and for any differences in key baseline characteristics (categories of age, sex, diabetes, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio and region) between treatment groups and weighted in proportion to the amount of time between follow-up visits. Each analysis includes all individuals with measurement of the outcome variable at baseline and at least once during follow-up.

Table 7-11: Safety outcomes by predicted risk of hospitalisation

Predicted risk of hospitalisation (%)	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events caused per 1000 patient-years (SE)	P_{trend}
Serious urinary tract infection						0.99		0.76
≤20%	7/983	3.7	8/1005	4.2			-0.3 (0.8)	
>20% ≤35%	13/1245	5.4	15/1259	6.0			-0.4 (1.1)	
>35% ≤45%	12/487	12.4	13/481	13.7			-0.9 (2.5)	
>45%	20/589	17.0	18/560	16.3			-1.0 (3.0)	
Overall	**52/3304**	**8.1**	**54/3305**	**8.4**	**0.94 (0.64-1.37)**			
Serious genital infection						-		-
≤20%	0/983	0.0	0/1005	0.0			-	
>20% ≤35%	0/1245	0.0	0/1259	0.0			-	
>35% ≤45%	1/487	1.0	0/481	0.0			-	
>45%	0/589	0.0	1/560	0.9			-	
Overall	**1/3304**	**0.2**	**1/3305**	**0.2**	-			
Serious hyperkalaemia						0.59		0.27
≤20%	11/983	5.9	17/1005	9.1			-1.6 (1.1)	
>20% ≤35%	32/1245	13.3	41/1259	16.7			-2.9 (2.0)	
>35% ≤45%	21/487	21.8	16/481	17.0			-2.9 (2.0)	
>45%	28/589	24.3	35/560	32.6			-5.6 (3.8)	
Overall	**92/3304**	**14.4**	**109/3305**	**17.2**	**0.83 (0.63-1.09)**			
Serious acute kidney injury						0.28		0.01
≤20%	7/983	3.7	13/1005	6.9			-1.5 (0.7)	
>20% ≤35%	37/1245	15.4	41/1259	16.6			-3.7 (1.7)	
>35% ≤45%	14/487	14.4	28/481	29.9			-6.6 (3.0)	
>45%	49/589	42.8	53/560	48.6			-10.7 (4.9)	
Overall	**107/3304**	**16.7**	**135/3305**	**21.1**	**0.78 (0.60-1.00)**			
Serious dehydration						0.84		-
≤20%	0/983	0.0	0/1005	0.0			0.0 (0.0)	
>20% ≤35%	12/1245	4.9	8/1259	3.2			0.8 (1.1)	
>35% ≤45%	5/487	5.1	6/481	6.3			1.6 (2.1)	
>45%	13/589	11.0	10/560	9.0			2.3 (3.1)	
Overall	**30/3304**	**4.6**	**24/3305**	**3.7**	**1.25 (0.73-2.14)**			
Liver injury						0.67		0.88
≤20%	2/983	1.1	2/1005	1.1			0.1 (0.5)	
>20% ≤35%	6/1245	2.5	3/1259	1.2			0.1 (0.5)	
>35% ≤45%	2/487	2.0	3/481	3.1			0.3 (1.4)	
>45%	3/589	2.5	4/560	3.6			0.3 (1.6)	
Overall	**13/3304**	**2.0**	**12/3305**	**1.9**	**1.09 (0.50-2.38)**			
Ketoacidosis						-		-
≤20%	0/983	0.0	0/1005	0.0			-	
>20% ≤35%	3/1245	1.2	0/1259	0.0			-	
>35% ≤45%	0/487	0.0	1/481	1.0			-	
>45%	3/589	2.5	0/560	0.0			-	
Overall	**6/3304**	**0.9**	**1/3305**	**0.2**	**6.25 (0.75-52.03)**			
Lower limb amputation						0.40		0.93
≤20%	1/983	0.5	1/1005	0.5			0.2 (0.2)	
>20% ≤35%	7/1245	2.9	1/1259	0.4			0.2 (0.2)	
>35% ≤45%	8/487	8.2	7/481	7.4			3.2 (3.1)	
>45%	12/589	10.2	10/560	9.0			3.9 (3.8)	
Overall	**28/3304**	**4.3**	**19/3305**	**2.9**	**1.43 (0.80-2.57)**			
Bone fracture						0.29		0.77
≤20%	23/983	12.4	28/1005	15.0			1.1 (2.0)	
>20% ≤35%	38/1245	15.8	34/1259	13.8			1.0 (1.9)	
>35% ≤45%	35/487	36.9	22/481	23.4			1.8 (3.2)	
>45%	37/589	32.2	39/560	36.0			2.7 (4.8)	
Overall	**133/3304**	**20.9**	**123/3305**	**19.3**	**1.08 (0.84-1.38)**			
Severe hypoglycaemia‡						0.45		0.96
≤20%	4/983	2.1	2/1005	1.1			-0.0 (0.2)	
>20% ≤35%	21/1245	8.7	29/1259	11.7			-0.1 (1.9)	
>35% ≤45%	17/487	17.6	20/481	21.3			-0.1 (3.4)	
>45%	35/589	30.5	26/560	24.1			-0.1 (3.9)	
Overall	**77/3304**	**12.0**	**77/3305**	**12.1**	**<1.00 (0.73-1.37)**			
Symptomatic dehydration§						0.995		0.59
≤20%	10/983	5.4	10/1005	5.3			0.5 (0.9)	
>20% ≤35%	30/1245	12.5	27/1259	10.9			1.1 (1.9)	
>35% ≤45%	14/487	14.6	12/481	12.7			1.3 (2.2)	
>45%	29/589	25.1	27/560	24.7			2.6 (4.3)	
Overall	**83/3304**	**13.0**	**76/3305**	**11.9**	**1.10 (0.81-1.51)**			

* See section 2.7.5 for methods. ‡ Defined as low blood sugar causing severe cognitive impairment which requires assistance from another person for recovery.
§ Defined as whether or not a participant has experienced symptoms they attribute to dehydration, such as feeling faint or fainting.

Table 7-12: Safety outcomes by multimorbidity

No. of conditions (excluding CKD)	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events caused per 1000 patient-years (SE)	P_{trend}
Serious urinary tract infection						0.42		0.87
≤1	18/1924	4.9	23/1940	6.2			-0.4 (1.1)	
2	13/706	9.2	15/663	11.3			-0.7 (2.1)	
≥3	21/674	15.8	16/702	11.5			-0.7 (2.1)	
Overall	**52/3304**	**8.1**	**54/3305**	**8.4**	**0.94 (0.64-1.37)**			
Serious genital infection								-
≤1	0/1924	0.0	0/1940	0.0		-	-	
2	0/706	0.0	0/663	0.0			-	
≥3	1/674	0.7	1/702	0.7			-	
Overall	**1/3304**	**0.2**	**1/3305**	**0.2**	**-**			
Serious hyperkalaemia						0.61		0.66
≤1	50/1924	13.6	51/1940	13.9			-2.4 (1.6)	
2	22/706	15.8	30/663	23.2			-4.0 (2.7)	
≥3	20/674	15.2	28/702	20.2			-3.5 (2.4)	
Overall	**92/3304**	**14.4**	**109/3305**	**17.2**	**0.83 (0.63-1.09)**			
Serious acute kidney injury						0.64		0.15
≤1	35/1924	9.5	51/1940	13.8			-3.0 (1.4)	
2	35/706	25.1	36/663	27.5			-6.1 (2.8)	
≥3	37/674	28.3	48/702	34.7			-7.6 (3.5)	
Overall	**107/3304**	**16.7**	**135/3305**	**21.1**	**0.78 (0.60-1.00)**			
Serious dehydration						0.77		0.60
≤1	8/1924	2.2	8/1940	2.1			0.5 (0.7)	
2	9/706	6.3	5/663	3.7			0.9 (1.3)	
≥3	13/674	9.7	11/702	7.9			2.0 (2.7)	
Overall	**30/3304**	**4.6**	**24/3305**	**3.7**	**1.25 (0.73-2.14)**			
Liver injury						0.55		0.88
≤1	7/1924	1.9	4/1940	1.1			0.1 (0.5)	
2	3/706	2.1	3/663	2.2			0.2 (1.0)	
≥3	3/674	2.2	5/702	3.6			0.3 (1.5)	
Overall	**13/3304**	**2.0**	**12/3305**	**1.9**	**1.09 (0.50-2.38)**			
Ketoacidosis						-		-
≤1	1/1924	0.3	0/1940	0.0			-	
2	2/706	1.4	0/663	0.0			-	
≥3	3/674	2.2	1/702	0.7			-	
Overall	**6/3304**	**0.9**	**1/3305**	**0.2**	**6.25 (0.75-52.03)**			
Lower limb amputation						0.33		0.20
≤1	7/1924	1.9	2/1940	0.5			0.2 (0.2)	
2	11/706	7.8	6/663	4.5			1.9 (1.9)	
≥3	10/674	7.5	11/702	7.9			3.4 (3.4)	
Overall	**28/3304**	**4.3**	**19/3305**	**2.9**	**1.43 (0.80-2.57)**			
Bone fracture						0.09		0.77
≤1	68/1924	18.6	52/1940	14.1			1.1 (1.9)	
2	23/706	16.4	33/663	25.2			1.9 (3.4)	
≥3	42/674	32.4	38/702	27.7			2.1 (3.7)	
Overall	**133/3304**	**20.9**	**123/3305**	**19.3**	**1.08 (0.84-1.38)**			
Severe hypoglycaemia[‡]						0.34		0.97
≤1	19/1924	5.1	13/1940	3.5			-0.0 (0.6)	
2	26/706	18.7	23/663	17.6			-0.1 (2.8)	
≥3	32/674	24.5	41/702	30.2			-0.1 (4.9)	
Overall	**77/3304**	**12.0**	**77/3305**	**12.1**	**<1.00 (0.73-1.37)**			
Symptomatic dehydration[§]						0.74		0.73
≤1	31/1924	8.4	30/1940	8.1			0.8 (1.4)	
2	23/706	16.5	16/663	12.1			1.3 (2.1)	
≥3	29/674	22.2	30/702	21.8			2.3 (3.8)	
Overall	**83/3304**	**13.0**	**76/3305**	**11.9**	**1.10 (0.81-1.51)**			

* Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. [‡] Defined as low blood sugar causing severe cognitive impairment which requires assistance from another person for recovery. [§] Defined as whether or not a participant has experienced symptoms they attribute to dehydration, such as feeling faint or fainting.

Table 7-13: Safety outcomes by concomitant medication count

No. of concomitant medications	Empagliflozin		Placebo		Relative effects		Estimated absolute effects*	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events caused per 1000 patient-years (SE)	P_{trend}
Serious urinary tract infection						0.40		0.87
≤5	8/1128	3.6	12/1121	5.5			-0.3 (1.0)	
≥6 <9	14/1010	7.1	17/1004	8.7			-0.5 (1.6)	
≥9	30/1166	13.2	25/1180	10.8			-0.7 (2.0)	
Overall	**52/3304**	**8.1**	**54/3305**	**8.4**	**0.94 (0.64-1.37)**			
Serious genital infection						-		-
≤5	0/1128	0.0	0/1121	0.0			-	
≥6 <9	0/1010	0.0	1/1004	0.5			-	
≥9	1/1166	0.4	0/1180	0.0			-	
Overall	**1/3304**	**0.2**	**1/3305**	**0.2**	-			
Serious hyperkalaemia						0.97		0.64
≤5	24/1128	11.0	29/1121	13.5			-2.3 (1.6)	
≥6 <9	24/1010	12.2	30/1004	15.5			-2.7 (1.8)	
≥9	44/1166	19.6	50/1180	22.1			-3.8 (2.6)	
Overall	**92/3304**	**14.4**	**109/3305**	**17.2**	**0.83 (0.63-1.09)**			
Serious acute kidney injury						0.26		0.12
≤5	16/1128	7.3	23/1121	10.6			-2.3 (1.1)	
≥6 <9	25/1010	12.6	42/1004	21.7			-4.8 (2.2)	
≥9	66/1166	29.5	70/1180	30.6			-6.7 (3.1)	
Overall	**107/3304**	**16.7**	**135/3305**	**21.1**	**0.78 (0.60-1.00)**			
Serious dehydration						0.30		0.57
≤5	1/1128	0.5	3/1121	1.4			0.3 (0.5)	
≥6 <9	4/1010	2.0	5/1004	2.6			0.6 (0.9)	
≥9	25/1166	11.0	16/1180	6.9			1.7 (2.4)	
Overall	**30/3304**	**4.6**	**24/3305**	**3.7**	**1.25 (0.73-2.14)**			
Liver injury						0.22		0.97
≤5	6/1128	2.7	3/1121	1.4			0.1 (0.6)	
≥6 <9	4/1010	2.0	2/1004	1.0			0.1 (0.4)	
≥9	3/1166	1.3	7/1180	3.0			0.3 (1.3)	
Overall	**13/3304**	**2.0**	**12/3305**	**1.9**	**1.09 (0.50-2.38)**			
Ketoacidosis						-		-
≤5	2/1128	0.9	0/1121	0.0			-	
≥6 <9	3/1010	1.5	0/1004	0.0			-	
≥9	1/1166	0.4	1/1180	0.4			-	
Overall	**6/3304**	**0.9**	**1/3305**	**0.2**	**6.25 (0.75-52.03)**			
Lower limb amputation						0.96		0.22
≤5	2/1128	0.9	1/1121	0.5			0.2 (0.2)	
≥6 <9	10/1010	5.1	7/1004	3.6			1.5 (1.5)	
≥9	16/1166	7.0	11/1180	4.7			2.0 (2.0)	
Overall	**28/3304**	**4.3**	**19/3305**	**2.9**	**1.43 (0.80-2.57)**			
Bone fracture						0.29		0.75
≤5	36/1128	16.6	25/1121	11.6			0.9 (1.6)	
≥6 <9	42/1010	21.6	34/1004	17.5			1.3 (2.4)	
≥9	55/1166	24.5	64/1180	28.2			2.1 (3.8)	
Overall	**133/3304**	**20.9**	**123/3305**	**19.3**	**1.08 (0.84-1.38)**			
Severe hypoglycaemia[‡]						0.50		0.98
≤5	8/1128	3.6	5/1121	2.3			-0.0 (0.4)	
≥6 <9	20/1010	10.2	16/1004	8.2			-0.0 (1.3)	
≥9	49/1166	21.8	56/1180	24.8			-0.1 (4.0)	
Overall	**77/3304**	**12.0**	**77/3305**	**12.1**	**<1.00 (0.73-1.37)**			
Symptomatic dehydration[§]						0.60		0.80
≤5	13/1128	6.0	16/1121	7.4			0.8 (1.3)	
≥6 <9	18/1010	9.2	16/1004	8.2			0.9 (1.4)	
≥9	52/1166	23.2	44/1180	19.2			2.0 (3.4)	
Overall	**83/3304**	**13.0**	**76/3305**	**11.9**	**1.10 (0.81-1.51)**			

* Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. Hazard ratios are not presented for outcomes with <10 events. [‡] Defined as low blood sugar causing severe cognitive impairment which requires assistance from another person for recovery. [§] Defined as whether or not a participant has experienced symptoms they attribute to dehydration, such as feeling faint or fainting.

Table 7-14: Safety outcomes by health-related quality of life

EQ-5D index value*	Empagliflozin		Placebo		Relative effects		Estimated absolute effects†	
	n/N	Rate per 1000 patient-years	n/N	Rate per 1000 patient-years	Hazard Ratio (95% CI)	P_{het}	Events caused per 1000 patient-years (SE)	P_{trend}
Serious urinary tract infection						0.55		0.88
>0.987	8/1064	3.9	11/1083	5.3			-0.3 (1.0)	
>0.811 ≤0.987	17/1117	7.8	13/1142	5.9			-0.4 (1.1)	
≤0.811	27/1123	12.1	30/1080	14.0			-0.9 (2.5)	
Overall	**52/3304**	**8.1**	**54/3305**	**8.4**	**0.94 (0.64-1.37)**			
Serious genital infection						-		-
>0.987	0/1064	0.0	0/1083	0.0			-	
>0.811 ≤0.987	0/1117	0.0	0/1142	0.0			-	
≤0.811	1/1123	0.4	1/1080	0.5			-	
Overall	**1/3304**	**0.2**	**1/3305**	**0.2**	-			
Serious hyperkalaemia						0.87		0.81
>0.987	25/1064	12.4	33/1083	16.0			-2.8 (1.9)	
>0.811 ≤0.987	22/1117	10.1	28/1142	12.9			-2.2 (1.5)	
≤0.811	45/1123	20.4	48/1080	22.8			-3.9 (2.7)	
Overall	**92/3304**	**14.4**	**109/3305**	**17.2**	**0.83 (0.63-1.09)**			
Serious acute kidney injury						0.11		0.22
>0.987	26/1064	12.8	27/1083	13.0			-2.9 (1.3)	
>0.811 ≤0.987	23/1117	10.6	45/1142	20.7			-4.6 (2.1)	
≤0.811	58/1123	26.4	63/1080	29.6			-6.5 (3.0)	
Overall	**107/3304**	**16.7**	**135/3305**	**21.1**	**0.78 (0.60-1.00)**			
Serious dehydration						0.25		0.62
>0.987	4/1064	2.0	4/1083	1.9			0.5 (0.7)	
>0.811 ≤0.987	7/1117	3.2	10/1142	4.6			1.1 (1.6)	
≤0.811	19/1123	8.5	10/1080	4.6			1.2 (1.6)	
Overall	**30/3304**	**4.6**	**24/3305**	**3.7**	**1.25 (0.73-2.14)**			
Liver injury						0.41		0.89
>0.987	5/1064	2.5	2/1083	1.0			0.1 (0.4)	
>0.811 ≤0.987	3/1117	1.4	5/1142	2.3			0.2 (1.0)	
≤0.811	5/1123	2.2	5/1080	2.3			0.2 (1.0)	
Overall	**13/3304**	**2.0**	**12/3305**	**1.9**	**1.09 (0.50-2.38)**			
Ketoacidosis						-		-
>0.987	1/1064	0.5	1/1083	0.5			-	
>0.811 ≤0.987	1/1117	0.5	0/1142	0.0			-	
≤0.811	4/1123	1.8	0/1080	0.0			-	
Overall	**6/3304**	**0.9**	**1/3305**	**0.2**	**6.25 (0.75-52.03)**			
Lower limb amputation						0.17		0.28
>0.987	8/1064	3.9	1/1083	0.5			0.2 (0.2)	
>0.811 ≤0.987	6/1117	2.7	4/1142	1.8			0.8 (0.8)	
≤0.811	14/1123	6.3	14/1080	6.5			2.8 (2.8)	
Overall	**28/3304**	**4.3**	**19/3305**	**2.9**	**1.43 (0.80-2.57)**			
Bone fracture						0.39		0.79
>0.987	37/1064	18.4	27/1083	13.0			1.0 (1.8)	
>0.811 ≤0.987	37/1117	17.1	38/1142	17.5			1.3 (2.4)	
≤0.811	59/1123	27.0	58/1080	27.4			2.1 (3.7)	
Overall	**133/3304**	**20.9**	**123/3305**	**19.3**	**1.08 (0.84-1.38)**			
Severe hypoglycaemia[§]						0.20		0.98
>0.987	11/1064	5.4	6/1083	2.9			-0.0 (0.5)	
>0.811 ≤0.987	22/1117	10.2	17/1142	7.8			-0.0 (1.2)	
≤0.811	44/1123	19.9	54/1080	25.7			-0.1 (4.1)	
Overall	**77/3304**	**12.0**	**77/3305**	**12.1**	**<1.00 (0.73-1.37)**			
Symptomatic dehydration[ǀ]						0.82		0.58
>0.987	12/1064	5.9	9/1083	4.3			0.4 (0.8)	
>0.811 ≤0.987	25/1117	11.6	25/1142	11.5			1.2 (2.0)	
≤0.811	46/1123	20.9	42/1080	19.8			2.0 (3.5)	
Overall	**83/3304**	**13.0**	**76/3305**	**11.9**	**1.10 (0.81-1.51)**			

* Weighted index of 5 EQ-5D domain scores (mobility, self-care, usual activities, pain/discomfort and anxiety/depression); lower values indicate poorer quality of life. † Absolute events avoided per 1000 patients treated with empagliflozin for 1 year (SE) were estimated by applying the overall hazard ratio to the subgroup-specific event rate per 1000 patient-years in the placebo group. § Defined as low blood sugar causing severe cognitive impairment which requires assistance from another person for recovery. ǀ Defined as whether or not a participant has experienced symptoms they attribute to dehydration, such as feeling faint or fainting.

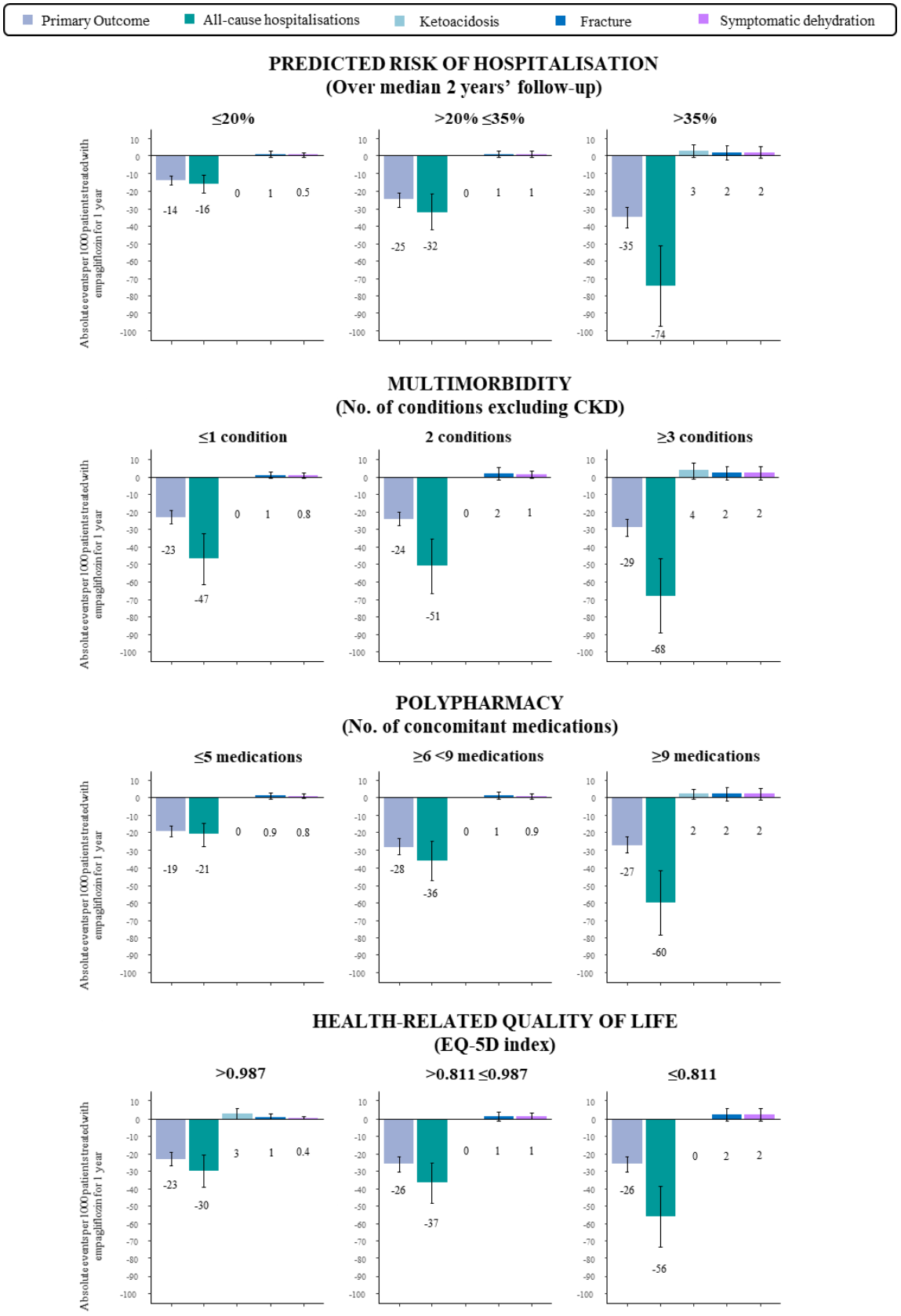
7.2.6.2 ABSOLUTE EFFECTS ON SAFETY OUTCOMES

Uncertainty exists around the estimates of the effect of empagliflozin on safety outcomes due to low event numbers overall. However, on an absolute scale, there were numerically more excess occurrences of fracture and symptomatic dehydration in participants in the highest (versus lowest) categories of frailty, multimorbidity and polypharmacy and in those with poorest (versus greatest) HRQoL although there was no statistical evidence of trend and even in the highest risk participants, these events were infrequent (Table 7-11 to Table 7-14).

7.2.7 AGGREGATED ABSOLUTE EFFECTS OF EMPAGLIFLOZIN BY FRAILTY INDICATORS

When estimated absolute benefits of empagliflozin versus placebo were plotted alongside estimated absolute potential harms, across frailty indicator levels, it could be seen that the estimated absolute benefits substantially outweighed any potential serious harms in the studied population (Figure 7-9).

Figure 7-9: Absolute benefits and harms of empagliflozin per 1000 patient-years by frailty



Absolute events avoided per 1000 patients treated with empagliflozin per 1 year (and SE represented by error bars) were estimated by applying the overall hazard ratio (since no significant trend was observed across subgroups) to the event rate per 1000 patient-years in the placebo group. Pre-specified analyses of all-cause hospitalisations include first and recurrent events; all other events are time-to-first event analyses.

7.2.8 EFFECTS OF EMPAGLIFLOZIN ON BIOIMPEDANCE PARAMETERS BY FRAILTY INDICATORS

All of the indicators of frailty and related metrics showed clear patterns of association with bioimpedance parameters at baseline in the 635 participants with a valid baseline bioimpedance measurement. Increased absolute "Fluid Overload", increased adipose tissue mass and decreased lean tissue mass were associated with greater levels of frailty (by predicted risk of hospitalisation, Figure 7-10), greater numbers of comorbid conditions (Figure 7-11) and concomitant medications (Figure 7-12) and poorer HRQoL (Figure 7-13).

Figure 7-10: Associations between predicted risk of hospitalisation and bioimpedance parameters at baseline

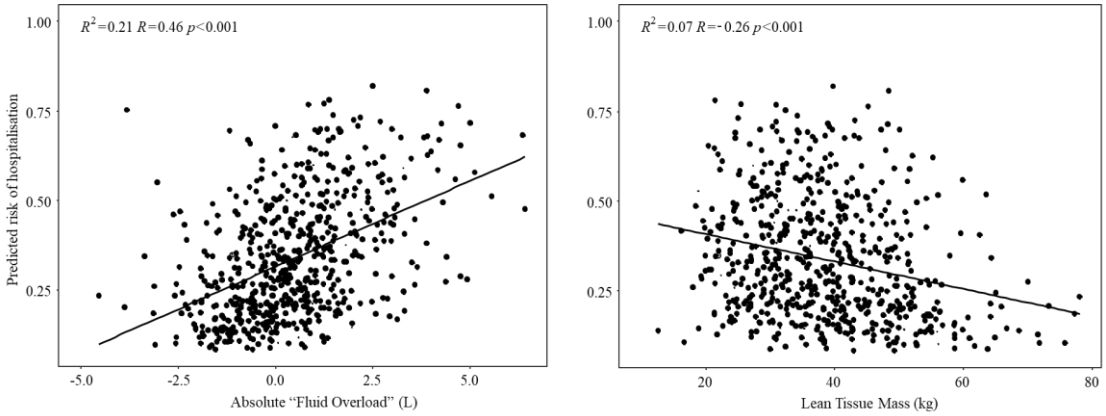


Figure 7-11: Associations between multimorbidity and bioimpedance parameters at baseline

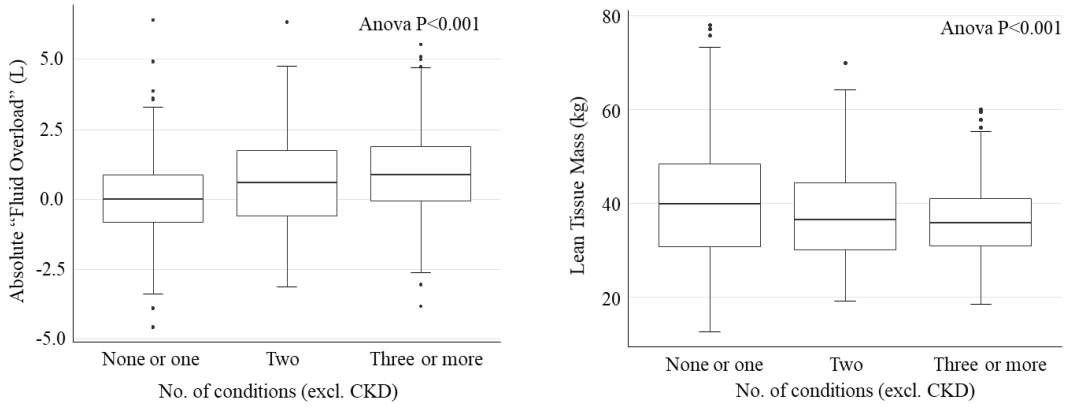


Figure 7-12: Associations between polypharmacy and bioimpedance parameters at baseline

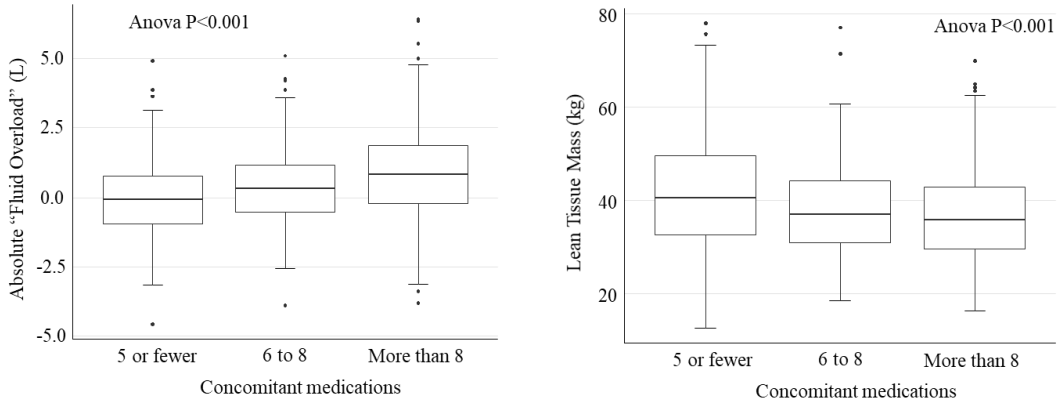
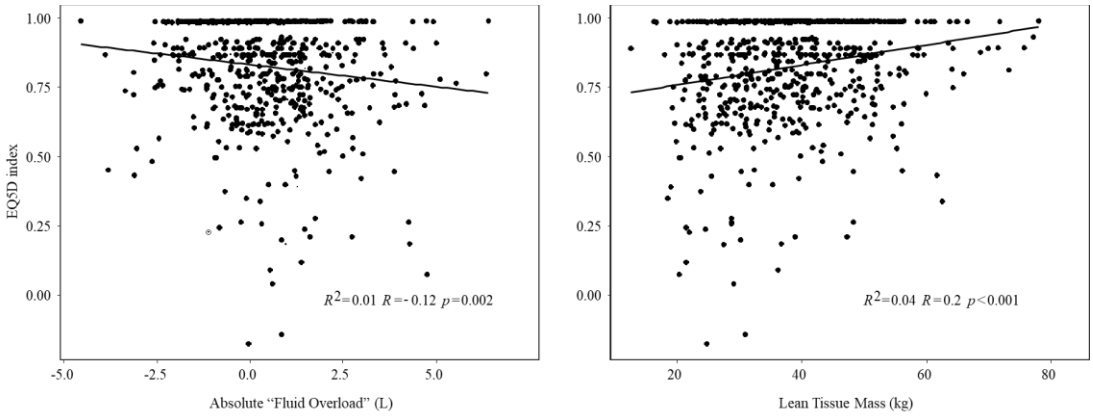
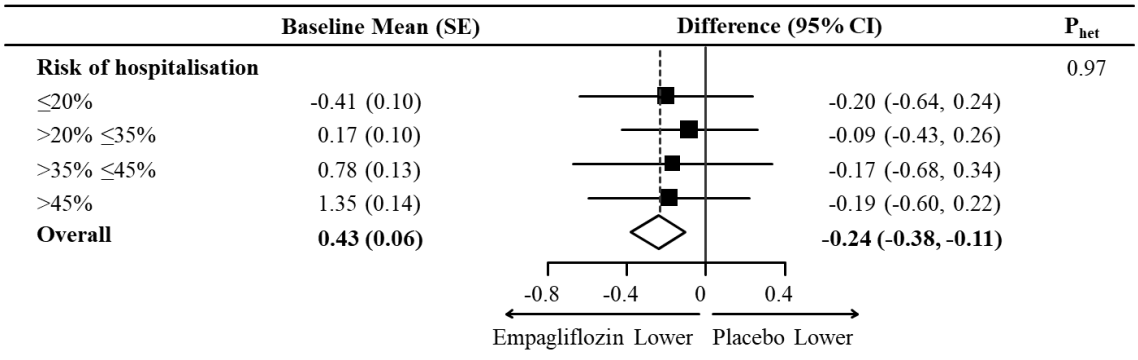


Figure 7-13: Associations between HRQoL (EQ5D index) and bioimpedance parameters at baseline



The effect of empagliflozin on "Fluid Overload" in the bioimpedance substudy (reported in Chapter 4) was not modified by predicted risk of hospitalisation at baseline (Figure 7-14).

Figure 7-14: Effect of empagliflozin on absolute "Fluid Overload" (L) by predicted risk of hospitalisation



7.3 DISCUSSION

In EMPA-KIDNEY, there was no evidence of effect modification on a relative scale for the primary outcome, hospitalisation or any of the safety assessments by any of the indicators of frailty (or related characteristics). Adverse events and treatment discontinuation occurred more frequently in patients with a greater degree of frailty but neither adverse events nor treatment discontinuation were more common with SGLT2 inhibition than with placebo irrespective of levels of frailty. In absolute terms, since those with indicators of frailty were generally at higher baseline risk of kidney disease progression/cardiovascular death and hospitalisation, these participants experienced the largest absolute net benefits.

The results of these analyses demonstrate that consistent treatment effects on the relative scale do not imply lack of difference in treatment effects on the absolute scale. Understanding such heterogeneity of treatment effect is critical to inform evidence-based medicine and individualised patient care. It is essential to conduct these analyses for both efficacy and safety outcomes since the group which derived the largest absolute benefits on kidney outcomes were also the group with the highest frequency of adverse events. Presenting both the estimated absolute benefits and potential harms (Figure 7-9) allows clear communication of benefits versus risks of treatment and can be used in patient and family discussions.

Frailty assessments can broadly be split into either frailty phenotypes or frailty indices. The former (such as the Fried frailty phenotype (Fried et al., 2001)) requires physical assessments (e.g. grip strength) which are not available in streamlined trials such as EMPA-KIDNEY and include components which can be subjective (Clark et al., 2021). Commonly used frailty indicators are derived from general populations and not widely validated across the spectrum of CKD (Hurst et al., 2022, Anderson et al., 2021, Nixon et al., 2019). Furthermore, frailty phenotypes and frailty indices have been found to produce vastly different estimates of frailty prevalence in CKD (Worthen et al., 2021). Within the limitations of randomised controlled trials designed to answer a different question, analyses of frailty have typically been conducted using the Rockwood Frailty Index (Searle et al., 2008). This approach has been used in exploratory analyses of the DELIVER (Butt et al., 2022c), DAPA-HF (Butt et al., 2022b) and DAPA-CKD (Vart et al., 2023) trials to

assess whether the effects of SGLT2 inhibition in patients with heart failure or proteinuric CKD (mainly diabetic kidney disease) may vary by level of frailty at recruitment. The Rockwood Frailty Index has several limitations in this context: it was developed in community-dwelling adults aged over 70 years in the United States of America and applies weights to each comorbidity (Searle et al., 2008) which may not be generalisable across diseased populations like in CKD. Therefore, a bespoke approach was used in analyses of the EMPA-KIDNEY trial population based upon baseline predicted risk of hospitalisation during follow-up due to the established association between hospital use and clinical frailty. The "predicted risk of hospitalisation" frailty indicator derived in EMPA-KIDNEY was an effective discriminator of risk of adverse outcomes, specifically hospitalisation and mortality, which are the most commonly reported outcomes in studies of frailty in CKD (Hurst et al., 2022). Predicted risk of hospitalisation correlated with other concepts related to frailty, namely multimorbidity, polypharmacy and health-related quality of life. Clear evidence of greater absolute benefits was observed when clinical risk reflected multiple domains (using predicted risk of hospitalisation) which was less apparent when considering multimorbidity, polypharmacy or health-related quality of life in isolation. Developing a multivariable model predicting risk of hospitalisation using numerous clinical characteristics follows the approach described as "predictive heterogeneity of treatment effect (HTE) analysis" in the PATH Statement (Kent et al., 2020). This more finely characterises individual participants' combined risk of adverse outcomes based on multiple characteristics and may expose important heterogeneity not manifested in conventional subgroup analyses stratifying by a single variable (which may underestimate true clinical heterogeneity) (Kent and Hayward, 2007). Predictive HTE analysis using an internal model (developed using trial data) is an accepted approach though carries the risk of exaggerating the extent of heterogeneity due to overfitting data. Use of an externally-developed model is preferred to overcome this limitation however a validated risk prediction model for all-cause hospitalisations in non-dialysis chronic kidney disease does not exist.

Analyses according to concomitant medication count may be more difficult to interpret than frailty (predicted risk of hospitalisation) or multimorbidity. Numerical polypharmacy categorisations do not take into account appropriateness of treatment and polypharmacy is expected in CKD, particularly with implementation of recent advances in disease-modifying treatments for CKD and cardiovascular disease. Polypharmacy may therefore

reflect individuals on appropriate medical therapy with multiple agents which can be expected to improve prognosis; yet polypharmacy also reflects extent of disease burden with cumulative deleterious impacts upon prognosis. The number of medications a patient is taking is likely therefore to be a poorer discriminant than other metrics related to frailty or multimorbidity. It is commonly reported that as the number of prescribed drugs increases, so do the chances of adverse drug events and likelihood of harm (Hanlon et al., 2020). In EMPA-KIDNEY, adverse events were numerically more frequent in those taking more medications but importantly and reassuringly, there were no significant increased harms of empagliflozin associated with polypharmacy, compared with placebo.

These analyses have some limitations. The assessment of frailty is limited by lack of standardised frailty assessment tools which have been validated in CKD populations. A formal frailty index was not applied in EMPA-KIDNEY due to the limitations of these tools and data availability however the bespoke frailty indicators derived in the EMPA-KIDNEY population were employed to derive a scientifically robust assessment of frailty using predicted risk of hospitalisation based on its established associations with clinical frailty. This tool also performed well for prediction of death. Although considered an appropriately robust assessment of frailty, this approach means results are not directly comparable to data from other populations. Furthermore, the selected clinical trial population is likely to exhibit lower levels of frailty and hospitalisation risk than the general CKD population and results may not be extrapolated to the extremes of frailty in a general CKD population. Furthermore, measures of multimorbidity in EMPA-KIDNEY are an underestimate since retrospectively derived based on pre-specified data collected (based upon questions asked at randomisation which largely capture cardiovascular disease and do not include, for example, respiratory or neuropsychiatric disease).

In conclusion, empagliflozin safely lowered risk of progression of CKD or death from cardiovascular causes among a broad range of patients with CKD, irrespective of baseline frailty (or related metrics). Absolute benefits for the primary outcome were greater in frailer patients and far outweighed potential harms. Clinical guidelines should therefore encourage evidence-based prescribing of SGLT2 inhibitors even in frail individuals with CKD and emphasise that these patients may stand to gain most from treatment.

CHAPTER 8 – FINAL DISCUSSION

This chapter assimilates the key findings from Chapters 3-7 and discusses implications of the work.

8.1 SUMMARY OF FINDINGS

The main conclusions from each research question are summarised in this section.

Research question 1: Is bioimpedance spectroscopy a valuable tool in clinical and research settings in non-dialysis chronic kidney disease (CKD) - what are the associations between bioimpedance-derived fluid overload and clinical outcomes?

Chapter 3 reports the first systematic review assessing associations between bioimpedance indices of fluid excess and cardiorenal outcomes in non-dialysis CKD and heart failure cohorts. Bioimpedance indices of fluid excess were consistently positively associated with mortality and adverse cardiovascular outcomes in included populations with non-dialysis CKD and/or heart failure. These associations were analogous to previously well-established associations in patients with kidney failure requiring dialysis in whom clinically apparent fluid excess is much more prevalent. From the systematic review, thresholds of moderate and severe "Fluid Overload" (>7%, ≤15 and >15% relative "Fluid Overload", respectively) were proposed and applied in analyses of the EMPA-KIDNEY bioimpedance substudy.

The systematic review confirmed that a variety of bioimpedance devices are in use in clinical practice and research settings. This presented a challenge in aggregating outcomes using different methods of assessment therefore conclusions were qualitative only. This heterogeneity also acts as a barrier to the efficient adoption of bioimpedance in clinical and research contexts. A key advantage of the BCM device, which was selected for use in the EMPA-KIDNEY bioimpedance substudy, over all other commercially-available bioimpedance devices is the ability to quantify fluid status independent of body composition (i.e. lean and adipose tissue mass). This device therefore seems to be the optimum currently-available method to assess fluid excess for patients with CKD and/or heart failure and is the most widely used in dialysis practice, favouring its adoption in earlier stage CKD. The success of the EMPA-KIDNEY bioimpedance substudy (research questions 2 and 3; Chapters 4 and 5) demonstrates that bioimpedance spectroscopy can be

successfully incorporated into a streamlined randomised trial design and provide valuable outcome data and mechanistic insight.

Research question 2: What are the effects of empagliflozin on fluid status estimated by bioimpedance spectroscopy?

Empagliflozin, compared with placebo, caused a sustained reduction in body water which occurred early (by 2 months) and was maintained until at least 18 months. Quantitatively, the between-group difference in the primary outcome measure of absolute "Fluid Overload" was -0.24 (-0.38, -0.11) L. This "Fluid Overload" parameter represents *excess* ECW; the difference in *total* ECW between the treatment groups was -0.49 (-0.69, -0.30) L. Added to a between-group difference in ICW of -0.30 (-0.57, -0.03) L; the difference in total body water between the groups was ~0.8 L. Substudy average total body weight was 0.7 kg lower in participants allocated to empagliflozin therefore, in EMPA-KIDNEY, sodium-glucose cotransporter 2 (SGLT2) inhibitor-induced weight loss was largely explained by fluid loss. The diuretic effects of empagliflozin appear to be maintained across the spectrum of eGFR studied in EMPA-KIDNEY and no significant heterogeneity of treatment effect was noted in any of the studied subgroups including by sex, diabetes and NT-proBNP at baseline.

Research question 3: What are the effects of empagliflozin on body composition and is weight lost due to fluid or reduced adiposity?

Empagliflozin had no statistically significant observable effect on fat or lean tissue parameters in EMPA-KIDNEY. This finding makes sense in CKD since at lower levels of glomerular filtration, glycosuric effects are attenuated (particularly in patients without diabetes) resulting in lower propensity for caloric and fat loss. Taken together with the effects on fluid parameters which equate to the between-group difference in body weight, it can therefore be concluded that in patients with CKD, the weight lost during SGLT2 inhibitor treatment is accounted for by loss of fluid and not body tissue (fat or lean tissue). Like the effects on "Fluid Overload", the effects of empagliflozin on total body weight occurred early and were sustained across the follow-up period and neither was there any significant heterogeneity in subgroup analyses, supporting the conclusion that weight loss is explained by diuresis since similar patterns were observed. It had previously been suggested that SGLT2 inhibitors may cause a degree of breakdown of skeletal muscle and loss of lean tissue mass (Zhang et al., 2023, Sasaki et al., 2019), a theoretical concern in

underweight or malnourished patients. This theory was based on gluconeogenesis as a result of reduced glucose reabsorption leading to lipolysis of adipose tissue and proteolysis in skeletal muscle (Sasaki, 2019) so may be less relevant in patients with CKD without diabetes (in whom glycosuria is attenuated). There was no significant difference in lean tissue mass between participants allocated to empagliflozin or placebo in EMPA-KIDNEY, providing reassurance that SGLT2 inhibitors can be safely used without concern of loss of lean tissue. The effects of empagliflozin on weight translated into minor differences in body mass index and waist-to-hip ratio however these are imperfect markers of body composition which cannot distinguish changes in body water from tissue mass, evidencing the value of bioimpedance in this work.

Research question 4: What are the effects of empagliflozin on blood pressure and how do these relate to effects on fluid status?

Empagliflozin caused modest reductions in both systolic and diastolic blood pressure which were sustained throughout the follow-up period but particularly marked early during follow-up, consistent with the acute haemodynamic effect of SGLT2 inhibition. The between-group differences in blood pressure were -2.6 mmHg (95% CI -3.3, -1.9) and -0.5 mmHg (95% CI -0.9, -0.1) for systolic and diastolic blood pressure, respectively. Subgroup analyses revealed larger antihypertensive effects in patients with diabetes but preserved blood pressure lowering across eGFR and other key subgroups. Accepting the possibility of a chance finding considering multiplicity of testing, the exaggerated blood pressure lowering effect in patients with diabetes cannot be easily explained and requires further research into the differing mechanisms of SGLT2 inhibition in patients with and without diabetes. What these differing patterns in antihypertensive effects suggest, compared with consistent reductions in weight and “Fluid Overload” across time and subgroups, is that the antihypertensive effects of SGLT2 inhibitors cannot be solely or largely explained by diuresis and other mechanisms must be contributing, such as effects on endothelial function and vascular stiffness (Lytvyn et al., 2017, Lytvyn et al., 2022, Cherney et al., 2014).

Research question 5: What is the impact of frailty, multimorbidity, polypharmacy and health-related quality of life on the effects of empagliflozin on clinical outcomes and physical measurements?

In EMPA-KIDNEY, the relative effects of empagliflozin on kidney disease progression were maintained across the studied population irrespective of baseline levels of frailty (based upon predicted risk of hospitalisation at baseline), multimorbidity, polypharmacy or health-related quality of life (HRQoL). Estimating the absolute effects of treatment, participants with the highest levels of frailty actually received the greatest absolute benefits owing to higher baseline risk of adverse outcomes. These patients are also those most at risk of adverse effects of treatment however there was no significant excess of adverse events in the empagliflozin group relative to placebo in any frailty category. It can therefore be concluded that the benefits of empagliflozin treatment considerably outweigh the harms even in those with the highest burden of frailty amongst patients studied. Analyses were broadly consistent when considering multimorbidity, polypharmacy and HRQoL (which overlapped considerably with predicted risk of hospitalisation) but with less clear trends in absolute effects, likely because these metrics reflect a single domain whereas predicted risk of hospitalisation as a surrogate for frailty likely more completely captures risk.

Predicted risk of hospitalisation was effectively used as a surrogate for frailty in analyses of the EMPA-KIDNEY trial based on its established associations with clinical frailty, aiming to address limitations of other available methods, in the absence of a gold standard method of assessment of frailty within clinical trial data. A model was derived using logistic regression which satisfactorily discriminated between participants who were hospitalised versus not and between participants who died versus survived. The strongest predictor of hospitalisation of the variables available for analysis was found to be N-terminal pro B-type natriuretic peptide (NT-proBNP), a surrogate (albeit non-specific) for fluid overload; and self-reported ankle swelling was also a significant independent predictor of all-cause hospitalisation and therefore retained in the final model. Taken together, the effects of empagliflozin in reducing fluid excess may therefore be considered to indirectly have beneficial effects on clinical frailty since markers of fluid excess (NT-proBNP, ankle swelling) were important determinants of hospitalisation risk inferring clinical frailty. These data also suggest that the diuretic effects of SGLT2 inhibitors are likely to contribute to the reductions in hospitalisations and heart failure which have been observed in meta-analyses of the SGLT2 inhibitor trials including EMPA-KIDNEY.

In addition, the effects of empagliflozin on weight, blood pressure and "Fluid Overload" were not modified by baseline predicted risk of hospitalisation. This provides reassurance to prescribing clinicians that these additional benefits of SGLT2 inhibition prescribed for kidney function preservation can be achieved safely even in individuals at greater risk of adverse effects due to low body weight, frailty and associated increased risks of postural hypotension and falls. The finding from the bioimpedance substudy that empagliflozin had no significant effect on lean tissue mass also further supports the safe use of these drugs in patients with frailty in whom muscle mass preservation and risk of falls are important clinical considerations. These supplementary analyses focusing on frailty, multimorbidity, polypharmacy and HRQoL therefore support the use of empagliflozin for its kidney protective effects and additional diuretic and blood pressure lowering effects in a broad range of patients with CKD, irrespective of indicators of frailty. Since diuretic and antihypertensive effects may reduce the need for additional therapeutic agents, the use of SGLT2 inhibitors may even have the potential to reduce polypharmacy in CKD, along with their beneficial effects on other common comorbidities such as anaemia and gout which typically require additional drug therapy in CKD.

8.2 IMPLICATIONS

8.2.1 PATIENTS

For patients with CKD, the findings from this thesis demonstrate that the benefits of SGLT2 inhibitor treatment extend beyond the main effects on slowing kidney disease progression. Fluid overload commonly manifests as swelling and breathlessness as CKD advances which cause patients discomfort and erodes quality of life. Slowing of kidney disease progression may be difficult for individual patients to conceptualise since they do not feel this effect of treatment nor see the alternative trajectory in their estimated kidney function were they not on treatment. On the other hand, improvement in symptoms such as fluid overload is more tangible and education on such effects may support compliance. Furthermore, treatment with empagliflozin reduces the need for hospitalisation which is a major event that many patients with CKD will have experienced and wish to avoid wherever possible therefore including this observation in patient counselling may also aid patients' understanding of the extent of benefit afforded by SGLT2 inhibitor treatment. For patients with frailty, multimorbidity and/or polypharmacy, it may be helpful for them to know that empagliflozin affords them the same (if not greater) benefits and is safe. Patients

should not be deterred by the addition of another daily medication and should be counselled on the expected benefits when polypharmacy or pill burden is a concern. Clinical practice guidelines should be updated to reflect this and encourage SGLT2 inhibitor use in patients with CKD and frailty as they are the most likely to benefit in absolute terms. Current UK guidelines on SGLT2 inhibitor use from the UK Kidney Association infer reticence in recommending "an approach to care that takes account of frailty and multimorbidity… (and) consideration of the balance of disease and treatment burden." (UK Kidney Association, 2023).

The willingness of patients to participate in randomised controlled trials is absolutely critical to the generation of such reliable evidence. The dissemination of trial results to participants (and other patients) is critically important to communicate the impact of their contribution to advancing the care of people with CKD. This can be expected to motivate patients to continue to contribute to clinical research. Patients cite very positive experiences of participating in clinical trials and it is the vision of many organisations such as charities like Kidney Research UK that all patients have the opportunity to participate in research. Trialists and clinicians have a responsibility to ensure all patients are afforded such opportunities where available and to ensure appropriate recruitment of underrepresented groups such as women and ethnic minorities to future trials to ensure generation of widely applicable evidence.

8.2.2 CLINICIANS

Following EMPA-KIDNEY, SGLT2 inhibitors have now been cemented as standard of care for the majority of patients with CKD and updated clinical guidelines reflect this. The work of this thesis expands upon the range of established benefits and should further support prescribing of SGLT2 inhibitors in CKD. Findings from this thesis can be used to support patient counselling regarding the tangible benefits of SGLT2 inhibitor therapy in addition to the invisible slowing of kidney disease progression. In EMPA-KIDNEY, empagliflozin caused significant reductions in interstitial fluid and while bioimpedance spectroscopy does not estimate intravascular fluid, the reassuring lack of excess dehydration adverse events supports the safe reduction in interstitial fluid without the plasma volume contraction seen with conventional diuretic agents. These data therefore provide clinically valuable information to support the notion that SGLT2 inhibitors can

safely relieve congestion with little impact on arterial filling and perfusion, an observation that is likely to be of particular importance in patients with CKD and concomitant heart failure (in whom reduced cardiac output reduces arterial filling pressures which can be exacerbated by conventional diuretics). Analyses within this thesis additionally ought to provide particular reassurance to support prescribing of SGLT2 inhibitors in patients with frailty, multimorbidity and/or polypharmacy, an area where there was previously uncertainty and potential for perceived altered risk-benefit ratio.

In addition to the effects of empagliflozin in CKD which are the findings from this thesis which have the greatest clinical impact, this work also provides rationale for the expanded use of bioimpedance spectroscopy in CKD. Although interventional trials in dialysis populations have failed to demonstrate meaningful benefits on hard endpoints, the use of bioimpedance has been shown to improve blood pressure, left ventricular mass and arterial stiffness (Scotland et al., 2018, Hur et al., 2013, Onofriescu et al., 2014, Huan-Sheng et al., 2016). Bioimpedance is routinely used in dialysis settings but not in the earlier stages of CKD. Bioimpedance is able to detect subclinical levels of fluid excess at which patients would not be expected to manifest clinical signs or symptoms and the observed associations with adverse outcomes in this thesis highlight a potential opportunity for early intervention with diuretic agents to avoid complications such as development of, and hospitalisation for heart failure in CKD.

8.2.3 RESEARCHERS

Results within this thesis from the EMPA-KIDNEY trial reflect considerable advancement in the field of nephrology research in recent years. EMPA-KIDNEY is an example of how large properly conducted randomised clinical trials in nephrology can impact the treatment of CKD and lives of people with kidney disease. Research efforts should be focused on such methodology as the gold standard of evidence generation, wherever possible, to ensure continued progress in the treatment of CKD.

Understanding of the effects of SGLT2 inhibitors continues to grow and the mechanisms by which their benefits are achieved remain incompletely understood. There is a need for

basic and translational science to better understand the physiology underlying the clinical outcome data in order to ensure the benefits of SGLT2 inhibitor treatment are realised by all who might benefit.

The bioimpedance substudy of EMPA-KIDNEY is also an example of how a mechanistic substudy can be incorporated into such a trial to provide valuable insights into more detailed effects of the intervention in a subset of the trial population. The application of bioimpedance spectroscopy in the setting of a randomised controlled trial assessing the effect of another intervention (such as empagliflozin) is relatively novel. Consistent use of bioimpedance parameter terminology and application of moderate and severe thresholds of "Fluid Overload" used in the EMPA-KIDNEY bioimpedance substudy will aid utility.

8.3 FUTURE DIRECTIONS

Several key groups have been understudied in SGLT2 inhibitor trials. Patients requiring dialysis or with kidney transplants were excluded from trials conducted to date and the efficacy of SGLT2 inhibition in these populations, particularly with little or no residual kidney function in patients receiving dialysis, is unknown. It is conceivable that continued SGLT2 inhibition after initiation of dialysis therapy may afford cardiovascular protection even if there is little to be gained in terms of kidney protection. The ongoing RENAL LIFECYCLE trial (ClinicalTrials.gov Identifier: NCT05374291) is testing the effects of dapagliflozin in patients with advanced CKD including with a kidney transplant or receiving dialysis with residual urine output and is due to report in 2027. Patients with CKD due to polycystic kidney disease were also excluded from completed trials due to potential safety concerns yet there is reason to believe they may also benefit from SGLT2 inhibition in slowing of the "final common pathway" stage of the disease characterised by tubulointerstitial fibrosis. Also excluded due to fears related to excessive risk of ketoacidosis and perhaps hypoglycaemia were patients with type 1 diabetes mellitus. Reassuring safety data from large numbers of patients studied with type 2 diabetes may provide rationale to pursue investigation of the effects of SGLT2 inhibition in this group with significant risks of nephropathy and cardiovascular disease.

The slowing of kidney disease progression achieved by SGLT2 inhibition is insufficient to halt the disease process or completely avoid progression to kidney failure in all patients. For that reason, there is a need for continued efforts to expand the treatment landscape of CKD. Both the non-steroidal mineralocorticoid receptor antagonist finerenone and the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide have also been shown to be efficacious in slowing kidney disease progression in patients with CKD and diabetes (Agarwal et al., 2022, Perkovic et al., 2024). Trials of both agents are ongoing in patients without diabetes and promising evidence of beneficial effects of semaglutide on kidney outcomes has recently emerged from the SELECT trial (Colhoun et al., 2024). A challenge associated with the improving treatment of CKD is falling event rates in clinical trials meaning future trials need to be sufficiently large, long and make use of efficient trial designs and in some situations, surrogate endpoints to feasibly, reliably and efficiently test new treatments.

8.4 CONCLUSIONS

Bioimpedance devices - and particularly the BCM - have a range of potential clinical and research applications in a range of patient groups, not just kidney failure with replacement therapy and may have particular value where CKD and heart failure coexist.

EMPA-KIDNEY was the largest placebo-controlled CKD progression trial of an SGLT2 inhibitor in CKD and reported a 28% reduction in the risk of kidney disease progression or cardiovascular death in its population of 6609 patients at risk of progression. The 660-participant embedded bioimpedance substudy demonstrated that, compared to placebo, empagliflozin reduced bioimpedance-derived “Fluid Overload” by about a quarter of a litre, and weight by nearly 1 kg. This effect was consistent across the broad range of patients studied, including those with and without diabetes, with different levels of hydration status at baseline, and even at low levels of kidney function. Empagliflozin reduced systolic blood pressure by about 2 mmHg, with effects that may be larger in people with diabetes than without diabetes. This is perhaps unexpected given that diabetes status did not modify the effect on fluid status and suggests additional distinct mechanisms underlying blood pressure lowering.

Empagliflozin had no significant effect on fat mass, consistent with the minimal effect of SGLT2 inhibition on glycaemic control among those with decreased kidney function.

SGLT2 inhibition is well-tolerated in patients with CKD. In *post-hoc* exploratory analyses, this was found to be true in EMPA-KIDNEY participants with the highest levels of frailty. In fact, estimates of the absolute benefits of empagliflozin on the primary outcome and risk of all-cause hospitalisation suggested large benefits in those who were frail, and the benefits outweighed any absolute risks of adverse events.

In conclusion, SGLT2 inhibitors should be widely used in patients with CKD. They are well-tolerated; favourably reduce fluid overload and lower blood pressure; modify risk of kidney disease progression and cardiovascular disease; and have demonstrable large net absolute benefits, even in individuals with indicators of frailty.

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Effects of empagliflozin on fluid overload, weight and blood pressure in chronic kidney disease

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CHAPTER 9 APPENDICES

Bioimpedance-measured fluid overload and cardiorenal outcomes in chronic kidney disease and heart failure: a systematic review

Review methods were amended after registration. Please see the revision notes and previous versions for detail.

Citation

Kaitlin Mayne, Richard Shemilt, Jennifer Lees, Paddy Mark, Will Herrington, David Keane, Richard Haynes. Bioimpedance-measured fluid overload and cardiorenal outcomes in chronic kidney disease and heart failure: a systematic review. PROSPERO 2022 CRD42022316312 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022316312

Review question

The purpose of this review is to investigate the association between fluid overload, measured by bioimpedance spectroscopy/analysis and mortality, cardiovascular disease and progression of chronic kidney disease (CKD) in those with chronic kidney disease and heart failure.

Searches [1 change]

Sources: MEDLINE (Ovid), Embase (Ovid) and the Cochrane Central Register of Controlled Trials (CENTRAL).

Dates will be searched from the beginning of database records until February 2022.

No language restriction will be applied to the initial search. Efforts will be made to source potentially relevant papers in English or translation support used, where available, to assess eligibility for inclusion. If papers cannot be sourced in English and adequately assessed, they will be excluded.

Additional search strategy information can be found in the attached PDF document (link provided below).

Types of study to be included [1 change]

The systematic review will include observational study designs (including prospective and retrospective cohort studies) as well as interventional studies/trials.

Case series/reports/narrative reviews will be excluded.

Condition or domain being studied [1 change]

Fluid overload in people with chronic kidney disease or heart failure and its associations with mortality, cardiovascular outcomes and association with progression of CKD.

Participants/population [1 change]

Studies including adult participants aged over 18 years will be included. Studies of participants with and without CKD and heart failure will be included. CKD will be defined according to the KDIGO staging system and will include CKD stages 1-5, not yet requiring kidney replacement therapy (dialysis or transplant). Studies of people with functioning kidney transplants or on maintenance dialysis will be excluded.

(The protocol and therefore search strategy originally included dialysis populations however this was revised on the basis of the number of search results and due to existing systematic reviews in dialysis populations but not in non-dialysis CKD and heart failure).

Intervention(s), exposure(s) [1 change]

The exposure to be measured is fluid overload, or overhydration, measured by bioimpedance techniques - bioimpedance spectroscopy or bioimpedance analysis. The exposure may be defined in different ways:

• Relative overhydration or overhydration index:

o Ratio of overhydration to extracellular water, expressed as %

o ≥7% generally considered = relative overhydration (based on 90th percentile of healthy population)

o Comparisons between overhydrated and non-overhydrated reported as hazard ratios

• Overhydration index expressed in litres:

o Reported either as a continuous variable or in quantiles - different cutpoints

o Studies generally consider >1L = overhydration however one study defines overhydration as >0L

o Comparisons between highest and lowest quantiles

• Other parameters may include:

o Ratios of measured extracellular water (ECW), intracellular water (ICW), total body water (TBW)

o Phase angle

o Bioimpedance vector analysis measurements

• Studies may use single or repeated measurements of overhydration

o In studies using repeated measurements, all data will be extracted and the baseline measurement may be favoured (more recent measurements may have been affected by treatment)

o If repeated measurements are common to several studies, a sensitivity analysis will be performed comparing baseline versus latest measurements to assess the potential impact of treatment for overhydration on CKD progression.

Comparator(s)/control [1 change]

Not required - expected to include largely observational studies. In interventional studies with a comparator, this is likely to be standard clinical fluid status assessment using clinical examination and dry weight assessment.

Context [1 change]

The purpose of this review is to investigate the association between fluid overload, measured by bioimpedance spectroscopy or analysis and adverse cardiovascular and kidney outcomes. Fluid overload, firstly, is common in chronic kidney disease (CKD), particularly in the advanced stages of the disease, and has been found to predict mortality and cardiovascular morbidity. Studies have generally focused on the kidney failure population receiving kidney replacement therapy, specifically haemodialysis, a population more likely to have problematic fluid overload than those with early CKD. Bioimpedance spectroscopy has been specifically validated in kidney failure but is not routinely used in earlier stages of CKD. The significance of fluid overload in the earlier stages of CKD is less clear however it has been suggested that extracellular water excess is evident in very early CKD. Some studies have suggested a link between fluid overload and cardiorenal outcomes in early CKD however to our knowledge, this has not been addressed by existing systematic reviews. There is a need to understand the risk associated with fluid overload in all stages of CKD as a potentially modifiable risk factor for adverse cardiorenal outcomes. The heart failure population are another patient group in whom fluid overload is common but not well studied using bioimpedance techniques - we seek to summarise the existing literature.

Main outcome(s) [2 changes]

1. All-cause mortality.

2. Cardiovascular outcomes: fatal and nonfatal incident cardiovascular disease requiring hospitalisation:

• Myocardial infarction

• Stroke

• Cardiovascular death

• Coronary heart disease including unstable angina and revascularisation procedures

• Hospitalisation for heart failure

Study definitions are expected to differ, especially in observational studies. For the purposes of this review, study definitions of cardiovascular events and composite outcomes will be used and heterogeneity assessed to consider whether aggregation is appropriate.

3. Progression of chronic kidney disease:

• Incident end-stage kidney disease requiring initiation of renal replacement therapy (RRT) (haemodialysis, peritoneal dialysis or renal transplantation including pre-emptive transplantation)

• By percentage decline in glomerular filtration rate (GFR) or eGFR (definitions vary eg. ≥25, ≥30%, ≥40% or ≥50%)

• By decline in GFR or eGFR per year (definitions vary eg. ≥3ml/min/1.73m2 per year)

• Doubling of serum creatinine from baseline

• Onset of self-reported persistent anuria (definitions vary eg. urine volume <100mL/24 hours)

Study definitions are expected to differ, especially in observational studies. All of the above will be summarised and heterogeneity assessed to consider whether aggregation is appropriate.

Measures of effect

Hazard ratios for risk of death, cardiovascular outcomes or kidney disease progression.

Additional outcome(s)

Depending on availability of data, secondary outcomes may include hospitalisations or urgent visits for heart failure or other. Data may also be summarised on related cardiac markers such as NTpro-BNP and echocardiographic/magnetic resonance markers of cardiac function, where reported.

Data extraction (selection and coding) [1 change]

Two reviewers will independently screen studies for inclusion, blinded to each other's decisions. Studies which are clearly not relevant to the review question will be removed based on title alone. Abstracts will then be screened to identify studies for full-text review. Reviewers' included studies will be compared and where there is disagreement, this will be resolved by discussion or, where necessary, a third author will be consulted. Hand searches of reference lists of eligible studies and review articles will be performed and any further identified studies reviewed for eligibility. Unpublished studies will not be sought and only full-text papers will be screened (conference abstracts excluded), studies with only abstracts available will not be included. If eligibility is unclear, contact with investigators will be attempted and contribution acknowledged accordingly. Papers which are not available in English will be excluded. Searches will be stored within each platform (Ovid and CENTRAL) to allow them to be re-run prior to final analysis and/or repeated in future. Covidence software will be used to import the search results directly and remove duplicates. Title and abstract screening, full text review and data extraction will all be performed within Covidence by two reviewers, independently.

Covidence allows generation of a bespoke data extraction form which the same two reviewers will use to independently extract relevant data. Data will be extracted on study design, methodology (including bioimpedance technique studied), participant demographics, baseline characteristics, kidney disease progression and how this is defined/measured.

Risk of bias (quality) assessment [1 change]

Two reviewers will independently assess the risk of bias for each study individually and then compare their assessments, with disagreement resolved by discussion (involvement of a third reviewer if consensus cannot be reached). Quality in Prognostic Studies (QUIPS) tool will be used to assess risk of bias for cohort studies and the Cochrane risk-of-bias 2 tool used for randomised trials. This will include assessment of the following:

Cohort studies (QUIPS)

1. Study participation

2. Study attrition

3. Prognostic factor measurement

4. Outcome measure

5. Study confounding

6. Statistical analysis reporting

Randomised trials (Cochrane ROB 2)

• Study design – randomisation/allocation process, deviations from intended intervention

• Analysis – appropriate analysis to estimate effect of intervention, pre-specified data analysis plan

• Outcome – completeness of follow-up data/missingness, appropriateness of outcome ascertainment method, influence of allocation

No validated threshold score exists for the QUIPS. Trials assessed as high risk of bias according to the Cochrane risk-of-bias assessment will be de-emphasised (tabulated but excluded from meta-analysis).

Strategy for data synthesis [1 change]

If sufficiently similar studies (in outcome measurement) are identified, meta-analysis may be useful. A statistical analysis plan will be developed depending on the available data and will consider the most appropriate methods to allow aggregation of data and testing for between study differences. We anticipate conducting meta-analyses for the mortality and kidney disease progression outcomes, cardiovascular outcomes will depend on search results/data availability. A proposed plan is outlined here:

Fixed-effect and random-effects models will be assessed, depending on the heterogeneity of included studies, assessed using the I^2 statistic. Hazard ratios (and 95% confidence intervals) and odds ratios will be the preferred estimates of effect size. Statistical analysis will depend on outcome definition and measurement in included studies and statistician advice will be sought if combining binary and continuous outcome data in meta-analysis is deemed necessary. Meta-analyses may include:

• Time-to-event analyses for progression to ESKD requiring renal replacement therapy

• Logistic regression models for initiation of renal replacement therapy

• Linear regression for changes in eGFR or serum creatinine using mean difference (or standardised mean difference if creatinine reported using different scales)

o Where change-from-baseline measurements are reported (eg. ANCOVA), these will be combined in meta-analysis using the generic inverse-variance method

o If data are skewed and studies not sufficiently large to address this, transformation will be applied

Regression analyses may will be performed to assess additional exposures/baseline characteristics associated with the exposure (fluid overload) and outcome where sufficient data exists. Results will be summarised in tables and forest plots created to illustrate results. Trim and fill analysis and forest plots will be used to assess publication bias. All analyses will be conducted using Stata software.

Analysis of subgroups or subsets

If sufficient data are available, subgroup analysis will be performed by:

• CKD status: absence of CKD; by CKD stage (1-4 and stage 5 not yet requiring RRT); ESKD requiring dialysis

• CKD aetiology

• Diabetes mellitus status

• History of cardiovascular disease.

Contact details for further information

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https://www.gla.ac.uk/researchinstitutes/icams/

Review team members and their organisational affiliations [1 change]

Dr Kaitlin Mayne. University of Oxford

Dr Richard Shemilt. University of Glasgow

Dr Jennifer Lees. University of Glasgow

Dr Paddy Mark. University of Glasgow

Assistant/Associate Professor Will Herrington. University of Oxford

Dr David Keane. Leeds Teaching Hospitals NHS Trust

Professor Richard Haynes. University of Oxford

Type and method of review

Epidemiologic, Meta-analysis, Systematic review

Anticipated or actual start date

01 February 2022

Anticipated completion date

01 October 2022

Funding sources/sponsors

No funding required for the review.

Conflicts of interest [1 change]

KM, WH & RH acknowledge institutional funding and sponsorship from Boehringer Ingelheim for the EMPA-KIDNEY trial. Fresenius Medical Care have provided body composition monitor (BCM) devices for use in EMPA-KIDNEY.

Yes

Language

English

Country

England, Scotland

Published protocol

https://www.crd.york.ac.uk/PROSPEROFILES/316312_PROTOCOL_20220622.pdf

Stage of review [1 change]

Review Completed published

Details of final report/publication(s) or preprints if available [1 change]

Mayne KJ, Shemilt R, Keane DF, Lees JS, Mark PB, Herrington WG. Bioimpedance Indices of Fluid Overload and Cardiorenal Outcomes in Heart Failure and Chronic Kidney Disease: a Systematic Review. J Card Fail. 2022 Nov;28(11):1628-1641. doi: 10.1016/j.cardfail.2022.08.005. Epub 2022 Aug 28. PMID: 36038013; PMCID: PMC7613800.

https://www.onlinejcf.com/article/S1071-9164(22)00684-4/fulltext

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Adult; Body Fluids; Cardiovascular Diseases; Dielectrc Spectroscopy; Disease Progression; Electric Impedance; Heart Failure; Humans; Mortality; Organism Hydration Status; Renal Insufficiency, Chronic

Date of registration in PROSPERO

16 March 2022

Date of first submission

11 March 2022

Stage of review at time of this submission [2 changes]

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	Yes

Revision note

Updated status to completed and details of publication provided

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions

16 March 2022

16 March 2022

23 June 2022

18 February 2023

Fluid overload by bioimpedance methods in chronic kidney disease and heart failure - mortality, cardiovascular and kidney outcomes: a systematic review and meta-analysis

Kaitlin J. Mayne [1-2], Richard Shemilt [2], Jennifer S. Lees [2], Patrick B. Mark [2], William G. Herrington [1]

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MEDLINE (Ovid) search strategy

1. overhydration.ab,ti.
2. hyperhydration.ab,ti.
3. hypervol?emia.ab,ti.
4. fluid overload.ab,ti.
5. fluid status.ab,ti.
6. volume overload.ab,ti.
7. volume status.ab,ti.
8. (dry adj2 weight).ab,ti.
9. hydration.ab,ti.
10. congestion.ab,ti.
11. body composition.ab,ti.
12. bioimpedance.ab,ti.
13. bio-impedance.ab,ti.
14. bioelectrical impedance.ab,ti.
15. bio-electrical impedance.ab,ti.
16. extracellular.ab,ti.
17. phase angle.ab,ti.
18. exp electric impedance/
19. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
20. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18
21. 19 and 20
22. (cardiovascular adj2 outcome$).ab,ti.
23. (cardiovascular adj2 event$).ab,ti.
24. (cardiovascular adj2 endpoint$).ab,ti.
25. (cardiovascular adj2 disease$).ab,ti.
26. (cardiac adj2 outcome$).ab,ti.
27. (cardiac adj2 event$).ab,ti.
28. (cardiac adj2 endpoint$).ab,ti.
29. (cardiac adj2 disease$).ab,ti.
30. (coronary adj2 disease$).ab,ti.
31. isch?emic heart disease$.ab,ti.
32. myocardial infarction.ab,ti.
33. myocardial isch?emia.ab,ti.
34. acute coronary syndrome.ab,ti.
35. heart failure.ab,ti.
36. cardiac failure.ab,ti.
37. stroke.ab,ti.
38. cerebrovascular accident.ab,ti.
39. cerebrovascular disease.ab,ti.
40. survival.ab,ti.
41. mortality.ab,ti.
42. death.ab,ti.

43. exp cardiovascular diseases/
44. exp heart diseases/
45. exp coronary disease/
46. exp myocardial ischemia/
47. exp heart failure/
48. exp stroke/
49. exp survival/
50. exp mortality/
51. exp death/
52. 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51
53. ((egfr or gfr) adj slope).ab,ti.
54. ((egfr or gfr) adj3 decline).ab,ti.
55. ((egfr or gfr) adj3 change).ab,ti.
56. glomerular filtration rate slope.ab,ti.
57. (glomerular filtration rate adj3 decline).ab,ti.
58. (glomerular filtration rate adj3 change).ab,ti.
59. (kidney disease adj2 progression).ab,ti.
60. end stage kidney disease.ab,ti.
61. (kidney adj3 failure).ab,ti.
62. (kidney adj3 outcome$).ab,ti.
63. (kidney adj3 event$).ab,ti.
64. (kidney adj3 endpoint$).ab,ti.
65. (renal disease adj2 progression).ab,ti.
66. end stage renal disease.ab,ti.
67. (renal adj3 failure).ab,ti.
68. (renal adj3 outcome$).ab,ti.
69. (renal adj3 event$).ab,ti.
70. (renal adj3 endpoint$).ab,ti.
71. renal insufficiency.ab,ti.
72. anuria.ab,ti.
73. residual kidney function.ab,ti.
74. residual renal function.ab,ti.
75. (doubl$ adj3 creatinine).ab,ti.
76. renal replacement.ab,ti.
77. kidney replacement.ab,ti.
78. dialysis.ab,ti.
79. h?emodialysis.ab,ti.
80. renal transplant$.ab,ti.
81. kidney transplant$.ab,ti.
82. exp glomerular filtration rate/
83. exp renal insufficiency, chronic/
84. exp creatinine/
85. exp renal dialysis/
86. exp kidney transplantation/
87. 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86
88. 52 or 87
89. 21 and 88

FINAL EMBASE 140322 EXPLODING ALL

1. overhydration.ab,ti.
2. hyperhydration.ab,ti.
3. hypervol?emia.ab,ti.
4. fluid overload.ab,ti.
5. fluid status.ab,ti.
6. volume overload.ab,ti.
7. volume status.ab,ti.
8. (dry adj2 weight).ab,ti.
9. hydration.ab,ti.
10. congestion.ab,ti.
11. body composition.ab,ti.
12. bioimpedance.ab,ti.
13. bio-impedance.ab,ti.
14. bioelectrical impedance.ab,ti.
15. bio-electrical impedance.ab,ti.
16. extracellular.ab,ti.
17. phase angle.ab,ti.
18. exp impedance/
19. exp body water/ or exp total body water/
20. exp hypervolemia/
21. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
22. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
23. 21 and 22
24. (cardiovascular adj2 outcome$).ab,ti.
25. (cardiovascular adj2 event$).ab,ti.
26. (cardiovascular adj2 endpoint$).ab,ti.
27. (cardiovascular adj2 disease$).ab,ti.
28. (cardiac adj2 outcome$).ab,ti.
29. (cardiac adj2 event$).ab,ti.
30. (cardiac adj2 endpoint$).ab,ti.
31. (cardiac adj2 disease$).ab,ti.
32. (coronary adj2 disease$).ab,ti.
33. isch?emic heart disease$.ab,ti.
34. myocardial infarction.ab,ti.
35. myocardial isch?emia.ab,ti.
36. acute coronary syndrome.ab,ti.
37. heart failure.ab,ti.
38. cardiac failure.ab,ti.
39. stroke.ab,ti.
40. cerebrovascular accident.ab,ti.
41. cerebrovascular disease.ab,ti.
42. survival.ab,ti.
43. mortality.ab,ti.
44. death.ab,ti.
45. exp cardiovascular disease/
46. exp heart disease/

47. exp coronary artery disease/
48. exp heart muscle ischemia/
49. exp heart failure/
50. exp cerebrovascular accident/
51. exp survival/
52. exp mortality/
53. exp death/
54. ((egfr or gfr) adj slope).ab,ti.
55. ((egfr or gfr) adj3 decline).ab,ti.
56. ((egfr or gfr) adj3 change).ab,ti.
57. glomerular filtration rate slope.ab,ti.
58. (glomerular filtration rate adj3 decline).ab,ti.
59. (glomerular filtration rate adj3 change).ab,ti.
60. (kidney disease adj2 progression).ab,ti.
61. end stage kidney disease.ab,ti.
62. (kidney adj3 failure).ab,ti.
63. (kidney adj3 outcome$).ab,ti.
64. (kidney adj3 event$).ab,ti.
65. (kidney adj3 endpoint$).ab,ti.
66. (renal disease adj2 progression).ab,ti.
67. end stage renal disease.ab,ti.
68. (renal adj3 failure).ab,ti.
69. (renal adj3 outcome$).ab,ti.
70. (renal adj3 event$).ab,ti.
71. (renal adj3 endpoint$).ab,ti.
72. renal insufficiency.ab,ti.
73. anuria.ab,ti.
74. residual kidney function.ab,ti.
75. residual renal function.ab,ti.
76. (doubl$ adj3 creatinine).ab,ti.
77. renal replacement.ab,ti.
78. kidney replacement.ab,ti.
79. dialysis.ab,ti.
80. h?emodialysis.ab,ti.
81. renal transplant$.ab,ti.
82. kidney transplant$.ab,ti.
83. exp glomerulus filtration rate/
84. exp kidney failure/ not acute kidney failure/
85. exp creatinine blood level/
86. exp hemodialysis/
87. exp peritoneal dialysis/
88. exp kidney graft/
89. 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53
90. 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 87 or 88
91. 89 or 90

92. 23 and 91
93. conference*.pt.
94. 92 and 93
95. 92 not 93

CENTRAL 140322

#1 (overhydration):ab,ti
#2 (hyperhydration):ab,ti
#3 (hypervol?emia):ab,ti
#4 (fluid overload):ab,ti
#5 (fluid status):ab,ti
#6 (volume overload):ab,ti
#7 (volume status):ab,ti
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#9 (hydration):ab,ti
#10 (congestion):ab,ti
#11 (body composition):ab,ti
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#14 (bioelectrical impedance):ab,ti
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#17 (phase angle):ab,ti
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#19 MeSH descriptor: [Body Water] explode all trees
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#21 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19
#22 #20 AND #21
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#29 (cardiac NEAR/2 endpoint*):ab,ti
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#31 (coronary NEAR/2 disease*):ab,ti
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#34 (myocardial isch?emia):ab,ti
#35 (acute coronary syndrome):ab,ti
#36 (heart failure):ab,ti
#37 (cardiac failure):ab,ti
#38 (stroke):ab,ti
#39 (cerebrovascular accident):ab,ti
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#46 MeSH descriptor: [Coronary Artery Disease] explode all trees
#47 MeSH descriptor: [Myocardial Ischemia] explode all trees
#48 MeSH descriptor: [Heart Failure] explode all trees
#49 MeSH descriptor: [Stroke] explode all trees

#50 MeSH descriptor: [Survival] explode all trees
#51 MeSH descriptor: [Mortality] explode all trees
#52 MeSH descriptor: [Death] explode all trees 222
#53 #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52
#54 ((egfr or gfr) NEXT slope):ab,ti
#55 ((egfr or gfr) NEAR/3 decline):ab,ti
#56 ((egfr or gfr) NEAR/3 change):ab,ti
#57 (glomerular filtration rate slope):ab,ti
#58 (glomerular filtration rate NEAR/3 decline):ab,ti
#59 (glomerular filtration rate NEAR/3 change):ab,ti
#60 (kidney disease NEAR/2 progression):ab,ti
#61 (end stage kidney disease):ab,ti
#62 (kidney NEAR/3 failure):ab,ti
#63 (kidney NEAR/3 outcome*):ab,ti
#64 (kidney NEAR/3 event*):ab,ti
#65 (kidney NEAR/3 endpoint*):ab,ti
#66 (renal disease NEAR/2 progression):ab,ti
#67 (end stage renal disease):ab,ti
#68 (renal NEAR/3 failure):ab,ti
#69 (renal NEAR/3 outcome*):ab,ti
#70 (renal NEAR/3 event*):ab,ti
#71 (renal NEAR/3 endpoint*):ab,ti
#72 (renal insufficiency):ab,ti
#73 (anuria):ab,ti
#74 (residual kidney function):ab,ti
#75 (residual renal function):ab,ti
#76 (doubl* NEAR/3 creatinine):ab,ti
#77 (renal replacement):ab,ti
#78 (kidney replacement):ab,ti
#79 (dialysis):ab,ti
#80 (h?emodialysis):ab,ti
#81 (renal transplant*):ab,ti
#82 (kidney transplant*):ab,ti
#83 MeSH descriptor: [Glomerular Filtration Rate] explode all trees
#84 MeSH descriptor: [Renal Insufficiency, Chronic] explode all trees
#85 MeSH descriptor: [Creatinine] explode all trees
#86 MeSH descriptor: [Renal Dialysis] explode all trees
#87 MeSH descriptor: [Kidney Transplantation] explode all trees
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#89 #53 OR #88
#90 #22 AND #89
#91 (conference*):pt
#93 #90 NOT #91

Quality in Prognostic Studies (QUIPS) tool for risk of bias

1. Study Participation: The study sample adequately represents the population of interest

Consider the following:
a) The source population or population of interest is adequately described for key characteristics (CKD/HF history/eGFR, proteinuria, diabetes, CVD).
b) The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)
c) Period of recruitment is adequately described
d) Place of recruitment (setting and geographic location) are adequately described
e) "Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or “zero time” description)."
f) There is adequate participation in the study by eligible individuals
g) The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (CKD/HF history/eGFR, proteinuria, diabetes, CVD).

High risk of bias: The relationship between fluid overload and outcome is very likely to be different for participants and eligible nonparticipants
Moderate risk of bias: The relationship between fluid overload and outcome may be different for participants and eligible nonparticipants
Low risk of bias: The relationship between fluid overload and outcome is unlikely to be different for participants and eligible nonparticipants

2. Study Attrition: The study data available (i.e., participants not lost to follow-up) adequately represent the study sample

Consider the following:
a) Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.
b) Attempts to collect information on participants who dropped out of the study are described.
c) Reasons for loss to follow-up are provided.
d) Participants lost to follow-up are adequately described for key characteristics (CKD/HF history/eGFR, proteinuria, diabetes, CVD).
e) There are no important differences between key characteristics (CKD/HF history/eGFR, proteinuria, diabetes, CVD) and outcomes in participants who completed the study and those who did not.

High risk of bias: The relationship between fluid overload and outcome is very likely to be different for completing and noncompleting participants
Moderate risk of bias: The relationship between fluid overload and outcome may be different for completing and noncompleting participants
Low risk of bias: The relationship between fluid overload and outcome is unlikely to be different for completing and noncompleting participants

3. Prognostic Factor (fluid overload) Measurement: The prognostic factor (fluid overload) is measured in a similar way for all participants

Consider the following:
a) A clear definition or description of fluid overload measurement is provided (method, device, parameter used, timing of measurement).
b) Method of fluid overload measurement is adequately valid and reliable to limit misclassification bias
c) Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.
d) The method and setting of measurement of fluid overload is the same for all study participants.
e) Adequate proportion of the study sample has complete data for fluid overload variable.
f) Appropriate methods of imputation are used for missing fluid overload data.

High risk of bias: The measurement of fluid overload is very likely to be different for different levels of the outcome
Moderate risk of bias: The measurement of fluid overload may be different for different levels of the outcome
Low risk of bias: The measurement of fluid overload is unlikely to be different for different levels of the outcome

4. Outcome(s) Measurement: The outcome(s) of interest is measured in a similar way for all participants

Consider the following:
a) A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.
b) The method of outcome measurement used is adequately valid and reliable to limit misclassification bias

c) The method and setting of outcome measurement is the same for all study participants.

High risk of bias: The measurement of the outcome is very likely to be different by the baseline level of fluid overload
Moderate risk of bias: The measurement of the outcome may be different by the baseline level of fluid overload
Low risk of bias: The measurement of the outcome is unlikely to be different by the baseline level of fluid overload

5. Study Confounding: Important potential confounding factors are appropriately accounted for

Consider the following:
a) All important confounders (eGFR/CKD stage, HF type/EF, diabetes, proteinuria, BP etc), are measured.
b) Clear definitions of the important confounders measured are provided
c) Measurement of all important confounders is adequately valid and reliable
d) The method and setting of confounding measurement are the same for all study participants.
e) Appropriate methods are used if imputation is used for missing confounder data.
f) Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).
g) Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).

High risk of bias: The observed effect of fluid overload on the outcome is very likely to be distorted by another factor related to fluid overload and outcome
Moderate risk of bias: The observed effect of fluid overload on outcome may be distorted by another factor related to fluid overload and outcome
Low risk of bias: The observed effect of fluid overload on outcome is unlikely to be distorted by another factor related to fluid overload and outcome

6. Statistical Analysis and Reporting: The statistical analysis is appropriate, and all primary outcomes are reported

a) There is sufficient presentation of data to assess the adequacy of the analysis.
b) The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.
c) The selected statistical model is adequate for the design of the study.
d) There is no selective reporting of results.

High risk of bias: The reported results are very likely to be spurious or biased related to analysis or reporting
Moderate risk of bias: The reported results may be spurious or biased related to analysis or reporting
Low risk of bias: The reported results are unlikely to be spurious or biased related to analysis or reporting

BCM substudy
Primary aim

- To use bioimpedance spectroscopy to assess the effect of empagliflozin 10mg *vs* placebo on "Fluid overload" (at 2 & 18 Months)
- In a subset of at least 400 EMPA-KIDNEY participants

BCM substudy
Secondary aims

- To assess effects of empagliflozin 10mg vs placebo on:
  - Extracellular water (ECW)
  - Intracellular water (ICW)
  - % Fat/adipose tissue mass
  - % Lean tissue mass

BCM substudy
Power

≥400 EMPA-KIDNEY participants with bioimpedance spectroscopy measurements will provide sufficent power to detect +/- 300 mL difference in Fluid Overload

Aiming for as many participants as possible to give power for subgroup analyses

BCM substudy
Design

- Any participants at collaborating sites with an existing Fresenius Body Composition Monitor (BCM) machines will be invited to consent
- **Exclusion criterion:** None
- Must not have completed their Randomization visit

BCM substudy
Methods and procedures

- **Method:** A BCM measure will need to be made as part of 3 scheduled study visits:
  - Randomization Visit
  - 2 and 18 month Follow-up Visits

BCM substudy
Methods and procedures

- **Method:** A BCM measure will need to be made as part of 3 scheduled study visits:
  - Randomization Visit
  - 2 and 18 month Follow-up Visits
- **Procedures:**
  - Simple
  - Integrated into *Livia*
  - BCM Kits provide electrodes and pre-prepared BCM datacard (the source of the data)

EMPA-KIDNEY Body Composition Measurement Substudy

Study Title: A multicentre international randomized parallel group double-blind placebo-controlled clinical trial of EMPAgliflozin once daily to assess cardio-renal outcomes in patients with chronic KIDNEY disease

Sponsor protocol number: 1245-0137

Protocol identifier: CTSUEMPA-KIDNEY1.4 2018-04-25

EudraCT number: 2017-002971-24

Summary

This document provides the rationale and design of an EMPA-KIDNEY substudy to measure body composition in a subset of the 5000 EMPA-KIDNEY participants using bioimpedenace spectroscopy. The substudy does not alter the main protocol in any respect.

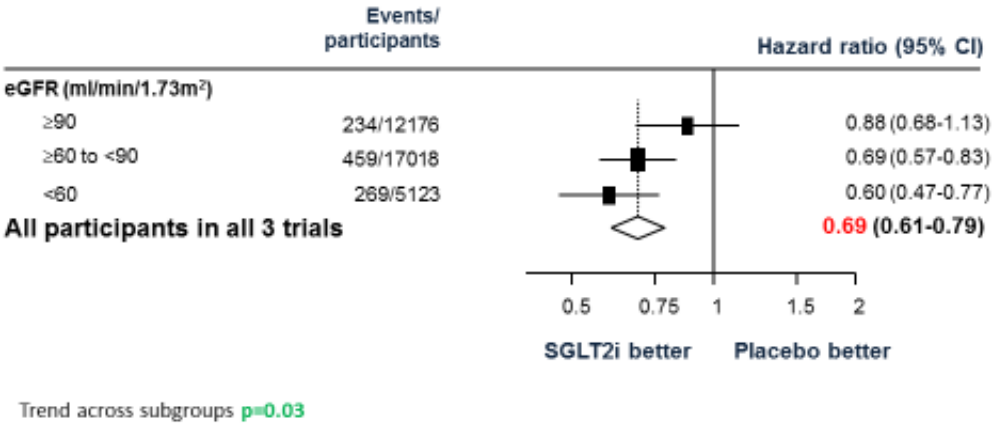
Background

In the EMPA-REG OUTCOME trial, empagliflozin 10-25mg was shown to reduce the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke by 14% compared to placebo (hazard ratio [HR] 0.86, 0.74-0.99) in 7020 people with type 2 diabetes mellitus (T2DM) and prior atherosclerotic cardiovascular disease.[1] This effect was in large part the result of a highly significant 38% (HR 0.62, 0.49-0.77) reduction in cardiovascular death. The pre-specified secondary outcome of hospitalization for heart failure was reduced by 35% (HR 0.65, 0.50-0.85).[1] Exploration of EMPA-REG OUTCOME data has suggested that the increase in haematocrit caused by empagliflozin, a possible surrogate for reductions in plasma volume, was the intermediate clinical parameter with the largest mediating effect on the reduction in cardiovascular death.[2] These observations may have particular relevance in people with chronic kidney disease (CKD) who have disturbed salt and water homeostasis which may cause chronic fluid overload which in turn contributes to the observed excess of structural heart disease and heart failure.[3]

In EMPA-REG OUTCOME, allocation to empagliflozin led to a sustained loss of weight (of about 2Kg from a mean of 86Kg) and a 2cm reduction in waist circumference (from a mean of 105cm).[1] How much of this weight change reflected reduction in total body water versus adipose tissue is unknown. A previous trial suggested that, after 2 years, weight loss resulting from SGLT-2 inhibition in people with T2DM appears almost completely attributable to reduced adipose tissue (measured using dual energy X-ray absorptiometry).[5] Lower kidney function substantially reduces glycosuric effects of SGLT-2 inhibition, and so reduced calorie loss at lower levels of kidney function may attenuate any loss of adipose tissue. However, no attenuation of the weight-lowering effects of SGLT-2 inhibition

was identified in those with CKD compared to those without (within the range of kidney function studied to date).[6-8] Furthermore, meta-analysis of three large placebo-controlled trials suggests effects of SGLT-2 inhibition on heart failure are at least as large among those with reduced kidney function.[9] Part of the preserved effect of SGLT-2 inhibition on body weight and heart failure in CKD may therefore result from reductions in excess extracellular water (ECW) being preserved in those with CKD, despite attenuated effects on glycosuria. This raises a hypothesis that the effects of empagliflozin on excess ECW and fat levels may be different in people with different levels of kidney function.

Figure 1: Effect of SGLT-2 inhibition versus placebo on hospitalization for heart failure, by baseline kidney function (meta-analysis EMPA-REG OUTCOME, CANVAS and DECLARE) [9]



Bioimpedance spectroscopy can assess different resistance patterns in the body which are affected by the amount of water present. Low frequency current exclusively passes extracellularly, whereas high frequencies can pass through all body water compartments. Comparing spectroscopy readings over a range of frequencies it is possible to derive total body water in Litres and separately the volume of ECW. From such measurements it is also possible to estimate normally hydrated adipose tissue and lean tissue mass, from which an index referred to as "Fluid Overload" (or overhydration) can be algorithmically calculated.[10] Sustained "Fluid Overload" measured by bioimpedance spectroscopy has been associated with increased risk of mortality among people on dialysis,[4] and some dialysis units are now using bioimpedance spectroscopy measurements clinically to guide patients' fluid management and dialysis prescription.

At each Follow-up Visit, EMPA-KIDNEY participants will have their weight measured and central plasma/serum blood samples collected. At Randomization, 2 & 18 months and Final Follow-up Visit, they will also have a measure of waist and hip circumference. A substudy using bioimpedance-based body composition measurements will ensure uncertainty about the effects of empagliflozin on ECW, adipose tissue and particularly "Fluid Overload" will be assessed in a CKD population.

Aims

The primary aim of this substudy is to use bioimpedance spectroscopy to assess, in a subset of EMPA-KIDNEY participants, the effect of empagliflozin 10mg versus matching placebo on "Fluid Overload" at the 2 month and 18 month Follow-up Visits.

Secondary aims are to use bioimpedance spectroscopy to assess:

1. Whether any effects of empagliflozin 10mg versus matching placebo on "Fluid Overload" are modified by baseline factors, in particular by level of kidney function, glycosylated haemoglobin, body mass index, NT-proBNP, age, sex, RAS inhibitor use, and different diuretics
2. The effects of empagliflozin 10mg versus matching placebo early and later during follow-up on:
    - ECW
    - Intracellular water (ICW)
    - Adipose tissue mass indexed to weight (i.e. %)
    - Lean tissue mass indexed to weight (i.e. %)

Exploratory aims are to:

- Assess if changes in ECW, ICW, % adipose tissue mass, % lean tissue mass and "Fluid Overload" correlate with changes in blood pressure and relevant other biomarkers.

Sample size estimates

The study will start a vanguard phase in a small number of sites in which bioimpedance spectroscopy will be performed at Randomization, 2 months and 18 months of Follow-up Visits. This vanguard phase will be expanded to other sites once feasiblity of adding a bioimpedance spectroscopy measurement is demonstrated. Feasibility will be based on feedback from the participating sites, successful completion of the other protocol-specified procedures and logistical considerations. It is estimated that at least 400 (of the 5000) EMPA-KIDNEY participants with follow-up bioimpedance spectroscopy measurements will provide ample power (>90%, 2p=0.05) to detect at least a ±300mL difference in "Fluid Overload" (reference range in healthy adults is ± 1100mL with a standard deviation of 900mL) based on an independent 2-sided t-test (Table 1).

Table 1: Sample size calculations for a study with Randomization Visit measurements

Outcome	Effect size	Standard deviation	Required sample size
"Fluid Overload" (ref range: ±1.1L)	±300mL	900mL	382

Note: An estimate of the correlation between successive bioimpedance spectroscopy measurements would be required to calculate the reduction in sample size that could be achieved by using ANCOVA analyses, but no such longitudinal data has yet been collected in a CKD population.

If a bioimpedance spectroscopy measurement at the Randomization Visit is shown to be infeasible, the substudy will be modifed to exclude the measurement at the Randomization Visit and only be performed at the relatively less busy phases of the study (i.e. measurements will be restricted to the 2 and 18 month Follow-up Visits). In this design, the sample size would need to increase to 850. This is because the absence of a bioimpedance spectroscopy measurement at the Randomization Visit means any imbalances in "Fluid Overload" between treatment arms at baseline cannot be corrected for. These imbalances could result in either the treatment effect being overestimated or a smaller than expected difference in mean "Fluid Overload" at follow-up. However, with a sample size of 850, the probability of large baseline imbalances is small, making it unlikely that the treatment effect would be overstated by more than 100mL (Table 2). With a sample size of 850, there would be sufficient power to detect a reduced difference in mean "Fluid Overload" of ± 200 mL at follow-up. This calculation is based on an independent 2-sided t-test using data from a healthy population (Note: sample size estimates differ little if dialysis population data are used).

Table 2: Sample size calculations for a study without Randomization Visit measurements

Sample size	Assumed possible baseline imbalance in Fluid Overload (mL)	Probability of a baseline imbalance at least this size due to chance (1-sided)	Difference between groups at follow-up (mL) after subtracting possible baseline imbalance from assumed treatment effect of 300 mL	Power to detect reduced difference in follow-up values at 2p=0.05
850	0	Not applicable	300	>99%
	50	12.6%	250	98%
	100	1.1%	200	90%
	150	0.03%	150	68%
	200	0.0002%	100	37%

Data Analysis Plan

The primary analysis will estimate the differences in "Fluid Overload" between treatment groups across all time points, regardless of whether a participant received all, some or none of their allocated treatment (i.e. intention-to-treat analyses). Secondary outcomes include ECW, ICW, % adipose tissue mass, and % lean tissue mass“. Differences in "Fluid Overload” and the secondary outcomes between treatment groups overall, and separately at 2 and 18 months, will be calculated using linear regression adjusted (or stratified) for the elements included in the minimization algorithm. The primary analysis will focus on a weighted average of the values at the two time points (with weights proportional to the amount of time between visits). Missing measurements will be imputed. Results from the imputed analyses will be compared with those from equivalent “complete-case” analyses, but primary emphasis will be placed on the results after multiple imputation. More complete details of statistical methods, including definitions of subgroups, methods of imputation, approaches to adjustments and

weighting of averages will be set out in a separate full Data Analysis Plan which will be consistent with the main study Data Analysis Plan.

Flowchart of Substudy Activities

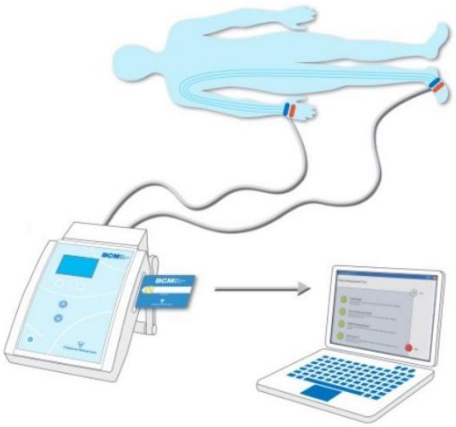
INVITATION
• Invite potential participants shortly before or at the time of the Randomization Visit • Written informed consent is sought from willing individuals at the first visit when a bioimpedance spectroscopy measurement is offered
RANDOMIZATION VISIT AND AT 2 & 18 MONTHS OF FOLLOW-UP
• A bioimpedance spectroscopy measurement is added to the protocol-specified study follow-up visit procedures

Design

Eligibility: In selected regions, EMPA-KIDNEY Local Clinical Centres (LCCs) with a Fresenius Medical Care Body Composition Monitor (BCM) machine will be invited to join this optional substudy. All those participants at these LCCs who have yet to attend the relevant scheduled study visit are eligible for invitation. There are no exclusion criteria.

Invitation and methods: Potential participants will be invited to join this substudy at before or around the time of their Randomization Visit. At the relevant visit, clinic staff will explain the substudy to potential participants using the Participant Information Leaflet and Consent Form. Consenting participants will have a measure of bioimpedance made in addition to the protocol-specified follow-up procedures. Bioimpedance measurements take about 2 minutes to record and pose no risk to health (although it is conceivable the 4 self-adhesive pads could rarely cause a skin reaction).

Body Composition Measurement



Training materials on how to perform Body Composition Measurements will be provided. The measurement requires four disposable self-adhesive electrode pads (2x on a wrist and the other 2x on an ankle) to be attached to a portable machine whilst a participant is lying supine. Bioimpedance spectroscopy readings are made automatically across about 50 frequencies over a range of 5-1000 kHz. The measurements take about 2 minutes to make. Data are then automatically transferrable onto a Storage Card which is linked securely to the participant by means of a unique Storage Card ID entered onto the relevant study visit form on trial's web-based data entry system (i.e. Storage Cards containing results are pseudonymised). The Storage Card will be stored securely before being transferred securely to the Central Coordinating Office in Oxford for downloading into the study database. Data may be transferred securely to specialists in bioimpedance for Quality Control review.

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Participant ID:	9								
Site ID:	9								

<u>Optional</u> Body Composition Measurement Substudy
Information Leaflet & Consent Form

You are invited to join an EMPA-KIDNEY substudy.

With your permission, we would like to measure your body water and fat levels using a body composition machine during the study so scientists can assess whether or not empagliflozin affects water and/or fat levels in people with kidney problems.

This substudy is entirely optional and does not affect any aspect of the consent you provided to join the main trial. As before, you are entirely free to decide to take part, and can withdraw at any time without affecting your participation in the main trial and without affecting your medical care or legal rights.

What does the substudy involve?
We would like to measure your body water & fat levels upto 3 times during the substudy.

At your second, third and fifth scheduled study follow-up visits, your study nurse may ask you if you are willing to undergo a body composition measurement. This is performed by connecting a bioimpedance machine to sticky pads placed over one of your wrists (two pads) and over one of your ankles (two pads) whilst you are lying down. The measurement, which is much like having an ECG heart trace, takes about 2 minutes to record. Body Composition Machines pose no major risk to your health (although rarely, the sticky pads could cause a mild skin reaction).

Body composition recordings will be stored securely on data cards which do not contain your name (i.e the cards are “de-identified”). The cards need a special reader and computer program to download and interpret the data. We will therefore not be able to provide you with results of your body water or fat levels from the study data cards.

Full details on how information about you is stored/handled and your data protection rights were provided in the main EMPA-KIDNEY Participant Information Leaflet you received at the study start.

Consent
I understand that I have already consented to join the EMPA-KIDNEY study and I agree to take part in the optional Body Composition Measurement Substudy

……	…….	**D D / M M M / Y Y Y Y**
PRINTED name of consenting patient	Signature	Today’s date
……	…….	**D D / M M M / Y Y Y Y**
PRINTED name of consent taker	Signature	Today’s date

Body Composition Monitor (BCM) Substudy Instruction Leaflet

Preparation

1. Each EMPA-KIDNEY BCM kit contains:
    i. 4x BCM self-adhesive electrodes;
    ii. 1x BCM chip card;
    iii. 1x skin wipe (optional use); and
    iv. Instruction leaflet.

2. Use a new EMPA-KIDNEY BCM kit for each visit.

3. Only use the EMPA-KIDNEY BCM chip-card provided to the participant during their EMPA-KIDNEY study visit (as it has been linked specifically to them within *Livia*).

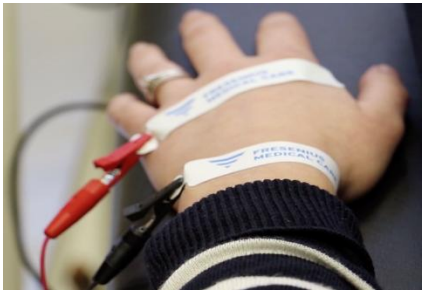
Fitting electrodes

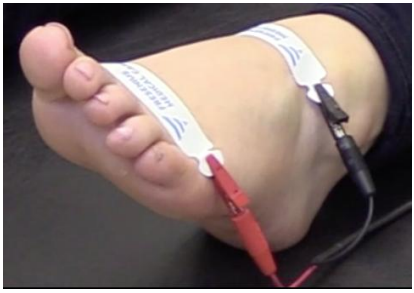
1. Remove all wrist/ankle jewellery (including watches) on the relevant side.

2. If moisturizing cream has been applied, clean an area on the back of the wrist and the ankle with the skin wipe and allow time for the skin to dry.

3. Whilst sitting or lying on a bed, stick 2x electrodes on the wrist and 2x on the ankle on the same side of the body as shown below (try to use the same side of the body at each visit).

4. Attach the red and black wire clips to the electrodes (red clips are always nearest the fingernails/toenails), then connect to the Fresenius BCM machine.





Making a BCM measurement

1. Insert the EMPA-KIDNEY BCM chip card into the Fresenius BCM machine.

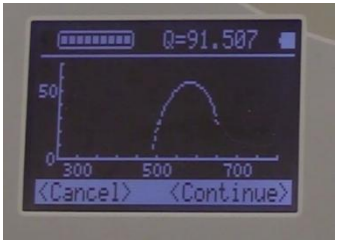
2. DO NOT CHANGE the information on the pre-prepared EMPA-KIDNEY BCM chip card (it has been preloaded with dummy data).

3. Press <Continue>, <New meas> and <Confirm> until "Start measurement" appears on the screen. There is also no need to change the weight or enter blood pressure or UF-volume.

4. Ensure the participant's:
   i. Legs are not touching each other;
   ii. Arms and hands are away from the body; and
   iii. Whole body is not touching any metal objects (e.g. the bedside).

5. When positioned correctly, ask the participant to remain still and silent, and then <Start> the measurement.

6. The measurement takes about 20 seconds, and the results are displayed after 1-2 minutes.



7. A single reliable measurement is required. It needs to have a:
   i. Q value of 80 or over; and a
   ii. Cole plot which looks like an inverted cone (as depicted here).

8. If an unreliable measurement is recorded, check the electrodes and participant's position before making a new measurement on the same EMPA-KIDNEY BCM chip card (there is no need to delete the unreliable measurement).

Storing the BCM chip card

1. Do not provide displayed BCM results to the participants as dummy age, sex and height data have been used on EMPA-KIDNEY BCM chip cards (the coordinating centre will re-process these data to get the true reading using the information on *Livia*).

2. Once the measurement has been automatically saved onto the EMPA-KIDNEY BCM chip card, remove and store the EMPA-KIDNEY BCM chip card safely in a locked cupboard awaiting collection by the coordinating centre.

EMPA-KIDNEY
The study of heart and kidney protection with empagliflozin

MRC Population Health Research Unit

UNIVERSITY OF OXFORD

EMPA-KIDNEY Local Research Coordinator

Body Composition Monitor (BCM) Substudy Training Signature Form

Local Research Coordinator (LRC) Name:	
Local Clinical Centre (LCC) Name:	
LCC Site Number (if known):	

- I have read the Ethics Committee approved EMPA-KIDNEY BCM Substudy protocol supplement
- I have read the Ethics Committee approved EMPA-KIDNEY BCM Substudy information leaflet & consent form
- I have read the EMPA-KIDNEY BCM Substudy kit leaflet
- I have watched the EMPA-KIDNEY BCM Substudy training video

Signature: …… Date: ……/……/20……..

(Traditional hand-written signature) (DD/MMM/20YY)

Original copy of this completed form to be kept in the LCC site file (investigator site file).

Scanned copy to be sent to Shraddha Shah at cco.empakidney@ndph.ox.ac.uk

EMPA-KIDNEY LRC BCM substudy training signature form 03-May-2019 version 1.0

Internal Operating Procedure:

BCM Measurement Substudy Datacard Collection & Data Extraction

EMPA-KIDNEY

EDMS #6433

Version History

Version	Version Date	Author	Description
1.0	08 Aug 2019	Shraddha Shah, Richard Brown, Will Herrington	Initial version

This is a controlled document. Distribution and approval is to be managed using the Electronic Document Management System.

TABLE OF CONTENTS

1 Purpose

This Internal Operating Procedure (IOP) describes the procedures to be followed within the Central Coordinating Office (CCO) for BCM datacard collection and data extraction.

2 Intended Readership

This is a controlled document. Distribution and approval is to be managed using the Electronic Document Management System in accordance with SOP0 (Development and Maintenance of Project Specific Operating Procedures). This document and any updates are to be made available to all CTSU based trial-specific staff. The main readership is the CCO administrative staff who are responsible for BCM measurement substudy datacard collection and data extraction. This document may be made available to the relevant teams within the external organisation(s).

3 Definitions and Abbreviations

BCM	Body Composition Monitor, Fresenius's bioimpedance machine
CCO	Central Coordinating Office: responsible for the overall coordination of the trial internationally, based at CTSU in Oxford
CTSU	Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, home of the CCO
EDMS	Electronic Document Management System utilized by the CCO
FMT	Fluid Management Tool, the software used to create and download data from the BCM datacards
IOP	Internal Operating Procedure: a document used within the CCO to describe a procedure to be followed in the CCO
LCC	Local Clinical Centre
RCC	Regional Coordinating Centre.

4 Scope

EMPA-KIDNEY will assess the clinical effects of empagliflozin versus matching placebo in over 5,000 participants with pre-existing chronic kidney disease who have been treated with renin-angiotensin system blockade (wherever indicated and tolerated). Follow-up for about 3-4 years will allow reliable assessments of any beneficial or adverse effects of empagliflozin on the primary composite endpoint of kidney disease progression or cardiovascular death.

The BCM measurement substudy is assessing the effect of empagliflozin versus placebo on "overhydration" measured using bioimpedance on a BCM measurement machine in a subset of about 400 participants. BCM measurements are made at randomization, 2 month and 18 month follow-up visits, using a datacard linked to the participant in the study's web-based IT system called *Livia.*

The setup of BCM substudy datacards is detailed in EDMS6242. This IOP's scope includes guidance on the approximate 6 monthly process of:

1. Selection of BCM datacards for collection from an LCC (section 5.1);
2. Secure couriering of the BCM card to the CCO (section 5.2);
3. BCM datacard receipt at the CCO (section 5.3);
4. BCM data download (section 5.4);
5. BCM data backup (section 6);
6. BCM data quality checks (section 7);
7. Return of the datacards to LCCs for archiving (section 8) and
8. BCM Substudy Datacard tracker and BCM Substudy Download Datacard Tracker (appendices 1 & 2)

5 Guidance on BCM datacard collection and data extraction

5.1 *Selection of BCM datacards for collection from an LCC*

A site-specific 'BCM Substudy Datacard Tracker' report from *Livia* (or its mirror database) will be used to identify BCM datacards, which have been linked to a participant ID and used for measurement (see appendix 1 for a template of this tracker report).

This report will be generated (approximately) every few months and sent to relevant LCCs and BCM datacards requested. The site will be asked to check all BCM datacards are available and document any lost card(s) on the tracker report. Once completed the tracker report should be signed off and returned to the CCO with the datacards.

5.2 *Secure couriering of the BCM cards to the CCO*

The CCO is responsible for arranging secure couriering (where necessary) to the CCO. In certain countries (e.g. Germany), it may be more appropriate for the BCM datacards to be collected at the RCC and then sent as a batch to the CCO.

5.3 *BCM datacard receipt at the CCO*

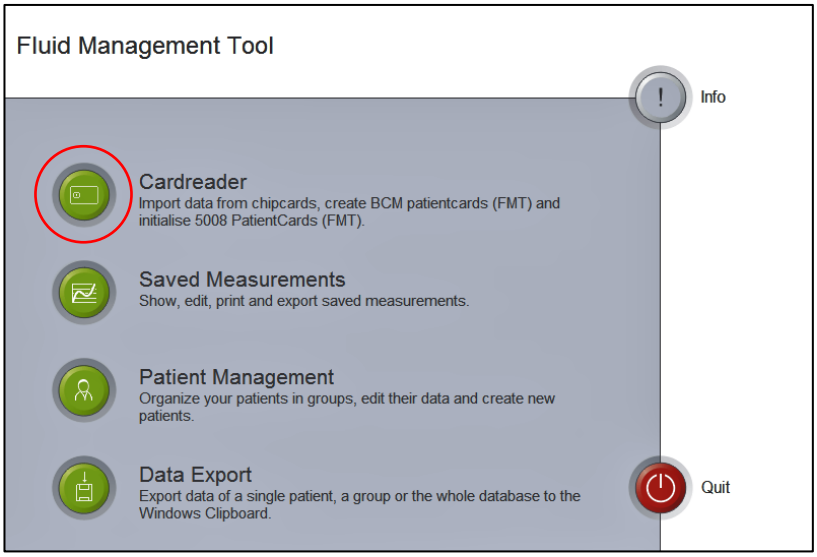
On receipt of the BCM substudy datacards at the CCO, the completeness of the shipment and date of the delivery should be entered on the 'BCM Substudy Datacard Tracker' report (appendix 1).

5.4 *BCM data download*

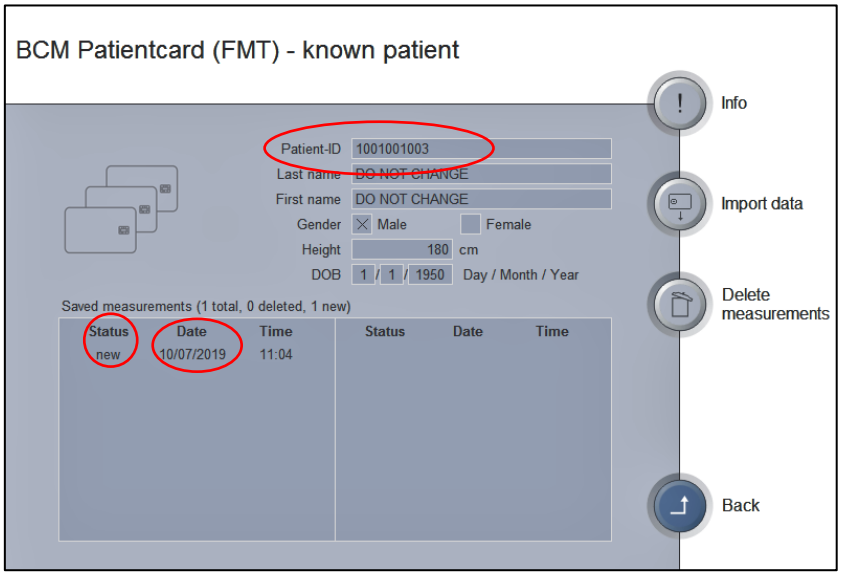
A separate site-specific 'BCM Substudy Download Datacard Tracker' report from *Livia* (or its mirror database) will be used to track the downloading of data from the BCM datacards (see appendix 2 for a template of this tracker report).

BCM datacard download is performed using the BCM substudy encrypted laptop running the Fluid Management Tool (FMT) program:

- Open the FMT program



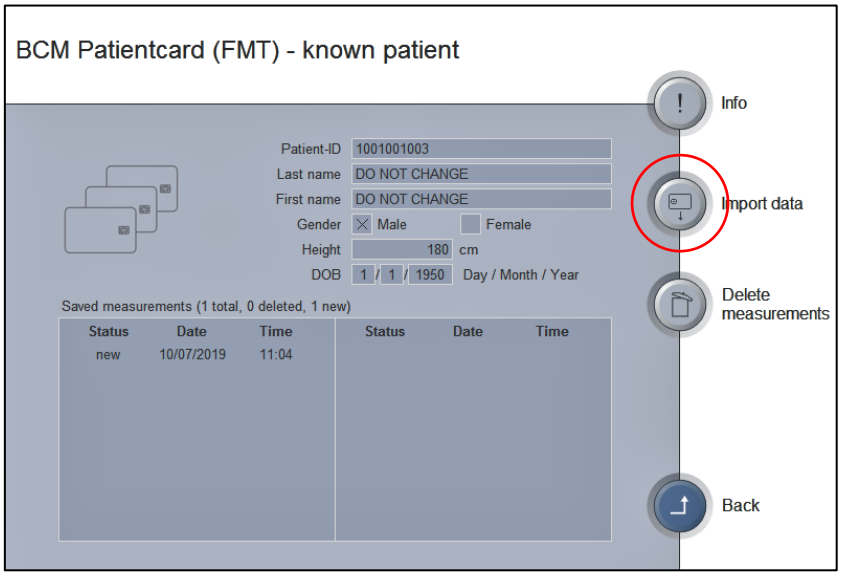
- Insert the card into the card reader and click on the ‘Cardreader’ button



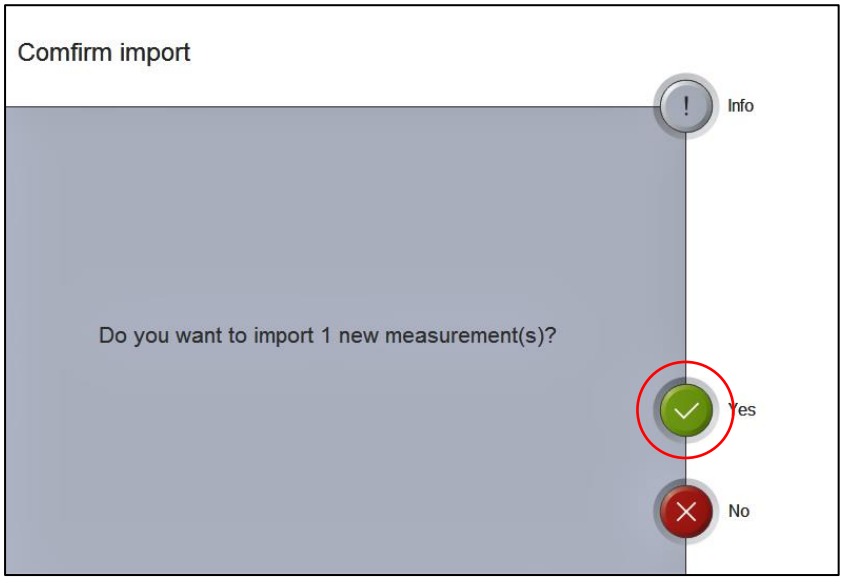
- The status of the saved measurement will be shown as ‘new’, as it contains the measurements, which are yet to be downloaded into the FMT database. There may be more than one measurement. All measurements should be downloaded

- Before the data are downloaded, check the FMT displayed Patient ID matches the BCM datacard ID on the sticker affixed to the BCM card and record this on the ‘BCM Substudy Download Datacard Tracker’

- Confirm the date of measurement matches the date in the tracker report. This check should be recorded on the ‘BCM Substudy Download Datacard Tracker’. If there are any discrepancies, record them on the tracker report and inform the EMPA-KIDNEY Chief Investigator without downloading the data. Any discrepancies will need to be recorded on a spreadsheet on the K:EMPA drive together with the advice given by the Chief Investigator

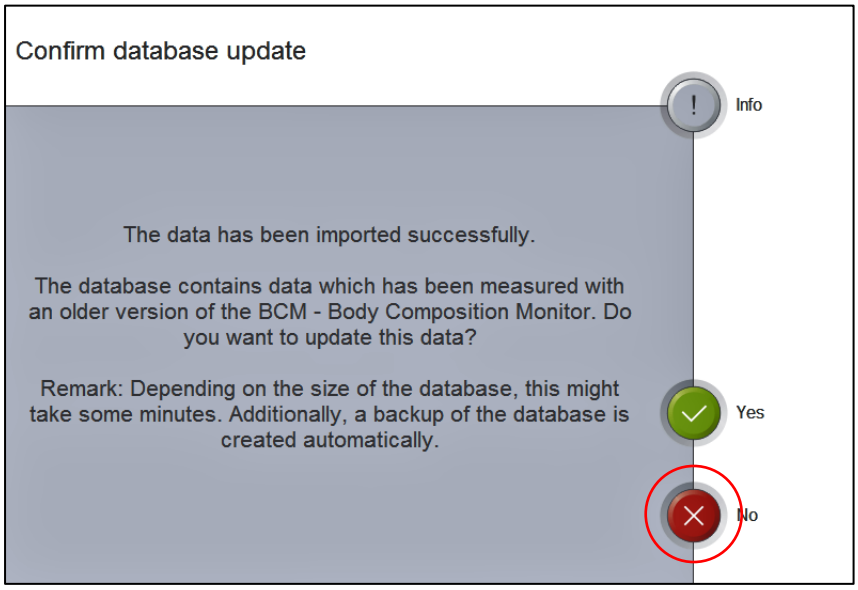


- If the Patient ID and date of measurement are confirmed, download the data by clicking on the 'Import data' button



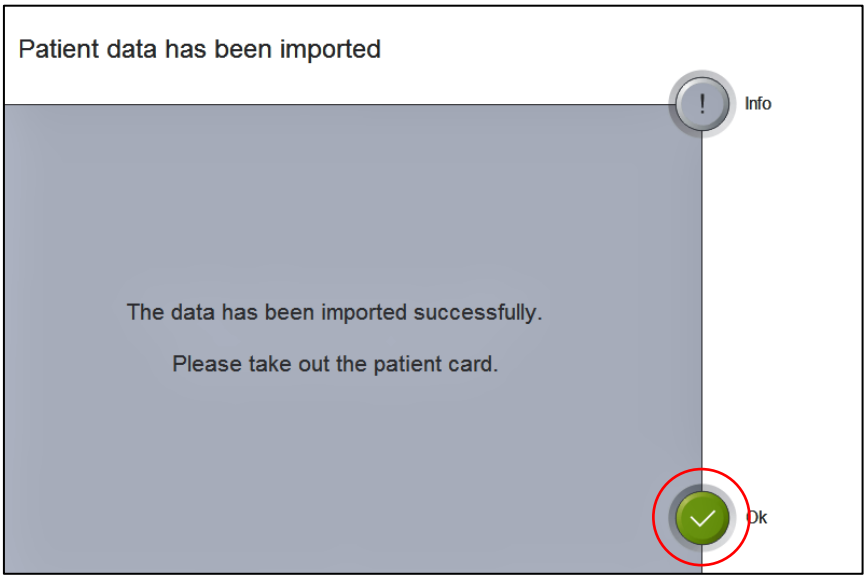
- Ensure the number of imported measurements displayed matched the number on the previous screen (it will usually be one)

- Confirm data download by clicking the 'Yes' button

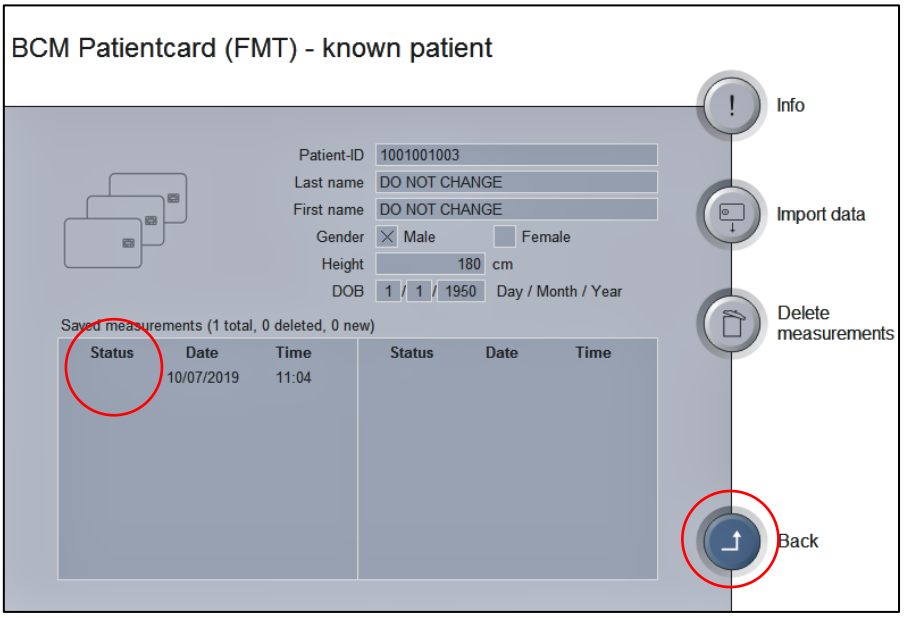


- The confirm database update screen will be displayed, confirming the data has been imported successfully

- There is no need to update the data if this was measured with an older version of the BCM, click 'No' if this message is displayed



- After the data has been imported successfully, click the 'OK' button



- After a successful download, the status now appears as being blank
- Record the data download as successful on the 'BCM Substudy Download Data Card Tracker' and then click the 'Back' button to return to the FMT main menu
- The next BCM datacard can now be inserted in the cardreader and the steps detailed in section 5.4 can be repeated for each BCM datacard to be downloaded

Notes

Never delete any measurements from the BCM datacard

If there are any problems during downloading, record the affected BCM datacard ID on the tracker record (in comments) and contact the EMPA-KIDNEY Chief Investigator for advice.

6 Data backup

After completing data downloading, and before turning off the BCM substudy laptop, **you must** perform a database backup. This is performed by connecting the encrypted external backup hard drive and clicking on the 'BACKUP' icon (as shown below) on the desktop. This icon will only appear after the hard drive has connected to the laptop.

Once the backup is complete, the data on the hard drive **must** be transferred onto the K:drive. Full details on how this is performed are provided in EDMS6432.

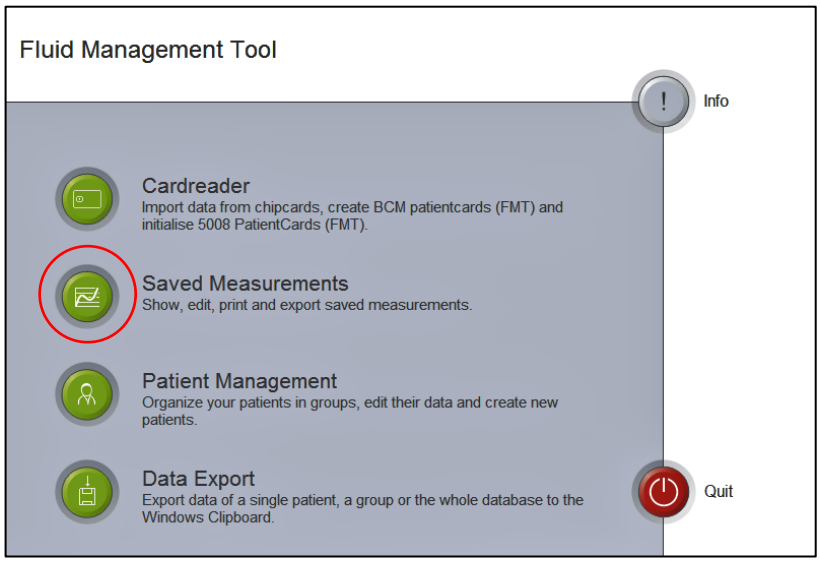
7 Data quality control checks

After completing the data backup, the Q score should be checked for each patient and added to the tracker.

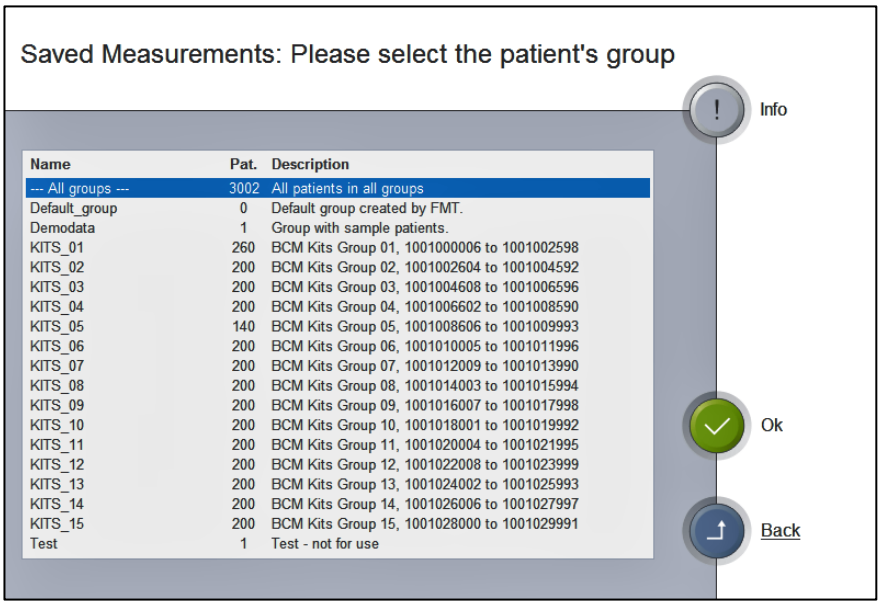
Sites with a substantial proportion of measurements with a Q score of <85% will be contacted and asked to review the training video.

Obtaining Q score from the FMT program.

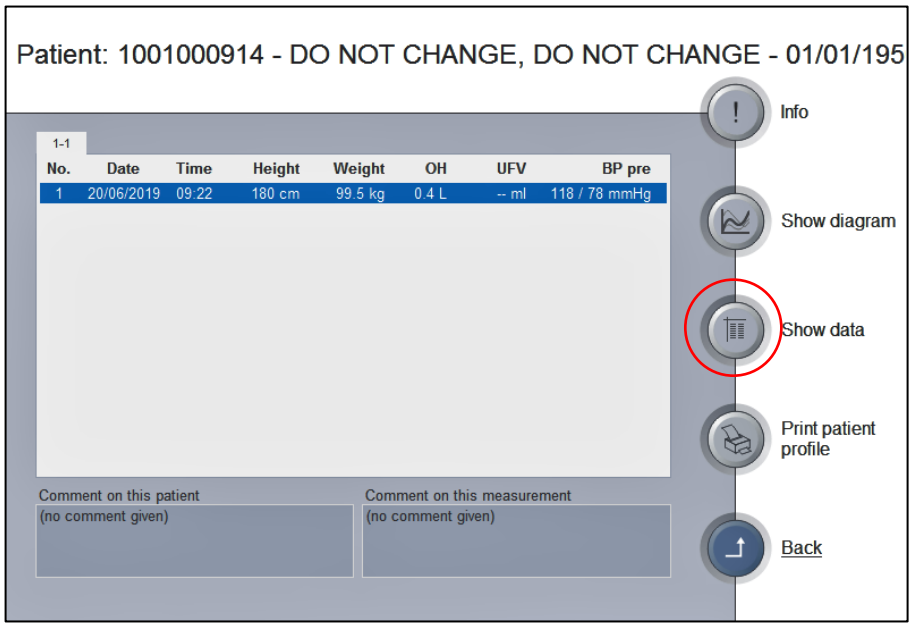
- Open the FMT program



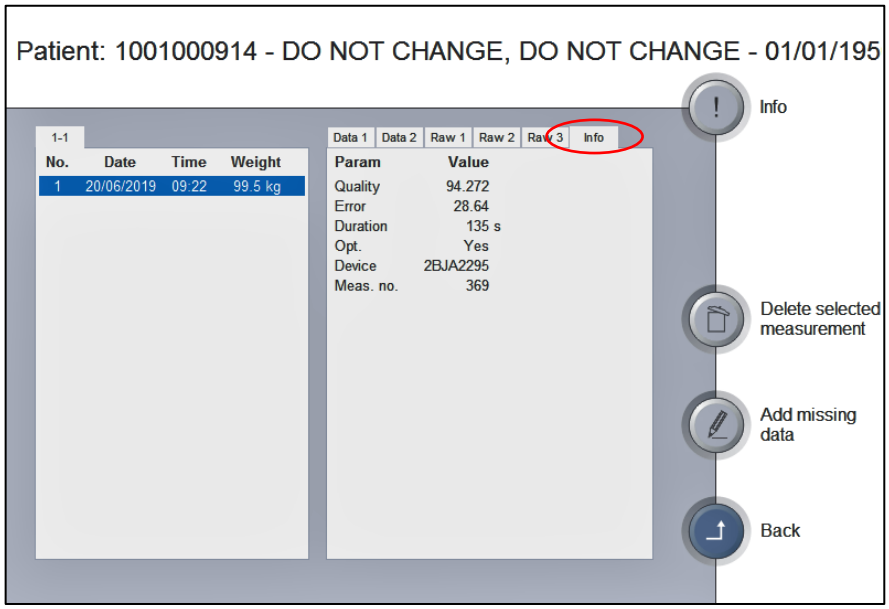
- Click on the Saved Measurements



- Select the relevant Patient ID from the list (screenshot not shown)
- Once you click on the Patient ID, the page below will be displayed showing the patient's measurements, and the date and time when they were taken (at site)



- Click on show data



- Select the 'Info' tab to display the patient's Quality 'Q' value. Enter this Q value on the 'BCM Substudy Download Datacard Tracker'. You do not need to record the numbers after the decimal point on the tracker, just record the initial two numbers (without rounding)

8 Return of the BCM datacards to LCCs for archiving

Once the 'BCM Substudy Datacard Tracker' and 'BCM Substudy Download Datacard Tracker' have been completed, and signed off, the BCM datacards can be returned to their originating LCC for archiving as source data, with the site file.

A copy of the 'BCM Substudy Datacard Tracker' should be returned to the LCC with the BCM datacards. The LCC should acknowledge receipt of the cards on the tracker, and once signed off, return a scanned copy to the CCO and file the LCC copy at site.

The original completed 'BCM Substudy Download Datacard Tracker' does not need to be sent to the LCC and should be retained at the CCO.

Appendix 1: Example BCM Substudy Datacard Tracker

BCM Substudy
-Datacard Tracker.xls

Appendix 2: Example BCM Substudy Download Datacard Tracker

BCM Substudy - Download Datacard

EMPA-KIDNEY BCM Substudy:
BCM Card Data Transfer for Outcome Derivation

Internal Operating Procedure

EDMS #7248

Version	Date	Author(s)	Summary
1.0	18th Aug 2021	Will Herrington/Will Stevens/John Nolan/David Keane/Dani Trinca	First issued version

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Purpose

This document sets out the procedure for collating and transferring BCM study related data to and from the Central Coordinating Centre based at the University of Oxford ("Oxford") to and from collaborators at the Leeds Teaching Hospital NHS Trust ("Leeds") where derivation of the BCM study outcomes is performed. A study-specific Data Analysis Plan will be specified before the trial results are unblinded to the Chief Investigator.

Summary of process

1. Data preparation Oxford

Data from BCM cards received from EMPA-KIDNEY sites in Oxford are downloaded and backed up according to EDMS #6433. The backup process is performed after any work has been done on the BCM study laptop computer which has (or may have) changed the data (see EDMS 6432 for backup procedure details). All measurements are downloaded (including multiple measurements on the same day) as the Data Analysis Plan will specify which is the optimum BCM measurement (or measurements) to use.

At periodic intervals, Oxford will export a backup of the Fluid Management Tool (FMT) database .pat files into a .zip file using the FMT software.

In addition, the *Livia* database will be queried for the following fields (after running Erato):

1. Age
2. Weight
3. Height
4. Sex
5. BCM card ID

The following fields will be added to an Excel spreadsheet for each BCM measurement:

New field	LIVIA_Identifiers	Notes
BCM card ID	LIVIA_BCM	Recorded directly into relevant visit form or via Erato
Age	LIVIA_AGE	Record as whole years on day of BCM measurement
Weight	LIVIA_WT	Recorded to 1 decimal place in kg as entered into *Livia* (or via ERATO) on day of BCM measurement (i.e. Randomization or Follow-up visit). Missing weights will be entered as 0.0 kg

Height	LIVIA_HT	Recorded in cm from Randomization Visit. Missing height will be entered as 0.0 cm and sought from site
Sex	LIVIA_SEX	F = Female, M = Male, U = Unknown

2. Data transfer to and from Oxford-Leeds

The Excel spreadsheet containing *Livia* data and the database backup will be transferred to Leeds for BCM Outcome derivation. According to the Service Agreement, data transfer needs to be using secure methods (i.e. encrypted via 7-zip and sent using Oxfile [or similarly secure method]). Similar secure methods could include encrypted via 7-zip between NHS.net email addresses.

Oxford team email addresses:

Will Stevens	will.stevens@ndph.ox.ac.uk
Cc: Will Herrington (Chief Investigator) Mobile number: 07970 520390	will.herrington@ndph.ox.ac.uk will.herrington@nhs.net

Leeds team email addresses:

Dani Trinca	daniele.trinca@nhs.net
David Keane	david.keane@nhs.net

3. Data processing in Leeds

The age, sex, height and weight entered on the BCM cards are dummy data and should be ignored. The BCM measurement plus the LIVIA_ fields_described above should be used to derive the BCM outcome value.

Each time data is sent to Leeds, the following steps outline the process of re-deriving BCM outcome variables:

- A macro written in Microsoft Excel will be used to extract all summary BCM measurement data needed for data processing (Re and Ri) from the .pat files (see appendix 1 for validation of the macro)
- The ID from the BCM card, corresponding to the ID in the *Livia* spreadsheet, will be used match the two data sources
- Body composition parameters are calculated using the *Livia* demographic parameters and the impedance information from the BCM

4. Processed data transfer from Leeds to Oxford

Data should be returned to Oxford using the secure methods described above (i.e. encryption and Oxfile).

Appendix I: Pat file importer validation documentation

1 Introduction

This document aims to describe the process of importing the BCM .pat file into a Microsoft Excel™ spreadsheet and the validation of the data compute using a VBA Macro.

2 Data import

The VBA Macro imports all the .pat files present in the folder the Excel file is located.
The code reads each .pat file and imports the following:

- Patient ID (which in the case of the BCM substudy will be the BCM card ID)
- Date & time of measurement (to allow a check between *Livia* data and BCM card data)
- Birthday Year
- Age
- Gender
- Height cm
- Pre weight Kg
- BMI
- Re
- Ri
- ECW_L
- ICW_L
- OHPre_L
- ATM
- LTM

A number of rows are created based on the amount of measurements present in the file.
Once the data from the .pat files are imported the spreadsheet computes the following variables.

- K_ECW
- K_ICW
- ECW
- ICW

The above variables are calculated using the formulas listed in the Moissl's paper from 2006. (formula 9-12)

LTM, ATM and OH are derived from the Chamney's paper. These formulae have been adjusted since publication and the new coefficients haven't been released to the public.

A total of 892 measurements taken from 141 patients has been analysed. The Bland Altman plot (Figure 1) , shows the level of agreement of the two measurements. The bias (Avg (OH Excel- OH BCM)) is 0.02 liters.

Figure 2 shows the current flow of the data in the Excel spreadsheet.

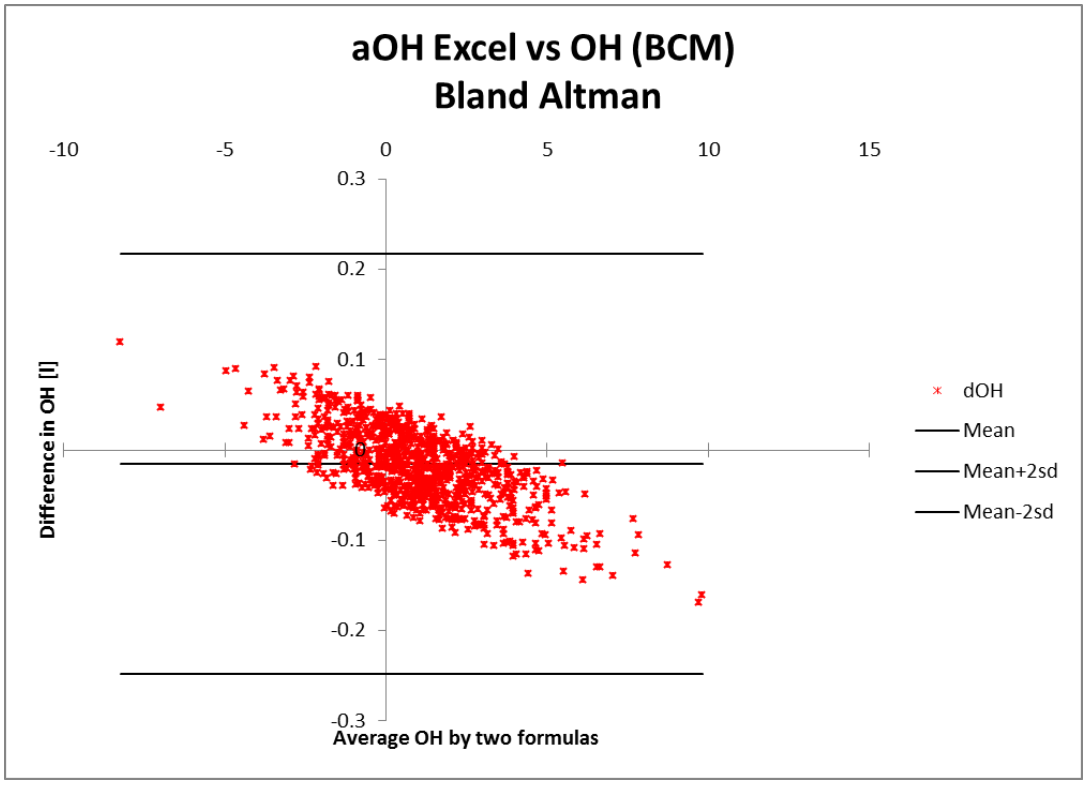


Figure 1 Bland-Altman plot based on 892 measurements (141 patients)

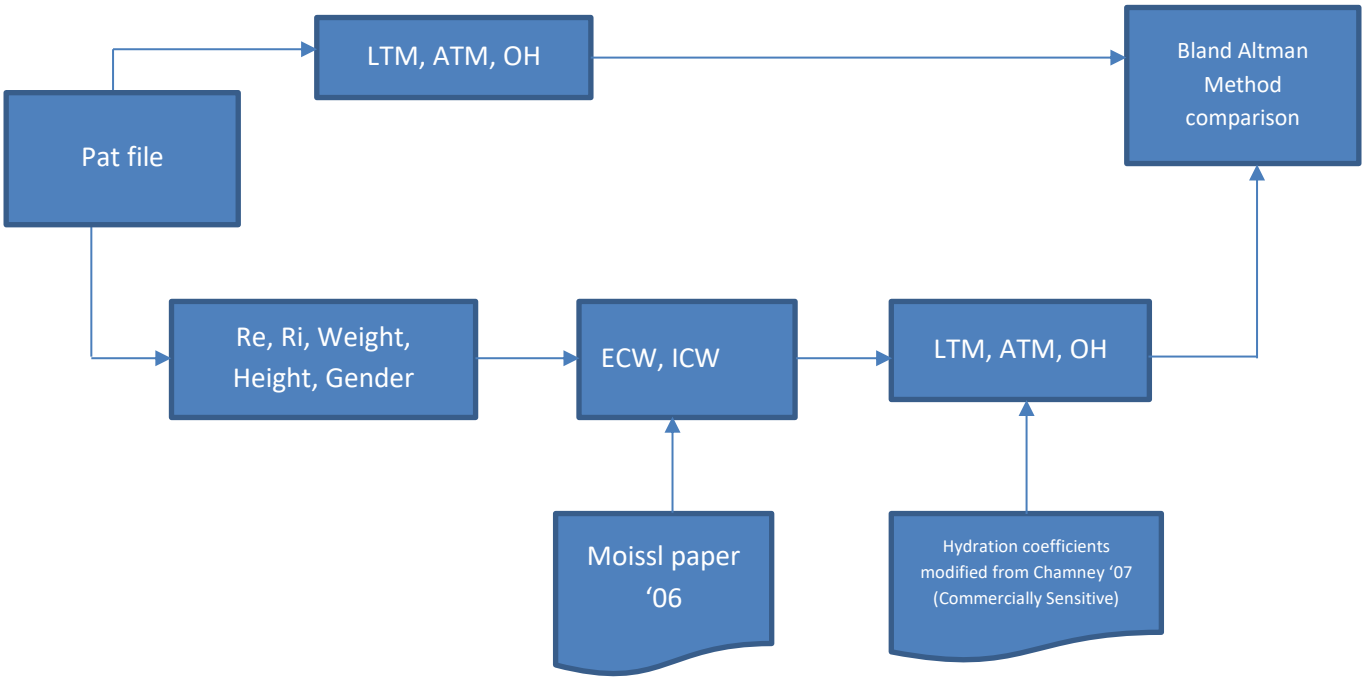


Figure 2 Current Excel import procedure.

EMPA-KIDNEY Body Composition Monitor Substudy Extended Data Analysis Plan

Version History

Version	Description	
1.0	First released version	Created by: Kaitlin J. Mayne, Natalie Staplin, Richard Haynes & William G. Herrington (JAN-MAR2022) Reviewed by Rebecca Sardell, Will Stevens, Karl Wallendszus, Jonathan Emberson, John Nolan, Dani Trinca, David Keane, Simon Davies, Rejive Dayanandan, Akiko Omata, Parminder Judge, Ryonfa Lee, Patrick Mark, Jennifer Lees, Vladimir Cejka, Christoph Wanner (MAR2022)
1.1	Revision to Appendix 8.1: Definition of a valid BCM measurement Addition of Appendices 8.4 and 8.5.	Revised by: Kaitlin J. Mayne, David Keane, & William G, Herrington (18OCT-16NOV2022) Released for use by William G. Herrington (17NOV2022)

This document is an extension to EDMS #7635, the primary Data Analysis Plan (DAP) for the EMPA-KIDNEY Body Composition Monitor (BCM) Substudy which is subject to review by the Steering Committee. This extended DAP adds background information supporting the rationale for the primary and secondary assessments and lays out, in detail, the planned exploratory analyses which are not fully described in EDMS #7635.

TABLE OF CONTENTS

1 RELEVANT PROCEDURAL DOCUMENTS

Document title	EDMS#
EMPA-KIDNEY Protocol	5434
EMPA-KIDNEY BCM Substudy Protocol Supplement	6251
EMPA-KIDNEY Data Analysis Plan (SOP11)	6290
EMPA-KIDNEY BCM datacard download IOP	6433
EMPA-KIDNEY Leeds BCM Card Data Transfer for Outcome Derivation	7248
EMPA-KIDNEY BCM kit leaflet	6240
EMPA-KIDNEY BCM Substudy Data Analysis Plan	7635

2 ABBREVIATIONS

Abbreviation	Definition
ACR	Albumin-to-creatinine ratio
ATM	Adipose tissue mass
BCM	Body composition monitor
BMI	Body mass index
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
DPP-4	Dipeptidyl peptidase-4
ECW	Extracellular water
EDMS	Electronic document management system
eGFR	Estimated glomerular filtration rate
FTI	Fat tissue index
GLP-1 agonist	Glucagon-like peptide-1
HbA1c	Glycosylated haemoglobin
ICW	Intracellular water
LTI	Lean tissue index
LTM	Lean tissue mass
MMRM	Mixed model repeated measures
NT-proBNP	N-terminus prohormone of brain natriuretic peptide
PD	Peritoneal dialysis
RAS	Renin-angiotensin system
SOP	Standard operating procedure
TBW	Total body water

3 INTRODUCTION

This document provides a Data Analysis Plan for the EMPA-KIDNEY substudy, which has measured body composition of a subset of approximately 650 EMPA-KIDNEY participants recruited from the UK and Germany using bioimpedenace spectroscopy on a body composition monitor (BCM). An outline BCM data analysis plan was provided in the BCM substudy's Protocol Supplement (EDMS#6251). The purpose of this BCM Data Analysis Plan is to define, before unblinding of the treatment allocation, detail of pre-specified randomized analyses to be presented in initial publication(s) of the substudy. The nature of all analyses (randomized or observational) including those related to subsequent publications and exploratory analyses cannot be specified in detail but, where appropriate, a general analytical approach is set out. Approaches, wherever possible, will follow those set out in EMPA-KIDNEY's main data analysis plan (SOP11; EDMS#6290).

Note: this pre-specified Data Analysis Plan re-orders the priority of some of the assessments set out in the BCM substudy Protocol Supplement (EDMS#6251). Certain assessments have been moved from secondary to tertiary assessments, and a new key secondary assessment introduced. This follows a more detailed review of data whilst compiling this plan. This pre-specified Data Analysis Plan therefore supersedes the proposed assessments set out in the Protocol Supplement and prevails in the event of any discrepancies between the two documents. In addition to the pre-specified comparisons, other post-hoc analyses may be performed with due allowance for their exploratory and, perhaps, data-dependent nature.

This extended version of the BCM DAP is an extension to EDMS #7635, the primary Data Analysis Plan (DAP) for the EMPA-KIDNEY Body Composition Monitor (BCM) Substudy which is subject to review by the Steering Committee. This extended DAP adds background information supporting the rationale for the primary and secondary assessments and lays out, in detail, the planned exploratory analyses which are not fully described in EDMS #7635. The extended DAP forms the basis of a statistical plan for a doctoral research project.

4 KEY FLUID OVERLOAD DEFINITIONS & JUSTIFICATION FOR CLINICAL COMPOSITE OUTCOMES

There is no standard nomenclature for BCM-derived fluid overload parameters in existing literature, with a range of terminology and threshold values to infer clinical significance employed. We have used the following approach to report the EMPA-KIDNEY BCM substudy

EMPA-KIDNEY Terminology		Definition	Alternative Terminology
Fluid Overload		Overhydration in litres, computed as the difference between expected (based upon weight and body composition) versus measured extracellular water (ECW) volume (1), with positive values representing excess fluid Fluid Overload = $ECW_{measured}$ - $ECW_{expected}$	Overhydration (2) Hydration status (1) † Absolute tissue hydration (3)
Relative Fluid Overload		Overhydration index relative to measured ECW volume, expressed as a percentage (4) Relative Fluid Overload = Fluid Overload ÷ $ECW_{measured}$	Overhydration index (2, 5) * Relative hydration status (4, 6) † Relative tissue hydration (3)
Clinically Significant Fluid Overload	Moderate	Relative Fluid Overload >7%, ≤15% where 7% reflects the 90th percentile in a healthy reference population (3) and is approximately equivalent to absolute Fluid Overload of +1.1L (3)	
	Severe	Relative Fluid Overload >15% which represents the highest quartile in a haemodialysis reference population (1, 4); approximately equivalent to absolute Fluid Overload of +2.5L (3, 4, 7, 8)	Hyperhydration (4)

** Although scientific literature has used the term "overhydration index" to refer to both absolute Fluid Overload in litres and Relative Fluid Overload* (5, 9)*, we consider it to most accurately describe overhydration indexed to ECW.*

† Hydration status expressed as ΔHS has also been used to refer to both absolute Fluid Overload in litres (1) as well as Relative Fluid Overload (4, 6).

Fluid overload is a hallmark of decompensated heart failure (10, 11) and therefore BCM-derived fluid overload could be a surrogate for decompensated heart failure. Observational studies in both dialysis and non-dialysis chronic kidney disease (CKD) have demonstrated that bioimpedance-derived fluid overload is strongly associated with mortality (5, 6, 12). Data in heart failure, although limited to small cohorts, consistently demonstrate the association between fluid parameters derived from bioimpedance analysis - a less precise technique than bioimpedance spectroscopy employed by the BCM - and mortality and hospital readmission for decompensated heart failure (13-17).

The key secondary assessment of the BCM substudy is a composite outcome combining BCM-derived Clinically Significant Fluid Overload with the clinical outcomes of death from

heart failure or hospitalization for heart failure (see **section 6.3**). Important data on fluid overload captured by BCM measurements is missed when remote follow-up visits are necessary (e.g. as a result of the COVID-19 pandemic) or after death, so the composite outcome serves to capture all recorded data on fluid overload and its clinical consequences (whether measured by BCM or reflected in reported adverse events).

The Fluid Overload parameters defined above are based upon the three-compartment model described by Chamney *et al*. comprising normally hydrated adipose tissue, normally hydrated lean tissue and excess fluid (18). The excess fluid compartment is derived from extracellular water (ECW), intracellular water (ICW) and total body water (TBW) however ECW is used as standard and forms the basis of the more recently developed Fluid Overload and Relative Fluid Overload parameters derived using the Fresenius BCM. This measurement of fluid overload has been validated against gold standard techniques (19) and reproducibility demonstrated (20, 21). The Clinically Significant Fluid Overload BCM-derived component of the composite secondary outcome is defined based upon established thresholds values of Relative Fluid Overload reported in existing literature. Relative Fluid Overload is considered to be more clinically meaningful than Fluid Overload because normalization to ECW facilitates comparison between patients (4). Wizemann *et al*. established a 15% threshold value of Relative Fluid Overload (referred to as relative hydration status) based upon the highest quartile of a reference haemodialysis population (4). In EMPA-KIDNEY, the threshold of >15% Relative Fluid Overload is referred to as “severe” as the study population can be expected to exhibit lower levels of fluid overload than dialysis populations. This threshold is approximately equivalent to >+2.5L absolute Fluid Overload in patients on haemodialysis (1, 4, 22). In the Wizemann *et al.* cohort of 269 patients on haemodialysis, 86 died during 3.5 years of follow-up and the adjusted hazard ratio (HR) for all-cause mortality associated with pre-dialysis Relative Fluid Overload >15% was 2.1 (95% confidence interval [CI] 1.4-3.2) compared to ≤15% (4). Pre-dialysis Relative Fluid Overload >15% was more strongly associated with death than age or systolic blood pressure (4). The >15% threshold (or equivalent in litres) has subsequently been widely used in confirmatory studies in haemodialysis populations (5, 7, 8, 23-27).

Other studies have also used a lower threshold of >7% Relative Fluid Overload, which in a healthy reference population is equivalent to approximately +1.1L absolute Fluid Overload and to the 90th percentile (3, 28, 29). In EMPA-KIDNEY, this level of fluid overload is referred to as “moderate”. Fluid Overload of >7% is also associated with risk of death in dialysis cohorts (8, 27-29). For example, Dekker *et al.* reported that, compared with patients considered to be euvolaemic pre-dialysis (defined as Fluid Overload -1.1L to +1.1L), those with pre-dialysis

values of >+1.1, ≤+2.5L (equivalent to 7-15% Relative Fluid Overload) and >+2.5, ≤+5.0L (equivalent to >15% Relative Fluid Overload), hazards of death were increased by 1.6- and 2.7-times, respectively (HR 1.6 [95% CI 1.4-2.0] & 2.7 [95% CI 2.3-3.4]) (8). Similar sized HRs were also reported for BCM assessments of Fluid Overload made after completing a dialysis session (8). Siriopol *et al.* report similar findings comparing haemodialysis patients with moderate (>+1.1L, <+2.5L) and severe (>2.5L) Fluid Overload pre-dialysis to those considered normovolaemic (-1.1L to +1.1L) (HR 1.5 [95% CI 1.2–1.9] & 2.0 [95% CI 1.6–2.6]; respectively) (27).

In peritoneal dialysis (PD) populations, a study by Jotterand Drepper *et al.* applied the same 15% threshold to a cohort of 54 PD patients and demonstrated a significant association with hazards of death (HR for each for 1-SD [11%] increase in Relative Fluid Overload 7.8 [95% CI 1.1–29.1]) (30). In the IPOD-PD study, van Biesen *et al.* studied the association between serial measurements of Relative Fluid Overload and mortality in a cohort of 1054 incident PD patients (31). Moderate Relative Fluid Overload was defined as >7% and severe >17.3% (31). The severe threshold value of >17.3% is derived from the 75th percentile of their population at 1 month since commencing PD. It was associated with a 59% increased hazards of all-cause mortality (HR 1.6 [95% CI 1.1-2.3], compared with Relative Fluid Overload ≤17.3%) (31). In a subgroup analysis of the cohort who developed PD technique failure (composite of death or transfer to haemodialysis), they used the same principle of a cut-off value based on the 75th percentile of the study population (who developed technique failure) and therefore a value of >14.4% was used. This was associated with a significantly higher risk of PD technique failure (HR 2.7 ([95% CI 1.8-4.3) (32). O'Lone *et al.* applied another alternative threshold value of ≥10% (representing the top 30% of the studied cohort), and reported that Relative Fluid Overload of ≥10% was strongly associated with risk of death in 529 patients on PD (HR 2.1 [95% CI 1.4-3.2]) compared to those with <10% Fluid Overload (9). Other cohorts have not assessed the same 17.3% or 10% threshold used in these studies. Of note, it is generally considered that BCM measurements are not affected by presence or absence of peritoneal dialysate, (3) although measurements are generally obtained with dialysate *in situ* (9, 30), and there is some uncertainty (33).

Data in the non-dialysis CKD population are limited to assessing the relevance of the >7% threshold because of lower levels of fluid overload compared to patients on dialysis. A study by Tsai *et al.* demonstrated that in patients with CKD stages G4-5 not requiring dialysis, Relative Fluid Overload ≥7% was associated with about a doubling of the hazards of the composite outcome of death or cardiovascular event (incident myocardial infarction, stroke, peripheral artery disease, or hospitalization for heart failure or arrhythmia) compared to <7%

Fluid Overload (HR 1.9 [95% CI 1.0-3.7]) (6). Associations were similar in a study by Hung *et al.* conducted in patients with CKD stages G3-5 (not requiring dialysis): Relative Fluid Overload ≥7% was associated with significantly increased risk of a composite outcome of myocardial infarction, hospitalization for congestive heart failure or unstable angina, or death from cardiovascular causes (HR 2.7 [95% CI 1.1-6.5]) (34). These and other studies have also reported associations between Relative Fluid Overload >7% and kidney disease progression (35, 36), but these may simply reflect Fluid Overload as a marker of risk rather than be directly responsible for CKD progression.

5 BASELINE CHARACTERISTICS

In order to assess balance of baseline characteristics between randomized arms of BCM substudy, the following variables recorded at Randomization (or at Screening) will be presented for each of the empagliflozin and placebo groups. All participants with at least one valid BCM measurement will be included, with missing baseline BCM values imputed using methods set out in section 7.1.

Note that these are a subset of the characteristics pre-specified in the main Data Analysis Plan (SOP11; EDMS#6290) plus other measures of anthropometry and BCM measurement variables. Categories will be consistent with those from the main trial publications or subgroup analyses:

a. History of prior disease:
    i. Diabetes mellitus (presence *vs* absence);
    ii. Self-reported heart failure (presence *vs* absence);
    iii. Primary renal diagnosis (diabetic kidney disease, hypertensive/renovascular disease, glomerular disease, other or unknown [1])
b. Patient characteristics;
    i. Age (continuous and categorised: <60; ⩾60 <70; ⩾70 years);
    ii. Sex (male *vs* female);
    iii. Race (White, Black/African American, South Asian, Southeast Asian, Mixed or Other);
    iv. Smoking status (ever smoked regularly at Randomization, yes *vs* no);
    v. Weight in kg*;
    vi. Body mass index (BMI) (continuous and categorised: <25; ≥25 <30; ≥30 kg/m^2);
    vii. Waist-to-hip ratio*;
    viii. Extracelllular water (ECW) in litres*;
    ix. Intracellular water (ICW) in litres*;
    x. Fluid Overload in litres*;
    xi. Relative Fluid Overload (%)*;
    xii. Clinically Significant Fluid Overload (%, presence *vs* absence)*;
        - Moderate

[1] Other includes tubulointerstitial disease, familial/hereditary nephropathies, other systemic disorders and miscellaneous renal disorders. Glomerular disease is subcategorised as follows: focal segmental glomerulosclerosis, IgA nephropathy, membranous nephropathy, minimal change disease and other glomerular disease.

- Severe (see section 4 for definitions)

xiii. Lean tissue index (LTI) (lean tissue mass [LTM] indexed to height) *;
xiv. Fat tissue index (FTI) (adipose tissue mass [ATM] indexed to height) *;
xv. Systolic blood pressure (continuous and categorised: <130; ≥130 <145; ≥145 mmHg);
xvi. Diastolic blood pressure (continuous and categorised: <75; ≥75 <85; ≥85 mmHg);

c. Laboratory values at Randomization:
   a. CKD-EPI estimated glomerular filtration rate (eGFR) (continuous and categorised: <30, ≥30 <45, ≥45 mL/min/1.73m^2 estimated from central enzymatic creatinine [or local creatinine where central value unavailable])
   b. Urinary albumin:creatinine ratio (ACR): (continuous and categorised: <30, ≥30 ≤300, >300 mg/g)
   c. Glycosylated haemoglobin (HbA1c) (continuous and categorised: <39 [normoglycaemia], ≥39<48 [pre-diabetes], ≥48<75 [well-controlled diabetes], ≥75 [poor glycaemic control] mmol/mol, or missing
   d. N-terminus prohormone of brain natriuretic peptide (NT-proBNP) (continuous and categorised: <110, ≥110 <330, ≥330 ng/L)
   e. Haematocrit (continuous and categorised: <37%; ≥37% <41%; ≥41%)
d. Medication use at randomization:
   i. RAS inhibition (yes *vs* no);
   ii. Diuretics (yes *vs* no, and analyses by type [loop *vs* thiazide *vs* mineralocorticoid receptor antagonist *vs* other potassium-sparing].
   iii. Antidiabetic medications (yes *vs* no, and analyses by type [biguanide *vs* sulphonylurea *vs* insulin *vs* DPP-4 inhibitor *vs* GLP-1 agonist *vs* other]

* continuous and categorized into approximate thirds of the distribution.

In general, baseline characteristics presented in publications will include all those listed above, with those provided in main versus subsidiary tables selected based upon relevance to the publication. For continuous variables, mean (standard deviation) will be presented unless the variable has a skewed distribution, in which case median (interquartile range) will be used. For all categorical variables, the number and percentage of participants in the category will be presented. All possible categories will be displayed, zero-filled where necessary, the category 'missing' will only be displayed (e.g. in footnotes) if there are actually missing values.

6 DEFINITIONS OF KEY RANDOMIZED ASSESSMENTS

BCM measurements were specified to be performed at Randomization, 2 and 18 months of Follow-up Visits (EDMS#6251). At these visits, weight, waist circumference, and hip circumference were measured together with blood and urine for central analysis and storage. The COVID-19 pandemic caused a substantial proportion of face-to-face Follow-up Visits to be delayed, however BCM measurements were permitted at later attended Follow-up Visit appointments, as outlined in the table below. Unless otherwise specified, all analyses will involve an intention-to-treat comparison among all randomized participants with at least one valid BCM measurement during Follow-up of the effects of allocation to empagliflozin versus placebo during the scheduled treatment period (i.e. all participants will be included irrespective of whether they take none, some or all of their allocated treatment) (8-10). Handling of missing valid BCM measurements is described in section 7.1.

Scheduled Follow-up Visits relative to the Randomization Visit date

Trial visit number	Follow-up month	Follow-up period	Ideal Follow-up day
1	2	≥30, <400 days	60 days
4	18	≥400 days, until Final Follow-up*	540 days

* Assume <680 days for maximum window for purposes of calculating weighting.

6.1 Hypotheses

For all statistical tests (other than tests for heterogeneity or trend), the null hypothesis will be that the effect of allocation to empagliflozin on the parameter of interest (e.g. Fluid Overload) in the target population is the same as the effect of allocation to placebo (and hence the alternative hypothesis will be that the effect of allocation to empagliflozin is not the same as the effect of allocation to placebo).

6.2 Primary randomized assessment

The primary assessment will be the effect of allocation to empagliflozin on mean absolute Fluid Overload in litres. Effects on Relative Fluid Overload (overhydration indexed to ECW, expressed as a percentage) will be presented alongside. Effects will be averaged over the two Follow-up time points (with weights proportional to the amount of time between visits, see section 7.2.1), adjusted for Randomization Fluid Overload values. The details of analysis methods for the primary assessment are described in section 7.2.1.

6.3 Key secondary randomized assessment

The key secondary composite outcome combines clinical outcome data with BCM measurements. Important data on fluid overload captured by BCM measurements is missed when remote Follow-up visits are necessary (e.g. as a result of the COVID-19 pandemic) or after death, so the composite outcome serves to capture all recorded data on fluid overload and its clinical consequences (whether measured by BCM or reflected in reported adverse events). The key secondary assessment is time-to-first development or worsening of Clinically Significant Fluid Overload. The composite outcome is defined as:

- Death from Heart Failure;
- Hospitalization for Heart Failure (as defined for the main trial analyses in SOP11; EDMS#6290); or
- Development of moderate Clinically Significant Fluid Overload (defined as >7% to ≤15% Relative Fluid Overload) among those without any Clinically Significant Fluid Overload at baseline; or
- Development of severe Clinically Significant Fluid Overload (defined as >15% Relative Fluid Overload) among those without this outcome at baseline.

The analysis method is described in section 7.2.2.

6.4 Other secondary randomized assessment

The other secondary assessment is to test whether the effects of empagliflozin 10mg versus matching placebo on Fluid Overload vary with time – in addition to the primary randomized assessment, analyses will be presented for the separate early (2-month) versus late (18-month) time points. The analysis method is described in section 7.2.3.

6.5 Tertiary randomized assessments including subgroup analyses

Tertiary assessments include:

i. Whether any effects of empagliflozin 10mg versus matching placebo are modified by baseline factors listed in section 5 for the primary assessment (absolute Fluid Overload). Subgroups based on sex, diabetes status, NT-proBNP, and eGFR will be the key subgroups and will be emphasised in presentation and interpretation. The sensitivity of subgroup assessments to indexing to ECW will be assessed by repeating subgroup analyses for the outcome of Relative Fluid Overload.

ii. The effects of empagliflozin 10mg versus matching placebo overall, and also early versus later during follow-up on:

a. Extracellular water (ECW)
b. Intracellular water (ICW)
c. Lean tissue index (LTI) (lean tissue mass [LTM] indexed to height)
d. Fat tissue index (FTI) (adipose tissue mass [ATM] indexed to height)
e. Body weight
f. BMI
g. Waist circumference
h. Hip circumference
i. Waist-to-hip ratio

iii. The effects of empagliflozin 10mg versus matching placebo on the four separate components of the key secondary outcome of development or worsening of Clinically Significant Fluid Overload.

iv. The effects of empagliflozin 10mg versus matching placebo on regression of Clinically Significant Fluid Overload from Severe (>15%) to Moderate (>7%); Severe to normal (≤7%); or Moderate to normal.

The analysis method for tertiary assessments is described in section 7.2.4.

6.6 Exploratory observational analyses using baseline data

Descriptive cross-sectional analyses correlating baseline characteristics with baseline anthropometry and with baseline BCM measurements will also be performed which will include assessing correlations between:

- Absolute and Relative Fluid Overload and:
  - Age; sex; race; BMI; eGFR; NT-proBNP; urinary ACR; haematocrit; blood pressure; (systolic and diastolic separately); diabetes status; self-reported history of heart failure; primary renal diagnosis; RAS inhibitor use; diuretic (any and by subtype) use; smoking status (ever smoked regularly at Randomization)
- Lean tissue index (LTI), fat tissue index (FTI); and:
  - Age; sex; race; body weight; BMI; waist circumference; hip circumference; waist-to-hip ratio; urinary ACR; blood pressure (systolic and diastolic separately); heart failure (self-reported history of heart failure; NT-proBNP); diabetes mellitus status; HbA1c; diabetes therapy (by class); smoking status (ever smoked regularly at Randomization).

A predictive model for Fluid Overload will be developed using characteristics recorded at baseline (methods outlined in section 7.2.5).

6.7 Exploratory analyses using randomized assessments

Exploratory assessments are planned to better understand how any effects of empagliflozin versus placebo on Fluid Overload compare with related measures. These may include, for example, examining the relationship between effects of empagliflozin versus placebo on BCM parameters (e.g. Fluid Overload, ECW, and ICW) with effects on:

- Blood pressure
- eGFR

Exploratory assessments are planned to better understand how any effects of empagliflozin versus placebo on adiposity compare with related measures. These may include, for example, examining the relationship between effects of empagliflozin versus placebo on relevant BCM parameters (lean tissue index and fat tissue index) with its effects on:

- Body weight; BMI; Waist circumference; Hip circumference; Waist-hip ratio
- HbA1c

For the potential surrogate endpoint proposed here to be validated in future, this requires confirmation that relative effects of an intervention on the surrogate mirror the size of relative effects on the clinical outcome the surrogate purports to measure. In order to assess if either of the BCM-derived outcomes which are included in the composite outcome of development or worsening of Clinically Significant Fluid Overload could be useful surrogates of the clinical components of the composite (i.e. death or hospitalization for heart failure), the relative effect sizes will be compared. This will require exploratory analyses of the effect of empagliflozin versus placebo on the BCM-derived measures of the development or worsening of Clinically Significant Fluid Overload from this BCM substudy to be compared to results of randomized assessments of the effect of empagliflozin versus placebo on the composite of death from heart failure or hospitalization for heart failure from the full EMPA-KIDNEY trial cohort of 6609 participants.

7 STATISTICAL METHODOLOGY

7.1 Handling of missing and extreme values

Participants with a missing baseline BCM measurement will still be included in analyses if subsequent BCM measurements are obtained within the 2- and/or 18-month Follow-up windows. Missing baseline BCM measurements will be imputed with the average observed value (in both treatment groups combined). Sensitivity analyses will be performed limited to participants with complete baseline BCM data. Participants with missing baseline values relevant to subgroup analyses will be included in the subgroup containing the average value (or the most frequent category for a binary variable). Missing Follow-up BCM measurements including Fluid Overload at 2 and 18 months will be handled in the mixed model repeated measures (MMRM) approach (as outlined in section 7.2.1).

7.2 Methods of analysis

7.2.1 Primary randomized assessment

Absolute Fluid Overload in litres will be analysed as a continuous variable. Extreme outliers (defined as >2 standard deviations from the mean) will be reviewed prior to unblinding to assess data quality and plausibility (see Appendix section 8.1). These analyses will be completed before any randomized comparisons are conducted. Differences in Fluid Overload between treatment groups will be assessed using a mixed model repeated measures (MMRM) approach adjusted for the elements included in the minimization algorithm which determined treatment allocation (age, sex, prior diabetes, eGFR, and urinary ACR [but not region as the BCM substudy was only conducted in Europe]).

The primary assessment will focus on a weighted average of the values at the two Follow-up time points with weighting based on the relative size of each Follow-up window as set out in section 6. As the first Follow-up window (2-month Follow-up) is 370 days (days 30-400 post-Randomization) and the second window (18-month Follow-up) assumed to be 280 days (days 400-680 post-Randomization), this effectively weights information at the first Follow-up visits as 55% compared to 45% at the second. This is appropriate as we hypothesise that there will be a greater effect of empagliflozin versus placebo on Fluid Overload at 2 months versus 18 months as the effect of empagliflozin on Fluid Overload is expected to develop rapidly and diminish over time. Additionally, changes to other medication which can influence fluid balance may occur over time. Time will be included in the model as a categorical variable to avoid assuming a linear association between treatment allocation and Fluid Overload over time. The model will include fixed, categorical effects of treatment allocation, treatment-by-time interaction, and the prognostic variables used in the minimization algorithm (in the same

categories used in the minimization process) along with continuous effects of baseline (randomization) measurements and baseline-by-time interaction. The within-person error correlations will be assumed to be unstructured.

7.2.2 Assessment for key secondary randomized assessment

Time-to-first event analyses will use adjusted Cox regression. The general statistical methods and approaches to subgroup analyses are set out in the main Data Analysis Plan (SOP11; EDMS#6290). Follow-up for the clinical components of the composite outcome will be censored according to the main Data Analysis Plan. Follow-up for the BCM-derived components of the development or worsening of Fluid Overload outcomes (see section 4 for definitions) will be censored on the day after the last valid BCM measurement (but these individuals may remain at risk of clinical outcomes) or at death/withdrawal of consent.

7.2.3 Other secondary randomized assessment

The effect of treatment allocation on Fluid Overload separately at 2 and 18 months (see section 6.4) will be analysed using the same MMRM approach outlined in 7.2.1.

7.2.4 Tertiary randomized assessments including subgroup analyses

The same MMRM approach outlined in section 7.2.1 will be used for tertiary assessments (i) and (ii) as described in section 6.5. Tertiary assessment (i) is an analysis of the primary outcome by subgroup. Subgroup analysis will be performed by fitting relevant interaction terms for subgroups in the MMRM model with the aim of assessing whether the proportional effects in specific subgroups are statistically different from the overall effect. Interpretation will take into account the number of subgroups assessed as well as biological rationale. Tertiary assessment (ii) will use the same MMRM approach as for the primary assessment (section 7.2.1). Tertiary assessments (iii) and (iv) which analyse effects of treatment allocation on the components of the composite key secondary outcome and regression of Clinically Significant Fluid Overload will be analysed according to the same time-to-event approach outlined in section 7.2.2.

7.2.5 Exploratory observational analyses using baseline data

While the trial is ongoing and unblinded randomized data not yet available, exploratory analyses of baseline data will be possible and may generate hypotheses for subsequent analyses and allow checking of the assumptions made in this Data Analysis Plan. Correlations between Fluid Overload, ECW and ICW; LTI and FTI at baseline with the baseline

characteristics and measurements outlined in section 5 will be assessed using univariable and multivariable linear regression models considering confounding and effect modification.

Before unblinding, a predictive model for moderate and severe Clinically Significant Fluid Overload will be developed using characteristics recorded at baseline using linear regression, with predictors selected by backward elimination, with factors remaining in the model if they were statistically significant at the 5% level, and with age, sex and treatment allocation forced to remain irrespective of statistical significance.

7.2.6 Exploratory analyses using randomized assessments

Exploratory assessments are planned to better understand how any effects of empagliflozin versus placebo on BCM-derived measures (Fluid Overload, ECW, ICW, LTI and FTI) compare with effects on related measures (such as blood pressure, eGFR, HbA1c and anthropometric measures, as outlined in section 6.7). Effects will be estimated using the MMRM approach outlined in section 7.2.1, using baseline-adjusted mean follow-up values.

Validation of surrogate endpoints (see key secondary randomized assessment, sections 6.3 and 7.3.2) will use the results of time-to-event analyses of the BCM-derived components of the primary outcome (see tertiary randomized assessment (iii) described in sections 6.5, 7.2.2 and 7.2.4) and compare these to results of randomized assessments of the effect of empagliflozin versus placebo on the composite of death from, or hospitalization for heart failure from the full EMPA-KIDNEY trial cohort of 6609 participants (using methods set out in SOP11; EDMS#6290).

Further technical documentation to accompany this Data Analysis Plan may also be added as an appendix, if additional methodological details for the approaches described in section 7 are found to be required. In addition to the pre-specified comparisons, other post-hoc analyses may be performed with due allowance for their exploratory and, perhaps, data-dependent nature.

8 APPENDIX: DEFINITION OF VALID BCM MEASUREMENTS AND DATA HANDLING CONSIDERATIONS

8.1 Definition of a valid BCM measurement

To be included in analyses, an EMPA-KIDNEY participant must have at least one valid BCM measurement during Follow-up and been allocated to empagliflozin 10mg or matching placebo. To be included in analyses, each BCM measurement must have a corresponding weight measurement recorded at the same visit, from which BCM parameters can be derived according to the procedure set out in EDMS#7248.

Validity of BCM measurements will be assessed, prior to unblinding. Measurements with an absolute Fluid Overload value more negative than -5 litres will be excluded due to implausibility[1]. Measurements with a Q value[2] of <80 (site staff were trained to repeat BCM measurements if the Q value was <80; EDMS#6240) will be identified for visual inspection of the associated Cole-Cole plot[3] to assess data quality and determine inclusion in analyses. Two observers blind to treatment allocation will independently assess Cole-Cole plots by visual inspection, applying pre-specified criteria (outlined in section 8.4), with any differences resolved by consensus discussion.

Information on completeness of valid BCM data at each visit (i.e. number of participants with at least one valid BCM measurement at each visit, no valid BCM measurement but at least one invalid measure, or no BCM measurement) will be presented in the substudy CONSORT flow diagram. Statistical comparisons by treatment will be presented for the following parameters:

- The distribution of Q values for measurements included in the main comparison and sensitivity analyses
- The distribution of time-to-measurements from Randomization for each Follow-up window.

[1] In pilot work, Cole-Cole plots were reviewed for all measurements with absolute Fluid Overload values >2 standard deviations from the mean in a preliminary dataset to inform this cut-off. Values more negative than -5 litres were consistently associated with poor quality Cole-Cole plots. Conversely, outlying positive values were found to consistently have good quality Cole-Cole plots (and are considered plausible results).
[2] The Q score is an assessment of data quality generated by the BCM where 100 is a perfect Q value. In pilot work, a random subset of 50 measurements with a Q score ≥80 were selected for Cole-Cole plot review. Q scores above this threshold were confirmed to be a reliable indicator of good data quality in the cohort.
[3] The Cole-Cole plot generated by the BCM device fits a curve to the measured impedance data and defines the extracellular and intracellular resistances upon which all body composition data are based. Visual inspection of Cole-Cole plots identifies artefact within the impedance data.

8.2 Handling multiple BCM measurements

8.2.1 Multiple valid BCM measurements at the same visit

In all analyses, if more than one valid BCM measurement is available at a single Follow-up visit (i.e. date), the measurement with the highest Q value will be used and additional measurements ignored. In the situation where >1 valid measurements are obtained with an identical Q value, the first measurement will be used.

8.2.2 Multiple valid BCM measurements within a Follow-up window

In all analyses, if valid BCM measurements are made on more than one day within a Follow-up period, then the valid BCM measurement made on the day nearest the ideal follow-up day will be used and other BCM measurement excluded (see section 6 for Follow-up days). In the situation where >1 valid BCM measurements are obtained within the Follow-up window on dates which are equidistant from the ideal Follow-up date, a mean value will be calculated and used in analyses. This is considered a more scientifically robust approach in this unique situation due to the hypothesised interaction of time in the association between treatment allocation and Fluid Overload which means that selecting one or other equidistant measurement on the basis of Q values could introduce bias.

8.2.3 Multiple measurements at different visits on a single BCM card

Where data for two separate visits is recorded on a single BCM card, valid BCM results will be derived for the separate visits, wherever possible.

8.3 Data processing: BCM variables

The BCM provides measurement of:

- Extracellular water (ECW) resistance (denoted as R_e)
- Intracellular water (ICW) resistance (denoted as R_i)

BCM data are downloaded to study-specific laptops in a .pat file format and imported into a Microsoft Excel™ spreadsheet according to the procedure set out in EDMS#7248.

The following data are extracted from the analysis database to allow processing of the BCM data:

- Age, recorded in whole years at the time of each BCM measurement
- Weight, measured in kilograms, at the time of each BCM measurement
- Height, measured in centimetres, at Randomization
- Sex, recorded as male or female, at Randomization

along with Re and Ri reported by the BCM

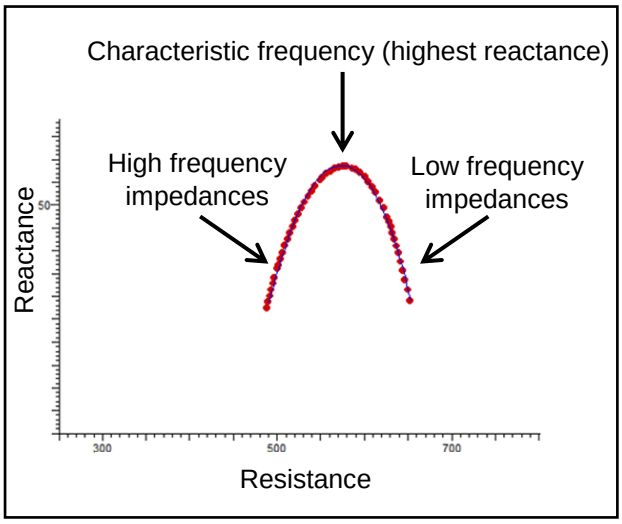
Standard formulae will be applied to methodology described by Moissl and Chamney et al (11, 12) [1] to derive the following:

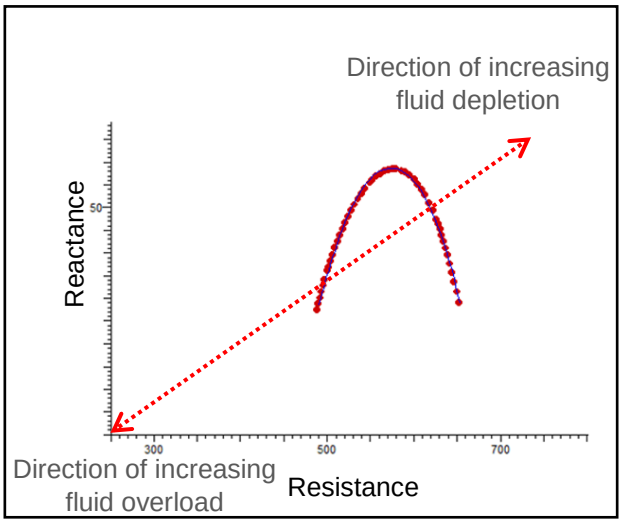
- Body mass index (BMI) in kg/m^2 using height and weight
- Extracellular water (ECW) in litres
- Intracellular water (ICW) in litres
- Total body water (TBW) in litres, by addition of ECW and ICW values
- Absolute Fluid Overload in litres
- Relative Fluid Overload (indexed to ECW), expressed as %
- Lean tissue index (LTI)
- Fat tissue index (FTI)

[1] Methods will use different coefficients to those available in published the current literature (coefficients which have been shared with permission).

8.4 Criteria for rejecting BCM measurements by Cole-Cole plot visual inspection

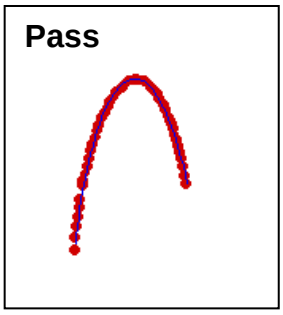
The two diagrams below provide a basic interpretation of the Cole-Cole plot:



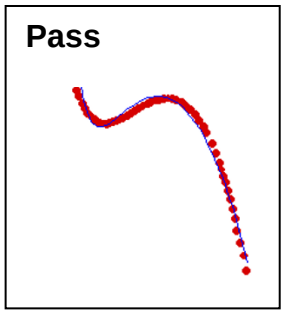


When manually reviewing Cole-Cole plots generated by the BCM for quality assurance, the following rule will be used to classify measurements as having poor data quality.

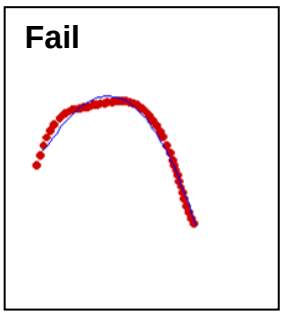
KEY CRITERION: In the opinion of the observer blind to treatment allocation, a good quality Cole-Cole plot should have the basic structure of a parabola, ignoring any artefacts at the high and low frequency end, and the plotted blue curve should closely fit the raw data red.
Examples of good ("pass") and poor ("fail") quality bioimpedance data are provided below:



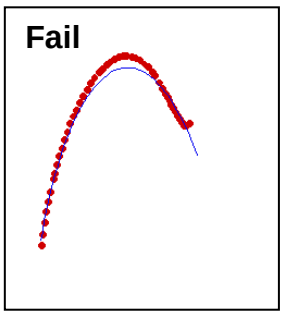
Parabola with good fit of plotted curve against raw data



Ignoring artefact at high frequency, acceptable parabola with good fit



Raw data is not parabolic in shape & consequently poor fit



Unacceptable fit of plotted curve against raw data

Note: review of the Cole-Cole plot is not affected by the height or width of the plot, length of either end of a parabola, nor its position in the plot region.

8.5 Sensitivity analyses

Data quality assessment outlined in section 8.1 will be used to determine data inclusion in the primary analysis. Sensitivity analyses will also be conducted to assess the impact of the data quality assessments on the effects of empagliflozin versus placebo on the primary randomized assessment. These include analyses:

1. Of all single BCM measurements, irrespective of quality assessment or outlying values (i.e. the complete “unreviewed” set)
2. Restricted to single BCM measurements with a Q value ≥80 (i.e. a stricter criterion than the primary approach)

The criteria outlined in section 8.1 are thought to represent the optimal data quality assessment procedure to determine inclusion in the primary analysis and these sensitivity analyses represent the two alternative most extreme approaches.

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UNIVERSITY OF GLASGOW

Data Management Plan template for PGR students

1. Overview	
Student name	Kaitlin Mayne (KM)
Supervisor name	Prof Paddy Mark (PM) & Dr Jennifer Lees (JL) [& Associate Prof Will G. Herrington (WGH), University of Oxford, EMPA-KIDNEY Co-Chief Investigator] [Support from Professor Richard Haynes (RH), University of Oxford, EMPA-KIDNEY Co-Chief Investigator]
Project title	The effect of empagliflozin on overhydration and adiposity in chronic kidney disease measured by bioimpedance spectroscopy
Funder & award number	Nuffield Department of Population Health, University of Oxford
Project Summary	Study of the effect of empagliflozin on fluid overload measured by bioimpedance spectroscopy [using Fresenius Body Composition Monitor (BCM) device] in people with chronic kidney disease used randomised controlled trial data (EMPA-KIDNEY). Further analyses will also be conducted examining associations between fluid overload (measured by bioimpedance spectroscopy) and various measured characteristics and clinical outcomes.

2. Data
What types of data will be collected or created?
Study identifier, age, sex, anthropometry, serum and urine biomarkers, other clinical data including medication use and kidney disease status and BCM measurements.
What formats will you use?
Data is already being collected using a bespoke online trial data collection system (*Livia*). From this, a data management team will derive relevant fields into CDISC STDM domains for analyses. BCM-specific data are collected on BCM cards linked to the participant and transferred using a standardised process into a backed up substudy laptop system, from which BCM measurements will be derived for analysis.
How much data will you collect?
Data on 664 trial participants participating in BCM substudy. Recruitment is complete (6609 participants in main EMPA-KIDNEY trial) and follow-up ongoing. BCM data has been collected at randomisation and 2 month follow-up and 18 month data collection is ongoing.

3. Documentation
How will the data be documented and described?
Data dictionary created according to standards outlined below
Are there any standards for this in your field of research?
Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) used in clinical trials

4. Ethics and Intellectual Property
Who owns the data in your project?
Co-owned by Nuffield Department of Population Health (NDPH), University of Oxford and trial sponsor, Boehringer Ingelheim.
Detail any ethical, legal or commercial considerations relating to your research data

Ethical approval is in place for EMPA-KIDNEY [Oxford Research Ethics Committee (REC) reference number: 18/SC/0155; IRAS project ID: 236211]. The substantial amendment pertaining to the BCM substudy is dated 14 March 2019. The trial sponsor is Boehringer Ingelheim who provide the study drug and any publications resulting from the BCM substudy will need to be approved by the Steering Committee however the data is co-owned by NDPH.
How will these concerns be dealt with?
Data will remain within NDPH and only be shared externally (with PM & JL) in a limited manner where required for this project – access to analyses but not direct access to raw data. We will explore whether a Confidential Disclosure Agreement is required or whether existing permissions will suffice as the University of Glasgow is an EMPA-KIDNEY site (Glasgow Clinical Research Facility, Queen Elizabeth University Hospital, Glasgow).

5. Storage and organisation
How will the data be named, organised and structured?
A pseudonmymised dataset ready for analysis will be derived from the SDTM data in a suitable format to be used with statistical software (eg. dta file for use with Stata). This will be named such as "EMPA-KIDNEY_BCM_dataset_MASTER_date" with subsequent copies created during analysis and stored in a systematic manner, preserving the original data file.
How will the data be stored for the duration of the project?
A specific folder will be requested on the NDPH network drive to which only myself and the Chief/Co-Principal Investigators (WGH and RH) will have access. This will be accessed via a departmental encrypted laptop or desktop only.
How will the data be backed up during the project?
Raw BCM data and analysis data will be stored on K:EMPA network drive and subject to daily back up and "EMPA-KIDNEY Disaster Recovery Plan" [Electronic Document Management System (EDMS) reference number 6154] which includes an outline of the strategy used in recovery of the *Livia* database were this ever required.
Does access to the data need to be controlled for the duration of the project?
Yes (see earlier), especially until unblinded data from the trial is published. Unblinded analyses will not be possible until the completion of the main trial (due in 2022) but analyses of baseline data can begin at the time of writing.
Who has the right to access the data during the project?
KM, WGH and RH.

6. Deposit and long-term preservation
Which data should be retained long-term?
All data will need to be maintained for at least 25 years from trial completion to be consistent with consent and trial regulations.
How long will data be retained for?
At least 25 years.
Where will the data be archived at the end of the project?
NDPH servers.
What formats will the data be archived in?
As earlier described using CDISC SDTM formats.

7. Data sharing
Is any of the data suitable for sharing?

Data sharing will not be possible during the course of this PhD, but once all planned publications are complete, open access sharing of data will be implemented according to local internal policy.
How will the data be shared?
According to local internal policy.
Who should be able to access and use the shared data?
Open access.

8. Implementation
Who is responsible for implementing this plan?
Already implemented under the responsibility of Chief Investigator and co-Principal Investigator (WGH/RH).
How will this plan be kept up-to-date?
Reviewed annually by KM & WGH as required by UoG however established trial processes are not expected to change.
What actions are necessary to implement this plan?
KM to maintain documentation and storage of data according to DMP with oversight from WGH.
What training or further information are needed to implement this plan?
UoG Introduction to Research Data Management for PGRs course to be completed by end of first year.