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Investigation of frailty markers including a novel biomarker panel in patients undergoing emergency laparotomy

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

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Abstract

Emergency laparotomy (EmLap) is one of the commonest operations performed in the UK, about 30,000 annually. When frailty, a clinical syndrome of increased vulnerability that results from age associated multi-system physiological decline is present, the morbidity and mortality post-EmLap is high. Insights into the mechanisms of frailty are urgently needed to develop interventions and improve outcomes for patients living with frailty undergoing EmLap. This thesis aims to compare the ability of the established pre-operative frailty markers in predicting 30-day morbidity and 30-day and 90-day mortality post-EmLap, develop a robust sample preparation workflow for Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS)-based metabolomic analysis of plasma samples from EmLap patients, explore novel metabolites associated with frailty and their changes in peri-operative period and analyse the influence of peri-operative frailty on quality of life (QoL) post-EmLap.

A prospective, single centred, observational study was conducted on 150 patients ≥40 years of age who underwent EmLap from May 2022 to April 2023 according to the established National Emergency Laparotomy Audit (NELA) criteria with 3 months follow up. Morbidity and mortality were assessed and compared using established frailty markers such as sarcopenia (psoas muscle measurement on CT scan), nutrition (Malnutrition Universal Screening Tool (MUST) score), cognition (Abbreviated Mental Test score) and the Rockwood Clinical Frailty Score (CFS score >4 as frail). When determining novel biomarkers, plasma samples were collected pre-operatively and post-EmLap day 1, 7, 30 and 90 and metabolic signatures correlated to frailty status were determined using UHPLC-MS-based metabolomics. Finally, quality of life was assessed using the EQ-5D-5L questionnaire on the 30- and 90-day follow-up. The patients were divided into groups of frail and non-frail, and the Mann-Whitney test was used for comparison analysis. Univariate analysis using Chi-square test or Fisher's exact test followed by multivariable analysis using logistic regression were performed. The metabolomic data collected across the five time points were analysed using Principal Component Canonical Variance Analysis (PC-CVA) and the identified metabolites were classified with Multivariate Marginal Hierarchical

Cluster Analysis (MMHCA) and univariate generalised linear model (GLM) was used to analyse the metabolites differing between the non-frail to mortality group.

Of the 150 recruited patients, 55% were female with median age of 69 years and the 90-day mortality was 13.3%. In this cohort, 10% were living with frailty pre-EmLap and 10.1% became frail post-EmLap. Across the 5 peri-EmLap time points, distinct metabolites differed between groups (ANOVA FDR ≤ 0.05), largely associated with subsequent mortality in patients who were non-frail pre-operatively rather than subsequent frailty. MMHCA revealed discrete clusters of metabolites that suggested post-EmLap metabolic dysregulation while GLM detected 26 metabolites (from biochemical class of amino acid, acylcarnitine, peptides and nucleoside metabolism) that were associated with 90-day mortality. Quality of life was found to be worse in the frail group but there was a positive trajectory of recovery over the follow-up period.

In this study, the established frailty markers (CFS, sarcopenia, cognition and nutrition) were not associated with 30-day morbidity and 30 or 90-day mortality. This finding needs to be interpreted with caution due to small sample size. Despite no metabolites identified that predicted frailty, metabolites associated with post-operative mortality were identified in EmLap patients using UHPLC-MS-based metabolomics. These metabolites may be candidate biomarkers for further investigation that may offer a promising approach for assessing vulnerability in emergency setting. Quality of life findings also affirmed that recovery is possible even among frail patients, reinforcing the value of integrated, multidisciplinary post-operative care. Further studies with larger patient cohorts are required to validate these findings.

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Author's declaration

I hereby declare that this thesis is the result of my own work and is being submitted for the degree of PhD at the University of Glasgow. This work has not been submitted for any other degree at this or other institution. This thesis is the result of my own independent work, unless otherwise stated. All sources of information and contributions to the work have been specifically acknowledged in the text.

Hwei Jene Ng

Publications and published abstracts

Publications

1. **HJ Ng**, T Quasim, NJW Rattray, S Moug. Investigation of frailty markers including a novel biomarker panel in emergency laparotomy: protocol of a prospective cohort study. BMC Surgery. 2023;23:190. doi: 10.1186/s12893-023-02093-5.

2. **HJ Ng**, NJW Rattray, T Quasim, S Moug. Changes in frailty status and discharge destination post emergency laparotomy. World J Emerg Surg. 2025;20(1):37. doi: 10.1186/s13017-025-00612-8.

3. Thu MM, **HJ Ng**, Moug S. The influence between frailty, sarcopenia and physical status on mortality in patients undergoing emergency laparotomy. World J Emerg Surg. 2025;20:38. doi: 10.1186/s13017-025-00588-5.

4. Silva L, L, Abbas Mohamed S, Meggy A, **HJ Ng** et al. Psychosocial outcomes following emergency laparotomy (POLO) study: a study protocol for a multicentre mixed-methods prospective cohort study assessing the psycho-social outcomes following emergency laparotomy in adults. BMJ Open 2024;14:e081821. doi: 10.1136/bmjopen-2023-081821.

5. Ambrose S, Silva L, John- Charles R, **HJ Ng** et al. A description of patient experience after undergoing an emergency laparotomy. (Accepted by World Journal of Emergency Surgery on 25/2/26)

Published abstracts

1. **HJ Ng**, T Quasim, NJW Rattray, S Moug. Outcome of polypharmacy in frail patient post emergency laparotomy. BJS. 2023;110(6). doi: 10.1093/bjs/znad241.285.

2. **HJ Ng**, T Quasim, NJW Rattray, S Moug. Mortality rate for frail and sarcopenic patients post emergency laparotomy. BJS. 2023;110(6). doi: 10.1093/bjs/znad241.015.

3. **HJ Ng**, T Quasim, NJW Rattray, S Moug. Changes in frailty and discharge destination post emergency laparotomy. BJS. 2024;111(8). doi: 10.1093/bjs/znae197.100.

4. **HJ Ng**, T Quasim, NJW Rattray, S Moug. 90-day mortality rate post emergency laparotomy for frail and sarcopenic patients. BJS. 2024;111(8). doi: 10.1093/bjs/znae197.403.

5. **HJ Ng**, T Quasim, NJW Rattray, S Moug. Novel frailty biomarkers in emergency laparotomy patients. BJS. 2025;112(13). doi: 10.1093/bjs/znaf166.318.

Presentations

Invited speaker

1. Association of Surgeons of Great Britain & Ireland (ASGBI) conference 2024, Belfast. Research@ASGBI- Investigation of frailty markers and outcomes in patients requiring emergency laparotomy.

2. Emergency Laparoscopic and Laparotomy Scottish Audit (ELLSA)/ National Enhanced Recovery in Colorectal Initiative (NERCI) conference 2024, Glasgow. Investigation of frailty markers and outcomes in patients requiring emergency laparotomy.

International

Poster

1. Investigating frailty biomarkers in patients requiring emergency laparotomy. **HJ Ng,** T Quasim, S Moug, NJW Rattray. 20th Annual conference of the Metabolomics Society. Japan (Osaka) 2024.

Abbreviations

A&E- Accident and Emergency

AMT- Abbreviated Mental Test

ASA- American Society of Anesthesiologists

ASGBI- Association of Surgeons of Great Britain and Ireland

ATP- Adenosine Triphosphate

BIA- Bioelectrical Impedance Analysis

BMI- Body Mass Index

BNF- British National Formulary

CCI- Charlson Co-morbidities Index

CI: Confidence interval

CSHA- Canadian Study of Health and Aging

CFS- Clinical Frailty Scale

CGA- Comprehensive Geriatric Assessment

CT- Computed Tomography

DXA- Dual-energy X-ray Absorptiometry

DNA- Deoxyribonucleic Acid

EFS- Edmonton Frailty Scale

EGS- Emergency General Surgery

ELF- Emergency Laparotomy and Frailty study

ELFUS- Emergency Laparotomy Follow Up Study

ELLSA- Emergency Laparoscopic and Laparotomy Scottish Audit

ELN- Emergency Laparotomy Network

EmLap- Emergency Laparotomy

EQ-VAS- EuroQol Visual Analogue Scale

ERAS- Enhanced Recovery After Surgery

EWGSOP- European Working Group on Sarcopenia in Older People

FI- Frailty Index

GFI- Groningen Frailty Indicator

GLM- Generalised linear model

GP- General Practice

H_2O- Water

HDU- High Dependency Unit

HPLC-MS- High performance liquid chromatography-mass spectrometry

HQIP- Healthcare Quality Improvement Partnership's

ICU- Intensive Care Unit

LASSO- Least Absolute Shrinkage and Selection Operator

MeOH- Methanol

MMHCA- Multivariate Marginal Hierarchical Cluster Analysis

MoCA- Montreal Cognitive Assessment

MNA- Mini Nutritional Assessment

MUST- Malnutrition Universal Screening Tool

MRI- Magnetic Resonance Imaging

NHS- National Health Service

NIRT- Nucleoside reverse transcriptase inhibitor

NMR- Nuclear magnetic resonance

NoLap- Patients with acute abdomen with indication for emergency laparotomy but did not proceed to surgery

NICE- National Institute for Health and Care Excellence

NCAPOP- National Clinical Audit and Patient Outcomes Programme

NELA- National Emergency Laparotomy Audit

OR- Odds ratio

PCA- Principal Component Analysis

PIS- Patient Information Sheet

PROMs- Patient-Reported Outcomes Measures

QC- Quality Control

QCMXP- Quality Control and Matrix eXtraction Pipeline

QoL- Quality of Life

RNA- Ribonucleic Acid

RCT- Randomised Controlled Trial

SAU- Surgical Assessment Unit

SIMD- Scottish Index Multiple Deprivation

SPE- Solid Phase Extraction

SSRG- Scottish Surgical Research Group

TUG- Timed Up and Go

UHPLC-MS- Ultra High-Performance Liquid Chromatography-Mass Spectrometry

WHO- World Health Organisation

WSES- World Society of Emergency Surgery

Symbols

°C Degree Celsius

> Greater than

< Less than

≥ Equal to and more than

≤ Equal to and less than

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Chapter 1: Introduction

1.1 Background

Emergency laparotomy (EmLap) is the term where a surgical incision to the abdomen is performed for patients who are admitted under the emergency general surgery service with an acute abdominal condition. Compared to elective surgery, patients who require EmLap can be critically unwell and lack the time and opportunity to optimise their physiological function pre-operatively. As a consequence, EmLap results in higher rates of morbidity and mortality when compared to the elective setting (1). This was shown in a study conducted by the American College of Surgeons National Surgical Quality Improvement Program where they found both a higher morbidity rate (32.8% EmLap vs 12.74% elective) and mortality rate (12.5% EmLap vs 2.66% elective) in EmLap patients (2).

A large cohort of patients undergo EmLap in the United Kingdom (UK). According to the recent National Emergency Laparotomy Audit (NELA) report (3) looking into data from England and Wales, over 23,560 patients underwent EmLap from December 2023 to March 2024, with 55% aged 65 years and above. Given the high post-operative mortality associated with EmLap, the NELA group developed a 30-day mortality risk assessment model based on data from 38,000 EmLap patients in the NELA database. This prognostic tool uses the definition of high-risk as mortality risk ≥ 5% (4). In addition, this patient cohort was found to carry the highest risk of developing post-operative complications; especially when pre-operative frailty was present (5).

Frailty, which is prevalent in the older adults, is a reduction in physiological reserve, compromising the ability to recover promptly after health stresses which leads to long-term impact on fitness and well-being. However, frailty is not unique to the older population and can be present in the younger adults (6, 7). As the outcomes of EmLap affects a large cohort of patients living with frailty, there are increasing number of researchers looking into improving the outcomes of emergency surgery.

This thesis sets out to assess the ability of pre-operative frailty markers to predict post-EmLap outcomes and quality of life and to provide much needed insights into the biochemical mechanisms behind frailty in emergency surgery in older adults as well as the younger cohort from 40 years of age. This introduction provides a general background on emergency laparotomy, frailty assessments including blood-based frailty biomarkers and outcomes of frailty in emergency laparotomy patients.

1.2 Emergency laparotomy

Laparotomy is a term originated from the Greek words 'lapara' and 'tomy' which means "flank" and "cut" respectively. It is a major procedure that allows the surgeon to explore the peritoneal cavity via an abdominal incision to examine and manage the underlying pathology involving the bowel or abdominal organs. In the emergency setting, it is often a life-saving procedure when there are no other feasible non-surgical alternatives such as treating perforated bowel with faecal peritonitis.

Evidence of high mortality rates in patients undergoing general surgical procedures was obtained from 94 National Health Service hospitals. (8). The first report from the collaborative Emergency Laparotomy Network (9) highlighted the wide range of mortality rates up to 41.7% and the heterogeneity in the treatment and management of EmLap patients across England and Wales. Following this, the National Emergency Laparotomy Audit (NELA) was launched in 2011. It was part of the National Clinical Audit and Patient Outcomes Programme (NCAPOP), commissioned by the Healthcare Quality Improvement Partnership's (HQIP), and an important national quality improvement project implemented to explore the clinical effectiveness of an integrated peri-operative care pathway for EmLap patients. Following the success of NELA, the Scottish Government initiated the formation of Emergency Laparoscopic and Laparotomy Scottish Audit (ELLSA) via the Modernising Patient Pathways Programme in 2017. It aimed to recruit all Scottish Hospitals into this audit. Based on the first ELLSA report (2017-2018), 17 Scottish hospitals out of acute 18 sites were successfully recruited and a total of 2204 patients underwent EmLap (10).

In the second ELLSA report (2022), 12 out of 18 Scottish hospitals had completed ELLSA data collection and a total of 1430 patients underwent EmLap (11). In the latest third ELLSA report (2025), 14 out of 18 Scottish hospitals participated and 1927 patients underwent EmLap (12).

1.2.1 Morbidity in emergency laparotomy

Morbidity is derived from the Latin word "*morbidus*" which means unhealthy. It is commonly used to describe the state of being symptomatic of poor health due to any disease or condition (13). In order to have a consistent standardised process to classify post-operative adverse events, in 1992, Clavien et al. (14) proposed a surgical complications grading (Table 1) to differentiate complications from failure to cure and sequelae.

Table 1: The first classifications of complications of surgery, proposed by Clavien et al in 1992 (14).

Grade	Description
I	Variations from the ideal post-operative course, non-life-threatening, and with no lasting disability, necessitate only bedside procedures and do not exceed twice the median length of hospital stay for the procedure.
II	Potentially life-threatening but without residual disability.
IIa	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusion and total parenteral nutrition are also included. Any patient with a complication and hospital stay exceeding twice the median length of stay for the procedure and not falling into a higher category.
IIb	Requiring invasive procedure; surgical, endoscopic or invasive radiological intervention.
III	Those with residual or lasting disability, including organ resection or persistence of life-threatening conditions.
IV	Death

Subsequently, the Clavien-Dindo classification (15) was proposed and became an internationally recognised method for categorising post-operative adverse events. This classification has been widely used by most surgical specialties as well as in this study to categorise morbidity (Table 2).

Table 2: Clavien-Dindo Classification of surgical complications (15).

Grade	Definition
Grade 1	Any deviation from the normal post-operative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Allowed therapeutic regimens include drugs such as antiemetic, antipyretics, analgesics, diuretics, and electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade 2	Requiring pharmacological treatment with drugs other than such allowed for grade 1 complications. Blood transfusions and total parenteral nutrition are also included.
Grade 3 Grade 3a Grade 3b	Requiring surgical, endoscopic or radiological intervention. Intervention not under general anaesthesia. Intervention under general anaesthesia.
Grade 4 Grade 4a Grade 4b	Life-threatening complication (including CNS complications such as brain haemorrhage, ischemic stroke, subarachnoid bleeding, but excluding transient ischemic attacks) requiring ICU. Single organ dysfunction (including dialysis). Multiorgan dysfunction.
Grade 5	Death of a patient.
Suffix “d”	If the patient suffers from a complication at the time of discharge, the suffix “d” (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

Complications following emergency laparotomy are variable but are important post-operative outcome measures. Tengberg et al. performed a retrospective multicentre study on 1139 EmLap patients and found 47% with Clavien-Dindo Classification of grade ≥3. Interestingly, NELA and ELLSA excluded post-operative morbidity data due

to data collection burden and to streamline the audit process, ensuring consistent quality of data collection. This led to the feasibility study Emergency Laparotomy Follow Up Study (ELFUS), founded by Saunders et al. (16); it aimed to record post-operative morbidity in post-EmLap patients as well as assess post-operative quality of life up to 1 year. The Accordion Severity Grading of complications (17) were used and the authors reported 27.1% with Grade 3 and above complications highlighting a significant concern for EmLap patients.

Sorensen et al. (18) analysed 5,255 post-open gastrointestinal operations and included both elective and emergency patients. They found 11.5% in the elective group had major complications, but in the emergency group, this rose up to 30.1%. This study demonstrated the huge gap in post-operative complications between elective and emergency cohorts, highlighting the need to improve this outcome in emergency laparotomy patients. In the elective setting, a multimodal peri-operative care pathway, enhanced recovery after surgery (ERAS), was developed to improve surgical outcomes by reducing recovery time, complications and hospital stays (19, 20). This developed protocol has been adopted and applied to various other elective surgical specialties. Recognising the potential benefits in emergency care, the first consensus guideline applying ERAS principles to EmLap was introduced in 2021 (21), aiming to improve outcomes in this high-risk patient group. However, due to the limited availability of prospective trials and robust evidence-based research in the emergency setting, these recommendations remain largely consensus-based rather than supported by high quality clinical evidence.

1.2.2 Quality of life post emergency laparotomy

The need to address the effect on quality of life (QoL) after EmLap has been recognised. Clinicians are primarily outcome focused, reporting on morbidity and mortality. These outcome measures are important, but the patient/ person centred outcomes are more important. It is essential to consider what patients prioritise and value in their life, as we know that QoL, especially after EmLap with post-operative complications can be poor (22). Law et al. (23) highlighted the discrepancy of

outcome measures between patients and clinicians *via* a focus group (Patient and Public Involvement, PPI). Instead of the traditional morbidity rates such as hospital length of stay or mortality, patients were found to place more value on their QoL such as stoma formation, independence and return to their own home as some of them has accepted that they may 'die at any point at this stage of life'. Some research studies such as the ELF study (24) have reported data on discharge destination (home with no carers, home with carers, intermediate care, residential home and nursing home) and they found 37.4% had an increased level of care at the time of hospital discharge. This data is yet to be included in national audits.

Patient-reported outcomes measures (PROMs) are multidimensional assessments that are completed by the patients and may include functional status or health related QoL. One of the commonly used generic health questionnaires is EQ-5D. It was introduced by the EuroQol group in 2009 (https://euroqol.org/) and is a validated measurement tool which evaluates 5 dimensions of QoL including mobility, self-care, activity of daily living, pain / discomfort and psychological status. The options included are no, slight, moderate, severe and extreme, increased from three choices (no, moderate, extreme on EQ-5D-3L). The EQ-5D-5L has been recommended by the National Institute for Health and Care Excellence (NICE) as their preferred health related QoL measurement tool from 2009 and in the position statement in 2019, NICE continued to support prospective clinical studies to use the EQ-5D-5L version to collect information on QoL (25).

A range of studies (16, 26-32) have assessed QoL following emergency laparotomy but most of them were assessing QoL from a specific disease process such as acute mesenteric ischemia (27), small bowel obstruction (28) or upper gastrointestinal perforation (33). A feasibility study assessing QoL after EmLap with 3 months follow up from 11 hospitals was conducted by Kwong et al. (30) in 2018; they achieved a 74.1% response rate when questionnaires were mailed at 3 months post-EmLap. Another feasibility study in collecting data on PROMs and QoL up to a year following EmLap was performed by the ELFUS study (16). The study used EQ-5D and WHODAS

2.0 questionnaires to collect patient-reported outcomes at 1-, 3-, 6-, and 12-months post-EmLap and they found good response rates, with 100% answering the baseline questionnaire; 87% at 1 month, 77% at 3 months, 75% at 6 months and 69% at 12-month (response rates compared to number of survivors). This showed that QoL post-EmLap can be assessed successfully and EQ-5D is a sensitive, consistent and reliable assessment tool which has been widely used as a PROM.

1.2.3 Mortality in emergency laparotomy

Mortality was derived from the Latin word "*mortalitem*" which is the state of being mortal, subjection to death. It is one of the commonest methods for assessing outcomes following treatments such as an emergency laparotomy. In 2007, the Association of Surgeons of Great Britain and Ireland (ASGBI) recognised that emergency surgery care was lacking and was described as 'Cinderella service' as it was overlooked and underfunded. This prompted the establishment of the Emergency Laparotomy Network (ELN) (www.networks.nhs.uk) in 2010 with the objective of improving the service and care to emergency admission patients. The ELN conducted a multicentred audit in 2012 to determine the baseline of non-risk adjusted outcomes; data from 35 UK hospitals were included. The preliminary result found a mortality rate of 14.9% for all patients and 24.4% in patients ≥80 (9).

In the 10th NELA report published in 2023, the 30-day mortality rate in England and Wales was 9.2%, a significant reduction from the 1st NELA report in 2013 where the mortality rate was 12.7% (3). When compared to EmLap result from other studies from the world, the 30-day mortality rate ranges from 7% to 21% (34-41). These mortality rates are very high when contrasted to the elective setting where the 30-day mortality rate post elective colorectal surgery was 1.8% (42). There is an ongoing push to reduce the mortality rate and provide a better service to the under-served EmLap population.

1.3 What is frailty

Frailty is a common term used in society to describe the state of vulnerability of a person to illness, but it has been challenging to define in the medical literature, reflecting its complex and multidimensional nature. Although the term was used informally to describe vulnerable older adults before the 1980s, frailty was not initially a clearly defined clinical concept. Historically, older adults with frailty were often described using terms such as chronically ill, debilitated, disabled or functionally dependent, with frailty frequently associated with institutionalisation, physical weakness and dependence on others for activities of daily living (43, 44).

The concept of frailty appeared in the research literature in 1968, when O'Brien et al. (45) described a heterogenous group of 48 community-dwelling older adults using the term 'frail and elderly'. Their work explored issues experienced by this population and contributed to the early discussion of vulnerability in older adults. However, frailty remained relatively uncommon in the medical literature until the late 1980s, when greater attention was directed towards identifying and characterising frail older adults. In 1988, Winograd et al. (46) contributed to the operationalisation of frailty by identifying frail older patients according to the presence of one or more of 15 common geriatric clinical conditions. During this period, frailty was frequently discussed alongside disability and comorbidity, and these terms were sometimes used interchangeably. Subsequently, frailty, disability and comorbidity became increasingly recognised as distinct, although interrelated, clinical constructs, highlighting the need for specific criteria and assessment methods to identify frailty (47).

The conceptualisation of frailty subsequently evolved from a broad description of vulnerability and functional dependence towards models recognising its multidimensional and dynamic nature. Woodhouse et al. (48) described frailty in relation to social and functional criteria, alongside physiological and psychological differences. Other authors, including Gillick MR (49), also explored frailty, although early descriptions frequently emphasised dependence on others for activities of daily

living. Rockwood et al. (50) in 1994 subsequently proposed a dynamic model based on the balance between an individual's assets and deficits, which influences their ability to maintain independence within the community (Figure 1). In this model, assets represent resources that support health and function, whereas deficits reflect impairments, illnesses and limitations that may reduce physiological and functional reserve. When deficit outweigh available assets, an individual may become more vulnerable to adverse health outcomes and loss of independence. This conceptualisation is particularly relevant to older adults, in whom reduced physiological reserve may limit the ability to compensate for additional health challenges.



Figure 1: Modified illustration of the dynamic model proposed by Rockwood et al. (50), depicting a person's ability to maintain independence in the community as a balance between the assets (left) and deficits (right). The figure is distributed under the CC BY-NC-ND 4.0 license.

This dynamic model was further developed (Figure 2) by Clegg et al. in 2013 (51) where it showed the changes in functional abilities or status after a minor stress event on a fit and frail older person. For the fit older adult, due to adequate physiological reserve, they can return to their pre-stress event baseline following a

minor illness, whereas for the frail older adult, a minor stress event will tip the balance affecting their functional ability causing them to recover but not back to their baseline.

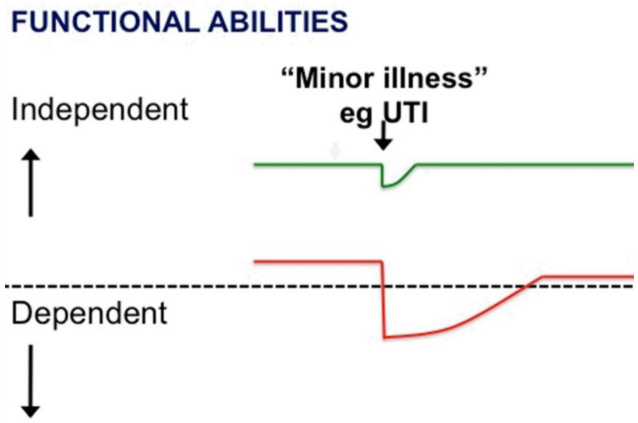


Figure 2: Vulnerability of a frail older person to their health status following a minor illness. The green line represents a fit older adult, after a minor illness such as UTI (Urinary Tract Infection), develops a small deterioration to functional status but returns to baseline. The red line represents a frail older adult, after a similar minor illness, develops a significant deterioration resulting in a prolonged recovery, affecting the functional dependency with no return to baseline. Figure by Clegg et al. (51), reprinted with permission, copyright © 2013 Elsevier Ltd.

In 2001, a phenotypic model of frailty was proposed by Fried et al. (52). After reviewing the prospective cohort data from the Cardiovascular Health Study (53), five variables, which included unintentional weight loss, self-reported exhaustion, weakness (grip strength), slow walking speed and low physical activity (Table 3), were used. The analysis excluded people with Parkinson's disease, previous stroke, cognitive impairment and depression; the term frail was used when three of the five variables were present. When one or two variables were present, the term 'pre-frail' was used while 'robust' was used when no variables were present. This concept was a stepping stone in framing physical frailty and assisting in the detection of frailty in daily care. Nevertheless, this concept did not include other important factors such as cognitive impairment which is an important condition associated with functional decline.

Table 3: Fried's frailty phenotype (52), Copied with permission, copyright © 2001 Oxford University Press.

Factors	**Characteristics**
Shrinking: Weight Loss (unintentional)	Weight loss of ≥10 pounds unintentionally in prior year or at follow-up, unintentional weight loss ≥5% of previous year body weight (by direct measurement of weight).
Exhaustion; poor endurance	Self-reported exhaustion, identified by two questions from the Centre for Epidemiological Studies depression (CES-D) scale.
Weakness	Grip strength: lowest 20% at baseline, adjusted for gender and body mass index.
Slowness	Walking time/ 15 feet: slowest 20% of the population defined at baseline, adjusted for gender and standing height.
Low physical activity	Lowest 20% of weighted score of kilocalories expended per week (Kcals/week). Male: <383 Kcals/week, female: <270 Kcals/week.

In 2001, Mitnitski and Rockwood introduced the concept that frailty could be assessed by measuring the health deficits, including chronic diseases, cognitive and psychological issues, laboratory abnormalities and functional disabilities (deficit accumulation model of frailty) (54). They suggested that frailty worsens as more deficits accumulate. Through this concept, the Frailty Index (FI) (54) was developed and validated using data from the Canadian Study of Health and Aging (CSHA) (55). The CSHA, a large prospective 5-year cohort study involving 10,263 older Canadians (>65 years old), collected data on 92 baseline parameters, comprising of signs and symptoms, laboratory results and deficits related to disease and disabilities. These parameters were used to construct the FI, which shifted the understanding of frailty from a categorical syndrome of frail or not frail, to a continuum of accumulated health deficits.

The biopsychosocial model of frailty, proposed by Gobbens et al. (56) expanded the traditional view of frailty beyond physical health to include biological and psychosocial domains. They examined the definitions of frailty and consulted a panel

of experts who had scientific publications on definition of frailty in the elderly from different specialties (geriatric, gerontology, nursing, biostatistics, psychology, general practice, health care and social inclusion) in two meetings. This resulted in a consensus that defined frailty as ‘‘a dynamic state affecting individual who experiences losses in one or more domains of human functioning (physical, psychological, social), due to influence of a range of variables which increases the risk of adverse outcomes’’ (56). This proposed biopsychosocial model recognised that frailty is predisposed by biological factors (chronic diseases, inflammation or sarcopenia), psychological factors (depression, stress) and social factors (social isolation, financial hardship and lack of support network), providing a more holistic understanding of frailty. In 2017, Bunt et al. (57) expanded the concept of social frailty via a scoping review, analysing 42 studies on social frailty and structured their findings using the social needs concept of Social Production Function theory. They defined social frailty as ‘a state where an individual is at an increased risk of adverse health outcomes due to diminished social resources, participation and support’. By addressing social frailty as part of a broader frailty framework, interventions can be designed to focus on both medical and social domains of health.

The concept of cognitive frailty was first put forward as a clinical term in 2006 by Panza et al. (58) when age-related changes in cognitive function and the progression from pre-dementia syndromes to dementia were examined. While physical frailty has been widely acknowledged in frailty research, cognitive impairment in frail older adults has gradually gained recognition as a vital component in defining frailty. This prompted the International Academy on Nutrition and Aging (A.I.N.A) and the International Association of Gerontology and Geriatrics (I.A.G.G) to establish an International Consensus Group on cognitive frailty in 2013 (59). The group suggested that cognitive frailty should have both physical frailty and cognitive impairment, and must be reviewed independently, excluding dementia or other pre-existing brain disorders (59). This emerging concept aligns with the deficit accumulation model, recognising that cognitive decline increases the risk of disability, hospitalisation and mortality (60). By incorporating both physical weaknesses and cognitive decline, the definition of frailty has evolved to better reflect the complexity of ageing.

Currently, Fried's phenotype of frailty and Rockwood's Frailty Index are the most used approaches to define frailty. However, many emerging models build upon these foundational concepts (summarised in Table 4). In an effort to standardise the definition of frailty, a consensus conference in 2012 in Orlando, Florida (61) was held. In this consensus conference, the group defined frailty as "A medical syndrome with multiple causes and contributors that is characterised by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death." In the UK, the British Geriatrics Society in association with Age UK and the Royal College of General Practitioners produced a Fit for Frailty Consensus Guidance in 2014 (62). In the report, they defined frailty as "a distinctive health state related to the ageing process in which multiple body systems gradually lose their in-built reserves." Additionally, in 2016, an international expert panel convened by the World Health Organization (WHO) discussed frailty at the "Clinical Consortium on Healthy Ageing" (63). They defined frailty as a concept of "clinically recognisable state in which the ability of older people to cope with every day or acute stressors is compromised by an increased vulnerability brought by age associated declines in physiological reserve and function across multiple organ systems". Despite all these consortiums, a universally agreed-upon gold standard definition remains elusive, highlighting the complexity of frailty as a multidimensional condition.

Table 4: Key conceptual models in defining frailty from different perspectives by different authors and years.

Conceptual Frailty Model	Authors (year)	Definition
Rockwood's Dynamic Frailty Model	Rockwood et al. (1994) (50)	A dynamic process that fluctuates over time based on balance between assets (compensatory mechanisms) and health deficits.
Fried's Phenotypic Model	Fried et al. (2001) (52)	A physical syndrome with five criteria: unintentional weight loss, self-reported exhaustion, weakness, slow walking speed and low physical activity. Frailty is present when 3 of the 5 criteria are present.
Deficit Accumulative Model- Rockwood's Frailty Index	Rockwood et al. (2005) (54)	Frailty based on accumulation of health deficits, worsens as more deficits accumulate.
Biopsychosocial Model	Gobbens et al. (2010) (56)	A dynamic state resulting from losses in one or more domains (physical, biological, psychological, social), which increases the risk of adverse outcomes.
Cognitive frailty	Kelaiditi et al. (2013) (59)	A combination of physical frailty and cognitive impairment, excluding dementia or other pre-existing brain disorders.
Social frailty	Bunt et al. (2017) (57)	A state of vulnerability due to diminished social resources, participation and support.

It is evident that frailty is not a standalone concept, but rather a multifaceted syndrome that encompasses physical, cognitive, and psychosocial components. It is part of a Geriatric or frailty syndrome, which includes conditions such as disability, multiple long-term conditions (multi-morbidity) and sarcopenia. These conditions are strongly linked and interact bidirectionally, contributing to poor outcomes in the older adults, resulting in higher morbidity and mortality as well as negative impact on QoL (64-66). The continued evolution of frailty definitions further demonstrates the intricacy in defining frailty, reinforcing the need for comprehensive assessments and multidisciplinary involvement.

1.4 Assessment of established frailty markers in clinical settings

As previously demonstrated, frailty can be assessed using questionnaires and measurements of physical capacity to assess loss of physical function, cognitive status (memory, orientation, and processing), social and psychological well-being. However, the availability of multiple assessment tools reflects the ambiguity of the term frailty and the complexity in categorising it. Nevertheless, the ability to assess frailty is important as it provides insights to population health and in the clinical setting, allows early identification which leads to preventive and targeted interventions, improvements in shared decision-making as well as development of strategies post hospital discharge.

1.4.1 Questionnaires

Comprehensive Geriatric Assessment (CGA) (67) is the internationally accepted gold standard in the assessment and management of frailty in older adult over the age of 65 in the clinical setting. It is defined as "a multidimensional, multidisciplinary, diagnostic process to screen an older person's medical, psychological and functional capability in order to form treatment and follow-up plan" (Figure 3). Collaboration between the multidisciplinary team and family members or carers is key in an accurate CGA and may take up to two hours to complete the assessment. The first study to validate CGA was based on CSHA data by Rockwood et al. (55) where the result affirmed that CGA was reliable and sensitive in identifying frailty. This result was supported by meta-analyses by Shuck et al. (68) and Ellis et al. (67) and subsequent analysis found older patients to be alive and community dwelling at 12 months follow-up after discharged from hospital (69). Despite being a standardised robust assessment tool and a good predictor of adverse outcome, the application is limited, especially in the emergency surgical setting as it is complex, time consuming and requires specialist input which is often lacking in the community setting.

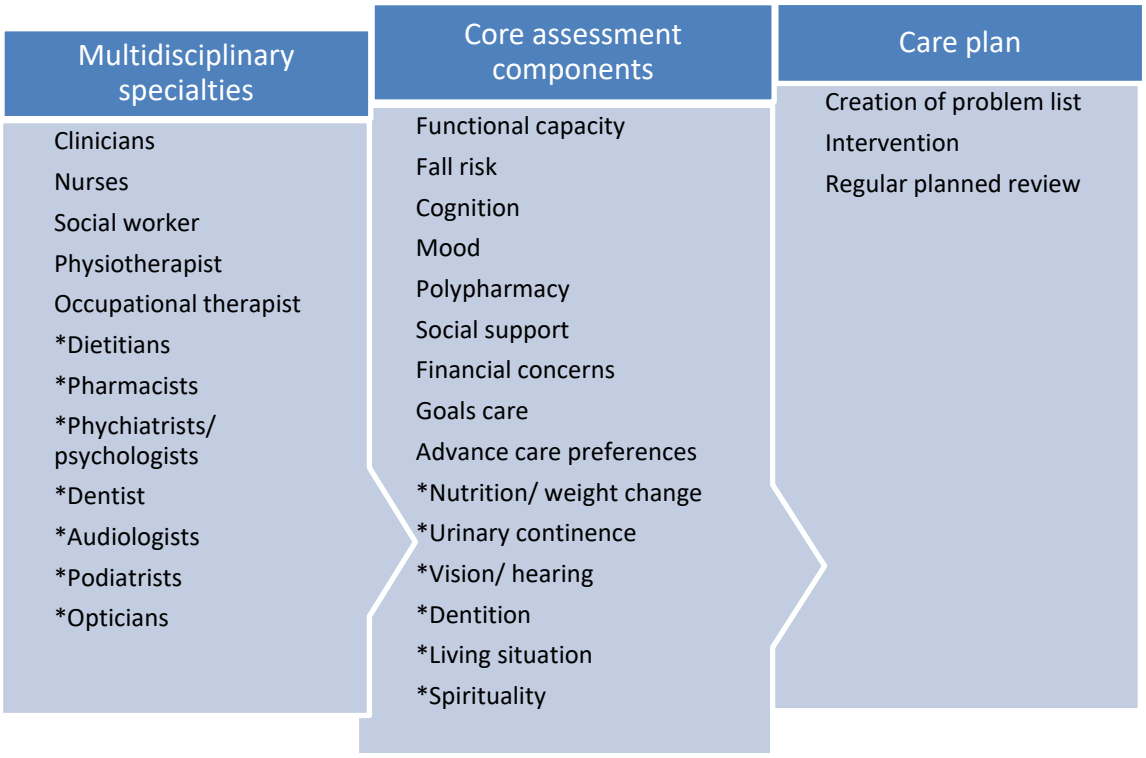


*Figure 3: Overview of a Comprehensive Geriatric Assessment (67) to assess and formulate treatment and follow up plan in frail patients. * Involvement of these multidisciplinary specialties and core assessment components if appropriate.*

To address the issues arising from CGA, multiple easy to use questionnaires for utilisation in the community settings have emerged in the past decade. One of them is the Groningen Frailty Indicator (GFI) (Figure 4) (70). It is a multidimensional 15 self-report item screening tool for frailty that assesses four domains: Physical, cognitive, social, and psychological. The score ranges from 0 to 15 and a score of ≥4 is considered frail. The GFI has been widely used and has shown good feasibility; in the Netherlands, 84% of the older adults in the community were able to successfully complete the questionnaire (71) and this result was mirrored in Romania, one of the fastest ageing populations in Europe (72). The GFI was also found to be reliable (73) and comparable to FI in identifying frailty (74). However, it is important to note that GFI is a self-reported screening tool and older adults with cognitive impairment might report fewer problems, misrepresenting the frailty level (74).

GFI (Groningen Frailty Index)

Circle the appropriate answer and add scores

	YES	NO	
Mobility. Can the patient perform the following tasks without assistance from another person (walking aids such as a can or a wheelchair are allowed)			
1. Grocery shopping	0	1	
2. Walk outside house (around house or to neighbour)	0	1	
3. Getting (un)dressed	0	1	
4. Visiting restroom	0	1	
Vision			
5. Does the patient encounter problems in daily life because of impaired vision?	1	0	
Hearing			
6. Does the patient encounter problems in daily life because of impaired hearing?	1	0	
Nutrition			
7. Has the patient unintentionally lost a lot of weight in the past 6 months (6kg in 6 months or 3kg in 3 months)?	1	0	
Co-morbidity			
8. Does the patient use 4 or more different types of medication?	1	0	
	YES	**NO**	**SOMETIMES**
Cognition			
9. Does the patient have any complaints on his/her memory (or diagnosed with dementia)?	1	0	0
Psychosocial			
10. Does the patient ever experience emptiness around him? *e.g. You feel so sad that you have no interest in your surroundings. Or if someone you love no longer love you, how do you feel?*	1	0	1
11. Does the patient ever miss the presence of other people around him? *Or do you miss anyone you love?*	1	0	1
12. Does the patient ever feel left alone? *e.g. You wish there is someone to go with you for something important.*	1	0	1
13. Has the patient been feeling down or depressed lately?	1	0	1
14. Has the patient felt nervous or anxious lately?	1	0	1
Physical Fitness			
15. How would the patient rate his/her own physical fitness? (0-10 ; 0 is very bad, 10 is very good) 0 – 6 = 1 7 – 10 = 0	1	0	
TOTAL SCORE GFI			

Appendix 6 – PS (Performance Status)

0		Normal activity without restriction.
1		Restricted in physically strenuous activity but ambulatory and able to carry out light work.
2		Ambulatory and capable for all self-care, unable to carry out any work and about >50% of waking hours.
3		Capable only limited self-care, confined to bed or chair and about <50% of waking hours.
4		Completely disabled, cannot carry on any self-care, totally confined to bed or chair.

Figure 4: Groningen Frailty Indicator questionnaire (70) consisting 15 self-report items to assess frailty looking into four domains (physical, cognitive, social, and psychological), with score ranging from 0 to 15 and a score of ≥4 considered frail. Figure by Schuurmans H et al. (75), reprinted with permission, copyright © 2004 Oxford University Press.

Another proposed simple questionnaire to assess and diagnose frailty in older adults includes the FRAIL scale (76, 77) (Table 5). The lack of agreement of definition of frailty led to the creation of this 5, simple question scale by the Geriatric Advisory Panel of the International Academy of Nutrition and Aging. Out of the 5 domains in this FRAIL scale, 4 questions are part of the Cardiovascular Health Study Frailty Index and 1 question is directed at the component of Rockwood Scale. As it is a self-reporting tool that is straightforward, it allows frailty to be assessed remotely. It has been recommended as the frailty assessment tool in the Australian primary care setting (78). Multiple studies have validated this tool and have predicted post-operative outcomes including mortality (79, 80).

Table 5: FRAIL scale (79), self- reporting questionnaire consisting of 5 questions to assess frailty. Table copied under CC BY-NC-ND 4.0 license.

Component	Question	Score
Fatigue	How much time during the previous 4 weeks did you feel tired?	all the time, most of the time= 1 point Some of the time, a little of the time and none = 0 point
Resistance	Do you have any difficulty walking up 10 steps alone without resting and without aids?	Yes= 1 point
Ambulation	Do you have any difficulty walking several hundred yards alone without aids?	Yes= 1 point
Illness	How many illnesses do you have out of a list of 11*?	≥5= 1 point
Loss of weight	Have you had weight loss of 5% or more for the last 1 year? % Weight change= (weight 1 year ago-current weight)/ weight 1 year ago	Yes = 1point
* The illnesses include hypertension, diabetes, cancer (other than a minor skin cancer), chronic lung disease, heart attack, congestive heart failure, angina, asthma, arthritis, stroke and kidney disease		
Score 0= Robust health Score 1-2: Pre-frail Score 3-5: Frail		

In the outpatient surgical setting, Edmonton Frail Scale (EFS) has been advocated as the assessment tool in national guidelines (62). The EFS was produced by Rolfson et al. and was first presented at the Canadian Geriatric Society in Edmonton in 2000 (81). The assessment includes 9 domains which comprised of cognition, general health status (frequency of hospital admissions), functional independence (meal preparation, shopping, transportation, telephone, housekeeping, laundry, managing money, taking medications), social support, medication use (≥5 different prescription medications), nutrition (weight loss), mood, urinary continence, and functional performance (Timed Up and Go (TUG) test). The maximum score is 17 and all components are summed with score of ≤5 as not frail; 6-7 as apparently vulnerable; 8-9 as mildly frail; 10-11 as moderately frail and 12-17 as severely frailty. They tested the EFS in the inpatient and outpatient settings, and found the scale to be valid, feasible and reliable (82). With only 9 components, it is simpler and quicker to complete when compared to FI or CGA. It is highly applicable in the outpatient setting as there is opportunity to optimise certain aspects that contribute to frailty such as nutrition or polypharmacy pre-operatively. Furthermore, the TUG test is part of the EFS where gait, balance and risk of falls are all assessed, which also provide an opportunity to patients access to pre-operative rehabilitation. Dasgupta et al. (83) found higher post-operative complications and increased hospital stay in the elective non-cardiac surgical setting when EFS score was >7. To improve these post-operative outcomes, they recommended a CGA referral when the EFS score was >7. Overall, EFS is a compelling screening tool for frailty in the elective setting. However, it is not suitable in the emergency surgical setting as some of the components such as TUG test is impractical especially in patients with acute abdomen.

1.4.2 Frailty Index

The Frailty Index (FI) developed by Mitnitski and Rockwood (54) was part of their study developing on the concept of deficit accumulation model of frailty at the population level. It was designed to measure frailty as a continuous variable rather than a binary classification, by calculating the presence or absence of parameters (regardless of the combination of parameters) as a proportion of the total (frailty

score= sum of health deficit present divided by total number of health deficits measured). The FI formula represents the cumulative effect of individual deficits, rated by a continuous score between 0 and 1, where a higher score indicating greater frailty (frailty is present when the score is ≥0.25, Figure 5) (84). The FI was developed to be an attractive mathematical model that could capture a more accurate assessment of an individual's overall health, as it was reproducible, more of a graded risk scale than just a term of presence or absence of frailty and highly correlated to the increased risk of mortality. Nonetheless, it was rather overwhelming to grade and interpret the 92 parameters, as it warranted a significant time and effort. Hence, subsequent work reduced the total number of parameters (ranged from 30 to 70 parameters) that were biologically sensible and accumulated with age (85, 86).

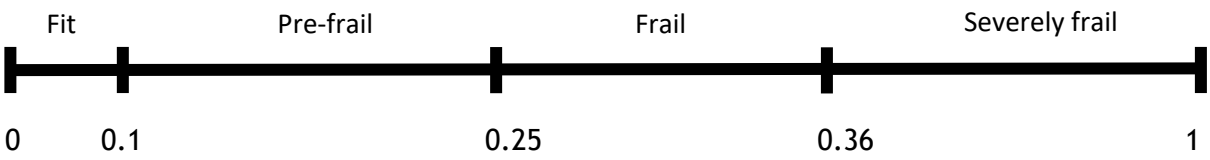


Figure 5: Score of frailty index to determine frailty status. Score <0.1 is fit, score 0.1-0.24 is pre-frail, score 0.25-0.35 is frail and score >0.35 is severely frail.

1.4.3 Clinical Frailty Scale

Rockwood et al. (55) developed the CSHA Clinical Frailty Scale (CFS) in 2005 which was a scale based on clinical judgement. This was their 3rd approach in defining frailty. In this approach, episodes of delirium, likelihood of falls and cognitive impairment were recorded and incorporated. This CFS ranged from 1 (robust) to 7 (complete functionally dependent on others) and was found to be easy to use especially in clinical setting; an improvement compared to previously developed tools. As clinical judgement can be subjective, Rockwood et al. have validated the CFS with FI and found the predictability of frailty to be the same. They felt that it provided flexibility which is not present in other rule-based assessments. The CFS has since been modified to include a scale up to 9 due to the complex nature of

severe frailty (87) (Figure 6). To date, CFS has been validated and used in many studies. The ELF study was the first multicentre prospective study to assess frailty in EmLap using CFS, further validating the feasibility in assessing frailty in acute setting which led to CFS being included in NELA from year 5 report (88) and ELLSA (10-12). In this study, the modified CFS (Figure 6) is used as a pragmatic way to assess frailty in the emergency laparotomy patients.

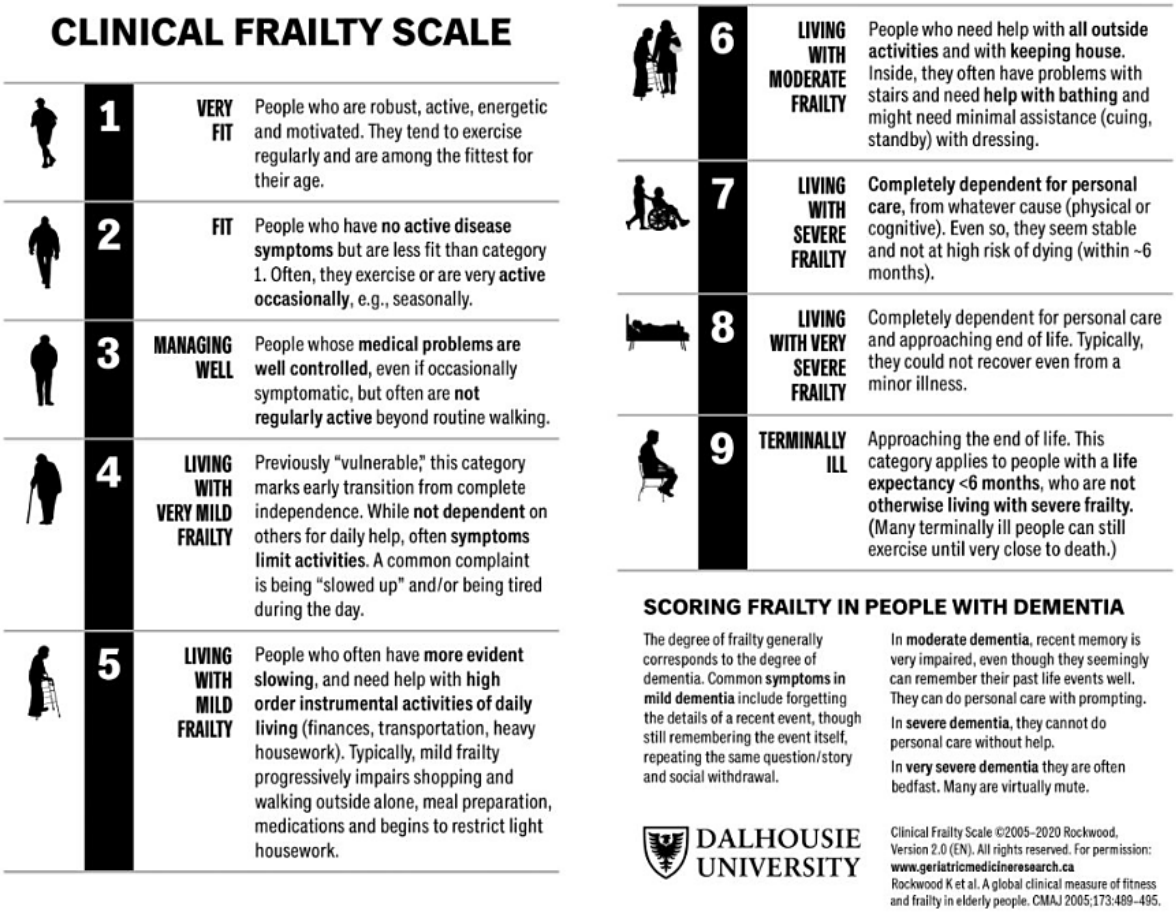


Figure 6: Rockwood Clinical Frailty Scale (87) consisting of 9 scores with worsening frailty as the score increases. Figure distributed under CC BY-NC-ND 2.5 CA DEED license.

1.4.4 Sarcopenia

Sarcopenia, which is part of the Geriatric syndrome, is correlated with frailty (89). Sarcopenia was first coined by Rosenberg in 1989 (90). In Greek, "Sarco" indicates "flesh (muscle)" and "paenia" denotes deficiency, therefore sarcopenia was loosely

translated to “deficiency of muscle”. Another way to define sarcopenia is “muscle mass of over two standard deviations below the mean of a typical healthy adult” (91). According to the European Working Group on Sarcopenia in Older People (EWGSOP), sarcopenia is present when there is both low muscle function and low muscle mass (92). For the assessment of muscle function or muscle strength, a range of methods have been established which include assessment of handgrip strength, lower limb muscle strength and repeated chair stand test. Some frailty questionnaires, such as the Edmonton Frail Scale, have incorporated sarcopenia measurement in the assessment tool, utilising the 400m walk test, gait speed or the timed up and go test. Other approaches to assess muscle mass include dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). However, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are the gold standard imaging methods to measure muscle mass as well as density and fatty infiltration (93, 94) as they are better in quantifying specific body compartments (95). Summary of different assessment methods of sarcopenia (www.cgakit.com/sarcopenia) is shown in Table 6 (96).

Table 6: Summary of different assessment methods of sarcopenia.

Assessment	Measurement
Muscle strength	
Handgrip strength	Using dynamometer on dominant hand.
Lower limb strength	Using isokinetic dynamometer on knee extension-flexion.
Repeated chair stand test	Rise from a standard height chair without using arms and return to seated position either consecutively for five times with action timed or the number of times action performed within 30 seconds.
Muscle function	
Gait speed	The time it takes to walk a specified distance on a level surface e.g. 4 metres with 1m for acceleration, 4m for testing and 1m for deceleration. Gait speed is total distance/time.
Timed Up and Go test	The time it takes to stand up from a chair, walk 3m, turn around, return and sit down again.
Standing balance test	4 stage test with each stage lasting for 10 s (1) Balance test: feet together side by side standing (2) Semi tandem stand: heel of one foot touching side of big toe of the other foot (3) Tandem stand: feet aligned heel to toe (4) Stand on one foot.
6 min walk test	The distance of walking on level surface for 6 min.
400m walk test	The time to walk 400m in 20m laps with maximum 2min rest between each lap.
Stair climb power test	The time it takes to climb 4-11 flights of stairs. Stair climb power in watt is [(body weight in kg) x ($9.8 m/s^2$) x (stair height in meter)]/time in seconds.
Short Physical Performance Battery (97)	Combination of (a) gait speed over 4m (b) chair stand test (c) standing balance test.
Muscle mass	
DXA	A total body scan using X-ray technology to measure appendicular skeletal muscle mass.
BIA	Using electric current to assess total body weight and total fat free mass is estimated.
CT	Cross sectional imaging of skeletal muscle mass.
MRI	Cross sectional imaging of skeletal muscle mass.

The skeletal muscle mass loss starts from age 30 for up to about 0.5% annually and this loss increases up to 30% after 65 years, and up to 50% after 80 years, affecting muscle function (94, 98). Malnutrition contributes to the development of sarcopenia especially in the frail older patients (98). Multiple studies have investigated nutritional supplements especially protein supplementation or physical activity/

resistance training as interventions to improve sarcopenia. The systematic review found most exercise trials showed improvements in muscle function but there was limited evidence with nutritional interventions due to small studies and diverse study design (99). Regardless of these findings, the main recommendation was to measure sarcopenia especially in the older population.

It is common to link sarcopenia with weight loss due to loss of muscle mass. Nonetheless, there are a cohort of patients who are obese and sarcopenic, termed 'sarcopenic obesity'(100). This term was first coined by Baumgartner (101). He presented two studies; the New Mexico Aging Process Study (NMAPS) which included 400 elderly men and women, and the New Mexico Elder Health Survey (NMEHS) which included 883 older population. He found that sarcopenic obesity increased with age, 2% in 60-69 years of age and up to 10% for >80 years (101). The relationship between obesity and sarcopenia are thought to be secondary from the obesity-related metabolic dysfunction such as insulin resistance, oxidative stress and inflammation which cause muscle loss (102). The group with sarcopenic obesity is at higher risk of developing frailty and disability (103, 104) and whilst there is no agreement on the diagnostic criteria of sarcopenic obesity, they should not be neglected.

1.4.5 Cognition

Cognitive decline has been considered as part of the frailty syndrome and majority of the frailty assessment questionnaires have included evaluation of this component (67, 70, 81). Rockwood et al. (105, 106) established a clinical frailty classification in 1999 and subsequently created the Frailty Index which incorporated an assessment of cognitive impairment (54).

Cognitive impairment is prevalent in the community. A systematic review found the presence of cognitive impairment in 20%-39% of the frail older population (aged >65 years), 12%-22% in the pre-frail older patient cohort and 5%-16% of the robust group in the community (107). It has also been studied in the emergency setting. Hewitt

et al. assessed cognitive impairment in the emergency General Surgery setting using the Montreal Cognitive Assessment tool (MoCA) and found 66.9% of the study cohort to have cognitive decline (108). It can occur acutely presenting as delirium, acute on chronic and chronically presenting as dementia (109). Delirium, a form of acute confusional state that is fluctuating (110), could be worsened by the presence of frailty (111). About a third of inpatients aged >70 years were found to have delirium; half of these patients presented with delirium on admission while the other half developed this condition during their hospital stay (112). The Abbreviated Mental Test 4 (AMT-4) is a quick 4 question assessment of cognition and is frequently used in clinical settings. It is a quick appraisal and is deliverable when time is limited. Score of <4 indicates impaired cognition. Hence, this test has been used frequently in the emergency department (113) and in the hospital ward (114), operating as a good screening tool for cognitive impairment.

Both physical frailty and cognitive frailty were found to be crucial elements in predicting poor outcome such as risk of mortality (115). Cognitive impairment itself also poses a huge impact on the healthcare service resources. Gordon et al. examined the health status and healthcare utilisation by residents in the UK care homes and found that majority (75%) were cognitively impaired and were using the NHS frequently (116). Another study also found worsening cognition increased societal costs by 8.7% which is approximately £2,200 per patient over 18 months period (117). It is therefore important to consider that cognitive frailty is in parallel with physical frailty and early identification may facilitate targeted care to treat acute cognitive impairment by addressing the reversible causes (118).

1.4.6 Malnutrition

Frailty is a multifactorial dynamic progression characterised by the transition from a state of fitness to weakness. The severity of weakness has the potential to fluctuate in any direction over time. Whilst distinct from frailty, malnutrition resulting from a deficiency in the consumption of nutrients and energy necessary to meet an individual's needs, can contribute to the development of frailty (119).

Prolonged malnutrition, particularly insufficient protein and energy intake can cause weight loss which leads to tiredness, weakness, slowness and low level of physical activity, all of which align with Fried's phenotype criteria for frailty (120). Lorenzo-Lopez et al. (121) conducted a systemic review looking into various nutrition assessment tools and their association with frailty. Their findings demonstrated a significant correlation between malnutrition and frailty. Such evidence underscores the importance of routine nutritional assessments in older adults, especially those at risk of frailty, to allow early identification and intervention.

Assessment of malnutrition is a critical step in understanding its role in frailty. Several validated tools are available for evaluating malnutrition status, including the Body Mass Index (BMI), the Mini Nutritional Assessment (MNA) and the Malnutrition Universal Screening Tool (MUST). The BMI is a widely used tool calculating an individual's body weight relative to their height. While it provides a general indicator of nutritional status, the BMI has limitations as it does not differentiate between muscle, fat or fluid retention (122). Low BMI value of <18.5 kg/m^2 indicates malnutrition. However, it is important to note that normal BMI values in some cases may mask underlying malnutrition. Among these assessment tools, the MNA has been validated for older adults. This tool consists of 18 questions, which are divided into four sections that assess anthropometric measurements (BMI, calf and arm circumferences measurement), self-reported state of health, dietary intake including recent weight loss, and clinical health conditions. Scores on MNA range from 0 to 30, with a score <17 indicating malnutrition. This tool combines both objective and subjective measurements to detect malnutrition. However, its usefulness is limited in the emergency environment. In contrast, the Malnutrition Universal Screening Tool (MUST) is a more suitable alternative in emergency setting. The MUST is a five steps screening tool including BMI, unintentional weight loss and the presence of acute disease affecting nutritional intake. Each parameter is scored, with a higher total score indicating greater risk. The MUST is easy to use, offers a rapid and effective assessment of nutritional risk and a routinely collected healthcare data in Scotland, hence was used in this study (123).

Nutrition is a modifiable factor that plays a pivotal role in both the prevention and treatment of frailty. Studies in a few systematic reviews (120, 121, 124) have suggested that addressing malnutrition through dietary interventions can reduce the risk of decline in physical function and reverse certain frailty-related symptoms. The included studies found protein intake, in particular, was essential for maintaining muscle mass and strength which are critical for physical performance and mobility, while energy-rich diets supplemented with essential vitamins and minerals support overall physiological function and reduce the risk of frailty progression. By prioritising nutritional health, particularly in older populations, the burden of frailty can be significantly reduced, ultimately improving health outcomes and QoL.

1.4.7 Polypharmacy

Polypharmacy is defined as the concurrent use of several different medications. The term is derived from the Greek word; 'Poly' which means 'more than one' and 'pharmacy' or 'pharmacon' representing 'drug'. While there is no universally standardised definition of polypharmacy in the clinical setting, it is generally recognised when a patient takes five or more daily medications (125, 126).

Polypharmacy is another significant element of the Geriatric syndrome and has been found strongly associated with frailty in numerous studies (127-131). However, the exact nature of the relationship between these two factors remains unclear. One critical question is whether the medications prescribed are to manage the co-morbidities contributing to frailty, or if the drug therapies themselves act as stressors that accelerate the development of frailty. What is well-established is that an increased number of medication prescriptions to treat chronic diseases is linked to an increased risk of adverse drug reactions, falls, and other negative consequences (132). In addition, studies (133-135) have demonstrated that increased medication use is associated with an increased risk of cognitive impairment, another key aspect of frailty syndrome.

The prevalence of polypharmacy among older frail adults is notably high. In the community setting, the Interdisciplinary Excellence Research Program on Healthy Aging in Chile found that 24.6% of participants were frail, and 32.9% experienced polypharmacy (131). In the emergency surgery setting, data from the ELF study revealed that 54% of the older adults had polypharmacy, with 28.5% being classified as frail (136). Additionally, a systematic review of 37 studies, which included 66.7% community-dwelling people, 25% hospitalised patients, and 8.3% nursing residents, found the pooled proportion of polypharmacy and frailty coexisted in 27% (137).

These findings underscore the intricate interconnections between polypharmacy and frailty. As part of the CGA, polypharmacy is routinely assessed, emphasising its importance in identifying and managing frailty. Given the associated risks, careful review of medication is crucial in older adults to minimise adverse outcomes. By addressing polypharmacy effectively, clinicians can reduce the burden of frailty and improve the QoL for older individuals.

1.4.8 Multiple long-term conditions (multi-morbidity)

The advances in medical care and longer life expectancy have resulted in an increased number of older people living with multiple chronic health conditions which has been referred as 'living with multiple long-term conditions or multi-morbidity'. In 2023, the term 'multi-morbidity' was changed to 'multiple long-term conditions' (138) as it conveys better clinical relevance and is a more acceptable terminology. Multiple long-term conditions can be defined as 'having the co-occurrence of two or more chronic conditions, where one is not necessarily more central than the others' (139). This description is mirrored in the definition of multiple long-term conditions by the British National Institute for Health and Care Excellence (NICE) guidelines (140) of 'two or more long-term, single count health condition'. Even though there is a broad agreement to this definition, there is no internationally accepted accord in the method of evaluation of multiple long-term conditions in the research setting. Thus, a modified study involving an international panel of 150 professionals and 25 public participants was conducted with the aim of

developing a consensus on the definition and multiple long-term conditions assessment (141). This study did not manage to reach an agreement on a standardised method to measure multiple long-term conditions but recommended the use of a weighted measurement tool depending on the study goals and to clearly justify the choice of assessment tool according to severity of disease or a particular outcome e.g. mortality.

At present, there are multiple measurement tools available to assess multiple long-term conditions (142). One of the commonly used assessment tools include Charlson Co-morbidities Index (CCI) (143). It was formulated to assess co-morbidities by taking into account the number of severities out of 19 pre-defined conditions and has been validated to predict short- and long-term outcomes such as mortality rate and hospital length of stay (144-147). In 1994, the CCI was validated and modified to include age as a factor, adding 1 point to each decade of age from 40 years old (148). As there is no weighted Charlson score specifically for emergency laparotomy patients, we used the age-adjusted Charlson Co-morbidity Index in this study to evaluate the participant's co-morbidities as it has been evaluated for predicting 30-day mortality in emergency general surgical patients (149). Details of the 19 health illnesses and the age adjusted weighted score is shown in Table 7.

Table 7: Age-adjusted Charlson Co-morbidities Index (CCI) (148) by scoring 19 pre-defined conditions and age.

Co-morbidities	Score
Age	
>50 years	0
50-59 years	+1
60-69 years	+2
70-79 years	+3
>80 years	+4
Myocardial infarction	+1
Congestive heart failure	+1
Peripheral vascular disease	+1
Dementia	+1
Stroke/ transient ischemic attack	+1
Chronic obstructive pulmonary disease	+1
Connective tissue disease	+1
Peptic ulcer disease	+1
Liver disease	
None	0
Mild	+1
Moderate to severe	+3
Diabetes mellitus	
None or diet-controlled	0
Uncomplicated	+1
End-organ damage	+2
Hemiplegia	+2
Moderate to severe chronic kidney disease	+2
Solid tumour	
None	0
Localised	+2
Metastatic	+6
Leukemia	+2
Lymphoma	+2
AIDS	+6

Frailty and multiple long-term conditions are interlinked. Studies have found a bidirectional relationship between these two factors (65) where the chronic illnesses contribute to the development of frailty and frailty causes deterioration in health conditions leading to the development of co-morbidities hence multiple long-term conditions (150). Various studies have explored the association between frailty and

multiple long-term conditions (65, 151) and the relationship between these factors with adverse outcomes such as mortality, unplanned readmissions, and prolonged length of stay (150, 152). The impact on healthcare utilisation and costs has been recognised, leading to the NICE guideline (140) to issue recommendations which included a multi-morbid approach when managing older patients with complex health issues and to provide additional support beyond standard care when the morbidities significantly affect daily life, especially when frailty is present.

1.4.9 Summary of all frailty assessment tools

Thus far, multiple alternative assessment tools (summarised in Table 8) have been developed to assess frailty as described in this chapter, yet there is a lack of consensus in which frailty assessment tool is the current best for all clinical settings and in particular, in the emergency surgery situation.

Even though frailty is commonly linked with older adults, it can also occur in the younger cohort. There are a few studies that demonstrated the presence of frailty in the younger cohort; Hanlon et al. (153) included 493,737 participants from the UK biobank database and using the frailty phenotype criteria, they reported 3.4% of patients age 37 to 65 years (399,481 patients) were frail and 39% of this younger cohort were pre-frail. Smart et al. (6) used the 7-points CFS to examine 169 patients admitted under emergency surgery unit, and found 16% of the 82 young patients (age <65 years) were frail while Blodgett et al. also reported increasing prevalence of frailty among the younger cohort of above age 35 years when 46-item frailty index was used (7). Another study by Bagshaw et al. where 197 critically ill patients aged 50- 64.9 years in intensive care units were recruited and 28% of this younger cohort were found frail. These findings reflect the risk of higher frailty among the future population as this young frail cohort ages, increasing risk of poorer outcomes in EmLap setting.

Nevertheless, the assessment tools available are not validated for the younger cohort and this was highlighted by Spiers et al. (154). Therefore, there remains a need for an objective measurement of frailty which this thesis aims to explore.

Table 8: Summary of frailty assessment tools used in clinical settings, grouped by type of tools.

Type of tools	Frailty classification
Physical phenotype-based tools	
Fried frailty phenotype (52)	5 criteria; Frail when ≥3 criteria, pre-frail when 1-2 criteria.
Short physical performance battery (SPPB) (97)	Score ranges from 0-12 (0-4 score each for balance test, gait speed test and chair stand test), lower scores indicate greater frailty.
Timed up and go (TUG) test (155)	>12 seconds indicate increased risk of frailty.
Grip strength (Hand dynamometry)	Low grip strength marker of frailty and sarcopenia.
Deficit Accumulation Models	
Frailty Index (54)	Continuous score from 0 (no deficit) to 1 (all deficits present).
Clinical Screening Tools	
Comprehensive Geriatric Assessment (CGA) (69)	
Rockwood Clinical Frailty Scale (CFS) (55)	Score 1 (fit) to 9 (terminally ill).
Groningen Frailty Indicator (GFI) (71)	Total score 15; Frail when score ≥4.
Edmonton Frail Scale (EFS) (81)	Total score 17; Score 8-9 as mildly frail; 10-11 as moderately frail and 12-17 as severely frailty.
PRISMA-7 (156)	Total of 7 questions; Frail when ≥3 yes answers.
FRAIL Scale (79)	Score 0-5; Frail when ≥3.
Tillburg Frailty Indicator (TFI) (157)	Total score 15; Frail when score ≥5.
G8 Geriatric Screening tool (158)	For older patients with cancer, score ≤14 needs evaluation with CGA (total score 17).
Vulnerable Elders Survey-13 (VES-13) (159)	Total score 13; score ≥3 indicate vulnerable.

1.5 Frailty in emergency laparotomy

The proportion of patients over the age of 65 is rapidly increasing. According to the recent 8th NELA report, of the EmLap patients who were frail (CFS> 4) (160), 18.6% of patients were ≥ 65 years old and 8.5% were ≥ 80 years. In the USA, 10,025 ≥ 65 years old EmLap patients were reviewed and 31.2% were found to be frail and 45.4% were mildly frail (161). This accounts for a large proportion of older patients undergoing EmLap with frailty resulting in a high post-operative morbidity and mortality risks. If frailty pre-EmLap is prevalent, then it must be remembered that surgery is itself a frailty stressor (162), inducing frailty in non-frail patients or worsening frailty in those that were already frail (surgical frailty). This was corroborated by Alder et al. where they found the average score of CFS in their study increased from 4 to 5 post-EmLap (29).

1.5.1 Frailty assessments in emergency settings

Frailty assessment in the emergency setting is different from the elective setting. Due to the severity of illness on presentation, most of the commonly used questionnaires assessing physical function are redundant. Frailty assessment tools used in emergency surgery need to be pragmatic and quick. In a systematic review and meta-analysis conducted by Tjeertes et al. in 2020 looking into influence of frailty on outcome post non-cardiac surgery, 56 studies (articles from year 1990-2017) were reviewed. They included studies where any type of frailty assessment tools was reviewed, and four papers were performed in the emergency general surgery setting. One of the papers included (163) in the systematic review consisted of patients that were admitted with general surgical pathology but did not undergo EmLap. The different frailty scores used in these four studies are shown in the Table 9 (164). Another systematic review and meta-analysis conducted in 2022 by Kennedy et al. (165) explored the impact of frailty on outcomes post-EmLap in patients ≥65 years (articles from 2009-2019 were reviewed). This review included 6 studies, with one study already included in the Tjeertes et al. analysis. The frailty assessment tool used in those included studies are shown in Table 9. Other studies also utilised the modified Frailty Index (161) and Rockwood CFS (136).

Table 9: Frailty scores used in emergency general surgery studies (164, 165).

Author	Year	Frailty score	Systematic review and meta-analysis
Farhat (166)	2012	Modified frailty Index	Tjeertes et al. (164)
Hewitt (163)	2015	Rockwood Clinical Frailty Scale	Tjeertes et al. (164)
Joseph (167)	2016	Rockwood Clinical Frailty Scale	Tjeertes et al. (164) Kennedy et al. (165)
Kenig (168)	2015	VES-13, Claims based frailty index, Rockwood clinical frailty scale, Balducci, Triage Risk-Screening Tool, Geriatric 8	Tjeertes et al. (164)
Jokar (169)	2015	EGS specific frailty index	Kennedy et al. (165)
Goeteyn (170)	2017	7-point Clinical Frailty Scale	Kennedy et al. (165)
Kenig (171)	2018	Geriatric 8	Kennedy et al. (165)
Parmar (136)	2019	Rockwood Clinical Frailty Scale	Kennedy et al. (165)
Lee (172)	2019	Claims based frailty index	Kennedy et al. (165)

As previously discussed, frailty is also present in younger individuals (<65 years). Loecker et al. (173) reviewed 12 articles and reported a range of frailty prevalence (3.9% to 63%) in the young and middle aged adults (18-65 years). However, most of the frailty assessment tools are not validated for the younger cohort such as the CFS (87) and was reported by Spiers et al. in a review on 85 studies (154). However, it is essential to have frailty assessment performed on all EmLap patients (174) which is the common recommendation by studies conducted on EmLap patients.

1.5.2 Morbidity and mortality in frail emergency laparotomy patients

According to the Hospital Episode Statistics (a national administrative dataset for England), 47% (about 600,000) of General Surgery admissions in 2020-2021 were

emergency admissions. This number includes a large population of high-risk patients and of the older populations. As reported by NELA (175), the percentage of older adults (≥65 years) undergoing EmLap remained the highest, reaching 55% over the last 4 years even when 70.7% were deemed frail (CFS >7) and identified with a high pre-operative risk of death. According to the 8th NELA report (160), the majority of patients in England and Wales that underwent an emergency laparotomy were in the age group of 60-79 years and around a third of all patients undergoing EmLap were recorded as frail. The report also stated that frailty approximately doubled the risk of mortality after EmLap. This finding was echoed in multiple studies (6, 29, 49, 55, 74, 85, 136, 153, 166, 176-185) and risk of high morbidity post-EmLap when frail was also reported.

The Emergency Laparotomy and Frailty (ELF) study (136) was the first multicentre observational study to explore the prevalence of frailty in older patients undergoing EmLap. The study included 937 patients who were >65 years from 49 UK sites across England, Wales and Scotland and found frailty in 20%. There was also similar association where the risk of complications, length of intensive care unit, overall hospital stay, change to discharge destination and mortality was higher as the frailty score increased. In addition, they found the scoring system was better at stratifying older patients into a greater number of prognostic groups allowing risk prediction of 30-day morbidity when compared to Portsmouth-physiological and operative severity score for the enumeration of mortality and morbidity (P-POSSUM) scoring system.

One of the factors that could improve the outcomes of frail older adults was post-operative geriatrician input. NELA found that post-operative geriatrician review reduced mortality post-EmLap (aged ≥65 years and frail had mortality rate decreased from 22.3% to 13% with geriatrician review) (160). This recommendation has been repeatedly emphasised for the last few years in the NELA reports and there is a slight improvement in the involvement of a geriatrician-led team (27.1% in NELA 5th year, 31.8% in NELA 8th year, 33.2% in NELA 9th year and 35.5.% in NELA 10th year) (3, 5, 88, 160). However, the data on this recommendation was still in red on the standard

of care rating (<55%) in the 10th NELA report, with less than a third of patients >65 years and frail or >80 years old having early geriatrician led multidisciplinary team input (3, 5). Compared to the recent 2nd ELLSA report, 50.8% of the EmLap group were ≥65 years, 18.7% of this group were frail and only 31.8% of these frail EmLap older group had Geriatrician input (11), which was similar to the NELA report.

The recommendation of geriatrician involvement in EmLap patients was echoed by the British Geriatrics Society (186) where the society stated (1) Clinical Frailty Scale (CFS) should be used to assess frailty in patients undergoing EmLap; (2) patients ≥65 years with CFS ≥5 or aged ≥80 years should be reviewed by a geriatrician led multidisciplinary CGA within 72 hours of admission to ward or step down to ward from critical care. The involvement of geriatricians in improving outcomes following EmLap has been widely acknowledged. However, several challenges hinder its implementation. These include limited hospital resources and funding, difficulties in recruiting geriatricians with a peri-operative interest and the absence of standardised EmLap care pathways (187). Furthermore, age restrictions on geriatric services exclude patients under the age of 65 with frailty, despite the potential benefit of targeted interventions to improve outcomes.

There are societal consequences to frailty or worsening frailty after emergency surgery. There have been studies examining how frailty influences the living state of frail patients post-EmLap after hospital discharge (176, 188-190). Joseph et al. (188) measured the adverse discharge disposition in their study which included discharge to skilled nursing facility in the geriatric trauma patients. They found 21.6% had adverse discharge disposition and frailty index of ≥0.25 was an independent predictor factor. Carter et al. (176) interrogated the data of the ELF study and reported 37% of older adults post-EmLap required increased care at discharge. These findings reaffirm that frailty affects morbidity not just in hospital, but also after discharge, which results in an increased burden on the already over-stretched National Health Service (NHS).

The 8th NELA report revealed a mortality rate of 13.0% for frail patients aged ≥65 years, compared to 5.9% for non-frail patients in the same age group, indicating that frailty more than doubles the risk of mortality (5). This finding was mirrored in the ELF study (136) where the mortality rate was higher when frailty score increased, after age and gender adjustment. A recent meta-analysis and systematic review was carried out by Leiner et al. (191) examining the effects of frailty on short term mortality and included patients aged over 18. They found the 30-day mortality increased nearly 4-fold over non-frail patients. This finding is supported by numerous studies focused on patients undergoing EmLap (9, 24, 86, 136, 153, 161, 163, 164, 166, 172, 181, 192-199), including a meta-analysis by Fehlmann et al. (193). Together, these studies provide compelling evidence that frail patients face a significantly higher mortality risk when undergoing emergency laparotomy.

Drawing from the wealth of data that is being recorded in NELA, the 30-day mortality rate for older adults that underwent EmLap with frailty (CFS ≥5) has steadily decreased from 21.7% in the 5th NELA report to 13.0% in the current 8th NELA report. This notable improvement can be attributed to several key factors including enhanced frailty assessments enabling early identification and management of high-risk patients, improved peri-operative care of frail patients supported by multidisciplinary team involvement, the adoption of standardised protocols for effective post-EmLap care such as chest physiotherapy, renal support, early mobilisation to maintain muscle mass, nutritional support, and wound management (136), regular feedback of outcomes to refine clinical practices, and the implementation of evidence-based strategies to address the unique challenges of EmLap (88, 160, 200). However, there is lack of evidence to show that it is the less frail older patient that undergoes an EmLap. Thus far, two studies have looked into patients presenting with acute abdomen but did not undergo laparotomy (NoLap) patients (201, 202) and NELA has now started collecting data on NoLap patients.

1.6 Novel frailty biomarkers in research studies

The flow of genetic information within a biological system was first coined by Francis Crick as 'Central Dogma of Molecular Biology' in his 1957 lecture (203). He stated that the transfer of information in genes flow unidirectionally: from DNA to RNA to proteins. This concept laid the foundation for understanding gene expression and molecular function. However, subsequent discoveries, such as reverse transcription (RNA back to DNA), RNA replication and the existence of non-coding RNAs, have revealed the important exceptions to this linear framework, disputing the rigid context of the original dogma (203). Despite these deviations, the Central Dogma remains the mainstream of science research in molecular biology. It has guided decades of research but also expanded and revolutionised by range of '-omics' technologies (Figure 7), ushering in a systems level understanding of biological complexity and disease mechanisms, allowing comprehensive, untargeted screening and profiling of biomolecules within a set of related samples.

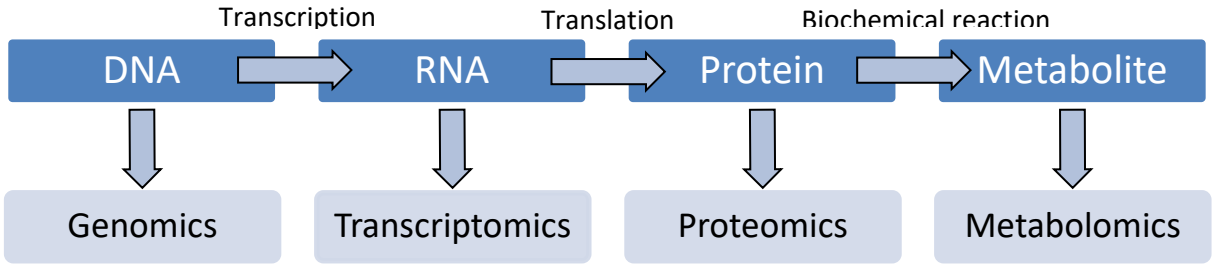


Figure 7: The central dogma of biology where information in genes flow in one direction, from DNA to proteins and metabolites which advanced the '-omics' technology.

The term biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" by the National Institutes of Health Biomarkers Definitions Working Group in 1998 (204). The World Health Organisation (WHO) subsequently defined a biomarker as "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction"

(205). Biomarkers can be used to diagnose, monitor, predict, prognosticate, assess safety, or assess response to medical treatment or drugs. When used in the context of frailty, it is challenging to define a 'frailty biomarker' as the pathophysiology of frailty is highly complex, interplays with other variables and the current definitions of frailty are still ambiguous.

As mentioned earlier, the repercussions of frailty extend from individual health, influencing a patient's health expectancy and QoL, to their family members, and simultaneously driving up healthcare cost and imposing additional strain on the healthcare system (206). Therefore, it is crucial that clinicians recognise and address frailty in all patients. In the context of emergency laparotomy, frailty status plays an important role in clinical decision-making within this "high stakes" circumstance. However, frailty assessment is often subjective, as it depends on the limited information gathered during the brief and constrained pre-operative assessment period. This highlights the need for an objective, measurable biomolecular marker of frailty.

Currently, the commonly investigated frailty biomarkers (207-209) include those associated with:

1. Inflammatory response-e.g., C-reactive protein (CRP], interleukin-6 (IL6), and tumour necrosis factor alpha (TNFα) (210-213)
2. Clinical parameters- eg., haemoglobin, glomerular filtration rate, and albumin (212, 214, 215)
3. Hormones- e.g., dehydroepiandrosterone [DHEA] sulphate, testosterone, insulin-like growth factor-1 (IGF1), and vitamin D (213-215)
4. Products of oxidative damage- eg., advanced glycation end products, protein carbonyls, oxidised low-density lipoproteins, and antioxidants (sirtuins and α-tocopherol) (211, 216)

Nonetheless, these biomarkers are not specific to frailty, as they can be affected by the systemic inflammatory process, ageing, and other co-morbidities (211).

1.6.1 Novel frailty biomarkers using metabolomic approach

The human metabolome consists of over 248,000 metabolites (https://hmdb.ca/metabolites) which have been quantified using mass spectrometry or nuclear magnetic resonance (NMR). Mass spectrometry is a technique of converting samples into gaseous ions by different ionisation processes to produce a mass spectrum based on the mass to charge ratio (m/z) and relative abundances of the ions (signal intensity). The gas phase ions produced, undergo molecular fragmentation in the mass spectrometer and a plot of ion abundance versus m/z is displayed as a mass spectrum. When a molecular ion of a compound is present on the mass spectrum, it will be illustrated at the highest value of m/z and the molecular mass of the compound will be provided. This information aids in obtaining the identity of the isotopes in the sample by comparing the findings with known masses or fragmentation patterns (217) (Figure 8).

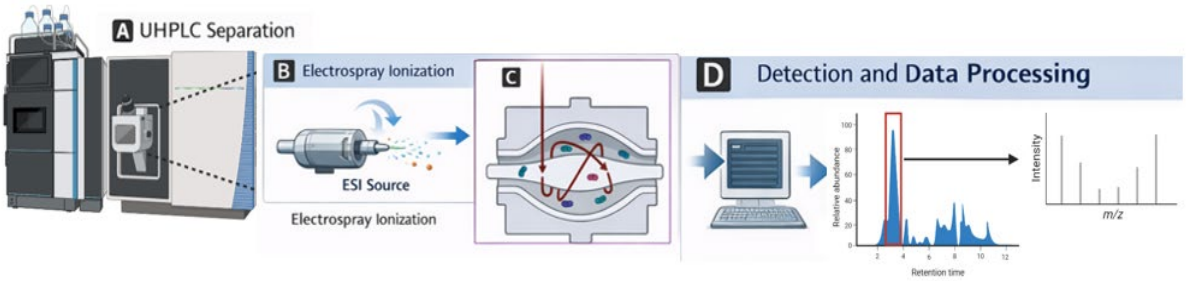


Figure 8: Illustration of ionisation and separation of metabolites in a UHPLC-MS. A: Autosampler separating metabolites prior to MS analysis. B: Electrospray ionisation (ESI) source with ion transfer capillary showing transmission of ions into the high vacuum region. C: Orbitrap analyser with red arrow depicting ion injection and trajectories of ion motion via centrifugal force around the central spindle electrode. The coloured dots represent separation of ions of different mass-to-charge ratio (m/z). D: Detection of current and generation of high-resolution mass spectra for qualitative and quantitative data analysis.

Currently, the ultra high-performance liquid chromatography-mass spectrometry (UHPLC-MS) is the most widely employed technology for clinical metabolomics analysis. It has been used as reliable platform to identify metabolic pathways in

frailty in the older adults (218). Metabolomics is the study of a set of low molecular weight compounds (under 1000 atomic mass units), which are the substrates and by-products of enzymatic reactions that directly affect the phenotype of the cell at a particular time and at a specific environmental condition, which can be translated up to tissue function and pathophysiology. Thus, metabolomics is a powerful approach to understand frailty at a molecular level especially when frailty is closely related with metabolic disorders (218). Recent metabolomics studies have shown strong associations between certain metabolites and frailty (218-222), where targeted and untargeted UHPLC-MS methods, used alongside Frailty Index (223) or frailty phenotype (222, 224, 225), have helped categorise the biomolecular signatures of frailty.

One of the largest metabolomic study in frailty in the UK using UHPLC-MS was performed by Rattray et al. (223). A longitudinal serum samples from 1191 older individuals from the English Longitudinal Study of Ageing (www.elsa-project.ac.uk) were chemically profiled and mapped onto associated Frailty Scores. Altered levels of 12 metabolites (including three tocotrienols (metabolites of vitamin E) and six carnitines) were found to differentiate between frail and non-frail patients. When mapping these metabolites onto the biomolecular pathway, they were dysregulated in the carnitine shuttle and antioxidant modulation which leads to decreased in energy production and in frail individuals, exhibited as symptoms of weakness, slowness, exhaustion and low activity. This evidence contributes to the growing body of data that supports modelling energy metabolism as the key to the development of frailty biomarkers.

The FRAILOMIC initiative, a European Union funded research programme between 2013-2018 (226) was established to identify biomarkers of frailty in the ageing population using different ‘omics’ technologies. They aimed to examine the genetic and proteomic markers, matched to serum and clinical biomarkers, to compare and predict the onset of frailty. This large-scale project consists of four phases; phase 1 focuses on identifying of potential frailty biomarkers while phase 2 serves as the

validation stage. In phase 1, Kochlik et al. (227) used metabolomic analysis to show that frail participants had significantly lower plasma levels of several carotenoids, including lutein + zeaxanthin, α-carotene, ß-carotene, lycopene, and ß-cryptoxanthin. These findings were subsequently validated in phase 2 by Henning et al. (228), who confirmed that low levels of specific carotenoids, particularly lutein + zeaxanthin, ß-carotene, and ß-cryptoxanthin, were strongly associated with frailty. Low carotenoid levels have been linked to elevated oxidative stress and inflammation, as carotenoids act as antioxidant and anti-inflammatory agents, scavenging reactive oxygen species that drive oxidative damage. High level of oxidative stress and inflammation have been associated to the development and progression of frailty (229, 230).

The participants in these studies (223, 227, 228) are from the community setting therefore replication and validation of this important initial finding in the surgical environment, as proposed in this research, would be a vital step forward in confirming these hitherto unseen biological aspects of frailty. We accept that the patient cohort is different (well community dwelling vs surgical pathology with inflammatory response), the results of this study would still allow us to understand frailty from the molecular level up to the level of patient outcomes.

1.7 Research in emergency laparotomy patients with frailty

In 1996, Prof. Horton questioned in *The Lancet* whether surgical research had a future, highlighting its perceived inadequacy at the time (231). Over the past 30 years, the quality of surgical research has improved drastically, with a notable increase in original studies and randomised controlled trials (232). However, research in emergency general surgery (EGS) remains an area of medicine that is still under-researched, despite this field encompassing a substantial portion of the NHS workload (233). The scarcity of high-quality research in EGS has resulted in limited evidence-based treatments and a lack of consensus and guidelines for best practices. In recent years, this gap has been increasingly acknowledged, leading to the production of several large multicentred studies focused on emergency surgery.

Examples include the National Audit of Small Bowel Obstruction (NASBO) (234), the DAMASCUS study (235), the ELF study (136) and the EPOCH trial (40). However, these studies have been observational or quality improvement projects rather than randomised controlled trials or prospective trials. The challenges of conducting trials prospectively in the EGS setting are significant. These include difficulties in identifying eligible patients, obtaining informed consent within tight time constraints, co-ordinating the intervention within a multidisciplinary team setting that is subjected to multiple rota changes and managing the clinical and emotional stress of acutely unwell patients (236). Despite these obstacles, successful trials in the emergency general surgical settings have been achieved. Notable examples include the CReST trial (237), the ROSSINI trial (238), the SUNRRISE trial (239), the LaCeS trial (laparoscopic versus open colorectal surgery in the acute setting) (240), and LaCeS2 trial (https://doi.org/10.1186/ISRCTN38815804, closed early due to poor recruitment). Additionally, there are several ongoing randomised controlled trials on emergency patients, such as TONIC trial (Timing Of Nutrition In emergenCy laparotomy), CLARITY (https://claritytrial.co.uk/), POP-I (Peri-op Iron and EPO Intervention Study, https://www.nottingham.ac.uk/POP-I/) and CAMELOT (https://doi.org/10.1186/ISRCTN15475290), aiming to address key unanswered questions in the emergency setting.

There has been a noticeable increase in publications focusing on frailty within the emergency surgery setting, reflecting growing interest in addressing this critical area of care. These developments highlight progress while emphasising the ongoing need for high-quality research to guide best practices in EGS. The rise of publications searched using the keywords ‘emergency general surgery’ and ‘frailty’ (Boolean search) is shown in Figure 9. The sharp rise in year 2021 coincides with the publication of large observational multicentre study by Parmar et al (136).

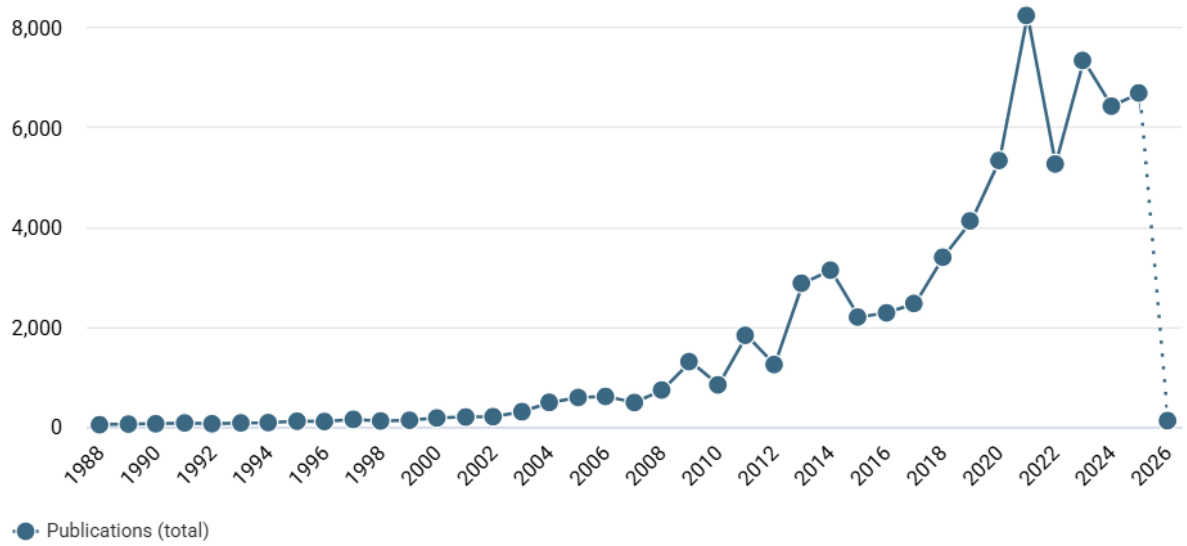


Figure 9: Timeline showing the number of publications (articles) from year 1988 to 2025 using keywords 'emergency general surgery' and 'frailty'. Source: https://app.dimensions.ai. © 2024 Digital Science and Research Solutions Inc. All rights reserved. Non-commercial redistribution / external re-use of this work is permitted subject to appropriate acknowledgement. This work is sourced from Dimensions® at www.dimensions.ai.

The Emergency General Surgical service is not standardised between hospitals and other hospital services. To improve patient outcomes and address challenges in delivering care in the emergency general surgical setting, a collaborative study (Emergency Surgery Delphi UK) between Scottish Surgical Research Group (SSRG), World Society of Emergency Surgery (WSES) and Association of Surgeons of Great Britain and Ireland (ASGBI) was conducted via a modified Delphi process. This process was to identify the highest priority areas in emergency surgery research (233). After three phases of prioritisation, 17 prioritised questions were agreed on from a total of 92 questions, posted by 108 stakeholders which included doctors, nurses, allied healthcare professionals, patients who had emergency general surgical procedure or were admitted as an emergency under the care of a general surgeon. Two of the top 5 priorities for emergency general surgery included the best predictors of adverse outcomes and mortality in the older patients presenting with emergency general surgery pathology; and the impact of emergency laparotomy on

QoL in frail patients. In this study, impact of frailty in emergency laparotomy will be addressed.

For the older patients that were identified as frail and deemed too high-risk for EmLap, the treatment options are limited to conservative management or palliation if the initial treatment fails. A multicentred audit, Perth Emergency Laparotomy Audit (PELA), examined outcomes for patients presenting with an acute abdomen. A convenience sampling of the database identified 8.1% of patients who met the criteria for EmLap but did not undergo laparotomy (NoLap) and the mortality rate of this NoLap group was 81% (241). These findings were corroborated by a prospective observational study of 252 patients, which included 21 NoLap patients with a 30-day mortality rate of 95% (242). Similarly, one of the largest single centred prospective cohort study conducted by McIlveen et al. reported a 30-day mortality rate of 63% among 100 (32%) NoLap patients (202) and another study conducted on data obtained from the Australia and New Zealand Emergency Laparotomy Audit-Quality Improvement (ANZELA-QI) found in-hospital mortality rate of 64.9% in the 188 NoLap group (243). The most recent multicentre cohort study on a 750 NoLap patient group also reported a high 30-day mortality rate of 71.3% and 90-day mortality rate of 79.1% (201). Across all three studies (202, 241, 242), bowel ischemia emerged as one of the predominant diagnoses for NoLap patients while Price et al. (201) and McIlveen et al. (202) found intestinal obstruction as the most common diagnosis within the NoLap group.

Despite high mortality in the NoLap group, some NoLap patients survived with conservative approaches such as antibiotics treatment. The NoLap group largely comprises individuals who are highly representative of advanced frailty with majority in the late 80s with significant functional dependence and limited physiological reserve. In such context, the decision not to proceed with EmLap is frequently aligned with the overall goal of care, given the high peri-operative risk and limited likelihood of meaningful recovery. Therefore, prioritising comfort, management of symptom control and holistic conservative management including

timely antibiotics, analgesia, and anticipatory care planning are essential. This approach recognises the underlying frailty of the NoLap population and focuses on maintaining dignity, minimising burden and optimising quality of life rather than pursuing invasive interventions with uncertain benefit.

Despite no gold standard tool currently exists for predicting post-EmLap outcomes in patients living with frailty in the acute setting, structured frailty measures have increasingly been incorporated into EmLap pathways. Frailty may develop or worsen sub-clinically and therefore remain undetected by pre-operative assessment alone. Molecular biomarkers offer a theoretically robust, objective and reproducible means of identifying and quantifying frailty and may enable precision or personalised interventions within the EmLap pathway; however, such approaches have not yet been established for emergency surgical patients, in contrast to elective cohort.

While the long- term objective of biomarker research is to improve the prediction of clinical outcomes, metabolomics also provides an opportunity to better understand the biological mechanisms underpinning frailty and impaired recovery. Accordingly, this thesis evaluates the performance of established clinical frailty measures while also exploring whether metabolic profiling can provide mechanistic insights into the biological processes associated with frailty and recovery following EmLap. This dual perspective recognises that biological characterisation represents an important step towards future biomarker development and clinical translation.

Importantly, this study was designed as a discovery-based exploratory investigation rather than a validation study aimed at replacing established frailty assessments. Given the complex and multidimensional nature of frailty, the potential role of metabolomic biomarkers is to complement existing clinical assessment by providing additional biological information regarding an individual's physiological vulnerability, recovery capacity and risk trajectory. Identification of a clinically applicable frailty biomarker will require subsequent validation in larger, multicentre cohorts.

Therefore, the current study represents an initial step towards understanding the molecular signatures associated with frailty and outcomes following EmLap.

At present, no clinically established frailty biomarkers exist and there are no published studies characterising frailty-related metabolites or their peri-operative dynamics in the EmLap population. Addressing these critical gaps forms the basis of the aims outlined in Chapter 2 and underpins the investigations presented throughout this thesis.

Chapter 2: Aims

The aims of this thesis are:

1. To evaluate and compare the predictive value of established pre-operative frailty markers (Rockwood clinical frailty scale, sarcopenia, and frailty biomarkers) to 30-day and 90-day post-operative mortality in patients undergoing emergency laparotomy (EmLap) (Chapter 4).

2. To assess the ability of established pre-operative frailty markers (Rockwood clinical frailty scale, sarcopenia, and frailty biomarkers) to predict 30-day post-operative morbidity after EmLap (Chapter 4).

3. To develop and optimise a robust sample preparation workflow for UHPLC-MS based metabolomic analysis of plasma samples from EmLap patients (Chapter 5- 7).

4. To identify metabolites associated with frailty using UHPLC-MS and examine the longitudinal changes of these novel frailty markers from the pre-EmLap to post-EmLap period (Chapter 8).

5. To investigate the impact of frailty on quality of life following EmLap, assessing the changes at 30- and 90-days post-operatively using EQ-5D-5L questionnaire (Chapter 9).

Chapter 3: Methodology

3.1 General methodology

This study was a prospective, single centred, observational cohort study conducted on consecutive patients ≥40 years old, who underwent emergency laparotomy (EmLap) according to the NELA inclusion/ exclusion criteria (Appendix 1.2) (244) in a busy NHS University Hospital with acute surgical services in the West of Scotland (Royal Alexandra Hospital, Paisley). The study was conducted in compliance with the ethical principles of the Research Governance Framework for Health and Community Care, Second Edition, 2006 and the principles of GCP and in accordance with all applicable legal and regulatory requirements. The protocol and related study documents were reviewed and approved by the Scotland A Research Ethics Committee (REC) IRAS 293392 on 27/05/2022. The REC and the relevant NHS management approval letter were received by the Investigators on 28/05/2022 and the recruitment commenced on 30/05/2022. The trial has been registered at ClinicalTrials.gov (NCT05416047). The risk management procedures were observed and attached in the appendix 1.3.

3.2 The Emergency General Surgery Department in Royal Alexandra Hospital

The Royal Alexandra Hospital (RAH), situated in Paisley, is part of NHS Greater Glasgow and Clyde health board. It serves a large catchment area of both urban and rural regions with a population of about 200,000, stretching from Oban to Argyll (https://www.nhsggc.scot/hospitals-services/) and includes Glasgow International Airport. There are 760 hospital beds and services provided include: trauma and emergency surgery, general medicine with acute receiving, medicine for the elderly, gynaecology, obstetrics and accident and emergency (A&E). To support these services there are seven Intensive Care Unit (ICU) beds and twelve High Dependency Unit (HDU) beds. Critical care physicians are the primary caregivers for the ICU beds with input from surgical or medical team whilst the HDU patients are cared by the parent surgical or medical team with input from the critical care team.

The General Surgery department consists of 10 consultants providing elective and emergency services. The consultant body covers the spectrum of the sub-specialties (colorectal, benign upper gastrointestinal and breast surgery) with 1 in 10 on-call.

The referral pathway for acute admissions include:

1. Referral from General Practice (GP)- these referrals are received by the Surgical Nurse Practitioners during day light hours and by the on-call team during out-of-hours.
2. Referral from Accident and Emergency department- these referrals are received by the on-call team.
3. Referral from other specialties (from within RAH or from other district/ rural hospitals)- these referrals are received by the on-call team.

The majority of the referrals are assessed in the six bedded Surgical Assessment Unit (SAU) ward during daylight hours or in the A&E department out-of-hours. The SAU serves as a combined assessment area for both acute general surgery and urology referrals. Prior to 2024, approximately 350 patients were reviewed in SAU per month. However, since 2024, this number has doubled to about 700. Patients requiring emergency laparotomy, identified in SAU, A&E or other specialty wards are routinely admitted to either HDU or ICU post-EmLap.

The Royal Alexandra Hospital is one of the 18 acute hospitals providing emergency general surgery data to the Emergency Laparoscopic and Laparotomy Scottish Audit (ELLSA). A dedicated ELLSA nurse specialist was employed in 2019 to collect EmLap data. The EmLap patients are identified by the ELLSA nurse specialist through theatre management software (Centricity™ OPERA), reviewed during their inpatient stay, and followed up for 30-days post-EmLap. This study was conducted at RAH as it was one of the top three hospital to contribute to ELLSA (172 laparotomies in 2022) (11), with a catchment area that would allow follow up for data collection, particularly for blood sample collection.

3.3 Recruitment

All patients who were assessed by the on-call General Surgical team (in A&E, SAU or other specialty wards) in RAH to require an EmLap were screened by the lead investigator (HJN) from 30th May 2022. The research team played no part in the EmLap decision-making. When deemed eligible for the study, the EmLap patients were approached, a Patient Information Sheet (PIS) (Appendix 1.8.1) was provided and written informed consent (Appendix 1.8.2) was obtained pre-operatively. For patients who lacked capacity to consent, their next of kin/ welfare attorney was approached and provided with participant information (Appendix 1.8.3) and written consent obtained pre-operatively, either in person or by telephone (Appendix 1.8.4). When the consent was obtained by telephone, an independent witness was present with the researcher during the consultation to ensure all queries were answered satisfactorily, and the consent form was discussed thoroughly (Appendix 1.8.5). The witness would then document their designation and signature on the declaration telephone consent form (Appendix 1.8.6) which was copied with one copy provided to the next of kin/ welfare attorney for them to return signed. The next of kin/ appointed power of attorney PIS was also delivered to them. When the study patients regained capacity, a 'participation after regaining capacity information sheet' was provided and consent was regained from the patient to allow continued post-operative participation (Appendix 1.8.7, appendix 1.8.8). All consent forms were copied to generate one copy for the participant; one copy scanned into participant's medical records and the original stored in the local site file. A letter was also sent to the participant's GP to inform them of the enrolment of their patient into this study (Appendix 1.8.9). All recruited patients had the right to withdraw from the study at any time. Withdrawal from the study did not result in exclusion of pre-existing data for that participant from analysis. These study documents have been approved by the NHS Research Ethics Committee (REC).

3.3.1 Sample size

Historically, Royal Alexandra Hospital (RAH) performed on average, approximately 100- 150 EmLap per year. Based on the data from UK Observational Emergency Laparotomy and Frailty (ELF) study and RAH ELLSA database in 2020, approximately

20% of older patients undergoing EmLap had frailty. This estimate was used to inform the anticipated proportion of participants with frailty in the present study. As no directly comparable study looking into frailty associated metabolites in patients undergoing emergency laparotomy was available at the time of this study design, the sample size calculation was based on the available clinical evidence and local data.

The sample size was calculated using 0.05 as two- tailed significance level (α), 0.05 as Type II error rate (β), corresponding to 95% statistical power, with an anticipated exposed-group proportion of 20% and an unexposed-group proportion of 80%. An effect size of 0.8 and a population standard deviation of 1.0 were specified, giving a standardised effect size of 0.8. Under these assumptions, the initial calculation indicated a total sample size of 130 participants, comprising 26 in the exposed group and 104 in the unexposed group. Allowing for an anticipated attrition rate of 20% to account for potential loss to follow-up, death or withdrawal from the study, the recruitment target was set at 150 participants. This target was intended to support the prospective investigation of clinical frailty measures, post-operative outcomes and exploratory metabolomic profiles within the available recruitment period and study resources.

Although the anticipated frailty prevalence informed the allocation of participants to the exposed and unexposed groups, the calculation was based on an assumed difference between group means rather than on a metabolomics-specific effect estimate derived from previous EmLap studies. Consequently, the assumed standardised effect size of 0.8 should not be interpreted as an established difference in metabolite abundance or as evidence that the study was adequately powered to detect associations between individual metabolites and mortality. The metabolomic analyses were therefore exploratory, aiming to characterise metabolic profiles associated with frailty and post-operative outcomes and to identify candidate biomarkers for further investigation.

3.4 Study design

For this study, recruited patients were approached at 5 time points for blood sampling and data collection to investigate peri-operative metabolic changes and assess the trajectory of frailty, recovery and quality of life post-EmLap. These 5 time points were selected to capture the baseline metabolic profile, the acute physiological response to surgery, and subsequent changes during hospitalisation and post-discharge recovery:

1. Pre-operative (within 24hrs before EmLap)- To establish the baseline metabolic profile before the surgical insult, providing a reference for comparison with subsequent post-operative metabolic changes. The CFS was the primary frailty measure because it is validated in EmLap patients, pragmatic assessment method and used in NELA and ELLSA. The other baseline measurements were markers covered the main frailty domains such as MUST (to assess nutritional status), AMT-4 (to assess cognition), CT (to assess muscle mass for sarcopenia), Charlson co-morbidity Index (to assess multi-morbidity), and medications (to assess polypharmacy). These were chosen as they were routinely collected without delaying emergency care.
2. Post-operative day 1- To characterise the early metabolic response to surgical stress and identify metabolic changes occurring during the immediate post-operative period.
3. Post-operative day 7/ discharge day, whichever occurred first- To assess metabolic changes during the early recovery phase and explore their relationship with the patient's clinical progress and recovery during hospitalisation.
4. Post-operative day 30- To evaluate ongoing metabolic changes and early recovery following discharge. This time point also enabled the assessment of 30-day morbidity and mortality which are the standard NELA outcomes, alongside health-related quality of life, providing an opportunity to examine the relationship between metabolic profiles and recovery from the acute surgical insult. EQ-5D-5L was used because it is a validated patient-reported measure with a UK value set. It was not collected from patients still in hospital at day 30.

5. Post-operative day 90- To assess longer-term metabolic changes and recovery, including the evolution of frailty status, health-related quality of life and survival. Comparison with earlier assessments enabled exploration of longitudinal metabolic trajectories and their potential associations with frailty progression and post-operative outcomes. The QoL questionnaire was not collected from patients still in hospital at day 90.

Figure 10 describes the workflow of the study.

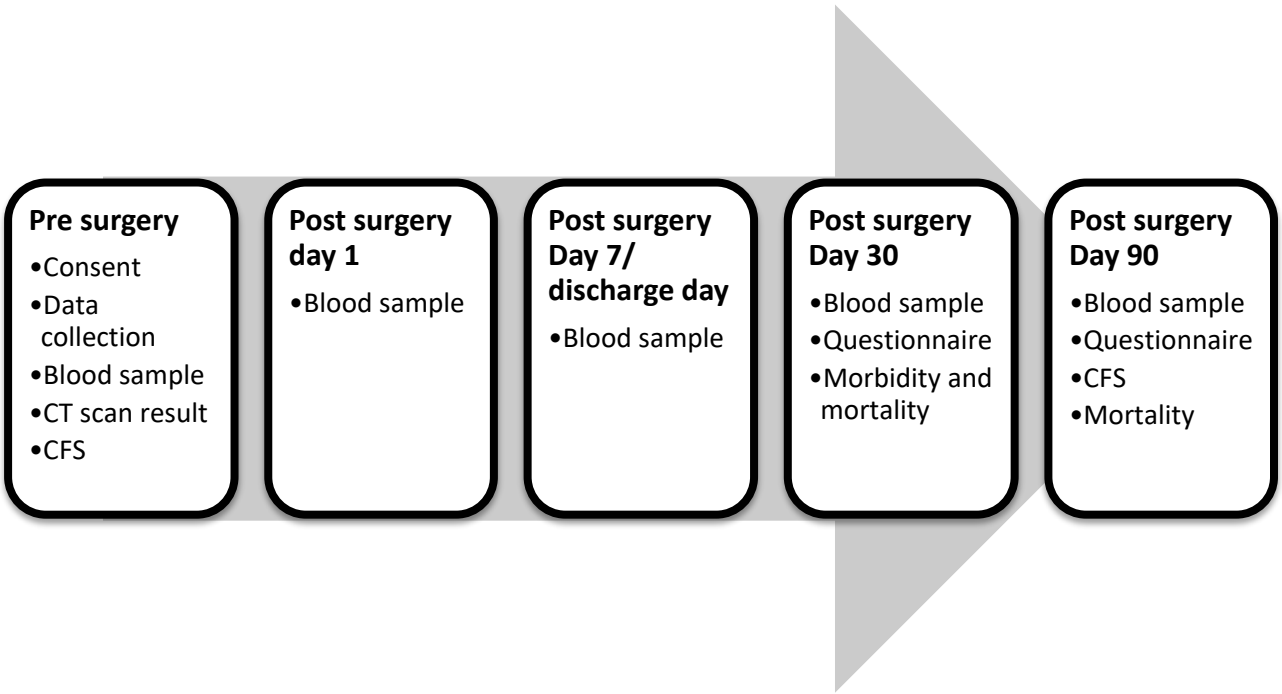


Figure 10: Workflow of the study (data and blood samples collection) from pre-operative time point to post emergency laparotomy day-90.

3.4.1 Data collection (peri-operative, frailty markers, and follow-up data)

Pre-operative data

The pre-operative collected data are shown in Table 10. Most of the information collected was obtained from the routinely collected data on each participant's admission documentation or electronic hospital record. Pre-operatively, the CFS was scored independently by the research team, while the ELLSA nurse specialist independently recorded her assessment of CFS score in the ELLSA database during

follow-up post-EmLap. To ensure reliability and consistency, the scores from the research team and ELLSA database were compared. In cases of discrepancy a third party, typically a General Surgical registrar from the parent team, was consulted to resolve the differences.

Table 10: Data collected at pre-operative timepoint.

Demographics
Age
Gender (Female or male)
Scottish Index Multiple Deprivation (SIMD) with score 1-2 as deprived, score 3-5 as not deprived (https://www.scotland.gov.uk/Topics/Statistics/)
Ethnic origin
Co-morbidities
Age-adjusted Charlson co-morbidity index (score) (Table 7)(148):
Mild ≤2
Moderate 3-4
Severe ≥5
Polypharmacy
≥5 regular medications, classification based on BNF classification
Lifestyle
Alcohol intake (safe alcohol intake ≤14units, excess >14 units)
Smoking status (currently, ex-smoker < 5-year, ex-smoker ≥5 years, never smoked)
Weight and height
Body Mass Index
Place of residence
Home and independent
Home with carers
Care home
Nursing home
Indication for and type of surgery
as per NELA criteria (Appendix 1.2)
Prognostic scores
NELA score (≥5% as high mortality risk)
ASA (American Society of Anaesthesiology) score (245)
ASA 1- Normal healthy patient
ASA 2- Patient with mild systemic disease
ASA 3- Patient with severe systemic disease
ASA 4- Patient with severe systemic disease that is a constant threat to life
ASA 5- Moribund patient not expected to survive without the operation
ASA 6- Declared brain dead patient whose organs are being removed for donor purposes
Frailty markers
Study blood sample
Sarcopenia on pre-operative CT abdomen pelvis
Malnutrition Universal Screening Tool (MUST) score (Score 0 low risk, score 1 medium risk, score ≥2 high-risk)
Abbreviated Mental Test 4 (AMT-4) score (score <4 cognition impaired)
Routine serum biochemistry and haematology: Hb, White cells, C-Reactive protein and Albumin.
Rockwood Clinical Frailty Scale (CFS; scores from 1 -9 as frailty increases with score of ≥5 as frail)

Follow-up data

The follow-up data collection was performed post-operatively on day-1, day-7 or discharge day, day-30 and day-90 (Table 11). The blood samples for the first 2 post-operative time points were obtained in the ward while the patients were still an inpatient. After discharge, all of the EmLap patients were invited back to a routine clinic in Royal Alexandra Hospital (RAH) outpatient department for 30-day post-operative follow-up. The collection of the blood samples was performed on that same day as well as conducting the EQ-5D-5L questionnaire to assess post-operative QoL. For the patients who were still an inpatient on day-30, the blood samples were collected with their routine blood tests and the EQ-5D-5L was not assessed. When the routine clinic follow-up dates did not coincide with the research follow-up dates, the participants were contacted 2 weeks prior to the follow-up date and invited to return to the RAH Surgical Assessment Unit. When the participant was unable to attend hospital for the 30-day and/or 90-day post-operative follow-up, consent was obtained by the research team member (HJ Ng) to collect the follow-up data (blood sample and questionnaire) at the participant's residence at an agreed time and date (as per ethical approval).

Table 11: Follow-up variables recorded on discharge day, day-30 and day-90 post-EmLap.

Discharge day
Place of residence
Total hospital stay including critical care stay
Day-30
Morbidity/ complications (according Clavien-Dindo classification Surgical Complications (14)
Mortality
EQ-5D-5L questionnaire
Day-90
Mortality
EQ-5D-5L questionnaire
Rockwood Clinical Frailty Scale

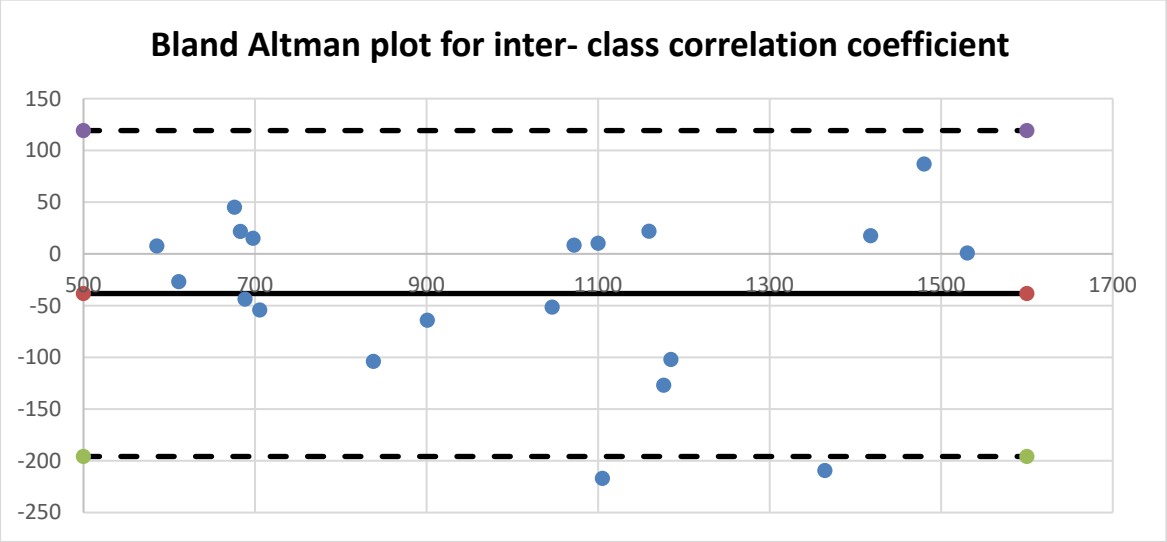
Day-30 morbidity and day-30 and day-90 mortality data were obtained from online medical records. This was assessed at 2 weeks prior to day-30 and -90 when participants were contacted to arrange collection of blood samples.

3.4.2 Sarcopenia measurement and validation

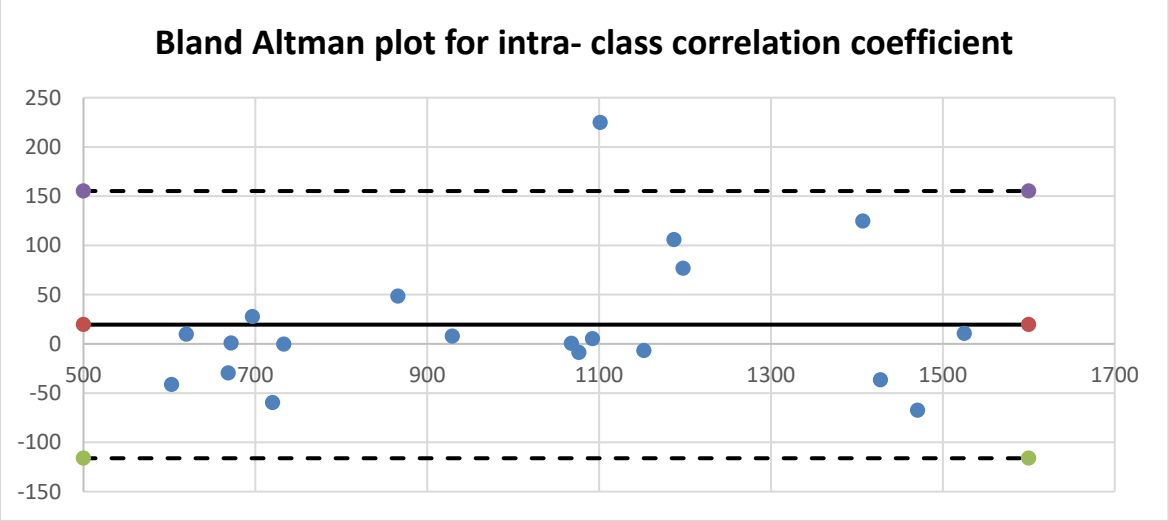
Participants of this study who underwent CT of abdomen and pelvis pre-operatively had their scans examined for sarcopenia. Based on NELA, more than 80% of EmLap patients underwent CT scanning pre-operatively. The measurement of total cross-sectional area of psoas muscle (total psoas area, TPA) at L3 vertebra level was performed using PACS (Picture Archiving and Communication System software). The area measured was standardised by measuring at the level where both L3 transverse processes were maximally in view on CT and using the freehand regions of interest (ROI) on PACS, the outline of the psoas muscle in the same CT slide was drawn. The result of cross-sectional area of each ROI which was calculated automatically was recorded. The TPA was standardised for patient height, using formula of TPA (mm^2)/ height (m^2) providing the total psoas index (TPI) (246). The TPI was stratified according to gender with sarcopenia being present in the lowest quartile (247-249). Patients who had post-operative scans were included and analysed for changes in sarcopenia, but no additional CT scans were performed as part of this research work.

Calculations of inter- and intra-class correlation coefficients (ICCC) were performed to ensure reliability and consistency of the sarcopenia measurement technique. A r^2 value of >0.8 indicates excellent correlation (250) and ICCC value close to 1 indicates high similarity and high reliability. The research department is experienced in this established measurement technique (246). The same assessment technique was applied to a total of 20 CT abdomen pelvis scans which were randomly selected. The CTs were anonymised and assessed by the original assessor twice (with 2 weeks break in between assessment) and again by another blinded trained research member. The r^2 value for the ICCC were excellent at 0.974 (inter-class) and 0.987 (intra-class) respectively. The Bland-Altman plots were used to plot the differences between the two different measurements for intra- and inter- class correlation coefficients, shown in Figure 11a and b. The Bland Altman plot for inter-class correlation showed moderate agreement with a small negative and a few outliers while the Bland Altman plot for intra-class correlation coefficient (Figure 11b) showed that the mean bias is small and most rating is within the line of agreement. Both figures indicated

consistency in measurement of the TPI by the same rater and between different raters.



11a



11b

Figure 11: The Bland Altman plots for inter (11a) and intra- (11b) correlation coefficient in measurement of sarcopenia. The blue dots are the data points representing the difference in total psoas index (TPI) measurements plotted against the mean between the 20 selected CT scans. The black solid line is the mean difference in TPI between the two raters and the black dashed line is the limits of agreement (mean difference ± 1.96 × standard deviation) indicating the range within which most differences in TPI measurements are expected to lie.

3.4.3 Classification of medications

The medications included in the data collection were categorised according to the British National Formulary (BNF) classification system. The BNF organises medications into therapeutic classes based on clinical use and pharmacological mechanism of action (https://bnf.nice.org.uk/about/how-to-use-bnf-publications-online/). It comprises 15 classes (in main chapters) and further divided into several subcategories. These chapters including examples of subcategories include:

- Chapter 1: Gastrointestinal system- proton pump inhibitors, laxatives
- Chapter 2: Cardiovascular system- antihypertensives, lipid- lowering drugs, antiplatelets
- Chapter 3: Respiratory system- bronchodilators, antihistamines
- Chapter 4: Nervous system- analgesics, antidepressants, antipsychotics, antiepileptic drugs
- Chapter 5: Infections- antibiotics, antivirals, antifungal
- Chapter 6: Endocrine system- diabetic drugs, corticosteroids, thyroid replacement hormone
- Chapter 7: Obstetrics, gynaecology, and urinary tract disorder- contraceptives
- Chapter 8: Malignant disease and immunosuppression- chemotherapy, immunosuppressants
- Chapter 9: Nutrition and blood- iron supplements, vitamins, anticoagulants
- Chapter 10: Musculoskeletal and joint diseases- NSAIDs, bisphosphonates
- Chapter 11: Eye- medications for glaucoma, anti- infective eye drops
- Chapter 12: Ear, nose and oropharynx- drugs for nasal congestions, ear infections
- Chapter 13: Skin- antifungal, emollients
- Chapter 14: Immunological products and vaccine- immunoglobulins, vaccines
- Chapter 15: Anaesthesia- drugs used during surgical procedures eg lignocaine, propofol

This method of classification is both comprehensive and promotes consistency, making the analysis of polypharmacy in this study more efficient and streamlined.

3.5 Collection and storage of plasma samples

Bloods were collected in green topped lithium heparin Vacutainers©, were labelled with study ID, centrifuged followed by the plasma aliquoted into a labelled 2mL Eppendorf tube. The residue samples beside plasma were discarded. The plasma samples were stored in the -40 °C freezer in RAH laboratory (Figure 12) before being transported in batches of 50 on dry ice to the -80 °C freezer in University of Strathclyde. To determine the optimal method of preparing the plasma samples prior to mass spectrometry analysis, optimisation method experiments were performed (Chapter 5).

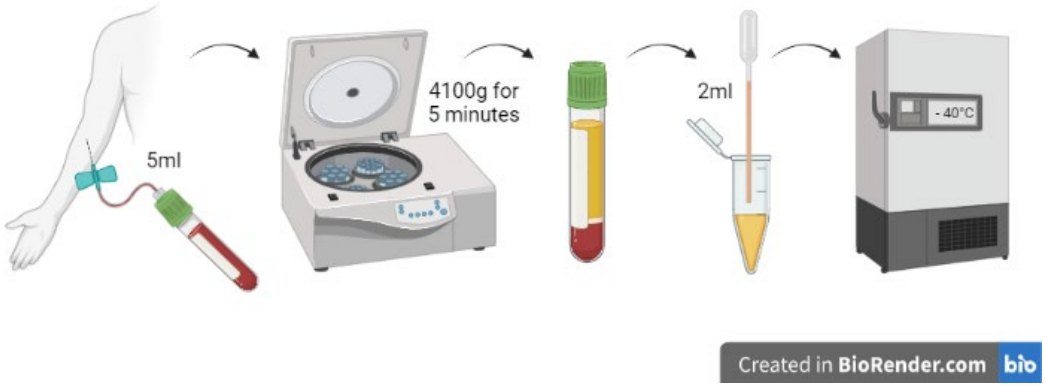


Figure 12: Schematic representation of blood sampling and plasma preparation process. 5ml of venous blood was collected from participants which was then centrifuged at 4100 x g for 5 minutes to separate plasma from cellular components. The upper plasma layer was then carefully aspirated, 2ml of plasma was transferred into an Eppendorf tube and stored at -40 °C for future analysis.

3.6 Data analysis

The statistical analysis was performed by HJN when analysing established clinical frailty markers, metabolomic data in pilot study, optimisation results and quality of life (in Chapter 4, Chapter 6, Chapter 7 and Chapter 9) while the bioinformatics statistics for metabolomic data (in Chapter 8) were performed by Prof. David Broadhurst (Professor of Biosystems Data Science, Edith Cowan University) using R Studio software V2024.4.2.764.

3.6.1 Statistics for clinical analysis

The collected pre-operative data were divided into groups of frail (CFS> 4) and non-frail (CFS ≤4) and compared using Mann-Whitney test for continuous or ordinal variables. Chi-square test or Fisher's exact test was performed on categorical variables and univariate analysis. The variables with outcome at p <0.05 on univariate analysis were entered into multivariable logistic regression models, with the aim of limiting the number of variables given the small number of events. Both unadjusted and adjusted results were reported. Because variable selection was data-driven rather than based on a causal model, adjusted analyses were considered exploratory. Sensitivity and specificity analyses were performed using the Receiver Operating Characteristic curve (ROC). Survival analysis using Kaplan-Meier survival curve was performed to compare 30- and 90-day survival between frail and non-frail group. Secondary outcomes were presented using descriptive analysis on time frame. The analysis was performed using IBM SPSS Statistics V29.0.1.0 (171).

3.6.2 EQ-5D-5L analysis

EQ-5D-5L questionnaire (Appendix 1.9) is a validated measurement tool which evaluates 5 dimensions of quality of life (QoL) including mobility, self-care, activity of daily living, pain/ discomfort and psychological status. This questionnaire was used to assess the participant's post-operative QoL. Descriptive summary statistics were performed for the 5 domains on the questionnaire and self-reported health status (EQ-VAS score). Utility scores were obtained by using the analysis tool (index value set calculator and analysis of observational descriptive systems data) provided by the EuroQol Office (https://euroqol.org/support/tools/analysis-tools/). Currently, there are no utility scores specific to patients who had emergency laparotomy however, value set for patients in UK from Devlin et al (251) have been used as reference. Variables in demographic data and EQ-5D-5L domains between day-30 and day-90 post-EmLap were compared using univariate analysis (ANOVA). EQ-5D-5L utility scores were compared between day-30 and day-90 post-EmLap using Wilcoxon Signed-Rank test and frail and non-frail groups were compared using Mann-Whitney U test. These analyses were unadjusted, as the small number of frail respondents precluded reliable multivariable modelling.

Chapter 4: Established clinical frailty markers results

All consecutive patients who underwent EmLap as per inclusion and exclusion criteria from 30/05/2022 to 13/04/2023 were approached and a total of 150 patients were recruited. The follow-up period was completed at 3 months post-EmLap (Figure 13).

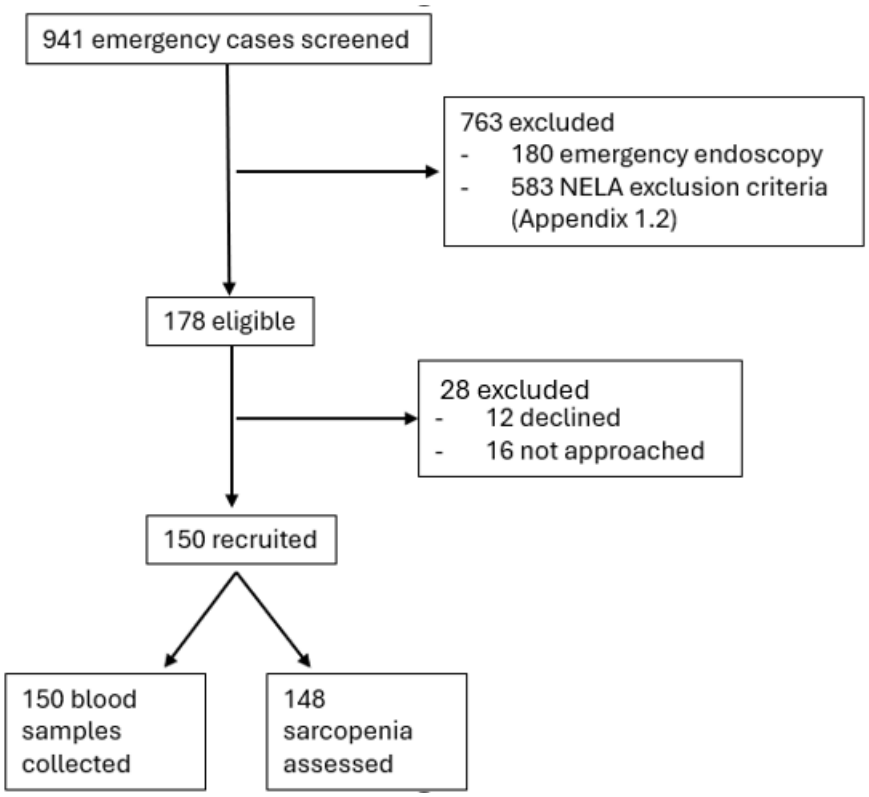


Figure 13: Flowchart showing the total number of emergency surgery screened, identified and recruited.

4.1 Patient demographics

The majority of the 150 emergency laparotomy patients were female (54.7%), Caucasian (99%) and from non-deprived population (SIMD score 3-5) (51.3%). The age range spanned from 40-91 years, with a median age of 69 years (Figure 14). The co-morbidities using the age-adjusted Charlson Co-morbidity Index (CCI), reported most patients having moderate score of 3-4 (40%) and 46.7% experiencing polypharmacy. A detailed summary of the demographic data is provided in Table 12.

Table 12: Demographic data on all 150 emergency laparotomy patients.

Demographics		N (%)	
Gender			
Male		68 (45.3%)	
Female		82 (54.7%)	
Ethnicity			
Caucasian		149 (99.3%)	
Asian		1 (0.7%)	
Age median (years)		69 years	
Scottish Index of Multiple Deprivation (Quintile)			
1	Deprived	37 (24.7%)	72 (48.0%)
2		35 (23.3%)	
3		26 (17.3%)	
4	Not deprived	27 (18.0%)	77 (51.3%)
5		24 (16.0%)	
N/A	N/A	1 (0.7%)	1 (0.7%)
Age-adjusted Charlson Co-morbidities Index			
None		11 (7.3%)	
Mild (score ≤2)		46 (30.7%)	
Moderate (score 3-4)		60 (40.0%)	
Severe (score ≥5)		33 (22.0%)	
Polypharmacy (≥5 daily medications)			
No		80 (53.3%)	
Yes		70 (46.7%)	
Smoking status			
Current smoker		31 (20.7%)	
Ex-smoker		50 (33.3%)	
Never smoked		69 (46.0%)	
Alcohol intake			
≤14 units/ week		38 (25.3%)	
>14 units/ week		14 (9.3%)	
Non-alcohol drinker		98 (65.3%)	
Body Mass Index			
Underweight (<18.5)		12 (8.0%)	
Normal (18.5-24.9)		57 (38.0%)	
Overweight (25-29.9)		39 (26.0%)	
Obese (≥30)		42 (28.0%)	
Residence prior to admission			
Home and independent		131 (87.3%)	
Home with carers		18 (12.0%)	
Care home		1 (0.7%)	

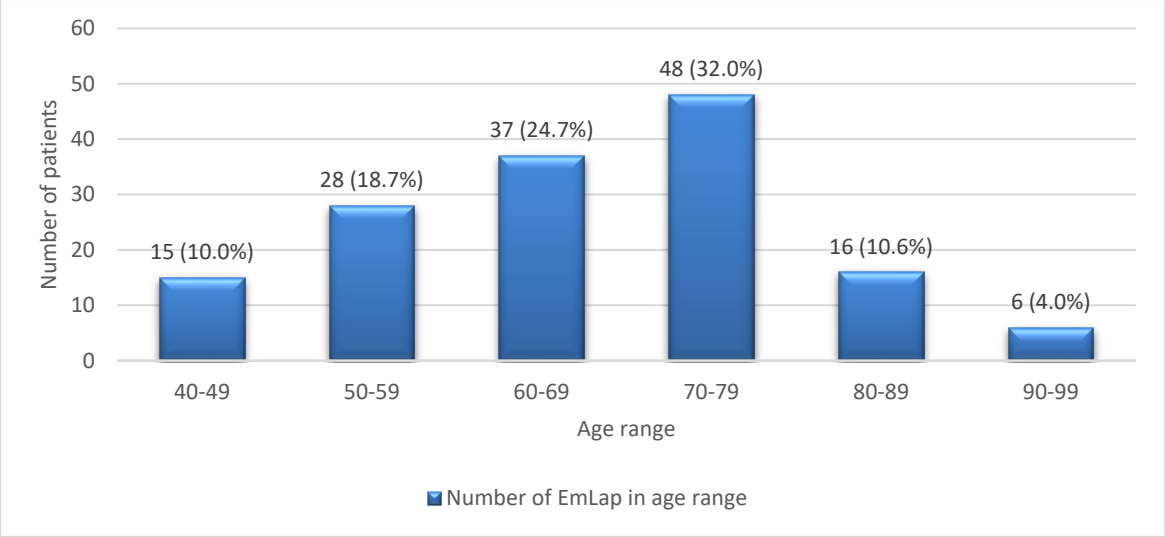
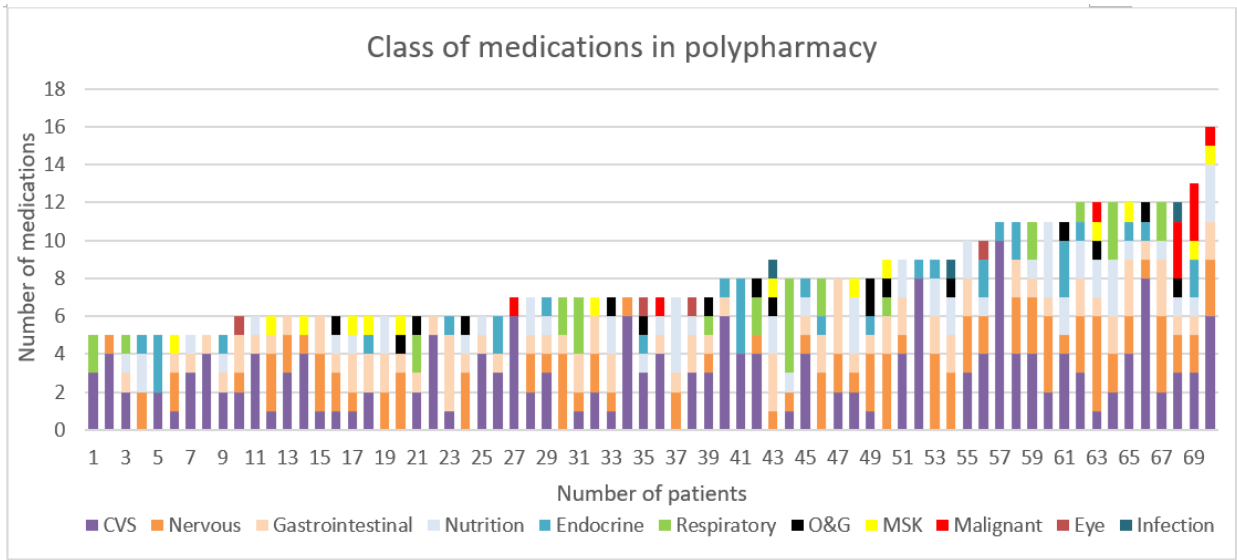


Figure 14: The age range of emergency laparotomy patients (EmLap).

Seventy-five of the 80 non-polypharmacy EmLap patients were not on any regular medications. Of the 70 EmLap patients with polypharmacy, the number of daily medications ranged from 5 to 16 medications. These medications were grouped according to the BNF class as shown in Figure 15. Majority of the medications are in the cardiovascular system (34.1%) followed by nervous system (19.2%) and gastrointestinal system (15.6%).



	CVS	Nervous	GI	Nutrition	Endocrine	Respiratory	O&G	MSK	Malignant	Eye	Infection
Total (%)	188 (34.1%)	106 (19.2%)	86 (15.6%)	61 (11.0%)	34 (6.2%)	29 (5.3%)	17 (3.1%)	14 (2.5%)	10 (1.8%)	4 (0.7%)	3 (0.5%)

Figure 15: Class of medications in patients with polypharmacy according to British National Formulary classification. x-axis represent the number of patients (total 70) while y-axis represent the number of different types of medications (according to the BNF class). The attached table showed the total number and percentages of different classes of medications.

4.2 Peri-operative data

The main indication for surgery in this cohort was small bowel obstruction (n= 66, 44%) (Table 13) and 22% (n= 33) were found to be caused by bowel cancer. 10.7% (n= 16) categorised as others included:

- 1 laparotomy for wound dehiscence
- 2 stoma complications
- 2 pancolitis
- 2 intra-abdominal collection
- 4 fistula complications
- 5 incarcerated hernia containing bowel.

The most common procedure performed in these 150 EmLap patients was adhesiolysis (22.7%). The other types of surgery performed (n= 12, 8%) included:

- 5 laparotomy and washout
- 1 laparotomy and thrombectomy
- 4 hernia repair with adhesiolysis
- 2 enterolithotomy for removal of gallstone

Majority of the EmLap patients were classified as ASA 3 (44.0%), NELA score >5% indicating high-risk for mortality (43.3%) and were admitted to high dependency unit (HDU) or intensive care unit (ICU) post-operatively (86%). Interestingly, 9 high-risk patients did not receive post-operative critical care stay (HDU/ ICU) and 7 EmLap patients required escalation from ward or HDU to ICU due to:

- 4 from HDU to ICU after undergoing second relaparotomy, remained intubated in ICU
- 2 from HDU to ICU due to pneumonia needing intubation
- 1 from ward care to HDU then ICU following a second relaparotomy from iatrogenic small bowel injury

The total length of hospital stay ranged from 2 days to 119 days. On discharge, a total of 22 patients (14.7%) had a change in discharge destination due to reduced independence. There were 13 (8.7%) patients who died within 30 days, and a further 7 who died between day 30 and 90 giving a 90-day mortality of 13.3%.

At 30 days post EmLap, 14 remained inpatients and 13 readmitted. The causes of readmission included:

- 1 with acute kidney injury
- 3 with abdominal collection needing drainage under radiological guidance
- 2 with wound infection requiring intravenous antibiotics
- 2 with laparotomy wound dehiscence where 1 needed to return to theatre

- 1 with high stoma output and acute kidney injury
- 1 with symptoms of bowel obstruction which was treated conservatively
- 1 with symptomatic incisional hernia
- 1 with enterocutaneous fistula
- 1 with unexplained abdominal pain where CT was normal.

The majority (54.7%) had no post-operative complications or did not meet Clavien-Dindo's morbidity classification within 30 days. Two patients (1.3%) were not assessed after discharge as they were not from the United Kingdom, but neither experienced post-operative complication during their inpatient stay. The peri-operative data are summarised in Table 13.

Table 13: The peri-operative data on all 150 emergency laparotomy patients.

Peri-operative data	**Total (%)**
Indication for surgery	
Small bowel obstruction	66 (44.0%)
Bowel perforation	27 (18.0%)
Large bowel obstruction	23 (15.3%)
Upper GI perforation	9 (6.0%)
Ischaemic bowel	5 (3.3%)
Anastomotic leak	4 (2.7%)
Others	16 (10.7%)
Type of emergency laparotomy	
Adhesiolysis	34 (22.7%)
Hartmann's procedure	32 (21.3%)
Small bowel resection	23 (15.3%)
Right hemicolectomy	17 (11.3%)
Omental patch repair	9 (6.0%)
Subtotal colectomy	9 (6.0%)
Resection of anastomosis	5 (3.3%)
Defunctioning stoma formation	5 (3.3%)
Bowel resection and refashioning of stoma	4 (2.7%)
Others	12 (8%)
ASA score	
1	0
2	50 (33.3%)
3	66 (44.0%)
4	33 (22.0%)
5	1 (0.7%)

NELA Score (%)	
Low risk (<5.0%)	85 (56.7%)
High risk (≥5.0%)	65 (43.3%)
5.0%- 9.9%	24 (36.9%)
10.0%- 24.9%	26 (40.0%)
25.0%- 49.9%	9 (13.8%)
≥50.0%	6 (9.2%)
Post-operative care	
Intensive care unit	15 (10.0%)
High dependency unit	114 (76.0%)
Ward	21 (14.0%)
NELA score and post-operative care	
NELA score 5.0-9.9%	24 (36.9%)
Critical care (ICU/ HDU)	21 (87.5%)
Ward	3 (12.5%)
NELA score ≥10.0%	41 (27.3%)
Critical care (HDU/ ICU)	37 (90.2%)
Ward	4 (9.8%)
Total length of hospital stay in days, median (range)	11 days (2-119 days)
Change in post-operative residence	22 (14.7%)
Home with extra care package	18 (81.8%)
Home with new care package	2 (9.1%)
Care home	2 (9.1%)
No change	113 (75.3%)
Not applicable	15 (10.0%)
Inpatient mortality	13 (86.7%)
Not in the United Kingdom	2 (13.3%)
30-days readmissions	
Yes	13 (8.7%)
No	110 (73.3%)
Still inpatient	14 (9.3%)
Not applicable	13 (8.7%)
30-days (Clavien-Dindo) morbidity	
No	83 (54.7%)
CD1	15 (10.0%)
CD2	25 (16.7%)
CD3a	2 (1.3%)
CD3b	9 (6.0%)
CD4a	2 (1.3%)
CD5	13 (8.7%)
N/A	2 (1.3%)
30-day mortality	13 (8.7%)
90-day mortality	20 (13.3%)

4.3 Established clinical frailty markers

Pre-EmLap

The EmLap patients were categorised as frail and non-frail using the Rockwood Clinical Frailty Scale (55). At admission, 15 patients (10.0%) were frail (CFS score ≥5). Of the 148 that had a CT scan, 23.3% were sarcopenic (19 female and 16 male) and 6 (4.0%) were frail and sarcopenic. The majority of the EmLap patients had no nutritional frailty (73.3%) or cognitive frailty (96.7%). The details of the established frailty markers are shown in Table 14.

Table 14: Established frailty markers pre-operatively.

Frailty markers	Number (%)
Rockwood Clinical Frailty Scale	
1 (non-frail)	0
2 (non-frail)	30 (20.0%)
3 (non-frail)	44 (29.3%)
4 (pre-frail)	61 (40.7%)
5 (frail)	12 (8.0%)
6 (frail)	3 (2.0%)
7-9 (frail)	0
Sarcopenia	35 (23.3%)
AMT-4 score (<4)	5 (3.3%)
MUST Score	
Low (Score 0)	110 (73.3%)
Medium (Score 1)	15 (10.0%)
High (Score ≥2)	25 (16.7%)

In the CFS >4 frail group (n= 15), the majority were female, older, living in the most deprived area (SIMD 1), had polypharmacy, were ex-smokers and lived at home independently. The detailed demographic data for frail and non-frail patients are shown in Table 15.

Table 15: Pre-EmLap demographic data for pre-EmLap frail and non-frail patients.

Demographics	Frail (n= 15, %)	Non-frail (n= 135, %)
Gender		
Male	1 (6.7%)	67 (49.5%)
Female	14 (93.3%)	68 (50.5%)
Ethnicity		
Caucasian	15 (100%)	134 (99.3%)
Asian	0	1 (0.7%)
Age median (years)	79 years	66 years
<65 years	3 (20.0%)	60 (44.5%)
≥65 years	12 (80.0%)	75 (55.5%)
Scottish Index of Multiple Deprivation (Quintile)		
1 (Deprived)	5 (33.3%)	32 (23.7%)
2 (Deprived)	2 (13.3%)	33 (24.4%)
3 (Not deprived)	1 (6.7%)	25 (18.5%)
4 (Not deprived)	3 (20.0%)	24 (17.8%)
5 (Not deprived)	4 (26.7%)	20 (14.8%)
N/A	0	1 (0.7%)
Age-adjusted Charlson Co-morbidities Index		
None	0	11 (8.1%)
Mild (score ≤2)	3 (20.0%)	43 (31.9%)
Moderate (score 3-4)	5 (33.3%)	55 (40.7%)
Severe (score ≥5)	7 (46.7%)	26 (19.3%)
Polypharmacy (≥5 daily medications)		
No	5 (33.3%)	75 (55.6%)
Yes	10 (66.7%)	60 (44.4%)
Smoking status		
Current smoker	1 (6.7%)	30 (22.2%)
Ex-smoker	8 (53.3%)	42 (31.1%)
Never smoked	6 (40.0%)	63 (46.7%)
Alcohol intake		
≤14 units/ week	3 (20.0%)	35 (25.9%)
>14 units/ week	1 (6.7%)	13 (9.6%)
Non-alcohol drinker	11 (73.3%)	87 (64.4%)
Body Mass Index		
Underweight (<18.5)	1 (6.7%)	11 (8.1%)
Normal (18.5-24.9)	10 (66.7%)	47 (34.8%)
Overweight (25-29.9)	3 (20.0%)	36 (26.7%)
Obese (≥30)	1 (6.7%)	41 (30.4%)
Residence prior to admission		
Home and independent	8 (53.3%)	123 (91.1%)
Home with carers	6 (40.0%)	12 (8.9%)
Care home	1 (0.7%)	0

Peri-EmLap and post-EmLap

Exploring the peri-operative data, majority of the patients living with frailty (CFS >4) had a small bowel obstruction requiring adhesiolysis, were ASA 3 or 4, had NELA score of ≥5% and were admitted to High Dependency Unit post-EmLap. They also had longer length of hospital stay and had a change in post-EmLap residence. The 30-day (13.3%) mortality rate was higher in the frail group. The detailed peri-operative data of frail and non-frail patients are shown in Table 16.

Table 16: Peri-operative data of pre-EmLap frail and non-frail patients.

Peri-operative data	Frail (%) (n= 15)	Non-frail (%) (n= 135)
Indication for surgery		
Small bowel obstruction	9 (60.0%)	57 (42.2%)
Bowel perforation	4 (26.7%)	23 (17.0%)
Large bowel obstruction	0	23 (17.0%)
Upper GI perforation	1 (6.7%)	8 (5.9%)
Ischaemic bowel	1 (6.7%)	4 (3.0%)
Anastomotic leak	0	4 (3.0%)
Others	0	16 (11.9%)
Type of emergency laparotomy		
Adhesiolysis	4 (26.7%)	30 (22.2%)
Hartmann's procedure	3 (20.0%)	29 (21.5%)
Small bowel resection	3 (20.0%)	20 (14.8%)
Right hemicolectomy	2 (13.3%)	15 (11.1%)
Omental patch repair	1 (6.7%)	8 (5.9%)
Subtotal colectomy	0	9 (6.7%)
Resection of anastomosis	0	5 (3.7%)
Defunctioning stoma formation	0	5 (3.7%)
Bowel resection and refashioning of stoma	0	4 (3.0%)
Others	1 (6.7%)	11 (8.1%)
ASA score		
1	0	0
2	0	50 (37.0%)
3	8 (53.3%)	58 (43.0%)
4	7 (46.7%)	26 (19.3%)
5	0	1 (0.7%)
NELA Score (%)		
Low risk (<5.0%)	3 (20.0%)	82 (60.7%)
High risk (≥5.0%)	12 (80.0%)	53 (39.3%)
5.0%- 9.9%	4 (33.3%)	20 (37.8%)
10.0%- 24.9%	7 (58.3%)	19 (35.8%)
25.0%- 49.9%	1 (8.3%)	8 (15.1%)
≥50.0%	0	6 (11.3%)

Post-operative care		
Intensive care unit	2 (13.3%)	13 (9.6%)
High dependency unit	11 (73.3%)	103 (76.3%)
Ward	2 (13.3%)	19 (14.1%)
NELA score and post-operative care		
NELA score 5.0-9.9%	4 (26.7%)	20 (14.8%)
Critical care (ICU/ HDU)	3 (75.0%)	18 (90.0%)
Ward	1 (25.0%)	2 (10.0%)
NELA score ≥10.0%	8 (53.3%)	33 (24.4%)
Critical care (HDU/ ICU)	7 (87.5%)	30 (90.9%)
Ward	1 (12.5%)	3 (9.1%)
Total length of hospital stay in days, median (range)	23 days (6- 119 days)	10 days (2- 112 days)
Change in post-operative residence	10 (66.7%)	12 (8.9%)
Home with extra care package	6 (60.0%)	12 (100%)
Home with new care package	2 (20.0%)	0
Care home	2 (20.0%)	0
No change	4 (26.7%)	109 (80.7%)
Not applicable	1 (6.7%)	14 (10.4%)
Inpatient mortality	1 (100%)	12 (85.7%)
Not in the United Kingdom	0	2 (14.3%)
30-days readmissions		
Yes	1 (6.7%)	12 (8.9%)
No	11 (73.3%)	99 (73.3%)
Still inpatient	1 (6.7%)	13 (9.6%)
Not applicable	2 (13.3%)	11 (8.1%)
30-days (Clavien-Dindo) morbidity		
No	8 (53.3%)	74 (54.8%)
CD1	2 (13.3%)	13 (9.6%)
CD2	3 (20.0%)	22 (16.3%)
CD3a	0	2 (1.5%)
CD3b	1 (6.7%)	8 (5.9%)
CD4a	0	2 (1.5%)
CD5	1 (6.7%)	12 (8.9%)
N/A	0	2 (1.5%)
30-day mortality	2 (13.3%)	11 (8.1%)
90-day mortality	2 (13.3%)	18 (13.3%)

Day-90 post-EmLap frailty

Due to mortality and declination of follow up, 119 patients were assessed at post-EmLap day-90. Of these, 25 (21.0%) were classified as frail with 12 (10.1%) developing new frailty; 10 were pre-frail pre-EmLap and 2 were non-frail pre-EmLap.

The changes in frailty status pre- and post-EmLap are illustrated in Figure 16 and detailed in Table 17.

Table 17: Rockwood Clinical Frailty Scale at pre-EmLap and day-90 post-EmLap.

Rockwood Clinical Frailty Scale	Pre-EmLap (%)	Post-EmLap day-90 (%)
1 (non-frail)	0	0
2 (non-frail)	30 (20.0%)	6 (5.0%)
3 (non-frail)	44 (29.3%)	47 (39.5%)
4 (pre-frail)	61 (40.7%)	41 (34.5%)
5 (frail)	12 (8.0%)	15 (12.6%)
6 (frail)	3 (2.0%)	9 (7.6%)
7 (frail)	0	0
8 (frail)	0	0
9 (frail)	0	1 (0.8%)



Figure 16: Distribution of frailty status including pre-frail status at pre-EmLap and post-EmLap day-90. Green indicates non-frail, orange indicates pre-frail, red indicates frail and grey indicates patients that were not assessed with causes.

For the 63 younger EmLap patients aged <65 years, 3 (4.8%) were deemed frail pre-EmLap. At post-EmLap, one had 30-day mortality and two developed new frailty. Majority of the new frailty post-EmLap were in the ≥65 years group (n= 10). The frailty changes according to <64 years and ≥65 years are shown in Figure 17.

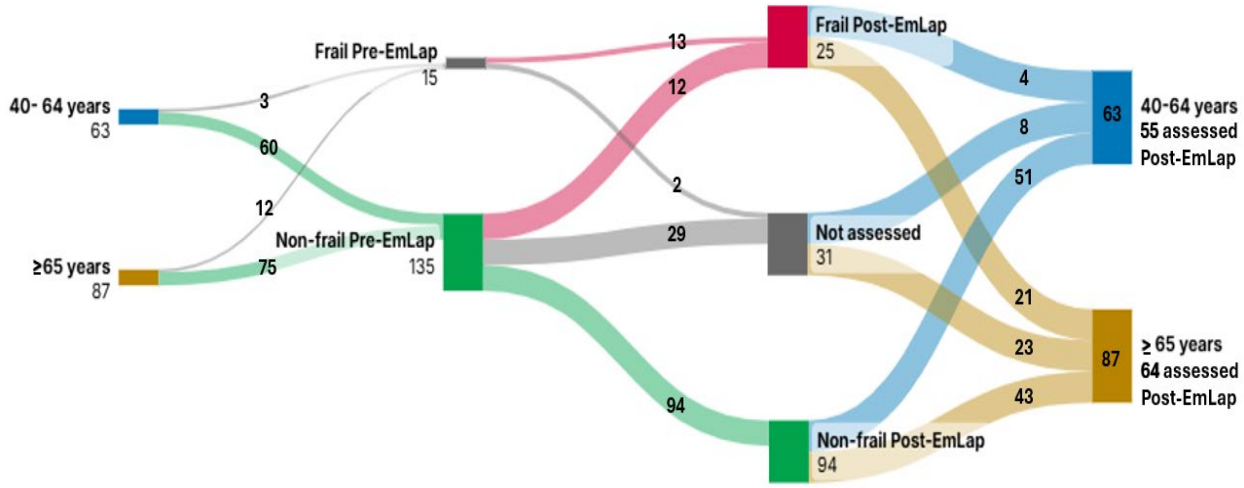


Figure 17: Sankey graph to illustrate the changes in frailty in the young (<65 years, blue) and older (≥65 years, brown) at pre- and post-EmLap day-90. The flow of non-frail pre- and post-EmLap day-90 is in green, frail pre-EmLap in grey, frail post-EmLap day-90 in red and not assessed in black.

4.3.1 Pre-EmLap clinical frailty markers with confounding factors

The association between Rockwood Clinical Frailty status and demographic as well as other established frailty markers were examined through univariate and multivariable analyses. In these models, frailty status is the dependent variable, describing correlates of frailty rather than estimating a confounder-adjusted effect of frailty.

At pre-EmLap, frailty was present in 10% (n=15). The univariate analysis using Chi-square test or Fisher's exact test demonstrated significant associations between pre-EmLap frailty status (CFS) and several factors, including being female (p= 0.002), pre-EmLap residence (p< 0.001), higher NELA risk score (p= 0.003), higher ASA score (p= 0.014) and impaired cognition (p= 0.023). When variables that were significant on univariate analysis were entered into a multivariable logistic regression model, none remained independently associated with pre-EmLap frailty. This finding should be considered within the context of the study's relatively small proportion of

patients living with frailty within the cohort and the close interrelationship between markers of clinical vulnerability, which may have reduced the statistical power and introduced collinearity between variables. Rather than suggesting that frailty lacks predictive value, these results suggest that pre-EmLap frailty is closely intertwined with multiple physiological and peri-operative risk factors, making it difficult to isolate single independent associations within this dataset. Overall, the findings remain consistent with the broader literature demonstrating the prognostic importance of frailty, while highlighting the methodological constraints of this specific cohort. Table 18 provides a detailed comparison of univariate and multivariable analyses for frailty pre-EmLap, incorporating key demographic and clinical variables.

Table 18: Univariate and multivariable analysis comparing pre-EmLap frailty status with demographic variables were performed. Multivariable analysis was not performed when univariate analysis was insignificant. Model limited by small event count.

Characteristics	Frailty at pre-EmLap (n= 150)		Univariate analysis p-value	Multivariable analysis p-value
	Frail (n= 15)	Non-frail (n= 135)		
Gender			**0.002**	0.113
Male	1	67		
Female	14	68		
Ethnicity			0.738	
Caucasian	15	134		
Non- Caucasian	0	1		
Deprivation			0.937	
Yes	7	65		
No	8	69		
N/A	0	1		
Age-adjusted CCI			0.084	
None	0	11		
Mild	3	43		
Moderate	5	55		
Severe	7	26		
Polypharmacy			0.102	
Yes	10	60		
No	5	75		

Safe alcohol			0.421	
Yes	4	34		
No	11	87		
Excess	0	14		
Smoking			0.156	
Yes	1	30		
No	6	63		
Ex	8	42		
Body Mass Index			0.087	
<18.5	1	11		
18.5-24.9	10	47		
25-29.9	3	36		
>30	1	41		
Pre-EmLap residence			**<0.001**	0.556
Home	8	123		
Home with carer	6	12		
Care home	1	0		
NELA score			**0.003**	0.804
High risk	12	53		
Low risk	3	82		
ASA score			**0.014**	0.992
1	0	0		
2	0	50		
3	8	58		
4	7	26		
5	0	1		
30-days readmission			0.891	
Yes	1	12		
No	11	99		
Not applicable	2	11		
Remained inpatient	1	13		
30-days morbidity			0.741	
Yes	7	57		
No	8	78		
Sarcopenia			0.256	
Yes	6	29		
No	9	104		
MUST score			0.089	
Low risk (Score 0)	7	104		
Medium risk (Score 1)	3	12		
High risk (Score ≥2)	5	19		
AMT (<4)			**0.023**	0.903
Yes	2	3		
No	13	132		

Pre-EmLap frailty was significantly associated with post-EmLap outcomes (Table 19). On univariate analysis, patients living with frailty were less likely to be discharged home directly and more likely to require new or increased package of care or care home placement after EmLap ($p<0.001$). However, this association did not persist in the multivariable analysis ($p= 0.896$), which suggest that discharge outcomes are influenced by multiple interrelated factors and frailty alone was not an independent predictor within this cohort. Patients living with frailty were also found to have fewer days alive outside hospital at day-30 post-EmLap, with a high proportion remaining inpatient or had died. This association did not reach statistical significance ($p= 0.072$) therefore multivariable analysis was not performed. In contrast, pre-EmLap frailty was strongly associated with frailty at 90-day post-EmLap. This association persisted after multivariable analysis (OR 2.28, 95% CI 1.23-4.2; $p= 0.009$), indicating that patients who were frail prior to surgery were more than twice as likely to remain frail at 90-days. While these finding may appear intuitive, given that frailty reflects reduced physiological reserve and vulnerability to stressors, it nevertheless quantifies that degree to which baseline frailty status tracks over time following EmLap. This result also support that frailty is not a transient peri-operative state but a persistent vulnerability. These findings therefore reinforce the importance of recognising pre-operative frailty, as it meaningfully informs longer-term functional trajectory after EmLap.

Table 19: Univariate and multivariable analysis comparing pre-EmLap frailty status with post-EmLap outcomes. Multivariable analysis was not performed when univariate analysis was insignificant.

Characteristics	Frailty at pre-EmLap (n= 150)		Univariate analysis p-value	Multivariable analysis p-value
	Frail (n= 15)	Non-frail (n= 135)		
Post-EmLap residence			**<0.001**	0.896
Home	3	111		
Home with increased package of care	6	12		
Home with new package of care	2	0		
Care home	3	0		
Not applicable	1	12		
Days alive outside hospital			0.072	
Inpatient at day-30 or mortality	4	29		
<10 days	2	7		
10-20 days	7	36		
>20 days	2	63		
Frailty post EmLap day-90			**<0.001**	**0.009**
Frail	13	12		
Non-frail	0	94		
Not applicable	2	29		

4.3.2 Post-EmLap day-90 frailty analysis

A total of 119 patients were included in the analysis of frailty status at 90 days post-EmLap, of which 25 (21.0%) were classified as frail. Given the dynamic nature of frailty and its potential to change following EmLap, frailty status at day-90 post-EmLap was explored in relation to baseline demographic and clinical characteristics with the aim of identifying trends and generating hypotheses, instead of establishing independent predictors.

On univariate analysis using Chi-square test or Fisher's exact test, frailty post-EmLap day-90 was significantly association with being female (p= 0.02), higher age-adjusted Charlson Co-morbidity Index (CCI) (p= 0.019), lower BMI (p= 0.006), living non-independently at home (p<0.01), higher NELA risk (p<0.01), higher ASA score

(p<0.01), impaired cognition (p<0.01) and post-EmLap residence (p<0.01). However, none of these variables remained significant in the multivariable analysis. Associations between post-EmLap frailty day-90 and demographic, clinical and peri-operative variables are detailed in Table 20.

Table 20: Univariate and multivariable analysis comparing demographic variables with frailty status at post-EmLap day-90. Multivariable analysis was not performed when univariate analysis was insignificant.

Characteristics	Frailty post-EmLap (n= 119)		Univariate analysis p-value	Multivariable analysis p-value
	Frail (n= 25)	Non-frail (n=94)		
Gender			**0.020**	0.140
Male	6	47		
Female	19	47		
Ethnicity			N/A	
Caucasian	25	94		
Non- Caucasian	0	0		
Deprivation			0.859	
Yes	12	47		
No	13	47		
Age-adjusted CCI			**0.019**	0.727
None	0	10		
Mild	5	35		
Moderate	10	34		
Severe	10	15		
Polypharmacy			0.145	
Yes	15	41		
No	10	53		
Safe alcohol			0.132	
Yes	5	24		
No	20	59		
Excess	0	11		
Smoking			0.486	
Yes	4	20		
No	14	40		
Ex	7	34		
Body Mass Index			**0.006**	0.413
<18.5	1	9		
18.5-24.9	17	28		
25-29.9	4	24		
>30	3	33		

Pre-EmLap residence			**<0.001**	1.000
Home	15	88		
Home with carer	9	6		
Care home	1	0		
NELA score			**<0.001**	0.057
High risk	20	26		
Low risk	5	68		
ASA score			**<0.001**	0.272
1	0	0		
2	4	40		
3	9	44		
4	12	9		
5	0	1		
30-days readmission			0.406	
Yes	2	10		
No	18	76		
Not applicable	1	1		
Remained inpatient	4	7		
30-days morbidity			0.123	
Yes	13	33		
No	12	61		
Sarcopenia			0.375	
Yes	7	16		
No	18	76		
N/A	0	2		
MUST score			0.152	
Low risk (Score 0)	15	74		
Medium risk (Score 1)	4	9		
High risk (Score ≥2)	6	11		
AMT (<4)			**<0.001**	0.999
Yes	3	0		
No	22	94		
Post-EmLap residence			**<0.001**	1.000
Home	11	87		
Home with increased package of care	9	7		
Home with new package of care	2	0		
Care home	2	0		
Not applicable	1	0		
Days alive outside hospital			0.083	
Inpatient at day-90 or mortality	2	15		
<10 days	0	2		
11-20 days	0	0		
21-30 days	0	0		
31-40 days	2	0		
41-50 days	2	1		
51-60 days	2	5		

61-70 days 71-80 days 81-90 days	4 9 4	3 29 52		

While univariate analysis identified several potential risk factors for frailty both before and after EmLap, none of these factors remained significant in the multivariable model. This suggests that frailty may be influenced by a complex interplay of multiple factors, which are not fully captured in this analysis due to small sample size.

4.4 Association with 30-day mortality

4.4.1 Association between peri-operative results and 30-day mortality

Within 30-days post-EmLap, there were 13 deaths (8.7%) with 12 occurring as an inpatient. One patient living with frailty was discharged back to care home post-EmLap where she came from pre-admission, but died within the 30-day outcome measure, surviving only 5 days in a care home. The demographic and peri-operative data were analysed using univariate analysis to assess associations with 30-day mortality. Deprivation ($p<0.001$) was significantly associated with 30-day mortality with deaths distributed across all quintiles but occurred more frequently in quintiles 1, 2, and 4. Operative risk assessed via NELA score ($p=0.004$) was significantly associated with 30-day mortality, with majority of deaths occurring in the high-risk group. In addition, all patients who died within 30 days experienced post-EmLap morbidity, resulting in a significant association on univariate analysis ($p<0.001$).

Variables demonstrating statistical significance on univariate were entered into multivariable logistic regression model. After adjustment, none remained independently associated with 30-day mortality. The results are summarised in Table 21.

Table 21: The association between 30-day mortality with demographic factors and peri-operative outcomes. Multivariable analysis was not performed when univariate analysis was insignificant.

Data	30-day mortality (%) (n=13)	Univariate analysis p-value	Multivariable analysis p-value
Scottish Index of Multiple Deprivation (Quintile)		**< 0.001**	0.600
1	4 (30.8%)		
2	3 (23.1%)		
3	1 (7.7%)		
4	4 (30.8%)		
5	1 (7.7%)		
Age-adjusted CCI		0.369	
Mild (score ≤2)	1 (7.7%)		
Moderate (score 3-4)	8 (61.5%)		
Severe (score ≥5)	4 (30.8%)		
Polypharmacy		0.104	
Yes	9 (69.2%)		
No	4 (30.7%)		
ASA score		0.123	
2	1 (7.7%)		
3	5 (38.5%)		
4	7 (53.8%)		
Indication for surgery		0.353	
Small bowel obstruction	3 (23.1%)		
Bowel perforation	4 (30.8%)		
Large bowel obstruction	3 (23.1%)		
Ischaemic bowel	2 (15.4%)		
Others	1 (7.7%)		
NELA score		**0.004**	0.103
Low risk	2 (15.4%)		
High risk	11 (84.6%)		
30-day morbidity		**<0.001**	0.994
Yes	13 (100%)		
No	0		

4.4.2 Association between established frailty markers and 30-day mortality

The relationship between established frailty markers and 30-day mortality is presented in Table 22. On univariate analysis, only cognitive impairment was significantly associated with 30-day mortality (p= 0.040). Patients with impaired

cognition accounted for 15.4% of the deaths. When included in multivariable analysis, cognitive impairment did not remain independently associated with 30-day mortality (p= 0.996).

Table 22: The association between 30-day mortality and frailty markers. Multivariable analysis was not performed when univariate analysis was insignificant.

Data	30-day mortality (%) (n= 13)	Univariate analysis p-value	Multivariable analysis p-value
Rockwood Clinical Frailty Scale		0.383	
1-2 (non-frail)	0		
3 (non-frail)	3 (23.1%)		
4 (non-frail)	8 (61.5%)		
5 (frail)	1 (7.7%)		
6 (frail)	1 (7.7%)		
7-9 (frail)	0		
Sarcopenia		0.873	
Yes	4 (30.8%)		
No	9 (69.2%)		
AMT <4		**0.040**	0.996
Yes	2 (15.4%)		
No	11 (84.6%)		
MUST		0.393	
Low (Score 0)	9 (69.2%)		
Medium (Score 1-2)	0		
High (Score ≥2)	4 (30.8%)		

Survival analysis using Kaplan-Meier survival curve was performed to compare 30-day survival between frail and non-frail group pre-EmLap (Figure 18). The frail patients had higher 30-day mortality, however the log rank test (p= 0.485) suggested no statistically significant difference in survival between the groups due to small sample size and low number of frail patients.

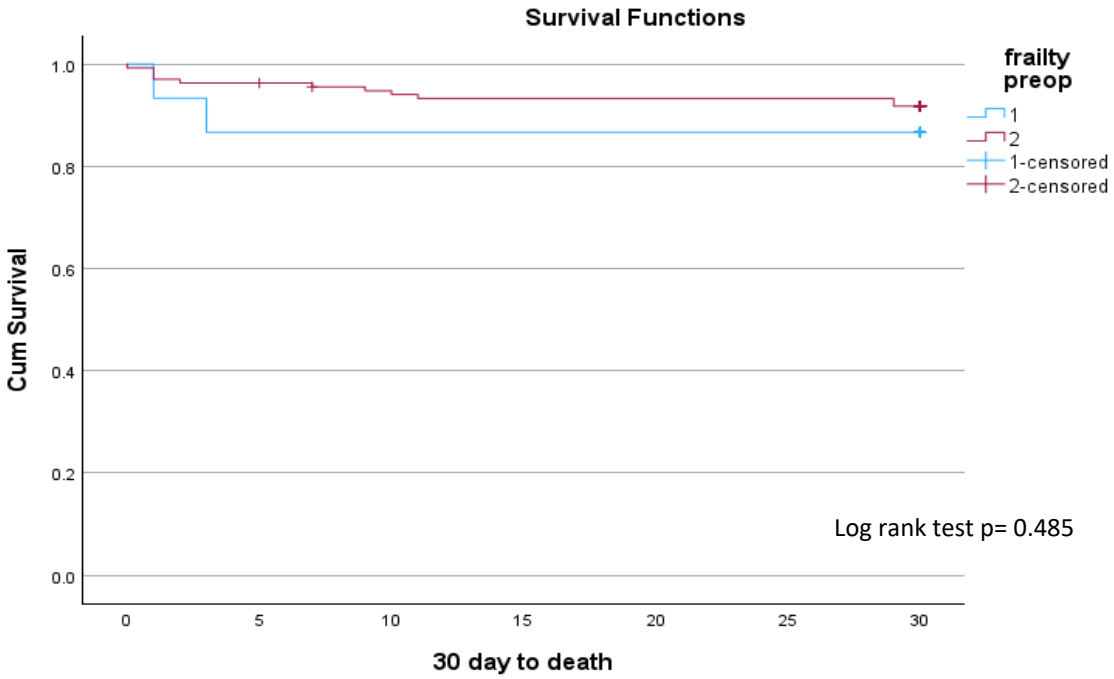


Figure 18: The Kaplan-Meier comparing 30-day survival with pre-EmLap frailty status (1 (blue line) as frail and 2 (red line) as non-frail) and censored as patients who were alive at the last follow-up.

4.5 Association with 90-day mortality

4.5.1 Association between peri-operative outcomes and 90-day mortality

There were 20 (13.3%) mortalities within 90-days post-EmLap, including 13 deaths within the first 30 days post-EmLap. Of these, 12 were inpatient mortalities, and 4 remained inpatients at day-90 post-EmLap. Associations between demographic data and peri-operative outcomes and 90-day mortality are presented in Table 23.

On univariate analysis, deprivation demonstrated a significant association with mortality ($p< 0.001$), with deaths distributed across all quintiles but occurring more frequently in quintiles 1 and 4. Higher operative risk was also associated with increased mortality, with significant associations observed for ASA score ($p= 0.013$) and NELA score ($p <0.001$). Post-EmLap variables were also strongly associated with 90-day mortality, including 30-day morbidity ($p<0.001$) and 30-day readmissions ($p <0.001$).

Variables that were statistically significant on univariate analysis were entered into a multivariable logistic regression model. Following adjustment, only 30-day readmission remained associated with 90-day mortality (p= 0.007). As this result was based on 20 mortalities, the result should be interpreted cautiously.

Table 23: The association between 90-day mortality with demographic variables and peri-operative outcomes.

Characteristics	90-day mortality (%) (n= 20)	Univariate analysis p-value	Multivariable analysis p-value
Scottish Index of Multiple Deprivation (Quintile)		**< 0.001**	0.504
1	6 (30.0%)		
2	4 (20.0%)		
3	1 (5.0%)		
4	7 (35.0%)		
5	2 (10.0%)		
Age-adjusted CCI		0.140	
Mild (score ≤2)	2 (10.0%)		
Moderate (score 3-4)	10 (50.0%)		
Severe (score ≥5)	8 (40.0%)		
Polypharmacy (>5 daily medications)		0.195	
Yes	12 (60.0%)		
No	8 (40.0%)		
ASA score		**0.013**	0.539
2	2 (10.0%)		
3	7 (35.0%)		
4	11 (55.0%)		
Indication for surgery		0.401	
Small bowel obstruction	5 (7.6%)		
Bowel perforation	6 (22.2%)		
Large bowel obstruction	5 (21.7%)		
Ischemic bowel	2 (40.0%)		
Others	2 (6.9%)		
NELA score		**< 0.001**	0.124
Low risk	3 (15.0%)		
High risk	17 (85.0%)		
30-day morbidity		**< 0.001**	0.621
Yes	17 (85.0%)		
No	3 (15.0%)		
30-day readmissions		**< 0.001**	**0.007**
Yes	1 (5.0%)		
No	6 (30.0%)		
Not applicable	11 (55.0%)		
Remained inpatient	2 (10.0%)		

4.5.2 Association between established frailty markers and 90-day mortality

The association between established frailty markers and 90-day mortality is summarised in Table 24. On univariate analysis, no statistically significant associations were observed between 90-day mortality and all the established frailty markers. As none demonstrated significant associations on univariate testing, multivariable analysis was not performed.

Table 24: The association between 90-day mortality and established frailty markers.

Frailty markers	90-day mortality (%) (n= 20)	Univariate analysis p-value
Rockwood Clinical Frailty Scale		0.144
1 (non-frail)	0	
2 (non-frail)	1 (5.0%)	
3 (non-frail)	3 (15.0%)	
4 (non-frail)	14 (70.0%)	
5 (frail)	1 (5.0%)	
6 (frail)	1 (5.0%)	
7-9 (frail)	0	
Sarcopenia		0.151
Yes	9 (45.0%)	
No	11 (55.0%)	
AMT <4		0.200
Yes	2 (10.0%)	
No	18 (90.0%)	
MUST score		0.209
Low (Score 0)	14 (70.0%)	
Medium (Score 1)	0	
High (Score ≥2)	6 (30.0%)	

Survival analysis using Kaplan-Meier survival curve was performed to compare 90-day survival between frail and non-frail group post-EmLap (Figure 19). The log rank test (p= 0.925) suggested that there is no statistically significant survival difference between the groups.

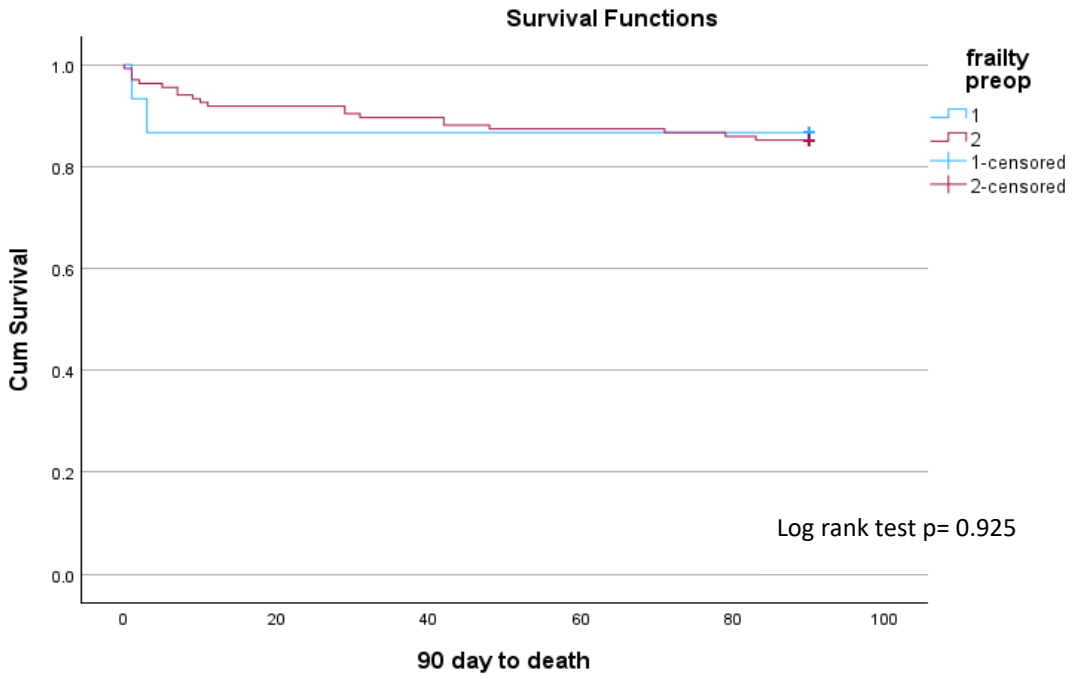


Figure 19: The Kaplan-Meier comparing 90-day survival with frailty status (1 (blue line) as frail and 2 (red line) as non-frail) and censored as patients who were alive at the last follow-up.

The results showed that 30-day mortality was primarily associated with markers of operative risk and early post-EmLap complications. Cognitive impairment demonstrated an association with 30-day mortality on univariate analysis but did not persist after multivariable adjustment. At 90-day post-EmLap, mortality was similarly associated with peri-operative risk and early post-operative outcomes. Importantly, only 30-day readmission remained independently associated with 90-day mortality following multivariable analysis, highlighting the prognostic importance of early post-operative deterioration and healthcare utilisation. Across both time points, established frailty markers were not independently associated with mortality. These findings suggest that mortality following EmLap may be more strongly driven by operative risk and early post-EmLap complications. However, given the small number of patients living with frailty and the limited number of deaths observed, these results should be interpreted cautiously. It may also raise the possibility that currently used frailty markers, while validated in broader surgical and medical populations, are not sufficiently sensitive to capture risk in the EmLap

population, where acute physiological insult and surgical pathology may outweigh baseline vulnerability measures.

4.6 Discussion

This study evaluated the predictive value of established pre-operative frailty markers for mortality and morbidity following emergency laparotomy. While several frailty-related variables demonstrated associations with adverse outcomes on univariate analysis, their independent predictor values were attenuated after adjustment for acute physiological and operative risk factors. This suggests that, within the EmLap population, acute illness severity may dominate early outcome prediction, potentially obscuring the independent contribution of baseline frailty. These findings highlight the complex interactions between frailty status and post-operative trajectories in the emergency surgical setting.

4.6.1 Predictors of mortality post-EmLap

In this study, the observed mortality rates of 8.7% at 30 days and 13.3% at 90 days were closely align with national benchmarks reported in the NELA database (30-day mortality rate of 9.8% and 90-day mortality rate of 13.1%) (5, 160), supporting the external validity of this cohort. On univariate analysis, deprivation score, ASA grade, and NELA score were significantly associated with mortality. However, in multivariable models, none of these factors remained statistically significant for predicting 30-day mortality. This finding suggests that acute physiological derangement and operative pathology may exert a stronger influence on early survival than baseline vulnerability markers such as frailty.

In contrast, 30-day readmission remained associated with 90-day mortality in the multivariable analysis. This finding should be interpreted cautiously as it was based on 20 mortalities who could not contribute to this variable and the model was at risk of instability or overfitting due to low numbers of mortalities. The readmission could also be a consequence of baseline vulnerability therefore could be regarded as

mediator than as a confounder. With these limitations, the association may suggest that readmission acts as a dynamic marker of unresolved physiological stress, rather than solely reflecting system level failures. Increasing evidence supports the view that readmissions reflect ongoing morbidity, diminished physiological reserve and failure to recover following discharge (252, 253). In the context of EmLap, readmissions frequently arise from complications such as wound infection, sepsis, ileus, dehydration, or nutritional decline (252-254). The persistence of 30-day readmission as a predictor may imply that readmission could represent an intermediate step in the frailty-mortality pathway. Frail individuals, characterised by diminished physiological reserve, may initially survive EmLap but subsequently experience subtle physiological deterioration after discharge, culminating in readmission and eventual mortality (51, 184). This hypothesis requires confirmation in larger cohorts with sufficient events to support mediation analysis.

Clinically, this finding underscores the importance of enhanced post-discharge care for high-risk patients. When frailty assessment is incorporated into discharge planning, it could enable early identification of high-risk patients and facilitate interventions such as community nurse follow-up, nutritional optimisation, and home physiotherapy, with the potential to mitigate mortality risk (69, 182).

Clinical Frailty Scale

The Rockwood Clinical Frailty Scale (CFS) (55) remains one of the most commonly used tools for frailty assessment in both elective and emergency surgical populations. In this study, CFS was associated with several baseline characteristics including gender, residence, ASA class, and cognitive impairment on univariate analyses, but did not independently predict mortality following multivariable adjustment. This finding contrasts with findings from large multicentre studies and systematic reviews demonstrating an independent association between CFS and both 30- and 90-day mortality (136, 185, 191, 193, 195, 255).

Several methodological and cohort-specific factors may explain this discrepancy. Firstly, the proportion of frail patients in this study (10%) was lower than previously reported, reducing statistical power, particularly as sample size calculation were based on an anticipated frailty prevalence of 20%. Secondly, there is substantial conceptual and statistical overlap between frailty, ASA grade and NELA score, all of which incorporate age, co-morbidities, and physiological derangement. This overlap may mask the independent effect of frailty in multivariable models. Importantly, despite the absence of statistical significance, mortality was markedly higher among frail patients (13.3%) on day 30 post-EmLap, a difference that remains clinically meaningful. Interestingly, 14 of the non-frail patients with score 4 (70%) had 90-day mortality. These findings suggest that, in the acute EmLap setting, the prognostic influence of frailty on mortality may be mediated through established physiological risk models rather than acting as an isolated predictor.

Age and gender precede frailty and are conventional confounders. BMI, ASA grade, NELA score, cognition and residence, however, may be components, consequences or mediators of frailty. Low BMI is part of the frailty phenotype, and cognition and dependence are captured by the CFS. Adjusting for them may therefore remove part of the association of interest (over-adjustment). As these variables also correlate strongly with the CFS and with each other, collinearity may inflate standard errors, particularly with few events. The adjusted estimates describe associations independent of the included variables rather than total effects, and loss of significance after adjustment does not demonstrate absence of association. Selection by univariate significance ignores causal structure, so these multivariable models in this chapter are descriptive and hypothesis-generating. The unadjusted and adjusted analyses are best regarded as complementary.

Sarcopenia

Sarcopenia represents a key physical manifestation of frailty (89, 92) and has been associated with increased mortality, complications, and prolonged length of stay in surgical populations (249, 256-258). In this study, radiologically defined sarcopenia

did not independently predict 30- or 90-day mortality. This finding aligns with emerging evidence suggesting that, in high acuity emergency settings, the prognostic value of sarcopenia may be diminished. Acute inflammatory catabolism and rapid post-operative physiological deterioration may override the baseline influence of muscle mass (259, 260). In addition, variation in imaging thresholds and the absence of functional strength assessments (e.g., handgrip strength) may have reduced the sensitivity for identifying clinically significant sarcopenia (261).

Despite the absence of statistical significance, sarcopenia remains biologically and clinically relevant. It reflects chronic inflammation, nutritional deficiency, and mitochondrial dysfunction, all are pathophysiological processes that overlap substantially with frailty that are prevalent in EmLap patients (216). Its prognostic value may therefore be better realised as a longitudinal biomarker, predicting recovery quality and longer-term outcomes, rather than immediate survival. This interpretation is supported by studies demonstrating associations between radiological sarcopenia and up to 1-year outcomes post-EmLap (257, 262-264).

Cognitive impairment

Cognitive impairment was the only established frailty marker associated with 30-day mortality on univariate analysis, although this association did not persist after multivariable adjustment. Cognitive impairment may exacerbate post-operative vulnerability through impaired communication of symptoms, reduced adherence to treatment, and increased susceptibility to delirium (107, 109).

In EmLap populations, cognitive impairment has been linked to poor outcomes and higher rates of institutionalisation (108, 185, 265). However, its predictive effect is often interdependent with physical frailty and co-morbidity burden. In this cohort, cognitive impairment likely functioned as a surrogate marker of advanced frailty, rather than an independent determinant of mortality. This observation is consistent with evidence demonstrating that multidimensional frailty indices outperform

isolated cognitive measures in predicting surgical outcomes (266). Future work should therefore explore integrated frailty models that incorporate cognitive assessment alongside physiological and functional measures to improve prognostic accuracy in EmLap pathways.

Nutritional status

Nutritional deficiency is an integral component of frailty and represents a potentially modifiable risk factor influencing surgical outcomes (119). Although malnutrition and BMI have historically been used as surrogates, they capture only limited aspects of nutritional frailty (267, 268). In this study, neither BMI nor MUST score independently predicted mortality. This may reflect the overriding effect of acute surgical pathology, sepsis, and physiological instability during the immediate post-EmLap period. As highlighted by Sharma et al (269) and Jayanama et al (268), frailty is a multidimensional syndrome, and early post-EmLap mortality may be driven predominantly by acute illness severity rather than baseline nutritional status. Nevertheless, nutritional status may exert a greater influence on recovery trajectory, complication burden, and functional outcomes beyond early survival.

4.6.2 Predictors of morbidity post-EmLap

Post-operative morbidity represents a crucial endpoint following EmLap, capturing both survival and quality of post-EmLap recovery. In this study, the 30-day morbidity rate was high (45.3%), consistent with previously published complication rates exceeding 45% (270, 271). This mirrors patterns observed in mortality analyses and existing literature, whereby frail patients often experience higher complication rates and slower functional recovery, yet frailty does not consistently emerge as an independent predictor once other clinical variables are considered (272, 273).

Univariate analysis identified associations between pre-EmLap frailty status and several clinical parameters, including gender, residence, ASA class, NELA score, cognitive impairment, and post-EmLap residence. However, none remained

statistically significant in multivariable analysis. Similarly, frailty status at day-90 was associated with BMI, Charlson Co-morbidity Index (CCI), and cognitive function on univariate analysis, but no independent predictors persisted after adjustment. These findings suggest that while frailty correlates strongly with co-morbidity and physiological derangement, its independent predictive value for short-term morbidity may be modest once acute illness severity is accounted for. This observation aligns with prior evidence demonstrating that acute physiological scoring systems such as NELA or APACHE II frequently outperform frailty measures when predicting early post-operative complications (188, 274).

Clinical Frailty Scale

Although CFS did not independently predict morbidity in the multivariable analysis, this finding should be interpreted carefully. The relatively small cohort size limits statistical power, reducing the ability to detect independent associations once multiple co-variates are included in the analysis in the model. In addition, frailty often overlaps with established peri-operative risk factors such as age, co-morbidities and physiological reserve, which may lead to collinearity in multivariable analyses thereby obscuring the apparent independent statistical contribution of frailty.

Importantly, the absence of an independent statistical association in this cohort does not diminish the broader clinical relevance of frailty. Numerous studies have demonstrated that frailty is associated with prolonged recovery, increased post-operative dependency, and discharge to higher levels of care (185, 255). Rather than predicting the immediate occurrence of complications alone, frailty may exert a more pronounced influence on the trajectory of recovery, including the ability to withstand physiological stress, resolve complications and regain baseline functional status.

Frailty may therefore be particularly important in determining resilience following surgical stress Supporting this, Mankowski et al. (275) demonstrated that frail patients experience delayed recovery from sepsis and prolonged organ dysfunction, even after resolution of the acute surgical insult. This suggests that frailty reflects an underlying biological vulnerability influencing recovery dynamics, rather than solely the initial development of complications.

With the limited predictive performance of CFS in the present multivariable model, this finding highlights the potential limitations of relying exclusively on clinical scoring systems to capture the biological complexity of frailty. This supports the rationale for exploring objective metabolomic biomarkers of surgical frailty, as undertaken in later chapters of this thesis.

Sarcopenia

Sarcopenia was not independently predictive of morbidity in this study. However, muscle loss is closely linked to delayed wound healing, respiratory weakness, and reduced mobility, all of which may complicate post-operative recovery (276, 277). A recent meta-analysis by Wang et al (278) reaffirmed an association between sarcopenia and post-operative morbidity across elective and emergency surgery. Nevertheless, in the EmLap setting, extreme physiological stress and acute inflammatory responses may overshadow baseline muscle mass. Additionally, early post-operative catabolism may result in acute sarcopenia, which may not be fully captured by pre-operative imaging (279).

Cognitive impairment

Cognitive impairment contributes substantially to frailty-related morbidity (174, 280). Although it was not independently predictive in this study, cognitive impairment has consistently been associated with post-operative delirium, infection, prolonged hospitalisation, and reduced functional recovery (109, 265, 280). Routine cognitive screening within EmLap pathways may therefore enhance peri-operative

risk stratification and facilitate early implementation of delirium prevention strategies, geriatric involvement, and enhanced post-operative support.

Nutritional status

While baseline BMI and MUST score were not independently associated with morbidity, nutritional frailty remains a critical and potentially modifiable determinant of recovery. Malnutrition contributes to immune dysfunction, muscle wasting, and impaired wound healing (119, 281). Emerging evidence suggests that post-operative nutritional decline, rather than pre-operative status alone, may be a more powerful predictor of recovery quality (282). Enhanced nutrition protocols and early enteral feeding have demonstrated improved outcomes in critically ill intensive care unit patients (283). Future research could therefore evaluate whether targeted peri-operative nutritional interventions reduce morbidity in frail surgical populations.

4.7 Conclusion

In this study, no frailty measure independently predicted 30-day mortality or morbidity in multivariable analysis. This likely reflects limited statistical power, given the modest sample size, low prevalence of frailty within the cohort, and the limited number of deaths, rather than a true absence of biological association. Because frailty is closely related to age, co-morbidity, and nutritional status, adjustment for these variables may have attenuated genuine frailty related effects through shared variance rather than removing confounding alone. The findings should therefore be interpreted as associations independent of these demographic factors, not as evidence against a biological link between frailty and post-operative outcomes. A true null effect cannot, however, be excluded on the basis of this dataset alone.

Importantly, this study provides a novel insight through the peri-operative mapping of frailty measures, demonstrating that frailty is not a static characteristic but may

fluctuate during the surgical and recovery period. These dynamic changes highlight the potential value of longitudinal frailty assessment, rather than reliance on a single pre-operative measurement. Within the EmLap pathway, CFS remains a practical tool for routine clinical assessment due to its multidimensional evaluation of functional status and physiological reserve. However, the limited predictive performance observed in this study underscores the need for more sensitive and biologically informed approaches to characterising surgical frailty. These findings therefore provide a strong rationale for the metabolomic biomarker analyses presented in subsequent chapters, which aim to better characterise the biological underpinnings of surgical frailty and vulnerability in emergency surgical populations that may improve peri-operative risk stratification.

Chapter 5: Optimisation method for sample preparation

Ultra High-Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS) is one of the emerged and widely used technique in metabolomic technology, as it is more sensitive and provides more coverage when compared to other techniques (284). Whilst multiple sample preparation protocols are available to extract the serum or plasma samples, there is still a need to distinguish the preparation method that is vigorous, provides good quality data, reproducible and cost-efficient.

To allow for appropriate sample preparation in UHPLC-MS based metabolomics, protein precipitation is required to remove protein build up on the LC column, allowing the LC column to maintain its efficacy in metabolite separation and avoid suppression of ionisation of the metabolites. The commonly used solvents in these extraction methods included MeOH, ethanol or acetonitrile (285) but these selections produce different metabolite detection depending on the solvents used. For this study, three extraction methods including five solvents based, spin column and solid phase extraction (SPE) techniques were evaluated to determine the optimal extraction method in detecting the metabolic differences between frail and non-frail patients.

5.1 Experimental samples and reagents

Extraction method experiment

Serum sample

For standardisation, pooled human serum samples were used for the optimisation experiments. 100ml of human pooled serum was purchased from MP Biomedicals, LLC (Ohio, USA) (Cat No. 2930149). On arrival, it was stored in the -20°C freezer and defrosted at room temperature. A total of 100µL of serum were used for each solvent. The samples were prepared in triplicates for each solvent and reconstitution solvent.

Chemical and reagents

Five different solvents were used in this optimisation study. This included 99% MeOH, 50:50 MeOH: Ethanol, 99% Acetonitrile, Isopropyl Alcohol (IPA) and 1:3:1 Chloroform: MeOH:H_2O. Three reconstitution solvents were used which included H_2O, 50:50 Acetonitrile: H_2O and Acetonitrile.

H_2O (Optima® LC/MS #W6-4), 99.8% MeOH (Code M/4056/17 LOT 2106574 HPLC grade), 99.8% ethanol (Code E/0650DF/17 LOT 2211390 Analytical reagent grade), Acetonitrile (Code A/0626/17 Lot 1850390 HPLC grade) and Isopropyl Alcohol were purchased from Fisher Chemical, USA. The 99.8% chloroform (ACS spectrophotometric grade) was purchased from Sigma-Aldrich, USA.

5.2 Experiment 1- Serum extraction methods (solvent precipitation)

A total of 100 µL of pooled human serum was mixed with 300 µL of solvent, vortexed and centrifuged followed by solvent evaporation using a SpeedVac for 18 hours as per the local laboratory protocol. The dried sample was reconstituted with 100 µL of reconstitution solvent, vortexed and centrifuged at 4°C with speed 15,800 x g for 15 minutes. A total of 85 µL of sample was transferred onto a vial and 2 µL was injected on to the UHPLC-MS for analysis. The detailed methodology for the procedure is presented in Figure 20.

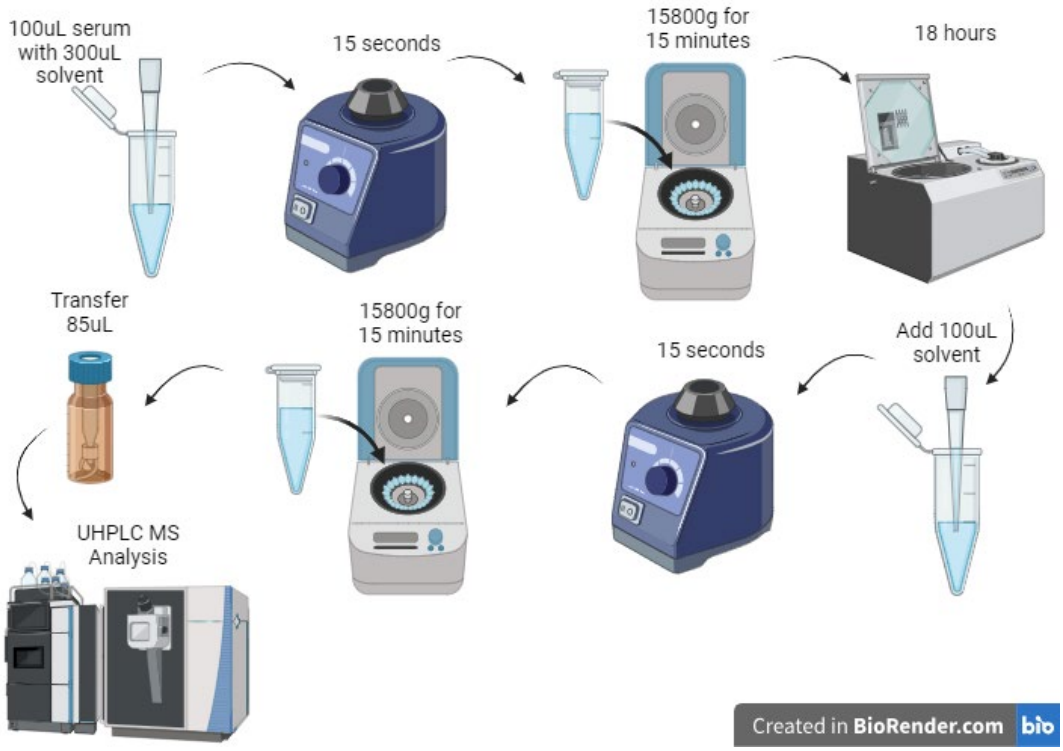


Figure 20: Schematic representation of the serum preparation procedure, where 100 µL of human serum was mixed with 300 µL of solvent, vortexed for 15 seconds, and then centrifuged at 15,800 x g for 15 minutes. The supernatant was then subjected to solvent evaporation using a SpeedVac for 18 hours. The dried sample was reconstituted with 100 µL of reconstitution solvent, vortexed for 15 seconds, and centrifuged at 15,800 x g at 4°C for 15 minutes. A total of 85 µL of the sample was transferred into a vial, and 2 µL was injected into the UHPLC-MS for analysis.

5.3 Experiment 2- Solid Phase Extraction (SPE)

96-well Hybrid SPE plates were purchased from Supelco (575601-U). The SPE wells were conditioned with two sequential additions of 300 µL of MeOH and then another two sequential additions of 300 µL of H_2O to activate the sorbent. A mixture of 100 µL of human serum and 300 µL of H_2O was loaded onto the conditioned SPE wells and drawn through the solid phase under vacuum. The SPE was then washed with 2 washes of 200 µL H_2O and eluted using two additions of 250 µL of MeOH. The eluates were dried down using SpeedVac for 12 hours and the dried samples were reconstituted in 85 µL of H_2O/ MeOH solution (95:5, v/v). The reconstituted solution

was vortexed and centrifuged. The clarified supernatant was then subjected to UHPLC-MS for analyte quantification (Figure 21).

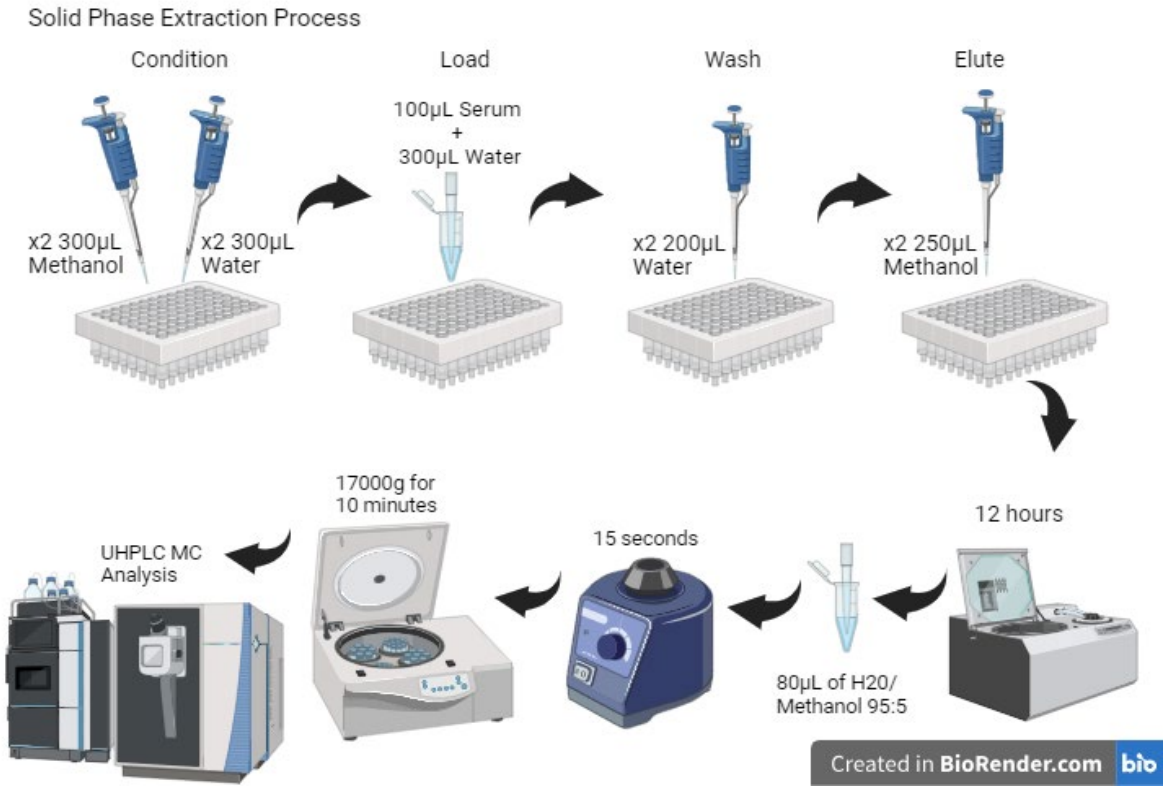


Figure 21: Schematic representation of serum preparation procedure using the solid phase extraction (SPE) method. The SPE wells were conditioned with two sequential additions of 300 µL MeOH, followed by two sequential additions of 300 µL H_2O. A total of 100 µL of serum was mixed with 300 µL of H_2O in an Eppendorf tube, and the resulting 400 µL of mixture was added to the conditioned SPE wells and drawn through the solid phase under vacuum. Interfering substances were removed with two washes of 200 µL H_2O, and target analytes were eluted using two additions of 250 µL MeOH. The eluted samples were dried under vacuum for 12 hours. The dried samples were then reconstituted in 80 µL of a H_2O/MeOH (95:5, v/v) solution, vortexed for 15 seconds, and centrifuged at 17,000 x g for 10 minutes. The supernatant was subsequently subjected to UHPLC-MS analysis.

5.4 Experiment 3-3kDA spin column

5.4.1 Experiment a: Serum amount

3kDa Pall Nanosep centrifugal tubes were purchased from Avantor™/ VWR Inc. The filter was first washed by adding 500 µL H_2O (Optima® LC/MS #W6-4) and centrifuged at speed 14,000 x g at temperature of 4°C for 4 minutes to precondition the membrane. The filtrate was discarded. The filter was then rinsed with H_2O eight times, discarding excess H_2O from the top of filter on each wash. A total of 100 µL of serum was added to filter and centrifuged at speed 14,000 x g at temperature of 4°C for 20 minutes. The filtrate was transferred to Eppendorf tube. Another 50 µL of H_2O was added to the centrifugal device, vortexed for 10 seconds and centrifuged at speed 14,000 x g at temperature of 4°C for 25 minutes. Additional filtrate was added to the Eppendorf tube and 100 µL of sample was transferred to LCMS vials and analysed using UHPLC-MS. This method was repeated using 250 µL and 500 µL of serum and performed in triplicates. H_2O was used as control sample to identify contaminants from the Nanosep filter. With the identified amount of serum that produced the highest number of metabolites, it was used for experiment b as below.

5.4.2 Experiment b: With solvent evaporation

As per experiment a, the Pall Nanosep centrifugal device with 3kDA molecular weight cut off was washed by adding 500 µL H_2O (Optima® LC/MS #W6-4) and centrifuged at speed 14,000 x g at temperature of 4°C for 4 minutes. The filtrate was discarded. The filter was then rinsed with H_2O eight times, discarding excess H_2O from the top of the filter on each wash. The amount of serum with highest metabolites from experiment 1 was added to filter and centrifuged at speed 14,000 x g at temperature of 4°C for 20 minutes. The filtrate was transferred to Eppendorf tube. Another 50 µL of H_2O was added to the centrifugal device, vortexed for 10 seconds and centrifuged at speed 14,000 x g at temperature of 4°C for 25 minutes. The additional filtrate was added to the Eppendorf tube. This was repeated to produce 2 triplicates. The first triplicate had 100 µL of sample analysed using UHPLC-MS and another triplicate was dried using a SpeedVac for 12 hours then reconstitute

with H_2O, vortexed for 10 seconds and 100 µL of sample analysed using UHPLC-MS. (Figure 22)

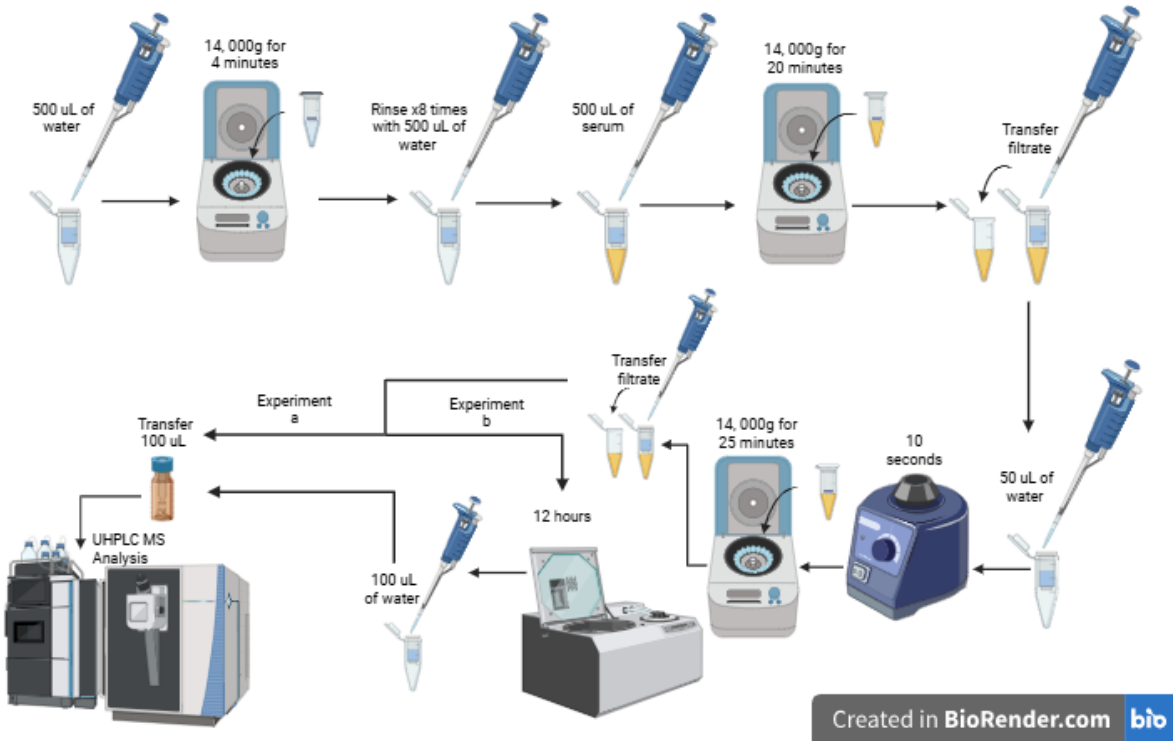


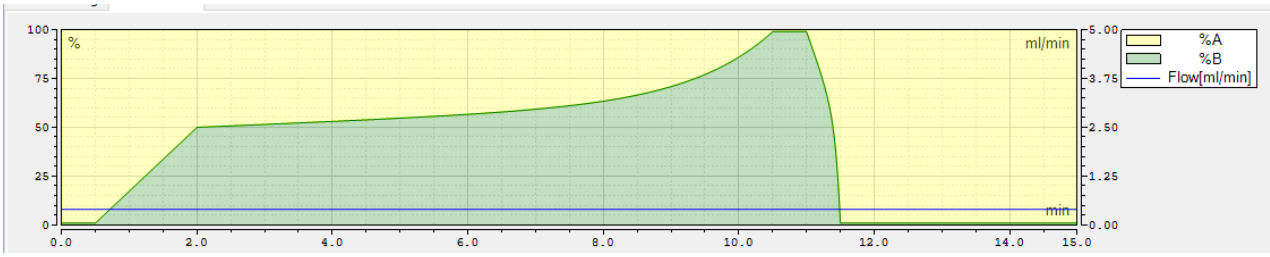
Figure 22: Schematic representation of serum preparation workflow using Nanosep centrifugal filters for protein removal and sample clean-up prior to UHPLC-MS analysis. Initially, 500 µL of H_2O was added to the Nanosep filter and centrifuged at 14,000 x g for 4 minutes to precondition the membrane. The filtrate was discarded, and the filter was then rinsed eight times with 500 µL of H_2O. Subsequently, 500 µL of serum was loaded into the preconditioned filter and centrifuged at 14,000 x g for 20 minutes. The filtrate was collected into an Eppendorf tube. 50 µL of H_2O is added to the centrifugal device, vortexed for 10 seconds and centrifuged at speed 14,000 x g for 25 minutes. The filtrate was added into the Eppendorf tube and the whole process was repeated to produce 2 triplicates. Each triplicate was divided into two experimental arms: Experiment a: 100 µL of filtrate was directly transferred for UHPLC-MS analysis; Experiment b: The filtrate was dried under vacuum for 12 hours, reconstituted in 100 µL of H_2O, vortexed for 10 seconds before injection into the UHPLC-MS system.

5.5 Mass spectrometry analysis

5.5.1 Analytical measurement

After serum sample extraction was performed, metabolomic analysis was performed with ultra high-performance liquid chromatography mass spectrometry (UHPLC-MS) on a Thermo Exploris 240 Orbitrap mass spectrometer coupled to a Thermo Vanquish binary liquid chromatography using positive and negative mode ionisation. The method extraction experiments were performed with blank, and QC run between each sample to prevent carryover. For each sample, 5 µL was injected onto a Accucore C18 UHPLC Column (2.6 µm, 100 mm × 2.1 mm I.D; Thermo). For both positive and negative ionisation modes, mobile phase A consisted of 0.1% formic acid in H_2O (v/v) while mobile phase B consisted of 0.1% formic acid in MeOH (v/v).

Each sample for C18 analysis (positive and negative mode) was ran on the solvent gradient as shown in Figure 23.



Retention time (Min)	Flow (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)	Curve
0.0	0.4	99.0	1.0	5
0.5	0.4	99.0	1.0	5
2.0	0.4	50.0	50.0	5
10.5	0.4	1.0	99.0	8
11.0	0.4	1.0	99.0	5
11.0	0.4	99.0	1.0	7
14.9	0.4	99.0	1.0	5
15.0	0.4	99.0	1.0	5

Figure 23: The solvent gradient changes according to the retention time and flow rate with different mobile phases (A and B) for analysis of mass spectrometer using the C18 column for positive and negative modes.

Subsequent mass spectrometry analysis was performed under the conditions as per Appendix 1.11. The targeted mass list for positive and negative mode is listed in Appendix 1.11.1 and 1.11.2.

5.5.2 Data processing and deconvolution post mass spectrometry analysis

After UHPLC-MS analysis, the raw data obtained was processed and analysed using Compound Discoverer 3.3 (Thermo Fisher Scientific), a software platform designed for untargeted metabolomics and small molecule workflows. This processing step was to deconvolute complex mass spectrometry (MS) datasets, identify relevant features, and annotate compounds based on spectral characteristics and database matching.

Raw MS data files (.RAW format) that were generated by the Thermo Fisher Scientific Orbitrap MS were directly imported into Compound Discoverer 3.3 using the workflow constructed via the software's Workflow Editor. The appropriate nodes for untargeted metabolomics were selected (Figure 24). The positive and negative mode used the same workflow, with the only difference in the polarity option of positive or negative. The detail of the workflow is in Appendix 1.12.

1. Select spectra: Filtering based on retention time and polarity.
2. Align retention times: Alignment across all samples using adaptive curve alignment and correcting retention time shifts due to chromatography variation.
3. Detect compounds: Detection of chromatographic peaks across aligned files, using a minimum peak intensity threshold (e.g., 1×10^6).
4. Group compounds: The Compound Discoverer grouped isotopes, adducts, and charge states into compound groups and this node used parameters to merge features across samples based on accurate mass (±5 ppm) and RT window (±0.2 min). Identified features then underwent putative annotation through online and local database searches (mzCloud and ChemSpider) and the MS2 fragmentation spectra were compared to reference spectra using cosine similarity scoring.

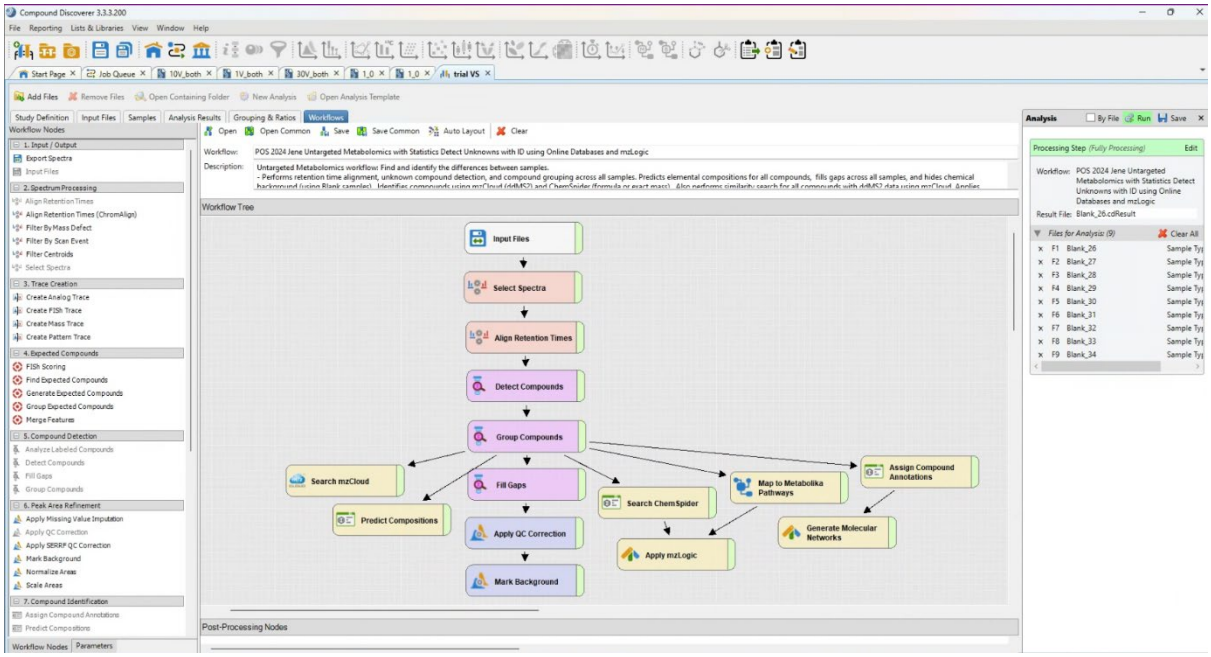


Figure 24: The workflow of the study edited using the software's Workflow Editor in Compound Discoverer 3.3.

After obtaining the compounds following analysis with Compound Discoverer 3.3, the results were filtered (Figure 25) to ensure only high-confidence identifications were retained. Filters included removal of blanked name, annotation status (full match with source from predicted compositions and ChemSpider), presence of MS2 data, and an annotation mass over charge (m/z) range of -5.00 to 5.00 ppm. This approach aimed to exclude low-confidence or unannotated features and prioritise biologically relevant metabolites for downstream analysis.

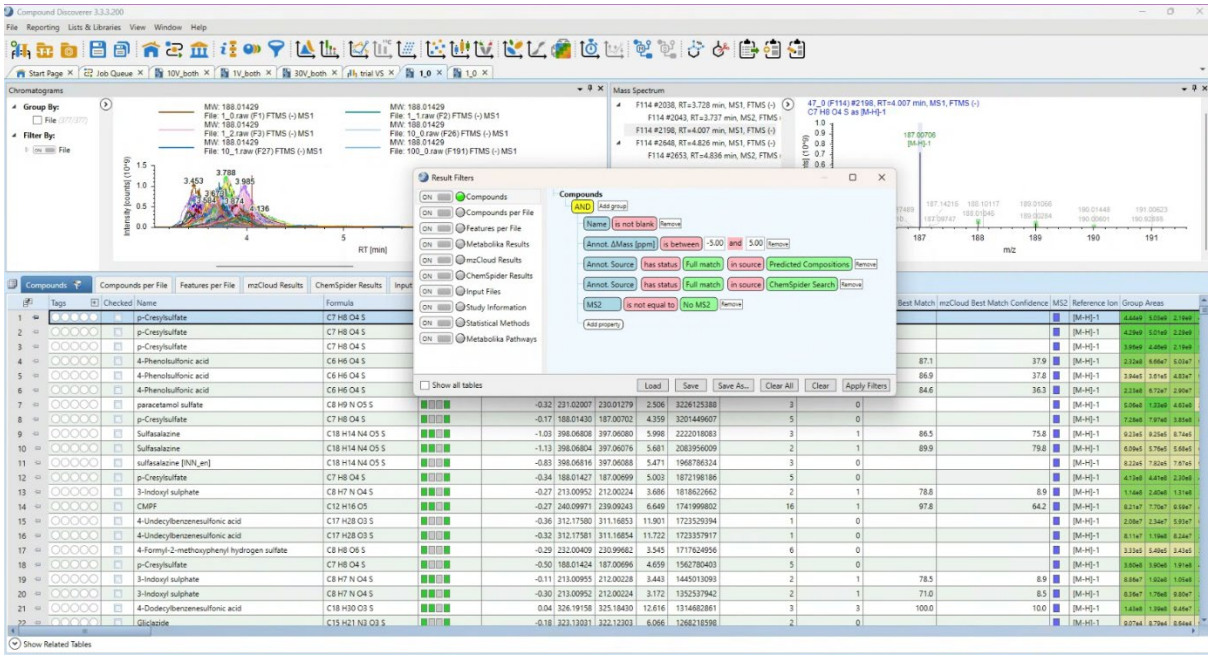


Figure 25: Filtering of detected compounds in Compound Discoverer 3.3 using specific criteria, including m/z range, annotation status, presence of MS2 data, and ChemSpider matches, to refine the dataset for downstream analysis.

The filtered result was then exported to Microsoft Excel, where duplicate metabolites were removed.

5.6 Results of optimisation experiments

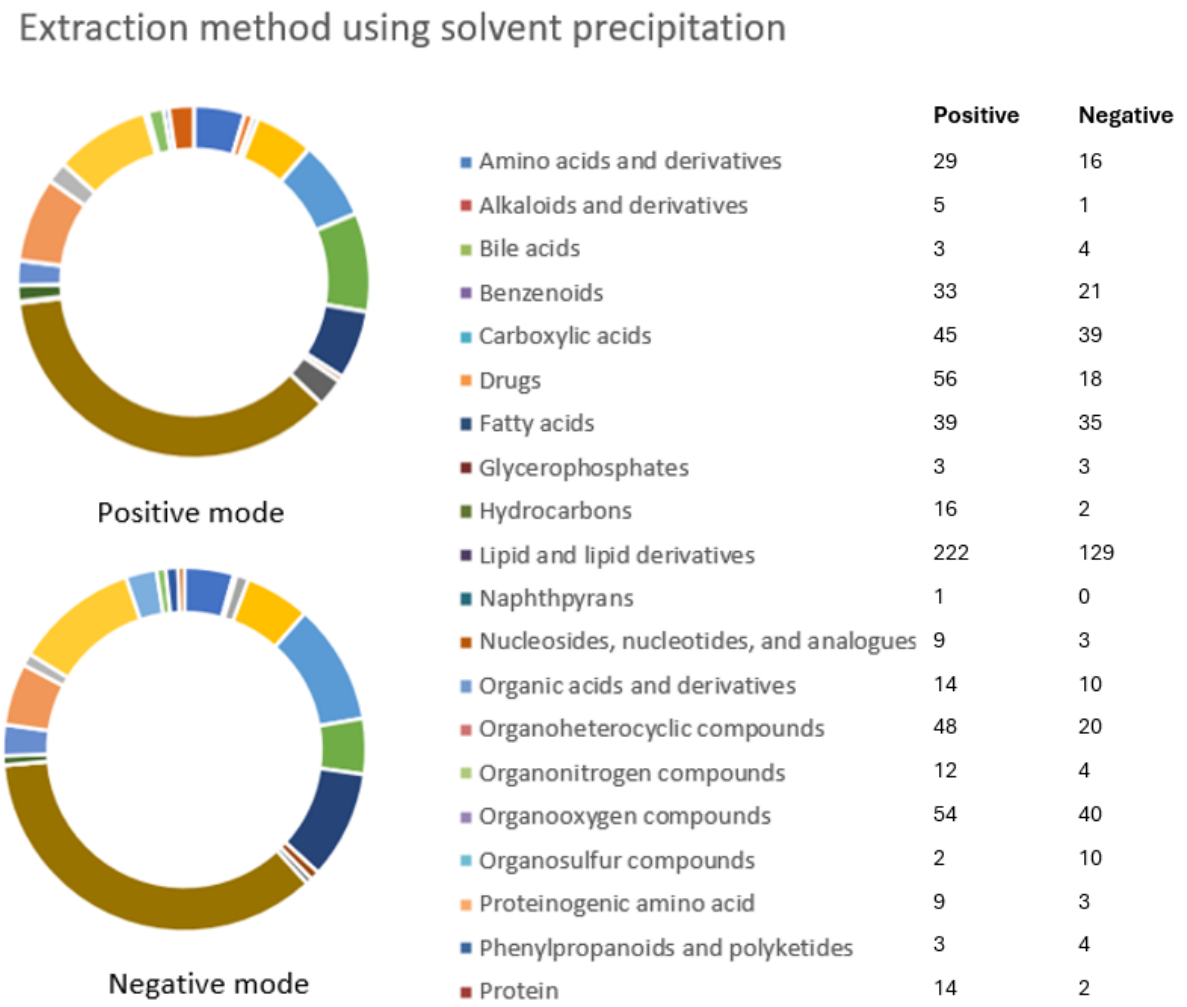
Following UHPLC-MS analysis and data deconvoluted on Compound Discoverer 3.3, the analysed results were exported to Microsoft Excel and comparison was made between the three extraction methods. The metabolomic profiles were evaluated for the total number of features, the duplicates were removed producing the identified metabolites. The results are shown in Table 25.

Table 25: Features and metabolites identified according to different extraction methods on UHPLC-MS C18 positive and negative mode.

		Total number of features		Total number of identified metabolites	
UHPLC-MS mode		**Positive**	**Negative**	**Positive**	**Negative**
Solvents	**Reconstitution**				
99% MeOH	99% H_20	3996	1422	**617**	**364**
	50:50 H_20: Acetonitrile	3725	1486	540	350
	99% Acetonitrile	2402	569	341	90
50:50 MeOH: Ethanol	99% H_20	4055	1395	601	410
	50:50 H_20: Acetonitrile	4076	1919	553	466
	99% Acetonitrile	2342	448	343	85
99% Acetonitrile	99% H_20	3626	1320	567	332
	50:50 H_20: Acetonitrile	3347	1577	481	342
	99% Acetonitrile	2285	534	314	104
Isopropyl Alcohol (IPA)	99% H_20	3685	1536	594	402
	50:50 H_20: Acetonitrile	3735	1969	557	442
	99% Acetonitrile	2291	655	347	117
1:3:1 Chloroform: MeOH: H_2O	99% H_20	3223	1441	506	397
	50:50 H_20: Acetonitrile	3306	1695	500	408
	99% Acetonitrile	2442	677	335	130
Solid Phase Extraction		5780	1073	578	210
Nanosep Amount of serum					
100 µL		2292	468	257	93
250 µL		2135	453	263	85

500 µL		2586	746	326	161
H_2O		1696	236	154	48
Nanosep Extraction methods					
500 µL serum		2319	813	394	202
500 µL serum Speedvac		1934	505	267	104
500 µL H_2O		1095	149	130	24
500 µL H_2O Speedvac		1530	174	130	27

The method producing the highest identified metabolites was found to be 99% MeOH as solvent and 99% H_20 as reconstitution solvent. The metabolites were then class quantified as shown in Figure 26 and in both positive and negative mode, majority of the metabolites identified were lipid and lipid derivatives.



	Positive	Negative
Amino acids and derivatives	29	16
Alkaloids and derivatives	5	1
Bile acids	3	4
Benzenoids	33	21
Carboxylic acids	45	39
Drugs	56	18
Fatty acids	39	35
Glycerophosphates	3	3
Hydrocarbons	16	2
Lipid and lipid derivatives	222	129
Naphthpyrans	1	0
Nucleosides, nucleotides, and analogues	9	3
Organic acids and derivatives	14	10
Organoheterocyclic compounds	48	20
Organonitrogen compounds	12	4
Organooxygen compounds	54	40
Organosulfur compounds	2	10
Proteinogenic amino acid	9	3
Phenylpropanoids and polyketides	3	4
Protein	14	2

Figure 26: The class quantification of highest metabolites identified using solvent precipitation (99% MeOH as solvent and 99% H_20 as reconstitution solvent) on Ultra High-Performance Liquid Chromatography (UHPLC-MS) C18 positive and negative mode.

In this optimisation method, 99% MeOH protein precipitation followed by H_2O reconstitution was selected as it produced the highest number of confidently identified metabolites in serum samples, particularly in the positive ionisation mode. MeOH, as an organic solvent, is highly efficient at denaturing and precipitating serum protein (286). The reduction in residual protein content would improve ionisation efficiency and cleaner spectra and higher quality of MS/MS data which improves metabolite identification confidence rather than raw feature count (287). In addition, serum sample is rich in polar and semi- polar metabolites (e.g. amino acids, organic acids, nucleosides) and MeOH efficiently extracts the small polar

metabolites and charged compounds, increasing the identification rates of metabolites (288). While mixed solvents may increase chemical diversity, they also extract lipids, phospholipids and plasticisers as well as additional low abundance or poorly ionised features (289, 290). These would increase the background noise and spectral complexity, inflating total feature counts, but do not translate into confident identifications.

The reconstitution solvent using H_2O after MeOH protein precipitation enhances the retention of polar metabolites on reversed phase UHPLC-MS columns, improves retention time reproducibility, produces sharper peak shapes and improved peak symmetry (291). These effects improve the spectral matching and reduce co-elution, both of which are critical for confident MS/MS based identification. Furthermore, starting in a highly aqueous solvent promotes stable electrospray formation, reduces early elution solvent mismatch effects and enhances signal-to-noise ratios for polar compounds (292). These results in cleaner MS/MS spectra, reproducible fragmentation patterns and increases library matching confidence.

The result showed that 99% MeOH + H_2O yields the highest number of identified metabolites, particularly in positive mode (617 identified metabolites) with strong performance in negative mode (364 identified metabolites) indicating superior annotation efficiency compared to mixed solvents.

Chapter 6: Pilot study (46 recruited patient samples)

Using the optimised extraction method established in Chapter 5, comprising of protein precipitation with 99% MeOH followed by reconstitution with H_2O, a pilot study was conducted on 20/06/2023 using the available collected clinical plasma samples. Although the optimisation experiments were performed using serum samples, this pilot study was undertaken to confirm the feasibility of applying the method to plasma samples and to ensure reliable metabolite detection. While plasma contains clotting factors and has a higher total protein content than serum, 99% MeOH is an effective protein precipitant capable of efficient protein removal. Furthermore, reconstitution in H_2O promotes the recovery and chromatographic performance of polar metabolites, supporting the suitability of this extraction method for plasma based UHPLC-MS analysis. In addition to method feasibility assessment, this pilot study would also provide an initial exploratory evaluation of metabolomic differences between frail and non-frail patients.

For this pilot study, plasma samples from 46 EmLap patients with full 5-time points follow-up, recruited as part of the ongoing study, were used for analysis using the optimised method. The 230 samples (46 patients at x5 time points) including quality control (QC) samples were separated into 4 batches as there was a maximum limit of 64 Eppendorf in one rotor of the SpeedVac.

After the extraction method and UHPLC-MS analysis, raw data obtained was processed and analysed using Compound Discoverer 3.3 (Thermo Fisher Scientific). The detailed data processing and deconvolution methods have been described in Chapter 5.5.2. In summary, the data produced by Compound Discoverer 3.3 was filtered according to predetermined properties, exported to Microsoft Excel, duplicate metabolites removed and end result underwent batch alignment via QCMXP (Quality Control and Matrix eXtraction Pipeline) software (https://broadhurstdavid.github.io/QC-MXP/) (293) and analysed using MetaboAnalyst (294).

6.1 Quality Control and Matrix eXtraction Pipeline (QCMXP) analysis

In mass spectrometry based metabolomic analysis of large cohort studies, batch alignment is a critical step. The data collected from multiple batches of experiments are safeguarded through this method which would lead to reduction in variability caused by instrument drift, minor inconsistency in sample processing or other external factors. Due to large datasets, many experiments are required to be divided into multiple batches where batch effects could affect data interpretation (295). In addition, raw metabolomics data can contain features from background noises, artifacts or instability across runs of different batches. These variations in retention times, ion intensities, and mass accuracies due to changes in the performance of the mass spectrometer can lead to inconsistencies causing biased results and losing the integrity of the data (296).

QCMXP is a specialised software platform that provides a reliable and efficient solution to ensure only stable metabolic signals are passed on to the statistical analysis to produce biologically meaningful results (https://broadhurstdavid.github.io/QC-MXP/) (293). For batch correction, quality control and data cleaning, the advanced algorithm used:

$$\sum_{i=1}^{n}\left(x_{QC}(i)-\hat{f}(t_{QC}(i))\right)^2+\gamma\int\left(\frac{d^2\hat{f}(t)}{dt^2}\right)^2 dt$$

Regularized Spline Regression

After acquiring result from Compound Discoverer, the data was processed using TidyMS (https://github.com/griquelme/tidyms) (297) and converted to data table (consisting of feature matrix (SampleID, SampleType (blank, QC, Sample), Order (injection order) and Batch number) and associated meta data) and peak table (metabolites data). These tables were stored as .csv files and uploaded onto QCMXP software (Figure 27). The software first removed features with less than 20% of QC values missing, then configurated the batch correction according to pooled QC (Figure 28). The Quality Control Regularised Spline Correction (QCRSC) engine was run, and a new corrected data and peak table was produced. After the QCRSC engine

finished, the metabolites were filtered by setting missing < 20%, QC-RSD <20%, D-Ratio 40%, blank ratio 1% and the cleaned data table and peak table were both saved for further analysis (293).

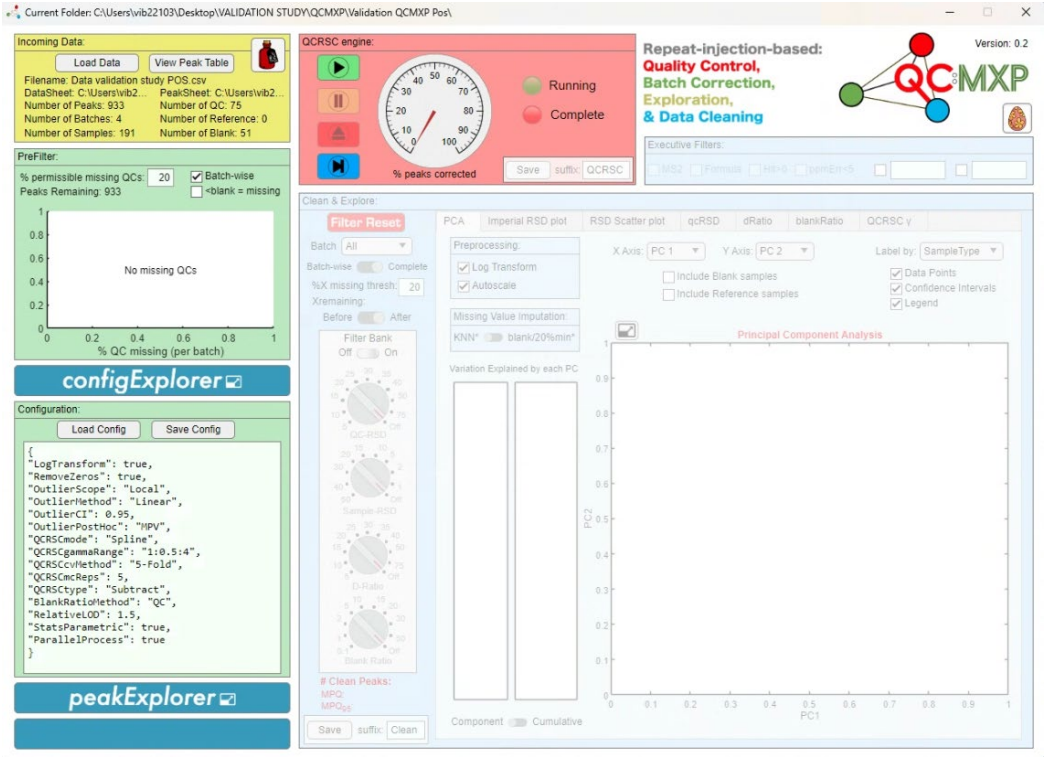


Figure 27: QCMXP software after uploading the table data and peak data files. To run the software, the green play button was clicked.

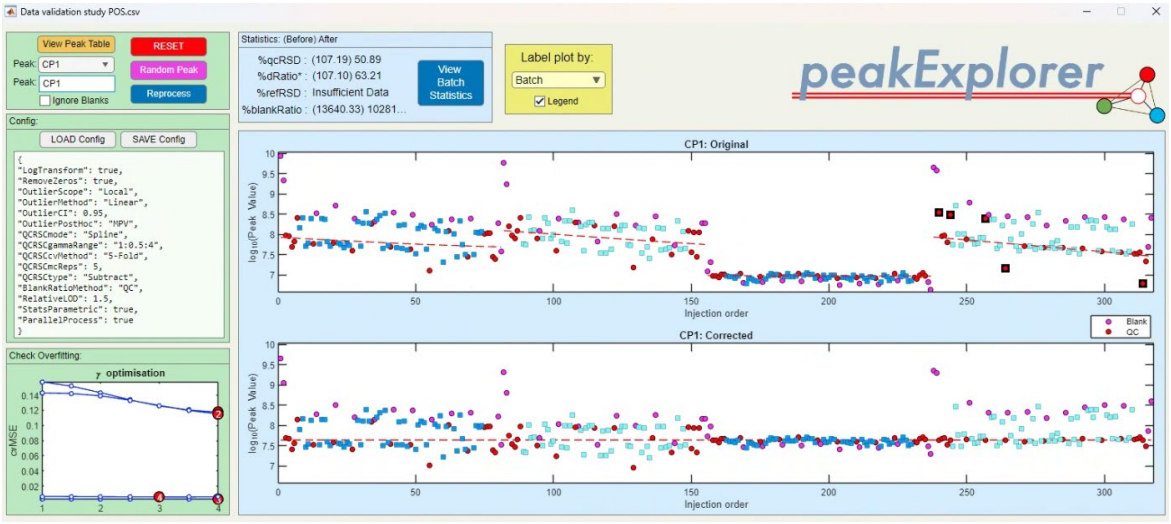


Figure 28: QCMXP software showing the within and between batch correction based on the pooled QC. The red dots were QC, pink dots were blank, blue and turquoise dots were batch 1-4 metabolites, and the dots with black squares were outliers, which were removed after correction. This illustration showed the batch correction of CP1.

6.2 Batch alignment result

The result from Compound Discoverer was arranged into Data table and Peak table, uploaded onto the QCMXP software producing principal component separation (PCA). After applying the recommended filters, the results showed alignment between the 4 batches, as shown in Figure 29.

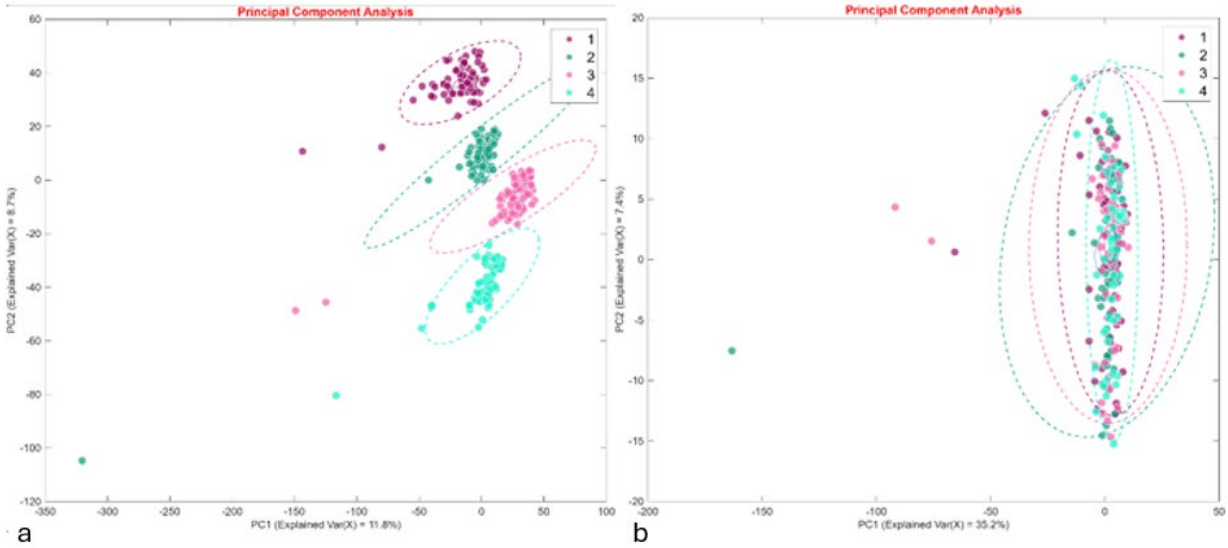


Figure 29: Alignment of samples. Figure 29a showed the PCA before alignment while figure 29b showed the PCA after application of filter for alignment.

6.3 Pilot study on frailty

The result following batch alignment was categorised according to frail and non-frail and further analysis using MetaboAnalyst (294) was performed. The data was uploaded (format used: samples as rows, metabolites as columns) and normalised (by log transformation and autoscaling) to reduce technical variability. Univariate (ANOVA test) was used to identify metabolites that were significantly different between frail and non-frail and multivariable analysis (PCA) was performed to visualise the separation between frail (CFS >4) and non-frail group (CFS ≤4).

When comparing frail versus non-frail patients at pre-operative time point, the principal component separation (PCA) showed partial separation between the frail and non-frail, particularly on component 2 (showing 22.5% variance), as shown in

Figure 30. This suggested potential differences in circulating metabolic profiles between clinically classified frail and non-frail patients. However, given the small pilot sample size and the overlap between groups, this finding was considered exploratory and did not establish the presence of a distinct frailty-associated metabolic signature.

Although the PCA showed partial separation between the frail and non-frail groups, some participants classified as non-frail were located within the overlapping region of the score plot. Of the 13 non-frail participants within this overlapping region, 8 (61.5%) had a CFS score of 4, corresponding to the pre-frail classification. In total, 12 patients in the pilot cohort had a pre-operative CFS score of 4. This observation raises the possibility that some patients classified as non-frail may have underlying metabolic differences that are not fully captured by clinical frailty assessment. However, the PCA overlap alone cannot establish that these individuals had an intermediate metabolic phenotype or that the observed pattern represented early metabolic changes associated with frailty. This hypothesis requires further investigation in larger cohorts.

The primary objective of the pilot study was to establish the feasibility of the analytical workflow and evaluate the quality of metabolomic data generation prior to expansion into the full study cohort. As such, the pilot was designed as an exploratory study to optimise sample preparation, data acquisition and bioinformatic processing rather than to identify or validate definitive metabolomic biomarkers of frailty. The physical samples of the 40 patients within this pilot study were subsequently randomised and reanalysed within the full 150 patient study. However, a formal comparison of metabolite-level agreement between the pilot and main analytical sweeps was not undertaken. The pilot findings should therefore be interpreted as preliminary evidence of feasibility rather than independent validation of frailty-associated metabolic signatures. The subsequent longitudinal study incorporated a larger cohort, repeated sampling and an optimised analytical and

bioinformatic workflow to investigate metabolomic alterations associated with frailty and post-operative outcomes.

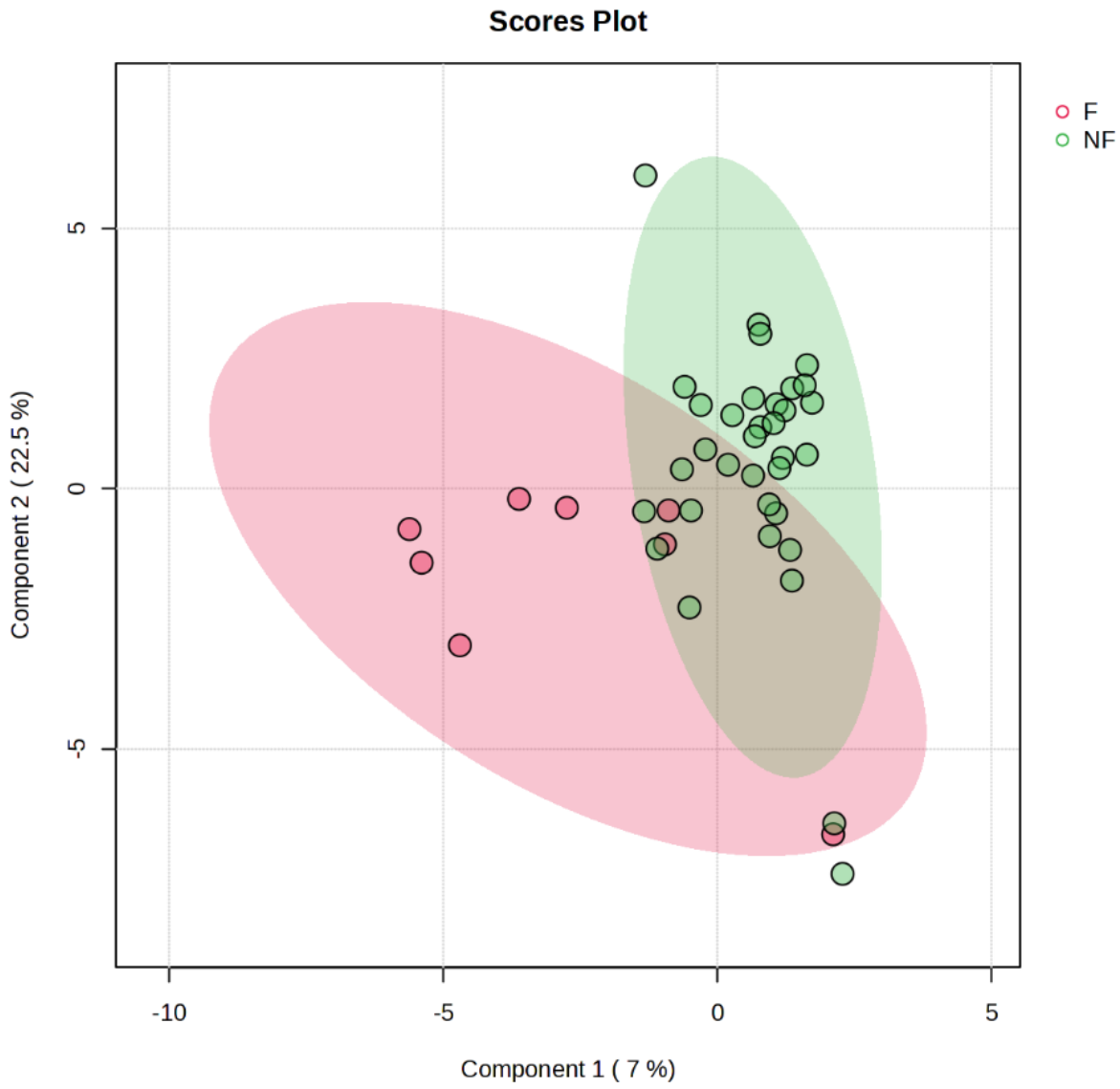


Figure 30: PCA score plot analysis in MetaboAnalyst to analyse the clustering between pre-operative frail and non-frail group. The frail group (in red) and non-frail group (in green) showed partial separation showing distinct metabolic changes associated with frailty. The overlapping area may indicate the group that are pre-frail. Coloured ellipses represent 95% confidence intervals for each group.

Chapter 7: Optimisation method for 150 patient samples

After the pilot study was conducted, it was noted that the time required for sample preparation and analysis on UHPLC-MS for one batch took 5 days. If this analysis method were to apply to the 664 samples (all 150 recruited patient samples), the analysis would involve up to 25 batches and take up to 4. 5 months. This length of time for analysis would cause a prominent batch effect (298) and impact on the results of the UHPLC-MS. To reduce this batch effect, the sample preparation method was optimised further with the aim of maintaining the number of metabolites identified and reducing time required for analysis. The changes included using:

1. 96 deep well plates instead of Eppendorf tubes
2. Nitrogen evaporator for sample drying instead of the use of a SpeedVac

By using 96 deep well plates, it allowed increased number of samples being processed as a batch. As observed in the pilot study method, the number of samples being processed was limited by the number of Eppendorf tubes in the centrifuge machine and SpeedVac. In addition, by using the nitrogen evaporator, the drying down time was reduced from 18 hours to 2 hours thus reducing the time for sample preparation and completing mass spectrometry analysis to 4 days. With both of these new changes, the time to analyse all 664 samples was reduced from 4.5 months to 1.5 months.

Prior to performing the sample preparation and analysis for the final study, further optimisation experiments were performed to ensure that the number of features and metabolites identified in pilot study were similar to the new method.

7.1 Experiment 1- Speedvac versus nitrogen evaporator

To ensure that the number of features and identified metabolites were similar between SpeedVac and nitrogen evaporator, an experiment was conducted to

compare the results. The protocol used for prepping the samples in both methods were similar (100 µL of human serum was used, mixed with 300 µL of MeOH, vortex and centrifuged). The differences were:

1. Eppendorf tubes were used and samples in triplicates were evaporated using SpeedVac for 18 hours.
2. 96 well plate was used and samples in triplicates were freeze dried for 1.5 hours in a nitrogen evaporator.

The dried down samples were reconstituted with 100 µL of 99% H_2O, vortexed and centrifuged at temperature of 4°C for 15 minutes. A total of 85 µL of sample was submitted for analysis and 2 µL was injected on to the UHPLC-MS system (Figure 31).

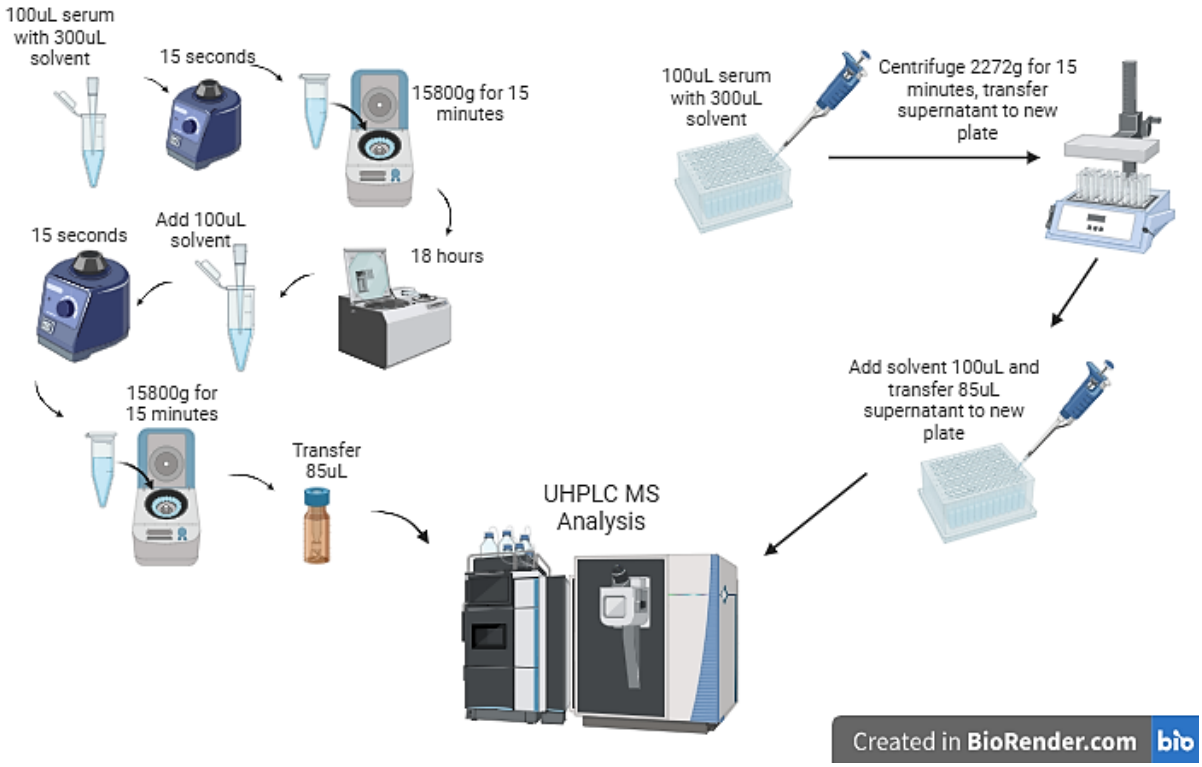


Figure 31: The workflow for plasma sample analysis in UHPLC-MS using the Eppendorf and Speedvac method vs 96 deep well plates and nitrogen evaporator.

The total number of features and number of metabolites identified were not lower when nitrogen evaporator and 96 deep well plates were used, in both positive and negative mode as shown in Table 26.

Table 26: Results of Speedvac vs nitrogen evaporator in positive and negative mode.

	Total number of features		Total number of identified metabolites	
UHPLC-MS mode	**Positive**	**Negative**	**Positive**	**Negative**
SpeedVac	2870	955	399	193
Nitrogen Evaporator	3397	1208	411	206

7.2 Experiment 2- Volume of solvent

When using the 96 deep well plates, the protein precipitants were found clogging the pipette during aliquoting, affecting the actual amount of supernatant getting aliquoted. This issue arose due to the differences in the base of the deep well plate and Eppendorf. The 96 deep well plate is made from polypropylene, restricting a clear view during pipetting and had a rounded base, while the Eppendorf tubes were clear with conical shaped base. To overcome this difficulty of protein precipitants affecting the pipetting technique, an optimisation experiment on the amount of solvent was performed.

In the 96 well plates, triplicates of human serum (100 µL) were used. Three different amounts of 99% MeOH solvent were used (300 µL, 400 µL and 500 µL) and after centrifuged at 4°C/2272g for 15 minutes, 60% of the supernatant were aliquoted (200 µL, 300 µL and 400 µL) into a new 96 deep well plate. All were freeze dried with the nitrogen evaporator for 2 hours. 100 µL of 99% H_2O was used as reconstitution solvent, which was subsequently centrifuged in at 4°C/2272g for 15minutes followed by transference of 85 µL of supernatant to a new 96 deep well plate which was analysed with UHPLC-MS. This was replicated using quality-controlled (QC) clinical patient plasma samples and the results are shown in Table 27.

Table 27: Comparison results between different amount of solvent using human serum and quality-controlled patient plasma sample.

Sample	99% MeOH volume	Supernatant volume	Total number of features	Total number of identified metabolites	Signal intensity
Human serum	300 µL 400 µL 500 µL	200 µL 300 µL 400 µL	1903 2223 2061	304 330 332	9.85E+08 9.65E+08 9.79E+08
Quality-controlled (QC) patient plasma	300 µL 400 µL 500 µL	200 µL 300 µL 400 µL	3193 3355 3131	511 516 486	1.27E+09 1.11E+09 1.07E+09

The highest number of features and metabolites identified were found consistently when 400 µL of 99% MeOH and 300 µL of supernatant were used. These volume of MeOH and supernatant were used as the new optimised protocol.

7.3 Experiment 3- Internal Standard

Three different compounds were used to compare the signal intensity as internal standard. The compounds included ^{13}C isotopically labelled L-Glutamine, ^{13}C isotopically labelled L-Glucose and N isotopically labelled L-tryptophan ($^{15}N_2$ 98%), bought from Goss Scientific. The compounds were dissolved in 1mL of 99% H_2O with formic acid. The stock (2mg/mL) was then diluted in 1: 100. The comparison was made between dilution of 1:100 and 1: 10. The signal intensity and retention time was explored on the UHPLC-MS.

When comparing the signal intensity, L-Glucose and L-Glutamine were found too high at 4.23E8 and 1.34E9. The signal intensity and retention time of L-tryptophan was 3.79E7. With the dilutional of 1:10 producing 20ng/ml, the signal intensity produced was lower at 10E6. This result was selected as the dilutional volume to ensure that the internal standard did not overwhelm the signal intensity of other metabolites. In addition, despite having different dilutional volume, the retention

time was consistent in both 200ng/ml and 20ng/ml at 2.78minutes, confirming that L-tryptophan was a reliable internal standard. The mass chromatogram of L-Glutamine, L-Glucose and L-tryptophan are shown in Figure 32, Figure 33 and Figure 34.

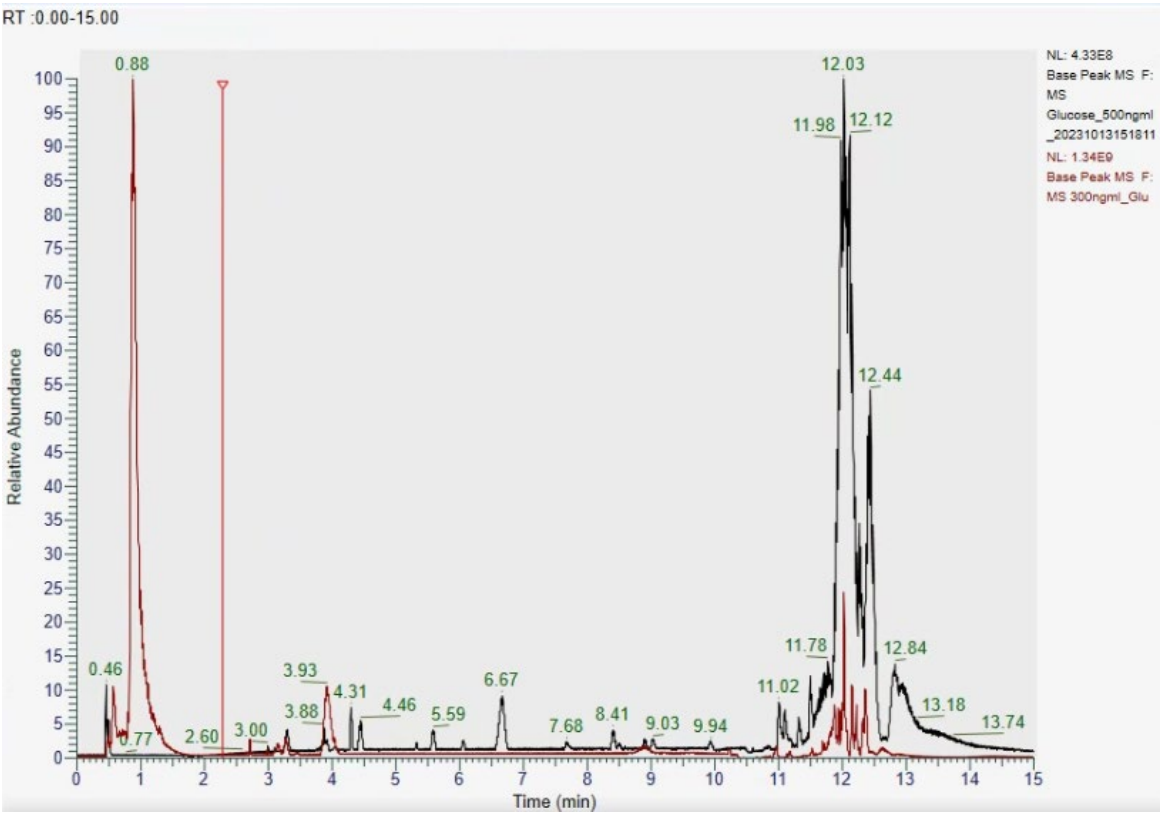


Figure 32: The chromatogram showing signal intensity of L-Glucose and L-Glutamine.

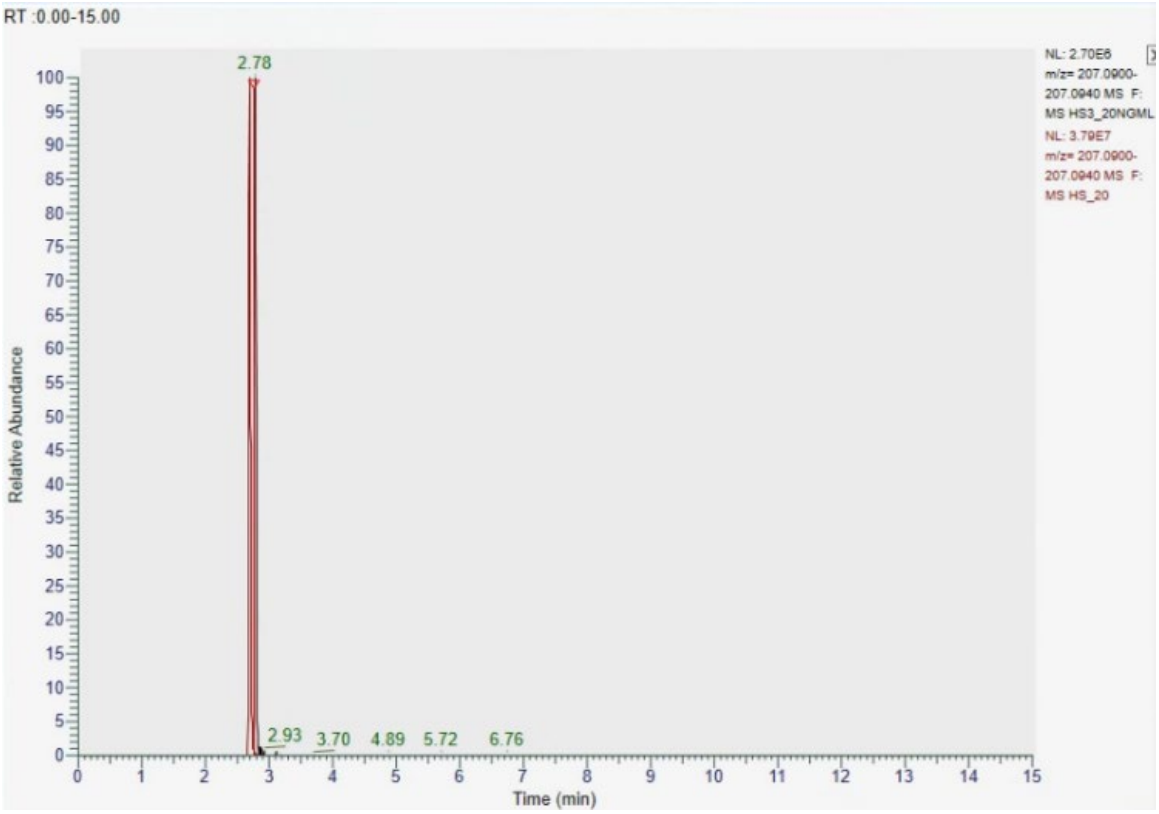


Figure 33: Extracted ion chromatogram of L-tryptophan showed signal intensity of 2.7E6 with dilutional volume of 200ng/ml. The retention time when L-tryptophan was identified at 2.78 minutes for both 200ng/ml and 20ng/ml.

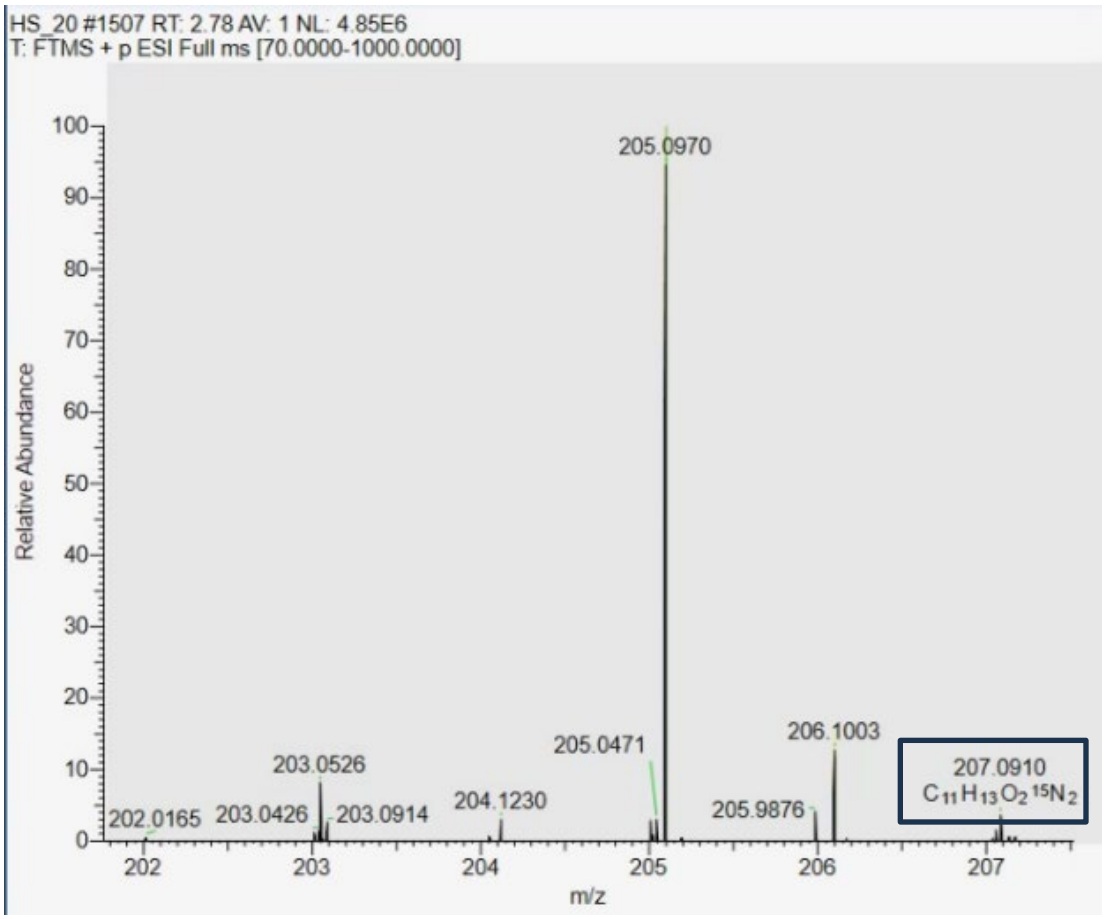


Figure 34: Mass spectrum confirming the mass of L-tryptophan at 207.0910.

7.4 Experiment 4- PAL System for sample handling

Consistent sample handling and preparation is vital to maintain the quality of the results. During the pilot study, it was noted when supernatant was being aliquoted after the centrifuge process, some protein precipitant was transferred and retained in the new 96 deep well plate. To reduce the effect of human error, the PAL sample handling robot named 'Frank' was used (Figure 35). A method was developed using the PAL Sample Control (Chronos) software.

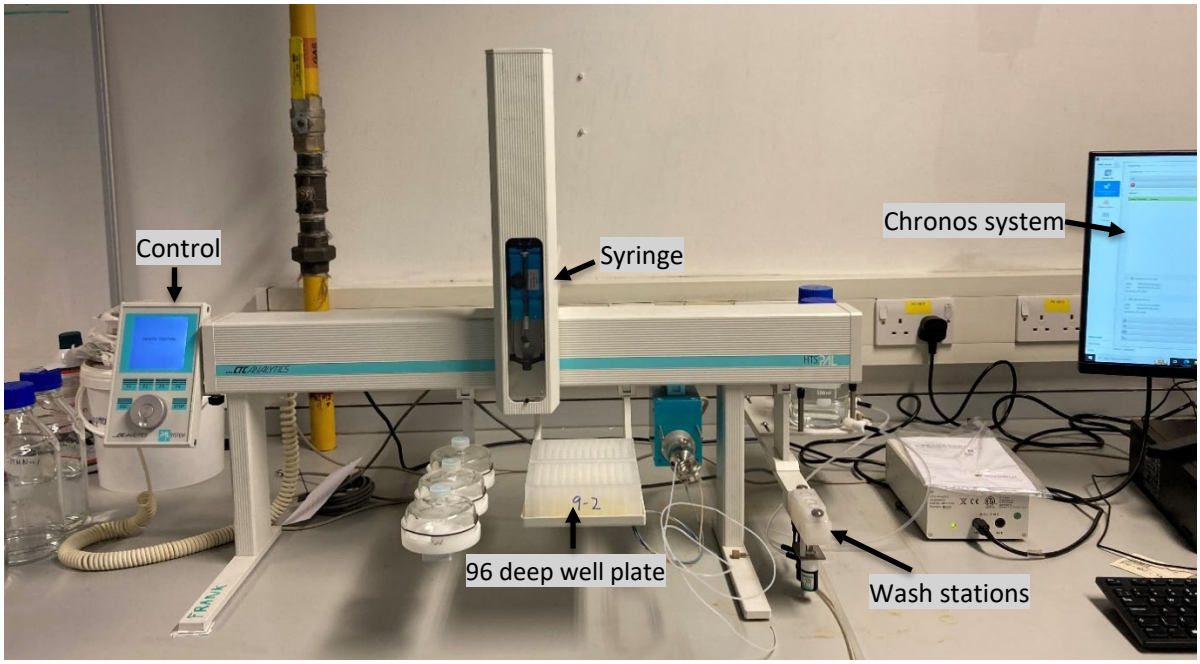


Figure 35: PAL System sample handling robot 'Frank' where the machine aliquots the supernatant from one plate to another.

Needle depth

The needle depth for the PAL system syringe ranges from 30mm to 49mm. As the syringe's needle need to be as low as possible in the 96 deep well plate but at the same time not aspirating protein precipitant, a few trials were conducted. The needle depth of 32mm was found to be the optimal depth for transferring supernatant with minimal protein precipitant.

Human vs PAL System

Comparison was made between human pipetting and PAL System 'Frank' using triplicates of quality-controlled (QC) clinical patient plasma samples. The total number of features and metabolites identified as well as amount of protein precipitant transferred were compared (Table 28).

Table 28: Comparison results of total number of features and metabolites identified between human and PAL system.

Type	Total number of features	Total number of identified metabolites	Signal intensity	Protein precipitant
Human	5792	705	1.07E+09	Present
PAL System	4121	560	1.14E+09	Absent

The comparison showed that the total number of features and identified metabolites were higher when samples were pipetted by human. However, the pump pressure was also higher compared to the PAL system (Figure 36). This has a higher risk of transferring precipitant that may clog the mass spectrometry column causing a high pump pressure leading to termination of analysis. This showed that the PAL System provided a cleaner sample for analysis while maintaining an acceptable number of metabolites identified.

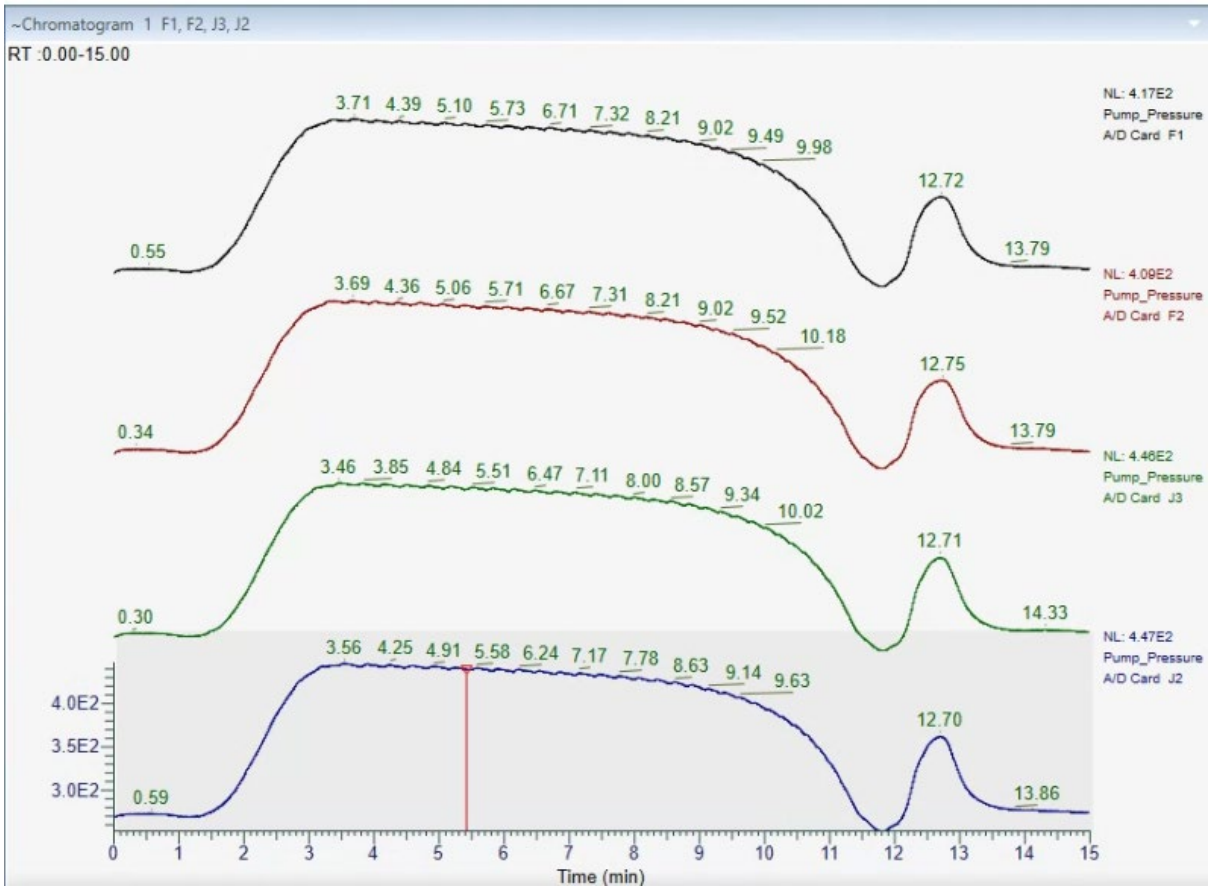


Figure 36: Comparison of pump pressure between human sample preparation and PAL System during the 15 minutes analysis on UHPLC-MS. The top two chromatograms are showing the pump pressure of 2 samples using PAL System (black F1 and red F2) while the bottom two chromatograms are showing the pump pressure of 2 samples pipetted by human (Green J3 and blue J2).

PAL System wash method

The method on the PAL Sample Control (Chronos) software was modified from the provided template. To ensure that no contamination occurred during transfer between samples, three different methods were developed and compared (Table 29).

Method 1: post sample transfer, x1 wash at wash station followed by x2 syringe washes.

Method 2: post sample transfer, x2 wash at wash station followed by x2 syringe washes.

Method 3: post sample transfer, x3 wash at wash station followed by x2 syringe washes.

Table 29: Comparison results of total number of features and identified metabolites between different methods of wash.

Methods	Total number of features	Total number of identified metabolites	Overall signal carryover (%)
1	385	46	11.9%
2	408	53	13.0%
3	466	53	11.4%

For an efficient and clean method, method 3 showed the best overall signal carryover with a clean transfer between samples.

7.5 Experiment 5: Metabolite differences between venous vs arterial samples

Studies have shown that the metabolites in venous system differ slightly from arterial system (299). As samples obtained in ICU and HDU included arterial samples from arterial line, a comparison of number of features and metabolites identified were performed using the protocol for pilot study. The samples were analysed in triplicates (Table 30).

Table 30: Comparison results of total number of features and identified metabolites between venous and arterial samples.

	Total number of features		Total number of identified metabolites	
UHPLC-MS mode	**Positive**	**Negative**	**Positive**	**Negative**
Venous	5467	2393	766	414
Arterial	5787	2593	764	421

The comparison showed that the total number of features and total number of identified metabolites were comparable.

7.6 Final optimised method

To minimise the possibility that analytical batch effects could become confounded with clinical variables, plasma samples were randomly distributed across the 11 analytical batches prior to sample preparation and UHPLC-MS analysis. This approach was intended to distribute patients with different frailty classifications, mortality outcomes and sampling time points across the analytical sequence rather than concentrating particular clinical groups within individual batches. Pooled quality control (QC) samples were analysed regularly throughout each batch to monitor analytical stability and facilitate subsequent batch correction using the QCMXP workflow.

The optimised method used for the analysis of 150 patient samples in 11 batches of 96 deep well plate is summarised below:

400 µL of 99% MeOH were added onto the 96 deep well plate that has been prepped with 100 µL of samples and quality control samples in each well. The deep well plate was then placed on the shaker (speed 750 for 2 minutes) and then centrifuged for 15 minutes at speed 2722 x g at 4 °C. 300 µL of supernatant was transferred to a new 96 deep well plate using the PAL System sample handling using the pre-set

protocol. The new 96 deep well plate containing 300 µL supernatant was placed on the nitrogen evaporator for 1.5 hours. The freeze-dried sample was reconstituted with 100 µL of 99% H_2O with internal standard, placed on shaker for 2 minutes with speed 750 then centrifuged at 4°C with speed 2722 x g for 15 minutes. A total of 85 µL of supernatant was transferred onto another new 96 deep well plate which was then transferred onto UHPLC-MS for analysis.

Chapter 8: Metabolomic characterisation of frailty and post-operative outcomes

Following the clinical analyses in Chapter 4, the emphasis of the thesis shifts from evaluating frailty measures to characterising the metabolomic features associated with frailty and outcome. The overarching aim of this chapter is to advance the development of metabolic markers of frailty in EmLap patients. Building on methodological optimisation and validation described in previous chapters, this section focuses on the identification of novel metabolites that may differentiate frail from non-frail EmLap patients. Having established a robust and reproducible analytical workflow, we now apply this method to large-scale sample analysis in order to uncover the metabolomic signatures associated with surgical vulnerability and physiological reserve.

Following the sample preparation and optimised method used (as demonstrated in chapter 7.6), employing 99% MeOH as extraction solvent and 99% H_20 as reconstitution solvent across 11 batches of 96 deep well plate, UHPLC-MS analysis was performed. The raw data obtained were processed and analysed using Compound Discoverer 3.3 (Thermo Fisher Scientific). The detailed data processing and deconvolution methods have been described in Chapter 5.5.2. The filtered data produced by Compound Discoverer 3.3 was exported to Microsoft Excel, duplicate metabolites were removed and the final dataset underwent batch alignment via QCMXP (Quality Control and Matrix eXtraction Pipeline) software (293), as described in chapter 6.1.

8.1 Batch alignment using QCMXP

The generated untargeted metabolomic raw data files from Compound Discoverer 3.3 (Thermo Fisher Scientific) were imported into QCMXP after the data was arranged in the accepted format, with data and peak files. No missing QCs were present during the pre-filter setting. The QCRSC engine was run where the algorithm corrected and optimised the best fit for each peak. The corrected data was saved

after cleaning using filter (QC-RSD 20%, sample-RSD 30%, D-ratio 40% and blank ratio 1%).

Examination of the batch allocation was consistent that no analytical batch corresponded preferentially with frailty status, mortality group or other major clinical outcomes most likely due to randomisation of the patient samples before analysis. Following QC-based correction using QCMXP, the PCA plots demonstrated improved clustering of pooled QC samples and reduced analytical drift, suggesting that the remaining variation was predominantly biological rather than technical. This provided confidence that subsequent metabolomic analyses reflected patient-related metabolic differences rather than systematic batch effects. However, randomisation and QC clustering do not, in themselves, establish the absence of associations between batch allocation and clinical variables or exclude all residual technical variation. This limitation has been considered in the interpretation of the metabolomic findings. The results of the batch alignments of the 11 batches in this study are shown in Figure 37 (a: pre-correction, b: post-correction).

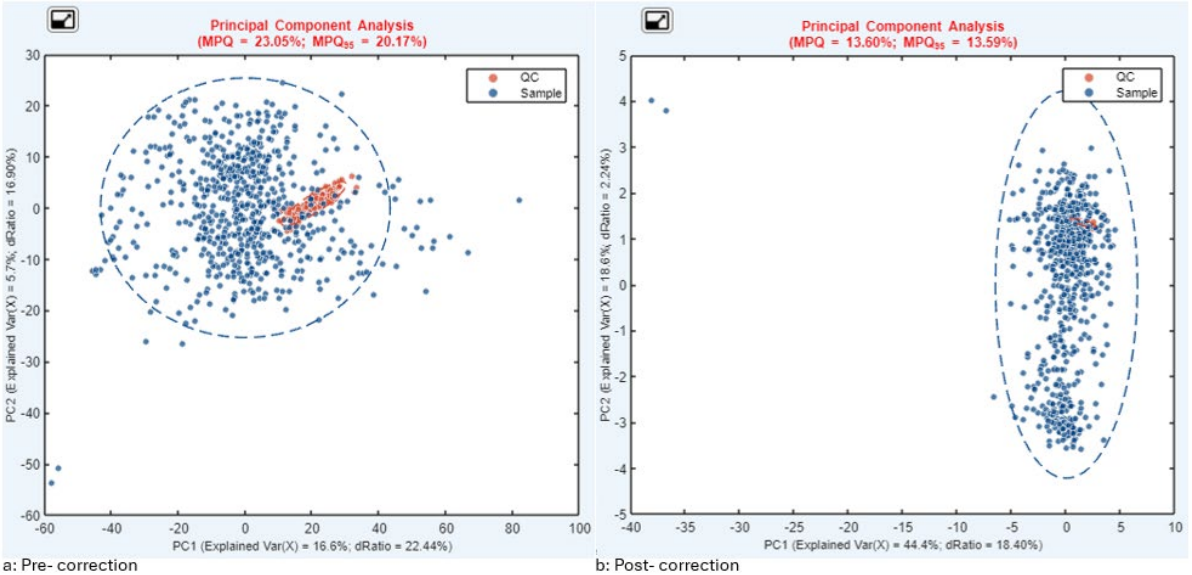


Figure 37: QCMXP result of batch alignment. Figure a: pre-correction PCA with clusters of Quality Control (QC, dots in red) among the samples (blue dots) showing good PCA variance of 16.6%. Figure b: post-correction PCA with clusters of QC among the samples showing relatively good PCA variance of 44.4%.

8.2 Bioinformatics (univariate and multivariate) analysis

Following batch alignment, the results were grouped according to frailty status (non-frail ≤4, frail >4) at baseline and other time points and univariate and multivariate analysis was performed. The bioinformatics statistics for metabolomic data in this chapter were performed by Prof. David Broadhurst (Professor of Biosystems Data Science, Edith Cowan University) with the R Studio Code appended in Appendix 1.14.

Principal Component Canonical Variance Analysis

The metabolomic data collected across the five time points were log- transformed, centred then analysed using Principal Component Canonical Variance Analysis (PC-CVA). PC-CVA is a multivariate technique that combines both the principal component analysis with canonical variance modelling to simultaneously evaluate the metabolomic differences in each group over time while factoring the intercorrelation and reducing dimensionality (300). Raw metabolite intensities are often right- skewed, with variability increasing as the mean increases. Log transformation was therefore applied to reduce right-skewness, stabilise variance and improve the suitability of the data for subsequent statistical modelling and multivariate analysis. It also facilitates the interpretation of differences in terms of relative changes, such as fold changes, rather than absolute differences in intensity. Applying the transformation to all metabolites assumes that a logarithmic scale provides a suitable representation of their distributions and that variability is more appropriately modelled on this scale, consistent with an approximately log-normal distribution and multiplicative error structure. However, this assumption may not hold equally for every metabolite, and log transformation does not guarantee normality or constant variance.

Multivariate Marginal Hierarchical Cluster Analysis (MMHCA)

Multivariate Marginal Hierarchical Cluster Analysis (MMHCA) was performed to identify metabolite groups associated with frailty. This approach was used as it allowed the simultaneous evaluation of multiple correlated metabolites while

accounting for within-cluster dependencies and marginal effects. Metabolite concentrations were log- transformed to reduce skewness and ensure comparability across metabolites. Hierarchical clustering was then performed to organise metabolites into clusters of biologically related features. The marginal modelling step was applied next to estimate associations between each cluster and frailty status while adjusting for potential confounders (age, gender, BMI). By modelling marginal means within each cluster, MMHCA reduces bias from correlation among metabolites and provides more stable estimates compared with univariate analyses. Clusters were evaluated using a combination of dendrogram inspection and statistical criteria (silhouette width) to determine the optimal grouping. Associations between metabolite clusters and frailty outcomes were expressed as effect estimates with 95% confidence intervals. Statistical significance was defined as a two-sided *p*-value < 0.05.

Univariate generalised linear model

The univariate generalised linear model (GLM) was used to analyse the metabolites that were significantly altered at the pre-operative and post-operative day-1, 7, 30 and 90 time points. The patients were divided into groups of (1) non-frail before and after EmLap (NFNF) (2) non-frail before but frail after EmLap (NFF) (3) non-frail before and 90-day mortality (NFM). The metabolic features identified in the UHPLC-MS were log- transformed to normalise the data prior to analysis. The following GLM design was performed over each metabolite to account for repeated measures and potential confounders such as gender, age and BMI and ultimately to assess temporal dynamics of the model:

$$\log(m) = 1 + NFF_{(0,1)} + NFM_{(0,1)} + Male_{(0,1)} + Age + BMI$$

** Baseline model = NFNF, Female, mean(Age), mean(BMI)*

Gender, age and BMI were included as established determinants of the plasma metabolome and of post-operative mortality. Models were fitted sequentially (unadjusted, adjusted for age, and adjusted for age and BMI) to assess the sensitivity of associations to covariate adjustment. Subsequent analysis of variance (ANOVA)

was conducted on the fitted models to evaluate the overarching hypothesis: "Is any group significantly different?" The ANOVA enabled comparison of mean metabolite levels across time points while controlling for intra-subject variability. Multiple testing correction was applied using the Benjamini-Hochberg procedure with a false discovery rate (FDR) threshold of 0.05 to identify statistically significant metabolites.

8.3 Discovery of metabolites associated with post-operative mortality and frailty

Principal Component Canonical Variance Analysis

The PC-CVA was performed to look at how the metabolic profiles change across the different frailty groups over four peri-operative time points (pre-EmLap, post-EmLap day 1, post-EmLap day 30 and post-EmLap day-90) in patients undergoing EmLap (Figure 38). The analysis captured the separation and clustering of groups based on changes of frailty status-frail to frail (FF), non-frail to non-frail (NFNF) and non-frail to frail (NFF) and non-frail to mortality at 90-day (NFM).

The result as shown in the PC-CVA in Figure 38 depicted substantial overlap between several groups across the peri-operative time points, indicating that their multivariate metabolic profiles were not completely distinct at the level of the component shown. However, relative separation of some groups was observed, suggesting differences in their overall metabolic profiles. The group that was frail pre-EmLap occupied a relatively distinct region of the multivariate space, although some overlap with other groups remained. The NFM4 group also occupied a relatively distinct position compared with several of the other groups. This pattern may indicate heterogeneity in the metabolic response within the cohort and raises the possibility of a distinct metabolic subphenotype in the NFM4 group. However, this interpretation should be considered exploratory, as separation in a PC-CVA plot alone cannot establish a biological mechanism or confirm a distinct metabolic trajectory, and validation in a larger independent cohort would be required.

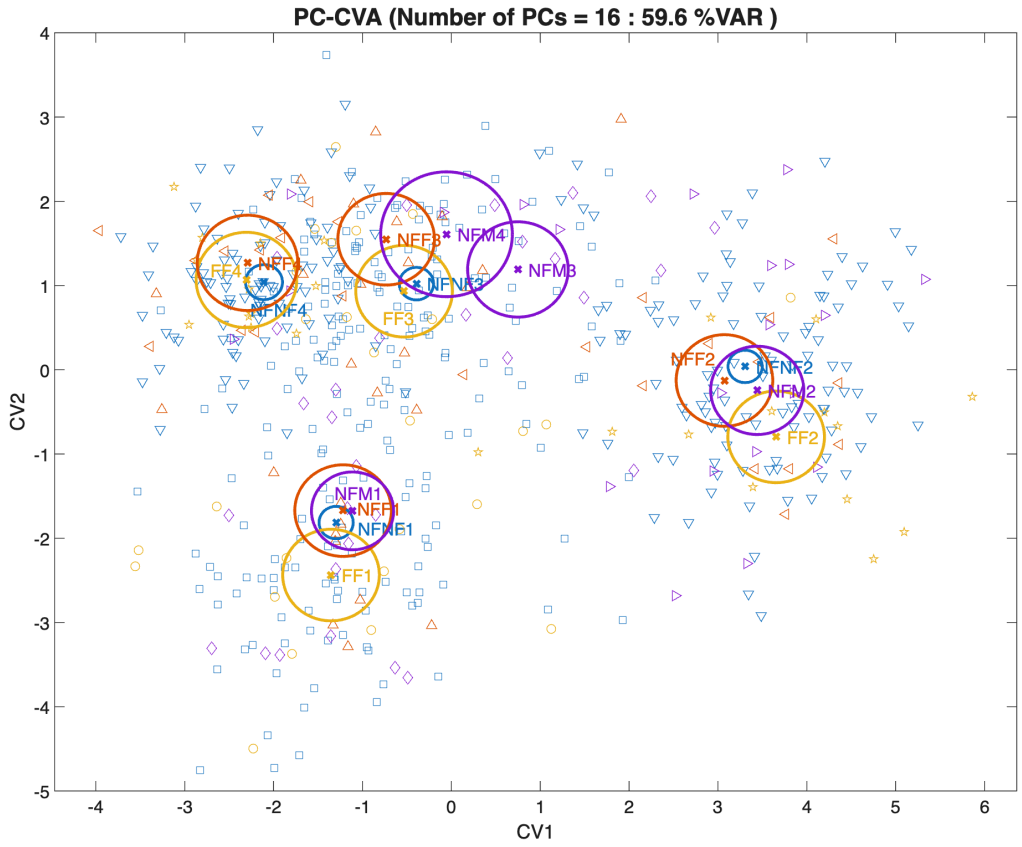


Figure 38: Principal component-canonical variate analysis (PC-CVA) of patient trajectories across four time points. Distinct clusters are observed among the groups apart from non-frail to 90-day mortality (NFM) where there was no progression of clusters beyond the 30-day time point. FF: frail pre- and post-EmLap, NFNF: non-frail pre- and post-EmLap, NFF: non-frail pre-EmLap and frail post-EmLap, NFM: non-frail pre-EmLap and mortality. Time points are denoted as 1: pre-EmLap, 2: post-EmLap day 1, 3: post-EmLap day 30 and 4: post-EmLap day 90. 16 PCAs were retained and total variance was 59.6%.

Multivariate Marginal Hierarchical Cluster Analysis (MMHCA)

The identified metabolites were subsequently classified using Multivariate Marginal Hierarchical Cluster Analysis (MMHCA). The dendrogram (Figure 39) was formed by grouping similar metabolic profiles (labelled A-L), representing metabolites with shared metabolic pathways associated with frailty (Figure 40).

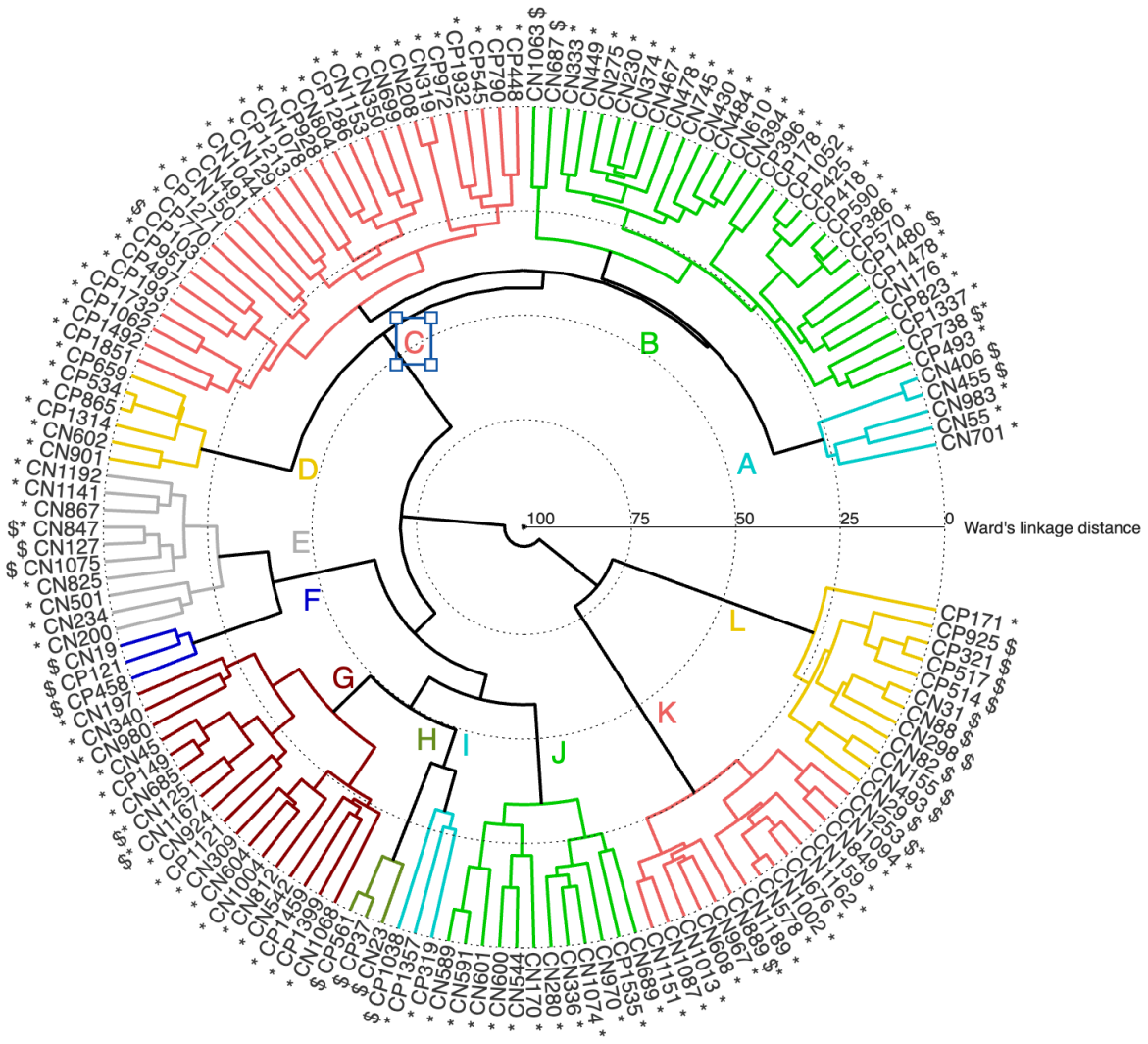


Figure 39: The Multivariate Marginal Hierarchical Cluster Analysis (MMHCA) showing the clustering of significant metabolites labelled A to L with different colours based on similarity of metabolic profiles. The asterisks (*) next to each metabolite suggest p<0.01 for significant difference in outcome across all time points (main effect), $ sign next to each metabolite suggest p<0.01 for significant difference in time and main effect (outcome trajectories significantly diverge across time points and $ sign next to each metabolite suggests significant difference in both. The name of each metabolite are listed in Appendix 1.13.*

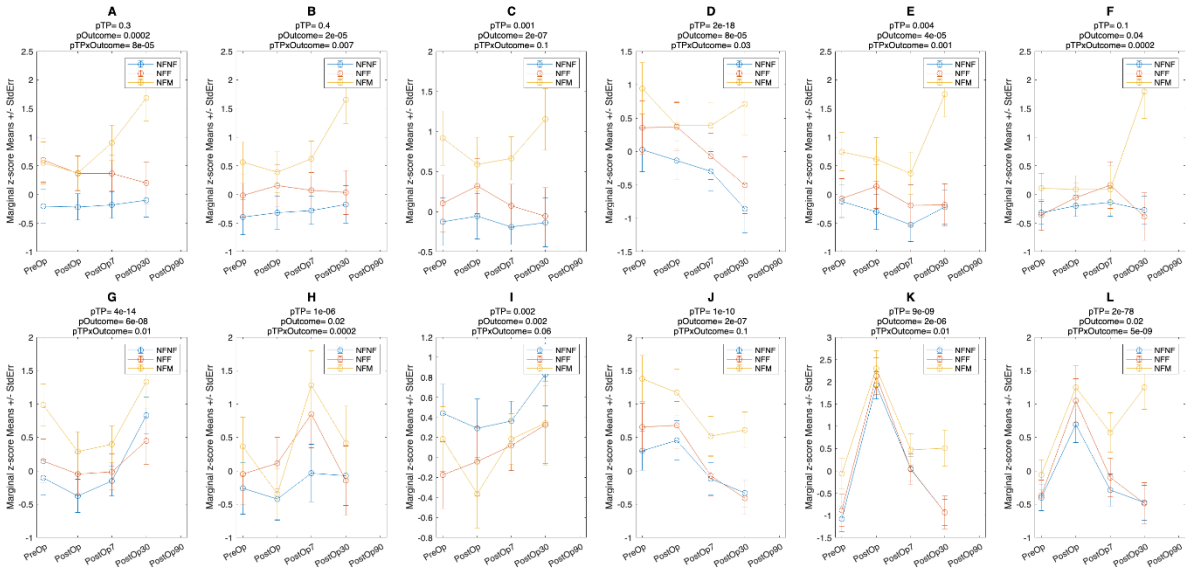


Figure 40: Longitudinal metabolite cluster trajectories in frail, mortality and non-frail groups identified by Multivariate Marginal Hierarchical Cluster Analysis (MMHCA). Panels A- L showed the marginal mean z scores in metabolite abundance relative to baseline across peri-operative time points (pre-op, post-op day 1 to day 90) stratified by frailty outcome changes. Red lines represent the frail group, blue lines represent the non-frail group, and yellow lines represent the mortality group. Error bars indicate standard error of the mean. p-values reflect the overall group differences for each cluster obtained from MMHCA on time (pTp), effect of frailty group (pOutcome) and the time x frailty interaction (pTp x Outcome). Clusters A, B, E, H, and L demonstrated highly significant divergence between frail and non-frail groups, whereas clusters C, I, and J showed no significant differences. These results suggest that frailty is associated with distinct, cluster-specific patterns of metabolic dysregulation over time.

Univariate generalised linear model

When individual metabolites were analysed across transition groups, including non-frail to frail (NFF) and non-frail to non-frail (NFNF), no metabolites reached statistical significance, likely reflecting limited statistical power due to small subgroup sizes. In contrast, analysis of the non-frail to non-frail (NFNF) to non-frail to mortality (NFM) group using a standard generalised linear model (GLM) identified 26 metabolites differed across groups with ANOVA FDR ≤0.05. The number of significant metabolites fell from 26 in the unadjusted comparison to 20 after adjustment for age and 2 after adjustment for age and BMI. The results are

demonstrated in the UpSet plot (Figure 41), which illustrates the overlap between metabolites associated with mortality and key demographic covariates, highlighting the limited number of metabolites that remain associated with adverse outcomes after multivariable adjustment.

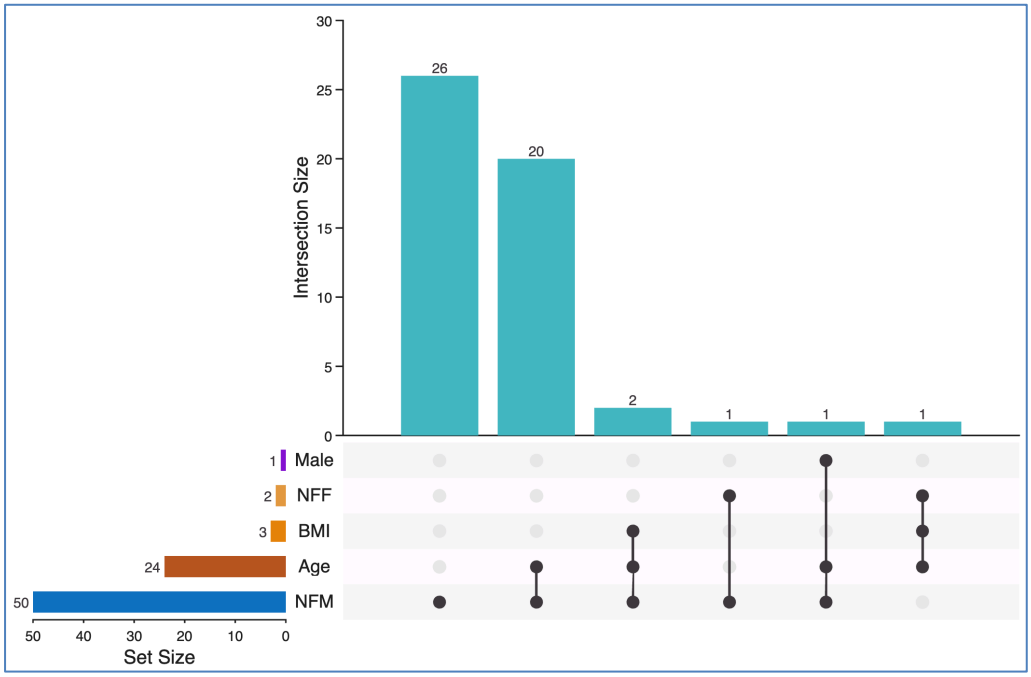


Figure 41: UpSet plot showing the intersections of significant metabolites across demographic and frailty related variables. The horizontal bars on the left indicate the total number of significant metabolites identified in each variable (set size) while the vertical bars represent the number of significant metabolites shared between the combinations of variables. The connected dots below the vertical bars indicate the specific variables involved in each intersection.

The 26 significant metabolites identified are listed in Table 31 and grouped according to their biochemical classes which included acylcarnitine, amino acids, peptides and nucleoside metabolism. For each metabolite, the regression coefficient (β) and standard error (SE) NFM vs NFNF group were reported. The β regression coefficient represents the estimated difference in metabolite abundance between the NFM and NFNF groups, with positive values indicating higher abundance in the NFM group and negative values indicating lower abundance, relative to NFNF. The biochemical pathways of these significant metabolites will be discussed in the next subchapter (Chapter 8.4).

Table 31: The identified metabolites (unadjusted model) with a significant ANOVA FDR (Benjamini-Hochberg) (≤0.05) across the generalised linear model comparing non-frail to non-frail (NFNF) to non-frail to mortality (NFM) group were categorised according to their biochemical classes. The β and standard error (SE) are on the log scale for each comparison against NFNF. Positive β indicates higher abundance than NFNF.

Class	Name of metabolites	ANOVA FDR	*β* (SE)
Acylcarnitine	(2E)-Octenoylcarnitine	0.033	0.252 (0.073)
	(R)-Crotonylcarnitine	0.0300	0.393 (0.114)
	C8-Carnitine (octanoyl-L-carnitine)	0.028	0.276 (0.076)
	Hexanoylcarnitine	0.037	0.237 (0.071)
	O-Suberoylcarnitine	0.033	0.404 (0.120)
Amino acids	(2S,3S,4S)-4-[(2Z,4E,6R)-6-Carboxylato-2,4-heptadien-2-yl]-3-(carboxylatomethyl)-2-pyrrolidiniumcarboxylate	0.003	0.271 (0.060)
	(3S,4S)-4-[(2Z,4E,6R)-6-Carboxy-2,4-heptadien-2-yl]-3-(carboxymethyl)-L-proline	0.003	0.340 (0.074)
	2'-alpha-mannosyl-L-tryptophan	0.026	0.258 (0.072)
	3-indole carboxylic acid glucuronide	0.008	0.459 (0.112)
	Ethyl L-methioninate	0.007	0.434 (0.104)
	N-Acetylvanilalanine	0.003	0.461 (0.101)
Peptides	Gamma-Glutamyl-N5-acetylornithine	0.049	0.289 (0.091)
	Hippuric acid	0.001	0.593 (0.120)
	L-Isoleucyl-L-tryptophan	0.026	-0.250 (0.076)
	Ophthalmic acid	0.031	0.382 (0.112)
	Phe-Tyr-Asp	0.043	0.248 (0.082)
Nucleoside	Uridine	0.002	0.269 (0.057)
	Dihydrouridine	0.012	0.236 (0.062)
	N2-Dimethylguanosine	0.017	0.276 (0.071)
	6-Hydroxypropylthymine	0.019	0.247 (0.074)
	1-(beta-D-ribofuranosyl)thymine	0.025	0.265 (0.072)
	Succinyladenosine	0.025	0.322 (0.087)
	1-Methylinosine	0.029	0.285 (0.079)
	Xanthosine	0.027	0.280 (0.080)
	5-Carboxymethylaminomethyluridine	0.027	0.252 (0.071)
	1-Methylguanine	0.036	0.148 (0.047)

Collectively, these findings suggest a distinct metabolic profile among emergency laparotomy patients who were non-frail pre-operatively but died within 90 days. No metabolites differed for subsequent frailty, so these data do not show a frailty-specific signature.

8.4 Discussion

An important finding of this study was that the significant metabolomic differences associated with adverse outcomes were observed in the non-frail to mortality group. This suggests that metabolic alterations associated with vulnerability following EmLap may not be fully captured by conventional clinical frailty assessment. Although the CFS provides a pragmatic assessment of baseline functional status and dependence, it may not capture all dimensions of physiological reserve, metabolic dysfunction or the capacity to withstand the substantial stress imposed by emergency surgery.

Importantly, further examination of the non-frail to mortality group revealed that 14 of the 20 patients who had 90-day mortality (70%) had a pre-operative CFS score of 4. Although these patients were classified as non-frail for the purposes of this study, CFS 4 represented a pre-frail category in the study's classification framework. This finding is important when interpreting the metabolic signatures associated with mortality, as the non-frail to mortality group was not composed predominantly of patients with the lowest levels of clinically assessed frailty. Instead, most patients who died had some degree of baseline vulnerability, despite not meeting the study's threshold for frailty. Consequently, the metabolic differences identified in the non-frail to mortality group may partly reflect the biological characteristics of patients with pre-frailty who subsequently died, rather than a distinct metabolic signature of mortality arising exclusively in clinically robust individuals.

One possible explanation is the presence of subclinical biological vulnerability in patients who appear non-frail according to the CFS. Such vulnerability may include

early metabolic dysregulation, impaired mitochondrial energy metabolism, altered amino acid utilisation or disturbances in lipid metabolism that precede clinically apparent functional decline. The predominance of patients with CFS 4 in the non-frail to mortality group also raises the possibility that the dichotomisation of CFS scores into frail and non-frail categories may have obscured clinically relevant variation in baseline vulnerability. The metabolic signatures identified in this study may therefore reflect aspects of biological vulnerability that are not readily apparent through a predominantly clinical assessment of frailty. Alternatively, these signatures may represent an individual's response to acute illness and surgical stress, underlying comorbidity or other pathophysiological processes associated with mortality, rather than frailty specifically. These explanations are not mutually exclusive, as pre-existing vulnerability may interact with the physiological demands of EmLap to influence post-operative outcomes.

These findings raise the possibility that metabolomic profiling could complement established clinical frailty assessment by providing additional information about biological vulnerability in patients who do not meet conventional CFS criteria for frailty. In this context, metabolomics may offer a means of identifying patients who appear clinically robust but nevertheless have limited physiological reserve or an altered metabolic response to acute surgical stress. However, given that most patients in the non-frail to mortality group had a CFS score of 4, the present findings may be more appropriately interpreted as reflecting vulnerability within a group classified as non-frail, rather than definitive evidence that metabolomics identifies hidden frailty in otherwise robust patients. The present findings do not establish that the CFS misclassified these patients as non-frail, nor do they demonstrate that the identified metabolites are specific biomarkers of frailty. The relatively small group sizes and the attenuation of metabolite associations following adjustment for age and BMI further warrant caution as it may reflect confounding by age and BMI, but it could equally reflect adjustment for variables on the causal pathway, collinearity between age, BMI and mortality status, or reduced power. The present data cannot distinguish between these explanations, and the loss of significance should not be taken as evidence that the unadjusted associations are spurious.

Consequently, these findings should be considered hypothesis-generating and require validation in larger, independent cohorts with detailed clinical, functional and longitudinal outcome assessments.

Taken together, these findings support the hypothesis that vulnerability following emergency laparotomy extends beyond clinically defined frailty and may involve biological processes that are not fully represented by the CFS. Integrating clinical frailty assessment with metabolic profiling may therefore offer a promising avenue for investigating this multidimensional vulnerability, although its incremental predictive value and clinical utility remain to be established.

Acylcarnitine metabolites

The acylcarnitine metabolites identified in this study collectively suggest disruption of mitochondrial fatty acid oxidation and impaired cellular energy metabolism. This bioenergetic dysfunction may be associated with vulnerability leading to post-operative mortality, and possibly frailty. Table 32 summarises the significant acylcarnitine metabolites identified and their biological relevance.

Table 32: The significant acylcarnitine metabolites identified with summary of relevance.

Name of metabolites	Relevance	ANOVA FDR	*β* (SE)
(2E)-Octenoylcarnitine	Medium chain acylcarnitine; accumulates with disrupted mitochondrial ß-oxidation.	0.033	0.252 (0.073)
(R)-Crotonylcarnitine	Short chain acylcarnitine; suggests altered fatty acid oxidation though not well studied in frailty.	0.0300	0.393 (0.114)
C8-Carnitine (octanoyl-L-carnitine)	Elevated in frailty cohorts; marker of impaired medium chain ß-oxidation and energy metabolism.	0.028	0.276 (0.076)
Hexanoylcarnitine	Short/ medium chain marker of mitochondrial dysfunction and metabolic stress.	0.037	0.237 (0.071)
O-Suberoylcarnitine	Longer chain acylcarnitine; reflects similar mitochondrial fatty acid oxidation disturbances.	0.033	0.404 (0.120)

This study supported an association between medium-chain and unsaturated acylcarnitine and subsequent mortality or subclinical frailty, consistent with findings from previous metabolomic studies of ageing and frailty (225, 301-303) including those found by Rattray et al (223) and the FRAILOMIC consortium (227) that associated these metabolites with frailty. The five dysregulated acylcarnitine identified (Table 32) could reflect incomplete mitochondrial ß-oxidation of fatty acids and impaired metabolic flexibility causing vulnerability in patients (225, 303, 304).

Under normal physiological conditions, mitochondrial ß-oxidation is essential for fatty acid breakdown and ATP generation, with acylcarnitine facilitating the transport of long-chain fatty acids into the mitochondrial matrix (305). However, mitochondrial dysfunction leads to accumulation of acylcarnitine species, signalling bottlenecks in mitochondrial transport or ß-oxidation enzyme activity (306, 307). In vulnerable individuals, this metabolic signature may suggest a systemic decline in mitochondrial oxidative capacity and diminished ATP production, forcing a compensatory shift towards glycolytic metabolism (223, 308).

Accumulation of acylcarnitine has also been associated with heightened oxidative stress, insulin resistance, and systemic inflammation (309, 310), processes that may compromise physiological reserve. Importantly, these metabolic changes may provide a biological explanation for several clinical features of frailty phenotype, including fatigue, muscle weakness and reduced physiological resilience. In the EmLap setting, where patients experience profound inflammatory and metabolic stress, impaired mitochondrial energy production may limit the ability to meet the increased metabolic demands of surgery. Consequently, frail individuals may demonstrate reduced capacity to respond to surgical insult, contributing to delayed recovery, impaired tissue repair, and increased susceptibility to post-operative complications.

Collectively, these findings are compatible with the hypothesis that frailty reflects a state of bioenergetic insufficiency or metabolic crisis. Mitochondrial dysfunction and impaired lipid oxidation contribute to muscle weakness, fatigue, and increased susceptibility to physiological stressors, which are hallmark features of the frailty phenotype (223, 302, 303, 311). As acylcarnitines are readily measurable plasma biomarkers, they may warrant evaluation for peri-operative risk stratification when integrated with functional, inflammatory, and clinical frailty measures.

Given the consistent association between carnitine dysregulation and mitochondrial dysfunction, the therapeutic potential of carnitine supplementation to enhance mitochondrial efficiency has been explored (312). However, pre-clinical studies on mouse models have demonstrated that excessive levels of carnitine may promote atherosclerosis through the generation of pro-atherogenic metabolites (313), raising concerns about unregulated supplementation, especially in the frail cohort who may already exhibit endothelial dysfunction and chronic inflammation. Two randomised placebo-controlled trial in community dwelling older adults have reported beneficial effects of carnitine supplementation on fatigue, muscle mass, frailty index scores, and hand-grip strength (314, 315). While these findings suggest that carnitine metabolism may represent a promising therapeutic avenue, any intervention aimed at modifying carnitine levels in the EmLap group must carefully balance the metabolic benefits against potential cardiovascular risk.

Amino acids metabolites

The amino acid metabolites identified in this study highlight disruptions in amino acid metabolism, microbiome-host interactions, and redox regulation, suggesting that impaired substrate utilisation and metabolic signalling may be associated with the biological basis of vulnerability that led to mortality, or possibly frailty. Table 33 summarises the significant amino acid metabolites identified and their biological relevance.

Table 33: The significant amino-acids metabolites identified with summary of relevance.

Name of metabolites	Relevance	ANOVA FDR	*β* (SE)
(2S,3S,4S)-4-[(2Z,4E,6R)-6-Carboxylato-2,4-heptadien-2-yl]-3-(carboxylatomethyl)-2-pyrrolidiniumcarboxylate	Highly functionalised proline derived zwitterion; may indicate altered proline/ornithine pathways and lipid amino acid conjugation.	0.003	0.271 (0.060)
(3S,4S)-4-[(2Z,4E,6R)-6-Carboxy-2,4-heptadien-2-yl]-3-(carboxymethyl)-L-proline	Stereo defined proline conjugate with polyunsaturated side chain; suggests coupling of proline metabolism to fatty acid chemistry.	0.003	0.340 (0.074)
2'-alpha-mannosyl-L-tryptophan	Glycosylated tryptophan; reflects modified tryptophan metabolism and potential host–microbiome glyco-conjugation.	0.026	0.258 (0.072)
3-indole carboxylic acid glucuronide	Gut microbiome–derived tryptophan catabolite conjugated for clearance; marks microbiota–host tryptophan flux.	0.008	0.459 (0.112)
Ethyl L-methioninate	Ethyl ester of methionine; a non-endogenous antimetabolite suggests altered methionine handling or exposure to esterified metabolites.	0.007	0.434 (0.104)
N-Acetylvanilalanine	N-acetylated alanine/vanillic acid hybrid; signals amino acid acetylation or phaseII metabolism alterations.	0.003	0.461 (0.101)

Among the identified 6 dysregulated amino acids metabolites (Table 33), two were complex proline derivatives. These conjugates play important roles in collagen turnover and tissue repair, linking proline metabolism to lipid peroxidation pathways (225, 316, 317). When altered, these pathways may impair extracellular matrix maintenance and tissue remodelling, thereby potentially contributing to oxidative damage and reduced regenerative capacity in these patients.

Alterations in tryptophan metabolism were also observed, consistent with previous studies (220, 225, 301). Tryptophan serves as a precursor for kynurenine and indole derivatives, which regulate immune tolerance, neuroinflammation and oxidative stress (318). The presence of conjugated forms such as 2′-α-mannosyl-L-tryptophan and 3-indolecarboxylic acid glucuronide suggests increased phase II detoxification and microbiota-mediated tryptophan catabolism (318). These findings are notable because growing evidence links alterations in the gut microbiome to systemic

inflammation, sarcopenia, and metabolic dysregulation, all core components of frailty (208). Disruption of microbiome-derived tryptophan metabolites may therefore represent an additional pathway through which frailty alters immune regulation and metabolic homeostasis.

Additional metabolites, including ethyl-L-methioninate and N-acetylvanilalanine, suggest disruption of one-carbon metabolism, methylation balance, and detoxification pathways (220). Methionine plays a central role in methylation reactions and glutathione synthesis, and impaired methionine handling may compromise redox buffering, epigenetic regulation, and cellular repair mechanisms, thereby accelerating senescence and inflammation (220, 319). Inflammation has long been hypothesised as a contributor to frailty pathogenesis, prompting investigations of anti-inflammatory therapies. Aspirin has shown no benefit in frailty prevention or treatment (320, 321), while, glucocorticoid, despite well recognised risks including infection, osteoporosis and insulin resistance (322), have shown preliminary improvements in frailty measures in selected populations (323). These findings reinforce the complexity of targeting inflammatory pathways in frailty and underscore the need for biologically informed, precision-based interventions.

Together, these amino acid dysregulations suggest reduced substrate availability for muscle maintenance and mitochondrial function, compatible with a frailty model driven by oxidative stress, impaired detoxification capacity, and microbiome host metabolic dysfunction. Such disturbances may contribute to key clinical manifestations of frailty, including sarcopenia, immune dysregulation, and impaired tissue repair. In the peri-operative context, such metabolic disturbances may limit the ability to sustain anabolic processes required for wound healing and recovery following EmLap, thereby increasing susceptibility to post-operative complications and prolonged functional decline. These findings also complement the mitochondrial energy deficits reflected in the acylcarnitine profile, suggesting that coordinated disruption of both energy production and substrate utilisation pathways leads to adverse outcomes. Collectively, this integrated metabolic dysfunction may reduce

the physiological resilience of vulnerable patients when exposed to acute physiological stressors such as major surgery.

Peptide- related metabolites

The peptide- related metabolites identified in this study are consistent with dysregulation of glutathione metabolism, detoxification pathways, and proteostasis, suggesting that impaired redox balance and protein turnover may be associated with vulnerability to adverse outcomes. Table 34 summarises the significant peptide-related metabolites identified and their biological relevance.

Table 34: The significant peptide- related metabolites identified with summary of relevance.

Name of metabolites	Relevance	ANOVA FDR	*B* (SE)
Gamma-Glutamyl-N5-acetylornithine	Marker of gamma glutamyl cycle activity; links to GGT, glutathione flux and metabolic stress.	0.049	0.289 (0.091)
Hippuric acid	Gut microbiome–diet metabolite; lower levels associate with frailty and reduced fruit/vegetable intake.	0.001	0.593 (0.120)
Ophthalmic acid	Glutathione analogue; indicator of hepatic GSH depletion and impaired antioxidant capacity.	0.031	0.382 (0.112)
L-Isoleucyl-L-tryptophan	Reflects essential amino acid availability (isoleucine, tryptophan); linked to muscle, cognition, redox status.	0.026	-0.250 (0.076)
Phe-Tyr-Asp	Small peptide reflecting proteolysis and altered aromatic/acidic amino acid metabolism reported in frailty.	0.043	0.248 (0.082)

The five significant peptide- related metabolites identified (Table 34) were closely linked to glutathione metabolism and phase II conjugation pathway, both of which are critical for detoxification and defence against oxidative stress (208, 324-328). Gamma-Glutamyl-N5-acetylornithine suggests increased activity of the gamma- glutamyl cycle and altered ornithine acetylation, reflecting shifts in amino

acid transport, glutathione precursor flux, or hepatic metabolic capacity. Hippuric acid, a major gut microbiota derived metabolite, is associated with metabolic resilience and antioxidant capacity (324-326). Dysregulation of this metabolite have been linked to gut dysbiosis, metabolic syndrome, and frailty (325, 326). Ophthalmic acid, a glutathione analogue formed under cysteine-deficient conditions, has been proposed as a marker of oxidative stress and depleted glutathione reserves. Its elevation suggests compensatory upregulation of antioxidant pathways in response to oxidative burden, consistent with mitochondrial dysfunction in frailty (208, 327). The presence of short peptides such as L-isoleucyl-L-tryptophan and Phe-Tyr-Asp may reflect enhanced proteolysis or incomplete peptide degradation, possibly reflecting disrupted proteostasis, muscle catabolism, and altered amino acid recycling (208, 216, 302, 328).

From a clinical perspective, these metabolic changes may underpin key aspects of the frailty phenotype, including muscle wasting, reduced oxidant capacity, and impaired recovery from physiological stress. During major surgical stress such as EmLap, impaired redox homeostasis and proteostatic imbalance may exacerbate tissue injury, delay wound healing, and contribute to organ dysfunction causing frailty and subsequent deaths.

Nucleoside metabolites

The nucleoside metabolites identified in this study suggest increased nucleic- acid turnover and genomic instability, possibly reflecting accelerated cellular ageing, with relevance to frailty yet to be established. Table 35 summarises the significant nucleoside metabolites identified and their biological relevance.

Table 35: The significant nucleoside metabolites identified with summary of relevance

Name of metabolites	Relevance	ANOVA FDR	*B* (SE)
Uridine	Uracil nucleoside; implicated in pyrimidine metabolism and cellular ageing.	*0.002*	*0.269 (0.057)*
Dihydrouridine	tRNA modification; marker of RNA turnover and stress.	0.012	*0.236 (0.062)*
N2-Dimethylguanosine	tRNA-derived methylated nucleoside; elevated in some disease states.	0.01*7*	*0.276 (0.071)*
6-Hydroxypropylthymine	Modified thymine; candidate marker of RNA/DNA oxidative damage.	0.01*9*	*0.247 (0.074)*
1-(beta-D-ribofuranosyl)thymine	Thymidine nucleoside involved in DNA metabolism.	0.025	*0.265 (0.072)*
Succinyladenosine	Reported in frailty metabolomic studies; linked to altered purine metabolism.	0.025	*0.322 (0.087)*
1-Methylinosine	Methylated inosine; tRNA modification associated with metabolic disturbance.	0.02*9*	*0.285 (0.079)*
Xanthosine	Ribose-conjugated xanthine; purine nucleoside linked to nucleotide turnover.	0.02*7*	*0.280 (0.080)*
5-Carboxymethylaminomethyluridine	Modified uridine reflecting RNA metabolism and turnover.	0.02*7*	*0.252 (0.071)*
1-Methylguanine	tRNA methylation product identified in frailty studies; linked to mitochondrial/carbohydrate metabolism.	0.03*6*	*0.148 (0.047)*

A distinct set of 10 nucleoside derivatives (Table 35) were significantly associated with post-operative mortality. These modified pyrimidine and purine nucleosides accumulate as a result of increased RNA and DNA turnover, defecting processing, and oxidative damage (228, 329). The presence of methylated and carboxymethylated, including 5-carboxymethylaminomethyluridine and 1-methylguanine, suggests enhanced RNA modification or degradation, potentially reflecting epigenetic drift and defective tRNA processing (319, 330). Such changes are consistent with accelerated biological ageing, characterised by defective repair mechanisms, genomic instability, and reduced translational efficiency. Furthermore, Succinyladenosine, a purine intermediate, may signal disruption of adenine nucleotide turnover and mitochondrial nucleotide metabolism, further underscoring bioenergetic instability in frailty (225).

These nucleoside alterations may therefore represent molecular footprints of cumulative cellular damage and impaired DNA repair capacity in patients who subsequently died. In the context of surgical stress, such genomic instability may compromise the ability of tissue to repair and regenerate following injury, potentially contributing to poorer recovery trajectories and increased mortality risk following EmLap.

Although previous studies have not directly linked nucleoside metabolites to frailty, nucleoside reverse transcriptase inhibitors (NRTI), used in oncology and human immunodeficiency virus (HIV) treatment, have been shown to slow ageing processes in animal models (331). Validation of these nucleoside metabolites alongside cellular markers of genomic damage may enable their use in risk stratification and inform novel therapeutic strategies aimed at improving surgical resilience.

Integrative metabolic interpretation

Taken together, the metabolomic signatures identified in this study may be associated with coordinated disruption across multiple interconnected biological systems. Acylcarnitine accumulation suggests impaired mitochondrial energy metabolism; amino acid abnormalities suggest altered substrate availability and microbiome-host metabolic interactions; peptide-related metabolites highlight oxidative stress and impaired detoxification pathways; and nucleoside derivatives reflect accelerated molecular turnover and genomic instability.

Collectively, these pathways may converge on a shared biological theme: reduced metabolic resilience. This diminished capacity to maintain energy homeostasis, repair cellular damage, and regulate inflammatory responses may explain why patients experience disproportionate physiological deterioration following acute stressors such as emergency surgery leading to death.

8.5 Conclusion

This study suggests that vulnerability to post-operative mortality, and possibly frailty, is associated with systemic metabolic dysregulation characterised by bioenergetic insufficiency, impaired redox (reduction and oxidation) homeostasis, disrupted proteostasis, and altered molecular turnover. These findings extend current understanding of frailty beyond a purely clinical construct, raising the possibility that it is a biologically heterogeneous syndrome with distinct metabolic signatures. In the clinical context, integration of these biochemical markers with established frailty assessment tools may refine peri-operative risk stratification and enable more personalised surgical care. Although therapeutic strategies in this area remain underdeveloped, the metabolic pathways identified here suggest potential avenues for intervention. For example, pharmacological approaches aimed at improving mitochondrial function and cellular energetics, such as metformin, modulation of oxidative stress pathways, or agents targeting metabolic and inflammatory signalling have all been explored in ageing and frailty related biology and may warrant consideration in the peri-operative setting as potential therapeutic targets for modifying frailty trajectories.

While these possibilities remain hypothesis generating, they illustrate how metabolic characterisation of frailty may ultimately inform targeted therapeutic strategies. Further validation in larger, multicentre cohorts with longitudinal sampling will be required to confirm these metabolic findings as candidate biomarkers. Such work will be essential to translate metabolomic discovery into clinically actionable strategies aimed at improving outcomes for frail EmLap patients.

Chapter 9: Quality of life post-EmLap

In this study, quality of life was assessed using the EQ-5D-5L on post-EmLap day-30 and day-90 to evaluate patient recovery and the effect of EmLap. Of the 150 recruited patients, 115 completed the questionnaires at day-30. The remaining 35 patients were excluded from the day-30 analysis because 13 remained in hospital, 13 had died and nine were lost to follow-up. At day 90, 119 patients completed the questionnaire, while the remaining 31 patients were excluded because 20 had died and 11 were lost to follow-up. A total of 108 patients completed the questionnaires at both time points and were therefore available for longitudinal analysis. The quality-of-life analyses consequently represent a selected subgroup of patients who survived and were able to participate in follow-up assessments. Patients who died were necessarily excluded, while those who remained in hospital at day 30 or were lost to follow-up were also not represented in the respective analyses. This may introduce selection and survivor bias, particularly if patients with greater illness severity, frailty or poorer functional outcomes were less likely to complete the assessments. Nevertheless, these data provide valuable insight into the recovery and quality of life of patients who survived emergency laparotomy and remained available for follow-up. The findings should therefore be interpreted in this context and should not be assumed to represent the quality of life of the entire recruited cohort.

On day post-EmLap day-30, majority of the patients reported no limitations in mobility, self-care and anxiety/ depression. These trends persisted on day-90, indicating stability in these domains of health. There was a significant improvement in patients performing usual activities and managing pain/ anxiety when comparing results from day-30 to day-90. This suggests a positive trajectory of recovery over the follow-up period. The EQ-VAS score, which ranged from 10 to 100, also reflected variability in patient's self-rated health. The median EQ-VAS score improved from 70 on day-30 to 80 on day-90, indicating an overall enhancement in perceived health as patients progressed in their recovery (Table 36). A comparison of EQ-VAS score is illustrated in Figure 42.

Table 36: Result of EQ-5D-5L score including the EQ-VAS score on 30-day and 90-day post-EmLap.

EQ-5D-5L score	30-day (N= 115) %	90-day (N= 119) %
Mobility		
No	71 (61.7%)	79 (66.4%)
Slight	27 (23.5%)	18 (15.1%)
Moderate	7 (6.1%)	13 (10.9%)
Severe	9 (7.8%)	7 (5.9%)
Unable	1 (0.9%)	2 (1.7%)
Self-care		
No	91 (79.1%)	100 (84.0%)
Slight	17 (14.8%)	10 (8.4%)
Moderate	4 (3.5%)	7 (5.9%)
Severe	2 (1.7%)	2 (1.7%)
Unable	1 (0.9%)	0
Usual activities		
No	26 (22.6%)	64 (53.8%)
Slight	46 (40.0%)	22 (18.5%)
Moderate	23 (20.0%)	17 (14.3%)
Severe	10 (8.7%)	6 (5.0%)
Unable	10 (8.7%)	9 (7.6%)
Pain/ discomfort		
No	44 (38.3%)	62 (52.1%)
Slight	48 (41.7%)	38 (31.9%)
Moderate	18 (15.7%)	13 (10.9%)
Severe	5 (4.3%)	6 (5.0%)
Extreme	0	0
Anxiety/ depression		
No	83 (72.2%)	85 (71.4%)
Slight	16 (13.9%)	23 (19.3%)
Moderate	11 (9.6%)	7 (5.9%)
Severe	5 (4.3%)	3 (2.5%)
Extreme	0	1 (0.8%)
EQ-VAS score (median)	70	80

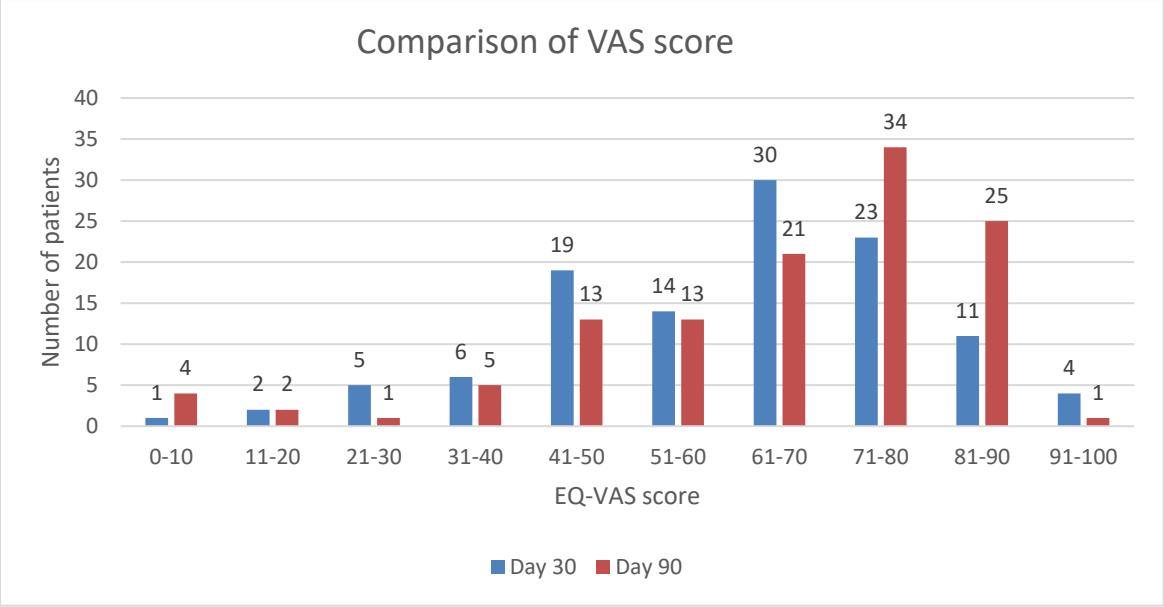


Figure 42: Comparison of EQ-VAS score between day-30 and day-90 post-EmLap.

9.1 Day-30 quality of life

At day-30 post-EmLap, 115 participants completed the EQ-5D-5L questionnaire. The median age of this group was 64 years (range: 40-91 years). The demographic data of the participants who responded to the questionnaire is detailed in Table 37.

The relationship between the five domains of the questionnaire at day-30 post-EmLap and demographic data revealed significant associations in several areas (Table 37):

- Frailty: Frail patients showed significant associations with three domains: mobility, self-care and usual activities.
- 30-day readmissions: Patients who required readmission within 30 days were significantly associated with difficulties in mobility, usual activities, and anxiety/ depression.
- Change in home environment: Patients requiring changes in their home care arrangements reported significant challenges with mobility and self-care.
- 30-day morbidity: Patients with post-operative complications were significantly associated with difficulties in usual activities and increased anxiety.

- Socioeconomic status: Patients from deprived areas showed no significant associations with any of the five domains.

Table 37: Univariate analysis (ANOVA) comparing variables in demographic data of all 115 emergency laparotomy patients post-EmLap day-30 in 5 domains of EQ-5D-5L questionnaire.

Demographics	N (%)	Mobility	Self-care	Usual activities	Pain/ discomfort	Anxiety/ depression
Gender Male Female	 51 (44.3%) 64 (55.7%)	p= 0.449	p= 0.204	**p= 0.022**	p= 0.253	p= 0.506
Scottish Index of Multiple Deprivation Score 1-2 (deprived) Score 3-5 (Not deprived)	 56 (48.7%) 59 (51.3%)	p= 0.502	p= 0.395	p= 0.435	p= 0.933	p= 0.133
Rockwood frailty Scale Non-frail (score ≤4) Frail (score >4)	 104 (90.4%) 11 (9.6%)	**p= 0.035**	**P< 0.001**	**p= 0.016**	p= 0.513	p= 0.642
30-days readmission	12 (10.4%)	**p= 0.003**	p= 0.428	**p< 0.001**	p= 0.153	**p< 0.001**
Discharge destinations Home Home with increased package of care Home with new package of care Care home	 100 (87.0%) 13 (11.3%) 1 (0.9%) 1 (0.9%)	**p= 0.036**	**p= 0.013**	p= 0.576	p= 0.829	p= 0.798
30-days morbidity	36 (31.3%)	p= 0.738	p= 0.322	**p= 0.002**	p= 0.787	**p= 0.008**

9.2 Day-90 quality of life

At day-90 post-EmLap, there were 119 participants who completed the EQ-5D-5L questionnaire. The median age of respondents was 65 years (range: 40-91 years). The demographic data of the participants who responded to the questionnaire is presented in Table 38.

The relationship between the five domains of the questionnaire at day-90 post-EmLap and demographic data were analysed, and it demonstrated significant associations in several areas:

- Frailty: Frail patients showed significant associations with four domains: mobility, self-care, usual activities and anxiety.
- Change in home environment: Patients requiring changes in home care arrangements reported significant challenges with mobility.
- 30-days morbidity: Post-operative complications at day-30 significantly associated with mobility, difficulties in usual activities and increased anxiety on day-90.
- 30-days readmissions: Patients who required readmissions within 30 days demonstrated significant associations mobility, self-care, usual activities and pain/ discomfort on day-90.
- Socioeconomic status: Similar to day-30, patients from deprived areas showed no significant associations with any of the EQ-5D-5L domains.

Table 38: Univariate analysis comparing variables in demographic data of all 119 emergency laparotomy patients post-EmLap day-90 in 5 domains of EQ-5D-5L questionnaire.

Demographics	N (%)	Mobility	Self-care	Usual activities	Pain/ discomfort	Anxiety/ depression
Gender Male Female	 53 (44.5%) 66 (55.5%)	p= 0.268	p= 0.190	p= 0.330	p= 0.022	p= 0.841
Scottish Index of Multiple Deprivation Score 1-2 (deprived) Score 3-5 (Not deprived)	 57 (47.9%) 62 (52.1%)	p= 0.484	p= 0.505	p= 0.168	p= 0.611	p= 0.808
Rockwood frailty Scale Non-frail (score ≤4) Frail (score >4)	 92 (77.3%) 24 (20.2%)	**p< 0.001**	**p< 0.001**	**p< 0.001**	p= 0.053	**p= 0.042**
Discharge destinations Home Home with increased package of care Home with new package of care Care home	 99 (83.2%) 15 (12.6%) 3 (2.5%) 2 (1.7%)	**p= 0.045**	p= 0.524	p= 0.219	p= 0.068	p= 0.714
Had 30 days morbidity	44 (37.0%)	**p= 0.004**	p= 0.163	**p< 0.001**	p= 0.432	**p= 0.019**
Had 30 days readmissions	11 (9.2%)	**p< 0.001**	**p= 0.001**	**p< 0.001**	**p< 0.001**	p= 0.071

EQ-5D-5L Utility scores

The utility scores for day-30 and day-90 were calculated using the value set from Devlin et al (251). These scores reflect each patient's overall quality of life on a scale from -0.594 to 1.000 where 1.000 represents perfect health, values < 1 represents poor health and < 0 represents a health state considered worse than death. The mean EQ-5D-5L utility score increased from 0.805 ± 0.175 at day-30 to 0.844 ± 0.200 at day-90, while the median values improved from 0.850 to 0.916, respectively. The interquartile range also shifted upward (IQR 0.732–0.937 at day-30 vs. 0.804–0.988 at day-90), indicating that the majority of patients experienced continued improvement in health related QoL during the recovery period. The observed mean increase of 0.039 falls within the range typically considered the minimal clinically important difference for EQ-5D-5L (approximately 0.03-0.07), suggesting that the change is likely to represent a clinically meaningful improvement

rather than random variation. The range of utility scores widened slightly by day-90, reflecting greater heterogeneity in post-operative recovery trajectories, with some patients demonstrating substantial improvement while others experienced persistent health limitations following EmLap.

When the EQ-5D-5L utility scores were compared between day-30 and day-90 post-EmLap using Wilcoxon Signed-Rank test, it showed 33 (30.6%) participants with lower scores (decreased QoL) (mean rank 51.6) and 70 (64.8%) with improved scores (improved QoL) (mean rank 52.2), and 5 (3.6%) with no change in scores. The difference was statistically significant ($Z = -4.12$, $p < 0.001$), indicating a significant improvement in post-operative QoL at day-90 compared to day-30 following emergency laparotomy.

Frail and non-frail utility scores

Mann–Whitney U test was conducted to compare the EQ-5D-5L utility scores at post-operative day-30 between frail and non-frail patients. The results showed a statistically significant difference between the two groups ($U = 294.50$, $Z = -2.43$, $p = 0.015$). The frail group (mean rank = 32.77) had lower EQ-5D-5L utility scores compared to the non-frail group (mean rank = 56.96), indicating poorer post-operative QoL among frail patients.

The same test was used to compare EQ-5D-5L utility scores at post-operative day-90 between frail and non-frail patients. There was no statistically significant difference between the groups ($U = 520.50$, $Z = -0.13$, $p = 0.894$). The mean ranks of EQ-5D-5L utility scores were similar for frail (mean rank = 53.32) and non-frail (mean rank = 54.63) patients, indicating that by day-90, the QoL had improved and was comparable between the two groups.

Overall, quality of life outcomes improved significantly between days-30 and -90 post-EmLap, particularly in the domains of usual activities and pain/discomfort, but they did not return to pre-EmLap baseline. The median utility score increased from 0.850 (IQR 0.732- 0.937) at day-30 to 0.916 (IQR 0.804- 0.988) at day-90 and mean scores also increased from 0.805 to 0.844. This upward shift across all percentiles suggests an overall improvement in health-related QoL.

Within-group analysis demonstrated significant improvement in EQ-5D-5L utility scores between day-30 and day-90 in both frail and non-frail patients. Comparison of change in EQ-5D-5L utility scores between day-30 and -90 demonstrated a significant greater improvement among frail patients compared to non-frail patients. Frail patients had higher mean ranks for change scores (72.8 vs 52.4), indicating a larger magnitude of improvement. This difference was statistically significant on Mann-Whitney U test (U= 332, z= -2.05, p= 0.041). This likely reflects lower baseline utility scores among frail patients, allowing greater scope for recovery over time.

Among patients who had post-EmLap critical care stay (HDU or ITU), the change in EQ-5D-5L utility scores between post-operative day-30 and day-90 were compared between frail and non-frail patients using the Mann–Whitney U test. Although frail patients demonstrated a higher mean rank for utility change compared to those in non-frail (61.25 vs 47.02), this difference was not statistically significant (U = 302.5, Z = –1.53, p = 0.126). This suggests that, despite differences in early post-operative quality of life, the magnitude of improvement over time was similar between frail and non-frail patients following a post-EmLap critical care admission.

Although frail patients demonstrated greater improvement, their absolute EQ-5D-5L scores remained lower than those of non-frail patients at both time points. Frailty, morbidity, and changes in home care arrangements were key factors influencing recovery. These findings underscore the importance of targeted interventions to

provide support and address specific needs as well as the need for an enhanced post-operative recovery for EmLap patients.

9.3 Discussion

Quality of life (QoL) improved significantly between day-30 and day-90 post-EmLap. Improvements were observed across several domains, including greater ability to perform usual activities and reductions in pain and anxiety. The mean EQ-5D-5L utility score increased from 0.805 ± 0.175 at day-30 to 0.844 ± 0.200 at day-90, while median EQ-VAS health scores improved from 70 to 80 over the same period. Additionally, the upward shift in the interquartile range of utility scores (from 0.732–0.937 at day-30 to 0.804–0.988 at day-90) suggests that most patients experienced continued recovery during this period. However, the slightly wider distribution at day-90 suggests increasing heterogeneity in recovery trajectories over time.

Despite these improvements, QoL had not fully returned to pre-EmLap levels by 90 days. Recovery post-EmLap was also slower than that reported in the elective surgical populations, where median utility scores approaching 1.0 at 90 days have been described (332), compared with a median utility score of 0.916 in this cohort. This difference highlights the sustained physiological and functional impact of both emergency surgery and the underlying acute illness on patient-perceived health and wellbeing.

When stratified by frailty status, frail patients had significantly lower EQ-5D-5L utility scores than the non-frail counterparts at day-30 ($p= 0.015$), whereas the difference at day-90 was not significant ($p= 0.894$). These comparisons were unadjusted. Given the small number of frail respondents, no adjusted analysis was undertaken, so differences in age, comorbidity or other baseline characteristics may contribute to the findings. Frail patients also reported poorer mobility, self-care, and ability to perform usual activities, and these deficits persisted at day-90, even though the overall utility difference was no longer significant. This may suggest

prolonged functional impairment in some frail survivors, although the analysis is exploratory. While few studies have specifically examined QoL outcomes post-EmLap in frail patients, the findings align with broader literature demonstrating that frailty is associated with delayed functional restoration and poorer post-operative QoL in an already vulnerable surgical population (254). These observations highlight the potential importance of structured peri-operative support for frail patients undergoing EmLap. Adaptation of enhanced recovery pathways for EmLap may support earlier functional recovery, while targeted post-operative rehabilitation programmes and strengthened social support mechanisms may facilitate recovery beyond the immediate hospital stay.

Encouragingly by 90-days post-operatively, the difference in utility scores between frail and non-frail patients, which included patients requiring critical care admission post-EmLap, was no longer statistically significant. This suggests that meaningful recovery is achievable even among frail patients, consistent with the findings of Purcell et al (31). Importantly, this reinforces the principle that frailty alone should not preclude emergency surgical intervention when the potential for recovery exists. Instead, it underscores the importance of optimising peri-operative and post-operative care through targeted rehabilitation, supportive care pathways and shared decision-making in post-operative supportive interventions. However, it is important to note that the result also may reflect convergence in recovery, limited statistical power or variability in individual recovery trajectories. The findings therefore suggest that the difference observed at day-30 was not demonstrated at day-90 in this cohort, rather than confirming that quality of life had become equivalent between frail and non-frail patients.

The improvements observed in anxiety and pain domains suggest effective post-operative psychological adaptation and effective symptom control during recovery. Psychological resilience, the capacity to recover swiftly from challenging experiences and return to healthy functional state, may play a role in shaping post-operative recovery trajectories, particularly in frail patients who are at higher risk

of post-operative depression or anxiety (333). These findings further highlight that survival alone represents an incomplete measure of surgical success, and emphasises the need for integrated psychological, rehabilitative, and social support as part of holistic EmLap aftercare (334). Improving post-EmLap QoL therefore requires multidisciplinary approach. This includes realistic pre-operative discussions regarding the risks of post-EmLap morbidity, consideration of less invasive surgical approaches where feasible (such as laparoscopic techniques), and coordinated post-EmLap management including pain control, nutritional optimisation, physiotherapy, stoma care, and early recognition and timely intervention of post-operative complications.

Quality of life outcomes complement traditional mortality and morbidity measures by capturing the functional and experiential aspects of recovery that are highly relevant to patients. Incorporating QoL assessment into EmLap audit and evaluation frameworks could therefore support more patient-centred care, guide allocation of rehabilitation and community resources, and better reflect meaningful recovery, particularly in an ageing and frail population.

9.4 Conclusion

Quality of life improves significantly between 30 and 90 days following EmLap, although recovery remains incomplete and slower than that observed after elective surgery. Frailty is associated with poorer early post-operative QoL and delayed functional recovery, although substantial improvement was observed post-EmLap. These findings highlight the importance of developing multidisciplinary, frailty-informed peri-operative pathways that prioritise rehabilitation, psychological wellbeing, and functional outcomes alongside survival. Incorporating QoL assessment into routine evaluation of EmLap outcomes may provide a more patient-centred measure of surgical success in this EmLap population.

Chapter 10: Final discussion

The QoL findings presented in Chater 9 suggested that although meaningful recovery is achievable following EmLap, recovery trajectories differ substantially according to frailty status, with frail patients experiencing slower improvement. These observations underscore that frailty is not merely a static pre-operative descriptor of risk, but a dynamic state that interacts with physiological stress and influences post-operative recovery. Integrating these clinical insights with plasma metabolomic profiling provides an opportunity to better understand the biological mechanisms underpinning vulnerability, identify metabolic pathways associated with impaired recovery and ultimately inform future personalised strategies to optimise peri-operative care.

This study was originally designed to investigate whether established clinical frailty markers, together with novel plasma metabolomic biomarkers, could improve the assessment of frailty and prediction of outcomes following emergency laparotomy. The overarching aim was to determine whether molecular biomarkers could complement established clinical assessment tools and contribute to more objective peri-operative risk stratification. As the analyses progressed, the characteristics of this single-centre cohort shaped the interpretation of the findings. The relatively small number of frail patients and mortalities limited the statistical power required for robust biomarker validation and independent prediction modelling. However, rather than limiting the contribution of the metabolomic analyses, this highlighted their complementary role in characterising the biological processes associated with frailty and post-operative outcomes.

Although this constrained the ability to establish novel biomarkers as independent clinical tools or replacement for existing frailty measures, the metabolomic analyses revealed patterns of metabolic perturbation associated with frailty status and post-operative mortality. Importantly, these findings may have provided biological insights that extended beyond conventional clinical risk prediction. Consequently, the emphasis of the thesis evolved from validating molecular biomarkers for clinical

prediction towards characterising the biological processes underlying frailty and recovery following emergency laparotomy. Rather than viewing metabolomics solely as a diagnostic tool, the findings suggest metabolomics may provide mechanistic insight into metabolic pathways associated with physiological vulnerability and resilience. In this context, metabolomics should be viewed as complementary to, rather than a replacement for, established clinical frailty assessment. Together, these approaches provide a more comprehensive understanding of frailty by combining clinical phenotyping with molecular characterisation.

This evolution reflects the exploratory nature of translational metabolomics research. Although the present study does not establish a clinically deployable biomarker panel, it identifies candidate metabolic pathways and metabolite signatures that warrant validation in larger multicentre cohorts. These findings provide a biological framework for future studies seeking to integrate molecular profiling with clinical frailty assessment, ultimately supporting the development of more personalised peri-operative risk stratification and targeted interventions for patients undergoing EmLap.

The following discussion integrates the principal findings from the clinical frailty analyses, metabolomic investigations and QoL assessment to evaluate their collective contribution to understanding frailty in EmLap patients. Particular emphasis is placed on how these complementary datasets advance current knowledge of the biological basis of frailty, the mechanisms influencing post-operative recovery, and the implications for future research and clinical practice.

10.1 Established frailty markers

Emergency laparotomy (EmLap) is a high-risk surgical procedure associated with substantial morbidity and mortality, particularly among older and physiologically compromised patients (160). Frailty has emerged as a critical determinant of outcomes across medical disciplines, reflecting diminished physiological reserve and

impaired homeostatic capacity (51, 266). However, the influence of frailty on outcomes following EmLap is complex, as the profound physiological stress associated with EmLap may overshadow the incremental effects of pre-existing vulnerability (136, 167, 195).

To our knowledge, this thesis represents one of the first studies to integrate metabolomic profiling with established clinical frailty markers in an EmLap cohort, enabling both clinical and biological characterisation of vulnerability. By combining longitudinal QoL data with metabolic analysis, this study provides observations consistent with the frailty trajectory model described by Clegg et al. (51), in which frailty is conceptualised as a progressive decline in physiological reserve over time with increasing vulnerability to stressors. In this model, EmLap represents a major physiological stressor that appears to precipitate stepwise deterioration in frailty status. Longitudinal assessment within this cohort may have demonstrated that frailty trajectories following EmLap were predominantly downward or static rather than improving, consistent with the pattern of accelerated declined proposed by Clegg's framework.

Among the frailty measures evaluated, the CFS emerged as the best suited tool for integration within the EmLap pathway. Unlike single-domain markers such as sarcopenia, cognitive impairment, or nutritional indices, the CFS captures multidimensional functional vulnerability and allows repeated longitudinal assessment, making it particularly well suited to monitoring peri-operative changes in frailty status. Importantly, the dynamic nature of CFS highlights several potential intervention points across the EmLap trajectory. Pre-operatively, frailty assessment may support risk stratification and shared decision-making. In the immediate post-operative period, repeated CFS assessment may help identify early functional deterioration and guide targeted rehabilitation strategies. Finally, longer term follow-up allows monitoring of recovery trajectories and identification of patients who may benefit from community-based support, physiotherapy or nutritional interventions. Although statistically significant differences between frail and non-

frail groups were not observed in this cohort, this likely reflects the limited sample size and the relatively small proportion of frail patients rather than the absence of clinically meaningful differences.

Collectively, these findings reinforce the concept that EmLap acts as an acute stressor point within the frailty trajectory. Frailty assessment using dynamic tools such as CFS provides a practical mechanism for identifying patients at risk of accelerated decline and implementing interventions aimed at improving outcomes for frail EmLap patients should therefore prioritise peri-operative risk stratification and multidisciplinary care. Frailty informed pathways incorporating early geriatric involvement, nutritional optimisation, physiotherapy, and enhanced discharge planning may improve functional recovery and reduce late morbidity. Larger prospective studies are required to deepen our understanding on peri-operative fluctuations in frailty and to determine whether early identification and targeted intervention in pre-frail patients can mitigate post-EmLap decline.

10.2 Metabolic signatures associated with frailty and post-operative mortality

A key novel aspect of this thesis was the integration of untargeted metabolomic profiling using UHPLC-MS to investigate biochemical signatures associated with frailty and post-operative outcomes. The most distinct metabolic differences were observed in the non-frail to mortality group, comprising patients classified as non-frail according to the CFS (score ≤4) before EmLap who subsequently died. These differences involved alterations in acylcarnitine, amino acids, peptides and nucleoside metabolism. These metabolic disturbances provide biological context for the clinical frailty trajectories observed following EmLap, suggesting that impaired mitochondrial function, oxidative stress dysregulation, and altered cellular repair pathways may contribute to reduced physiological resilience during surgical stress.

An important consideration when interpreting these findings is the composition of the non-frail to mortality group. Of the 20 patients who died within this group, 14 (70%) had a pre-operative CFS score of 4, which was classified as non-frail in this study despite representing a pre-frail state within the study's classification framework. This observation suggests that the metabolic signatures associated with mortality may partly reflect pre-existing vulnerability among patients at the upper end of the non-frail CFS category. Consequently, the distinction between non-frail patients who survived and those who died may not represent a straightforward contrast between biological robustness and mortality. Instead, it may reflect differences in physiological reserve and metabolic response among patients whose clinical frailty scores did not meet the threshold for frailty. The predominance of CFS 4 patients in the non-frail to mortality group therefore provides a plausible explanation for why substantial metabolic differences were observed in this transition group, although the present analysis cannot establish whether these differences are attributable to pre-frailty, acute illness severity, other clinical factors or processes directly associated with mortality.

This finding also highlights the potential influence of the study's CFS categorisation on the interpretation of metabolomic results. Combining patients with CFS scores of 1–4 into a single non-frail category may have grouped together individuals with different levels of baseline vulnerability. If metabolic profiles vary along this clinical spectrum, the non-frail to mortality signature could partly reflect this underlying heterogeneity. Future studies should therefore examine metabolite associations across the full range of CFS scores or compare patients with CFS 4 against those with lower scores, while accounting for relevant demographic and clinical covariates. Such analyses could help determine whether the observed metabolic differences are associated with a continuum of frailty, mortality risk independently of frailty, or an interaction between baseline vulnerability and the physiological stress of emergency surgery.

The metabolic findings highlight the substantial biological heterogeneity that exists within the EmLap group. EmLap is currently treated as a single clinical entity within research frameworks. However, the underlying pathologies leading to surgery, including perforation, obstruction, ischemia and sepsis, represent distinct biological states with potentially different metabolic consequences. Due to the relatively small number of patients within each pathology subgroup, metabolic signatures could not be analysed according to surgical diagnosis within this cohort. Nevertheless, the metabolites identified raise the possibility that future peri-operative strategies may move beyond treating EmLap as a homogeneous surgical cohort and instead adopt a more personalised approach based on underlying pathology, frailty phenotype, and metabolic profile.

From a translational perspective, these findings provide a rationale for investigating metabolically informed therapeutic strategies, although their clinical relevance remains to be established. The alterations in acylcarnitine levels suggest mitochondrial dysfunction, which may be amenable to therapies targeting cellular energetics. The amino acid and peptide dysregulation may reflect impaired proteostasis and muscle metabolism, which are biological processes closely linked to sarcopenia and frailty, suggesting potential roles for targeted nutritional or anabolic interventions. Perhaps an observation of potential interest was the prominent involvement of nucleoside pathways disturbances. The dysregulation of nucleoside turnover may suggest impaired cellular repair, altered immune responses and increased metabolic stress, highlighting potential links to ageing pathways and inflammatory signalling. However, the specific biological mechanisms underlying the observed metabolite differences cannot be established from the present observational data alone. Emerging research into interventions targeting inflammation, mitochondrial function and amino acid metabolism provides potential avenues for future investigation. For example, glucocorticoid therapy has been explored in relation to inflammatory responses and postoperative outcomes (322, 323), while interventions targeting mitochondrial function, oxidative stress, or amino acid metabolism such as carnitine supplementation (314, 315) may offer future therapeutic avenues. These approaches should not be interpreted as

established treatments for metabolic frailty in EmLap patients, and their potential benefits and risks require careful evaluation in appropriately designed studies.

In the longer term, integration of metabolomic biomarkers with clinical frailty assessment could enable stratification of EmLap patients into biologically defined subgroups, allowing discovery of clinically actionable applications via targeted pharmacological, nutritional or metabolic interventions, aimed at improving resilience in high-risk surgical patients. A larger cohort would allow further investigation of the potential value of molecular profiling in characterising frailty and predicting outcomes. The patients could be grouped by their molecular profiles alone, without incorporating their CFS classifications or clinical outcomes allowing identification of distinct metabolic phenotypes, while clustering could be compared with conventional CFS scores to assess concordance and identify discordant patients, such as patients classified as non-frail by CFS who fall within a cluster displaying a metabolic profile associated with frailty or adverse outcomes. Such discordance could help identify biological characteristics that are not fully represented by conventional clinical assessment, although a frail-like metabolic profile would not, in itself, establish the presence of subclinical frailty. Moreover, examining whether patients with CFS 4 demonstrate metabolic profiles that differ from those with CFS scores of 1–3 may help clarify whether the current binary categorisation obscures biologically meaningful differences in vulnerability. This would be especially relevant to understanding the non-frail to mortality findings, given the high proportion of patients with CFS 4 who died. In addition, the prospective outcome analyses described in Chapter 4 could be repeated using molecular groups as the exposure, to determine whether these groups provide additional prognostic information beyond the CFS. Such an approach could help establish whether molecular profiling captures aspects of biological vulnerability not fully reflected by conventional clinical frailty assessment. Any proposed replacement of conventional frailty categories would require evidence of superior predictive performance and validation in an independent cohort; initially, molecular profiling would be more appropriately evaluated as a complementary approach. Ultimately, such research could inform the development of targeted pharmacological, nutritional or metabolic

interventions for high-risk patients. Rather than applying uniform peri-operative management to all EmLap patients, future care pathways may involve personalised therapeutic strategies tailored to the patient's metabolic profile and surgical pathology. This approach aligns with the broader transition of national strategies promoting precision peri-operative medicine, in which treatment strategies are informed not only by clinical risk scores but also by underlying biological vulnerability and recovery.

10.3 Impact of frailty on post-operative quality of life

Beyond mortality and complications, this study highlights frailty as a key determinant of post-operative QoL. While overall QoL improved between 30- and 90-days post-EmLap, frail patients consistently experienced poorer early outcomes and slower functional restoration. Importantly, these findings suggest delayed recovery rather than an absence of improvement, as the differences in QoL between frail and non-frail patients had largely narrowed by 90 days. These observations challenge the nihilistic attitudes toward EmLap in frail patients, emphasising that meaningful recovery remains achievable even among vulnerable individuals.

These findings highlight the importance of recovery focused peri-operative care. Targeted strategies including proactive pain management, nutritional optimisation, physiotherapy-led rehabilitation, delirium prevention, and psychological support may be particularly beneficial for frail patients. Integration of geriatric expertise within EmLap pathways represents an important opportunity to address both physical and cognitive vulnerability and improve functional recovery and patient-reported outcomes.

10.4 Challenges (during recruitment)

Recruitment to EmLap research presents unique challenges, primarily due to the acute and unpredictable nature of emergency surgical presentation. Unlike elective surgical studies, where patients have time to consider participation, EmLap patients

often require urgent intervention while experiencing severe pain, sepsis or receiving opioid analgesia, all of which may impair decision-making capacity. In cases where patients lacked capacity, informed consent was obtained from the next of kin or guardian (appendix 1.8.3 and 1.8.4). This approach was necessary but could be challenging, as family members were frequently distressed by the acute clinical situation. Overall, 11 patients (7.3%) in this study were recruited through next of kin or guardian consent, with one patient later regained capacity post-EmLap and provided direct consent to continue participation. These challenges are well recognised in emergency surgical research, with LaCeS2 trial closed early due to difficulties with recruitment and participant retention, highlighting the complexity of conducting trials in this patient population.

Another significant challenge was the unpredictable timing of patient presentation. EmLap procedures occur at all hours, requiring rapid identification of eligible patients and timely collection of pre-operative blood samples. Because metabolomic analysis requires rapid centrifugation and freezing to preserve metabolite stability (335), timely sample processing was essential. When recruitment occurred during standard working hours, samples were processed by the research member (HJN). However, when EmLap occurred out of hours, trained members of the on-call surgical team assisted with consent and sample collection, while hospital laboratory staff supported sample processing. Despite these arrangements, reduced overnight laboratory staffing occasionally resulted in significant time delay in processing and freezing the samples, highlighting the logistic challenges inherent in conducting metabolomic research in emergency surgical settings. To mitigate this factor, the on-call team was encouraged to contact the research member (HJN) directly when feasible so that samples could be processed promptly.

10.5 Strengths and limitations

This study possessed several strengths and limitations that should be acknowledged when interpreting the findings.

Strengths

Despite the challenges associated with emergency surgical research, several strengths should be noted. Consecutive recruitment attempts helped in reducing selection bias, while prospective data collection ensured consistency in clinical and biochemical measurements. The incorporation of clinical frailty assessments, metabolomic profiling, and patient reported outcomes provided a comprehensive and multidimensional evaluation of frailty within the EmLap pathway. Furthermore, the use of untargeted metabolomics allowed exploration of novel biological mechanisms beyond traditional clinical metrics, linking phenotypic frailty to underlying metabolic pathways.

Limitations

A key limitation of this study is the relatively small sample size, which limited the statistical power and may have prevented detection of independent associations between frailty measures and clinical outcomes. The small number of frail patients also limited the covariate adjustment, and some adjustment variables may lie on the causal pathway between frailty and outcome. As this was a novel study, the sample size calculation was based on frailty data from EmLap studies rather than metabolomic studies. However, the prospective design and integration of clinical frailty assessment with metabolomic profiling provide an important foundation for larger multicentre studies.

Secondly, unmeasured confounders such as post-operative complications, rehabilitation intensity, nutritional support, and psychosocial factors may have influenced the recovery trajectories and long-term outcomes.

Thirdly, the single-centre design limits the external generalisability, although this approach ensured a high degree of methodological consistency, data completeness,

and uniform clinical management, thereby strengthening the internal validity of the study.

Finally, the metabolomic analysis was exploratory, and the identified metabolic signatures require validation in larger, independent cohorts before clinical application can be considered. Moreover, further in-vitro and in-vivo studies will also be necessary to clarify the biological mechanisms linking these metabolites to frailty.

10.6 Clinical implications and future research

Taken together, the findings of this thesis present a multidimensional cohesive narrative on the role of frailty in emergency laparotomy, integrating clinical vulnerability, biological mechanisms, and recovery trajectories. Although frailty markers were not independent predictors of mortality within this cohort, frailty remained strongly associated with patient vulnerability and functional reserve. Integration of frailty assessment, particularly CFS, into standard EmLap pathways, as demonstrated by national programmes such as NELA and ELLSA, should not be used primarily as a mortality prediction tool, but rather as a framework for personalised care. This would identify patients who may benefit from early geriatric involvement, tailored peri-operative decision-making and enhanced discharge planning. Dynamic reassessment in the post-EmLap period enables early identification of functional decline, facilitating timely rehabilitant, nutritional optimisation, and community support. Embedding frailty awareness into multidisciplinary decision-making may also improve alignment between treatment intensity and patient goals, supporting shared decision-making in high-risk emergency settings.

When considered alongside the frailty trajectory model described by Clegg et al (51), the findings in this study support a view of EmLap as a critical biological stressor capable of accelerating frailty progression, highlighting frailty as a dynamic and

potentially iatrogenic condition. While prehabilitation is not feasible in the emergency surgical setting, this finding underscores the need for research into early post-operative interventions aimed at preventing frailty progression. These may include multidisciplinary follow up, nutritional support, early mobilisation, delirium prevention strategies, and targeted rehabilitation programmes initiated immediately following EmLap.

Quality of life analyses may have demonstrated that, despite increased vulnerability, meaningful recovery is achievable following EmLap, including among frail patients. These findings reinforce the principle that frailty should not be viewed as a contraindication to EmLap, but rather as a key determinant in shaping post-operative needs, recovery potential, and long-term wellbeing. Frailty therefore has critical implications for personalised care, resource allocation, and patient counselling.

As the current cohort represents a limited subset of the UK population, future studies should prioritise the inclusion of larger, more diverse and nationally representative patient population to enhance generalisability. Strengthening data linkages with national registries such as NELA or ELLSA would enable more comprehensive longitudinal outcome tracking, facilitate evaluation of frailty informed care pathways, and support the development of evidence-based interventions across the entire EmLap trajectories.

A particular important implication of this study is the potential for personalised pharmacological interventions in high-risk surgical population. The identification of nucleoside metabolism as a significantly altered metabolic pathway represents a novel and important avenue for metabolomic discovery and highlights the potential of candidate biomarkers to inform post-operative risk stratification. As metabolomic technologies continue to evolve, it may become possible to identify specific metabolic vulnerabilities and target these pathways with tailored therapies. Further

investigation is warranted to deepen our understanding on the biological mechanisms linking these metabolic alterations to frailty and impaired recovery. Large-scale, multicentre studies are essential to validate the predictive value of metabolomic and other biomolecular markers for post-operative recovery, long-term functional outcomes, and mortality, and to determine their added value when integrated with established clinical frailty assessment tools.

As this is an exploratory study investigating potential candidate biomarkers associated with frailty which subsequently was found associated with adverse outcomes following EmLap instead, untargeted metabolomic analysis was undertaken to characterise a broad range of metabolic features without restricting the investigation to a predefined panel. The findings should be considered hypothesis-generating, as the metabolites identified require further analytical and clinical validation before their predictive performance or clinical utility can be established. Following validation in independent cohorts, specific candidate metabolites could be identified and incorporated into a targeted assay, potentially enabling more rapid and clinically practical measurement than comprehensive untargeted profiling.

If validated, such biomarkers could potentially contribute to clinical decision-making at three stages of the EmLap pathway. Pre-operatively, a biomarker panel could complement the CFS and other clinical assessments, particularly when frailty is difficult to assess reliably in the emergency setting, thereby supporting risk stratification and shared decision-making. It may also be useful in patients whose underlying disability or complex functional limitations make conventional frailty assessment difficult, although this application would require specific validation. During the post-operative hospital stay, the biomarker measurements could potentially help identify patients at risk of frailty progression or poor recovery who may benefit from early geriatric review, nutritional support, or targeted rehabilitation. At post-operative follow-up, particularly between days 30 and 90, biomarkers could potentially provide an objective measure of biological recovery

and serve as outcomes in intervention studies evaluating strategies such as nutritional or carnitine supplementation. However, their suitability for these different applications, including their ability to detect changes over time and respond to interventions, remains to be established.

The metabolic findings among patients classified as non-frail who subsequently died are also clinically important. These findings raise the possibility that metabolomic profiling could identify patients who appear relatively robust according to conventional clinical assessment but nevertheless have an underlying biological vulnerability associated with adverse outcomes. This potential application is particularly relevant given that clinical frailty classifications may not fully capture the biological heterogeneity within the non-frail group. However, further studies are required to determine whether these metabolic signatures provide independent prognostic information beyond established clinical and operative risk factors.

The present dataset can also inform the design of future metabolomic research. The observed effect sizes and variability provide preliminary empirical estimates to inform sample-size and power calculations for subsequent emergency laparotomy studies, although these estimates will require cautious interpretation and confirmation in independent cohorts. Candidate acylcarnitine and nucleoside-related metabolites could be prioritised for targeted analytical validation, followed by assessment of their predictive performance in an independent cohort. Covariates, including age and body mass index, should be pre-specified in future analyses in view of the sensitivity of some findings to these factors. The availability of serial samples across the peri-operative and follow-up periods also provides an opportunity to investigate metabolic trajectories and their relationships with frailty progression and recovery. Where appropriate, analysing frailty as a continuous measure, rather than relying solely on a binary CFS threshold, may preserve clinically relevant variation and improve the detection of early or intermediate changes in frailty status. Finally, the metabolic pathways identified in this thesis provide candidates for future intervention studies, including investigations of nutritional or carnitine

supplementation, in which changes in metabolomic profiles could be explored alongside clinical outcomes. Such interventions should be evaluated prospectively, rather than inferred to be effective from the observed metabolic associations alone.

In addition, EmLap research has traditionally treated the EmLap patients as a single clinical entity, however the underlying pathologies leading to EmLap represent distinct conditions with differing inflammatory and metabolic responses. Moreover, the candidate metabolites identified in this study should be validated across other surgical populations with a high prevalence of frailty, including emergency orthopaedic and trauma patients, as well as elective cohorts such as inflammatory bowel disease patients, and breast, colorectal and lung cancer surgery. Future research frameworks may therefore benefit from stratifying patients not only by operative risk or frailty status, but also by underlying pathology and metabolic phenotype. Such an approach may enable more precise identification of patients most likely to benefit from targeted peri-operative interventions and may ultimately facilitate the development of personalised treatment strategies, including nutrition supplementation (e.g. carnitine) and anti-inflammatory drugs, capable of slowing frailty progression or improving frailty status. The potential application of validated biomarkers in elective surgery also warrants investigation, particularly in patients for whom conventional frailty assessment is challenging. Nevertheless, biomarker performance and clinically appropriate thresholds may differ between elective and emergency settings, necessitating setting-specific validation before clinical use. This could help translate mechanistic discovery into actionable clinical strategies.

Finally, patients deemed unfit for surgery and managed non-operatively (NoLap) are typically excluded from operative cohorts, potentially leading to underestimation of frailty associated risk. This selection bias has been described previously (336). Notably, despite poor overall survival, a subset of NoLap patients survives and are discharged home. If validated biomarkers can be established in EmLap populations, it may open a novel research avenue to explore targeted medical or supportive interventions or therapies aimed at improving outcomes in the NoLap group,

potentially redefining management strategies for this highly vulnerable and historically under-researched population. However, the applicability of biomarkers identified in operative patients to the NoLap population would need to be investigated separately, given the potential differences in case mix, disease severity, treatment selection, and underlying metabolic responses.

Overall, this thesis contributes to the field in three keyways: by demonstrating the dynamic nature of frailty following EmLap, by identifying exploratory metabolomic signatures associated with dysregulation from EmLap, and by highlighting the potential for integrating clinical frailty assessment with biological markers to support personalised peri-operative care and potential pharmacological intervention. Importantly, the clinical translation of these findings will require a staged process of analytical validation, independent clinical validation, assessment of incremental predictive value beyond existing risk models, and prospective evaluation of clinical utility. This would establish whether metabolomic biomarkers can move beyond discovery to support meaningful improvements in risk stratification, recovery monitoring, and personalised interventions for patients undergoing EmLap.

Chapter 11: Final conclusion

This thesis provides a comprehensive evaluation of frailty in the EmLap population by integrating clinical frailty assessment, metabolomic profiling, and patient reported outcomes. The findings are consistent with that frailty reflects a state of biological vulnerability associated with impaired physiological reserve and delayed post-operative recovery.

Traditional frailty markers, including the Clinical Frailty Scale, sarcopenia, and circulating biomarkers, were associated with patient vulnerability and delayed recovery following EmLap. However, when incorporated into conventional peri-operative risk models, these frailty markers were not associated with 30- or 90-day mortality within this cohort, most likely due to the limited sample size and relatively low prevalence of frailty. Importantly, longitudinal assessment is consistent with that frailty status may evolve following major emergency surgery, supporting the concept that frailty is a dynamic rather than fixed condition.

A novel contribution of this study is the identification of exploratory metabolomic signatures, driven mainly by post-operative mortality in the EmLap group. The metabolite profile observed suggests multifaceted metabolic disturbance involving mitochondrial dysfunction, amino acid dysregulation, impaired detoxification pathways, and nucleic acid instability. These findings may provide new biological insight into the mechanisms underlying frailty and surgical vulnerability and highlight the potential role of metabolomics in identifying candidate biomarkers of vulnerability.

Importantly, the quality-of-life analysis supports that meaningful recovery is achievable following emergency laparotomy, even among frail patients. These findings challenge nihilistic attitudes towards surgery in frail individuals and instead support a model of care that prioritises multidisciplinary rehabilitation, functional recovery, and patient-centred outcomes alongside survival.

Together, these findings suggest that frailty in emergency surgery should be viewed not solely as a marker of risk, but as a biologically grounded and potentially modifiable state that can inform personalised peri-operative care. By integrating clinical frailty assessment with emerging metabolomic biomarkers, this study provides an early framework for biologically informed risk stratification and targeted intervention in high-risk surgical patients.

Beyond biomarker discovery, the results may be relevant to the wider movement towards personalised peri-operative medicine. Integration of metabolomic biomarkers with established clinical frailty assessments may improve risk stratification, guide peri-operative decision-making, and inform targeted interventions aimed at enhancing physiological resilience. In the future, metabolically informed strategies may enable targeted therapies, such as nutritional supplementation, mitochondrial support, or pharmacological modulation of inflammatory and metabolic pathways, to improve outcomes in vulnerable surgical patients.

In conclusion, this thesis advances the understanding of frailty in emergency surgery by linking clinical frailty, metabolic dysregulation, and recovery outcomes. These findings highlight the importance of recognising frailty as a biologically grounded and potentially modifiable state and provide a foundation for future translational research aimed at developing biomarker guided interventions to improve resilience and recovery in high-risk surgical populations.

Appendices

Appendix 1.1: Outcome measures

1) Primary outcome: Frailty biomarkers

Recruited patients were categorised into frail and non-frail group according to collected data on co-morbidities, CT measurement for sarcopenia and Rockwood CFS results. Blood samples collected were processed and plasma analysed via UHPLC-MS based untargeted metabolomics to establish the presence of a metabolite panel that can differentiate non-frail from frail patients alongside longitudinal assessment of metabolic changes as the patient recovers or subsequently died.

2) Secondary Outcomes: 30-day morbidity, 30-day and 90-day mortality and quality of life

Data on length of total hospital stay, length of stay in critical care, post-operative complications according to Clavien-Dindo Classification, time from surgery to medical discharge (i.e. when medically well, excluding social issues etc) were collected and compared among frail and non-frail group of patients. Data on date of death, cause of death and location will be collected at 30-day and 90-day to compare between frail and non-frail group of patients. Place of residence post discharge and changes in quality of life post-EmLap using EQ-5D-5L questionnaire were explored.

Appendix 1.2: NELA Inclusion and exclusion criteria

Inclusion Criteria

- Over 40 years of age
- NELA/ ELLSA inclusion criteria
  - Open, laparoscopic, or laparoscopically-assisted procedures
  - Procedures involving the stomach, small or large bowel, or rectum for conditions such as perforation, ischaemia, abdominal abscess, bleeding or obstruction

- Washout/evacuation of intra-peritoneal abscess (unless due to appendicitis or cholecystitis – excluded, see below)
- Washout/evacuation of intra-peritoneal haematoma
- Bowel resection/repair due to incarcerated incisional, umbilical, inguinal and femoral hernias (but not hernia repair without bowel resection/repair). E.g. Large incisional hernia repair with bowel resection
- Bowel resection/repair due to obstructing/ incarcerated incisional hernias provided the presentation and findings were acute. This will include large incisional hernia repair with division of adhesions
- Laparotomy/laparoscopy with inoperable pathology (e.g. peritoneal/hepatic metastases) where the intention was to perform a definitive procedure. This does not include purely diagnostic procedures
- Laparoscopic/Open adhesiolysis
- Return to theatre for repair of substantial dehiscence of major abdominal wound (i.e. "burst abdomen")
- Any reoperation/return to theatre for complications of elective general/upper GI surgery meeting the criteria above is included. Returns to theatre for complications following non-GI surgery are now excluded (see exclusion criteria below)

Exclusion Criteria

- Under 40 years of age
- CT scan or post-operative finding of inoperable disseminated peritoneal disease
- Open and close laparotomy (post-operative palliation, non-survivable global ischemia where there are <90cm from duodenojejunal junction to stoma)
- Complication from colonic stenting requiring laparotomy
- NELA exclusion criteria
  - Elective laparotomy / laparoscopy

- Diagnostic laparotomy/laparoscopy where no subsequent procedure is performed (NB, if no procedure is performed because of inoperable pathology, then include)
- Appendicectomy +/- drainage of localised collection unless the procedure is incidental to a non-elective procedure on the GI tract
- Cholecystectomy +/- drainage of localised collection unless the procedure is incidental to a non-elective procedure on the GI tract (All surgery involving the appendix or gallbladder, including any surgery relating to complications such as abscess or bile leak is excluded. The only exception to this is if carried out as an incidental procedure to a more major procedure. We acknowledge that there might be extreme cases of peritoneal contamination, but total exclusion avoids subjective judgement calls about severity of contamination)
- Non-elective hernia repair without bowel resection or division of adhesions
- Minor abdominal wound dehiscence unless this causes bowel complications requiring resection
- Non-elective formation of a colostomy or ileostomy as either a trephine or a laparoscopic procedure (NB: if a midline laparotomy is performed, with the primary procedure being formation of a stoma then this should be included)
- Vascular surgery, including abdominal aortic aneurysm repair
- Caesarean section or obstetric laparotomies
- Gynaecological laparotomy
- Ruptured ectopic pregnancy, or pelvic abscesses due to pelvic inflammatory disease
- Laparotomy/laparoscopy for pathology caused by blunt or penetrating trauma
- All surgery relating to organ transplantation (including returns to theatre for any reason following transplant surgery)
- Surgery relating to sclerosing peritonitis
- Surgery for removal of dialysis catheters
- Laparotomy/laparoscopy for oesophageal pathology

- Laparotomy/laparoscopy for pathology of the spleen, renal tract, kidneys, liver, gall bladder and biliary tree, pancreas or urinary tract
- Returns to theatre for complications (eg bowel injury, haematoma, collection) following non-GI surgery are now excluded. i.e returns to theatre following renal, urological, gynaecological, vascular, hepatic, pancreatic, splenic surgery are excluded

Appendix 1.3: Risk management procedures

This was an observational study. No serious adverse events were anticipated but plans were put in place for the Chief Investigator and Principal Investigator (PI) to take immediate safety measures to protect research participants against any hazard to their health or safety without prior authorisation from the Research Ethics Committee (REC). The Chief Investigator (CI) or Principal Investigator (PI) would notify the REC of the presenting issue within 3 days of the urgent measure setting out the reasons for the urgent measure and the plan for further action. The local R&D department would also be informed.

There were plans in place in the event a serious breach of Good Clinical Practice (GCP) was suspected. It would be reported to the REC immediately and the GCP breach would be investigated. Any corrective action required would be undertaken by the CI and REC informed, followed by amendment of protocol which would be submitted for review. During the study, no serious adverse events and breach of GCP were encountered.

The PI (HJ Ng) attended the participant's residence to obtain follow-up blood samples at day-30 and/or 90 post surgery. The NHS GG&C and University of Glasgow Lone Working policies were adhered to. Safety measures, including advising colleagues of time and place of visit, and calling colleagues before and after visit, was performed.

Appendix 1.4: Data recording and record keeping

Two password protected Excel spreadsheets was created- a Link spreadsheet and an Outcome spreadsheet.

Link Spreadsheet

Participant's personal details (name, date of birth, address) were stored on the link spreadsheet, with corresponding study ID and hospital number. The study ID was numerical and in order of recruitment. This Link Spreadsheet was kept within NHS computer system which is password protected.

Outcome Spreadsheet

The outcome spreadsheet was pseudo-anonymised with only the study ID recorded. The spreadsheet was stored within the University of Glasgow and uploaded from NHS system via secure email (NHS email to University of Glasgow email) and stored in OneDrive. Blood samples when collected were labelled with patient's hospital number. After processed, the plasma sample was transferred into Eppendorf tubes that were labelled with participant's study ID number and stored in RAH laboratory freezer prior to transport to University of Strathclyde for mass spectrometry analysis. The EQ-5D-5L questionnaires conducted on participant's quality of life were coded with the study ID with date questionnaires completed and kept in a folder in a locked filing cabinet in the coded room in the Royal Alexandra Hospital research department. Results from blood sample analysis was emailed from University of Strathclyde to University of Glasgow email account. The data and questionnaire results were inputted onto the Outcome spreadsheet.

Onward data management and availability

Clinical study data will be retained securely for up to 10 years from the end of the study. The clinical data are pseudonymised using unique study identifiers, with the

identification key held separately within NHS Greater Glasgow and Clyde (NHS GGC), as described in Appendices 1.4 and 1.5.

Raw mass spectrometry files and processed metabolite tables are stored on a password-protected desktop at the University of Strathclyde's Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS) and will be retained for up to 10 years. Subject to confirmation that sharing is consistent with the study's ethical approval, participant consent and applicable institutional data-governance requirements, de-identified clinical and metabolomic data may be made available to researchers upon reasonable request and appropriate approval. Public deposition of participant-level data in an open repository will not be undertaken unless the necessary permissions have been confirmed.

Appendix 1.5: Participant Confidentiality and Data Protection

Data collected during the research will be kept strictly confidential and will be accessed only by members of the research team. Personal data will not be kept for longer than is required for the purpose for which it has been acquired. All investigators and research members will comply with the requirements of the General Data Protection Regulation (GDPR) and Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and uphold the Act's core principles. Identifiable data (Link Spreadsheet) was backed up on the encrypted PCs/servers (OneDrive) at the sponsor/host site (NHS GGC) to enable data privacy and security. The Outcome Spreadsheet was stored and backed up within the University of Glasgow.

The CI was responsible for the data entry, quality of the data and data analysis. The NHS GGC was the data controller; The University of Glasgow was the data processor for the study. The Chief Investigator (CI) was responsible for data entry, data quality and data analysis. NHS GGC was the data controller, and the University of Glasgow was the data processor for the study. Pseudonymised research data will be retained

securely by the University of Glasgow for up to 10 years from the end of the study. The identification key linking study IDs to participants' personal details will remain stored separately within NHS GGC, restricting the ability to directly identify participants from the research dataset alone.

Raw and processed mass spectrometry data are stored separately at the University of Strathclyde's SIPBS on a password-protected desktop and will be retained for up to 10 years. Public deposition of the de-identified clinical data, raw mass spectrometry files and processed metabolomic data in an open-access repository was not explicitly covered or approved in the original ethical application. Therefore, approval must be sought from the study sponsor, data custodian and relevant institutional governance bodies before these data can be deposited in a public repository. Subject to these approvals, de-identified clinical and metabolomic data may be made available to researchers upon reasonable request. Any future data sharing will be undertaken in accordance with the study's ethical approval, participant consent and applicable data-governance requirements.

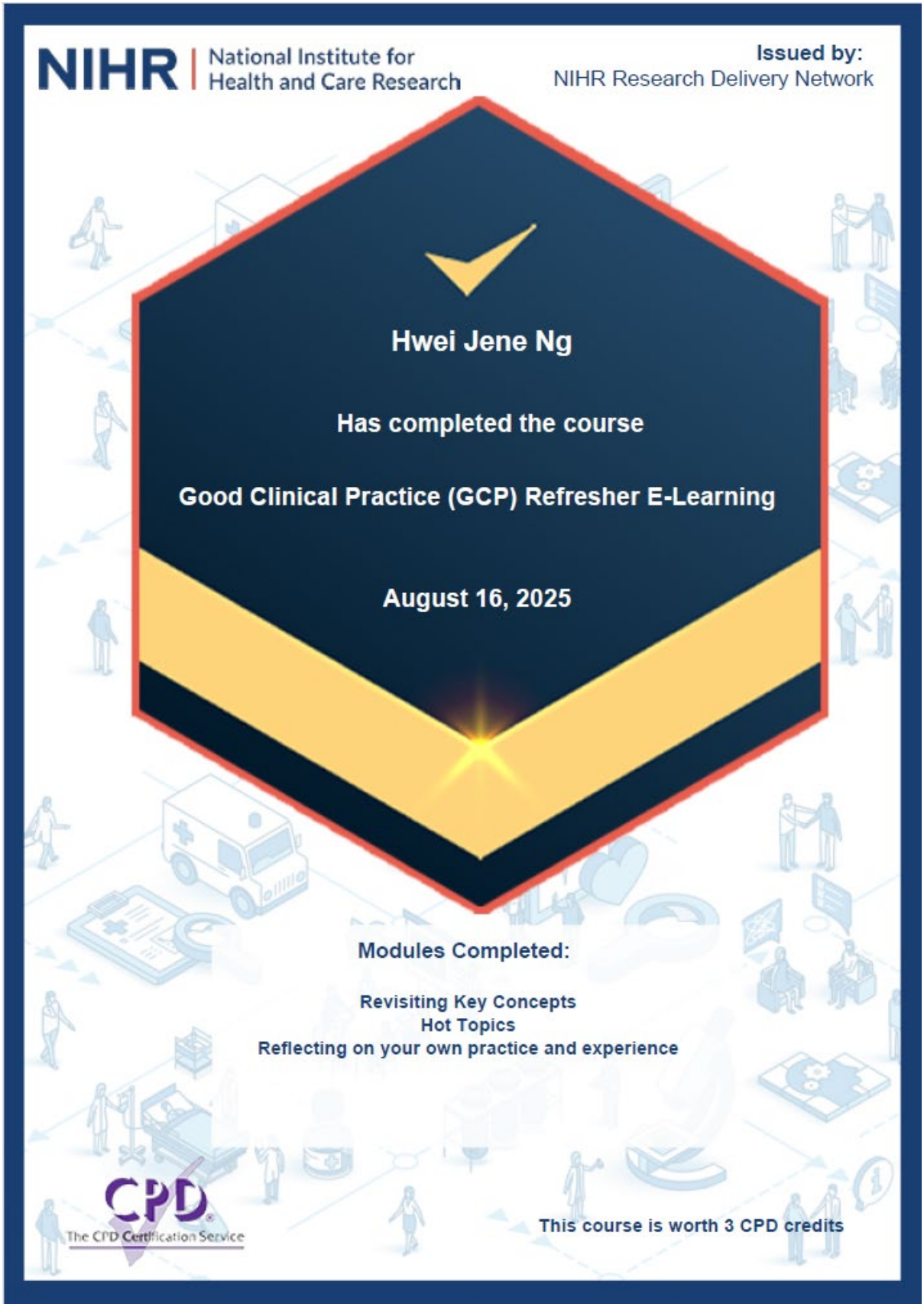
Appendix 1.6: Ethics and indemnity

The study was conducted in compliance with the ethical principles of the Research Governance Framework for Health and Community Care, Second Edition, 2006 and the principles of GCP and in accordance with all applicable legal and regulatory requirements.

Before the start of this study, the protocol and related study documents were reviewed and approved by an NHS Research Ethics Committee (REC). The REC and the relevant NHS management approval letter were received by the Investigator before any study procedures can commence.

The NHS CNORIS indemnity scheme was applied to meet the potential legal liability for harm to participants arising from the management, design and conduct of the research. University of Glasgow clinical trials insurance was also applied to meet the potential legal liability for harm to participants arising from the design of the research.

Appendix 1.7: Good Clinical Practice certificate



Appendix 1.8: Recruitment documentation

Appendix 1.8.1: Patient Information Sheet

Patient Information Sheet

(Self Consenting Patient Participants)

Department of General Surgery
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN

Investigation of biology and outcomes of frailty in emergency laparotomy

We would like to invite you to take part in this research study. This information sheet will provide you with a general understanding of the research that is being done and what it would involve for you. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?

The research is being carried out by:

- Miss H Jene Ng (Local Principal Investigator/ PhD Student) – University of Glasgow & Dept of General Surgery, Royal Alexandra Hospital, Paisley
- Professor Susan Moug (Chief Investigator/ Academic Supervisor)- Department of General Surgery, Royal Alexandra Hospital, Paisley, and Honorary Professor, University of Glasgow
- Professor Tara Quasim (Academic Supervisor)- Department of Anaesthesia, Critical Care & Perioperative Medicine, Glasgow Royal Infirmary, and Professor, University of Glasgow
- Dr. Nicholas Rattray (Academic Supervisor)- Lecturer of Clinical Metabolism, University of Strathclyde

What is the purpose of the study?

Emergency abdominal surgery (laparotomy) is one of the most common operations performed in the UK (30,000/ year) and is usually on older adults (>65 years). It does, however, carry a risk of developing problems after surgery (complications), including death, especially if there is frailty before the operation.

Frailty is a term used commonly in society but has been more challenging to define in a medical sense. Being frail makes a person more likely to have an infection or illness or to lose their independence and going through an emergency surgery could also cause frailty in a non-frail patient ('surgical frailty').

Although we usually think frailty exists only in older adults, the frailty problem is much bigger because frailty also exists in younger adults and patients can become frail after emergency surgery.

This research aims to

- Look at the changes in blood samples in patients undergoing emergency laparotomy and whether these changes are related to frailty for up to 90 days after surgery.
- Look at measures of frailty and see if they relate to patient morbidity (medical problems caused by the treatment) and mortality (death). These measures of frailty are the loss of muscle mass (seen on a CT scan) and how much a person can do. The measures are made by the doctors and nurses on all patients before they have surgery – they will not be done just for this research study.
- Find out about quality of life for patients after surgery.

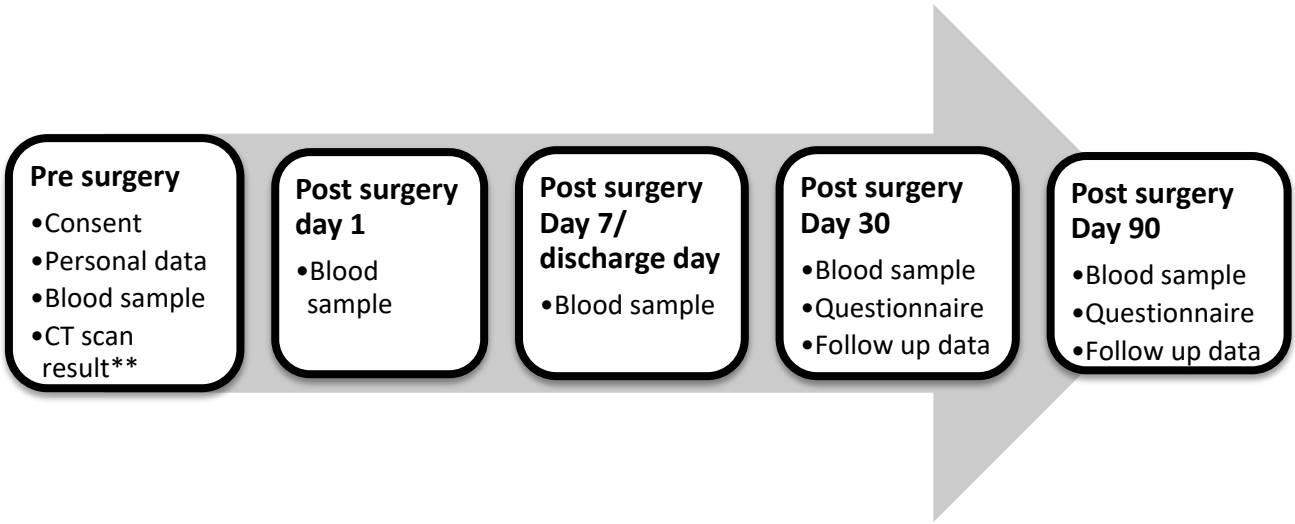
Why have I been invited?

If you are above 40 years old and undergoing emergency abdominal surgery, you are eligible to take part.

Do I have to take part?

No. It is up to you to decide whether to take part in the research or not. If you decide that you wish to take part you are free to change your mind at any time and without giving a reason and this will not alter your care in any way, now or at any stage in the future. The data collected up to the point of withdrawal will be retained and used for analysis.

What does taking part involve?



** Data on additional CT scan done after surgery will be collected but no extra CT scan will be done for research purposes.

- Details- We will collect your details including: age, gender, address including postcode, ethnic origin, medical history, lifestyle (physically active), alcohol intake, smoking status, height, weight, place of residence (home and independent, home with carers, home with family support, sheltered housing, nursing home), nursing assessment. We will also record your GP's name and contact details. We will obtain all of this information from your admission documents.

- Blood samples- An additional tube of your blood sample will be collected while in hospital (before surgery, after surgery day 1 and day 7/ discharge day) and will be used in this research. The result from your routine blood tests done during your stay in hospital will be recorded in this study (before surgery, after surgery day 1 and day 7/ discharge day). After discharge, we will require follow up blood tests to look at the changes in the blood that predict frailty. We will require you to get your blood sample taken at **day 30 and 90** after your emergency surgery. We will collect your day-30 blood test at the clinic when you attend for your routine 30-day check-up and at the surgical assessment unit in hospital on day 90. The clinic appointment at day 30 is part of usual care; the appointment at day 90 is an extra for the study only. We will contact and remind you 2 weeks before the follow up dates for these follow up data. If you are unable to attend hospital, let us know when we contact you and with your consent, we can arrange a date/ time that is suitable for the research team (HJ Ng) to collect the blood sample at your residence.

- CT scan- The CT scan that was used to diagnose your condition that required you to undergo surgery will be used in this research to assess sarcopenia (loss of muscle mass). We will be measuring the muscle bulk of your back on the CT scan. No extra scans will be performed as part of this research work. We will also collect data on any extra CT scans that are performed post-operatively.

- Questionnaire- We will ask you some questions at a face to face meeting during your hospital visit for your follow up blood sample collection to check your quality of life, especially changes in daily activity since your surgery (place of residence, mobility, self-care, pet caring, return to work, leisure activity and disability) at **30- and 90-day post-**

surgery. We will be using the EQ5D-5L questionnaire which is often used to ask people about their quality of life.

- Your health after your surgery - we will check your electronic medical records for information about complications and mortality on post surgery day 30 and day 90. The records will also be accessed 2 weeks prior date of follow up when you are contacted to arrange a date for follow up data collection.

Are there risks in taking part?

The main risk in taking part in this study is from the blood sampling which include pain and infection. We will take an extra amount of blood before surgery and then on day 1 and day 7 after surgery, at the same time as blood will be taken as part of standard care. Only the blood samples at day 30 and day 90 after surgery will be extra samples that would not be taken if you were not in this study. We will follow all the usual procedures to prevent pain and infection when taking blood samples. Other risks are associated with your data that are collected, but this information will be kept in a safe place and labelled using study ID. Some of the information collected are part of your standard care and will not be extra for this research therefore these risks are low.

Are there benefits to taking part?

There will be no direct benefit to you. This study may contribute to the understanding of frailty and lead to improvements in management of frail patients who undergo emergency surgery. This study may also lead to future treatment options to help frail patients.

What will happen to my data?

NHS Greater Glasgow and Clyde (NHS GG&C) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. NHS GG&C will keep identifiable information about you collected for this study for up to 3 years once the study ends.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. If you decide to withdraw at any time, you can contact the local principal investigator via email (hweijene.ng@ggc.scot.nhs.uk) or telephone (xxxx xxx xxxx).

Your identity and personal information will be completely confidential and known only to the primary researchers (HJN and SM) and representatives of the study Sponsor who audit the study to check the work of the researchers. A study ID will be allocated when your data are recorded and your personal information will only be accessed for follow up purposes. This means that your research data will only be labelled with a study ID, not your name (this is called pseudonymised data) and your name and other details will only be used when we need to contact you.

The blood samples collected will be stored using study ID only and may be used for future UK based studies that have research ethics approval.

The University of Glasgow will be the data processor for the study and will keep pseudonymised research data from the study for up to 10 years from the end of the study. This information will be stored within secure NHS and University electronic systems.

All research data will be held in accordance with The General Data Protection Regulation (2018).

Your research data (including pseudonymised medical information and questionnaire responses) will be combined with other participants' data and analysed together in order to understand more about the effects of emergency laparotomy. Some of this data will be analysed as part of a PhD educational qualification. All data will be anonymous when it is published – you will not be identified.

You can find out more about how we use your information at https://www.hra.nhs.uk/information-about-patients/ or by contacting NHS GG&C Information Governance Department.

Tel: 0141 355 2059

Email: data.protection@ggc.scot.nhs.uk

What will happen to the results of the study?

We plan to share the results of this study to help researchers, clinical team, scientists, third parties such as Royal College of Surgeons, Royal College of Medicine and Royal College of Anaesthetists and patients in the future. This will be done through presentation at medical conferences, publication in medical journals and PhD thesis. All data will be anonymous when it is published – you will not be identified.

We would also like to send you a summary at the end of the project by post or email. Let us know on the consent form if you would like to receive this.

Who has reviewed the study?

This study has been reviewed by the Scotland A Research Ethics Committee.

Who Can I Contact for More Information?:

The Local Principal Investigator is a General Surgery Registrar in the West of Scotland. If you have any queries relating to this research, please feel free to contact the research team using the details below:

Local Principal Investigator: hweijene.ng@ggc.scot.nhs.uk

Supervisor: susan.moug@ggc.scot.nhs.uk, tara.quasim@ggc.scot.nhs.uk, Nicholas.rattray@strath.ac.uk

Phone number (Prof Moug's secretary): xxxx xxx xxxx

Address:
Level 5, Department of Surgery,
Corsebar Road,
Royal Alexandra Hospital
Paisley
PA2 9PN

If you wish to discuss the study with someone who is not closely linked to the study, please contact: Maggie Clark, ELLSA nurse specialist xxxx xxxxxxx

Who Can I Contact If I Have a Complaint about the Study?

You are usually asked to put your complaint in writing so that the terms of the complaint are clear. You can ask someone else to do this for you, such as a family member, your GP or a member of the care team. The care team can help you if you are unsure about anything to do with the complaint's procedures.

You can find out more about the complaints process or send your complaint to NHS Greater Glasgow and Clyde using the following details:

NHS Greater Glasgow and Clyde

Complaints Department

North East Sector Offices
Stobhill Hospital
300 Balgrayhill Road
Glasgow
G21 3UR
Tel: 0141 201 4500
Email: complaints@ggc.scot.nhs.uk

Thank you for taking the time to read this information.

Appendix 1.8.2: Consent form

Consent Form (Patient)

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

Name of Researcher: Dr. Hwei Jene Ng

Supervisor: Prof Susan Moug, Prof Tara Quasim, Dr. Nicholas Rattray

If you agree to participate, please write your initials in the relevant box for each statement and sign, name and date below. Thank you.

Please initial box

1. I confirm that I have read and understood the Participant Information Sheet (IRAS 293392 Version 1.4, dated 07/05/22) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected. ☐

3. For follow up on day 30 and day 90:

a. I agree to have my follow up data (blood sampling and questionnaire) performed in hospital during clinic visit/ attend Surgical Assessment Unit. ☐

b. If option (a) above is not suitable, I agree to allow the research team member of this study to collect the follow up data at my residence at an arranged time and date. ☐

4. I acknowledge that there will be no effect on treatment arising from my participation or non-participation in this research. ☐

5. If I decide to withdraw, I agree that the information collected about me up to the point of my withdrawal will be used in the data analysis for this study. ☐

6. I agree to my pseudo- anonymised data/ blood sample being used for future ethically approved studies. ☐

7. I agree to my General Practitioner being informed of my participation in thi study. ☐

Confidentiality

1. I acknowledge that my personal details in this study will be kept confidential and will be referred to using a study ID. ☐

2. I understand that my personal data will be accessible to the research team as required where relevant to this study and to representatives of the Sponsor who audit the study to check the work of the researchers. ☐

Data usage and storage

1. All names and other material likely to identify individuals will be pseudo-anonymised and referred to using a study ID. ☐
2. The material will be treated as confidential and kept in secure storage at all times. ☐
3. The material may be used in future publications, both print and online. ☐

Consent

I agree to take part in this research study and this includes: ☐

- Personal and clinical data collection

- Pre and post surgery CT scan data collection

- Additional volume of blood collected during hospital stay (before surgery, post surgery day 1 and day 7/ discharge day), day 30 and day 90

- Answer questionnaire on day 30 and day 90

(Optional) I would like to receive a newsletter with the results of the study via

- Post ☐ Yes/No

- Email (please provide email:) ☐ Yes/No

I do not agree to take part in this research study ☐

Name of Participant _____

Signature _____ Date _____

Name of Researcher _____ Signature _____

Date _____

Appendix 1.8.3: Welfare Attorney/ Guardian/ Nearest Relative Information Sheet

Welfare Attorney/ Welfare Guardian/ Nearest Relative Information Sheet

Department of General Surgery
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN

Investigation of biology and outcomes of frailty in emergency laparotomy

We would like to invite your ward/ relative/ person you are consenting for to take part in this research study. Due to their lack of capacity, we are approaching you as their Welfare Attorney/ Welfare Guardian/ Nearest Relative to gain consent. This information sheet will provide you with a general understanding of the research that is being done and what it would involve for them. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information.

We would ask that you put your own views about the research aside and to consider and take into account, the past and present wishes and feelings of your ward/relative/person you are consenting for, had they been able to consent for themselves.

Who is conducting the research?

The research is being carried out by:

- Miss H Jene Ng (Local Principal Investigator/ PhD Student) – University of Glasgow & Dept of General Surgery, Royal Alexandra Hospital, Paisley
- Professor Susan Moug (Chief Investigator/ Academic Supervisor)- Department of General Surgery, Royal Alexandra Hospital, Paisley, and Honorary Professor, University of Glasgow

- Professor Tara Quasim (Academic Supervisor)- Department of Anaesthesia, Critical Care & Perioperative Medicine, Glasgow Royal Infirmary, and Professor, University of Glasgow
- Dr. Nicholas Rattray (Academic Supervisor)- Lecturer of Clinical Metabolism, University of Strathclyde

What is the purpose of the study?

Emergency abdominal surgery (laparotomy) is one of the most common operations performed in the UK (30,000/ year) and is usually on older adults (>65 years). Unfortunately, they carry the highest risk of developing problems after surgery (complications), including death, especially if there is frailty before the operation.

Frailty is a term used commonly in society but has been more challenging to define in a medical sense. Being frail makes a person more likely to have an infection or illness or to lose their independence and going through an emergency surgery could also cause frailty in a non-frail patient ('surgical frailty').

Although we usually think frailty exists only in older adults, the frailty problem is much bigger because frailty also exists in younger adults and patients can become frail after emergency surgery.

This research aims to

- Look at the changes in blood samples in patients undergoing emergency laparotomy and whether these changes are related to frailty for up to 90 days after surgery.
- Look at measures of frailty and see if they relate to patient morbidity (medical problems caused by the treatment) and mortality (death). These measures of frailty are the loss of muscle mass (seen on a CT scan) and how much a person can do. The measures are made by the doctors and nurses on all patients before they have surgery – they will not be done just for this research study.
- Find out about quality of life for patients after surgery.

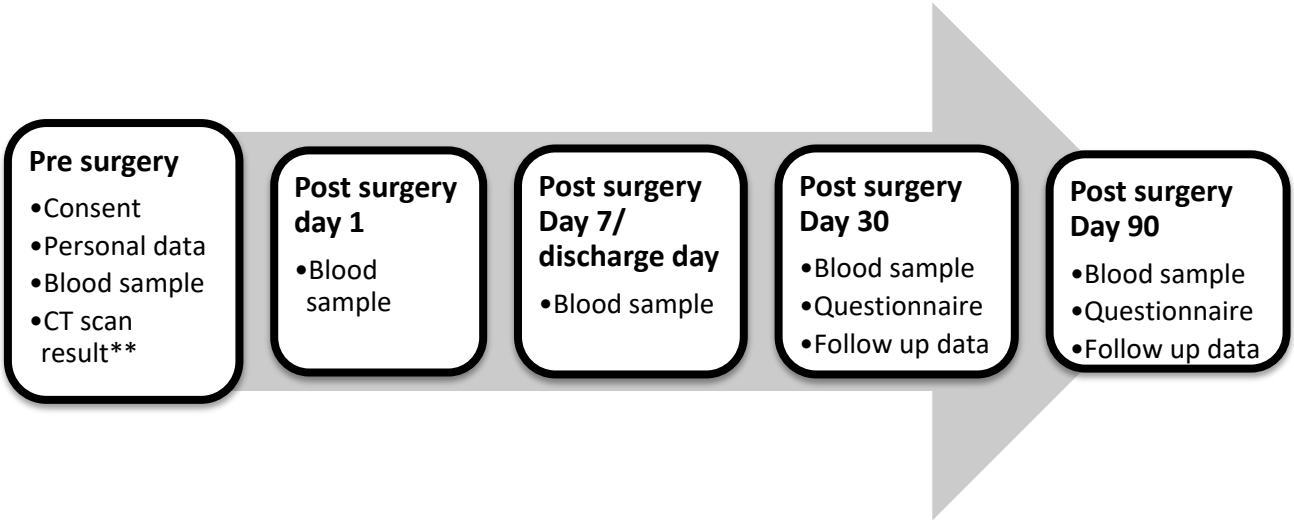
Why have your ward/ relative/ person you are consenting for been invited?

If your ward/ relative/ person you are consenting for is above 40 years old and undergoing emergency abdominal surgery, they are eligible to take part.

Do they have to take part?

No. It is up to you to decide whether they should take part in the research or not. If you decide for them to take part, you (or when they regain capacity) are free to change your mind at any time and without giving a reason and this will not alter their care in any way, now or at any stage in the future. We will ask for their consent to continue in this research when they regain capacity. If decision is made for withdrawal, the data collected up to the point of withdrawal will be retained and used for analysis.

What does taking part involve?



** Data on additional CT scan done after surgery will be collected but no extra CT scan will be done for research purposes.

- Details- We will collect their details including: age, gender, address including postcode, ethnic origin, medical history, lifestyle (physically active), alcohol intake, smoking status, height, weight, place of residence (home and independent, home with carers, home with family support, sheltered housing, nursing home) and nursing assessments. We will also record the GP's name and contact details. We will obtain all of this information from their admission documents.

- Blood samples- An additional tube of their blood sample will be collected while in hospital (before surgery, after surgery day 1 and day 7/ discharge day) and will be used in this research. The result from their routine blood tests done during their stay in hospital will be recorded in this study (before surgery, after surgery day 1 and day 7/ discharge day). After discharge, we will require follow up blood tests to look at the changes in the blood that predict frailty. We will require them to get their blood sample taken at **day 30 and 90** after their emergency surgery. We will collect their day 30 blood test at the clinic when they attend for their routine 30-day check-up and at the surgical assessment unit in hospital on day 90. The clinic appointment at day 30 is part of usual care; the appointment at day 90 is an extra for the study only. We will contact and remind you/ them 2 weeks before the follow up dates for these follow up data. If they are unable to attend hospital, let us know when we contact you/ them and with consent, we can arrange a date/ time that is suitable for the research team (HJ Ng) to collect the blood sample at their residence.

- CT scan- The CT scan that was used to diagnose their condition that required them to undergo surgery will be used in this research to assess sarcopenia (loss of muscle mass and function). We will be measuring the muscle bulk of the back on the CT scan. No extra scans will be performed as part of this research work. We will also collect data on any extra CT scans that are performed post-operatively.

- Questionnaire- We will ask them some questions at a face to face meeting during their hospital visit for their follow up blood sample collection to check their quality of life, especially changes in daily activity since their surgery (place of residence, mobility, self-care, pet caring, return to work, leisure activity and disability) at **30 and 90 day post-surgery**. We will be using the EQ5D-5L questionnaire which is often used to ask people about their quality of life.

- Their health after their surgery - we will check their electronic medical records for information about complications and mortality on post surgery day 30 and day 90. The records will also be accessed 2 weeks prior date of follow up when they are contacted to arrange a date for follow up data collection.

Are there risks in taking part?

The main risk in taking part in this study is from the blood sampling which include pain and infection. We will take an extra amount of blood before surgery and then on day 1 and day 7 after surgery, at the same time as blood will be taken as part of standard care. Only the blood samples at day 30 and day 90 after surgery will be extra samples that would not be taken if they were not in this study. We will follow all the usual procedures to prevent pain and infection when taking blood samples. Other risks are associated with the data that are collected, but this information will be kept in a safe place and labelled using study ID. Some of the information collected are part of the standard care and will not be extra for this research therefore these risks are low.

Are there benefits to taking part?

There will be no direct benefit to them. This study may contribute to the understanding of frailty and lead to improvements in management of frail patients who undergo emergency surgery. This study may also lead to future treatment options to help frail patients.

What will happen to their data?

NHS Greater Glasgow and Clyde (NHS GG&C) is the sponsor for this study based in the United Kingdom. We will be using information from your ward/ relative/ person you are consenting for and their medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after their information and using it properly. NHS GG&C will keep identifiable information about them collected for this study for up to 3 years once the study ends.

The rights to access, change or move the information are limited, as we need to manage their information in specific ways in order for the research to be reliable and accurate. If you withdraw them from the study, we will keep the information about them that we have already obtained. To safeguard their rights, we will use the minimum personally-identifiable

information possible. If you decide to withdraw them at any time, you can contact the local principal investigator via email (hweijene.ng@ggc.scot.nhs.uk) or telephone (xxxx xxx xxxx).

Your ward/ relative/ person you are consenting for's identity and personal information will be completely confidential and known only to the primary researchers (HJN and SM) and representatives of the study Sponsor who audit the study to check the work of the researchers. A study ID will be allocated when their data are recorded and their personal information will only be accessed for follow up purposes. This means that their research data will only be labelled with a study ID, not their name (this is called pseudonymised data) and their name and other details will only be used when we need to contact you/ them.

The blood samples collected will be stored using study ID only and may be used for future UK based studies that have research ethics approval.

The University of Glasgow will be the data processor for the study and will keep pseudonymised research data from the study for up to 10 years from the end of the study. This information will be stored within secure NHS and University electronic systems.

All research data will be held in accordance with The General Data Protection Regulation (2018).

Your ward/ relative/ person you are consenting for's research data (including pseudonymised medical information and questionnaire responses) will be combined with other participants' data and analysed together in order to understand more about the effects of emergency laparotomy. Some of this data will be analysed as part of a PhD educational qualification.

You can find out more about how we use your information at https://www.hra.nhs.uk/information-about-patients/ or by contacting NHS GG&C Information Governance Department.

Tel: 0141 355 2059 Email: data.protection@ggc.scot.nhs.uk

What will happen to the results of the study?

We plan to share the results of this study to help researchers, clinical team, scientists, third parties such as Royal College of Surgeons, Royal College of Medicine and Royal College of Anaesthetists and patients in the future. This will be done through presentation at medical conferences, publication in medical journals and PhD thesis. All data will be anonymous

when it is published – your ward/ relative/ person you are consenting for will not be identified.

We would also like to send you a summary at the end of the project by post or email. Let us know on the consent form if you would like to receive this.

Who has reviewed the study?

This study has been reviewed by the Scotland A Research Ethics Committee.

Who Can I Contact for More Information?:

The Local Principal Investigator is a General Surgery Registrar in the West of Scotland. If you have any queries relating to this research, please feel free to contact the research team using the details below:

Local Principal Investigator: hweijene.ng@ggc.scot.nhs.uk

Supervisor: susan.moug@ggc.scot.nhs.uk, tara.quasim@ggc.scot.nhs.uk, Nicholas.rattray@strath.ac.uk

Phone number (Prof Moug's secretary): xxxx xxx xxxx

Address:
Level 5, Department of Surgery,
Corsebar Road,
Royal Alexandra Hospital
Paisley
PA2 9PN

If you wish to discuss the study with someone who is not closely linked to the study, please contact: Maggie Clark, ELLSA nurse specialist xxxx xxxxxxx

Who Can I Contact If I Have a Complaint about the Study?

You are usually asked to put your complaint in writing so that the terms of the complaint are clear. You can ask someone else to do this for you, such as a family member, your GP or a member of the care team. The care team can help you if you are unsure about anything to do with the complaint's procedures.

You can find out more about the complaints process or send your complaint to NHS Greater Glasgow and Clyde using the following details:

NHS Greater Glasgow and Clyde
Complaints Department
North East Sector Offices
Stobhill Hospital
300 Balgrayhill Road
Glasgow
G21 3UR
Tel: 0141 201 4500
Email: complaints@ggc.scot.nhs.uk

Thank you for taking the time to read this information.

Appendix 1.8.4: Consent form welfare attorney/ guardian/ nearest relative

Consent Form

(Welfare Attorney/ Welfare Guardian/ Nearest Relative)

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

Name of Researcher: Dr. Hwei Jene Ng

Supervisor: Prof Susan Moug, Prof Tara Quasim, Dr. Nicholas Rattray

If you agree to participate on behalf of your ward/ relative, please write your initials in the relevant box for each statement and sign, name and dat[illegible]
Thank you.

Please initial box

1. I confirm that I have read and understood the Welfare Attorney/ Welfare Guardian/ Nearest Relative Information Sheet (IRAS 293392 Version 1.5, dated 07/05/22) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my ward/ relative/ person I am consenting for's participation is voluntary and that I am free to withdraw them at any time, without giving any reason and without their medical care or legal rights being affected. ☐

3. For follow up on day 30 and day 90 (if my ward/ relative/ person I am consenting for still does not have capacity):

a. I agree to have their follow up data (blood sampling and questionnaire) performed in hospital during clinic visit/ attend Surgical Assessment Unit. ☐

b. If option (a) above is not suitable, I agree to allow the research team member of this study to collect the follow up data at their residence at an arranged time & date. ☐

4. I acknowledge that there will be no effect on treatment arising from the participation or non-participation of my ward/ relative/ person I am consenting fo in this research. ☐

5. If I decide to withdraw my ward/ relative/ person I am consenting for from the study, I agree that the information collected about them up to the point of withdrawal will be used in the data analysis for this study. ☐

6. I agree to my ward/ relative/ person I am consenting for's pseudo-anonymised data/ blood sample being used for future ethically approved studies. ☐

7. I agree to my ward/ relative/ person I am consenting for's General Practitioner being informed of their participation in this study. ☐

Confidentiality

1. I acknowledge that my ward/ relative/ person I am consenting for's personal details in this study will be kept confidential and will be referred to using a study ID. ☐

2. I understand that my ward/ relative/ person I am consenting for's personal data will be accessible to the research team as required where relevant to this study and to representatives of the Sponsor who audit the study to check the work of the researchers. ☐

Data usage and storage

4. All names and other material likely to identify individuals will be pseudo-anonymised and referred to using a study ID. ☐
5. The material will be treated as confidential and kept in secure storage at all times. ☐
6. The material may be used in future publications, both print and online. ☐

Consent

I agree that my ward/ relative/ person I am consenting for will take part in this research study whilst he/ she has no capacity and this includes: ☐

- Personal and clinical data collection

- Pre and post surgery CT scan data collection

- Additional volume of blood collected during hospital stay (before surgery, post surgery day 1 and day 7/ discharge day), day 30 and day 90

- Answer questionnaire on day 30 and day 90

(Optional) I would like to receive a newsletter with the results of the study via

- Post ☐ Yes/No

- Email (please provide email:) ☐ Yes/No

I do not agree to my ward/ relative/ person I am consenting for taking part in this research study ☐

I confirm that I am the welfare attorney/ welfare guardian/ next of kin for _____

Name of person giving consent _____

Relationship to patient _____

Signature _____ Date _____

Name of Researcher _____ Signature _____

Date _____

Appendix 1.8.5: Consent form (telephone) Welfare attorney/ guardian/ nearest relative

Telephone Consent Form

(Welfare Attorney/ Welfare Guardian/ Nearest Relative)

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

Name of Researcher: Dr. Hwei Jene Ng

Supervisor: Prof Susan Moug, Prof Tara Quasim, Dr. Nicholas Rattray

If the Welfare Attorney/ Welfare Guardian/ Nearest Relative agree to participate on behalf of your ward/ relative, please write their initials in the relevant box for each statement and sign, name and date below. Than *Please initial box*

1. I confirm that I have been explained the main points of the above study to understand the Welfare Attorney/ Welfare Guardian/ Nearest Relative Information Sheet (IRAS 293392 Version 1.5, dated 07/05/22) and have had the opportunity to ask questions. ☐

2. I understand that my ward/ relative/ person I am consenting for's participation is voluntary and that I am free to withdraw them at any time, without giving any reason and without their medical care or legal rights being affected. ☐

3. For follow up on day 30 and day 90 (if my ward/ relative/ person I am consenting for still does not have capacity):

a. I agree to have their follow up data (blood sampling and questionnaire) performed in hospital during clinic visit/ attend Surgical Assessment Unit. ☐

b. If option (a) above is not suitable, I agree to allow the research team member of this study to collect the follow up data at their residence at an arranged time & date. ☐

4. I acknowledge that there will be no effect on treatment arising from the participation or non-participation of my ward/ relative/ person I am consenting fo in this research. ☐

5. If I decide to withdraw my ward/ relative/ person I am consenting for from the study, I agree that the information collected about them for up to the point of withdrawal will be used in the data analysis for this study. ☐

6. I agree to my ward/ relative/ person I am consenting for's pseudo-anonymised data/ blood sample being used for future ethically approved studies. ☐

7. I agree to my ward/ relative/ person I am consenting for's General Practitioner being informed of their participation in this study. ☐

8. I confirm that the Welfare Attorney/ Welfare Guardian/ Nearest Relative Information Sheet (IRAS 293392 Version 1.5, dated 07/05/22) and Welfare Attorney/ Welfare Guardian/ Nearest Relative Consent Form (IRAS 293392 Version 1.5, dated 07/05/22) will be posted/ emailed to me.

☐

Confidentiality

1. I acknowledge that my ward/ relative/ person I am consenting for's personal details in this study will be kept confidential and will be referred to using a study ID. ☐

2. I understand that my ward/ relative/ person I am consenting for's personal data will be accessible to the research team as required where relevant to this study and to representatives of the Sponsor who audit the study to check the work of the researchers. ☐

Data usage and storage

7. All names and other material likely to identify individuals will be pseudo-anonymised and referred to using a study ID. ☐
8. The material will be treated as confidential and kept in secure storage at all times. ☐
9. The material may be used in future publications, both print and online. ☐

Consent

I agree that my ward/ relative/ person I am consenting for will take part in this research study whilst he/ she has no capacity and this includes: ☐

- Personal and clinical data collection

- Pre and post surgery CT scan data collection

- Additional volume of blood collected during hospital stay (before surgery, post surgery day 1 and day 7/ discharge day), day 30 and day 90

- Answer questionnaire on day 30 and day 90

(Optional) I would like to receive a newsletter with the results of the study via

- Post ☐ Yes/No

- Email (please provide email:) ☐ Yes/No

I do not agree to my ward/ relative/ person I am consenting for taking part in this research study ☐

I confirm that I am the welfare attorney/ welfare guardian/ next of kin for _____

_____

Name of person giving consent _____

Relationship to patient _____

Signature _____ Date _____

(Signed on behalf of Welfare Attorney/ Welfare Guardian/ Nearest Relative)

Name of Researcher _____ Signature _____

Date _____

Witness declaration telephone consent

I confirm that consent was obtained voluntarily, information on the study was provided, queries have been answered satisfactorily and that the articles on this consent form were discussed. The conversation details have/ will be documented in medical notes. The patient and Welfare Attorney/ Welfare Guardian/ Nearest Relative information sheet and consent forms will be provided in writing.

Name of witness _____ Signature _____

Designation _____ Date _____

Appendix 1.8.6: Record of telephone consent form

Welfare Attorney/ Welfare Guardian/ Nearest Relative
Record of Telephone Consent

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

Name of Researcher: Dr. Hwei Jene Ng

Supervisor: Prof Susan Moug, Prof Tara Quasim, Dr. Nicholas Rattray

This sheet is to assist with the process of taking telephone consent from a Welfare Attorney or Welfare Guardian or Nearest Relative (Personal Legal Representative, PLR)

To note: A Personal Legal Representative can be asked to give consent on behalf of an adult who lacks the capacity (is unable) to do so themselves. In Scotland, this can be the adult's welfare guardian, welfare attorney or if these are not appointed, the adult's nearest relative.

The telephone call to seek consent from the PLR should always be witnessed by an independent
member of staff.

1. Introduce yourself and ensure that you are speaking to the contact listed for the patient in question.
2. Confirm that the person you are speaking to is the appropriate PLR.
3. Confirm that the PLR has already spoken to the clinical team regarding the patient's diagnosis and treatment and clarify that this call is not to discuss their relative's condition.
4. Explain the purpose of the call and check the nominated PLR is happy to discuss the research study and consenting on behalf of the identified patient. Explain that telephone consent would not normally be the preferred option for consent but in these extraordinary circumstances the research team have decided it is the best way forward.

5. Explain that the PLR should feel under no obligation to discuss the research study with you and confirm it is a convenient time to discuss the study. If not, the study should be discussed with the PLR at a more convenient time.
6. Ensure that the PLR is aware that they will be on speaker phone (in a private area) or that the independent witness will be placed on the phone at the end of the conversation to verify consent.
7. During this global pandemic, the majority of relatives will not be allowed in the hospital so this telephone call could be the first time the PLR has heard about the study (this is even more likely in the case of WA/WG). It is highly likely the PLR has not read an information sheet. You should therefore confirm a preferred method for sending an information sheet to the PLR (email, text or mail). Record the PLR's wishes at the end of this document.
8. Then explain that the PLR can have up to 24 hours to consider the study and that that if they wish to review a copy of the information sheet and consent form prior to consent, these documents can be sent via email or text or can be viewed on the website. If this is the case, confirm a convenient time to phone the PLR once the documents have been reviewed and the PLR has had the opportunity to consider the information.
9. Explain that if the PLR does not wish to see the information sheet and consent form before consent but wishes more time to think about the study and called back, an appropriate time for this can be arranged.
10. Please ensure the PLR is aware that they can withdraw at any point after consenting.

If the PLR is happy to continue with the conversation regarding consent:

1. Briefly outline the main points of the study to ensure that the PLR understands the patient information sheet.
2. Emphasise that there is no obligation for the PLR to agree to their relative taking part in this study.
3. Ensure that the PLR has considered whether their ward/ relative would have agreed to be part of a research study if they were able to consent for themselves.
4. The PLR should be given ample opportunity to ask questions and clarify any details of the study.
5. Once the PLR is happy that they understand what they are required to do, the consent statements on Welfare Attorney/ Welfare Guardian/ Nearest Relative Telephone Consent (IRAS 293392 Version 1.0, dated 16/02/22) should be asked in order. At this point, the researcher should initial the appropriate boxes that the PLR agrees to and then must sign the consent form.
6. Ensure that the PLR is fully aware that the researcher will be providing a signature on their behalf (and that of the patient). The independent member of staff must sign the consent form and witness declaration.
7. Ensure that the PLR is aware that we will also ask (if it's possible) for a signature from them (via post or an electronic signature). It is also possible for the PLR to send an email to the study team detailing their consent. The PLR will be sent the template below.
8. Ensure the PLR is aware that however the consent form is signed they will receive a copy of it with the information sheet in the post.
9. Please ensure the PLR are aware that they can withdraw at any point including once they receive a copy of the information sheet and consent.

10. Ensure the details below are completed and sign to state that this guidance was used during the consent process.

Please record:

Patient name:…….. Participant ID:……..

Name of PLR
(WA/WG/NR) ……

Date of phone call: …… Time of phone call: ……

Preferred method of receiving the information sheet (email, text or mail):……..

Email address:…….……..

Postal address:……..

……..

……..

……..

Name of researcher…….……..

Signature of researcher…….……..

Name of Witness……

Signature of Witness……

++

Template for e-mail

Title: Investigation of biology and outcomes of frailty in emergency laparotomy

Personal Legal Representative's Name-

Patients Name-

I (*PLR name entered here*) give consent for (*patient name entered here*) to take part in the Investigation of biology and outcomes of frailty in emergency laparotomy research study. I can confirm that I have had the study explained to me in full and had the opportunity to ask and have any questions answered.

Signed (PLR's name)

Date (Today's date)

Appendix 1.8.7: Patient Information Sheet (self-consenting patient participants after regaining capacity)

Patient Information Sheet

(Self-Consenting Patient Participants After Regaining Capacity)

Department of General Surgery
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN

Investigation of biology and outcomes of frailty in emergency laparotomy

We would like to invite you to take part in this research study. Your Welfare Attorney/ Welfare Guardian/ Nearest Relative has agreed on your behalf to participate in this study when you were too unwell to make the decision. This information sheet will provide you with a general understanding of the research that is being done and what it would involve for you. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?

The research is being carried out by:

- Miss H Jene Ng (Local Principal Investigator/ PhD Student) – University of Glasgow & Dept of General Surgery, Royal Alexandra Hospital, Paisley
- Professor Susan Moug (Chief Investigator/ Academic Supervisor)- Department of General Surgery, Royal Alexandra Hospital, Paisley, and Honorary Professor, University of Glasgow
- Professor Tara Quasim (Academic Supervisor)- Department of Anaesthesia, Critical Care & Perioperative Medicine, Glasgow Royal Infirmary, and Professor, University of Glasgow
- Dr. Nicholas Rattray (Academic Supervisor)- Lecturer of Clinical Metabolism, University of Strathclyde

What is the purpose of the study?

Emergency abdominal surgery (laparotomy) is one of the most common operations performed in the UK (30,000/ year) and is usually on older adults (>65 years). It does, however, carry a risk of developing problems after surgery (complications), including death, especially if there is frailty before the operation.

Frailty is a term used commonly in society but has been more challenging to define in a medical sense. Being frail makes a person more likely to have an infection or illness or to lose their independence and going through an emergency surgery could also cause frailty in a non-frail patient ('surgical frailty').

Although we usually think frailty exists only in older adults, the frailty problem is much bigger because frailty also exists in younger adults and patients can become frail after emergency surgery.

This research aims to

- Look at the changes in blood samples in patients undergoing emergency laparotomy and whether these changes are related to frailty for up to 90 days after surgery.
- Look at measures of frailty and see if they relate to patient morbidity (medical problems caused by the treatment) and mortality (death). These measures of frailty are the loss of muscle mass (seen on a CT scan) and how much a person can do. The measures are made by the doctors and nurses on all patients before they have surgery – they will not be done just for this research study.
- Find out about quality of life for patients after surgery.

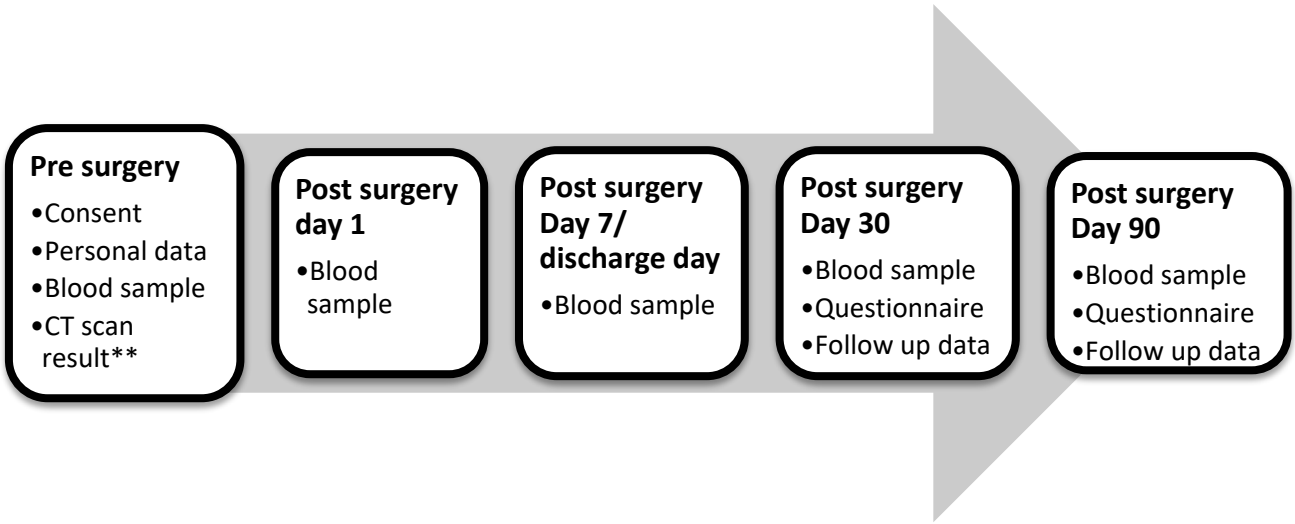
Why have I been invited?

If you are above 40 years old and undergoing/ underwent emergency abdominal surgery, you are eligible to take part.

Do I have to take part?

No. It is up to you to decide whether to take part in the research or not. If you decide that you wish to take part you are free to change your mind at any time and without giving a reason and this will not alter your care in any way, now or at any stage in the future. The data collected up to the point of withdrawal will be retained and used for analysis. Some of the data/samples will already have been collected because your Welfare Attorney/ Welfare Guardian/ Nearest Relative agreed to you taking part. You can decide if you want to stay in the study; if you decide you do not, no more data or samples will be collected from you.

What does taking part involve?



** Data on additional CT scan done after surgery will be collected but no extra CT scan will be done for research purposes.

- Details- We will collect your details including: age, gender, address including postcode, ethnic origin, medical history, lifestyle (physically active), alcohol intake, smoking status, height, weight, place of residence (home and independent, home with carers, home with family support, sheltered housing, nursing home), nursing assessment. We will also record your GP's name and contact details. We will obtain all of this information from your admission documents.

- Blood samples- An additional tube of your blood sample will be collected while in hospital (before surgery, after surgery day 1 and day 7/ discharge day) and will be used in this research. The result from your routine blood tests done during your stay in hospital will be recorded in this study (before surgery, after surgery day 1 and day 7/ discharge day). After discharge, we will require follow up blood tests to look at the changes in the blood that predict frailty. We will require you to get your blood sample taken at **day 30 and 90** after your emergency surgery. We will collect your day 30 blood test at the clinic when you attend for your routine 30-day check-up and at the surgical assessment unit in hospital on day 90. The clinic appointment at day 30 is part of usual care; the appointment at day 90 is an extra for the study only. We will contact and remind you 2 weeks before the follow up dates for these follow up data. If you are unable to attend hospital, let us know when we contact you and with your consent, we can arrange a date/ time that is suitable for the research team (HJ Ng) to collect the blood sample at your residence.

- CT scan- The CT scan that was used to diagnose your condition that required you to undergo surgery will be used in this research to assess sarcopenia (loss of muscle mass). We will be measuring the muscle bulk of your back on the CT scan. No extra scans will be performed as part of this research work. We will also collect data on any extra CT scans that are performed post-operatively.

- Questionnaire- We will ask you some questions at a face to face meeting during your hospital visit for your follow up blood sample collection to check your quality of life, especially changes in daily activity since your surgery (place of residence, mobility, self-care, pet caring, return to work, leisure activity and disability) at **30 and 90 day post-**

surgery. We will be using the EQ5D-5L questionnaire which is often used to ask people about their quality of life.

- Your health after your surgery - we will check your electronic medical records for information about complications and mortality on post-surgery day 30 and day 90. The records will also be accessed 2 weeks prior date of follow up when you are contacted to arrange a date for follow up data collection.

Are there risks in taking part?

The main risk in taking part in this study is from the blood sampling which include pain and infection. We will take an extra amount of blood before surgery and then on day 1 and day 7 after surgery, at the same time as blood will be taken as part of standard care. Only the blood samples at day 30 and day 90 after surgery will be extra samples that would not be taken if you were not in this study. We will follow all the usual procedures to prevent pain and infection when taking blood samples. Other risks are associated with your data that are collected, but this information will be kept in a safe place and labelled using study ID. Some of the information collected are part of your standard care and will not be extra for this research therefore these risks are low.

Are there benefits to taking part?

There will be no direct benefit to you. This study may contribute to the understanding of frailty and lead to improvements in management of frail patients who undergo emergency surgery. This study may also lead to future treatment options to help frail patients.

What will happen to my data?

NHS Greater Glasgow and Clyde (NHS GG&C) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. NHS GG&C will keep identifiable information about you collected for this study for up to 3 years once the study ends.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. If you decide to withdraw at any time, you can contact the local principal investigator via email (hweijene.ng@ggc.scot.nhs.uk) or telephone (xxxx xxx xxxx).

Your identity and personal information will be completely confidential and known only to the primary researchers (HJN and SM) and representatives of the study Sponsor who audit the study to check the work of the researchers. A study ID will be allocated when your data are recorded and your personal information will only be accessed for follow up purposes. This means that your research data will only be labelled with a study ID, not your name (this is called pseudonymised data) and your name and other details will only be used when we need to contact you.

The blood samples collected will be stored using study ID only and may be used for future UK based studies that have research ethics approval.

The University of Glasgow will be the data processor for the study and will keep pseudonymised research data from the study for up to 10 years from the end of the study. This information will be stored within secure NHS and University electronic systems.

All research data will be held in accordance with The General Data Protection Regulation (2018).

Your research data (including pseudonymised medical information and questionnaire responses) will be combined with other participants' data and analysed together in order to understand more about the effects of emergency laparotomy. Some of this data will be analysed as part of a PhD educational qualification. All data will be anonymous when it is published – you will not be identified.

You can find out more about how we use your information at https://www.hra.nhs.uk/information-about-patients/ or by contacting NHS GG&C Information Governance Department.

Tel: 0141 355 2059

Email: data.protection@ggc.scot.nhs.uk

What will happen to the results of the study?

We plan to share the results of this study to help researchers, clinical team, scientists, third parties such as Royal College of Surgeons, Royal College of Medicine and Royal College of Anaesthetists and patients in the future. This will be done through presentation at medical conferences, publication in medical journals and PhD thesis. All data will be anonymous when it is published – you will not be identified.

We would also like to send you a summary at the end of the project by post or email. Let us know on the consent form if you would like to receive this.

Who has reviewed the study?

This study has been reviewed by the Scotland A Research Ethics Committee.

Who Can I Contact for More Information?:

The Local Principal Investigator is a General Surgery Registrar in the West of Scotland. If you have any queries relating to this research, please feel free to contact the research team using the details below:

Local Principal Investigator: hweijene.ng@ggc.scot.nhs.uk

Supervisor: susan.moug@ggc.scot.nhs.uk, tara.quasim@ggc.scot.nhs.uk, Nicholas.rattray@strath.ac.uk

Phone number (Prof Moug's secretary): xxxx xxx xxxx

Address:
Level 5, Department of Surgery,
Corsebar Road,
Royal Alexandra Hospital
Paisley
PA2 9PN

If you wish to discuss the study with someone who is not closely linked to the study, please contact: Maggie Clark, ELLSA nurse specialist xxxx xxxxxxx

Who Can I Contact If I Have a Complaint about the Study?

You are usually asked to put your complaint in writing so that the terms of the complaint are clear. You can ask someone else to do this for you, such as a family member, your GP or a member of the care team. The care team can help you if you are unsure about anything to do with the complaint's procedures.

You can find out more about the complaints process or send your complaint to NHS Greater Glasgow and Clyde using the following details:

NHS Greater Glasgow and Clyde

Complaints Department
North East Sector Offices
Stobhill Hospital
300 Balgrayhill Road
Glasgow
G21 3UR
Tel: 0141 201 4500
Email: complaints@ggc.scot.nhs.uk

Thank you for taking the time to read this information.

Appendix 1.8.8: Consent form regained capacity

Consent Form (Regained Capacity)

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

Name of Researcher: Dr. Hwei Jene Ng

Supervisor: Prof Susan Moug, Prof Tara Quasim, Dr. Nicholas Rattray

This consent form is for the purpose of obtaining consent after patient regained capacity (after emergency laparotomy).

If you agree to participate, please write your initials in the relevant box for each statement and sign, name and date below. Thank you.

Please initial box

1. I confirm that I have read and understood the Participant Information Sheet (Recovered Capacity, IRAS 293392 Version 1.4, dated 07/05/22) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected. ☐

3. I acknowledge that there will be no effect on treatment arising from my participation or non-participation in this research. ☐

4. I understand that my Welfare Attorney/ Welfare Guardian/ Nearest Relative consented on my behalf while I did not have capacity. ☐

And

5. I understand that I am being asked to agree to continue participating in this study. ☐

6. If I decide to withdraw, I agree that the information collected about me up to the point of my withdrawal will be used in the data analysis for this study. ☐

7. For follow up on day 30 and day 90:

a. I agree to have my follow up data (blood sampling and questionnaire) performed in hospital during clinic visit/ attend Surgical Assessment Unit. ☐

b. If option (a) above is not suitable, I agree to allow the research team member of this study to collect the follow up data at my residence at an arranged time and date. ☐

8. I agree to my pseudo- anonymised data/ blood sample being used for future ethically approved studies. ☐

9. I agree to my General Practitioner being informed of my participation in this study. ☐

Confidentiality

1. I acknowledge that my personal details in this study will be kept confidential and will be referred to using a study ID. ☐

2. I understand that my personal data will be accessible to the research team as required where relevant to this study and to representatives of the Sponsor who audit the study to check the work of the researchers. ☐

Data usage and storage

1. All names and other material likely to identify individuals will be pseudo-anonymised and referred to using a study ID. ☐

2. The material will be treated as confidential and kept in secure storage at all times. ☐

3. The material may be used in future publications, both print and online. ☐

Consent

I agree to continue to take part in this research study and this includes ☐

- Personal and clinical data collection

- Pre and post surgery CT scan data collection

- Additional volume of blood collected during hospital stay (before surgery, post surgery day 1 and day 7/ discharge day), day 30 and day 90

- Answer questionnaire on day 30 and day 90

(Optional) I would like to receive a newsletter with the results of the study via

- Post Yes/No

- Email (please provide email:) Yes/No

I do not agree to continue to take part in this research study ☐

Name of Participant _____

Signature _____ Date _____

Name of Researcher _____ Signature _____

Date _____

Appendix 1.8.9: GP letter

GP LETTER

Dear Dr Date:

Re: Patient's name:

Date of birth:

Address:

Title of Project: Investigation of biology and outcomes of frailty in emergency laparotomy

This patient has consented to participate in the above research study.

This is a prospective single centred observational study that involves patients ≥40 years of age that undergo emergency laparotomy (EmLAP). This study aims to compare pre-operative frailty markers in their ability to predict 30 and 90-day mortality post-EmLAP. The secondary aims are to define peri-operative changes in frailty in patients undergoing EmLAP and to analyse the influence of peri-operative frailty on morbidity and quality of life post-EmLAP.

Your patient will have their blood samples collected (when they have their routine blood samples collected in hospital) on pre-operative, post-operative day 1 and day 7/ discharge day. Follow up blood samples will be collected at day 30 and 90 post-EmLAP where your patient is invited to return to hospital for their routine clinic review on Day 30 and to Surgical Assessment Unit on Day 90. If they are unable to attend hospital for follow up, the research team member will collect the follow up data at the patient's residence with consent. Questionnaire (EQ5D-5L) on quality of life post-EmLAP will be assessed on Day 30 and 90 when follow up blood samples are collected. Your patient will be followed up for 90 days.

A copy of the patient information sheet has been provided to your patient.

Should you have any questions concerning the patient's participation or require further information about this study, please contact myself on hweijene.ng@ggc.scot.nhs.uk.

Please ensure that a copy of this letter is kept in the patient's file at your practice and that the patient is flagged for taking part in this study. The results for this patient and/or overall study results will not be sent to the GP.

Many thanks and best wishes.

Dr. Hwei J Ng

Appendix 1.9: EQ-5D-5L questionnaire

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

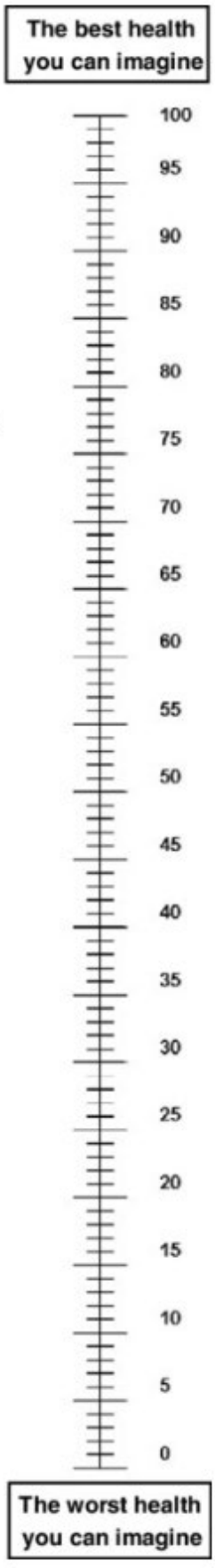
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is **TODAY**.
- This scale is numbered from **0** to **100**.
- 100 means the best you can imagine.
- Mark an **X** on the scale to indicate how your health is **TODAY**.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Appendix 1.10: Mass spectrometry analysis conditions

Ion Source

Ion Source Type: **H-ESI**
Spray Voltage: **Static**
Positive Ion (V): **3900**
Negative Ion (V): **2500**
Gas Mode: **Static**
Sheath Gas (Arb): **40**
Aux Gas (Arb): **5**
Sweep Gas (Arb): **1**
Ion Transfer Tube Temp (°C): **300**
Vaporizer Temp (°C): **280**
APPI Lamp: **Not in Use**
Use Ion Source Settings from Tune: **False**
FAIMS Mode: **Not Installed**

MS Global Settings

Infusion Mode: **Liquid Chromatography**
Expected LC Peak Width (s): **6**
Advanced Peak Determination: **False**
Mild Trapping: **True**
Default Charge State: **1**
Enable Xcalibur AcquireX method modifications: **False**
Internal Mass Calibration: **EASY-IC™**
Mode: **Run Start**

Experiment#1 [MS]

Start Time (min): **0**
End Time (min): **15**

Full scan filters

Full Scan

Orbitrap Resolution: **60000**
Scan Range (m/z): **70-1000**
RF Lens (%): **70**
AGC Target: **Standard**
Maximum Injection Time Mode: **Auto**
Microscans: **1**
Data Type: **Profile**
Polarity: **Positive**
Source Fragmentation: **Disabled**
Use EASY-IC™: **On**
Scan Description:

Filters:

Intensity

Intensity Threshold: **5.0e4**

Precursor Fit

Fit Threshold (%): **70**
Fit Window (m/z): **0.7**

Dynamic Exclusion

Dynamic Exclusion Mode: **Custom**
Exclude after n times: **1**
Exclusion duration (s): **60**
Mass Tolerance: **ppm**
Low: **3**
High: **3**
Exclude isotopes: **True**
Perform dependent scan on single charge state per precursor only: **False**

The ddMS2 filters:

ddMS^2

Multiplex Ions: **False**
Isolation Window (m/z): **0.7**
Isolation Offset: **Off**
Collision Energy Type: **Normalized**
HCD Collision Energy (%): **30**
Orbitrap Resolution: **30000**
Scan Range Mode: **Auto**
AGC Target: **Standard**

Appendix 1.11: Mass spectrometry target mass inclusion list

Appendix 1.11.1: C18 Positive mode

Compound	m/z	RT Time (min)	Window (min)	HCD Collision Energies (%)
1-METHYL-L-HISTIDINE	170.0923	6.37	2	20
1-METHYLADENOSINE	282.1196	1	2	10
1-phenylethanol	123.0805	1	2	30
1-PHENYLETHANOL	123.0805	1	2	30
2-AMINOISOBUTYRATE	104.1071	3.08	2	40
2-AMINOPHENOL	110.06	1	2	60
2-DEOXY-D-GLUCOSE	129.0547	3.6	2	10
2-HYDROXYPYRIDINE	96.0444	1	2	100
2-METHYLPROPANAL	73.0648	1	2	30
2-QUINOLINECARBOXYLATE	174.055	1	2	20
2,3-DIAMINOPROPIONATE	105.0659	9.82	2	10
2,6-DIHYDROXYPYRIDINE	112.0393	1	2	70
3-AMINO-4-HYDROXYBENZOATE	154.0503	1	2	50
3-AMINO-5-HYDROXYBENZOATE	154.0506	1	2	40
3-HYDROXYANTHRANILATE	136.0393	1	2	10
3-METHOXYTYRAMINE	168.1021	3.73	2	10

3-METHOXYTYROSINE	212.0943	2.71	2	10
3-Methyloxindole	148.0757	1	2	40
3-METHYL-2-OXINDOLE	148.0757	1	2	40
3-METHYL-2-OXOVALERATE	131.1624	1	2	40
3-Methyladenine	150.0775	3.84	2	80
3-METHYLADENINE	150.0775	3.84	2	80
3-NITRO-L-TYROSINE	227.0664	2.13	2	10
3,5-DIIODO-L-THYRONINE	525.9011	1	2	10
3,5-DIIODO-L-TYROSINE	433.874	2.13	2	10
4-ACETAMIDOBUTANOATE	86.06	1	2	10
4-AMINOBENZOATE	138.055	1	2	50
4-GUANIDINOBUTANOATE	146.0924	4.01	2	40
4-IMIDAZOLEACETATE	127.0502	3.91	2	20
4-PYRIDOXATE	184.0606	1	2	10
4-QUINOLINECARBOXYLATE	174.0551	1	2	50
5-AMINOLEVULINATE	132.0656	4.86	2	10
5-AMINOPENTANOATE	118.0864	1	2	60
5-HYDROXYTRYPTOPHAN	204.0657	4.24	2	10

5-METHYLCYTOSINE	126.0661	2.16	2	70
5,6 DIMETHYLBENZIMIDAZOLE	147.0918	1	2	80
5'-DEOXYADENOSINE	252.1092	1	2	10
6-HYDROXYNICOTINATE	140.0344	1	2	90
ACETOIN	72.0808	1	2	10
ADENINE	136.0618	1	2	70
ADENOSINE	268.1039	1	2	10
ADENOSINE 2',3'-CYCLIC PHOSPHATE	330.0598	1	2	20
ADENOSINE-MONOPHOSPHATE	348.0704	4.35	2	10
ADP-GLUCOSE	428.0366	6.89	2	10
AICAR	339.0689	5.52	2	10
AMINOADIPATE	162.076	4.17	2	10
AMINOISOBUTANOATE	104.0707	3.18	2	20
ARGININE	175.119	1	2	30
ASPARAGINE	133.0608	4.79	2	10
ASPARTATE	134.0449	3.71	2	10
BENZYL ALCOHOL	109.0648	1	2	10
BENZYLAMINE	91.0542	3.82	2	10
BETA-ALANINE	90.055	1	2	10
BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE	664.1159	5.47	2	10
BETAINE	118.0863	1	2	60
BIOTIN	245.0952	1	2	10

Decyl octyl phthalate	227.0614	5.22	2	10
BIS(2-ETHYLHEXYL) PHTHALATE	227.0674	1	2	10
BUTANOATE	89.0597	1	2	10
CAFFEINE	195.0876	1	2	40
CARNOSINE	227.1141	8.97	2	10
CDP-ETHANOLAMINE	324.0591	6.73	2	10
CIS-4-HYDROXY-D-PROLINE	132.0655	4.22	2	20
CITRULLINE	159.0764	4.99	2	10
CORTISOL	363.2166	1	2	20
CORTISOL 21-ACETATE	405.2268	1	2	20
CREATINE	132.0768	4.01	2	20
CREATININE	114.0662	2.17	2	40
CYCLIC AMP	330.0598	2.44	2	20
CYSTATHIONINE	223.0748	6.91	2	10
CYSTEATE	170.0117	5.83	2	10
CYSTINE	241.031	6.98	2	10
CYTIDINE	112.0506	1	2	10
CYTIDINE 2',3'-CYCLIC PHOSPHATE	112.0506	4.28	2	10
CYTIDINE MONOPHOSPHATE	112.0506	6.01	2	10
CYTOSINE	112.0506	1	2	70
D-(-)-Mannitol	69.0336	3.56	2	20
D-ALANINE	44.0495	1	2	40
D-MANNOSAMINE	180.0867	7.64	2	10
D-ORNITHINE	133.0972	9.48	2	10
DEOXYADENOSINE	252.1091	1	2	10
DEOXYADENOSINE	332.0757	3.53	2	10

MONOPHOSPHATE				
DEOXYCARNITINE	146.1176	2.3	2	30
Desoxycorticosterone acetate	227.0657	1	2	10
DEOXYCYTIDINE	112.0506	1	2	20
DEOXYCYTIDINE MONOPHOSPHATE	112.0506	5.17	2	10
DEOXYGUANOSINE	268.104	1	2	10
DEOXYGUANOSINE-MONOPHOSPHATE	348.0708	4.8	2	10
DEOXYURIDINE-MONOPHOSPHATE	227.0596	3.72	2	10
DETHIOBIOTIN	215.139	1	2	10
DIAMINOPIMELATE	191.1025	7.04	2	10
DIDECANOYL-GLYCEROPHOSPHOCHOLINE	566.3815	1	2	10
DIETHANOLAMINE	106.0863	1	2	30
DIHYDROBIOPTERIN	240.1103	2.59	2	20
DIHYDROFOLATE	178.0722	3.55	2	10
DOPAMINE	137.0598	4.81	2	10
EPINEPHRINE	166.0862	4.75	2	10
ETHANOLAMINE	62.0601	1	2	10
ETHYL 3-UREIDOPROPIONATE	161.0294	14	2	10
FOLATE	295.0937	3.38	2	10
GALACTITOL	129.0548	3.57	2	10
GALACTOSAMINE	180.102	7.84	2	40
GAMMA-AMINOBUTYRATE	104.0706	3.61	2	10
GLUCOSAMINATE	196.0816	5.85	2	20
GLUCOSAMINE	180.1022	7.96	2	30

GLUCOSAMINE 6-PHOSPHATE	260.1615	8.3	2	10
GLUCOSAMINE 6-SULFATE	242.033	8.2	2	10
GLUTAMATE	148.0604	3.24	2	10
GLUTAMINE	147.0765	3.58	2	10
GLUTARYLCARNITINE	276.1441	1	2	30
GLUTATHIONE REDUCED	308.0911	5.02	2	10
GLYCEROL	57.0335	5.18	2	10
GLYCEROL 3-PHOSPHATE	98.9841	5.45	2	10
GLYCINE	76.0393	4.98	2	10
GLYCOCHENODEOXYCHOLATE	432.3106	1	2	10
GLYCOCHOLATE	430.2948	1	2	10
GUANIDINOACETATE	118.0611	4.78	2	30
GUANINE	152.0568	3.25	2	40
GUANOSINE	284.3314	2.75	2	10
GUANOSINE MONOPHOSPHATE	152.0566	6.5	2	10
HIPPURATE	180.1021	1	2	20
HISTAMINE	112.0505	3.35	2	10
HOMOCYSTEINE THIOLACTONE	118.0321	3.87	2	10
HOMOCYSTINE	268.9997	6.26	2	10
HOMOSERINE	120.0808	1	2	10
HYPOTAURINE	110.0271	4.34	2	20
HYPOXANTHINE	137.0458	1	2	80
INDOLE-3-ETHANOL	162.0913	1	2	10
Methyl 3-indolylacetate	130.0652	1	2	10

INDOLE-3-METHYL ACETATE	130.0652	1	2	10
INDOLE-3-PYRUVATE	204.0653	1	2	10
INDOXYL-GLUCOSIDE	296.1143	1	2	10
INOSINE	268.9996	1	2	10
INOSINE-MONOPHOSPHATE	137.0458	5.58	2	10
ISOLEUCINE	132.102	1	2	10
Ketoleucine	131.1625	1	2	40
KETOLEUCINE	131.1625	1	2	40
KYNURENATE	190.0499	1	2	20
KYNURENINE	192.0655	2.05	2	10
L-ALANINE	90.055	1	2	10
L-CARNITINE	162.1126	1	2	50
L-DOPA	198.0763	4.66	2	10
L-ORNITHINE	133.0973	9.44	2	10
L-TRYPTOPHANAMIDE	204.1132	4.15	2	10
LACTOSE	227.0626	4.61	2	10
LAUROYLCARNITINE	344.2797	1	2	20
LEUCINE	132.102	1	2	10
LIPOAMIDE	206.0668	1	2	10
LITHOCHOLYLTAURINE	466.2981	1	2	10
LUMICHROME	243.0875	1	2	40
LYSINE	147.1129	9.13	2	10
MELATONIN	233.1286	1	2	10
METHIONINE	150.0584	1	2	10
METHIONINE SULFOXIMINE	181.0644	4.77	2	10

METHYL 4-AMINOBUTYRATE	118.0863	1	2	60
Methyl Jasmonate	225.1487	1	2	20
METHYL JASMONATE	225.1487	1	2	10
METHYLTHIOADENOSINE	298.0967	1	2	10
MEVALOLACTONE	131.1624	1	2	30
mevalonate	149.0234	11.97	2	50
MEVALONATE	149.0234	11.97	2	50
N-ACETYLALANINE	132.1019	1	2	10
N-ACETYLASPARAGINE	175.119	2.65	2	10
N-ACETYLASPARTATE	134.045	2.34	2	10
N-ACETYLCYSTEINE	164.1279	1	2	10
N-ACETYLGALACTOSAMINE	227.0677	1	2	20
N-ACETYLGLUCOSAMINE	227.0617	2.08	2	10
N-ACETYLGLUTAMATE	130.05	1	2	10
N-ACETYLLEUCINE	174.1278	1	2	30
N-ACETYLMETHIONINE	144.0656	1	2	10
N-ACETYLNEURAMINATE	274.0921	3.64	2	10
N-ACETYLPHENYLALANINE	162.0914	1	2	10
N-ACETYLPROLINE	158.0812	1	2	10

N-ACETYLPUTRESCINE	131.118	1	2	10
N-ACETYLSERINE	148.0604	2.27	2	10
N-ACETYLSEROTONIN	219.1128	1	2	10
N-ACETYLTRYPTOPHAN	247.1078	1	2	10
N-ALPHA-ACETYLLYSINE	189.1234	4.07	2	20
N-METHYLASPARTATE	148.0605	3.57	2	20
N-METHYLGLUTAMATE	162.0761	3.51	2	20
N-METHYLTRYPTAMINE	175.123	3.69	2	10
N-METYL-ALANINE	104.0706	2.77	2	10
N,N-DIMETHYLARGININE	203.1502	6.79	2	30
N,N,N-TRIMETHYLLYSINE	189.1598	7.11	2	20
N6-(DELTA2-ISOPENTENYL)-ADENINE	204.1242	1	2	20
NICOTINAMIDE	123.0553	1	2	60
NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	543.0522	6.06	2	10
NICOTINAMIDE MONONUCLEOTIDE	123.0553	5.26	2	10
NICOTINATE	124.0394	1	2	70
NORADRENALINE	170.0967	6.67	2	50
NORLEUCINE	132.1019	1	2	10

NORMETANEPHRINE	184.1695	4.13	2	10
NORVALINE	118.0864	1	2	60
O-ACETYLCARNITINE	204.1231	1	2	20
O-ACETYLSERINE	148.0606	2.83	2	10
O-SUCCINYL-HOMOSERINE	220.0818	3.17	2	10
OXOPROLINE	130.0499	2.14	2	20
P-OCTOPAMINE	154.0444	4.77	2	40
PALMITOYLCARNITINE	400.342	1	2	20
PANTOLACTONE	131.1623	1	2	40
PANTOTHENATE	220.118	1	2	20
PARAXANTHINE	181.0722	1	2	40
PHENYLALANINE	166.0863	1	2	10
PHENYLETHANOLAMINE	120.0809	3.88	2	10
PHOSPHORYLCHOLINE	184.0733	1	2	30
PIPECOLATE	130.0864	1	2	20
PROLINE	116.0706	1	2	20
Protoporphyrin IX	563.265	1	2	40
PROTOPORPHYRIN	563.265	1	2	40
PTERIN	164.0568	1	2	50
PURINE	121.0509	1	2	80
PUTRESCINE	72.0808	1	2	10
PYRAZOLE	69.0448	1	2	90
PYRIDOXAL-PHOSPHATE	248.0317	1	2	20
PYRIDOXAMINE	169.0973	5.22	2	10
PYRIDOXINE	170.0812	1	2	10
PYROGLUTAMATE	130.05	1	2	20

RIBOFLAVIN	377.1453	1	2	30
S-ADENOSYLHOMOCYSTEINE	385.1284	3.95	2	10
S-CARBOXYMETHYLCYSTEINE	180.0325	4.92	2	10
SALSOLINOL	180.1019	1	2	30
SARCOSINE	90.055	1	2	10
SERINE	106.0499	1	2	10
SEROTONIN	160.0757	4.51	2	10
SUBERATE	175.1115	1	2	20
THEOBROMINE	181.0721	1	2	50
THEOPHYLLINE	181.072	1	2	40
THIAMINE MONOPHOSPHATE	345.0781	4.93	2	10
THIOPURINE S-METHYLETHER	167.0386	1	2	60
THREONINE	120.0808	1	2	10
THYMIDINE-MONOPHOSPHATE	81.0336	3.45	2	10
THYMINE	127.0503	1	2	50
THYROTROPIN RELEASING HORMONE	363.1774	3.08	2	20
TRANS-4-HYDROXY-L-PROLINE	132.0656	3.96	2	20
TRIGONELLINE	138.0551	1	2	70
TRIMETHYLAMINE	60.0808	3.81	2	80
TRYPTAMINE	161.1073	3.75	2	40
TRYPTOPHAN	188.0707	1	2	10
TYRAMINE	138.0915	3.74	2	40
TYROSINE	182.0812	1	2	10
UREIDOPROPIONATE	115.0503	1	2	10

URIDINE DIPHOSPHATE-N-ACETYLGALACTOSAMINE	204.0867	6.84	2	10
URIDINE MONOPHOSPHATE	325.1048	5.03	2	10
UROCANATE	139.0502	1	2	30
VALINE	118.0862	1	2	60
XANTHINE	153.041	1	2	40
XANTHOSINE	153.0408	2.25	2	10
XANTHOSINE-MONOPHOSPHATE	365.1571	6.28	2	10
XANTHURENATE	206.0449	2.3	2	30
DEOXYCORTICOSTERONE ACETATE	227.0657	1	2	10
biotin	245.0952	1	2	10
benzyl alcohol	109.0648	1	2	20
L-Alanine-13C3,15N	94.0626	7.5	15	10
L-Arginine-13C6,15N4_hydrochloride	184.1199	7.5	15	20
L-Asparagine-15N2	135.0554	7.5	15	10
L-Aspartic acid-13C4,15N4	139.0558	7.5	15	10
L-Glutamic acid-13C5,15N	154.0748	7.5	15	10
L-Glutamine-15N2	149.071	7.5	15	10
Glycine-13C2,15N	79.0436	7.5	15	10
L-Histidine-15N3	159.0684	7.5	15	10
L-Isoleucine-13C6,15N	139.1196	7.5	15	10
L-Leucine-13C6,15N	139.1196	7.5	15	10
L-Lysine-13C6,15N2_hydrochloride	155.1275	7.5	15	10

L-Methionine-15N	151.0559	7.5	15	10
L-Phenylalanine-13C9,15N	176.114	7.5	15	10
L-Proline-13C5,15N	122.085	7.5	15	20
L-Serine-13C3,15N	110.0575	7.5	15	10
L-Threonine-13C4,15N	125.0765	7.5	15	10
L-Tryptophan-15N2	207.0918	7.5	15	10
L-Tyrosine-13C9,15N	192.1089	7.5	15	10
L-Valine-13C5,15N	124.1006	7.5	15	10

Appendix 1.11.2: C18 Negative mode

Compound	m/z	RT Time (min)	Window (min)	HCD Collision Energies (%)
1-METHYL-L-HISTIDINE	168.0766	6.37	2	20
1-METHYLADENOSINE	280.1039	1	2	10
1-phenylethanol	121.0648	1	2	30
1-PHENYLETHANOL	121.0648	1	2	30
2-AMINOISOBUTYRATE	102.0914	3.08	2	40
2-AMINOPHENOL	108.0443	1	2	60
2-DEOXY-D-GLUCOSE	127.039	3.6	2	10
2-HYDROXYPYRIDINE	94.0287	1	2	100
2-METHYLPROPANAL	71.0491	1	2	30
2-QUINOLINECARBOXYLATE	172.0393	1	2	20
2,3-DIAMINOPROPIONATE	103.0502	9.82	2	10
2,6-DIHYDROXYPYRIDINE	110.0236	1	2	70
3-AMINO-4-HYDROXYBENZOATE	152.0346	1	2	50
3-AMINO-5-HYDROXYBENZOATE	152.0349	1	2	40
3-HYDROXYANTHRANILATE	134.0236	1	2	10
3-METHOXYTYRAMINE	166.0864	3.73	2	10

3-METHOXYTYROSINE	210.0786	2.71	2	10
3-Methyloxindole	146.06	1	2	40
3-METHYL-2-OXINDOLE	146.06	1	2	40
3-METHYL-2-OXOVALERATE	129.1467	1	2	40
3-Methyladenine	148.0618	3.84	2	80
3-METHYLADENINE	148.0618	3.84	2	80
3-NITRO-L-TYROSINE	225.0507	2.13	2	10
3,5-DIIODO-L-THYRONINE	523.8854	1	2	10
3,5-DIIODO-L-TYROSINE	431.8583	2.13	2	10
4-ACETAMIDOBUTANOATE	84.0443	1	2	10
4-AMINOBENZOATE	136.0393	1	2	50
4-GUANIDINOBUTANOATE	144.0767	4.01	2	40
4-IMIDAZOLEACETATE	125.0345	3.91	2	20
4-PYRIDOXATE	182.0449	1	2	10
4-QUINOLINECARBOXYLATE	172.0394	1	2	50
5-AMINOLEVULINATE	130.0499	4.86	2	10
5-AMINOPENTANOATE	116.0707	1	2	60
5-HYDROXYTRYPTOPHAN	202.05	4.24	2	10

5-METHYLCYTOSINE	124.0504	2.16	2	70
5,6 DIMETHYLBENZIMIDAZOLE	145.0761	1	2	80
5'-DEOXYADENOSINE	250.0935	1	2	10
6-HYDROXYNICOTINATE	138.0187	1	2	90
ACETOIN	70.0651	1	2	10
ADENINE	134.0461	1	2	70
ADENOSINE	266.0882	1	2	10
ADENOSINE 2',3'-CYCLIC PHOSPHATE	328.0441	1	2	20
ADENOSINE-MONOPHOSPHATE	346.0547	4.35	2	10
ADP-GLUCOSE	426.0209	6.89	2	10
AICAR	337.0532	5.52	2	10
AMINOADIPATE	160.0603	4.17	2	10
AMINOISOBUTANOATE	102.055	3.18	2	20
ARGININE	173.1033	1	2	30
ASPARAGINE	131.0451	4.79	2	10
ASPARTATE	132.0292	3.71	2	10
BENZYL ALCOHOL	107.0491	1	2	10
BENZYLAMINE	89.0385	3.82	2	10
BETA-ALANINE	88.0393	1	2	10
BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE	662.1002	5.47	2	10
BETAINE	116.0706	1	2	60
BIOTIN	243.0795	1	2	10

Decyl octyl phthalate	225.0457	5.22	2	10
BIS(2-ETHYLHEXYL) PHTHALATE	225.0517	1	2	10
BUTANOATE	87.044	1	2	10
CAFFEINE	193.0719	1	2	40
CARNOSINE	225.0984	8.97	2	10
CDP-ETHANOLAMINE	322.0434	6.73	2	10
CIS-4-HYDROXY-D-PROLINE	130.0498	4.22	2	20
CITRULLINE	157.0607	4.99	2	10
CORTISOL	361.2009	1	2	20
CORTISOL 21-ACETATE	403.2111	1	2	20
CREATINE	130.0611	4.01	2	20
CREATININE	112.0505	2.17	2	40
CYCLIC AMP	328.0441	2.44	2	20
CYSTATHIONINE	221.0591	6.91	2	10
CYSTEATE	167.996	5.83	2	10
CYSTINE	239.0153	6.98	2	10
CYTIDINE	110.0349	1	2	10
CYTIDINE 2',3'-CYCLIC PHOSPHATE	110.0349	4.28	2	10
CYTIDINE MONOPHOSPHATE	110.0349	6.01	2	10
CYTOSINE	110.0349	1	2	70
D-(-)-Mannitol	67.0179	3.56	2	20
D-ALANINE	42.0338	1	2	40
D-MANNOSAMINE	178.071	7.64	2	10
D-ORNITHINE	131.0815	9.48	2	10
DEOXYADENOSINE	250.0934	1	2	10
DEOXYADENOSINE	330.06	3.53	2	10

MONOPHOSPHATE				
DEOXYCARNITINE	144.1019	2.3	2	30
Desoxycorticosterone acetate	225.05	1	2	10
DEOXYCYTIDINE	110.0349	1	2	20
DEOXYCYTIDINE MONOPHOSPHATE	110.0349	5.17	2	10
DEOXYGUANOSINE	266.0883	1	2	10
DEOXYGUANOSINE - MONOPHOSPHATE	346.0551	4.8	2	10
DEOXYURIDINE-MONOPHOSPHATE	225.0439	3.72	2	10
DETHIOBIOTIN	213.1233	1	2	10
DIAMINOPIMELATE	189.0868	7.04	2	10
DIDECANOYL-GLYCEROPHOSPHOCHOLINE	564.3658	1	2	10
DIETHANOLAMINE	104.0706	1	2	30
DIHYDROBIOPTERIN	238.0946	2.59	2	20
DIHYDROFOLATE	176.0565	3.55	2	10
DOPAMINE	135.0441	4.81	2	10
EPINEPHRINE	164.0705	4.75	2	10
ETHANOLAMINE	60.0444	1	2	10
ETHYL 3-UREIDOPROPIONATE	159.0137	14	2	10
FOLATE	293.078	3.38	2	10
GALACTITOL	127.0391	3.57	2	10
GALACTOSAMINE	178.0863	7.84	2	40
GAMMA-AMINOBUTYRATE	102.0549	3.61	2	10
GLUCOSAMINATE	194.0659	5.85	2	20
GLUCOSAMINE	178.0865	7.96	2	30

GLUCOSAMINE 6-PHOSPHATE	258.1458	8.3	2	10
GLUCOSAMINE 6-SULFATE	240.0173	8.2	2	10
GLUTAMATE	146.0447	3.24	2	10
GLUTAMINE	145.0608	3.58	2	10
GLUTARYLCARNITINE	274.1284	1	2	30
GLUTATHIONE REDUCED	306.0754	5.02	2	10
GLYCEROL	55.0178	5.18	2	10
GLYCEROL 3-PHOSPHATE	96.9684	5.45	2	10
GLYCINE	74.0236	4.98	2	10
GLYCOCHENODEOXYCHOLATE	430.2949	1	2	10
GLYCOCHOLATE	428.2791	1	2	10
GUANIDINOACETATE	116.0454	4.78	2	30
GUANINE	150.0411	3.25	2	40
GUANOSINE	282.3157	2.75	2	10
GUANOSINE MONOPHOSPHATE	150.0409	6.5	2	10
HIPPURATE	178.0864	1	2	20
HISTAMINE	110.0348	3.35	2	10
HOMOCYSTEINE THIOLACTONE	116.0164	3.87	2	10
HOMOCYSTINE	266.984	6.26	2	10
HOMOSERINE	118.0651	1	2	10
HYPOTAURINE	108.0114	4.34	2	20
HYPOXANTHINE	135.0301	1	2	80
INDOLE-3-ETHANOL	160.0756	1	2	10
Methyl 3-indolylacetate	128.0495	1	2	10

INDOLE-3-METHYL ACETATE	128.0495	1	2	10
INDOLE-3-PYRUVATE	202.0496	1	2	10
INDOXYL-GLUCOSIDE	294.0986	1	2	10
INOSINE	266.9839	1	2	10
INOSINE-MONOPHOSPHATE	135.0301	5.58	2	10
ISOLEUCINE	130.0863	1	2	10
Ketoleucine	129.1468	1	2	40
KETOLEUCINE	129.1468	1	2	40
KYNURENATE	188.0342	1	2	20
KYNURENINE	190.0498	2.05	2	10
L-ALANINE	88.0393	1	2	10
L-CARNITINE	160.0969	1	2	50
L-DOPA	196.0606	4.66	2	10
L-ORNITHINE	131.0816	9.44	2	10
L-TRYPTOPHANAMIDE	202.0975	4.15	2	10
LACTOSE	225.0469	4.61	2	10
LAUROYLCARNITINE	342.264	1	2	20
LEUCINE	130.0863	1	2	10
LIPOAMIDE	204.0511	1	2	10
LITHOCHOLYLTAURINE	464.2824	1	2	10
LUMICHROME	241.0718	1	2	40
LYSINE	145.0972	9.13	2	10
MELATONIN	231.1129	1	2	10
METHIONINE	148.0427	1	2	10
METHIONINE SULFOXIMINE	179.0487	4.77	2	10

METHYL 4-AMINOBUTYRATE	116.0706	1	2	60
Methyl Jasmonate	223.133	1	2	20
METHYL JASMONATE	223.133	1	2	10
METHYLTHIOADENOSINE	296.081	1	2	10
MEVALOLACTONE	129.1467	1	2	30
mevalonate	147.0077	11.97	2	50
MEVALONATE	147.0077	11.97	2	50
N-ACETYLALANINE	130.0862	1	2	10
N-ACETYLASPARAGINE	173.1033	2.65	2	10
N-ACETYLASPARTATE	132.0293	2.34	2	10
N-ACETYLCYSTEINE	162.1122	1	2	10
N-ACETYLGALACTOSAMINE	225.052	1	2	20
N-ACETYLGLUCOSAMINE	225.046	2.08	2	10
N-ACETYLGLUTAMATE	128.0343	1	2	10
N-ACETYLLEUCINE	172.1121	1	2	30
N-ACETYLMETHIONINE	142.0499	1	2	10
N-ACETYLNEURAMINATE	272.0764	3.64	2	10
N-ACETYLPHENYLALANINE	160.0757	1	2	10
N-ACETYLPROLINE	156.0655	1	2	10

N-ACETYLPUTRESCINE	129.1023	1	2	10
N-ACETYLSERINE	146.0447	2.27	2	10
N-ACETYLSEROTONIN	217.0971	1	2	10
N-ACETYLTRYPTOPHAN	245.0921	1	2	10
N-ALPHA-ACETYLLYSINE	187.1077	4.07	2	20
N-METHYLASPARTATE	146.0448	3.57	2	20
N-METHYLGLUTAMATE	160.0604	3.51	2	20
N-METHYLTRYPTAMINE	173.1073	3.69	2	10
N-METYL-ALANINE	102.0549	2.77	2	10
N,N-DIMETHYLARGININE	201.1345	6.79	2	30
N,N,N-TRIMETHYLLYSINE	187.1441	7.11	2	20
N6-(DELTA2-ISOPENTENYL)-ADENINE	202.1085	1	2	20
NICOTINAMIDE	121.0396	1	2	60
NICOTINAMIDE HYPOXANTHINE DINUCLEOTIDE	541.0365	6.06	2	10
NICOTINAMIDE MONONUCLEOTIDE	121.0396	5.26	2	10
NICOTINATE	122.0237	1	2	70
NORADRENALINE	168.081	6.67	2	50
NORLEUCINE	130.0862	1	2	10

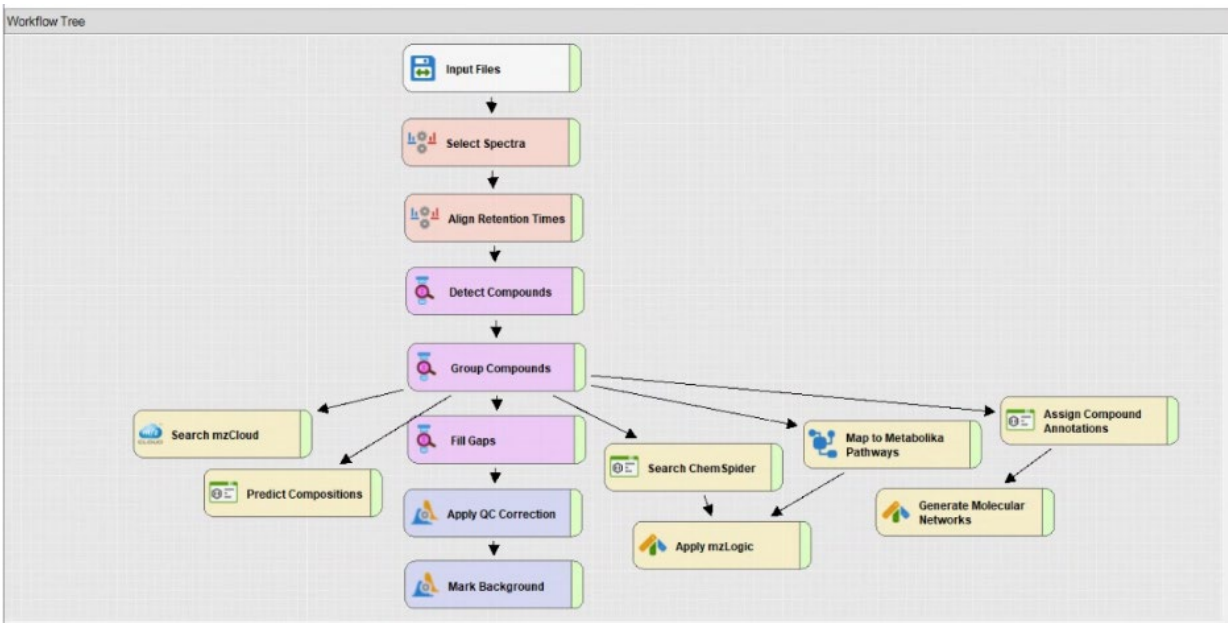
NORMETANEPHRINE	182.1538	4.13	2	10
NORVALINE	116.0707	1	2	60
O-ACETYLCARNITINE	202.1074	1	2	20
O-ACETYLSERINE	146.0449	2.83	2	10
O-SUCCINYL-HOMOSERINE	218.0661	3.17	2	10
OXOPROLINE	128.0342	2.14	2	20
P-OCTOPAMINE	152.0287	4.77	2	40
PALMITOYLCARNITINE	398.3263	1	2	20
PANTOLACTONE	129.1466	1	2	40
PANTOTHENATE	218.1023	1	2	20
PARAXANTHINE	179.0565	1	2	40
PHENYLALANINE	164.0706	1	2	10
PHENYLETHANOLAMINE	118.0652	3.88	2	10
PHOSPHORYLCHOLINE	182.0576	1	2	30
PIPECOLATE	128.0707	1	2	20
PROLINE	114.0549	1	2	20
Protoporphyrin IX	561.2493	1	2	40
PROTOPORPHYRIN	561.2493	1	2	40
PTERIN	162.0411	1	2	50
PURINE	119.0352	1	2	80
PUTRESCINE	70.0651	1	2	10
PYRAZOLE	67.0291	1	2	90
PYRIDOXAL-PHOSPHATE	246.016	1	2	20
PYRIDOXAMINE	167.0816	5.22	2	10
PYRIDOXINE	168.0655	1	2	10
PYROGLUTAMATE	128.0343	1	2	20

RIBOFLAVIN	375.1296	1	2	30
S-ADENOSYLHOMOCYSTEINE	383.1127	3.95	2	10
S-CARBOXYMETHYL CYSTEINE	178.0168	4.92	2	10
SALSOLINOL	178.0862	1	2	30
SARCOSINE	88.0393	1	2	10
SERINE	104.0342	1	2	10
SEROTONIN	158.06	4.51	2	10
SUBERATE	173.0958	1	2	20
THEOBROMINE	179.0564	1	2	50
THEOPHYLLINE	179.0563	1	2	40
THIAMINE MONOPHOSPHATE	343.0624	4.93	2	10
THIOPURINE S-METHYLETHER	165.0229	1	2	60
THREONINE	118.0651	1	2	10
THYMIDINE-MONOPHOSPHATE	79.0179	3.45	2	10
THYMINE	125.0346	1	2	50
THYROTROPIN RELEASING HORMONE	361.1617	3.08	2	20
TRANS-4-HYDROXY-L-PROLINE	130.0499	3.96	2	20
TRIGONELLINE	136.0394	1	2	70
TRIMETHYLAMINE	58.0651	3.81	2	80
TRYPTAMINE	159.0916	3.75	2	40
TRYPTOPHAN	186.055	1	2	10
TYRAMINE	136.0758	3.74	2	40
TYROSINE	180.0655	1	2	10
UREIDOPROPIONATE	113.0346	1	2	10

URIDINE DIPHOSPHATE-N-ACETYLGALACTOSAMINE	202.071	6.84	2	10
URIDINE MONOPHOSPHATE	323.0891	5.03	2	10
UROCANATE	137.0345	1	2	30
VALINE	116.0705	1	2	60
XANTHINE	151.0253	1	2	40
XANTHOSINE	151.0251	2.25	2	10
XANTHOSINE-MONOPHOSPHATE	363.1414	6.28	2	10
XANTHURENATE	204.0292	2.3	2	30
DEOXYCORTICOSTERONE ACETATE	225.05	1	2	10
biotin	243.0795	1	2	10
benzyl alcohol	107.0491	1	2	20
L-Alanine-13C3,15N	92.0469	7.5	15	10
L-Arginine-13C6,15N4_hydrochloride	182.1042	7.5	15	20
L-Asparagine-15N2	133.0397	7.5	15	10
L-Aspartic acid-13C4,15N4	137.0401	7.5	15	10
L-Glutamic acid-13C5,15N	152.0591	7.5	15	10
L-Glutamine-15N2	147.0553	7.5	15	10
Glycine-13C2,15N	77.0279	7.5	15	10
L-Histidine-15N3	157.0527	7.5	15	10
L-Isoleucine-13C6,15N	137.1039	7.5	15	10
L-Leucine-13C6,15N	137.1039	7.5	15	10
L-Lysine-13C6,15N2_hydrochloride	153.1118	7.5	15	10

L-Methionine-15N	149.0402	7.5	15	10
L-Phenylalanine-13C9,15N	174.0983	7.5	15	10
L-Proline-13C5,15N	120.0693	7.5	15	20
L-Serine-13C3,15N	108.0418	7.5	15	10
L-Threonine-13C4,15N	123.0608	7.5	15	10
L-Tryptophan-15N2	205.0761	7.5	15	10
L-Tyrosine-13C9,15N	190.0932	7.5	15	10
L-Valine-13C5,15N	122.0849	7.5	15	10

Appendix 1.12: Workflow of Compound Discoverer 3.3



Input files- Raw files selected after UHPLC-MS analysis.

Select Spectra

1. Spectrum Properties Filter

Lower RT Limit: 0

Upper RT Limit: 0

2. Scan Event Fillers

Polarity Mode: Is +ve (-ve in Negative mode)

Align Retention Times

Alignment Model: Adaptive curve

Maximum Shift (min): 2

Mass Tolerance: 5ppm

Detect Compounds

1. General Settings

Mass Tolerance: 5ppm

Min Peak Intensity: 1000000

Use Most Intense Isotope Only: True

Precursor Mass Tolerance: 0.025Da

2. Peak Detection

Chromatographic S/N Threshold: 1.5

Remove Baseline: False

3. Isotope Pattern Detection

Group Isotope for: Br; Cl

4. Compound Assembly

Ions: [M+2H]+2; [M+H]+1; [M+H+MeOH]+1; [M+K]+1; [M+Na]+1

Group Compounds

1. General Settings

Mass Tolerance: 5ppm

RT Tolerance [min]: 0.2

Minimum Valley [%]: 10

Align Peaks: False

Preferred Ions: [M+H]+1; [M+H+MeOH]+1; [M+H-H_2O]+1

Area Integration: Most common ion

2. Peak Rating Contributions

Area Contribution: 3

CV Contribution: 10

FWHM to base Contribution: 5

Jaggedness Contribution: 5

Zig- Zag Index Contribution: 5

3. Peak Rating Filter

Peak Rating Threshold: 4

Number of Files: 1

Fill Gaps

Mass Tolerance: 5ppm

S/N Threshold: 5

Apply QC Correction

Min. QC Coverage [%]: 30

Max. QC Coverage [%]: 30

Max. Corrected QC Area RSD [%]: 25

Mark Background

Max. Sample/Blank: 5

Max. Blank/Sample: 0

Hide Background: True

Search ChemSpider

Database(s): BioCyc; ChEBI; Human Metabolome Database; KEGG; LIPID MAPS

Search Mode: By Formular or Mass

Mass Tolerance: 5ppm

Max. # of results per compounds: 100

Max. # of Predicted Compositions to be searched per Compounds: 3

Map to Metabolika Pathways

1. Search Settings

Metabolika Pathways: All selected

Search mode: By Formula or Mass

2. By Mass Search Settings

Mass Tolerance: 5ppm

3. By Formula Search Settings

Max. # of Predicted Compositions to be searched per Compounds: 3

4. Display Settings

Max. # Pathways in ‘Pathways’ column: 20

Apply mzLogic

Max. # Compounds: 0

Max. # mzCloud Similarity Results to consider per Compounds: 10

Match Factor Threshold: 70

Predict Compositions

1. Prediction Settings

Mass Tolerance: 5ppm

Min. Element Counts: C90 H190 Br3 Cl4 N10 O18 P3 S5

Min. RDBE: 0

Max. RDBE: 40

Min. H/C: 0.1

Max. H/C: 4

Max. # Candidates: 10

2. Pattern Matching

Intensity Tolerance [%]: 30

Intensity Threshold [%]: 0.1

S/N Threshold [%]: 3

Use Dynamic Recalibration: True

3. Fragment Matching

Use Fragment Matching: True

Mass Tolerance: 5ppm

S/N Threshold: 5

Search mzCloud

1. General Settings

Compound classes: All

Library: Autoprocessed; Reference

Search MSn Tree: False

2. DDA Search

Identity Search: Cosine

Match Activation Type: True

Match Activation Energy: Match with Tolerance

Activation Energy Threshold: 20

Apply Intensity Threshold: True

Similarity Search: None

Match Factor Threshold: 60

3. DIA Search

Use DIA Scans for Search: False

Max. Isolation Width [Da]: 500

Match Activation Type: False

Match Activation Energy: Any

Activation Energy Threshold: 100

Apply Intensity Threshold: False

Match Factor Threshold: 20

Assign Compound Annotations

1. General Settings

Mass Tolerance: 5ppm

2. Data Sources

Data Sources #1: mzCloud Search

Data Sources #2: Predicted Compositions

Data Sources #3: MassList Search

Data Sources #4: ChemSpider Search

Data Sources #5: Metabolika Search

3. Scoring Rules

Use mzLogic: True

Use Spectral Distance: True

SFit Threshold: 20

SFit Range: 20

4. Reprocessing

Clear Names: False

Generate Molecular Networks

1. Spectral Similarity

Use Full MSn Tree: True

Match Mass Shift: True

Match Transformations: True

Variate Transformations: False

S/N Threshold: 3

Mass Tolerance: 2.5 mmu

Min. Fragment m/z: 50

2. Transformations

Phase I

Phase II

Others

Max. # Phase II: 1

Max. # All Steps: 3

3. Applied View Filters

Require Transformation: True

Require MSn: True

Min. MSn Score: 50

Min. MSn Coverage: 70

Min. Fragments: 3

4. Applied Thresholds

Require Transformation: False

Require MSn: False

Min. MSn Score: 20

Min. MSn Coverage: 20

Min. Fragments: 0

Appendix 1.13: MMHCA table

MM-HCA group	ID	Name
A	CN701	N-acetyl-9-O-acetylneuraminic acid
A	CN55	Gluconic acid
A	CN983	Asparaginyl-4-hydroxyproline
A	CN455	1-Aminocyclopropane carboxylic acid
A	CN406	4-Amino-4-cyanobutanoic acid
B	CP493	5-Hydantoinpropionic acid
B	CP738	D-Ribofuranosylcreatine
B	CP1337	3-AMINOADIPIC ACID
B	CP823	N-Benzoyl-DL-alanine
B	CN176	Cyano-3-hydroxycinnamic acid
B	CP1478	1,3,4-Trihydroxy-5-oxocyclohexanecarboxylic acid
B	CP1480	S-Adenosylhomocysteine
B	CP570	1-Methylinosine
B	CP386	N2-Dimethylguanosine
B	CP590	N6-threonylcarbamoyladenosine
B	CP418	2-(4-Methylphenoxy)-N'-[(5-phenyl-2H-tetrazol-2-yl)acetyl]acetohydrazide
B	CP425	1-Pentofuranosyl-2,4(1H,3H)-pyrimidinedione
B	CP1052	Dihydrouridine
B	CP178	3-amino-5-morpholinomethyl-2-oxazolidinone
B	CP396	6-Carbamimidamidohexanoic acid
B	CN394	3,3′-Sulfanediyldipropanoic acid
B	CN610	Succinyladenosine
B	CN484	2'-alpha-mannosyl-L-tryptophan
B	CN430	Xanthosine
B	CN745	3-Methylglutaconic acid
B	CN478	Vanillyl mandelic acid
B	CN467	3-Methoxy-4-hydroxyphenylglycol glucuronide
B	CN374	Propyl gallate
B	CN230	4-Acetamidobutanoic acid
B	CN275	3-indole carboxylic acid glucuronide
B	CN449	loganate
B	CN333	n-Propyl Gallate
B	CN687	(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid
B	CN1063	(4S)-4-{[2-O-(beta-L-Arabinofuranosyl)-beta-L-arabinofuranosyl]oxy}proline
C	CP448	N8-Acetylspermidine
C	CP790	N-{3-[(4-Acetamidobutyl)amino]propyl}acetamide
C	CP545	(Aminomethyl)phosphonate
C	CP1932	1,3,7-Trimethylisoguanine
C	CP972	1-Methylguanine
C	CN319	Isobutyric acid
C	CN208	Ethylmalonic acid
C	CN699	(+)-(7S,8S)-guaiacylglycerol-beta-vanillic acid ether
C	CN350	4-Hydroxy-3- methoxyphenylglycol sulfate
C	CN1153	2-Acetamido-4-O-[2-(carboxyamino)-2-deoxy-beta-D-glucopyranosyl]-2-deoxy-beta-D-glucopyranose
C	CP1286	2,2-Bis(hydroxymethyl)-1,3-propanediol

C	CN804	Aspartame
C	CP928	2-Acrylamido-2-methyl-1-propane sulfonic acid
C	CN1078	(2S)-2-[(1-Carboxyethyl)amino]-4-(dimethylsulfonio)butanoate
C	CP1213	(R)-crotonylcarnitine
C	CN129	3-[4-Hydroxy-3-(sulfooxy)phenyl]propanoic acid
C	CN1044	Ethyl 3-(3,4-dimethoxyphenyl)-1,2,4-oxadiazole-5-carboxylate
C	CN491	L-Ascorbic acid 2-sulfate
C	CN150	Diethylpyrocarbonate
C	CP1271	val-arg
C	CP770	2-deoxyglucose
C	CP103	Prolylleucine
C	CP951	N6,N6,N6-Trimethyl-L-lysine
C	CP497	3-Hydroxy-3-[(3-methylbutanoyl)oxy]-4-(trimethylammonio)butanoate
C	CP193	Propionylcarnitine
C	CP1732	glu-asp
C	CP1062	L-Dopa
C	CP1492	4-Amino-1-(beta-D-ribofuranosyl)-2(1H)-pyrimidinethione
C	CP1851	N-Acetyl cytosine
D	CP659	Hydroxyhexanoycarnitine
D	CP534	trihomomethionine
D	CP865	4,4'-Thiobis(butan-2-one)
D	CP1314	pentahomomethionine
D	CN602	Ethyl malate
D	CN901	O-suberoylcarnitine
E	CN1192	O-sebacoylcarnitine
E	CN1141	N-(2,6-dimethylphenyl)-1-methyl-2,2,4-trioxo-3-thieno[3,2-c]thiazinecarboxamide
E	CN867	Methyl {(1R,3R,4aS,9aR)-1-(hydroxymethyl)-6-[(4-pyridinylacetyl)amino]-3,4,4a,9a-tetrahydro-1H-pyrano[3,4-b][1]benzofuran-3-yl}acetate
E	CN847	2-Oxo-2-(1-pyrrolidinyl)ethyl 3-amino-4-chlorobenzoate
E	CN127	Phenyl D-glucopyranosiduronic acid
E	CN1075	Oxadixyl
E	CN825	guaiacylglycerol
E	CN501	7-Methoxy-4,5-dihydro-2H-benzo[g]indazole-3-carboxylic acid
E	CN234	Avenein
E	CN200	Vanillic acid 4-sulfate
F	CN19	N4-Acetylsulfamethoxazole
F	CP121	4-(Hydroxyamino)-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide
F	CP458	1-(1-azepanyl)-2-[(5-methyl-4-nitro-1H-pyrazol-3-yl)thio]ethanone
G	CN197	4-Ethyl-2,6-dihydroxyphenyl hydrogen sulfate
G	CN340	(2E)-3-[4-Methoxy-3-(sulfooxy)phenyl]acrylic acid
G	CN980	(2S,3S,4S)-4-[(2Z,4E,6R)-6-Carboxylato-2,4-heptadien-2-yl]-3-(carboxylatomethyl)-2-pyrrolidiniumcarboxylate
G	CN45	4-Acetamidobenzoic acid
G	CP149	Hippuric acid
G	CN685	Acetanilide
G	CN125	2-Methoxyethyl 6-amino-4-(1,3-benzodioxol-5-yl)-5-cyano-2-methyl-4H-pyran-3-carboxylate
G	CN1167	3-Chloro-L-tyrosine
G	CN924	phe-tyr-asp
G	CP1121	Z-Leu-OH
G	CN309	D-(+)-Xylose

G	CN604	porphobilinogen
G	CN1004	nicotianamine
G	CN812	N-Acetylvanilalanine
G	CN542	N-[(3S)-2-Oxotetrahydro-3-furanyl]pentanamide
G	CP1459	6-hydroxypropylthymine
G	CP1399	2-Methoxy-phloroglucinol
G	CN1068	4-Pyridoxate
H	CP561	Glycylglycylglycine
H	CP37	L-Pyroglutamic acid
H	CN23	4-Oxoproline
I	CP1038	Threonine
I	CP1357	L-Isoleucyl-L-tryptophan
I	CP319	3',4'-Dimethoxyacetophenone
J	CN589	4-coumaroylshikimic acid
J	CN591	13-Dihydrocarminomycin
J	CN601	Methyl 4-[(cyclohexylcarbamoyl)amino]benzoate
J	CN600	Hydroxycarteolol
J	CN544	Tricetamide
J	CN170	5-Methoxy-1H-indol-6-yl ß-D-glucopyranosiduronic acid
J	CN280	N-Phenylacetylglutamic acid
J	CN336	1-Deoxy-1-(7,8-dimethyl-2,4-dioxo-1,3,4,4a,5,10a-hexahydrobenzo[g]pteridin-10(2H)-yl)-D-ribitol
J	CN1074	2-[(2R,4aS,12aS)-8-[[(4-cyanoanilino)-oxomethyl]amino]-5-methyl-6-oxo-2,3,4,4a,12,12a-hexahydropyrano[2,3-c][1,5]benzoxazocin-2-yl]-N-methylacetamide
J	CN970	N~2~-(3-Hydroxy-3-methylhexanoyl)-L-glutamine
J	CP1535	Ethyl L-methioninate
J	CN689	4-Methoxyhydrocinnamic acid
K	CN1151	Methyl [(3R,6aR,8R,10aR)-3-hydroxydecahydropyrano[2,3-c][1,5]oxazocin-8-yl]acetate
K	CN1087	5',6'-Diamino-1,3'-bis(2-deoxy-beta-D-erythro-pentofuranosyl)-4',5'-dihydro-4,4'-bipyrimidine-2,2'(1H,3'H)-dione
K	CN1013	3-Hydroxymonoethylglycinexylidide
K	CN608	4-Hydroxy-5-(phenyl)-valeric acid-O-sulphate
K	CN967	Hexylresorcinol
K	CN889	Benzyl-primeveroside
K	CN1189	(3S,4S)-4-[(2Z,4E,6R)-6-Carboxy-2,4-heptadien-2-yl]-3-(carboxymethyl)-L-proline
K	CN578	3-beta-D-glucopyranuronosyloxy-5-methylisoxazole
K	CN1002	5-Methoxy-3-(4-methoxyphenyl)-8-methyl-4-oxo-4H-chromen-7-yl 6-deoxy-alpha-L-erythro-hexopyranoside
K	CN676	N-[3-Chloro-4-(4-morpholinyl)phenyl]-2-furamide
K	CN1162	2,2-dimethyl-3-oxo-2,3-dihydro-1-benzofuran-7-yl N-methylcarbamate
K	CN159	tyramine sulfate
K	CN849	(2S)-2-({N-[(2S)-2-Ammoniopropanoyl]-L-leucyl}amino)propanoate
K	CN1094	(2S,3S,3aR,9bR)-3-(hydroxymethyl)-6-oxo-N-(2-piperidin-1-ylethyl)-7-pyridin-3-yl-1,2,3,3a,4,9b-hexahydropyrrolo[2,3-a]indolizine-2-carboxamide
K	CN253	3-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)propanoic acid
L	CN29	(3beta,5alpha,16beta)-3-{[4-O-(beta-D-Glucopyranosyl)-beta-D-glucopyranosyl]oxy}-14,16-dihydroxy-19-oxobufa-20,22-dienolide
L	CN493	olivetolic acid

L	CN155	(E)-3,4,5-Trimethoxycinnamic acid
L	CN82	3-[(3aS,4S,7aS)-1,5-dioxo-octahydroinden-4-yl]propanoic acid
L	CN298	4-[(2E)-4-Hydroxy-3-methyl-2-buten-1-yl]-1,3-benzenediol
L	CN88	5-(3-Methoxybenzyl)dihydro-2(3H)-furanone
L	CN31	(5Z,8Z,10E)-12-Oxo-5,8,10-dodecatrienoic acid
L	CP514	3-{(E)-2-[3,5-Dihydroxy-4-(3-methyl-2-buten-1-yl)phenyl]vinyl}-2,6-dihydroxyphenyl hydrogen sulfate
L	CP517	4-[({1-[2-Carboxy-3-(2-methoxyethoxy)propyl]cyclopentyl}carbonyl)amino]cyclohexanecarboxylic acid
L	CP321	mono(7-carboxy-2-methyloctyl) phthalate
L	CP925	(5Z,8Z,11Z,13E,15S)-N-[2-(3,4-Dihydroxyphenyl)ethyl]-15-hydroxy-5,8,11,13-icosatetraenamide
L	CP171	10,11-Dihydro-10,11-dihydroxycarbamazepine

Appendix 1.14: R Studio code for chapter 8 results

Load the Peak & Data tables from Excel

```
cd '/Users/dvdbrdhrst/LocalData/JeneStrathclyde'
%cd '/Users/davidbroadhurst/LocalData/JeneStrathclyde'
[DataS,PeakS] = loadXLS2022('Pilot50Clean.xlsx',Data='Data',Peak='PeakStats');
```

```
Loading PeakSheet: PeakStats
Loading DataSheet: Data
TOTAL SAMPLES: 294 TOTAL PEAKS: 645
Done!
```

```
DataS.FF = double(ismember(DataS.Class,'FF'));
DataS.M = double(ismember(DataS.Sex,'M'));
DataS2 = DataS(DataS.keep==1,:);
DataS3 = DataS(DataS.keep2==1,:);
```

Prepare the X matrix.

```
keep = PeakS.TPFF < 0.05 | PeakS.FF < 0.05;% | PeakS.TP < 0.005;
Peak2 = PeakS(keep,:);
% DataX2 = DataX(keep,:);
% Class = DataX2.Class2;
X = DataS2{:,Peak2.UID};
%ZZ = X;
%[ZZ,~] = scaleImpute(X,logTransform=false,impute="none");
[ZZ,~] = scaleImpute(X);
```

Plot the HCA Polar Dendrogram

```
temp = abs(round(log(Peak2.TPFF)));
temp(temp>4) = 5;
Peak2.SigTPFF = temp;
AnnotateDict = dictionary( ...
    0,'', ...
    1,'', ...
    2,'*', ...
    3,'**', ...
    4,'***', ...
    5,'****');

close all;
[PeakX,cmap] =
polardendrogram2023(ZZ,Peak2,cmap='moog',pDistType='euclidean',userLabelFontSize=10,ColorCutWeight=0.28,
Annotate='SigTPFF',AnnotateDict=AnnotateDict,LabelCol='UID');
```

```
Warning: Colon operands must be real scalars. This warning will become an error in a future release.
```

```
close all;
[PeakX,cmap] =
polardendrogram2023(ZZ,Peak2,cmap='moog',pDistType='euclidean',userLabelFontSize=10,ColorCutWeight=0.28,
Annotate='SigTPFF',AnnotateDict=AnnotateDict);%,LabelCol='UID');
```

```
Warning: Colon operands must be real scalars. This warning will become an error in a future release.
```

```
[DataHCA,PeakHCA,arglist] =
genHCAgroupTables2024(DataS2,PeakX,GroupColumn='HCAgroup',CentralTendency='mean',logTransform=true,scale
='zscore');
```

```
X = DataHCA{:,PeakHCA.UID};
peakGrps = unique(PeakHCA.UID);
numOfgrps = numel(peakGrps);
[Xm,SEm,Grp] = grpstats(X,DataHCA.Class,{'mean','sem','gname'});
grpIdx = grp2idx(Grp);
tiledlayout(2,ceil(numOfgrps/2))
for i = 1:numOfgrps
    nexttile
    p = anova1(X(:,i),DataHCA.Class,'off');
    warning('off')
    hold('on');
    in = ismember(DataHCA.Class,'FF');
    errorPlot(X(in,i),DataHCA.TimePoint(in),plotType='sem-line',color=[0,0,0.8],title=
[PeakHCA.Name{i},' (pval:',num2str(p,1),')'],ylabel='mean
log2(fold)',orderedlist={'preop','postop','postop7','postop30','postop90'});
    in = ismember(DataHCA.Class,'NFNF');
    errorPlot(X(in,i),DataHCA.TimePoint(in),plotType='sem-line',color=[0,0.8,0],title=
[PeakHCA.Name{i},' (pval:',num2str(p,1),')'],ylabel='mean
log2(fold)',orderedlist={'preop','postop','postop7','postop30','postop90'});
    %in = ismember(DataHCA.Class,'NFF');
    %errorPlot(X(in,i),DataHCA.TimePoint(in),plotType='sem-line',color=[0.8,0,0],title=
[PeakHCA.Name{i},' (pval:',num2str(p,1),')'],ylabel='mean
log2(fold)',orderedlist={'preop','postop','postop7','postop30','postop90'});
    hold('off');
    warning('on')
end
```

```
mdl =
Linear regression model (robust fit):
    Y ~ 1 + Age + BMI + Male + Outcome

Estimated Coefficients:
                    Estimate        SE         tStat       pValue
                    ________    _________    _______    ____________

    (Intercept)       4.9262     0.044893     109.73    3.0133e-129
    Age            0.0015457    0.0025572    0.60444        0.54661
    BMI            0.0014136    0.0043678    0.32364        0.74674
    Male            0.075872     0.059398     1.2773        0.20378
    Outcome_NFF     0.060052      0.10354    0.57996        0.56295
    Outcome_NFM      0.56735     0.091404     6.2071     6.8226e-09


Number of observations: 135, Error degrees of freedom: 129
Root Mean Squared Error: 0.344
R-squared: 0.266,  Adjusted R-Squared: 0.237
F-statistic vs. constant model: 9.34, p-value = 1.29e-07
```

Load Data & Peak Sheet

```
cd /Users/davidbroadhurst/LocalData/JeneJune2024
[DataX,PeakX] = loadXLS2022('Data_1-11_PosClean_UpdatedX.xlsx',Data='Data',Peak='Peak');
```

```
TOTAL SAMPLES: 996 TOTAL PEAKS: 532
```

Create new Data Table for comparison of (NFF & NFM) vs NFNF

```
keep1 = ismember(DataX.ClassX,{'NFF','NFM','NFNF'});
keep2 = ismember(DataX.TimePoint,'preop');
keep = keep1 & keep2;

Data = DataX(keep,:);
Peak = PeakX(PeakX.Drug == 0,:);
keep = Peak.dRatioMax < 40;
Peak = Peak(keep,:);
```

```
135 subjects in the Data Table
246 metabolites in the Peak Table
```

Extract X matrix & create binary Y vector

(NFF & NFM = 1 & NFNF = 0)

```
X = Data{:,Peak.UID};
X = [X,Data.Age,Data.BMI];
[ZZ,stats] = scaleImpute(X,"scale","zscore");
Outcome = Data.ClassX;
Y = double(ismember(Outcome,{'NFF','NFM'}));
```

```
Dataset has 31 Cases & 104 Controls
```

Determine optimal number of variables for regression model

```
[B,FitInfo] = lassoglm(ZZ,Y,'binomial','NumLambda',30,'CV',7,'MCReps',50);
```

```
idx = FitInfo.Index1SE;
mincoefs = length(find(B(:,idx)))
```

```
mincoefs = 16
```

```
B0 = FitInfo.Intercept(idx);
coef = [B0; B(:,idx)];
yhat = glmval(coef,ZZ,'logit');
close all;
scrsz = get(0,'ScreenSize');
figure('Position',[(scrsz(3)*0.1) scrsz(4)/8 scrsz(3)*0.7 scrsz(4)*0.4]);
included = find(B(:,idx));
BB = B(included,idx);
labels = [Peak.Name',{'Age','BMI'}];
labels = labels(included);
stem(B(:,idx));
hold('on');
text(included,BB,labels);
```

```
close all;
keep = ismember(DataX.Class,{'NFF','NFM30','NFM90','NFNF'});
Data2 = DataX(keep,:);
XT = Data2{:,Peak.UID};
XT = [XT,Data2.Age,Data2.BMI];
XTlog = log10(XT);
ZZtest = (XTlog-stats.center)./stats.scale;
yhatm = glmval(coef,ZZtest,'logit');
boxJitter(yhatm,Data2.ClassT,ylabel='PLS Score');
hold('on')
line([0 30],[0.32 0.32],'Color','k');
```

References

1. Mullen MG, Michaels AD, Mehaffey JH, Guidry CA, Turrentine FE, Hedrick TL, et al. Risk Associated With Complications and Mortality After Urgent Surgery vs Elective and Emergency Surgery: Implications for Defining "Quality" and Reporting Outcomes for Urgent Surgery. JAMA Surg. 2017;152(8):768-74.
2. Havens JM, Peetz AB, Do WS, Cooper Z, Kelly E, Askari R, et al. The excess morbidity and mortality of emergency general surgery. J Trauma Acute Care Surg. 2015;78(2):306-11.
3. NELA. NELA Tenth Patient Report 2025. Royal College of Anaesthetists (RCoA). 2025.
4. Eugene N, Oliver CM, Bassett MG, Poulton TE, Kuryba A, Johnston C, et al. Development and internal validation of a novel risk adjustment model for adult patients undergoing emergency laparotomy surgery: the National Emergency Laparotomy Audit risk model. Br J Anaesth. 2018;121(4):739-48.
5. NELA. The ninth patient report of the national emergency laparotomy audit. Royal College of Anaesthetists (RCoA). 2024.
6. Smart R, Carter B, McGovern J, Luckman S, Connelly A, Hewitt J, et al. Frailty Exists in Younger Adults Admitted as Surgical Emergency Leading to Adverse Outcomes. J Frailty Aging. 2017;6(4):219-23.
7. Blodgett JM, Rockwood K, Theou O. Changes in the severity and lethality of age-related health deficit accumulation in the USA between 1999 and 2018: a population-based cohort study. Lancet Healthy Longev. 2021;2(2):e96-e104.
8. Pearse RM, Harrison DA, James P, Watson D, Hinds C, Rhodes A, et al. Identification and characterisation of the high-risk surgical population in the United Kingdom. Crit Care. 2006;10(3):R81.
9. Saunders DI, Murray D, Pichel AC, Varley S, Peden CJ, Network UKEL. Variations in mortality after emergency laparotomy: the first report of the UK Emergency Laparotomy Network. Br J Anaesth. 2012;109(3):368-75.
10. ELLSA. The First National Report of the Emergency Laparoscopic and Laparotomy Scottish Audit (ELLSA) 2019 [Available from: https://learn.nes.nhs.scot/13211/scottish-government-health-and-social-care-resources/modernising-patient-pathways-programme/enhanced-recovery-after-surgery-eras-in-scotland/emergency-laparotomy-and-laparoscopic-scottish-audit-ellsa.
11. ELLSA. The Second National Report of the Emergency Laparoscopic and Laparotomy Scottish Audit (ELLSA) 2023 [Available from: https://learn.nes.nhs.scot/74354.
12. ELLSA. ELLSA Third annual report 2025 [Available from: https://learn.nes.nhs.scot/13211/scottish-government-health-and-social-care-resources/whole-system-patient-flow-improvement-programme/emergency-laparotomy-and-laparoscopic-scottish-audit-ellsa.
13. Hernandez JBR, Kim PY. Epidemiology Morbidity And Mortality. StatPearls; 2021.
14. Clavien PA, Sanabria JR, Strasberg SM. Proposed classification of complicatons of surgery with examples of utility in cholesystectomy. Surgery. 1992;111(5):518-26.
15. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2):205-13.
16. Saunders DI, Sinclair RCF, Griffiths B, Pugh E, Harji D, Salas B, et al. Emergency Laparotomy Follow-Up Study (ELFUS): prospective feasibility investigation into postoperative complications and quality of life using patient-reported outcome measures up to a year after emergency laparotomy. Perioper Med (Lond). 2021;10(1):22.
17. Strasberg SM, Linehan DC, Hawkins WG. The accordion severity grading system of surgical complications. Ann Surg 2009;250(2):177–86
18. Sorensen LT, Malaki A, Wille-Jorgensen P, Kallehave F, Kjaergaard J, Hemmingsen U, et al. Risk factors for mortality and postoperative complications after gastrointestinal surgery. J Gastrointest Surg. 2007;11(7):903-10.

19. Kehlet H, Mogensen T. Hospital stay of 2 days after open sigmoidectomy with a multimodal rehabilitation programme. BJS. 1999;86:227-30.
20. Kehlet H, Wilmore D. Multimodal strategies to improve surgical outcome. The American Journal of Surgery. 2002;183:630-41.
21. Peden CJ, Aggarwal G, Aitken RJ, Anderson ID, Bang Foss N, Cooper Z, et al. Guidelines for Perioperative Care for Emergency Laparotomy Enhanced Recovery After Surgery (ERAS) Society Recommendations: Part 1-Preoperative: Diagnosis, Rapid Assessment and Optimization. World J Surg. 2021;45(5):1272-90.
22. Tolstrup MB, Thorup T, Gogenur I. Chronic Pain, Quality of Life and Functional Impairment After Emergency Laparotomy. World J Surg. 2019;43(1):161-8.
23. Law J, Welch C, Javanmard-Emamghissi H, Clark M, Bisset CN, O'Neil P, et al. Decision-making for older patients undergoing emergency laparotomy: defining patient and clinician values and priorities. Colorectal Dis. 2020;22(11):1694-703.
24. Parmar KL, Pearce L, Farrell I, Hewitt J, Moug S. Influence of frailty in older patients undergoing emergency laparotomy: a UK-based observational study. BMJ Open. 2017;7(10):e017928.
25. National Institute for Health and Care Excellence. Position statement on use of the EQ-5D-5L value set for England (updated October 2019) 2019 [Available from: https://www.nice.org.uk/about/what-we-do/our-programmes/nice-guidance/technology-appraisal-guidance/eq-5d-5l.
26. Scheingraber S, Kurz T, Dralle H. Short- and long-term outcome and health-related quality of life after severe peritonitis. World J Surg. 2002;26(6):667-71.
27. Witte M, Neese M, Leuchter M, Philipp M, Klar E, Schafmayer C. Acute Mesenteric Ischemia: Preexisting Comorbidity Determines Short-Term Outcome and Quality of Life in Long-Term Survivors. Visc Med. 2022;38(6):393-9.
28. Jeppesen M, Tolstrup MB, Gogenur I. Chronic Pain, Quality of Life, and Functional Impairment After Surgery Due to Small Bowel Obstruction. World J Surg. 2016;40(9):2091-7.
29. Alder L, Mercer SJ, Carter NC, Toh SK, Knight BC. Clinical frailty and its effect on the septuagenarian population after emergency laparotomy. Ann R Coll Surg Engl. 2021;103(3):180-5.
30. Kwong E, Neuburger J, Murray D, Black N. Feasibility of collecting and assessing patient-reported outcomes for emergency admissions: laparotomy for gastrointestinal conditions. BMJ Open Gastroenterol. 2018;5(1):e000238.
31. Purcell LN, Reiss R, Mtalimanja M, Kuyenda P, Msosa V, Westmoreland KD, et al. Patient-Reported Quality of Life Following Laparotomy in a Resource-Limited Setting. World J Surg. 2021;45(7):1971-8.
32. Boer KR, van Ruler O, Reitsma JB, Mahler CW, Opmeer BC, Reuland EA, et al. Health related quality of life six months following surgical treatment for secondary peritonitis--using the EQ-5D questionnaire. Health Qual Life Outcomes. 2007;5:35.
33. Li JGS, X. H., Zheng XB. Laparoscopy versus laparotomy for upper gastrointestinal perforation in elerly patients. Int J Clin Exp Med. 2017;10(8):11991-7.
34. Jansson Timan T, Hagberg G, Sernert N, Karlsson O, Prytz M. Mortality following emergency laparotomy: a Swedish cohort study. BMC Surg. 2021;21(1):322.
35. Barazanchi A, Bhat S, Wells CI, Taneja A, MacCormick AD, Hill AG. Short and long-term impact of sarcopenia on outcomes from emergency laparotomy. Eur J Trauma Emerg Surg. 2022.
36. Temimi MA-, Rangarajan S, Chandrasekaran B, Kim E, Trujillo C, Mousa A, et al. Predictors of failed transcystic laparoscopic common bile duct exploration: analysis of multicenter integrated health system database. J Laparoendosc Adv Surg Tech A. 2018;29(3):360-5.
37. Aggarwal G, Peden CJ, Mohammed MA, Pullyblank A, Williams B, Stephens T, et al. Evaluation of the Collaborative Use of an Evidence-Based Care Bundle in Emergency Laparotomy. JAMA Surg. 2019;154(5):e190145.
38. Sankar A, Johnson S, Beattie W, Tait G, Wijeysundera D. Reliability of the American Society of Anaesthesiologists physical status scale in clinical practice. BR J Anaesth. 2014;113(3):424-32.

39. Tengberg LT, Bay-Nielsen M, Bisgaard T, Cihoric M, Lauritsen ML, Foss NB, et al. Multidisciplinary perioperative protocol in patients undergoing acute high-risk abdominal surgery. Br J Surg. 2017;104(4):463-71.
40. Peden CJ, Stephens T, Martin G, Kahan BC, Thomson A, Rivett K, et al. Effectiveness of a national quality improvement programme to improve survival after emergency abdominal surgery (EPOCH): a stepped-wedge cluster-randomised trial. Lancet. 2019;393(10187):2213-21.
41. Vester-Andersen M, Lundstrom LH, Moller MH, Waldau T, Rosenberg J, Moller AM, et al. Mortality and postoperative care pathways after emergency gastrointestinal surgery in 2904 patients: a population-based cohort study. Br J Anaesth. 2014;112(5):860-70.
42. Murray AC, Mauro C, Rein J, Kiran RP. 30-day mortality after elective colorectal surgery can reasonably be predicted. Tech Coloproctol. 2016;20(8):567-76.
43. Warren MW. Care of Chronic Sick. Br Med, J. 1943;2:822-3.
44. Stamford BA. Physiological effects of training upon institutionalized geriatric men. J Gerontol. 1972;27(4):451-5.
45. O'Brien T Roberts J, Brackenridge GR, Lloyd WH. Some aspects of community care of the frail and elderly: the need for assessment. Gerontol Clin (Basel). 1968;10(215–227).
46. Winograd CH, Gerety Mb Fau - Chung M, Chung M Fau - Goldstein MK, Goldstein Mk Fau - Dominguez F, Jr., Dominguez F Jr Fau - Vallone R, Vallone R. Screening for frailty: criteria and predictors of outcomes. J Am Geriatr Soc. 1991;39(8):778-84.
47. Winograd CH. Targeting strategies: an overview of criteria and outcomes. J Am Geriatr Soc. 1991;39(9):25-35.
48. Woodhouse KW, Wynne H, Baillie S, James OF, Rawlins MD. Who are the frail elderly? Q J Med. 1988;68(255):505-6.
49. Gillick MR. Long-term care options for the frail elderly. J Am Geriatr Soc. 1989;37:1198-203.
50. Rockwood K, Fox RA, Stolee P, Robertson D, Beattie BL. Frailty in elderly people: an evolving concept. CMAJ. 1994;150(4):489-95.
51. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. The Lancet. 2013;381(9868):752-62.
52. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56(3):146–56.
53. Fried LP, Borhani NO, Enright P, Furgerg CD, Gardin JM, Kronmal RA, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol. 1991;1(3):263-76.
54. Mitnitski AB, Mogilner AJ, Rockwood K. Accumulation of deficits as a proxy measure of aging. ScientificWorldJournal. 2001;1:323-36.
55. Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ. 2005;173(5):489-95.
56. Gobbens RJ, Luijkx KG, Wijnen-Sponselee MT, Schols JM. Towards an integral conceptual model of frailty. J Nutr Health Aging. 2010;14(3):175-81.
57. Bunt S, Steverink N, Olthof J, van der Schans CP, Hobbelen JSM. Social frailty in older adults: a scoping review. Eur J Ageing. 2017;14(3):323-34.
58. Panza F, D'Introno A, Colacicco AM, Capurso C, Parigi AD, Capurso SA, et al. Cognitive frailty: Predementia syndrome and vascular risk factors. Neurobiol Aging. 2006;27(7):933-40.
59. Kelaiditi E, Cesari M, Canevelli M, Abellan Van Kan G, Ousset PJ, Gillette-Guyonnet S, et al. Cognitive frailty: rational and definition from an (I.A.N.A/I.A.G.G.) international consensus group. J Nutr Health Aging. 2013;17(9):726-34.
60. Panza F, Lozupone M, Solfrizzi V, Sardone R, Dibello V, Di Lena L, et al. Different Cognitive Frailty Models and Health- and Cognitive-related Outcomes in Older Age: From Epidemiology to Prevention. J Alzheimers Dis. 2018;62(3):993-1012.
61. Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, et al. Frailty consensus: a call to action. J Am Med Dir Assoc. 2013;14(6):392-7.

62. Fit for Frailty - consensus best practice guidance for the care of older people living in community and outpatient settings - a report from the British Geriatrics Society. 2014.
63. Ageing. WCCoH. Frailty and intrinsic capacity 2016 [Available from: https://iris.who.int/server/api/core/bitstreams/b8cc03c9-07e4-4bb1-ba21-3ccb0f030ec2/content.
64. Laskou F, Fuggle NR, Patel HP, Jameson K, Cooper C, Dennison E. Associations of osteoporosis and sarcopenia with frailty and multimorbidity among participants of the Hertfordshire Cohort Study. J Cachexia Sarcopenia Muscle. 2022;13(1):220-9.
65. Vetrano DL, Palmer K, Marengoni A, Marzetti E, Lattanzio F, Roller-Wirnsberger R, et al. Frailty and Multimorbidity: A Systematic Review and Meta-analysis. J Gerontol A Biol Sci Med Sci. 2019;74(5):659-66.
66. Fried LP, Ferrucci L, Darer J, Williamson JD, Anderson G. Untangling the concepts of disability, frailty and comorbidity freid. J Gerontol A Biol Sci Med Sci. 2004;59:255–63.
67. Ellis G, Whitehead MA, O'Neill D, Langhorne P, Robinson D. Comprehensive geriatric assessment for older adults admitted to hospital. Cochrane Database Syst Rev. 2011(7):CD006211.
68. Stuck AE, L. SA, Wieland GD, Rubenstein LZ, Adams J. Comprehensive geriatric assessment: a meta-analysis of controlled trials. Lancet. 1993;342:1032–6.
69. Ellis G, Gardner M, Tsiachristas A, Langhorne P, Burke O, Harwood RH, et al. Comprehensive geriatric assessment for older adults admitted to hospital. Cochrane Database Syst Rev. 2017;9:CD006211.
70. Steverink N, Slaets J, Schuurmans H, Van Lis M. Measuring frailty: developing and testing of the Groningen Frailty Indicator (GFI). Gerontologist 2001;41(1):236-7.
71. Peters LL, Boter H, Buskens E, Slaets JP. Measurement properties of the Groningen Frailty Indicator in home-dwelling and institutionalized elderly people. J Am Med Dir Assoc. 2012;13(6):546-51.
72. Olaroiu M, Ghinescu M, Naumo M, Brinza I, van den Heuvel I. The psychometric qualities of the Groningen Frailty Indicator in Romanian community-dwelling old citizens. Family Practice. 2014;31(4):490–5
73. Bielderman A, van der Schans CP, van Lieshout MR, de Greef MH, Boersma F, Krijnen WP, et al. Multidimensional structure of the Groningen Frailty Indicator in community-dwelling older people. BMC Geriatr. 2013;13:86.
74. Drubbel I, Bleijenberg N, Kranenburg G, Eijkemans RJ, Schuurmans MJ, de Wit NJ, et al. Identifying frailty: do the Frailty Index and Groningen Frailty Indicator cover different clinical perspectives? a cross-sectional study. BMC Fam Pract. 2013;14:64.
75. Schuurmans H, Steverink N, Lindenberg S, Frieswijk N, Slaets J. Old or frail: What tells us more? Journal of Gerentology: Medical Sciences. 2004;59A(9):962-5.
76. Abellan Van Kan G, Rolland Y, Bergman H, Morley JE, Kritchevsky SB, Vellas B. The I.A.N.A. task force on frailty assessment of older people in clinical practice. J Nutr Health Aging. 2008;12(1):29-37.
77. Abellan van Kan G, Rolland YM, Morley JE, Vellas B. Frailty: toward a clinical definition. J Am Med Dir Assoc. 2008;9(2):71-2.
78. Burgess S, Hercus C. Frailty in community dwelling older people - Using frailty screening as the canary in the coal mine. Melbourne, Vic.: Benetas; 2017.
79. Morley JE, Malmstrom TK, Miller DK. A simple frailty questionnaire (FRAIL) predicts outcomes in middle aged african americans. J Nutr Health Aging. 2012;16(7):601-8.
80. Gleason LJ, Benton EA, Alvarez-Nebreda ML, Weaver MJ, Harris MB, Javedan H. FRAIL Questionnaire Screening Tool and Short-Term Outcomes in Geriatric Fracture Patients. J Am Med Dir Assoc. 2017;18(12):1082-6.
81. Rolfson DB, Majumdar SR, Taher A, Tsuyuki RT. Development and validation of a new instrument for frailty. Clin Invest Med. 2000;23(336).
82. Rolfson DB, Majumdar SR, Tsuyuki RT, Tahir A, Rockwood K. Validity and reliability of the Edmonton frail scale. Age Ageing. 2006;35(5):526–9.

83. Dasgupta M, Rolfson DB, Stolee P, Borrie MJ, Speechley M. Frailty is associated with postoperative complications in older adults with medical problems. Arch Gerontol Geriatr. 2009;48:78-83.
84. Rockwood K, Andrew M, Mitnitski A. A comparison of two approaches to measuring frailty in elderly people. J Gerontol A Biol Sci Med Sci. 2007;62(7):738-43.
85. Rockwood K, Mitnitski A, Song X, Steen B, Skoog I. Long-term risks of death and institutionalization of elderly people in relation to deficit accumulation at age 70. J Am Geriatr Soc. 2006;54(6):975-9.
86. Song X, Mitnitski A, Rockwood K. Prevalence and 10-year outcomes of frailty in older adults in relation to deficit accumulation. J Am Geriatr Soc. 2010;58(4):681-7.
87. Rockwood K, Theou O. Using the Clinical Frailty Scale in Allocating Scarce Health Care Resources. Can Geriatr J. 2020;23(3):210-5.
88. NELA. The Fifth Patient Report of the NELA 2019 - Full Patient Report. 2019.
89. Cesari M, Landi F, Vellas B, Bernabei R, Marzetti E. Sarcopenia and physical frailty: two sides of the same coin. Front Aging Neurosci. 2014;6:192.
90. Rosenberg I. Epidemiologic and methodologic problems in determining nutritional status of older persons. Am J Cli Nutr 1989;50:1231–3.
91. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147:755–63. .
92. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412-23.
93. Beaudart C, McCloskey E, Bruyere O, Cesari M, Rolland Y, Rizzoli R, et al. Sarcopenia in daily practice: assessment and management. BMC Geriatr. 2016;16(1):170.
94. Liguori I, Russo G, Aran L, Bulli G, Curcio F, Della-Morte D, et al. Sarcopenia: assessment of disease burden and strategies to improve outcomes. Clin Interv Aging. 2018;13:913-27.
95. Sizoo D, de Heide LJM, Emous M, van Zutphen T, Navis G, van Beek AP. Measuring Muscle Mass and Strength in Obesity: a Review of Various Methods. Obes Surg. 2021;31(1):384-93.
96. Guttikonda D, Smith A. Sarcopenia assessment techniques. CLD. 2021;18(4):189–92.
97. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A Short Physical Performance Battery Assessing Lower Extremity Function: Association With Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. Journal of Gerontology. 1994;49(2):M85-M94.
98. Greig CA. Nutritional approaches to the management of sarcopenia. Nutrition Bulletin. 2013;38(3):344-8.
99. Cruz-Jentoft A, Landi F, Schneider S, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and Ageing*. 2014;43(6):748–59.
100. Poggiogalle E, Parrinello E, Barazzoni R, Busetto L, Donini L. Therapeutic strategies for sarcopenic obesity: a systematic review. Current Opinion in Clinical Nutrition and Metabolic Care. 2021;24(1):23-41.
101. Baumgartner RN. Body composition in healthy aging. Ann N Y Acad Sci. 2000;904:437-48.
102. Donini LM, Busetto L, Bauer JM, Bischoff S, Boirie Y, Cederholm T, et al. Critical appraisal of definitions and diagnostic criteria for sarcopenic obesity based on a systematic review. Clin Nutr. 2020;39(8):2368-88.
103. Roubenoff R. Sarcopenic obesity: does muscle loss cause fat gain? Lessons from rheumatoid arthritis and osteoarthritis. Ann N Y Acad Sci. 2000;904:553-7.
104. da Silva LSL, Abdalla PP, Marcos-Pardo PJ, Romo-Perez V, Garcia-Soidan JL, Mota J, et al. Sarcopenic obesity does not impair lower limb strength and physical performance in sufficiently active older adults: a cross-sectional study. Sci Rep. 2024;14(1):3061.

105. Rockwood K, Stadnyk K, MacKnight C, McDowell I, Hebert R, Hogan DB. A brief clinical instrument to classify frailty in elderly people. Lancet. 1999;353(9148):205-6.
106. Rockwood K, Hogan DB, MacKnight C. Conceptualisation and measurement of frailty in elderly people. Drugs & Aging. 2000;17:295-302.
107. Robertson DA, Savva GM, Kenny RA. Frailty and cognitive impairment--a review of the evidence and causal mechanisms. Ageing Res Rev. 2013;12(4):840-51.
108. Hewitt J, Williams M, Pearce L, Black A, Benson E, Tarrant M, et al. The prevalence of cognitive impairment in emergency general surgery. Int J Surg. 2014;12(10):1031-5.
109. Moug SJ, Stechman M, McCarthy K, Pearce L, Myint PK, Hewitt J, et al. Frailty and cognitive impairment: Unique challenges in the older emergency surgical patient. Ann R Coll Surg Engl. 2016;98(3):165-9.
110. (NICE). NIfHaCE. Delirium: prevention, diagnosis and management in hospital and long-term care (Clinical guideline [CG103]). London : National Institute for Health and Care Excellence (UK). 2010.
111. Verloo H, Goulet C, Morin D, von Gunten A. Association between frailty and delirium in older adult patients discharged from hospital. Clinical Interventions of Ageing. 2016;11:55-63.
112. Cechinel C, Lenardt M, Rodgrigues J, Binotto M, Aristides M, Kraus R. Frailty and delirium in hospitalized older adults: A systematic review with meta-analysis. Rev Latino-Am Enfermagem. 2022;30:e3687.
113. Schofield I, Stott D, Tolson D, McFadyen A, Monaghan J, Nelson D. Screening for cognitive impairment in older people attending accident and emergency using the 4-item Abbreviated Mental Test. Eur J Emerg Med. 2010;17(6):340-2.
114. Swain DG, O'Brien AG, Nightingale PG. Cognitive assessment in elderly patients admittedto hospital: the relationship between the shortenedversion of the Abbreviated Mental Test and theAbbreviated Mental Test and Mini-Mental StateExamination. Clin Rehabil. 2010;14:608-10.
115. Pilotto A, Rengo F, Marchionni N, Sancarlo D, Fontana A, Panza F, et al. Comparing the prognostic accuracy for all-cause mortality of frailty instruments: a multicentre 1-year follow-up in hospitalized older patients. PLoS One. 2012;7(1):e29090.
116. Gordon AL, Franklin M, Bradshaw L, Logan P, Elliott R, Gladman JR. Health status of UK care home residents: a cohort study. Age Ageing. 2014;43(1):97-103.
117. Lenox-Smith A, Reed C, Lebrec J, Belger M, Jones RW. Potential cost savings to be made by slowing cognitive decline in mild Alzheimer's disease dementia using a model derived from the UK GERAS observational study. BMC Geriatr. 2018;18(1):57.
118. Ruan Q, Yu Z, Chen M, Bao Z, Li J, He W. Cognitive frailty, a novel target for the prevention of elderly dependency. Ageing Res Rev. 2015;20:1-10.
119. Ni Lochlainn M, Cox NJ, Wilson T, Hayhoe RPG, Ramsay SE, Granic A, et al. Nutrition and Frailty: Opportunities for Prevention and Treatment. Nutrients. 2021;13(7).
120. Artaza-Artabe I, Saez-Lopez P, Sanchez-Hernandez N, Fernandez-Gutierrez N, Malafarina V. The relationship between nutrition and frailty: Effects of protein intake, nutritional supplementation, vitamin D and exercise on muscle metabolism in the elderly. A systematic review. Maturitas. 2016;93:89-99.
121. Lorenzo-Lopez L, Maseda A, de Labra C, Regueiro-Folgueira L, Rodriguez-Villamil JL, Millan-Calenti JC. Nutritional determinants of frailty in older adults: A systematic review. BMC Geriatr. 2017;17(1):108.
122. McKinlay J. Nutritional assessment: Identifying patients needs. J R Coll Physicians Edinb. 2004;34(1):28-31.
123. Stratton RJ, Hackston A, Longmore D, Dixon R, Price S, Stroud M, et al. Malnutrition in hospital outpatients and inpatients: prevalence, concurrent validity and ease of use of the 'malnutrition universal screening tool' ('MUST') for adults. Br J Nutr. 2004;92(5):799-808.
124. Hernandez Morante JJ, Gomez Martinez C, Morillas-Ruiz JM. Dietary Factors Associated with Frailty in Old Adults: A Review of Nutritional Interventions to Prevent Frailty Development. Nutrients. 2019;11(1).

125. Masnoon N, Shakib S, Kalisch-Ellett L, Caughey GE. What is polypharmacy? A systematic review of definitions. BMC Geriatr. 2017;17(1):230.
126. Taghy N, Cambon L, Cohen JM, Dussart C. Failure to Reach a Consensus in Polypharmacy Definition: An Obstacle to Measuring Risks and Impacts-Results of a Literature Review. Ther Clin Risk Manag. 2020;16:57-73.
127. Gutierrez-Valencia M, Izquierdo M, Cesari M, Casas-Herrero A, Inzitari M, Martinez-Velilla N. The relationship between frailty and polypharmacy in older people: A systematic review. Br J Clin Pharmacol. 2018;84(7):1432-44.
128. Rolland Y, Morley JE. Frailty and polypharmacy. The Journal of Nutrition, Health and Aging. 2016;20:645-6.
129. Rochon PA, Stall NM, Holmes HM. Drug Therapy and Frailty: Chicken or the Egg? J Am Geriatr Soc. 2019;67(12):2441-3.
130. Nwadiugwu MC. Frailty and the Risk of Polypharmacy in the Older Person: Enabling and Preventative Approaches. J Aging Res. 2020;2020:6759521.
131. Arauna D, Cerda A, Garcia-Garcia JF, Wehinger S, Castro F, Mendez D, et al. Polypharmacy Is Associated with Frailty, Nutritional Risk and Chronic Disease in Chilean Older Adults: Remarks from PIEI-ES Study. Clin Interv Aging. 2020;15:1013-22.
132. Hajjar ER, Cafiero AC, Hanlon JT. Polypharmacy in elderly patients. The American Journal of Geriatric Pharmacotherapy. 2007;5(4):345-51.
133. Cheng CM, Chang WH, Chiu YC, Sun Y, Lee HJ, Tang LY, et al. Association of Polypharmacy With Mild Cognitive Impairment and Cognitive Ability: A Nationwide Survey in Taiwan. J Clin Psychiatry. 2018;79(6).
134. Aljeaidi MS, Tan EC. The association between polypharmacy and cognitive ability in older adults: A national cohort study. Res Social Adm Pharm. 2022;18(3):2505-9.
135. Khezrian M, McNeil CJ, Myint PK, Murray AD. The association between polypharmacy and late life deficits in cognitive, physical and emotional capability: a cohort study. Int J Clin Pharm. 2019;41(1):251-7.
136. Parmar KL, Law J, Carter B, Hewitt J, Boyle JM, Casey P, et al. Frailty in Older Patients Undergoing Emergency Laparotomy: Results From the UK Observational Emergency Laparotomy and Frailty (ELF) Study. Ann Surg. 2021;273(4):709-18.
137. Palmer K, Villani ER, Vetrano DL, Cherubini A, Cruz-Jentoft AJ, Curtin D, et al. Association of polypharmacy and hyperpolypharmacy with frailty states: a systematic review and meta-analysis. Eur Geriatr Med. 2019;10(1):9-36.
138. Khunti K, Sathanapally H, Mountain P. Multiple long term conditions, multimorbidity, and co-morbidities: we should reconsider the terminology we use. BMJ. 2023;383:p2327.
139. Boyd CM, Fortin M. Future of multimorbidity research: How should understanding of multimorbidity inform health system design? Public Health Rev. 2010;32(2):451-74.
140. (NICE). NIfHaCE. Multimorbidity: clinical assessment and management . London : National Institute for Health and Care Excellence (UK). 2016.
141. Ho I, Azcoaga-Lorenzo A, Akbari A, Davies J, Khunti K, Kadam U, et al. Measuring multimorbidity in research : Delphi consensus study. BMJ Medicine. 2022;1(1):e000247.
142. Suls J, Bayliss E, Berry J, Bierman A, Chrischilles E, Farhat T, et al. Measuring Multimorbidity: Selecting the Right Instrument for the Purpose and the Data Source. Med Care. 2022;59(8):743-56.
143. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of Chronic Diseases. 1987;40(5):373-83.
144. Charlson M, Carrozzino D, Guidi J, Patierno C. Charlson Comorbidity Index: A Critical Review of Clinimetric Properties. Psychother Psychosom. 2022;9(1):8-35.
145. Klabunde C, Legler J, Warren J, Bladwin L, Schrag D. A refined comorbidity measurement algorithm for claims-based studies of breast, prostate, colorectal, and lung cancer patients. Ann Epidemiol. 2007;17:584–90.

146. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali W. New ICD-10 version of the Charlson comorbidity index predicted in-hospital mortality. J Clin Epidemiol. 2004;57:1288–94.
147. Romano PS, Roos LL, Jollis JG. Adapting a clinical comorbidity index for use with ICD-9-CM administrative data: Differing perspectives. J Clin Epidemiol. 1993;46:1075–9.
148. Charlson M, Szatrowski T, Peterson J, Gold J. Validation of a combined comorbidity Index. J Clin Epidemiol. 1994;47(11):1245-51.
149. St-Louis E, Iqbal S, Feldman LS, Sudarshan M, Deckelbaum DL, Razek TS, et al. Using the age-adjusted Charlson comorbidity index to predict outcomes in emergency general surgery. J Trauma Acute Care Surg. 2015;78(2):318-23.
150. Carrasco-Ribelles LA, Roso-Llorach A, Cabrera-Bean M, Costa-Garrido A, Zabaleta-Del-Olmo E, Toran-Monserrat P, et al. Dynamics of multimorbidity and frailty, and their contribution to mortality, nursing home and home care need: A primary care cohort of 1 456 052 ageing people. EClinicalMedicine. 2022;52:101610.
151. Vinjerui KH, Boeckxstaens P, Douglas KA, Sund ER. Prevalence of multimorbidity with frailty and associations with socioeconomic position in an adult population: findings from the cross-sectional HUNT Study in Norway. BMJ Open. 2020;10(6):e035070.
152. Lujic S, Randall DA, Simpson JM, Falster MO, Jorm LR. Interaction effects of multimorbidity and frailty on adverse health outcomes in elderly hospitalised patients. Sci Rep. 2022;12(1):14139.
153. Hanlon P, Nicholl BI, Jani BD, Lee D, McQueenie R, Mair FS. Frailty and pre-frailty in middle-aged and older adults and its association with multimorbidity and mortality: a prospective analysis of 493 737 UK Biobank participants. Lancet Public Health. 2018;3(7):E323-E32.
154. Spiers GF, Kunonga TP, Hall A, Beyer F, Boulton E, Parker S, et al. Measuring frailty in younger populations: a rapid review of evidence. BMJ Open. 2021;11(3):e047051.
155. Podsiadlo D, Richardson S. The Timed “Up & Go”: A Test of Basic Functional Mobility for Frail Elderly Persons. Journal of the American Geriatrics Society. 1991;39(2):142-8.
156. Raiche M, Hebert R, Dubois MF. PRISMA-7: a case-finding tool to identify older adults with moderate to severe disabilities. Arch Gerontol Geriatr. 2008;47(1):9-18.
157. Gobbens RJ, van Assen MA, Luijkx KG, Wijnen-Sponselee MT, Schols JM. The Tilburg Frailty Indicator: psychometric properties. J Am Med Dir Assoc. 2010;11(5):344-55.
158. Bellera CA, Rainfray M, Mathoulin-Pelissier S, Mertens C, Delva F, Fonck M, et al. Screening older cancer patients: first evaluation of the G-8 geriatric screening tool. Ann Oncol. 2012;23(8):2166-72.
159. Saliba D, Elliott M, Rubenstein LZ, Solomon DH, Young RT, Kamberg CJ, et al. The Vulnerable Elders Survey: a tool for identifying vulnerable older people in the community. J Am Geriatr Soc. 2001;49(12):1691-9.
160. NELA project team. Eighth patient report of the national emergency laparotomy audit. Royal College of Anaesthetists (RCoA). 2023.
161. Simon HL, Reif de Paula T, Profeta da Luz MM, Nemeth SK, Moug SJ, Keller DS. Frailty in older patients undergoing emergency colorectal surgery: USA National Surgical Quality Improvement Program analysis. Br J Surg. 2020;107(10):1363-71.
162. McIsaac DI, MacDonald DB, Aucoin SD. Frailty for Perioperative Clinicians: A Narrative Review. Anesth Analg. 2020;130(6):1450-60.
163. Hewitt J, Moug SJ, Middleton M, Chakrabarti M, Stechman MJ, McCarthy K, et al. Prevalence of frailty and its association with mortality in general surgery. Am J Surg. 2015;209(2):254-9.
164. Tjeertes EKM, van Fessem JMK, Mattace-Raso FUS, Hoofwijk AGM, Stolker RJ, Hoeks SE. Influence of Frailty on Outcome in Older Patients Undergoing Non-Cardiac Surgery - A Systematic Review and Meta-Analysis. Aging Dis. 2020;11(5):1276-90.
165. Kennedy CA, Shipway D, Barry K. Frailty and emergency abdominal surgery: A systematic review and meta-analysis. Surgeon. 2022;20(6):e307-e14.

166. Farhat JS, Velanovich V, Falvo AJ, Horst HM, Swartz A, Patton JH, Jr., et al. Are the frail destined to fail? Frailty index as predictor of surgical morbidity and mortality in the elderly. J Trauma Acute Care Surg. 2012;72(6):1526-30; discussion 30-1.
167. Joseph B, Zangbar B, Pandit V, Fain M, Mohler MJ, Kulvatunyou N, et al. Emergency General Surgery in the Elderly: Too Old or Too Frail? J Am Coll Surg. 2016;222(5):805-13.
168. Kenig J, Zychiewicz B, Olszewska U, Barczynski M, Nowak W. Six screening instruments for frailty in older patients qualified for emergency abdominal surgery. Arch Gerontol Geriatr. 2015;61(3):437-42.
169. Jokar T, Ibraheem K, Rhee P, Kulavatunyou N, Haider A, Phelan HA, et al. Emergency general surgery specific frailty index: A validation study. J Trauma Acute Care Surg. 2016;81(2):254-60.
170. Goeteyn J, Evans LA, De Cleyn S, Fauconnier S, Damen C, Hewitt J, et al. Frailty as a predictor of mortality in the elderly emergency general surgery patient. Acta Chir Belg. 2017;117(6):370-5.
171. Kenig J, Mastalerz K, Lukasiewicz K, Mitus-Kenig M, Skorus U. The Surgical Apgar Score predicts outcomes of emergency abdominal surgeries both in fit and frail older patients. Arch Gerontol Geriatr. 2018;76:54-9.
172. Lee KC, Streid J, Sturgeon D, Lipsitz S, Weissman JS, Rosenthal RA, et al. The Impact of Frailty on Long-Term Patient-Oriented Outcomes after Emergency General Surgery: A Retrospective Cohort Study. J Am Geriatr Soc. 2020;68(5):1037-43.
173. Loecker C, Schmaderer M, Zimmerman L. Frailty in Young and Middle-Aged Adults: An Integrative Review. J Frailty Aging. 2021;10(4):327-33.
174. Tian B, Stahel PF, Picetti E, Campanelli G, Di Saverio S, Moore E, et al. Assessing and managing frailty in emergency laparotomy: a WSES position paper. World J Emerg Surg. 2023;18(1):38.
175. NELA Project Team. Seventh Patient Report of the National Emergency Laparotomy Audit. RCoA London 2021.
176. Carter B, Law J, Hewitt J, Parmar KL, Boyle JM, Casey P, et al. Association between preadmission frailty and care level at discharge in older adults undergoing emergency laparotomy. Br J Surg. 2020;107(3):218-26.
177. Ramsay EA, Carter B, Soiza RL, Duffy S, Moug SJ, Myint PK. Frailty is associated with increased waiting time for relevant process-of-care measures; findings from the Emergency Laparoscopic and Laparotomy Scottish audit (ELLSA). Br J Surg. 2022;109(2):172-5.
178. Birkelbach O, Morgeli R, Spies C, Olbert M, Weiss B, Brauner M, et al. Routine frailty assessment predicts postoperative complications in elderly patients across surgical disciplines - a retrospective observational study. BMC Anesthesiol. 2019;19(1):204.
179. Costa G, Massa G, Group ECS. Frailty and emergency surgery in the elderly: protocol of a prospective, multicenter study in Italy for evaluating perioperative outcome (The FRAILESEL Study). Updates Surg. 2018;70(1):97-104.
180. Costa G, Bersigotti L, Massa G, Lepre L, Fransvea P, Lucarini A, et al. The Emergency Surgery Frailty Index (EmSFI): development and internal validation of a novel simple bedside risk score for elderly patients undergoing emergency surgery. Aging Clin Exp Res. 2021;33(8):2191-201.
181. Dent E, Kowal P, Hoogendijk EO. Frailty measurement in research and clinical practice: A review. Eur J Intern Med. 2016;31:3-10.
182. Dhesi JK, Lees NP, Partridge JS. Frailty in the perioperative setting. Clin Med (Lond). 2019;19(6):485-9.
183. McCarthy K, Hewitt J. Special needs of frail people undergoing emergency laparotomy surgery. Age Ageing. 2020;49(4):540-3.
184. Rockwood K, Howlett SE. Fifteen years of progress in understanding frailty and health in aging. BMC Med. 2018;16(1):220.
185. Swarbrick CJ, collaborators S-, Williams K, Evans B, Blake HA, Poulton T, et al. Postoperative outcomes in older patients living with frailty and multimorbidity in the UK: SNAP-3, a snapshot observational study. Br J Anaesth. 2025;135(1):166-76.

186. Society. BG. BGS Position Statement: Older patients undergoing emergency laparotomy 2020 [Available from: https://www.bgs.org.uk/bgs-position-statement-older-patients-undergoing-emergency-laparotomy.
187. Partridge J, Collingridge G, Gordon A, Martin F, Harari D, Dhesi J. Where are we in perioperative medicine for older surgical patients? A UK survey of geriatric medicine delivered services in surgery. Age and ageing. 2014;43(5):721–4.
188. Joseph B, Pandit V, Zangbar B, Kulvatunyou N, Hashmi A, Green DJ, et al. Superiority of frailty over age in predicting outcomes among geriatric trauma patients: a prospective analysis. JAMA Surg. 2014;149(8):766-72.
189. Makary MA, Segev DL, Pronovost PJ, Syin D, Bandeen-Roche K, Patel P, et al. Frailty as a predictor of surgical outcomes in older patients. J Am Coll Surg. 2010;210(6):901-8.
190. Rose M, Pan H, Levinson MR, Staples M. Can frailty predict complicated care needs and length of stay? Intern Med J. 2014;44(8):800-5.
191. Leiner T, Nemeth D, Hegyi P, Ocskay K, Virag M, Kiss S, et al. Frailty and Emergency Surgery: Results of a Systematic Review and Meta-Analysis. Frontiers in Medicine. 2022;9.
192. Arteaga AS, Aguilar LT, Gonzalez JT, Boza AS, Munoz-Cruzado VD, Ciuro FP, et al. Impact of frailty in surgical emergencies. A comparison of four frailty scales. Eur J Trauma Emerg Surg. 2021;47(5):1613-9.
193. Fehlmann CA, Patel D, McCallum J, Perry JJ, Eagles D. Association between mortality and frailty in emergency general surgery: a systematic review and meta-analysis. Eur J Trauma Emerg Surg. 2022;48(1):141-51.
194. Haas LEM, Boumendil A, Flaatten H, Guidet B, Ibarz M, Jung C, et al. Frailty is associated with long-term outcome in patients with sepsis who are over 80 years old: results from an observational study in 241 European ICUs. Age Ageing. 2021;50(5):1719-27.
195. Hewitt J, Carter B, McCarthy K, Pearce L, Law J, Wilson FV, et al. Frailty predicts mortality in all emergency surgical admissions regardless of age. An observational study. Age Ageing. 2019;48(3):388-94.
196. Howell SJ, Nair S. Measuring frailty in the older surgical patient: the case for evidence synthesis. Br J Anaesth. 2021;126(4):763-7.
197. Li Y, Pederson JL, Churchill TA, Wagg AS, Holroyd-Leduc JM, Alagiakrishnan K, et al. Impact of frailty on outcomes after discharge in older surgical patients: a prospective cohort study. CMAJ. 2018;190(7):E184-E90.
198. Panayi AC, Orkaby AR, Sakthivel D, Endo Y, Varon D, Roh D, et al. Impact of frailty on outcomes in surgical patients: A systematic review and meta-analysis. Am J Surg. 2019;218(2):393-400.
199. Shinall MC, Jr., Arya S, Youk A, Varley P, Shah R, Massarweh NN, et al. Association of Preoperative Patient Frailty and Operative Stress With Postoperative Mortality. JAMA Surg. 2020;155(1):e194620.
200. Huddart S, Peden CJ, Swart M, McCormick B, Dickinson M, Mohammed MA, et al. Use of a pathway quality improvement care bundle to reduce mortality after emergency laparotomy. Br J Surg. 2015;102(1):57-66.
201. Price A, McLennan E, Knight SR, Reeves N, Chandler S, Boyle J, et al. Characterisation of older patients that require, but do not undergo, emergency laparotomy: a multicentre cohort study. Br J Anaesth. 2024;133(5):973-82.
202. McIlveen EC, Wright E, Shaw M, Edwards J, Vella M, Quasim T, et al. A prospective cohort study characterising patients declined emergency laparotomy: survival in the 'NoLap' population. Anaesthesia. 2020;75(1):54- 62.
203. Cobb M. 60 years ago, Francis Crick changed the logic of biology. PLoS Biol. 2017;15(9):e2003243.
204. Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Therapeutics. 2001;69:89-95.

205. WHO International Programme on Chemical Safety. Biomarkers and Risk Assessment: Concepts and Principles 1993 [Available from: http://www.inchem.org/documents/ehc/ehc/ehc155.htm.
206. Hoogendijk EO, Afilalo J, Ensrud KE, Kowal P, Onder G, Fried LP. Frailty: implications for clinical practice and public health. Lancet. 2019;394(10206):1365-75.
207. Saedi AA, Feehan J, Phu S, Duque G. Current and emerging biomarkers of frailty in the elderly. Clin Interv Aging. 2019;14:389-98.
208. Kameda M, Teruya T, Yanagida M, Kondoh H. Frailty markers comprise blood metabolites involved in antioxidation, cognition, and mobility. Proc Natl Acad Sci U S A. 2020;117(17):9483-9.
209. Kondoh H, Kameda M, Yanagida M. Whole Blood Metabolomics in Aging Research. Int J Mol Sci. 2020;22(1).
210. Picca A, Calvani R, Cesari M, Landi F, Bernabei R, Coelho-Junior HJ, et al. Biomarkers of Physical Frailty and Sarcopenia: Coming up to the Place? Int J Mol Sci. 2020;21(16).
211. Mitnitski A, Collerton J, Martin-Ruiz C, Jagger C, von Zglinicki T, Rockwood K, et al. Age-related frailty and its association with biological markers of ageing. BMC Med. 2015;13(1):161.
212. Hubbard RE, O'Mahony MS, Savva GM, Calver BL, Woodhouse KW. Inflammation and frailty measures in older people. J Cell Mol Med. 2009;13(9B):3103-9.
213. Leng SX, Cappola AR, Anderson RE, Blackman MR, Koenig K, Blair M, et al. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. Aging Clin Exp Res. 2004;16:153-7.
214. Sanchis J, Nunez E, Ruiz V, Bonanad C, Fernandez J, Cauli O, et al. Usefulness of Clinical Data and Biomarkers for the Identification of Frailty After Acute Coronary Syndromes. Can J Cardiol. 2015;31(12):1462-8.
215. Wu IC, Lin XZ, Liu PF, Tsai WL, Shiesh SC. Low serum testosterone and frailty in older men and women. Maturitas. 2010;67(4):348-52.
216. Calvani R, Marini F, Cesari M, Tosato M, Anker SD, von Haehling S, et al. Biomarkers for physical frailty and sarcopenia: state of the science and future developments. J Cachexia Sarcopenia Muscle. 2015;6(4):278-86.
217. Faull KF, Dooley AN, Halgand F, Shoemaker LD, Norris AJ, Ryan CM, et al. Chapter 1 An Introduction to the Basic Principles and Concepts of Mass Spectrometry. Protein Mass Spectrometry. Comprehensive Analytical Chemistry2008. p. 1-46.
218. Picca A, Coelho-Junior HJ, Cesari M, Marini F, Miccheli A, Gervasoni J, et al. The metabolomics side of frailty: Toward personalized medicine for the aged. Exp Gerontol. 2019;126:110692.
219. Livshits G, Malkin I, Bowyer RCE, Verdi S, Bell JT, Menni C, et al. Multi- OMICS analyses of frailty and chronic widespread musculoskeletal pain suggest involvement of shared neurological pathways. Pain. 2018;159(12):2565– 25 72.
220. Calvani R, Picca A, Marini F, Biancolillo A, Gervasoni J, Persichilli S, et al. A distinct pattern of circulating amino acids characterizes older persons with physical frailty and sarcopenia: results from the BIOSPHERE study. Nutrients. Nutrients. 2018;10(11):1691.
221. Kochlik B, Stuetz W, Peres K, Feart C, Tegner J, Rodriguez-Manas L, et al. Associations of plasma 3-methylhistidine with frailty status in French cohorts of the FRAILOMIC initiative. J Clin Med. 2019;8(7):1010.
222. Pujos-Guillot E, Petera M, Jacquemin J, Centeno D, Lyan B, Montoliu I, et al. Identification of Pre-frailty Sub-Phenotypes in Elderly Using Metabolomics. Front Physiol. 2018;9:1903.
223. Rattray NJW, Trivedi DK, Xu Y, Chandola T, Johnson CH, Marshall AD, et al. Metabolic dysregulation in vitamin E and carnitine shuttle energy mechanisms associate with human frailty. Nat Commun. 2019;10(1):5027.
224. Westbrook R, Zhang C, Yang H, Tian J, Guo S, Xue Q, et al. Metabolomics-Based Identification of Metabolic Dysfunction in Frailty. *J Gerontol A Biol Sci Med Sci*. 2021:1-6.

225. Pan Y, Li Y, Liu P, Zhang Y, Li B, Liu Z, et al. Metabolomics-Based Frailty Biomarkers in Older Chinese Adults. Frontiers in Medicine. 2022;Volume 8 - 2021.
226. Erusalimsky JD, Grillari J, Grune T, Jansen-Duerr P, Lippi G, Sinclair AJ, et al. In Search of 'Omics'-Based Biomarkers to Predict Risk of Frailty and Its Consequences in Older Individuals: The FRAILOMIC Initiative. Gerontology. 2016;62(2):182-90.
227. Kochlik B, Stuetz W, Peres K, Pilleron S, Feart C, Garcia Garcia FJ, et al. Associations of fat-soluble micronutrients and redox biomarkers with frailty status in the FRAILOMIC initiative. J Cachexia Sarcopenia Muscle. 2019;10(6):1339-46.
228. Henning T, Kochlik B, Ara I, Gonzalez-Gross M, Fiorillo E, Marongiu M, et al. Patterns of Dietary Blood Markers Are Related to Frailty Status in the FRAILOMIC Validation Phase. Nutrients. 2023;15(5).
229. Mu L, Jiang L, Chen J, Xiao M, Wang W, Liu P, et al. Serum Inflammatory Factors and Oxidative Stress Factors Are Associated With Increased Risk of Frailty and Cognitive Frailty in Patients With Cerebral Small Vessel Disease. Front Neurol. 2021;12:786277.
230. El Assar M, Angulo J, Rodriguez-Manas L. Frailty as a phenotypic manifestation of underlying oxidative stress. Free Radic Biol Med. 2020;149:72-7.
231. Horton R. Surgical research or comic opera: questions, but few answers. The Lancet. 1996;347:984- 5.
232. Hutchinson PJ, Pinkney T, Mohan M, Cromwell D, van der Meulen J, Coomer M, et al. From opera buffa to opera seria: anniversaries of Royal College of Surgeons of England research initiatives. Br J Surg. 2024;111(1).
233. Vaughan EM, Pearson R, Wohlgemut JM, Knight SR, Spiers H, Damaskos D, et al. Research priorities in emergency general surgery (EGS): a modified Delphi approach. World J Emerg Surg. 2022;17(1):33.
234. Lee MJ, Sayers AE, Drake TM, Marriott PJ, Anderson ID, Bach SP, et al. National prospective cohort study of the burden of acute small bowel obstruction. BJS Open. 2019;3(3):354-66.
235. Damascus Study Management Group. Diverticulitis Management, a Snapshot Collaborative Audit Study (DAMASCUS): Protocol for an international, multicentre, prospective observational study. Colorectal Dis. 2021;23(8):2182-8.
236. Rowlands C, Rooshenas L, Fairhurst K, Rees J, Gamble C, Blazeby JM. Detailed systematic analysis of recruitment strategies in randomised controlled trials in patients with an unscheduled admission to hospital. BMJ Open. 2018;8(2):e018581.
237. CReST Collaborative Group. Colorectal Endoscopic Stenting Trial (CReST) for obstructing left-sided colorectal cancer: randomized clinical trial. Br J Surg. 2022;109(11):1073-80.
238. Pinkney TD, Calvert M, Bartlett DC, Gheorghe A, Redman V, Dowswell G, et al. Impact of wound edge protection devices on surgical site infection after laparotomy: multicentre randomised controlled trial (ROSSINI Trial). BMJ. 2013;347:f4305.
239. Sunrrise Study Group on behalf of the Northwest Research Collaborative and the West Midlands Research. An international pragmatic randomised controlled trial to compare a single use negative pressure dressing versus a surgeon's preference of dressing to reduce the incidence of surgical site infection following emergency laparotomy: the SUNRRISE Trial Protocol. Colorectal Dis. 2020.
240. Harji DP, Marshall H, Gordon K, Twiddy M, Pullan A, Meads D, et al. Laparoscopic versus open colorectal surgery in the acute setting (LaCeS trial): a multicentre randomized feasibility trial. Br J Surg. 2020;107(12):1595-604.
241. Broughton KJ, Aldridge O, Pradhan S, Aitken RJ. The Perth Emergency Laparotomy Audit. Australia and New Zealand Journal of Surgery. 2017;87(11):893-7
242. Ebrahim M, Lauritsen ML, Cihoric M, Hilsted KL, Foss NB. Triage and outcomes for a whole cohort of patients presenting for major emergency abdominal surgery including the No-LAP population: a prospective single-center observational study. Eur J Trauma Emerg Surg. 2023;49(1):253-60.

243. Pule LM, Kopunic H, Aitken RJ. Low mortality rate after emergency laparotomy in Australia is a reflection of its national surgical mortality audit influencing futile surgery. Br J Surg. 2023;110(10):1367-73.
244. NELA Team Project. NELA Inclusion Exclusion Criteria Updated 12 November 2021 2021 [Available from: https://www.nela.org.uk/Criteria.
245. Saklad M. Grading of patients for surgical procedures. *Anesthesiology*. 1941;2:281–4.
246. Dolan DR, Knight KA, Maguire S, Moug SJ. The relationship between sarcopenia and survival at 1 year in patients having elective colorectal cancer surgery. Tech Coloproctol. 2019;23(9):877-85.
247. Peng P, Hyder O, Firoozmand A, Kneuertz P, Schulick RD, Huang D, et al. Impact of sarcopenia on outcomes following resection of pancreatic adenocarcinoma. J Gastrointest Surg. 2012;16(8):1478-86.
248. Amini N, Spolverato G, Gupta R, Margonis GA, Kim Y, Wagner D, et al. Impact Total Psoas Volume on Short- and Long-Term Outcomes in Patients Undergoing Curative Resection for Pancreatic Adenocarcinoma: a New Tool to Assess Sarcopenia. J Gastrointest Surg. 2015;19(9):1593-602.
249. Joglekar S, Asghar A, Mott SL, Johnson BE, Button AM, Clark E, et al. Sarcopenia is an independent predictor of complications following pancreatectomy for adenocarcinoma. J Surg Oncol. 2015;111(6):771-5.
250. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychological Assessment. 1994;6(4):284-90.
251. Devlin NJ, Shah KK, Feng Y, Mulhern B, van Hout B. Valuing health-related quality of life: An EQ-5D-5L value set for England. Health Econ. 2018;27(1):7-22.
252. Kongkaewpaisan N, El Hechi MW, Naar L, Kaafarani HMA, Group EMES. Unplanned readmission after emergency laparotomy: A post hoc analysis of an EAST multicenter study. Surgery. 2021;169(6):1434-40.
253. Rossi IR, Ross SW, May AK, Reinke CE. Readmission After Emergency General Surgery: NSQIP Review of Risk, Cause and Ideal Follow-Up. J Surg Res. 2021;260:359-68.
254. L IS, Kokotovic D, Gogenur I, Ekeloef S, Burcharth J. Short and long-term readmission after major emergency abdominal surgery: a prospective Danish study. Eur J Trauma Emerg Surg. 2024;50(1):295-304.
255. Park B, Alani Z, Sulistio E, Barazanchi AWH, Koea J, Vandal A, et al. Frailty using the Clinical Frailty Scale to predict short- and long-term adverse outcomes following emergency laparotomy: meta-analysis. BJS Open. 2024;8(4).
256. Rangel EL, Rios-Diaz AJ, Uyeda JW, Castillo-Angeles M, Cooper Z, Olufajo OA, et al. Sarcopenia increases risk of long-term mortality in elderly patients undergoing emergency abdominal surgery. J Trauma Acute Care Surg. 2017;83(6):1179-86.
257. Trotter J, Johnston J, Ng A, Gatt M, MacFie J, McNaught C. Is sarcopenia a useful predictor of outcome in patients after emergency laparotomy? A study using the NELA database. Ann R Coll Surg Engl. 2018;100(5):377-81.
258. Knoedler S, Schliermann R, Knoedler L, Wu M, Hansen FJ, Matar DY, et al. Impact of sarcopenia on outcomes in surgical patients: a systematic review and meta-analysis. Int J Surg. 2023;109(12):4238-62.
259. Thu MM, Ng HJ, Moug S. The influence between frailty, sarcopenia and physical status on mortality in patients undergoing emergency laparotomy. World J Emerg Surg. 2025;20(1):38.
260. Park B, Bhat S, Wells CI, Barazanchi AWH, Hill AG, MacCormick AD. Short- and long-term impact of sarcopenia on outcomes after emergency laparotomy: A systematic review and meta-analysis. Surgery. 2022;172(1):436-45.
261. Mao X, Lv K, Qi W, Cheng W, Li T, Sun Y, et al. Research progress on sarcopenia in the musculoskeletal system. Bone Res. 2025;13(1):78.
262. Ming YJ, Howley P, Holmes M, Gani J, Pockney P. Sarcopenia 'made simple' and outcomes from emergency laparotomy. ANZ J Surg. 2022;92(12):3198-203.

263. Ng ZQ, Weber D. One-Year Outcomes Following Emergency Laparotomy: A Systematic Review. World J Surg. 2022;46(3):512-23.
264. Simpson G, Manu N, Magee C, Wilson J, Moug S, Vimalachandran D. Measuring sarcopenia on pre-operative CT in older adults undergoing emergency laparotomy: a comparison of three different calculations. Int J Colorectal Dis. 2020;35(6):1095-102.
265. Ablett AD, McCarthy K, Carter B, Pearce L, Stechman M, Moug S, et al. Cognitive impairment is associated with mortality in older adults in the emergency surgical setting: Findings from the Older Persons Surgical Outcomes Collaboration (OPSOC): A prospective cohort study. Surgery. 2019;165(5):978-84.
266. Rockwood K, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin Geriatr Med. 2011;27(1):17-26.
267. Vadlakonda A, Chervu N, Verma A, Sakowitz S, Bakhtiyar SS, Sanaiha Y, et al. Moving beyond frailty: Obesity paradox persists in lung resection. Surgery. 2023;174(4):964-70.
268. Jayanama K, Theou O, Godin J, Mayo A, Cahill L, Rockwood K. Relationship of body mass index with frailty and all-cause mortality among middle-aged and older adults. BMC Med. 2022;20(1):404.
269. Sharma Y, Avina P, Ross E, Horwood C, Hakendorf P, Thompson C. Validity of the Malnutrition Universal Screening Tool for Evaluation of Frailty Status in Older Hospitalised Patients. Gerontol Geriatr Med. 2022;8:23337214221107817.
270. Tengberg LT, Cihoric M, Foss NB, Bay-Nielsen M, Gogenur I, Henriksen R, et al. Complications after emergency laparotomy beyond the immediate postoperative period - a retrospective, observational cohort study of 1139 patients. Anaesthesia. 2017;72(3):309-16.
271. Ylimartimo AT, Nurkkala J, Koskela M, Lahtinen S, Kaakinen T, Vakkala M, et al. Postoperative Complications and Outcome After Emergency Laparotomy: A Retrospective Study. World J Surg. 2023;47(1):119-29.
272. Snitkjaer C, Rehne Jensen L, L IS, Hauge C, Kvist M, Jensen TK, et al. Impact of clinical frailty on surgical and non-surgical complications after major emergency abdominal surgery. BJS Open. 2024;8(3).
273. Murphy PB, Savage SA, Zarzaur BL. Impact of Patient Frailty on Morbidity and Mortality after Common Emergency General Surgery Operations. J Surg Res. 2020;247:95-102.
274. Ruiz de Gopegui Miguelena P, Martinez Lamazares MT, Claraco Vega LM, Gurpegui Puente M, Gonzalez Almarcegui I, Gutierrez Ibanes P, et al. Evaluating frailty may complement APACHE II in estimating mortality in elderly patients admitted to the ICU after digestive surgery. Med Intensiva (Engl Ed). 2022;46(5):239-47.
275. Mankowski RT, Anton SD, Ghita GL, Brumback B, Cox MC, Mohr AM, et al. Older Sepsis Survivors Suffer Persistent Disability Burden and Poor Long-Term Survival. J Am Geriatr Soc. 2020;68(9):1962-9.
276. Gariballa S, Alessa A. Impact of poor muscle strength on clinical and service outcomes of older people during both acute illness and after recovery. BMC Geriatr. 2017;17(1):123.
277. Deutz NEP, Ashurst I, Ballesteros MD, Bear DE, Cruz-Jentoft AJ, Genton L, et al. The Underappreciated Role of Low Muscle Mass in the Management of Malnutrition. J Am Med Dir Assoc. 2019;20(1):22-7.
278. Wang H, Yang R, Xu J, Fang K, Abdelrahim M, Chang L. Sarcopenia as a predictor of postoperative risk of complications, mortality and length of stay following gastrointestinal oncological surgery. Ann R Coll Surg Engl. 2021;103(9):630-7.
279. Damanti S, Senini E, De Lorenzo R, Merolla A, Santoro S, Festorazzi C, et al. Acute Sarcopenia: Mechanisms and Management. Nutrients. 2024;16(20).
280. Deiner S, Silverstein JH. Postoperative delirium and cognitive dysfunction. Br J Anaesth. 2009;103 Suppl 1(Suppl 1):i41-6.
281. Boccardi V, Marano L. Improving geriatric outcomes through nutritional and immunonutritional strategies: Focus on surgical setting by a comprehensive evidence review. Ageing Res Rev. 2024;96:102272.

282. Martinez-Ortega AJ, Pinar-Gutierrez A, Serrano-Aguayo P, Gonzalez-Navarro I, Remon-Ruiz PJ, Pereira-Cunill JL, et al. Perioperative Nutritional Support: A Review of Current Literature. Nutrients. 2022;14(8).
283. Xu W, Ding X. The application effectiveness of an early enteral nutrition protocol constructed based on the enhanced recovery after surgery concept in ICU patients. Medicine (Baltimore). 2025;104(18):e42292.
284. Perez de Souza L, Alseekh S, Scossa F, Fernie AR. Ultra-high-performance liquid chromatography high-resolution mass spectrometry variants for metabolomics research. Nat Methods. 2021;18(7):733-46.
285. Musharraf SG, Mazhar S, Siddiqui AJ, Choudhary MI, Atta ur R. Metabolite profiling of human plasma by different extraction methods through gas chromatography-mass spectrometry--an objective comparison. Anal Chim Acta. 2013;804:180-9.
286. Griebenow K, Klibanov AM. On Protein Denaturation in Aqueous−Organic Mixtures but Not in Pure Organic Solvents. Journal of the American Chemical Society. 1996;118(47):11695-700.
287. Polson C, Sarkar P, Incledon B, Raguvaran V, Grant R. Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography–tandem mass spectrometry. Journal of Chromatography B. 2003;785(2):263-75.
288. Fonseca TAH, Von Rekowski CP, Araújo R, Oliveira MC, Justino GC, Bento L, et al. The Impact of the Serum Extraction Protocol on Metabolomic Profiling Using UPLC-MS/MS and FTIR Spectroscopy. ACS Omega. 2023;8(23):20755-66.
289. Vitale CM, Price EJ, Miller GW, David A, Antignac J-P, Barouki R, et al. Analytical strategies for chemical exposomics: exploring limits and feasibility. Exposome. 2021;1(1).
290. Taylor PJ. Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. Clinical Biochemistry. 2005;38(4):328-34.
291. Lindahl A, Sääf S, Lehtiö J, Nordström A. Tuning Metabolome Coverage in Reversed Phase LC–MS Metabolomics of MeOH Extracted Samples Using the Reconstitution Solvent Composition. Analytical Chemistry. 2017;89(14):7356-64.
292. Geue N. Modern Electrospray Ionization Mass Spectrometry Techniques for the Characterization of Supramolecules and Coordination Compounds. Anal Chem. 2024;96(19):7332-41.
293. Broadhurst DI. *QC:MXP Repeat Injection based Quality Control, Batch Correction, Exploration & Data Cleaning* (Version 2) Zendono. 2024 [Available from: https://github.com/broadhurstdavid/QC-MXP.
294. Pang Z, Xu L, Viau C, Lu Y, Salavati R, Basu N, et al. MetaboAnalystR 4.0: a unified LC-MS workflow for global metabolomics. Nature Communications. 2024;15.
295. Worley B, Powers R. Multivariate Analysis in Metabolomics. Curr Metabolomics. 2013;1(1):92-107.
296. Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR, et al. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabolomics. 2018;14(6):72.
297. Riquelme G, Zabalegui N, Marchi P, Jones CM, Monge ME. A Python-Based Pipeline for Preprocessing LC–MS Data for Untargeted Metabolomics Workflows. Metabolites. 2020;10:416.
298. Altman N. Batches and blocks, sample pools and subsamples in the design and analysis of gene expression studies. In: Scherer A, editor. Batch effects and noise in microarray experiments: sources and solutions. Chichester: Wiley; 2009.
299. Ivanisevic J, Elias D, Deguchi H, Averell PM, Kurczy M, Johnson CH, et al. Arteriovenous Blood Metabolomics: A Readout of Intra-Tissue Metabostasis. Sci Rep. 2015;5:12757.
300. Krzanowski W. Principles of Multivariate Analysis: A User's Perspective.: OUP Oxford; 2000.
301. Marron MM, Yao S, Shah RV, Murthy VL, Newman AB. Metabolomic characterization of vigor to frailty among community-dwelling older Black and White men and women. Geroscience. 2024;46(2):2371-89.

302. Shekarchian A, Bandarian F, Hadizadeh AA-O, Amirsardari ZA-O, Sharifi YA-O, Ayati AA-O, et al. Exploring the metabolomics profile of frailty- a systematic review. J Diabetes Metab Disord. 2024;13(1):289–303.
303. Meng L, Shi H, Wang D-g, Shi J, Wu W-b, Dang Y-m, et al. Specific Metabolites Involved in Antioxidation and Mitochondrial Function Are Correlated With Frailty in Elderly Men. Frontiers in Medicine. 2022;Volume 9 - 2022.
304. Wishart DS, Guo A, Oler E, Wang F, Anjum A, Peters H, et al. HMDB 5.0: the Human Metabolome Database for 2022. Nucleic Acids Research. 2021;50(D1):D622-D31.
305. Li S, Gao D, Jiang Y. Function, Detection and Alteration of Acylcarnitine Metabolism in Hepatocellular Carcinoma. Metabolites. 2019;9(2):36.
306. Tyni T, Pourfarzam M, Turnbull DM. Analysis of Mitochondrial Fatty Acid Oxidation Intermediates by Tandem Mass Spectrometry from Intact Mitochondria Prepared from Homogenates of Cultured Fibroblasts, Skeletal Muscle Cells, and Fresh Muscle. Pediatric Research. 2002;52(1):64-70.
307. Wanders R, Visser G, Ferdinandusse S, Vaz F, Houtkooper R. Mitochondrial Fatty Acid Oxidation Disorders: Laboratory Diagnosis, Pathogenesis, and the Complicated Route to Treatment. . J Lipid Atheroscler. 2020;9:313-33.
308. Smith DM, Choi J, Wolfgang MJ. Tissue specific roles of fatty acid oxidation. Advances in Biological Regulation. 2025;95:101070.
309. Aguer C, McCoin CS, Knotts TA, Thrush AB, Ono-Moore K, McPherson R, et al. Acylcarnitines: potential implications for skeletal muscle insulin resistance. The FASEB Journal. 2015;29(1 %@ 0892-6638):336-45.
310. Sergi D, Naumovski N, Heilbronn LK, Abeywardena M, O'Callaghan N, Lionetti L, et al. Mitochondrial (Dys)function and Insulin Resistance: From Pathophysiological Molecular Mechanisms to the Impact of Diet. Frontiers in Physiology. 2019;10:532.
311. Xiang F, Zhang Z, Xie J, Xiong S, Yang C, Liao D, et al. Comprehensive review of the expanding roles of the carnitine pool in metabolic physiology: beyond fatty acid oxidation. J Transl Med. 2025;23(1):324.
312. Sharma S, Black SM. Carnitine Homeostasis, Mitochondrial Function, and Cardiovascular Disease. Drug Discov Today Dis Mech. 2009;6(1-4):e31-e9.
313. Vallance HD, Koochin A, Branov J, Rosen-Heath A, Bosdet T, Wang Z, et al. Marked elevation in plasma trimethylamine-N-oxide (TMAO) in patients with mitochondrial disorders treated with oral l-carnitine. Mol Genet Metab Rep. 2018;15:130-3.
314. Badrasawi M, Shahar S, Zahara A, Nor Fadilah R, Singh D. Efficacy of L-carnitine supplementation on frailty status and its biomarkers, nutritional status, and physical and cognitive function among prefrail older adults: a double-blind, randomized, placebo-controlled clinical trial. . Clin Interv Aging. 2016;11:1675-86.
315. Pistone G, Marino AD, Leotta C, Dell, apos, Arte S, et al. Levocarnitine administration in elderly subjects with rapid muscle fatigue: effect on body composition, lipid profile and fatigue. Drugs & Aging. 2003;20:761+.
316. Karna E, Szoka L, Huynh TYL, Palka JA. Proline-dependent regulation of collagen metabolism. (1420-9071 (Electronic)).
317. Palka J, Oscilowska I, Szoka L. Collagen metabolism as a regulator of proline dehydrogenase/proline oxidase-dependent apoptosis/autophagy. Amino Acids. 2021;53(12):1917-25.
318. Lu Z, Zhang C, Zhang J, Su W, Wang GA-O, Wang ZA-O. The Kynurenine Pathway and Indole Pathway in Tryptophan Metabolism Influence Tumor Progression. Cancer med. 2025;18(14):2045-7634 (Electronic).
319. Wu Z, Qu J, Zhang W, Liu G-H. Stress, epigenetics, and aging: Unraveling the intricate crosstalk. Molecular Cell. 2024;84(1):34-54.
320. Handono AP, Ryan J, Setiawan F, Espinoza SE, Phyo AZZ, Zhu C, et al. The effect of low-dose aspirin on reversing frailty status in older prefrail and frail adults: a secondary analysis of the ASPREE randomised clinical trial. Age and Ageing. 2025;54(9).

321. Gewurz D, Zhou G, Endo Y, Sinha I, Gaziano JM, Sesso HD, et al. Inflammation, Frailty, and Aspirin Use in the Physicians' Health Study: A Pilot Study. The Journal of Frailty & Aging. 2024;13(4):582-5.
322. Auger J-P, Zimmermann M, Faas M, Stifel U, Chambers D, Krishnacoumar B, et al. Metabolic rewiring promotes anti-inflammatory effects of glucocorticoids. Nature. 2024;629(8010):184-92.
323. Tada M, Matsumoto Y, Koike T, Mamoto K, Nakamura T, Anno S, et al. Glucocorticoid usage is involved in the improvement and development of frailty in patients with rheumatoid arthritis: Results from the PRESENT study. Geriatrics & Gerontology International. 2025;25(9):1231-8.
324. Brunelli L, Davin A, Sestito G, Mimmi MC, De Simone G, Balducci C, et al. Plasmatic Hippuric Acid as a Hallmark of Frailty in an Italian Cohort: The Mediation Effect of Fruit–Vegetable Intake. The Journals of Gerontology: Series A. 2021;76(12):2081-9 %@ 1079-5006.
325. De Simone G, Balducci C, Forloni G, Pastorelli R, Brunelli L. Hippuric acid: Could became a barometer for frailty and geriatric syndromes? Ageing Res Rev. 2021;72:101466.
326. Pallister T, Jackson MA, Martin TC, Zierer J, Jennings A, Mohney RP, et al. Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. Scientific Reports. 2017;7(1):13670.
327. Sepúlveda M, Arauna D, García F, Albala C, Palomo I, Fuentes E. Frailty in Aging and the Search for the Optimal Biomarker: A Review. Biomedicines. 2022;10(6):1426.
328. Zhou M, Sun W, Chu J, Liao Y, Xu P, Chen X, et al. Identification of novel biomarkers for frailty diagnosis via serum amino acids metabolomic analysis using UPLC-MS/MS. PROTEOMICS – Clinical Applications. 2024;18(3):2300035.
329. Gao S, Sun Y, Chen XA-O, Zhu C, Liu X, Wang W, et al. Pyrimidine catabolism is required to prevent the accumulation of 5-methyluridine in RNA. (1362-4962 (Electronic)).
330. Zhao Y, Chen X, Zhang X, Liu H. RNA epigenetic modifications as dynamic biomarkers in cancer: from mechanisms to clinical translation. Biomark Res. 2025;13(1):e81.
331. Brochard T, McIntyre RL, Houtkooper RH, Seluanov A, Gorbunova V, Janssens GE. Repurposing nucleoside reverse transcriptase inhibitors (NRTIs) to slow aging. Ageing Research Reviews. 2023;92:102132.
332. Xu X, Xu J, Xu G, Lv T, Yang J. A prospective longitudinal cohort study for quality of life in patients undergoing fourth level liver surgery. Scientific Reports. 2025;15(1):21035.
333. Ye B, Li Y, Bao Z, Gao J. Psychological Resilience and Frailty Progression in Older Adults. JAMA Network Open. 2024;7(11):e2447605-e.
334. Khanderia E, Aggarwal R, Bouras G, Patel V. Quality of life after emergency laparotomy: a systematic review. BMC Surg. 2024;24(1):73.
335. Stevens VL, Hoover E, Wang Y, Zanetti KA. Pre-Analytical Factors that Affect Metabolite Stability in Human Urine, Plasma, and Serum: A Review. Metabolites. 2019;9(8).
336. Suskind AM, Finlayson E. A Call for Frailty Screening in the Preoperative Setting. JAMA Surgery. 2017;152(3):240-1.