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**Diagnosis by comprehensive Cardiovascular Imaging for Stroke and
Transient Ischaemic Attack (D-CCIST):
A Randomized Controlled Pilot Study**

by
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A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Medicine (MD)

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Abstract

Accurate identification of stroke mechanism is essential to guide secondary prevention, yet conventional diagnostic pathways frequently remain incomplete or delayed. This pilot randomised controlled trial tested whether a fast-track of comprehensive cardiovascular imaging (CCI) protocol could improve diagnostic completeness and timeliness compared with routine care in acute ischaemic and transient ischaemic attack.

Participants were randomised in a 1:1 ratio to CCI protocol group, which integrated ECG-gated cardiac computed tomography, extended aortic-to-vertex computed tomography angiography, and 3T brain MRI performed during a single hospital session, or to the routine diagnostic pathway delivered according to standard clinical practice. The primary outcome was the proportion of patients with incomplete diagnostic work-up (ASCO-D 9) at Day 30, defined as any unresolved aetiological category. Secondary outcomes included time to final classification, change in ASCO-D certainty, imaging utilisation, treatment modification, and new clinical events within 30 days.

CCI was successfully completed in most patients and was safe, with radiation exposure within national reference limits. At Day 30, incomplete classification was markedly lower with CCI, while definite aetiological assignment nearly doubled. Diagnostic improvement was observed across the large artery, small vessel and cardioembolic domains, demonstrating that integrated multimodal imaging enhances mechanistic resolution and accelerates diagnostic completion.

Analysis of secondary outcomes showed that CCI shortens the time to definitive aetiological classification and facilitated earlier alignment between diagnostic certainty and therapy choices. Mechanism-specific treatment was initiated more frequently in CCI group, whereas generic single antiplatelet use predominated in routine care. No recurrent stroke or death occurred in the CCI arm during 30-day follow-up, compared with three non-fatal events and one death under routine care. Importantly, the CCI protocol neither prolonged nor shortened hospital stay, confirmed that early comprehensive imaging can be implemented efficiently within existing inpatients pathways.

Subsequent mediation analysis demonstrated that diagnostic benefits of CCI were primarily mediated through increased cardiac imaging use, rather than timing of investigation, thereby highlighting the mechanistic link between protocol design and improved diagnostic certainty.

This randomised pilot study provide evidence that comprehensive cardiovascular imaging substantially improves diagnostic completeness and timeliness in acute stroke evaluation. By reducing undetermined aetiological and promoting earlier, mechanism-aligned secondary prevention without increasing length of stay, the CCI approach offers a practical, patient-centred model for bridging the diagnostic-treatment gap in secondary stroke prevention.

Dedication

To the soul of my mother, who taught me my first words and whose love, strength, and faith continue to guide me at every step of life.

To my father, my first teacher and lifelong mentor, whose wisdom, discipline, and example have shaped my character and inspired my pursuit of knowledge.

And to my wife, whose unwavering support, patience, and encouragement have sustained me through every challenge of this demanding and often stressful journey.

Author's Declaration

I declare that this thesis is the result of my own work and has not been submitted for any other degree or qualification at the University of Glasgow or at any other institution.

All research involving human participants and clinical data was conducted in accordance with the ethical standards of the relevant institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments.

Ethical approval for the D-CCIST study was granted by the West of Scotland Research Ethics Committee (REC Ref 18/WS/0068) and NHS Greater Glasgow and Clyde Research Data Management authorisation (GN17ST585).

All data were collected, analysed and stored in accordance with the University of Glasgow's Research Data Management Policy and NHS governance framework.

As English is not my first language, I used language-editing tools, including an artificial intelligence-based application (OpenAI ChatGPT, 2024-2025), to improve clarity, structure, and formatting in the final preparation of this thesis. All intellectual content, data interpretation, and analytical decisions are entirely my own, and no such tools were used to generate data or perform analysis.

Portions of this work have been presented at professional meetings and are being prepared for publication.

The study was conceived and designed in collaboration with my supervisor, and the interpretation of findings was undertaken with his guidance. However, the writing, integration, and presentation of this thesis are entirely my own.

Author contributions

Study conception and design: The author conceived the research question and designed the overall study. This included development of the study aims, methodology, imaging strategy, outcome measures, and analytical framework underpinning the MD thesis. The study design and overall research direction were developed under supervisory guidance, with specific input from Radiology and Neurology colleagues during refinement of the imaging strategy and feasibility considerations.

Regulatory approval and governance: The author prepared the full study protocol, patient information sheet, consent materials, and data collection forms. The IRAS and Research Ethics committee (REC) applications were completed with formal input and guidance from Dr Azmil and the research governance team.

Data collection and management: The author was actively involved alongside with research nurse, in arranging imaging slots, coordinating with radiology departments, and ensuring participants received investigations according to their randomised allocation. The author coordinated patient identification and recruitment in collaboration with the clinical team and research nurse. Clinical, imaging, and outcome data were independently collected by the author, including supplementary data obtained from NHS electronic systems where required. The author curated, cleaned, and maintained the final study dataset.

Aetiological classification and clinical outcomes: The author performed stroke aetiological classification using the ASCO-D framework at both Day 7 and Day 30 time points for all participants. These classifications formed the basis for the diagnostic, treatment, medication, mediation, and validation analyses presented in the thesis. All classifications were performed independently by the author using pre-specified criteria.

Systematic Review: The author conducted the systematic review, including search strategy development, study selection, data extraction, and quality assessment. A medical librarian provided technical advice on search optimisation. A secondary reviewer (Huda Elsharkasi) independently checked study selection

and data extraction, with disagreements resolved by consensus. Data synthesis and interpretation were led by the author.

Statistical analysis: All statistical analyses, generation of tables and figures across all chapters, analytic decisions, and interpretation of findings, were performed by the author, with supervisory input regarding statistical appropriateness and presentation.

Imaging integration and interpretation: Imaging protocols were developed collaboratively with radiology and neuroradiology colleagues, including Dr Giles and others. However, integration of imaging findings into aetiological classification, statistical analysis, and interpretation across thesis chapters was led by the author.

Thesis preparation: The thesis was written in full by the author. Supervisors provided critical review, comments, and suggestion on draft version, however, the final content, structure, and interpretation were the responsibility of the author.

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Definitions/Abbreviations

Abbreviation	Definitions
AA	Aortic atheroma
AF	Atrial fibrillation
ASA	Atrial septal aneurysm
ASCO-D	Aetiological classification (A Atherosclerosis, S Small vessel disease, C Cardioembolic, O Others, D Dissection)
ASD	Atrial septal defect
CAP	Chest-Abdomen- Pelvis
CCI	Comprehensive cardiovascular imaging
CCS	Causative Classification of Stroke
CCT	Cardiac Computed Tomography
CE	Cardio-embolism/embolic
CEA	Carotid endarterectomy
CI	Confidence intervals
CMB	cerebral microbleed
CMR	Cardiac Magnetic Resonance
CT	Computed Tomograph
CTA	Computed Tomography Angiography
DAPT	Dual antiplatelet therapy
DLP	Dose-length product
DOACs	Direct oral anticoagulants
DWI	Diffusion-weighted imaging
EHRA	European Heart Rhythm Association
ESC	European Society of Cardiology
ESO	European Stroke Organisation
ESUS	Embolic stroke of undetermined source
FLAIR	Fluid-Attenuated Inversion Recovery
ICA	Internal carotid artery
ICAD	Intracranial atherosclerotic disease
ICD	Ischemic heart disease
IQR	Interquartile ranges
IS	Ischaemic stroke
LAA	Left atrial appendage
LAAs	Large artery atherosclerosis
LAAT	Left atrial appendages thrombus

LAT	Left atrial thrombus
LVH	Left ventricular hypertrophy
MIP	Maximum intensity projections
MPR	Multiplanar reconstructions
MRI	Magnetic resonance imaging
NPV	Negative predictive value
OLS	Ordinary least squares
OR	Odds ratio
PHE	Public Health England
PPN	Positive predictive value
PV	Periventricular (white matter region)
PVD	Peripheral vascular disease
QUADAS-2	Quality Assessment Tool for Diagnostic Accuracy Studies
SAPT	Single antiplatelet therapy
SD	Standard deviation
SE	Standard errors
SEC	Spontaneous echo contrast
SEM	Structural equation modelling
SIGN	Scottish intercollegiate Guidelines Networks
SVD	Small vessels diseases
SWI	Susceptibility-weighted imaging
TIA	Transient ischemic attack
TOE	Transoesophageal echocardiography
TSE	Turbo spin echo
TTE	Transthoracic echocardiography
WMH	White matter hyperintensity

Chapter 1 Thesis introduction

1.1 Stroke burden and the importance of secondary prevention.

1.1.1 Global and UK incidence

Stroke is considered as the second most common cause of death worldwide and the fourth most common killer in the UK. According to the World Stroke Organization, over twelve million people experience their first stroke each year, with approximately 6.5 million deaths resulting from stroke annually. Between 2011 and 2022, stroke prevalence increased by 14.6% among individuals aged 18 to 44 years and by 15.7% among those aged 45 to 64 years, highlighted the significant impact of stroke (Imoisili et al., 2024). About 34000 patients die from stroke annually in the UK, a life every 15 minutes. Among stroke survivors, 26% experience stroke recurrence within five years, and 39% within the decade, most of them during the first year (Mohan et al., 2011, Stroke, 2018). Costs for newly diagnosed stroke in the UK are estimated at about £45.000 in the first year and about £25.000 in subsequent years (Patel et al., 2020).

While stroke has traditionally been seen as a disease of older age, there is increasing concern about its rise in younger adults. The Oxford Vascular Study reported a 67% increase in stroke incidence among people under 55 years between 2010-2018 compared with 2002-2010, whereas incidence in those aged 55 and older fell by 15% over the same period (Li et al., 2022). Globally, the burden shows similar patterns, with a 36% increase in new stroke cases among young adults between 1990 and 2021 (Feigin et al., 2025). These shifts highlight that stroke is no longer confined to older populations.

1.1.2 Early recurrence risk

Recurrent events are also common and represent one of the greatest threats in the acute phase after stroke or TIA. Several studies have shown that the risk of recurrence is front-loaded, peaking within the first 48 hours and declining, therefore. A large population-based study reported that the 7-day risk of stroke

following TIA or minor stroke was approximately 8-10% in unselected patients, with half of these recurrent events occurring within the first 48 hours (Johnston et al., 2000). Coull et al., found that the cumulative risk of stroke after TIA was 5.2% at Day 7 and 12% at Day 30 (Coull et al., 2004). Consistent with this, Johnston et al., showed in emergency department settings that patients with limb weakness or longer lasting symptoms, had nearly a 10% risk of recurrence within 7 days (Johnston et al., 2000). Together, these data illustrate that recurrence risk is concentrated early and falls with the time, underscoring the importance of prompt investigation and secondary prevention.

1.1.3 Determinants of Stroke recurrence

The risk of recurrent stroke is not uniform but varies markedly according to the underlying mechanism and time-window after index event. Recognising these differences is essential for guiding mechanism-specific secondary prevention.

Large artery atherosclerosis is one of the leading mechanisms of recurrent stroke and carries a distinctly high early risk. The recurrence profile is front loaded, with the most events occurring within the first few days after symptoms onset. Pooled analyses showed recurrence rates of approximately 11-12% within two weeks and up to 19% within the 90 days for extracranial carotid stenosis, and observational studies report similar patterns for intracranial atherosclerotic disease (ICAD) (Ois et al., 2009, Strömberg et al., 2015, Yaghi et al., 2023). Lavalley` et al. demonstrated that over half of all recurrent strokes after TIA occurs within 48 hours, emphasizing the narrow therapeutic window for intervention (Lavallée et al., 2007)

Within the broader large artery disease, ICAD is especially prevalent in south-east Asian and Chinese population, where it may account for up to 30% of ischaemic stroke (Kang et al., 2024, Panagiotopoulos et al., 2024). Modern management , exemplified by SAMMPRIS trial, has evolved from single-agent therapy in the WASID era to intensive multimodal regimens combining short-term DAPT, high-intensity statins, and strict vascular risk control, which have markedly reduced recurrence rates in ICAD (Chimowitz et al., 2005, Chimowitz et al., 2011) .

Cardioembolic sources represent another major mechanism of recurrent stroke and are associated with high early recurrence risk (Arboix and Alió, 2010). Atrial fibrillation (AF) is the most frequent cause, but other cardiac conditions such as left atrial appendage thrombus, post-myocardial infarction mural thrombus, valvular disease, patent foramen oval, and dilated cardiomyopathy also contribute significantly to the overall burden (Ntaios, 2020, Hart et al., 2017). The presence of AF confers markedly higher early recurrence risk of stroke compared with non-cardioembolic mechanism and is associated with larger infarcts, early mortality, and poorer functional outcomes, contemporary data highlighted that recurrence risk in cardioembolic stroke is also front-loaded, with the greatest hazard occurring in the first two weeks (Paciaroni et al., 2015). Early identification of AF or other high-risk cardiac sources is therefore essential to enable timely anticoagulant or device-based intervention. Randomized trials such as CHANCE and POINT have shown that short-term dual antiplatelet therapy (DAPT) may reduce early recurrence in non-cardioembolic events, but in patients with cardioembolism, direct oral anticoagulants (DOACs) remain the treatment of choice (Johnston et al., 2018, Wang et al., 2013).

Diagnostic tools such as prolonged cardiac monitoring and integrated ABCD3-I, which incorporates imaging and cardiac findings, can improve early risk stratification and guide therapy (Song et al., 2013, Kelly et al., 2016). Together, these findings underline the critical importance of rapid detection and mechanism-specific treatment to prevent early recurrence in cardioembolic stroke.

The clinical risk profile also matters, patients with minor stroke or high-risk TIA face the greatest risk of early recurrence, with approximately 8-10% experiencing a recurrent stroke within 7 days and nearly half of those events occurring in the first 48 hours (Coull et al., 2004, Bourand and Brorson, 2022).

Small vessel diseases (SVD), typically manifesting as lacunar infarction, is generally associated with a lower short-term recurrence than large artery or cardioembolic mechanism. Nonetheless, recent imaging-confirmed cohorts demonstrated one-year recurrence rates of approximately 4-5%. And longer-term follow-up shows event rates exceeding 20% in patients with high SVD burden (Haidegger et al., 2024, Clancy et al., 2024, Pasi and Cordonnier, 2020). Optimal

control of hypertension, diabetes and other vascular risk factors remains the foundation of secondary prevention in this group.

In summary, stroke recurrence is frequent, front-loaded, and strongly mechanism dependent. Delayed or incomplete work-up risks misaligned treatment at the period of highest vulnerability. By capturing overlapping causes, certainty levels, and incomplete investigations, directly addresses this challenge. Coupled with comprehensive early imaging, it provides a practical pathway to timely, mechanism-specific prevention.

1.1.4 Why mechanism-specific therapy matters

The choice of secondary prevention after stroke depends on the underlying mechanism since different causes require different therapeutic strategies. Cardioembolic stroke, most likely due to AF, is best prevented with oral anticoagulant. Current international guidelines recommend the use of direct oral anticoagulant in preference to warfarin where suitable and advise extended rhythm monitoring to identify patients with otherwise undetected AF (Kleindorfer et al., 2021). In contrast, patients with non-cardioembolic minor ischemic stroke or high-risk TIA benefit from short course of dual antiplatelet therapy (DAPT), which reduces 90-day recurrence compared to aspirin alone (Johnston et al., 2018, Wang et al., 2013).

In cases of symptomatic extracranial carotid artery stenosis, particularly when narrowing is between 70-99%, optimal management is with carotid endarterectomy performed as early as possible and ideally within two weeks of the index event (Rothwell et al., 2003). For intracranial atherosclerosis disease, however, the SAMMPRIS trial demonstrated that intensive medical therapy including short term DAPT and strict risk-factor control was effective and safer than stenting, although it remains uncertain whether this strategy is superior to conventional medical care, which served as the control arm (Chimowitz et al., 2011).

Other mechanisms also require tailored treatment. In the case of cervical artery dissection, the CADISS trial found no clear superiority of anticoagulation over

antiplatelet therapy in prevention recurrence, indicating that either approach is reasonable depending on the clinical context (Markus et al., 2019).

Together, these findings highlight that early and accurate identification of stroke aetiology is essential, as the benefit of prevention therapy depends on matching treatment to the underlying mechanism. Failure to do so risks undertreatment, overtreatment, or inappropriate therapy during the period of high risk of recurrence.

1.2 Mechanisms and stroke classification

Ischaemic stroke can arise from multiple underlying causes, including large artery atherosclerosis, small vessel disease, cardioembolic and less common causes such as vasculitis, hypercoagulable states, or arterial dissection (Donnan et al., 2008, Sacco et al., 2013). However, despite modern diagnostic tools, a substantial proportion of cases remain unexplained. These are often termed cryptogenic stroke, and the more recent proposed category of embolic stroke of undetermined source (ESUS) highlights those with suspected embolic origin but no identified source (Hart et al., 2014). In practice, however, ESUS is still a broad and uncertain construct. Large, randomised trials comparing anticoagulation with antiplatelet therapy such as NAVIGATE-ESUS and RESPECT-ESUS, failed to demonstrate a reduction in recurrent stroke with direct oral anticoagulants (DOACs) compared with aspirin (Hart et al., 2018, Diener et al., 2019). This lack of benefits may reflect the heterogeneity and incomplete evaluation with the ESUS population, where many patients undergo only short-term ECG monitoring or limited cardiac and intracranial imaging. As a result, the ESUS concept has been criticized for grouping together patients with different underlying mechanisms, and for highlighting the need for more comprehensive diagnostic strategies before assigning aetiological uncertainty.

Over time several classification systems have been developed to standardize the attribution of stroke mechanisms. The most widely used are TOAST, CCS, and ASCO-D. Each offers strengths and limitations, as outlined below.

1.2.1 TOAST classification

The TOAST system (Trial of Org 10172 in Acute stroke Treatment, 1993) is the most widely used method for classifying stroke (Adams Jr et al., 1993). It divides patients into five categories: large artery atherosclerosis, cardioembolism, small vessel occlusion, other determined causes, and undetermined cause. Its strength lies in its simplicity and reproducibility, making it suitable for clinical trials and large-scale registries.

However, TOAST obliges the clinician to assign a single dominant cause, even when multiple plausible mechanisms exist. As a result, patients with overlapping aetiologies are reduced to one label, masking the true complexity of their stroke. In practice, this often leaves a large proportion classified as undetermined, reflecting the system's limited ability to capture diagnostic ambiguity or incomplete work-up.

1.2.2 Causative Classification of Stroke (CCS)

The CCS system was developed to improve on TOAST by providing a structured, computer-assisted algorithm (Ay et al., 2007). It integrated clinical, imaging, and laboratory data, and applies a probabilistic model to determine the most likely cause. This reduces subjectivity and improves inter-rater reliability compared with TOAST.

Yet, CCS still shares a key weakness with TOAST, it still forces the clinician to assign a single causative mechanism, which can obscure the reality of patients with mixed pathology. It does not fully resolve the problem of stroke mechanism overlap, and incomplete evaluations remain difficult to represent.

1.2.3 ASCO-D classification

The ASCO-D classification system marked an important shift in stroke classification (Amarenco et al., 2013). Unlike earlier systems that force a single label, ASCO-D takes a multidimensional approach by evaluating five domains separately: Atherosclerosis (A), Small vessel disease (S), Cardiac source (C), Other causes (O), and Dissection (D). Each domain is graded according to the level of certainty: 1 = definite, 2 = probable, 3 = possible, 0 = no disease, and 9 =

incomplete work-up. This structure recognises that multiple mechanisms can contribute to the same event and that not all investigations are always complete.

Its strengths lie in capturing the complex reality of stroke, providing graded certainty rather than a binary answer, and making diagnostic gaps explicit through the ASCO-D 9 category. However, ASCO-D system is more demanding than older systems, as it requires systematic data collection and training. In addition, inter-rater variability can still occur if structured protocols are not applied, which may limit its use in routine practice outside specialist centre.

1.2.4 Comparison and rationale

In summary, while TOAST remains simple and widely known, it reduces patients to a single cause and leaves a large proportion labelled as undetermined, which is not helpful in research focused on improving diagnosis. The CCS system improves reliability through its algorithmic approach but still forces a single mechanism and struggles with cases of overlap or incomplete investigation.

By contrast, the ASCO-D is the only framework that explicitly recognises the multifactorial nature of stroke, allows more than one mechanism to be recorded, grades the certainty of each domain, and identifies when diagnostic work-up is incomplete. These features make it uniquely suited to the aims of the thesis, which explores how comprehensive cardiovascular imaging can reduce diagnostic uncertainty and improve classification. Using ASCO-D allows a more accurate reflection of real-world complexity, supports mechanism-specific treatment decisions, and provides a transparent way of capturing both diagnostic gains and residual gaps.

1.3 Current diagnostic pathways and limitations

The diagnostic evaluation of patients with stroke or TIA usually follows a structured pathway. Brain imaging with CT or MRI is performed urgently to confirm the diagnosis and rule out haemorrhage. Vascular imaging, most often CT angiography (CTA), carotid ultrasound, or MR angiography, is used to assess for large artery disease. (Kleindorfer et al., 2021). However, comprehensive mechanism determination often requires integration of biochemical,

haematological, cerebrospinal-fluid, and increasingly, genetics investigations, which can reveal inflammatory, infection, or prothrombotic cause not evident on routine imaging (Saver, 2016). Cardiac evaluation may include ECG, prolonged rhythm monitoring, and echocardiography (TTE or TOE) to search for potential embolic sources.

Despite these established protocols, real-world practice remains variable. In many centres, a full work-up is not completed in a significant proportion of patients. Studies suggest that between 10% and 30% of patients are discharged with an incomplete evaluation, leaving the cause of stroke uncertain (Yaghi et al., 2017, Amarenco et al., 2013). Most diagnostic pathways and published trials emphasise extracranial carotid evaluation, whereas standards for imaging and grading of intracranial atherosclerosis remain less consistent across institutions (Arenillas, 2011).

Some of this reflects the limitations of available tests. For example, TOE is considered a gold standard for detection of cardiac sources such as left atrial thrombus or aortic arch atheroma, but it is semi-invasive, uncomfortable, and often poorly tolerated, resulting in variable uptake (Khariton et al., 2014). Rhythm monitoring is frequently shorter than guidelines recommend, reducing AF detection (Sposato et al., 2022). Resources and access disparities further limit implementation of prolonged monitoring or advance imaging, particularly in resource-constrained healthcare systems (Feigin et al., 2017, Feigin et al., 2025).

Timing is another weakness. Some embolic sources are transient and may disappear if imaging is delayed. LAA thrombus may resolve within days of an index event or after antithrombotic therapy, and spontaneous echo contrast can similarly vanish, leading to underdiagnosis (Hur et al., 2011a, Kim et al., 2010). These limitations mean many patients are classified as cryptogenic or undetermined which results in empirical rather than mechanism-specific prevention, a mismatch that is most concerning during the early period when recurrence risk is highest (Hart et al., 2017).

In addition to macrovascular and cardiac assessment, MRI biomarkers such as white matter hyperintensity, lacunae, and cerebral microbleeds provide

quantitative insight into small vessel disease burden. Their integration into mechanistic framework may enhance precision in future.

1.4 Advances in comprehensive imaging

In recent years, advances in cardiovascular and neurovascular imaging have broadened the diagnostic options available in stroke evaluation. Techniques such as cardiac CT (CCT), cardiac MR (CMR), and extended aorta-to-vertex CTA now allow more detailed assessment of potential embolic sources beyond the capabilities of routine echocardiography or standard vascular imaging. Likewise, new brain MRI sequences provide unique information on acute infarcts, small vessels diseases, and microbleeds, which are all relevant for aetiological classification (Wardlaw et al., 2013).

Crucially, the progress lies not in individual modalities but in their integration. Recent reviews stress that multimodal imaging is advancing faster than guideline updates and is now considered essential for both early diagnosis and long-term risk stratification (Abdalkader et al., 2023). Combining modalities also addresses the limitations of any single technique, leading to more precise classification and tailored treatment planning (Liu et al., 2024).

This makes the case for a comprehensive vascular imaging protocol, where cardiac, vascular, and brain imaging are brought together in a single structured work-up. By doing so, CCI has potential to reduce the number of patients labelled undetermined, detect high risk mechanisms before recurrence occurs, and enable mechanism-specific prevention at the time of greatest vulnerability.

1.5 Gaps in the literature

Despite progress in stroke classification and advances in multimodal imaging, several important gaps remain. There has been no comprehensive systematic review comparing the performance of alternative cardiac imaging against TOE across the full range of potential embolic sources. The feasibility of delivering a single- visit protocol that integrates brain, vascular, and cardiac imaging has rarely been tested, and its role in routine care is uncertain.

It is also unclear whether improved diagnostic certainty translates into meaningful changes in treatment decisions, therapy persistence, or clinical outcomes. Furthermore, the specific contribution of individual imaging components, and the impact of their timing, remain poorly defined. Finally, while vascular risk factors are known to influence stroke mechanisms, little is understood about how they align with graded certainty levels in multidimensional classification systems such as ASCO-D, or how the predicate multi-mechanism presentation.

Together, these gaps highlight the need for research that combines systematic review, randomized evaluation of structured imaging protocol, analysis of treatment impact, mediation of imaging pathways, and exploration of risk-factor distribution.

1.6 Rationale for this thesis

The central challenge in secondary prevention of stroke is that a significant proportion of patients remain without a clear aetiological classification, often labelled as cryptogenic or undetermined. This reflects both the limitations of conventional classification systems, which force attribution to single mechanism, and the shortcoming of current diagnostic pathways, which are frequently incomplete, delayed, or inconsistent across centres. As a result, many patients receive empirical rather mechanism-specific therapy during the period of greatest recurrence risk.

This thesis addresses these gaps in a stepwise fashion. First, although TOE remains the reference standard for identifying cardiac sources of embolism, its invasiveness and limited availability reduce its use in practice. At the same time, newer modalities such as TTE, CCT, and CMR are increasingly reported but have not been comprehensively reviewed against TOE within a single framework. A systematic review is therefore required to provide an integrated evaluation of their diagnostic yield and clinical value (chapter 2).

Second, while advances in imaging have created opportunities for broader aetiological assessment, the feasibility of implementing a structured, single visit protocol that combines brain MRI, CCT, and extended CTA has not been tested in a randomized design. A pilot trial comparing such a comprehensive vascular

imaging against routine diagnostic work-up is necessary to evaluate its practicality, diagnostic yield, and potential to reduce the number of patients left undetermined (chapter 3).

Third, improved diagnosis is only clinically meaningful if it leads to better treatment. It remains uncertain whether earlier and more precise identification of stroke mechanisms changes prescribing behaviour, improves treatment persistence, or leads to greater alignment decisions and outcomes therefore forms the next stage of this work (chapter 4).

Fourth, the mechanisms by which a structured CCI pathway improves classification are not well understood. It is unclear whether the observed benefits arise from the use of specific modalities, the timing of imaging or their integration. Mediation analysis offers a way to disentangle these components and clarify which elements of CCI contribute most to diagnostic certainty (chapter 5).

Finally, while vascular risk factors are known to cluster within certain stroke subtypes, their relationship to graded certainty levels in multidimensional classification systems such as ASCO-D is not well characterized. Exploring risk factor gradients across ASCO-D domains, and their association with single versus mixed mechanisms, can help validate the construct of classification system and inform earlier, targeted investigation in addition, later analyses examine whether quantity neuroimaging biomarkers such as white matter hyperintensity and cerebral microbleed provide complementary mechanistic evidence supporting ASCO-D validity (chapter 6).

Taken together, these lines of investigation provide a coherent rationale for the thesis. By linking systematic review, randomized evaluation, treatment analysis, mediation modelling, and risk-factor profiling, this work aims to determine whether early, comprehensive cardiovascular imaging can improve aetiological classification, support mechanisms-specific prevention, and ultimately reduces the burden of recurrent stroke.

1.7 Aim and Objectives

1.7.1 Overall Aim

To evaluate whether a structured comprehensive cardiovascular imaging protocol, combined with the ASCO-D classification system, can reduce diagnostic uncertainty and enable more precise, timely, and effective secondary prevention in patients with acute ischaemic stroke or TIA.

1.7.2 objectives

1.7.2.1 To appraise existing evidence

- Conducting a systematic review of cardiac imaging modalities (TTE, CCT, CMR) compared with TOE, to clarify their diagnostic yield and clinical value in detecting cardioembolic sources.

1.7.2.2 To test feasibility and diagnostic accuracy of CVI

- Implement a randomized pilot trial comparing a single-visit CCI protocol with routine diagnostic pathways.
- Assess whether CCI reduces the proportion of patients left undetermined and increase definite classification with the ASCO-D framework.

1.7.2.3 To evaluate impact on secondary prevention

- Examine whether early CCI influences therapeutic decision making, including choice of antithrombotic regimen, persistence with therapy, and alignment between treatment and identified mechanisms.

1.7.2.4 To investigate underlying mechanisms of CCI benefit

- Apply mediation analysis to determine whether improvements in diagnostic certainty are explained by use of specific modalities, by earlier timing of imaging, or by their integration as a combined pathway.

1.7.2.5 To explore the role of vascular risk factors within ASCO-D

- Analyse how vascular risk factors distribute across ASCO-D domains and certainty levels.
- Assess whether risk-factor burden predicts single versus mixed-mechanism presentation.
- Examine whether quantitative neuroimaging biomarkers of small vessel disease provide complementary mechanistic evidence supporting ASCO-D validity.

2 Chapter 2 The diagnostic Yield of Cardiac Imaging in Stroke and Transit Ischemic Attack Patients: Systematic Review

2.1 Abstract

2.1.1 Background

Transoesophageal echocardiography (TOE) has replaced transthoracic echocardiography (TTE) as the reference standard for identifying cardiac sources of embolism owing to its superior visualization of posterior cardiac structures such as the left atrial appendage (LAA). However, its invasiveness, limited availability, and patient tolerance have prompted the exploration of alternative imaging modalities such as transthoracic echocardiograph (TTE), cardiac computed tomography (CCT), and cardiac magnetic resonance imaging (CMR).

2.1.2 Objectives

To evaluate and compare the diagnostic yield of TTE, CCT and CMR against TOE in identifying cardioembolic sources in patients with ischemic stroke or TIA.

2.1.3 Methods

A systematic review was conducted according to PRISMA-DTA. MEDLINE and Embase were searched from 1946 to January 2025 for peer-reviewed studies comparing TTE, CCT, or CMR with TOE. Data extraction focused on diagnostic yield, sensitivity, specificity, positive predictive value (PPN), negative predictive value (NPV), and clinical impact. Risk of bias and applicability concerns were assessed using QUADAS-2.

2.1.4 Results

A total of 24 studies were included, encompassing 2992 patients. TTE studies demonstrated variable sensitivity (12.8-100%) and high specificity ($\geq 90\%$), particularly among patients with good acoustic windows, with highest yield for large PFO or LA enlargement. CCT studies showed consistent performance in

detecting left atrial appendages thrombus (LAAT), with pooled sensitivity of 84.2% and specificity of 96.1%. Dual-phase and delayed protocols enhanced thrombus differentiation. CMR studies were limited but showed high specificity (100%) and low sensitivity (16.7-56%) for detecting PFO or LAAT. PFO and AA were the most common identified embolic sources, with prevalence range from 39.8% and 25.6% respectively, depending on population and modality.

2.1.5 Conclusion

CCT, especially when optimized with multiphase protocols, offers high diagnostic accuracy for LAAT detection and is a viable non-invasive alternative to TOE. TTE remains a useful first-line tool, particularly in young or cryptogenic stroke patients when contrast and Valsalva protocols are used. Diagnostic performance varied according to the embolic sources: PFO and other structural septal anomalies were most reliably identified by echocardiography, whereas thrombus and aortic pathology were best detected by CT-based modalities. CMR is highly specific but limited by feasibility and low sensitivity. Imaging modality choice should consider patient risk profile, clinical urgency, and institutional resources.

2.2 Introduction and Aim

Ischemic strokes account for approximately 85% of all stroke causes and aetiologically may be due to large artery atherosclerosis, cerebral small vessel disease, cardio-embolism, or other determined uncommon pathologies such as vasculitis or hypercoagulable states, and arterial dissection. Stroke of uncertain cause, or cryptogenic stroke, constitutes a significant proportion of cases. Among these, cardioembolism constitutes about 20-30%, representing the most common determined aetiological cause in different age groups, and is associated with higher rates of mortality, recurrence and disability (Nacu et al., 2016, Adams et al., 2023). In addition, it is hypothesised that most cryptogenic ischemic stroke are embolic, and identifying the source of embolism may allow tailoring of secondary prevention strategies and improving patients outcomes and predicting future consequences such as fatality, disability, and recurrence (Hart et al., 2017).

In younger adults, cryptogenic strokes are particularly prevalent. Studies indicated that approximately 25% of ischemic stroke age 15 -49 are classified as cryptogenic according to the TOAST classification system (Egger et al., 2023). The high incidence of cryptogenic strokes in this demographic underscores the need for comprehensive diagnostic evaluations to detect potential underlying causes, since the treatment decision will be changed accordingly (Kirshner, 2009).

Cardioembolism sources are diverse, ranging from high-risk conditions such as AF, LAAT, and valvular heart disease, to lower-risk anomalies including PFO and ASA. Atrial fibrillation accounts for about 45% of all cardioembolic ischemic stroke (Topcuoglu et al., 2018).

Advances in diagnostic techniques have identified a substantial number of potential sources of embolism in patients with non-lacunar cryptogenic stroke (Hart et al., 2014). However, recent observational or registry data report that incomplete diagnostic evaluation compromises aetiological determination in 10-29% of cases, with use of cardiac or vascular imaging varying among centres (Ntaios et al., 2015, Perera et al., 2016, Camen et al., 2019).

Cardiac imaging plays a crucial role in detection of these embolic sources. Transthoracic echocardiogram (TTE) is widely available, non-invasive tool for assessing cardiac structure and function (Sharifkazemi et al., 2007), but has limited sensitivity for detection posterior cardiac structures (LAA) and small thrombi (Morris et al., 2009, Saric et al., 2016).

Transoesophageal echocardiogram (TOE) is considered as the gold standard technique for diagnosis of potential sources of cardiac embolism, especially PFO, LAAT. However, this technique has its drawbacks such as lack of cooperation especially in severely ill patients; the need to be conducted under sedation in most cases; limited availability and operator dependency (Bang et al., 2014). These disadvantages limit its feasibility and lead to the need for reliable alternative imaging technique which could assist clinicians on daily bases (Rosol et al., 2020).

Cardiac magnetic resonance imaging and computed tomography have been explored recently for diagnostic purposes in stroke workup, providing

supplementary or alternative to TOE in specific patient cohorts (Camen et al., 2019). However, the impact of systematic use of these imaging on post-stroke patients remains inconclusive (Camen et al., 2020). Cardiac Computed tomography (CCT) is a promising alternative to TOE, offering greater feasibility and patient tolerance in the stroke population (Boussel et al., 2011). It has demonstrated high accuracy in detection of high-risk cardioembolic sources, including LAAT and AA (Hur et al., 2009a). However, conventional CT protocols require dual contrast injection, beta blocker administration for heart rate control, and carry a relatively high radiation dose. Recent advances in scanner technology such as improved temporal resolution and artefact reduction may overcome these limitations and broaden its applicability (Yeo et al., 2017). Cardiac MRI provides excellent diagnostic accuracy for detection of intracardiac thrombi and AA and is a valuable tool for structural assessment (Chang et al., 2022, Meinel et al., 2021). However, its routine use is limited by contraindications like implanted devices, high cost, and availability.

Despite the array of imaging modalities, there is variability in their application across stroke centres, and the optimal imaging strategy remains a subject of debate. This systematic review aims to evaluate the diagnostic yield of TTE, CCT, and CMR compared to TOE in identifying cardiac sources of embolism in patients with ischemic stroke or transient ischemic attack (TIA). Additionally, it seeks to determine the most common underlying cause of cardiac embolism in this cohort and assess the impact of imaging findings on patients' management.

2.2.1 Review Question(s)

According to PICO (Population, Intervention, Comparison, and Outcome) criteria:

- 1/ What is the diagnostic yield (O) of a cardiac MRI, CT and TTE (I) in comparison to TOE (C) in the aetiological work up of stroke or TIA patients (P)?
- 2/ What was the predominant or most common underlying cause of cardiac embolism (O) in stroke or TIA patients (P)?
- 3/ What impact do the findings have for patient management (O).

2.3 Aim

This review aims to investigate the effectiveness of various cardiac imaging methods in diagnosing underlying cardiac conditions in individuals who have experienced a stroke or TIA.

2.4 Methods

2.4.1 Search Protocol and strategy

A systematic review was conducted in accordance with the published checklist and guidance on systematic reviews on diagnostic test accuracy studies (Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies, The PRISMA-DTA Statement (McInnes et al., 2018)). A comprehensive search of MEDLINE and Embase (via Ovid) databases was performed to identify clinical studies comparing TTE, CCT and CMR to TOE for detection of cardiac source of embolism in adults with TIA or acute ischemic stroke. The search was limited to studies published in peer reviewed journals between 1946 to January 15, 2025.

The complete search strategies for each database are attached in appendices. To enhance the sensitivity of the search, the strategy incorporated both Medical Subject Headings (MeSH) and text words, addressing each component of the review question (population, intervention, comparator, and outcome).

It is noteworthy that existing literature indicates that the inclusion of methodology research filter in the context of reviews focusing on diagnostic test accuracy (DTA) studies may reduce the overall sensitivity of the literature search. In the context of this review, my initial literature search without inclusion of diagnostic yield component (outcome) retrieved an unmanageable number of irrelevant citations, posing significant challenges in terms of manageability. To address that, a validated methodological filter for diagnostic component was applied, using the strategy by the Scottish intercollegiate Guidelines Networks (SIGN), which adapts earlier work designed by the Health Information Research Unit of the McMaster University, Ontario (Network, 2023).

In line with the recommendations outlined in the Cochrane Handbook for Systematic Reviews of DTA (Deeks et al., 2013), which suggests that the combination of methodological filter search terms with terms pertaining to diagnostic tests and the target condition should not serve as the sole approach for conducting formal searches in the context of systematic reviews of DTA, the electronic search was complemented by reference tracking of the final eligible MRI studies

2.4.2 Study Selection and screening

Study inclusion and exclusion criteria were developed based on the framework of PICO criteria. Population (Stroke or TIA patients), Intervention (TTE, cardiac CT or MRI), Comparator (TOE), Outcomes (sensitivity and specificity, positive and negative predictive value).

2.4.2.1 including Criteria

- Adults (≥ 18 years) with IS or TIA who underwent cardiac imaging (TTE, CCT, or CMR) as index test and TOE (reference standard) as part of the diagnostic evaluation for potential cardioembolic source.
- Clinical studies that compared the diagnostic accuracy of one or more of imaging modalities with TOE as a gold standard.
- Studies providing validity measurements such as sensitivity and specificity, positive and negative predictive value to TOE.

2.4.2.2 Exclusion Criteria

- Studies included haemorrhagic stroke or non-cardioembolic causes of IS and TIA.
- Absence of the comparison with TOE.

The studies were, review articles, case reports, clinical or conference report.

No restrictions based on primary studies' language or publication date were applied. However, if there is no available English translation version, the study was excluded.

References were arranged and duplicate papers were removed by EndNote® 21. The study selection was performed in two stages; firstly, titles and abstracts of all identified electronic database citations were reviewed. Articles that did not fulfil the selection criteria were removed. In the second stage, the full text of potentially relevant papers was read. The second stage of the study selection and the final list of articles were checked by another reviewer (HE). Any disagreement was resolved by consensus.

2.4.3 Data Extraction

The required information was collected into an Excel spread sheet. The followed data was extracted from each eligible study: first author; year of publication; place of study; aim of the study; recruitment procedure; sample size; age range; study design; setting of intervention; key findings (diagnostic accuracy of each modality, most prevalent cardiac pathology, time between index and reference tests, change in the management plan; author's conclusions. The data extraction process was reviewed by another reviewer (HE).

2.4.4 Risk of bias (quality) assessment

The quality of the retrieved papers was assessed independently by author and another reviewer (HE) using the Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2) (2021). This tool consists of four key domains: patient selection; index test; reference standard; flow and timing. We individually recorded the risk of bias and applicability concerns as “low risk,” _ “high risk,” _or “unclear risk”. The final quality assessment is shown in **Figure 2-2** . Disagreements were resolved by consensus.

2.5 Results

2.5.1 Literature search results and study selection

The initial search strategy identified 8957 papers from Embase (n = 4175), Medline (n = 4782). Of these, 1398 were duplicates and excluded by EndNote® 21. Titles and abstracts of 7559 papers were screened. 7503 were excluded because irrelevant. The remaining 56 papers were screened by reading through the full text from which 33 were excluded for the following reasons: conference report abstracts (n=13); full text not in English (n=4); Studies that either lacked diagnostic comparison against TOE or did not provide validity measurements (n=16). A further eligible study was identified from searching “references” and “cited by” lists of MRI included studies, giving a total of 24 studies which were relevant and met the inclusion criteria. The PRISMA flow diagram with the numbers of included and excluded articles at each step of the literature search is provided in Figure 2-1.

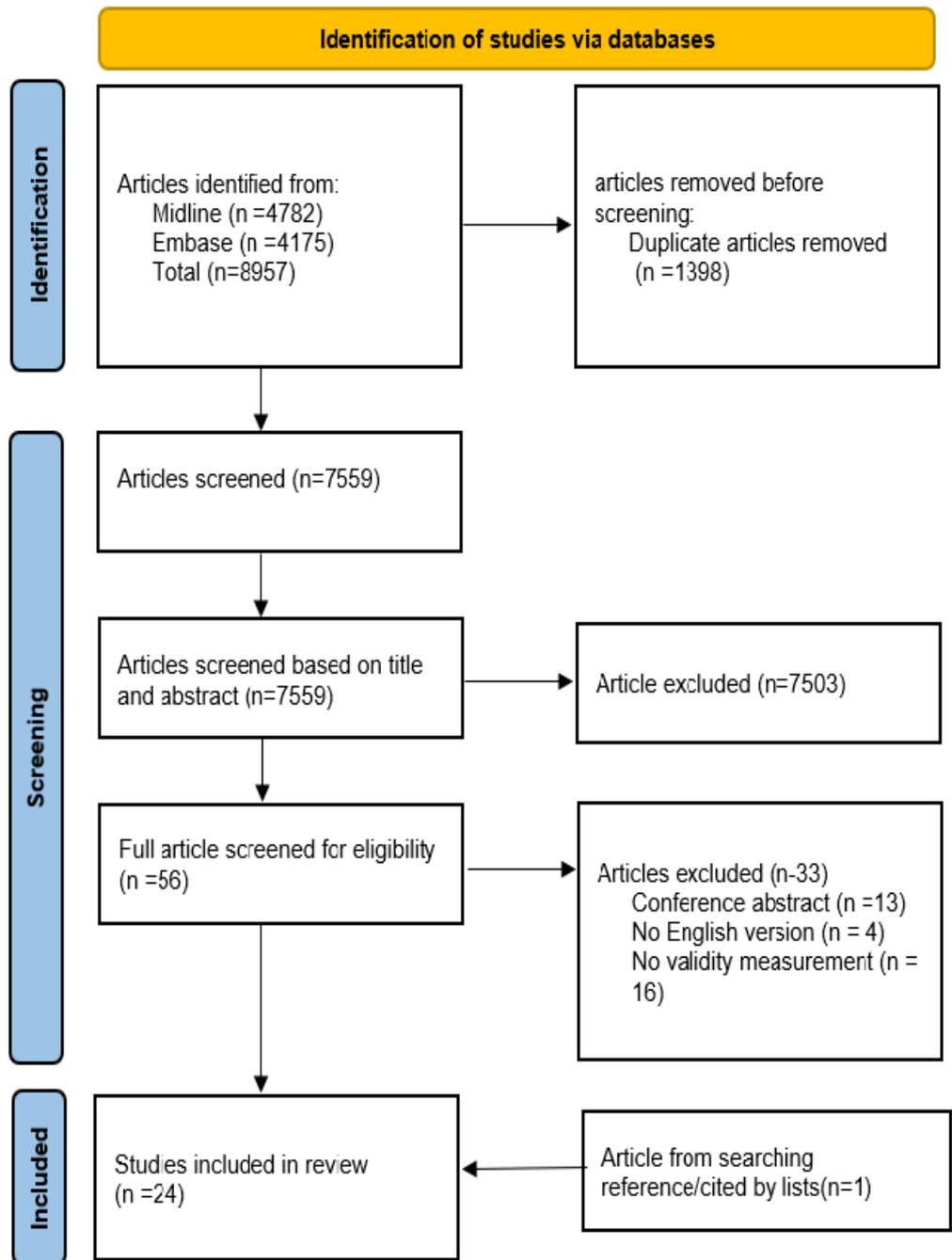


Figure 2-1 The PRISMA flow diagram with numbers of included and excluded articles at each step.

2.5.2 Quality assessment of the eligible studies

Across the 24 included studies, the highest proportion of high risk of bias or unclear risk bias was observed in the domains of patient selection (6/24, 25%), reference standard (6/24, 25%) and flow and timing (11/24, 46%). High or unclear risk of bias in patient selection domain was identified in (Pinho et al., 2023, Qiao et al., 2024, Shi et al., 2024, Zahuranec et al., 2012, Mohammad et al., 2020, Taina et al., 2016), where studies included highly selected subsets of patients from larger cohorts based on test feasibility, lacked random or consecutive sampling, or used retrospective designs and lack of details on exclusion criteria.

In the reference standard domain, one study (Pinho et al., 2023) was rated as high risk of bias because it did not state whether TOE was interpreted independently to the index test. Moreover, the author report that some results of TOE were changed after reviewing the index test results, while 5 studies were rated as unclear (Anaissie et al., 2016, Bousset et al., 2010, Hur et al., 2009b, Shi et al., 2024, Taina et al., 2016) due to insufficient reporting on blinding of reference test interpretation.

The index test domain showed strong methodological quality, with 21/24 (88%) rated as low risk of bias. Only two studies (Anaissie et al., 2016, Shi et al., 2024) were rated unclear due to lack of reporting on whether the index test were interpreted blinded to reference standard results and absence of pre-specified diagnostic thresholds.

In the flow and timing domain, 11 studies (46%) were rated as unclear. In several studies including (Bousset et al., 2010, Hur et al., 2009b, Hur et al., 2009c, Pinho et al., 2023, Taina et al., 2016) the meantime interval between the index and reference tests fell within acceptable range (1-3 days), but wide variability or outlier range (up to 27 days) raised the possibility of thrombus formation or resolution during the interim period. Other studies (Barazangi et al., 2011, Homsy et al., 2016, Kim et al., 2010, Mohammad et al., 2020, Tuncer et al., 2001, Zahuranec et al., 2012) either did not report exact interval between tests, or reported broad timeframes, or acknowledged potential discrepancies because of timing mismatch.

Only 7/24 studies (29%) were judged as having low risk of bias across all four domains, including (Hur et al., 2011b, Hur et al., 2012, Maffe et al., 2010, Omran et al., 1999, Shyu et al., 1994, Wai et al., 2017, Zito et al., 2009). Applicability concerns were minimal overall, however four studies raised unclear or high concern in the patient selection domain. (Hur et al., 2009b) and (Zahuranec et al., 2012) focused on selective age group which could limit generalization, (Mohammad et al., 2020) enrolled only cryptogenic stroke patients who are willing to do MRI which might lead to selection bias, and (Lee et al., 2021) lacked detail on sampling and exclusion criteria.

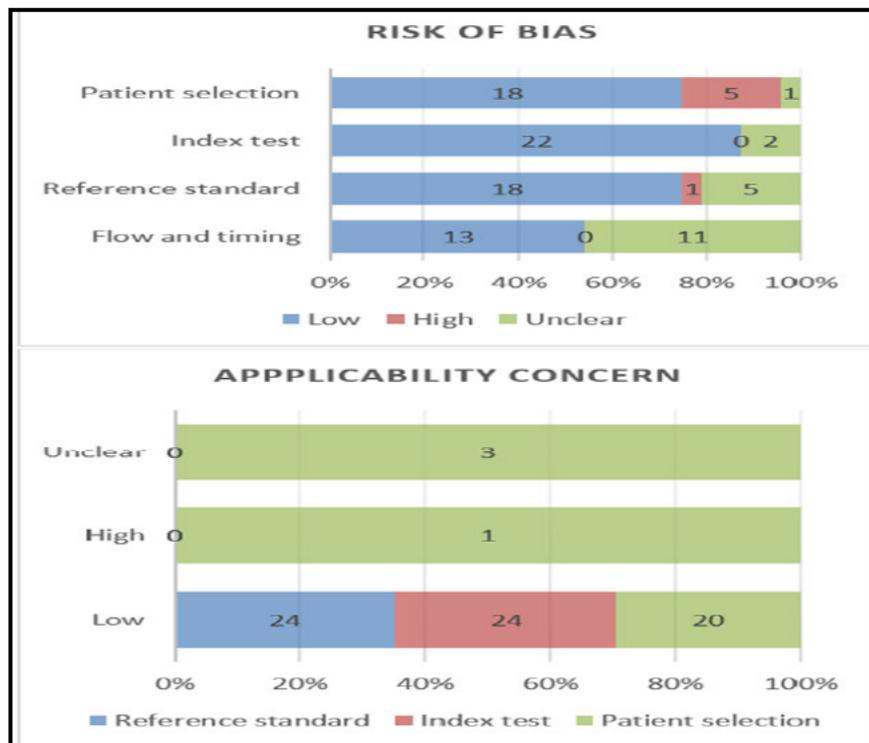


Figure 2-2 Quality assessment of eligible studies using QUADAS-2

Table 2-1 Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2)

Study	Risk bias				Applicability concerns		
	Patient selection	Index	Reference	Flow and timing	Patient selection	Index	Reference
Anaissie et al. (2016)	Low	Unclear	Unclear	Low	Low	Low	Low
Barazangi et al., 2011	Low	Low	Low	unclear	Low	Low	Low
Boussel et al., 2011	Low	Low	Low	Unclear	Low	Low	Low
González-Alujas et al., 2011	Low	Low	Unclear	Low	Low	Low	Low
Hamilton-Craig et al., 2011	Low	Low	Unclear	Low	Low	Low	Low
Homsy et al., 2016	Low	Low	Low	Unclear	Low	Low	Low
Hur et al., 2009 CTE	Low	Low	Low	Unclear	Unclear	Low	Low
Hur et al., 2009 LAA	Low	Low	Low	Unclear	Low	Low	Low
Hur et al., 2011	Low	Low	Low	Low	Low	Low	Low
Hur et al., 2012	Low	Low	Low	Low	Low	Low	Low
Kim et al., 2010	Low	Low	Low	Unclear	Low	Low	Low
Lee et al., 2021	Low	Low	Low	Low	Unclear	Low	Low
Maffè et al., 2010	Low	Low	Low	Low	Low	Low	Low
Mohammad et al., 2020	High	Low	Low	Unclear	Unclear	Low	Low
Omran et al., 1999	Low	Low	Low	Low	Low	Low	Low
Pinho et al., 2023	High	Low	High	Unclear	Low	Low	Low
Qiao et al., 2024	High	Low	Low	Low	Low	Low	Low
Shi et al., 2024	High	Unclear	Unclear	Low	Low	Low	Low
Shyu et al., 1994	Low	Low	Low	Low	Low	Low	Low
Taina et al., 2016	Unclear	Low	Unclear	Unclear	Low	Low	Low
Tuncer et al., 2001	Low	Low	Low	Unclear	Low	Low	Low
Wai et al., 2017	Low	Low	Low	Low	Low	Low	Low
Zahuranec et al., 2012	High	Low	Low	Unclear	High	Low	Low
Zito et al., 2009	Low	Low	Low	Low	Low	Low	Low

2.5.3 Study Characteristics

Across the 24 included studies (Table 2-2), mean participants age ranged from late 40s to early 60s, with roughly equal sex distribution and ischaemic stroke or TIA population. The 24 included studies were published between 1994 and 2024, Table 2-2. Collectively, these studies comprised a total of 2992 patients (50.1% males),

with sample sizes ranging from 20 participants (Zahuranec et al., 2012) to 687 patients (Qiao et al., 2024).

Most studies utilized a single-centre design, with 20 prospective studies. The study populations consisted of patients presenting with ischemic stroke and using TOE as the reference standard for investigation of cardioembolic causes of stroke. The imaging modalities evaluated as index tests included: TTE (9 studies) (Anaissie et al., 2016, Gonzalez-Alujas et al., 2011, Maffe et al., 2010, Omran et al., 1999, Shi et al., 2024, Shyu et al., 1994, Tuncer et al., 2001, Wai et al., 2017, Zito et al., 2009), CCT/CTA (12 studies) (Barazangi et al., 2011, Bousset et al., 2010, Homsy et al., 2016, Hur et al., 2009b, Hur et al., 2009c, Hur et al., 2011b, Hur et al., 2012, Kim et al., 2010, Lee et al., 2021, Pinho et al., 2023, Qiao et al., 2024, Taina et al., 2016), and CMR (3 studies) (Hamilton-Craig et al., 2011, Mohammad et al., 2020, Zahuranec et al., 2012). Regarding diagnostic objectives, 6 studies focused on the detection of PFO or right to left shunt, often in younger populations, 11 studies specifically assessed left atrial thrombus (LAT), left atrial appendage thrombus (LAAT) or spontaneous echo contrast (SEC).

One study evaluated the detection of aortic atheroma (AA), which included because of its close mechanistic and imaging overlap with cardioembolic stroke evaluation and its frequent assessment alongside cardiac imaging in embolic work-up. The remaining studies investigated any underlying cardioembolic source, aiming to improve stroke aetiology classification. Table 2-2 summarise the design and main demographic features of the included studies, provide an overview of sample size, mean age, sex distribution, and stroke population context for each imaging modality.

Table 2-2 Study and patient characteristics of included studies grouped by imaging modality

Study	Study design	No. patients	Mean age (years)	Men (%)	Population characteristics	Imaging modalities
Anaissie et al. (2016)	Retrospective	219	61.5	NR	AIS	TEE vs TTE
González-Alujas et al., 2011	Prospective	134	75	46.6	AIS/TIA/migraine	TEE vs TTE
Maffè et al., 2010	Prospective	75	28	49	AIS/TIA/migraine	TEE vs TTE

Omran et al., 1999	Prospective	117	69	54	AIS/TIA	TEE vs TTE
Shi et al., 2024	Retrospective	48	28	49	AIS/ migraine	TEE vs TTE
Shyu et al., 1994	Prospective	94	44	60	AIS	TEE vs TTE
Tuncer et al., 2001	Prospective	46	26	59	AIS/TIA	TEE vs TTE
Wai et al., 2017	Prospective	103	60	55.4	AIS/AF	TEE vs TTE
Zito et al., 2009	Prospective	72	33	49	cryptogenic stroke/migraine	TEE vs TTE
Homsi et al., 2016	Prospective	124	83	58.6	AIS/AF	TEE vs CCT
Hur et al., 2009 CTE	Prospective	137	95	61	AIS	TEE vs CCT
Hur et al., 2009 LAA	prospective	55	36	61	AIS	TEE vs CCT
Hur et al., 2011	Prospective	83	56	62.6	AIS	TEE vs CCT
Hur et al., 2012	Prospective	63	49	63.6	AIS	TEE vs CCT
Kim et al., 2010	Prospective	314	186	65	AIS/TIA	TEE vs CCT
Lee et al., 2021	Retrospective/ Prospective	66	42	61	IS/TIA	TEE vs CCT
Boussel et al., 2011	Prospective	46	38	61	AIS	TEE vs CCT
Barazangi et al., 2011	Retrospective	250	NR	NR	AIS	TEE vs CCTA
Pinho et al., 2023	Retrospective	88	54	64	AIS	TEE vs CCT
Qiao et al., 2024	Retrospective	687	709	62	AIS/AF	TEE vs CCT
Taina et al., 2016	Prospective	102	70	61.7	AIS	TEE vs CCT
Hamilton-Craig et al., 2011	Prospective	25	16	50	AIS/TIA	TEE vs CMRI
Zahuranec et al., 2012	Prospective	20	12	51	AIS	TEE vs CMRI
Mohammad et al., 2020	Prospective	24	13	62	IS	TEE vs CMRI

2.5.4 Pooled diagnostic yield of TOE across included studies.

Across the 23 studies that used TOE as reference standard, pooled prevalence estimates were calculated for the major cardioembolic sources, as shown in Table 2-3. PFO was the most frequently detected abnormality with a prevalence of 39.8% (266 cases out of 669 patients, across 10 studies). AA had the second highest prevalence at 25.6% (58 cases out of 227 patients, across 4 studies). LAAT and SEC together had prevalence of 21.1% (481 cases out of 2279 patients, across 15 studies). Barazangi et al. was excluded from pooled AA prevalence calculations as it reported AA per aortic segments rather than per patients, limiting comparability.

Table 2-3: Pooled prevalence of embolic sources detected by TOE

Embolic source	N. of study	Total N.	Total cases	Prevalence (%) (CI)
PFO	10	669	266	39.8 (36.1-43.5)
AA	4	227	58	25.6 (25.6-31.6)
SEC	12	1848	332	18 (16.0-19.8)
LAAT	14	2233	149	6.7 (5.7-7.8)
LAAT + SEC	15	2279	481	21.1 (19.5-22.8)

Table shows the pooled prevalence of major embolic sources detected by TOE across included studies. Confidence intervals (CI) represent 95% binomial estimate calculated from the aggregated totals.

2.5.5 Analysis of studies that compared TTE and TOE.

A total of nine studies comparing TTE with TOE as shown in Table 2-4. Of these, seven studies (Anaissie et al., 2016, Gonzalez-Alujas et al., 2011, Maffe et al., 2010, Omran et al., 1999, Shyu et al., 1994, Tuncer et al., 2001, Wai et al., 2017) were conducted in patients who presented with ischemic stroke and/or TIA. The remaining two studies included participants with broader cardiovascular profile, (Zito et al., 2009) included individuals with migraine, and (Shi et al., 2024) enrolled patients with either AF or suspected thromboembolic risk. These studies were retained because the stroke/TIA subgroup underwent identical imaging protocol and TOE reference testing as the broader cohort, allowing extraction of direct comparable diagnostic data relevant to the review question.

Four studies enrolled unselected ischemic stroke patients regardless of stroke mechanism (Anaissie et al., 2016, Omran et al., 1999, Shyu et al., 1994, Tuncer et al., 2001) and four others focused specially on suspected cardioembolic stroke (Gonzalez-Alujas et al., 2011, Maffe et al., 2010, Shi et al., 2024, Wai et al.,

2017), (Zito et al., 2009) excluded patients with known non-cardioembolic sources.

Details on echocardiographic technique and contrast protocols are summarized in Table 2-5. TOE was the reference standard in all studies, and multiplane TOE probes were utilised in 7/9, and biplane or monoplane used in (Anaissie et al., 2016, Shyu et al., 1994, Zito et al., 2009), imaging typically involved mid oesophageal views with different angles for comprehensive interatrial septum and LAA assessment. Contrast enhanced TTE was used in seven studies, with second harmonic imaging reported in (Gonzalez-Alujas et al., 2011, Maffe et al., 2010, Zito et al., 2009) and formal Valsalva manoeuvre protocols were used in six studies aiming to detect any Right to left shunts, TTE was typically performed using apical or parasternal views. most studies performed the two tests in the same sessions, one in the same day (Wai et al., 2017), two within a week (Tuncer et al., 2001, Zito et al., 2009), and one study did not report the time between tests (Anaissie et al., 2016).

Across a pooled sample of 908 patients, TTE demonstrated variable sensitivity in detecting PFO or intracardiac thrombus when compared to TOE. TTE sensitivity ranged from 12.8% to 100%, with notably high specificity $\geq 90\%$ in all studies. Diagnostic yield was highest for TTE in detecting large right to left shunts and larger atrial thrombi especially in patients with favourable acoustic windows. Gonzalez-Alujas et al. reported 100% in sensitivity and specificity for detection PFO and it was superior to TTE when contrast enhanced TTE was used with Valsalva and second harmonic imaging (Gonzalez-Alujas et al., 2011). Both Maffe` and Zito et al. showed high specificity for detection of large PFO 89%, 92% respectively, however diagnostic performance decline for mild to moderate shunts (Maffe et al., 2010, Zito et al., 2009). In the evaluation of LAA thrombi, Omran et al. found that TTE detect 91% of LAAT in patients with adequate imaging windows (Omran et al., 1999), and Anaissie et al showed that LA enlargement on TTE predicted thrombus risk with 88.9% sensitivity (Anaissie et al., 2016). However, TTE was consistently limited in detection SEC, small thrombi, and subtle septal abnormalities, all of which were more reliably seen by TOE.

Table 2-4 : Diagnostic Accuracy of TTE vs. TOE

Study	Time between tests	No patients with diagnostic objective by TOE (%)	Sensitivity	Specificity	PPV	NPV
Anaissie et al., 2006	NR	LAT:9/219 (4.1)	88.89	52.2	92.9	99
González-Alujas et al., 2011	5 min	PFO: 54/134 (40.3)	100	100	100	76
Maffè et al., 2010	5 min	PFO :62/75 (82.6)	89	100	100	65
Omran et al., 1999	5 min	LAAT:12/117(10.3), SEC:55/117(47)	91	100	100	99.1
Shi et al., 2024	5 min	PFO: 18/48 (37.5)	89	100	100	96
Shyu et al., 1994	5 min	CE:39/94(41.5)LAAT9/94(9.6), SEC11/94(11.7)	12.8	100	100	61.8
Tuncer et al., 2001	7 days	CE:32/46(69.5).LAAT10/46(21.7),AA10/46(21.7), SEC6/46(13),PFO 4/46(8.7)	37.5	100	100	41.2
Wai et al 2017	Same day	LAAT:3/104(2.9),LAAT&SEC 24/104 (23.3)	75	90	85.7	77.8
Zito et al., 2009	7 days	PFO:46/72(64%)	55	100	100	54

Table 2-5 : Echocardiographic technique and contrast protocols

Study	Contrast enhanced TTE	Second harmonic imaging	Valsalva manoeuvre	TOE probe type	TOE sedation	TTE imaging view	TOE imaging view
Anaissie et al., 2006	No	No	NR	NR	Yes	Apical 4-chamber	Biplane, LA volume
González-Alujas et al 2011	Yes	Yes	Yes	2.9-8 MHz multiplane	Yes	Apical 4 chamber	Sagittal
Maffè et al 2010	Yes	Yes	Yes	5 MHz multiplane	Yes	Apical 4 chamber	Mid-oesophageal , multiplane
Omran et al 1999	No	No	Yes	5 MHz multiplane	No	Parasternal short axis	Mid-oesophageal
Shi et al., 2024	Yes	No	Yes	2-7 MHz multiplane	Yes	Parasternal short axis	Mid-oesophageal multiple angles
Shyu et al., 1994	No	No	NR	5 MHz biplane	NR	Parasternal and apical	Mid-oesophageal biplane

Tuncer et al., 2001	Yes	No	Yes	5 MHz monoplane	NR	NR	Mid-oesophageal
Wai et al., 2017	Yes	No	Yes	2-7 MHz multiplane	yes	Parasternal short axis	Mid-oesophageal multiple angles
Zito et al., 2009	Yes	Yes	Yes	5 MNZ multiplane	NO	Apical 4 chamber	Mid-oesophageal , bicaval

2.5.6 Analysis of studies that compared CCT/CTA and TOE.

A total of 12 studies compared cardiac computed tomography (CCT/CTA) with TOE as shown in Table 2-6. 10/12 studies were conducted exclusively in patients with acute ischemic stroke or TIA, whereas (Boussel et al., 2010, Homsy et al., 2016) included broader cardiovascular populations. 5/12 enrolled unselected ischemic stroke patients regardless of embolic risk (Barazangi et al., 2011, Hur et al., 2009b, Hur et al., 2011b, Lee et al., 2021, Taina et al., 2016), where 7/12 focus specifically on patients with cardioembolic sources or known embolic risk (Boussel et al., 2010, Homsy et al., 2016, Hur et al., 2009c, Hur et al., 2012, Kim et al., 2010, Pinho et al., 2023, Qiao et al., 2024). 8/12 studies primarily evaluated LAA thrombus detection as diagnostic objective, (Boussel et al., 2010, Hur et al., 2009b) assessed various cardiac embolic sources, (Anaissie et al., 2016) focused on aortic atheroma or atherosclerosis, and (Lee et al., 2021) evaluated PFO as underlying cause of embolism.

Details on CCT/CTA acquisition protocol are summarized in Table 2-7 . 6/12 used retrospective ECG gating with or without a prospective delayed phase, two used prospective (Homsy et al., 2016, Hur et al., 2012), one employed full cycle multiphase retrospective gating (Lee et al., 2021) , and single-phase CTA without gating was used in two emergency studies (Barazangi et al., 2011, Pinho et al., 2023) Timing between CCT and TOE varied; in most studies, both tests were performed on the same day or within 5 days, (Barazangi et al., 2011, Pinho et al., 2023) had slightly wider timing windows.

Across a pooled diagnostic cohort of 2015 patients, CCT/CTA demonstrated variable sensitivity and specificity compared to TOE in detecting LAAT or high-risk embolic sources. In studies using dedicated multiphase cardiac CT protocol, CCT

achieved sensitivity between 71.4% and 100% and specificity range from 90% to 100%. Two studies that used non gated CTA (Barazangi et al., 2011, Pinho et al., 2023), sensitivity was much lower, 53.1% and 63.6% respectively, although specificity remained moderate to high (89.4%, 81.8%).

Dual-phase CT and dual-energy CT techniques used in (Hur et al., 2011b, Hur et al., 2012) improved thrombus differentiation by capturing delayed enhancement or iodine differences between thrombus and stasis. Kim et al reported 100% sensitivity and specificity when late phase persistent defects were interpreted as thrombus, supporting benefit of delayed imaging (Kim et al., 2010). Taina et al. showed a sensitivity of 71.4% and specificity of 100% for LAAT detection with dual phase CCT, emphasizing that CCT may miss very small thrombi (Taina et al., 2016). Qiao et al demonstrated that while early-phase CCT filling defects were highly sensitive (92.9%), the positive predictive value was low (47.4%), reflecting the frequent presence of slow flow phenomena mimicking thrombus (Qiao et al., 2024).

In terms of diagnostic interpretation methods, studies varied between qualitative and quantitative approaches. Quantitative methods were employed in (Hur et al., 2012, Kim et al., 2010, Qiao et al., 2024), using delayed enhancement analysis, iodine density mapping, or Hounsfield unit threshold to objectively differentiate thrombus from circulatory stasis. The other studies primarily relied on qualitative visual assessment of filling defects or detect PFO (Barazangi et al., 2011, Pinho et al., 2023).

Table 2-6 Diagnostic Accuracy of CCT/CTA vs. TOE

Study	Time between tests	Test order	diagnostic yield of TOE (%)	Sensitivity	Specificity	PPV	NPV
Barazangi et al 2011	≤7 days in 82%	NR	AA:277/494 (56.1)	53.1	89.4	86.5	59.5
Boussel et al 2011	2.5 ± 2.8 days	Varied	CE:25/46(54.3),PFO:6/46,SEC:3/46	72	95.2	94.7	74
Homsiet al 2016	Less 1 day	NR	LAAT:6/124(4.8)	83.3	100	100	99.2
Hur et al 2009 LA	5 days	NR	LAAT:14/55(25.4), SEC:10/55(18.2)	100	98	93	100
Hur et al 2009 ST	5 days	NR	CE:91/137(66.4)LAAT:12/91(13.2),AA:33/91(36.2),SEC:15/91(16.5),PFO:19/91(20.9)	89	100	100	92
Hur et al 2011	2 days	NR	LAAT+SEC:13/83(15.7).SEC:14/83(16.9)	96.3	100	100	98.8

Hur et al 2012	3 days	NR	LAAT:13/63(20.6), SEC:19/63(30.1)	97	100	100	97
Kim et al 2010	7 days	NR	LAAT:23/314(7.2),SEC:104/314(33.1)	100	98	79.3	100
Lee et al 2021	3 days	NR	PFO:35/66(53)	91.4	100	100	91.2
Pinho et al 2023	NR	NR	LAAT:22/88(25)	63.6	81.8	53.9	87.1
Qiao et al 2024	5 days	NR	SEC:61/687(8.9)	90.9	90.3	47.4	98.9
Taina et al 2016	NR (hrs/days)	NR	LAAT:3/102(2.9),SEC:10/102(9.8)	71.4	100	100	98

Table 2-7 CCT/CTA acquisition protocols

Study	CCT scanner type	ECG-gating	HR control used	slice thickness	CCT/CTA coverage
Barazangi et al., 2011	16-slice spiral CT	No	No	1.25 mm	Mid-chest/vertex
Boussel et al 2011	40-detector	Retrospective	Yes	1.5 mm	Heart/aorta/neck
Homs et al 2016	64-slice	prospective	Yes	0.75 mm	Heart +LAA
Hur et al 2009 LA	64-slice	Retro/EP +Pro/DP	Yes	0.75 mm	Heart +LAA
Hur et al 2009 ST	64-slice	Retro/EP + Pro/DP	Yes	0.80 mm	Heart + Aortic arch
Huret et al 2011	64-slice	Retro/EP +Pro/DP	Yes	0.75 mm	Heart + LAA
Hur et al 2012	Dual-source CT	Prospective	Yes	0.75 mm	Heart + LAA
Kim et al 2010	64-slice	Retro/EP + Pro/DP	Yes	0.625	Heart
Lee et al 2021	320-slice	Retro/full cycle	No	0.50	Heart
Pinho et al 2023	40-slice	No (CTA)	No	NR	Heart+ supra-aortic vessels
Qiao et al 2024	Doul-source CT	Retro/EP +Pro/DP	Yes	0.75 mm	Heart +LAA
Taina et al 2016	64-slice	Retrospective	NO	0.60	Heart

2.5.7 Analysis of studies that compared CMR and TOE:

A total of three studies compared CMR with TOE (Hamilton-Craig et al., 2011, Mohammad et al., 2020, Zahuranec et al., 2012), were conducted in 69 patients with ischemic stroke or TIA, two of which specifically included cryptogenic stroke populations (Hamilton-Craig et al., 2011, Zahuranec et al., 2012) Table 2-8. The imaging targets across studies detection of cardioembolic source of stroke. CMR protocols varied in scanner type, sequence selection, use of contract, and duration of imaging acquisition, but all studies used 1.5T scanners and were performed during the index hospitalization within one week of TOE, Table 2-9.

CMR demonstrated high specificity across studies but variable sensitivity depending on the diagnostic target. For detection PFO, CMR showed limited sensitivity 56%, and 16.7% in Hamilton-Craig et al and Zahurance et al

respectively, however, the specificity was 100% in both studies. For other findings, CMR performed more favourably: Mohammad et al. reports sensitivity of 93% for AA detection, and detected ASA not seen on TOE (Mohammad et al., 2020). No study reported treatment changes based on CMR findings, though Mohammad et al. noted that CMR more accurately graded AA severity in some cases, which could influence embolic risk assessment.

Table 2-8 Diagnostic Accuracy of CMR vs. TOE

Study	Time between tests	Test order	diagnostic yield of TOE	Sensitivity	Specificity	PPV	NPV
Hamilton-Craig et al 2011	7 days	NR	PFO:16/25(64)	50	100	100	31
Mohammad et al 2020	7 days	NR	AA:14/24(58.3) ASA:2/24(8.3) LVT:2/24 (8.3)	92.86 100 100	100 95.45 100	100	90.9
Zahuranec et al 2012	6 days	TTE	PFO:6/20(30), AA: 1/20 (5)	16.7%	100	100	73.7

Table 2-9 CMR protocols

study	CMR scanner	Sequences	Contrast	Duration
Hamilton-Craig et al 2011	1.5T GE	SSFP, cine GRE, perfusion, phase contrast	Yes	45 min
Mohammad et al 2020	1.5T GE	FIESTA cine, dark blood	No	45 min
Zahuranec et al 2012	1.5T GE	SSFP, delayed enhancement, phase mapping	Yes	60-90 min

2.6 Discussion

2.6.1 Overview of main findings

This systematic review assessed the diagnostic yield of TTE, CCT and CMR compared to TOE in patient with ischemic stroke or TIA. Across nine studies involving 763 patients and comparing TTE to TOE, TOE was consistently superior for detecting high-risk cardioembolic sources such as SEC, LAAT and atrial septal abnormalities. However, TTE, offered a highly specific, widely accessible and non-

invasive alternative that performed best when optimized imaging protocol were and assessing for large right to left shunts.

2.6.2 TTE vs. TOE

TTE sensitivity across studies ranged from 12.8% to 100%, with uniformly high specificity, 100% in most studies. The wide range of TTE sensitivity is diverse and influenced by different factors. Studies using contrast enhanced TTE, Valsalva manoeuvre, second harmonic imaging and 3D view (Gonzalez-Alujas et al., 2011, Maffe et al., 2010), achieved higher sensitivity than those used standard grayscale imaging without contrast or Valsalva (Shyu et al., 1994). Mojadidi et al found that the diagnostic performance of TTE improved when Valsalva and harmonic imaging were applied (Mojadidi et al., 2014). Using modern ultrasound systems with better resolution and advanced doppler feature, contributed to improve image quality (Maffe et al., 2010, Shi et al., 2024). Deep sedation could impair Valsalva effectiveness during TOE, which in turn can underestimate shunt detection. Shorter time intervals between two test minimize the chance of physiological changes affecting findings and improve comparability (Omran et al., 1999, Wai et al., 2017), in contrast, delays between imaging modalities might result in missed or resolved findings (Tuncer et al., 2001).

Variability in study population characteristics could affect diagnostic performance. Studies focusing on younger patients with suspected PFO (Gonzalez-Alujas et al., 2011, Zito et al., 2009) had a higher prevalence of detectable shunts, compared to those involving older, heterogenous populations (Shyu et al., 1994, Tuncer et al., 2001).

TTE findings may guide the decision to pursue or avoid TOE and used to stratify thromboembolic risk. In study conducted by Anaissie et al., LA enlargement was used as a predictor for thrombus presence (Anaissie et al., 2016). The author proposed that in patients without LA enlargement on TTE and no other high-risk features, the likelihood of LAAT was very low with sensitivity of 88.9%. Shi and Alujas et al. showed that contrast TTE with Valsalva had high sensitivity and specificity for detection PFO, supporting its used as screening tool (Gonzalez-Alujas et al., 2011, Shi et al., 2024). In Wai et al., a TTE velocity ≤ 30 cm/s predicted LAAT or SEC with high accuracy (75% sensitivity, 90%

specificity), suggesting potential utility in identifying patients who may benefit from anticoagulation, however, number or proportion of patients whose treatment changed based on imaging findings was not reported (Wai et al., 2017).

While TTE is an effective screening tool for detecting large embolic sources or large PFO, TOE remains essential for comprehensive evaluation especially in case of small thrombi, SEC, or subtle interatrial defects. In (Shyu et al., 1994), TOE detected previously unrecognized cardiac embolic sources in 39 patients, including 14 patients without clinical signs of heart diseases, none of them detected by TTE, and treatment of 11 of them were changed from antiplatelet to anticoagulant. Also, Tuncer et al. (2001) found that TOE identified high risk embolic sources in 69%, compared to only 26% with TTE. Though exact treatment changes were not quantified, the authors emphasized importance of TOE findings in influencing therapeutic decision.

2.6.3 CCT/CTA vs. TOE

Twelve of the 24 studies comparing CCT/CTA to TOE to detect LAAT and other potential cardioembolic sources. Across a pooled diagnostic cohort of 2015 patients. Dedicated multiphase and dual-energy CCT consistently outperformed non-ECG gated CTA, particularly for LAAT detection. Studies that employed quantitative methods achieved the highest diagnostic performance (Hur et al., 2012, Kim et al., 2010, Qiao et al., 2024), and provided more objective thrombus detection and reduced subjective errors compared to purely qualitative visual assessments. Another factor might impact diagnostic yield is patient selection. Several studies selectively included population with AF or high embolic risk patients (Homsy et al., 2016, Hur et al., 2009c, Hur et al., 2011b), likely increasing the prevalence of true LAAT and enhancing CCT performance. Variation in the timing between CCT and TOE could have introduced discrepancies. Ideally, both tests should be performed within 48-72 hours to minimize the potential for thrombus formation, evolution, or resolution between modalities (Hindricks et al., 2020). In this review, most studies performed CCT and EET within the recommended time window. Qiao and Bousset et al., maintained a slightly broader interval within 5 days, while Pinho et al. had more variable timing (Pinho et al., 2023). Barazangi et al., (Barazangi et al., 2011) allowed up to 90 days between CTA and TOE, which likely contributed to their lower sensitivity. However, none

of the included studies analysed the direct impact of timing on diagnostic accuracy.

Studies that compare CCT with TOE highlight several strengths of CCT in detection of LAAT and potential cardioembolic sources in ischemic stroke and TIA patients. They demonstrated excellent specificity and high sensitivity, particularly studies using dual-energy and multiphase techniques. CCT offers non-invasive, reliable alternative to TOE, and better tolerated by patients. Moreover, it enables simultaneous assessment of heart, aorta, and cerebral vessels within a single imaging session, which is particularly advantageous in acute stroke patients where rapid and comprehensive evaluation is critical. Given its high specificity, CCT may be useful as a first line screening tool to exclude LAAT before proceeding with interventions such as thrombectomy, especially in patients for whom TOE is contraindicated, poorly tolerated, or logistically delayed.

Despite its promising diagnostic performance, several of CCT/CTA limitations for LAAT detection must be acknowledged. CCT has reduced sensitivity for detecting very small thrombi or early-stage SEC. Taina et al., demonstrated that small thrombi can be missed even with dual-phase CCT, emphasizing that very fine anatomical detail remains better by TOE (Taina et al., 2016). Motion artifacts and suboptimal cardiac imaging can compromise imaging quality especially in patients with AF or without heart rate control. This limitation was evident in (Pinho et al., 2023), where non-ECG-gated CTA was associated with reduced sensitivity due to cardiac motion-related blurring of the LAAT. Homsí et al, noted that subjective evaluation of filling defects may vary between observers, particularly in the absences of quantitative threshold or standardised method (Homsí et al., 2016).

CCT carries inherent technical risks must be considered. although radiation exposure has been increasingly minimized with dose- reduction techniques, it remains higher especially with dual- energy or dual phase CT, as acknowledged by (Hur et al., 2012), also using of iodinated contrast agents introduces a small but relevant risk of contrast induced nephropathy particularly in elderly patients or those with renal impairment, as noted by (Hur et al., 2011b, Kim et al., 2010).

These findings align with prior studies demonstrated that CCT offers high specificity but variable sensitivity for LAAT detection compared to TOE (Romero

et al., 2013), (Yu et al., 2021) which also reported that delayed-phase imaging technique significantly improve diagnostic performances. Chen et al, claimed that although non-ECG gated CTA widely available, suffer from lower sensitivity due to motion artifacts and limited cardiac phase targeting.

2.6.4 CMR vs. TOE

Three of 24 studies compared the diagnostic performance between CMR and TOE, where CMR demonstrated high specificity but variable sensitivity. In all included studies, CMR consistently underperformed in detection PFO, with sensitivity ranging from 16.7% to 56%, despite 100% specificity. This limited yield likely reflects challenges in temporal resolution, contrast timing, and the inability to visualize real-time contrast passage or guide Valsalva manoeuvres during acquisition. In contrast, CMR was more reliable for evaluating AA and left ventricle thrombus, Mohammad et al reported high diagnostic accuracy, with improve grading of aortic plaques compared to TOE. These strengths highlight the role of CMR in detection embolic substrates, especially, when TOE is limited or contraindicated. However, the studies did not link patients' management with imaging findings. A systematic review included 27 studies comparing CMR performance with TOE, in stroke patients, concluded that: while CMR and TOE had similar diagnostic yield for most cardiac source of embolism, CMR was significantly lower in detection of PFO, with sensitivity of 29.3% (Meinel et al., 2021). In addition to its diagnostic limitations, CMR is associated with practical constrains. Scan duration ranges from 45 to 90 minutes, which can be burdensome for acutely ill stroke patients or those with cognitive impairment. Furthermore, CMR is less accessible and more costly than other imaging modalities, limiting its widespread use in urgent clinical sittings.

2.6.5 Population selection and diagnostic-pathways context

Across the 24 studies that utilized TOE as the reference standard, our pooled analysis found that, the most frequently identified cardioembolic source were PFO, followed by AA (39.8% and 25.6% respectively). LAAT has the lowest prevalence (6.7%), however with combined with SEC (18%) as a marker of left atrial stasis, their prevalence increased to (21.1%).

These findings were influenced by the patient selection criteria, imaging protocol, total patients' number, and modality used in each study. Studies enriched with AF or high-risk cardiac populations reported more LAAT. Enhanced imaging protocol, such as dual-phase CT or contrast-TOE, also increased detection of thrombus and shunts. The observed prevalence of PFO was higher than general stroke population, predominantly influenced by inclusion of younger patient cohort, cryptogenic stroke population, and patients with suspected paradoxical embolism. This aligns with previous studies indicated PFO is present approximately 40-50% of patients with cryptogenic stroke or younger populations (Alsheikh-Ali et al., 2009, Webster et al., 1988). The prevalence of AA was 25.6% this is consistent with other studies in which AA prevalence in stroke patients ranged between 14-42% (Zavala et al., 2006, Toyoda et al., 1992, Fasseas et al., 2002).

Taken together, these findings should be interpreted within the wider context of stroke aetiological work-up. The extent and timing of diagnostic work-up vary markedly between centres and health systems, particularly in the availability and use of advanced cardiac imaging (Capirossi et al., 2023, McMahon et al., 2020). TOE, while regarded as the most definitive test for cardioembolic substrates, is invasive and best reserved for selective patients, typically younger or cryptogenic stroke cases, rather than applied universally (Shah et al., 2021, Strecker et al., 2020).

Insights from ESUS studies reinforce this selective approach, demonstrating both the diagnostic value and the limited feasibility of routine TOE in all stroke populations (Hart et al., 2014). This perspective helps explain the observed variability in test performance across studies, which primarily reflects differences in patients' selection and case mix rather than intrinsic test accuracy.

2.7 Limitation:

This review has several limitations. First, heterogeneity across studies in terms of imaging protocols, scanner technology, patients' selection, and diagnostic targets prevented comparability. Many studies were small, single-centre cohort, reducing generalizability. Patient populations varied, with some studies recruiting patients with high-risk cardiac embolism and others enrolling unselected ischemic stroke or cryptogenic stroke cohort.

Secondly, imaging protocols of index test differed considerably with variation ECG-gating, sequence selectin, inconsistent use of contrast and Valsalva manoeuvres influenced diagnostic yield and sensitivity variability. Interpretation methods ranged from subjective using visual assessment to objective mapping, with limited reporting of interobserver variability or blinding, introducing risk of bias.

Additionally, timing between index stroke and imaging was often variably reported and discrepancies in the interval between tests may have affected findings, particularly for thrombus evolution. Lastly, advances in imaging technology over time, especially in CCT and CMR, may limit the applicability of older studies to current clinical practice.

2.8 Conclusion:

TOE remains essential for comprehensive evaluation, especially for detecting subtle high-risk features such as SEC, small thrombi, and complex septal abnormalities. However, TTE, CCT, and CMR each offer strengths. TTE is a highly specific, accessible, and non-invasive imaging modality that offers reliable detection of large shunts, particularly when enhanced with contrast, Valsalva manoeuvres, and advanced imaging techniques. CCT has a high accuracy in identifying LAAT and AA, especially with dual-phase or ECG-gated protocols, making it a valuable tool in acute stroke settings. CMR provides detailed assessment of structural thrombi and AA but lacks sensitivity for PFO.

These findings suggests that while TOE should remain central in embolic stroke workup, alternative modalities can support or substitute its use based on clinical context, patient tolerance, and diagnostic need. Future research should focus on optimizing and standardizing non-invasive, high yield imaging protocols, and assess diagnostic thresholds. Importantly, future research should link imaging yield to clinical outcomes and therapeutic decisions to determine their true impact on secondary stroke prevention.

3 Chapter 3- Diagnostic feasibility and Accuracy of CCI

3.1 Abstract

Background: Aetiology of acute stroke remains undefined in about 25% of patients after typical routine diagnostic work-up. Delayed investigation may reduce the likelihood of identifying relevant findings (such as intravascular thrombi) and hence contribute to suboptimal mechanistic diagnosis, which may delay initiation of ideal secondary prevention.

Aims: To evaluate a single-visit Comprehensive cardiovascular imaging (CCI) protocol based on combined cardiac CT and CT angiography (CTA) for aetiological diagnosis in acute stroke.

Methods: Patients with recent minor stroke or TIA were assigned randomly to undergo either CCI or routine investigations. The mechanism of stroke was assigned using the ASCO-D classification, a five-domain system (Atherosclerosis, Small vessel disease, Cardiac source, Other, Dissection). The primary endpoint was the proportion of patients with incomplete diagnostic work-up (ASCO-D 9) at Day 30; secondary endpoints included time to final classification, change in diagnostic certainty between Day 7 and Day 30, and distribution of definite or probable aetiologies (ASCO-D1-2).

Results: Of the 50 participants in each group, the CCI group had no patient with A9 at 30 day compared to 4 patients in the routine group ($p=0.010$) and one patient with C9 compared to 31 patients in routine group ($p<0.001$). The median time from admission to ASCO-D classification allocation was 51 hours for CCI vs. 82 hours for routine group, $p = 0.050$. The CCI group had more cases with aetiological phenotype score 1 across all subcategories, collectively (A1, S1, C1, D1) at day 7 compared to the routine investigation group ($p=0.03$). Small vessel disease (S1, S2) was present in 42% of patients of CCI vs. 20% of the routine ($p=0.002$). 22% of CCI group had some degree of atherosclerosis (A1, A2), and 24% in the routine group ($p = 0.480$). Markers of cardioembolism (C1, C2) were 16% and 18% for CCI

and routine groups, respectively ($p=0.610$). The CCI group showed a higher prevalence of PFO/ASD at day 30 ($p=0.020$).

Conclusion: These preliminary data demonstrate that a CCI protocol was feasible in stroke and TIA patients and significantly improved both time to diagnosis and definite diagnostic categorisation within 7 days of hospital admission. Findings also suggest that the cardiac imaging component may be less important than MRI brain-detected small vessel disease in this cohort.

3.2 Introduction

Stroke and TIA remain leading causes of disability and death, and their optimal management requires accurate and timely identification of the underlying aetiological mechanism. Although most ischaemic strokes and TIAs are caused by thromboembolism, a substantial proportion - estimated at up to 25% - remain cryptogenic despite standard investigation.

ESUS poses therapeutic challenges, as patients may harbour occult cardioembolic or aortic sources that remain undetected due to limitations in standard imaging protocols and the timing of their application: as a result, this population is often left without targeted therapy (for example anticoagulation rather than antiplatelet therapy), potentially increasing the risk of recurrence (Kolominsky-Rabas et al., 2001).

The risk of stroke recurrence is front loaded within the first days after a TIA and minor stroke, with up to 25% of patients experiencing recurrence within 7 days if not urgently treated (Coull et al., 2004, Rothwell et al., 2007). International guidelines emphasise the importance of early, mechanism-specific diagnosis in acute ischemic stroke to guide appropriate secondary preventive treatment, as therapeutic strategies vary significantly depending on underlying cause (Sacco et al., 2013, Warner et al., 2019, Dawson et al., 2022). For example, early surgical carotid endarterectomy is indicated for patients severe internal carotid artery disease, anticoagulants are recommended for cardioembolic stroke or arterial dissection (Powers et al., 2019), and dual antiplatelet therapy (DAPT) may be beneficial in certain non-cardioembolic minor strokes and TIAs, as evidenced by the POINT and CHANCE trials (Wong et al., 2013, Dawson et al., 2022).

In patients with intracranial atherosclerosis, evidence supporting DAPT comes primarily from trials such as SAMMPRIS or subgroup analysis of POINT (Johnston et al., 2018, Chimowitz et al., 2011). Early and accurate identification of stroke mechanisms is therefore a potentially important means of reducing recurrence risk and improving outcomes (Mendelson and Prabhakaran, 2021, Rothwell et al., 2007).

The diagnostic workup varies by locale and resource availability but generally includes brain imaging (CT or/and MRI), carotid ultrasound, cardiac imaging such as TTE, and TOE. However, these pathways are constrained by incomplete vascular coverage, logistical challenges, and delays, especially for cardiac investigations. (Gladstone et al., 2022, Camen et al., 2020, Hart et al., 2017). Although TOE is considered as the reference standard for identifying cardiac sources of embolism, it is semi-invasive, requires skilled operators, and is not always readily accessible. Evidence suggests that findings of TOE lead to changes in clinical management in a minority of cases and do not appear to consistently improve clinical outcome following stroke (Camen et al., 2020, Shah et al., 2021).

In addition, diagnostic tests may have lower sensitivity for relevant findings if undertaken at a delayed time point after stroke presentation (e.g. diffusion weighted imaging [DWI] lesions on MRI, or echo for detection of intracardiac thrombi), reducing the likelihood of successful aetiological investigation. These limitations contribute to a significant proportion of patients being classified as ESUS. Patients with ESUS have been reported to experience higher rates of recurrence ischaemic stroke than those with other clearly defined aetiological subtypes reflecting the impact of incomplete diagnostic clarification at initial evaluation (Mead et al., 2023, Kolmos et al., 2021).

Advances in diagnostic imaging, notably ECG-gated contrast CCT combined with comprehensive visualization of aorta and supra-aorta vasculature, have significantly enhanced the sensitivity, specificity, and feasibility in detection of high risk cardioembolic sources such as LAAT, left ventricular thrombi and AA in stroke and TIA patients (Boussel et al., 2011, Sposato et al., 2024). A meta-analysis of 43 studies reported that CCT achieved an overall sensitivity of 98.4% and specificity of 96.0% for detection of intracardiac thrombi, outperforming TTE and demonstrating diagnostic accuracy comparable to TOE (Ghozy et al., 2024).

Furthermore, the implementation of a delayed-phase CCT protocol was found to facilitate the early detection of major cardioembolic sources in acute ischemic stroke patients, thereby expediting decision-making processes for secondary prevention strategies (Larsen et al., 2023, Boussel et al., 2011). These findings underscore the potential of integrated CCT and CTA approaches to serve as first-line imaging modalities in the aetiological assessment of stroke.

The rationale for this pilot study was to explore whether a fast track, holistic imaging protocol could expedite the identification of stroke mechanisms and support timely initiation of evidence-based secondary prevention therapies. This approach combines three critical domains in a single encounter: ECG-gated CCT for cardiac sources of embolism, CTA from the aortic arch to vertex for vascular sources, and MRI for brain parenchymal and small vessel pathology, within a closely timed, same-admission workflow rather than as separate outpatient investigations.

We hypothesised that this protocol including comprehensive cardiovascular imaging could reduce the time to definitive stroke aetiology classification, increase the proportion of patients receiving appropriate secondary prevention earlier, and potentially reduce the proportion of patients classified as ESUS at Day 30. In doing so, this study aimed to address not diagnostic accuracy, but also system-level efficiency and treatment timeliness.

3.3 Method

3.3.1 Study design and setting

This was a prospective single centre, randomised, controlled pilot trial undertaken at a university hospital with a comprehensive stroke centre in the UK. The study aimed to assess the diagnostic feasibility, yield, and clinical impact of fast-track comprehensive cardiovascular imaging (CCI) protocol compared to routine diagnostic pathways in patients with acute ischaemic stroke or TIA.

3.3.2 Patient selection and randomisation

Participants were adults (≥ 18 years) with a clinical diagnosis of acute ischaemic stroke or TIA within the past seven days. Exclusion criteria included impaired renal

function, known hypersensitivity to contrast media, severe concurrent medical conditions, contraindications to MRI, or predictable unavailability for follow-up.

After obtaining informed consent from patients or their legal representatives, patients were randomised in a 1:1 ratio to either fast track CCI or routine investigation group using a computer-generated allocation sequence. Randomisation was conducted through a secure web-based platform hosted by the Robertson centre for biostatistics, University of Glasgow, which automatically assigned participants after data entry. This ensured allocation concealment and prevented investigators from influencing or predicting group assignment.

The study aimed to recruit a convenience sample of 100 patients (50 per group). Ethics approval was obtained from the West of Scotland research ethics committee TOE (reference 18/WS/0068).

3.3.3 Data collection and follow up

The initial study visit occurred within seven days of the event. At this point, the following were recorded: demographic data, medical and medication history, risk factors, and clinical assessment, baseline neurological exam (e.g. NIHSS) and imaging group allocation.

At day 30, a follow-up was conducted via review of electronic medical records. The following were recorded:

- Occurrence of New clinical events (stroke, TIA, or death).
- Completion and date of outstanding diagnostic tests.
- Final ASCOD classification.
- Changes or confirmation of secondary prevention therapy.

Imaging timing for diagnostic modalities was recorded from the time of hospital admission to the day of imaging to reflect operational feasibility, whereas timing of ASCOD classification at Day 7 and Day 30, was calculated from the time of event, to evaluate diagnostic timeliness in relation to the clinical event.

3.3.4 Imaging acquisition

3.3.4.1 CCI group

Patients randomised to the CCI group underwent a same-day, fast-track, single session comprehensive cardiovascular and cerebrovascular imaging protocol using a 320-detector row Aquilion One Vision CT scanner (Canon Medical Systems), and a 3 Tesla MRI scanner (Prisma, Siemens Healthineers). The protocol included ECG-gated CCT, non-gated CTA, and high-resolution brain MRI. The full imaging session was completed within a single hospital encounter, typically requiring 40 to 50 minutes including inter-modality transfer.

3.3.4.1.1 CT Acquisition

CT imaging was performed during a single contrast-enhanced session using a 76 ml bolus of Omnipaque 350 followed by 95 ml of saline, injected at 5 ml/s. Bolus tracking was triggered at 150 HU within a region of interest placed in the left ventricle. Scanning was conducted in a single volumetric helical pass, combining a prospectively ECG-gated CCT (heart base to carina), followed immediately by non-gated CTA (carina to skull vertex). The CCT and CTA protocol incorporated standard dose-reduction techniques including prospective ECG gating, low tube voltage (120 kVp), and adaptive tube current modulation. Imaging quality was optimised using thin collimation (0.5 mm) and fast rotation time (0.275 s). No rate-control medication was administered. Scanning was performed craniocaudally then caudocranially to optimise contrast timing across regions. All imaging data were transferred to dedicated workstations for post-processing, where multiplanar reconstructions (MPR), maximum intensity projections (MIP), and three-dimensional reconstructions were utilized for image analysis. Radiation exposure was documented using standard scanner-generated dosimetric metrics. These included the CTDI_{vol} (Computed Tomography Dose Index-volume), representing the average radiation dose per slice volume in milli-gray, and the DLP (Dose-Length Product), reflecting the total radiation dose across the scanned region (mGy.cm). All values were extracted from the CT does report by the research radiographer and reviewed for compliance with ethics-approved safety thresholds of 20 mSv.

3.3.4.1.2 MRI protocol

MRI brain was conducted on 3.0 Tesla scanner (Prisma, Siemens Healthineers) using a 20-channel head coil. The imaging protocol included a series of core sequences tailored for acute stroke evaluation and structural assessment. Diffusion-weighted imaging (DWI) was acquired using a Resolve 4-scan trace sequence in the axial plane to detect acute infarction. T2-weighted FLAIR sequences were obtained axially for chronic ischemic changes. Susceptibility-weighted imaging (SWI) was performed in 2D and 3D axial to identify microhaemorrhages. A dark-fluid T2-weighted turbo spin echo (TSE) was included for general structural evaluation. 3D T2 SPACE and T1 MPRAGE are also included. All sequences employed parallel imaging using GRAPPA to reduce acquisition time, along with distortion correction and pre-scan normalization to optimize image quality and spatial resolution.

3.3.4.2 Routine investigation group

Patients in this group underwent clinically indicated stroke imaging according to routine clinical practice at the study centre, following the standard diagnostic options available within the local NHS service. Non-contrast CT brain scans were acquired using a standard CT scanner (predominantly GE 660). When performed, MRI brain was acquired using clinical 1.5 Tesla scanners and included these sequences: Diffusion-Weighted Imaging (DWI), T2W Fluid-Attenuated Inversion Recovery (FLAIR), and Susceptibility-Weighted Imaging (SWI). Vascular imaging included carotid duplex ultrasound, CTA, or MRA of the cervical arteries, at the discretion of the clinical team. Cardiac investigation included 12-lead ECG, 24-72-hour ECG/or Holter monitoring, TTE, and/or TOE as indicated. The timing and type of the investigation were at the discretion of treating clinicians and typically occurred over multiple appointments.

3.3.4.3 Imaging post-processing and blinded review

All imaging data were transferred to a dedicated workstation for post-processing. Imaging interpretation was performed by a senior neuroradiologist and a cardiovascular radiologist, both are blind to clinical outcome. As patients in the CCI arm underwent cardiac CT not performed in the routine pathway, complete blinding to group allocation was not feasible. Aetiological classification (ASCO-D) was assigned by integrating imaging findings with clinical data and was performed

independently by a clinical researcher. All imaging and clinical data were pseudonymised and stored securely on encrypted research servers.

3.3.5 Stroke aetiological classification

Aetiological classification was performed using the ASCO-D system (Amarenco et al., 2013), a structured five-domain scheme that characterises potential stroke mechanisms based on clinical, radiological, and diagnostic evidence. The five domains represent: **A: Atherosclerosis** (extra or intra cranial large artery disease), **S: Small vessel disease** (lacunar infarct, white matter changes), **C: Cardiac embolism** (AF, LAAT, PFO), **O: Other causes** (e.g. vasculitis, coagulopathy), **D: Dissection** (e.g. carotid or vertebral artery dissection). Each domain is independently graded on a 0-3 or 9 score: 0= no abnormality, 1= definite aetiology, 2= probable cause, 3=unlikely cause, and 9= incomplete workup. Classifications were determined by the research team at two predefined time points: Day 7: initial classification based on early diagnostics imaging; and Day 30: final classification incorporating follow-up imaging and clinical data. This dual time-point assessment allowed for the evaluation of both diagnostic completeness and the timeliness of mechanism identification, which may influence early therapeutic decisions.

3.3.6 Study outcomes

The primary outcome was the proportion of patients with incomplete diagnostic workup (ASCO-D 9), defined as assignment of a category 9 in any ASCO-D domains at day 30, indicating that one or more aetiological categories remained unresolved.

Secondary outcomes included:

- Time from event to final aetiological classification (diagnostic timeliness).
- Change in ASCO-D certainty between Day 7 and Day 30 (conversion from uncertain to definite classification).
- Utilisation and timing of diagnostic imaging modalities across groups.
- Initiation or change of secondary prevention therapy (antiplatelet, anticoagulant, or lipid-lowering).

- Overall diagnostic findings across groups.
- Incidence of new clinical events (stroke / TIA or death) within 30 days

Endpoints were blindly adjudicated by a consultant neuroradiologist and cardioradiologist, based on presence or absence of the following findings relevant to ASCO-D domains:

- Atherosclerotic or large-artery disease: Intra or extracranial stenosis, atherosclerosis, or dissection.
- Evidence of small vessel disease (SVD), including new acute/chronic infarct (with number and size recorded), white matter hyperintensities, lacunes, microbleeds and enlarged perivascular spaces. SVD markers were defined and assessed according to STRIVE guidelines (Wardlaw et al., 2013).
- Cardioembolic sources (e.g. intracardiac thrombus, valvular diseases, septal defects, LV hypertrophy, and cardiac mass, aortic arch abnormalities such as thrombus, complex plaque \geq 4 mm).

Vascular stenosis was recorded based on radiology reports in the clinical records. As definitions varied across reporting clinicians, stenosis was recorded as present if either the degree of narrowing was explicitly quantified as \geq 50 %, or the report described it as moderate or severe. This pragmatic approach was adopted to ensure consistency given the variability in reporting format.

3.3.7 Statistical analysis

Statistical analysis was performed using SPSS (v24, IBM). Continuous variables were presented as median with interquartile ranges (IQR) and categorical variables as frequency (percentage). Between-groups comparisons were carried out using Mann-Whitney U tests for continuous variables and chi-square test for categorical variables. A $p < 0.05$ was considered significant. Kaplan-Meier survival analysis was conducted to compare time to events across different treatment groups including initiation of antiplatelet, dual antiplatelet, anticoagulant, statin and antihypertensive medications.

3.4 Results

3.4.1 Participant characteristics

Between October 2018 and September 2019, a total of 100 patients were enrolled and randomized into the study. Fifty patients were assigned to the fast-track CCI group and fifty to the routine investigation group. Demographic and clinical characteristics were well balanced between the two groups (Table 3-1). The median age was 64.6 years (range 37-88) in CCI group and 67.5 years (range 39-92) in the routine group. The sex distribution was identical in both groups, with 34 males and 16 females. Common vascular risk factors were similarly distributed, although hypertension was more prevalent in routine group (64% vs. 52%). Diabetes mellites was present in 18% of participants in both groups. Other variable, including AF, peripheral vascular disease(PVD), hyperlipidaemia, and pervious ischemic heart disease(ICD), showed no marked imbalances.

Table 3-1 Baseline characteristics of study participants

Variable	CCI (n=50)	Routine (n=50)
Age (years) median, (range)	64.6 (37,88)	67.5 (39 ,92)
Sex (M: F)	34:16	34:16
HTN (n) (%)	26 (52)	32 (64)
DM (n) (%)	9 (18)	9 (18)
PVD (n) (%)	4 (8)	5 (10)
AF (n) (%)	4 (8)	3 (6)
High blood cholesterol (n) (%)	11 (22)	6 (12)
ICD (n) (%)	4 (8)	11 (22)
TIA (n) (%)	6 (12)	7 (14)
IS (n) (%)	6 (12)	3 (6)
TIA & IS (n) (%)	1 (2)	2 (4)
Provisional diagnosis		
Minor stroke with NIHSS> 2 (n) (%)	20 (40)	20 (40)
Minor stroke with NIHSS 0-2 (n) (%)	17 (34)	17 (34)
TIA< than 1hour duration (n) (%)	5 (10)	5 (10)
TIA 1-24 duration (n) (%)	8 (16)	8 (16)

Regarding past cerebrovascular history, prior TIA was reported in 6 patients (12%) of CCI group and 7 (14%) of the routine group. Prior ischaemic stroke (IS) was slightly more frequent in CCI group (12% vs.6%), while small number of patients in both groups had a history of both TIA and IS.

At presentation, both groups had identical distribution of stroke severity. Minor stroke with NIHSS scores >2 , was recorded in 20 patients (40%) per group, and 17 patients (34%) scored 0-2. The median baseline NIHSS score was 2 (IQR 1-3) in both groups. Additionally, 5 patients (10%) in each group presented with a TIA <1 hour duration, and 8 patients (16%) presented with a TIA lasting 1-24 hours.

3.4.2 Stroke aetiology classification at Day 7 and Day 30

ASCO-D score 1 category, representing assignment of a definite stroke mechanism, was significantly more frequent in the CCI group than in the routine group at both Day 7 (41 vs. 18 patients, $p = 0.012$) and Day 30 (45 vs. 20 patients, $p = 0.013$).

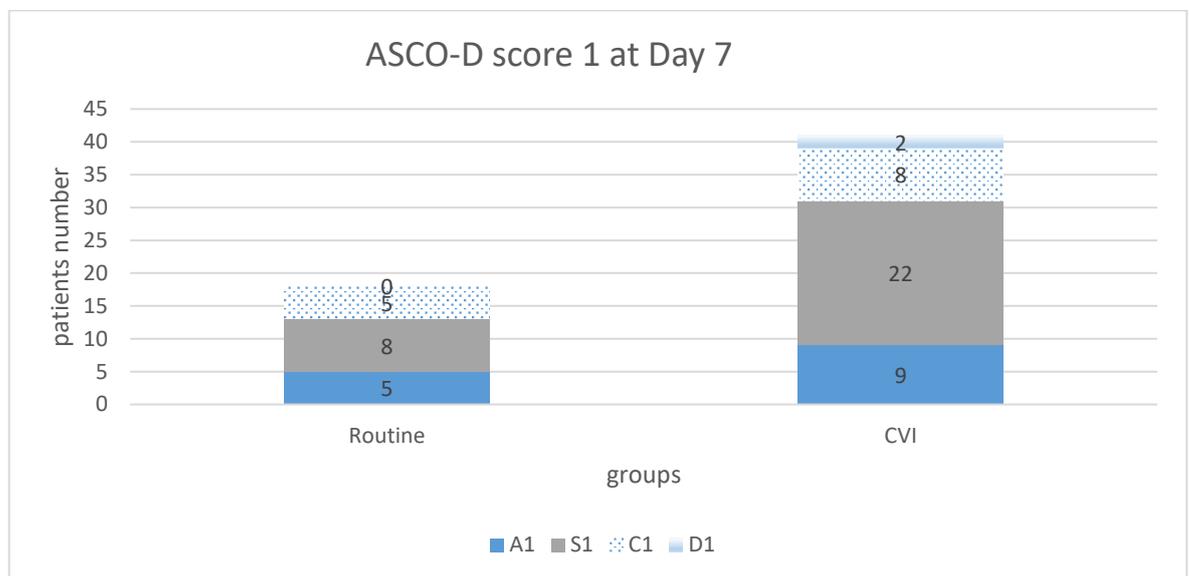


Figure 3-1 ASCO-D score 1 at Day 7

At Day 7, a probable stroke aetiology (ASCO-D score 1 or 2) was identified more frequently in the CCI group across several domains. The CCI group showed higher proportions of definite large artery disease (A1: 18% vs 10%, $p = 0.243$), small vessel disease (S1: 44% vs. 16%, $p = 0.002$), and definite cardiac embolism (C1: 16% vs. 10%, $p = 0.370$), though only the difference in SVD S1 reached statistical significance. Notably, definite dissection D1 was identified in two CCI patients compared to none in the routine group, $p = 0.152$. Probable large artery disease A2 was similar between groups (10% vs. 12%, $p = 0.741$), however, when combining A1 and A2, both groups had similar proportions (28% vs. 22%, $p = 0.482$). Small vessel disease classifications (S1+ S2) were significantly more common in the CCI

group at Day 7 (54% vs. 24%, $p = 0.002$), while combined categories such as C1+C2 and D1 + D2 showed no significant differences Table 3-2.

The rate of incomplete cardiac assessment C9 was markedly higher in the routine group 70% compared to only 4% in the CCI group ($p < 0.001$), Similarly, incomplete large artery evaluation A9 was more frequent in the routine group (16% vs. 2%, $p = 0.008$)(Table 3-2).

These patterns largely persisted at Day 30, with incomplete large artery (A9) decreased 12% in the routine group and to 0% in CCI group ($p=0.013$) and incomplete cardiac assessment C9 was still higher in the routine group 64% compared to only 2% in the CCI group ($p < 0.001$) Table 3-2.

In addition to better diagnostic completeness, the CCI protocol resulted in faster aetiological classification, with shorter median time to full ASCO-D phenotype assignment: 51 hours (IQR 25- 105) in CCI group versus 82 hours (IQR 46-180) in the routine group ($p= 0.056$).

Table 3-2. ASCOD aetiological classification at Day 7 and Day 30

	CCI	Routine	P value
Stroke aetiology classification day 7			
Probable Aetiology Identified	n (%)	n (%)	
A1	9 (18)	5 (10)	0.243
S1	22 (44)	8 (18)	0.002
C1	8 (16)	5 (10)	0.370
O1	0	0	0
D1	2 (4)	0	0.152
A2	5 (10)	6 (12)	0.741
S2	5 (10)	4 (8)	0.172
C2	3 (6)	4 (10)	0.69
A3	12 (24)	22 (44)	0.03
S3	7 (14)	25 (50)	< .001
C3	6 (12)	2 (4)	0.143
O3	1 (2)	0	0.491
D3	0	1 (2)	0.980
Incomplete Work-up			
A9	1	8 (16)	0.010
C9	2 (4)	35 (70)	< 0.001
S9	0	0	0
O9	1	3	0

D9	0	1(2)	0.311
A1, A2	14 (28)	11(22)	0.482
S1, S2	27 (54)	12 (24)	0.002
C1, C2	11 (22)	9 (18)	0.617
O1, O2	0	0	0
D1, D2	2(4)	0	0.152
Stroke aetiology classification day 30			
Probable Aetiology Identified			
A1	12(24)	7 (14)	0.201
S1	23(56)	8 (16)	0.001
C1	8 (16)	5 (10)	0.542
O1	0	0	0
D1	2 (4)	0	0.150
A2	5(10)	7 (12)	0.53
S2	4 (8)	5 (10)	0.722
C2	3 (6)	4 (8)	0.693
O2	0	0	0
D2	0	0	0
A3	12 (24)	22 (44)	0.031
S3	6 (12)	25 (50)	<.001
C3	6 (12)	4(8)	0.502
O3	0	2 (4)	0.491
D3	0	1(2)	0.310
Incomplete work-up			
A9	0	6(12)	0.013
C9	1 (2)	32(64)	<0.001
S9	0	0	0
O9	0	0	0
D9	0	1(2)	0.313
A1, A2	17 (34)	14 (28)	0.512
S1, S2	28 (65)	13(26)	0.004
C1, C2	11 (22)	9 (18)	0.792
O1, O2	0	0	0
D1, D2	2 (4)	0	0.491

3.4.3 Completion and timing of diagnostic investigations

In the CCI group, 45 of 50 patients (90%) completed the full protocol, which included ECG-gated cardiac CT, CTA and brain MRI. Five patients did not undergo all planned CCI imaging due to the following reasons: one developed renal

impairment after being randomized, two patients experienced an acute asthmatic attack during hospitalization, one patient refused scanning after being allocated, and one patient underwent CTA alone without the ECG-gated Cardiac CT component (due having earlier had CT pulmonary angiography thought to cover the required anatomy).

Table 3-3 shows the median time from admission to investigation for key imaging modalities across both groups, along with the number of patients who received each test. Overall, brain imaging with non-contrast CT was performed promptly in near all patients in both groups (median 3 hrs). Brain MRI was performed in 49 patients of CCI group versus 35 in routine group with earlier imaging in the CCI group (26 vs. 47 hours). TTE was completed more in CCI group (12 vs. 7 patients), and in shorter time (123 vs. 310 hrs), though these differences did not reach statistical significance. The Routine group had shorter times for Carotid Doppler (43 vs. 52 hrs), but again these differences were not statistically significant, all $p > 0.05$.

Table 3-3. Median time from admission to investigation

investigation	CCI group (n)	Median (IQR)hrs	Routine Group(n)	Median (IQR)hrs	P value
MRI brain	49	26(22,49)	35	47 (26,72)	0.891
CT brain	47	3(2,6)	50	3 (1,6)	0.654
CTA	46	26(21,45)	37	24 (22,46)	0.125
Carotid doppler	14	52(26,73)	14	43 (23,105)	0.963
TTE	12	123(83,283)	7	310 (151,684)	0.226
TCD	3	113(87,164)	2	71(47,95)	0.624

Time presented as median and interquartile range along with number of patients in each group. $P < 0.05$ consider significant.

3.4.4 Duration of hospitalization

The overall duration of hospital stay was similar between groups, with a median of 63.5 hrs in CCI group and 60.1 in the routine group ($p=0.69$), indicating that the fast-track imaging protocol did not prolong inpatient stay (Table 3-4). Although the median duration was slightly shorter in the routine group, the wider interquartile range (45.1-116.0 hrs vs. 41.4-95.4 hrs) may reflect variability in either the timing of diagnostic completion or treatment decision-making within the routine care pathway.

Table 3-4. Duration of hospitalisation

Group	Median(hrs)	IQR(hrs)
CCI	63.5	41.4-95.4
Routine	60.1	45.1-116.0
p-value		0.692

3.4.5 vascular and cardiac imaging findings

3.4.5.1 Acute infarct detection on brain imaging

An acute infarct on brain imaging was identified significantly more often in the CCI group compared to the routine group (68% vs. 40%, $p = 0.005$). among the 50 CCI patients, 34 had evidence of acute infarcts, whereas only 20 patients in the routine group had similar findings. Enhanced detection of acute infarcts supports the diagnostic advantage of consistent, early use of high resolution 3T brain MRI, which may improve diagnostic confidence, especially in patients with minor stroke

3.4.5.2 Aortic arch atheroma

Aortic arch plaque was evaluated in 96% of CCI patients and 72% of routine patients. The distribution of findings differed significantly between groups. Incomplete imaging was more frequent in the routine group (14 vs. 2 patients in CCI). Among patients with completed imaging, aortic arch atheroma was identified in 10 patients of routine group (7 with plaque < 4mm, and 3 with plaque \geq 4mm), compared to 5 patients in CCI group (4 with plaque < 4mm, 1 with plaque \geq 4mm), these differences were statistically significant, $p = 0.002$. most CCI patients (86%) had no detectable AA versus only 52% in the routine group, see Table 3-5. Although AA appeared more frequent in the routine group, it had a much higher rate of incomplete investigations raising the possibility that additional cases of AA may have gone undetected.

Table 3-5. Aortic arch plaque findings by group

AA category	CCI (n=50)	Routine (n=50)
Incomplete work-up	2	14
No AA	43	26
AA < 4 mm	4/50	7/50
AA \geq 4 mm	1/50	3/50

3.4.5.3 Cardiac imaging findings

Completion of cardiac imaging was higher in CCI group (98%) compared to the routine group (36%). At Day 30, imaging-identified cardiac abnormalities were

detected in 13 of 49 CCI patients (26.5%) and in 4 of 18 routine 22.2%. the overall distribution of cardiac imaging findings differed significantly between groups ($X^2 = 66.9$, $p = 0.001$), reflecting the markedly higher imaging completion in the CCI arm.

Among the CCI cohort, findings include five isolated PFOs, for valvular abnormalities, three with combined PFOs and valvular disease, one LAAT with a PFO, and one LAAT with valvular disease. In the routine group, two patients had PFO and two had valvular abnormalities, while no LAAT was detected. AF was present in four CCI and three routine participants; notably, one of the two LAAT cases occurred in a patient with AF and PFO. Percentages are based on the total number of randomised participants in each group, unless otherwise specified.

Table 3-6. Cardiac source findings by group

Cardiac source	CCI(n=50)	Routine(n=50)
Incomplete work-up	1/50	32/50
No cardiac pathology	34/50	6/50
PFO	5/50	2/50
Valvular disease	4/50	2/50
PFO + valvular disease	3/50	0
LAAT + valvular disease	1/50	0
LAAT + PFO	1/50	0
Left ventricular hypertrophy (LVH)	1/50	0
Atrial septal defect (ASD)	1/50	0

3.4.5.4 Extracranial internal carotid artery imaging findings

Internal carotid artery (ICA) imaging was completed in 98% of the CCI group and in 88% of the routine group Table 3-7. Most patients in both groups had no detectable stenosis (CCI 76% vs. 64%). The remaining patients exhibited various stenosis patterns: bilateral disease (n = 9), ipsilateral stenosis (n = 8), or contralateral stenosis (n = 6). The routine group had slightly higher number of ipsilateral and bilateral stenosis, while CCI had more contralateral stenosis. However, these differences were not statistically significant, $p = 0.252$

Table 3-7. ICA imaging findings

ICA stenosis category	CCI n=50 (%)	Routine n=50 (%)
Incomplete work-up	1 (2)	6 (12)
No stenosis	38 (76)	32 (64)
Bilateral stenosis	4 (8)	5 (10)
Ipsilateral stenosis	3 (6)	5 (10)
Contralateral stenosis	4 (8)	2 (4)

3.4.5.5 Intracranial arterial stenosis

Intracranial arterial imaging was completed in 96% of CCI patients compared to 74% of those in routine group (Table 3-8). Most of the patients had no detectable intracranial stenosis in their group (CCI: 76% vs. routine 64%). Among those successfully imaged any intracranial stenosis was observed in 10 of 48 CCI patients and 5 of 37 routine patients. The overall distribution of imaging-completion and stenosis categories differed significantly between groups ($X^2 = 10.38$, $p = 0.016$), reflecting the markedly higher rate of incomplete workups in routine arm rather than a true anatomical difference in stenosis location.

Table 3-8. Intracranial arterial stenosis by group

Intracranial stenosis category	CCI n=50 (%)	Routine n=50 (%)
Incomplete work-up	2 (10)	13 (26)
No stenosis	38 (76)	32 (64)
Stenosis both sides	3 (6)	2 (4)
Ipsilateral stenosis	7 (14)	3 (6)

3.4.6 Radiation dose analysis in CCI group

Radiation dose exposure in patients who underwent the full CCI protocol was evaluated to ensure adherence to diagnostic safety standards. Of the 50 patients in the CCI group, 34 (68%) patients completed both ECG-gated CCT and non-gated CTA in a single contrast-enhanced session were included in the analysis. Sixteen patients were excluded for different reasons: eight had CTA performed separately in clinical setting outside the research scanner, four underwent only partial CCI protocol, one had no available dose report, one had high heart rate persistently high heart rate (about 90 bpm) that required a modified scan protocol resulting in non-standard dose metrics, one experienced trigger error that prevented accurate bolus timing which invalidated dose record, and one had ECG changes during breath holding led to scan termination.

These exclusions ensure that dose estimates reflect patients who completed the full CCI protocol under standardised conditions, allowing accurate and ethically aligned evaluation of radiation exposure.

For the 34 included patients, the median effective dose was 17.6 mSv (IQR 9.2-24.5 mSv), with 71% receiving less than 20 mSv- below the predefined ethical

threshold. The corresponding median total DLP was 1261 mGy.cm and median CTDIvol 24.5 mGy (Table 3-9). Variability in radiation dose was primarily attributable to differences in patients body habitus and automatic exposure modulation Figure 3-2.

No equivalent dose metrics were available for the routine group because imaging protocol varied. For context, a standard chest-abdomen- pelvis (CAP) CT typically delivers approximately 15-18 mSv, and within the expected range for combining cardiac and vascular CT (12-20 mSv), indicating that the CCI protocol achieved comprehensive cardiac and vascular imaging at a dose comparable to national diagnostic reference levels.

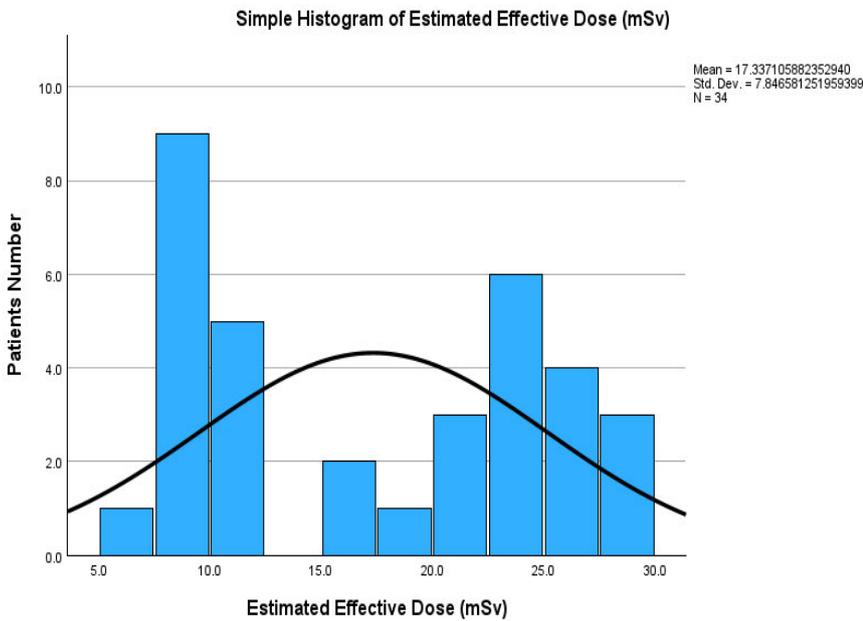


Figure 3-2. Estimate effective dose

Table 3-9 Descriptive statistics for radiation dose parameters

Measure	Total DLP (mGy.cm)	CTDIvol(mGy)	Estimated effective dose (mSv)
Mean	1238.4	22.5	17.3
Median	1260.7	24.5	17.6
Standard deviation	560.5	10.1	7.8
IQR (25%-75%)	659.0 - 1750.4	12.2 - 31.0	9.2 - 24.5

3.5 Discussion

3.5.1 Overview of the main findings

This randomised pilot trial demonstrated the feasibility and diagnostic utility of a fast-track, comprehensive cardiovascular imaging (CCI) protocol in delivering faster, more complete, and more informative aetiological assessment of acute ischemic stroke and TIA compared to routine investigation pathways.

The proportion of patients with incomplete diagnostic workup (ASCO-D 9) in any ASCO-D domain at Day 30 was significantly lower in the CCI group: only one patient (2%) in CCI arm was classified with C9, compared to 32 patients (64%) in the routine group ($p < 0.001$), and no patients in the CCI group had incomplete atherosclerosis (A9) versus six (12%) in the routine group ($p = 0.013$). The CCI group also demonstrated a higher proportion of patients with definite stroke mechanisms (ASCO-D1) remained higher in CCI group at both Day 7 (41 vs. 18, $p = 0.012$) and Day 30 (45 vs. 20, $p = 0.013$), including more frequent identification of mechanisms across A1, S1, C1 and D1.

Time to final aetiological classification was modestly shorter in the CCI arm, with a median of 51 hours (IQR 25-105) compared to 82 hours (IQR 46-180) in the routine group ($p = 0.056$), reflecting potentially earlier aetiological diagnosis relevant to secondary prevention decisions.

Across vascular, cerebral and cardiac territories, the CCI protocol achieved more complete evaluation than routine care. The CCI group demonstrated a consistently higher proportion with definite aetiological cause at both time points. Small vessel disease (S1 or S2) at Day 30 was more frequently identified in CCI group (56% vs. 26%, $p = 0.004$), likely reflecting both greater MRI utilization, and possibly the superior resolution of 3T MRI. Acute infarcts on brain imaging were also more frequently detected in the CCI group than in the routine group (68% vs. 40%, $p = 0.005$). Cardiac embolic sources, including PFO, LAAT, and valvular diseases, were identified in 26% of CCI patients compared to only 6% in the routine group ($p = 0.001$), reflecting a markedly higher completion rate of cardiac imaging (98% vs. 20%).

3.5.2 Interpretation and comparison with literature

The findings of this pilot randomized controlled trial underscore the potential advantages of a comprehensive cardiovascular imaging protocol in enhancing diagnostic completeness and timeliness in assessment of acute ischemic stroke and TIA. The substantial reduction in incomplete diagnostic work-up (ASCO-D 9), particularly in A9 and C9, align with recent guidelines, which emphasize the early identification of stroke aetiology as a critical step in guiding appropriate secondary prevention. Mead et al. highlighted that optimal management strategies depend on timely and accurate aetiological classification (Mead et al., 2023). Hart et al. also describe ESUS as a diagnostic category often resulting from incomplete work-up, noting its relatively high prevalence and recurrence risk (Hart et al., 2017). In this study, the CCI not only achieved higher rates of definitive stroke mechanisms across ASCO-D domains but also within a shorter time frame. Fontaine et al. demonstrated that early, structured imaging work-up, incorporating both brain and vascular imaging, facilitates accurate and refined stroke mechanistic classification using ASCO-D phenotype system (Fontaine et al., 2021).

The enhanced detection of small vessel disease (SVD) in the CCI group (56% vs. 26%, $p = 0.004$) likely reflects the systematic use of high resolution 3T brain MRI with advanced sequences. The HARNESS initiative advocates for a harmonised, high-resolution imaging protocol - best achieved at 3T MRI for detecting SVD features such as white matter hyperintensities, lacunes, and microbleeds (Smith et al., 2019). Wardlaw et al. concluded that higher MRI field strengths increase sensitivity for SVD, especially cerebral microbleeds, by improving susceptibility contrast, thereby offering greater sensitive for detection of subtle lesions compared to lower-field scanners (Wardlaw et al., 2012). Schmidt et al., demonstrated that a higher burden of MRI-defined SVD is independently associated with an increased risk of recurrent stroke and long-term disability (Schmidt et al., 2016).

Additionally, the CCI protocol's systematic use of high-resolution 3T brain MRI significantly improved the detection of acute infarcts, (68% vs. 40%, $p = 0.005$). Both groups underwent DWI sequence, but the CCI arm predominantly used 3T scanners, which may enhance lesion conspicuity compared with 1.5T systems

through improve signal-to-noise and susceptibility contrast. Comparative study showed that 3T DWI and perfusion imaging provide at least equivalent and, in some respects, superior image quality to 1.5 T in hyperacute stroke, with improved contrast and perfusion-map details (Kosior et al., 2007), suggesting that the higher detection rate here likely reflects more consistent MRI completion and modest technical gains at higher field strength, rather than field strength alone.

Higher MRI sensitivity for small vessel disease and minor infarcts can also influence aetiological classification. Greater visibility of lacunar or subcortical lesions may increase attribution of stroke to SVD (S1 or S2), but overclassification remains possible when concurrent cardioembolic or large artery mechanism coexist (Wardlaw et al., 2015). In this cohort, some infarct classified as lacunar could have occurred in patients with coexisting atherosclerotic or cardiac sources.

The CCT protocol demonstrated a significantly higher detection of high-risk cardiac embolic sources -including LAAT, PFO, and valvular disease- compared to the routine group (26% vs 6%, $p = 0.001$), highlighting the diagnostic value of CCT when integrated into a structured early protocol. This finding is supported by recent meta-analyses: Ghozy et al. confirmed that CCT offers high sensitivity and specificity for intracardiac thrombus detection, and achieved pooled values of 98.4% and 96.0% respectively, relative to TOE (Ghozy et al., 2024). Xu et al. also identified thrombi in 8% of stroke patients including many who did not have known AF, highlighting CCT capacity to uncover otherwise occult embolic sources (Xu et al., 2024, Ajlan et al., 2016). Nio et al. similarly demonstrated a significantly higher prevalence of high-risk embolic sources on CCT among ischemic stroke patients in a Dutch case-control study and emphasised that the comparative value of ECG-gated versus non-gated protocols remains a key consideration (Nio et al., 2025).

The diagnostic optimisation achieved in the CCT protocol reflects the advantage of ECG-gated acquisition, which synchronises image capture with cardiac cycle to minimize motion artefact and enhance visualization of LAA and cardiac valves. In contrast, non-gated CCT has lower diagnostic performance due to suboptimal temporal resolution. Yeo et al. reported that non-gated CCT reduced reliability in detecting embolic sources (Yeo et al., 2017). Further supporting the utility of ECG-

gated protocol, Tania et al. demonstrated that CCT can reliably differentiate between thrombus and circulatory stasis within the LAA (Taina et al., 2016).

The CCI protocol detected more cases of intracranial arterial stenosis than the routine (20% vs. 10%, $p = 0.016$), likely due to its higher CTA completion rate (96% vs. 74%), from aortic arch to intracranial vessels. This approach ensured consistent coverage and contrast timing, improving lesion detection across the vascular tree. These findings align with existing literature that recognizes intracranial stenosis as a common but often underdiagnosed stroke mechanism, particularly in patients with cryptogenic stroke or incomplete diagnostic work-up (Qureshi et al., 2009, Kim et al., 2005).

Sposato et al. and Ha et al. also highlighted that an extended CTA protocol improved detection of embolic sources, including complex aortic arch plaques, which may be missed with fragmented or incomplete imaging (Sposato et al., 2024, Ha et al., 2024). Aortic arch atheroma was identified in 10.4% of CCI group and in 27.8% of routine group. although the frequency appeared higher in the routine group, interpretation should be cautious given the differing completeness of imaging between arms (96% in CCI vs. 72% in routine). The apparent difference may reflect sampling variability or incomplete coverage in the routine group rather than a genuine disparity in disease prevalence. Overall, these results support the value of comprehensive and standardized vascular imaging for improve diagnostic accuracy and guiding stroke prevention strategies.

3.5.3 Clinical relevance and implications

The diagnostic gains achieved through the CCI protocol have several direct implications for clinical stroke management pathways. First, the protocol supports earlier aetiological clarity, enabling clinicians to make confident treatment decisions during the hospital admission window, rather than awaiting delayed outpatient investigations. In practice, this may accelerate the initiation of mechanism-specific secondary prevention while the patients are still under acute stroke unit care.

Second, CCI offers a pragmatic solution to limitation frequently encountered in real-world stroke work-up, such as delays in echocardiograph and fragmented

diagnostic pathways. By combining CCT, extended CTA, and MRI within fast-track session, it may streamline assessment and reduce the need for repeat investigation. However, its effectiveness remains dependent on local scanner capabilities and MRI access. Potential drawbacks included longer scan times, higher radiation exposure and over-reliance on CT for cardiac assessment. Similar one-stop CT approaches have demonstrated feasibility but also highlighted these operational and interpretive challenges (Austein et al., 2021).

The fast-track session design consolidates multiple diagnostic steps, potentially reducing time to diagnosis and the need for multiple outpatient visits. This is particularly valuable in system with resource constraints or fragmented referral pathways, where incomplete or delayed investigations contribute to therapeutic inertia.

Furthermore, the ability to detect multiple potential mechanisms within fast-track session allows for more comprehensive risk stratification. This may be especially beneficial in older patients or those with mixed vascular risk profile, where CT also offer an alternative to TOE, which despite being guidelines-recommended, is variably tolerated and inconsistently available.

While clinical outcomes were not significantly different within the 30-day follow-up of the study, the diagnostic efficiency demonstrated by CCI suggests its potential to influence long-term outcomes if integrated into routine pathways. A broader adoption of structured, comprehensive imaging at the point of initial stroke presentation could improve adherence to guideline-based secondary prevention and reduce the proportion of strokes classified as cryptogenic or ESUS in clinical practice. Early recurrence risk is highest within days of the index event, and evidence from EXPRESS study demonstrated that prompt initiation of secondary prevention reduces early recurrent stroke, even when treatment is initiated before complete aetiological clarification (Rothwell et al., 2007).

3.5.4 Feasibility and implementation considerations

The feasibility of CCI protocol was supported by several practical features observed during the study. First, 90% of participants in CCI group completed the full protocol-including brain MRI, CTA and CCT- within a single hospital encounter,

indicating high procedure tolerability and operational efficiency. However, as most participants had minor stroke or TIA and were clinically stable, generalisability to patients with more severe deficits or reduced capacity cannot be assumed.

Imaging was successfully acquired without sedation or heart rate control medications, and no major safety concerns or technical failures were encountered.

The use of a 320-detector row CT scanner and a 3T MRI system allowed rapid sequential acquisition, and all imaging components were interpreted by blind specialist radiologists. This confirmed that full diagnostic integration was achievable within routine workflow structure.

Importantly, the protocol did not increase hospital stay median inpatient duration was comparable between groups (63.5 vs. 60.1 hours, $p = 0.69$), suggesting that comprehensive imaging can be implemented without negatively affecting bed capacity or throughput. However, the absence of shorter stay despite earlier imaging completion suggested that other factors such as rehabilitation plane or discharge logistics were the determinants of the length of stay in this cohort.

Although these imaging platforms may not be universally available, they are increasingly present in tertiary stroke centres. The streamlined, predefined imaging workflow supported efficient scheduling and minimized redundant investigations. Patients' tolerance was high, and ECG-gated CCT avoided the need for TOE in many cases, reducing the burden on invasive testing. Scanning was completed in nearly all cases without motion artefact, and no participants required sedation.

The ability to complete brain cardiac and vascular imaging in a single session typically 40 to 50 minutes also offered notable operational efficiency during the inpatient stay. This streamline approach reduced the need for multiple separate imaging appointments and coordination across departments. While not formally assessed, this single-session model likely lessens procedural fatigue in older patients, particularly when multiple investigations are required during a short duration of time.

Staffing, access to advanced scanners, and workflow logistics will need consideration in wider implementation. Taking together, the results suggest that a streamlined, same-session CCI protocol is both clinically beneficial and practically workable within current acute stroke care system.

3.5.5 Radiation dose consideration

The inclusion of ECG-gated CCT and arch to vertex CTA in the comprehensive cardiovascular imaging (CCI) protocol introduces important considerations regarding patient radiation exposure. In this study, 34 of 50 patients in CCI group underwent both components within a single session under standardized research conditions, resulting in a mean effective dose of 17.3 mSv (median 17.6 mSv). Notably, 71% of patients received less than 20 mSv, remaining within the predefined ethical threshold.

These radiation exposure levels are consistent with recent literature on comprehensive cardiovascular CT protocols that incorporated cardiac and vascular imaging. Austein et al reported mean dose-length product (DLP) of 1834 mGy.cm in a similar setting using retrospective ECG-gating CCT, resulting in higher radiation exposure (Austein et al., 2021). In comparison, our use of prospective ECG gating and optimization techniques, including low tube voltage, adaptive current modulation, thin-slice collimation, rapid scan rotation and iterative dose reduction in 3D (AIDR 3D), enable dose efficiency without compromising diagnostic quality (McCollough et al., 2009, Kalender, 2014).

Our findings demonstrate effective dose management with standards recommended by UK Public Health England (PHE), which emphasises minimizing patient radiation exposure according to established national diagnostic reference levels (DRLs) (Public Health, 2018). Homsy et al. reported a median effective dose of 11.8 mSv using retrospective gated MDCT for LAAT assessment, although they used a narrower scan range and single-phase protocol (Homsy et al., 2016). Qiao et al., evaluating dual-phase CCT in over 1000 AF patients, estimated radiation doses of 4-7 mSv, reflecting a more focused cardiac-only scan range (Qiao et al., 2024).

Dose variability across our patient cohort (IQR 9.2 - 24.5 mSv) was influenced by anatomical differences and technical factors including BMI and heart rate variability. These observations are consistent with prior findings showing body habitus and cardiac output can significantly affect scan parameters and resultant radiation burden (Law et al., 2022, Sabarudin et al., 2019).

In summary, despite broader anatomical coverage, our CCI protocol-maintained radiation doses comparable to or lower than previously reported. These findings support feasibility and safety of integrating comprehensive CCT and CTA into stroke evaluation workflows without undue radiation risk when modern dose-reduction tools are employed.

3.5.6 Strengths and limitations

This study has several strengths. To our knowledge, this represents the first randomised controlled study to evaluate a single-session comprehensive cardiovascular and cerebrovascular imaging (CCI) protocol including brain MRI, CCT, and arch to vertex CTA in patients with acute stroke or TIA. This integrated imaging approach was designed to facilitate rapid, non-invasive, and comprehensive assessment of potential stroke aetiology within a single hospital encounter. The protocol demonstrated high feasibility with 90% of patients completing all imaging components in a single hospital visit and interpretation was performed by blinded subspecialty radiologist, without increasing hospital stay. The use of predefined ASCO-D classification and protocolised imaging pathways further strengthened the reliability and reproducibility of findings.

However, several limitations must be acknowledged. The study was conducted at a single centre with access to advanced imaging infrastructure, which may limit generalizability to non-tertiary or resource-limited settings. The sample size was modest, as appropriate for a pilot study, and the follow up period was limited to 30 days, preventing assessment of long term stroke recurrence or disability outcomes. Patient-reported outcomes such as satisfaction, comfort, or burden were not formally evaluated, and the study did not include cost-effectiveness analysis. Furthermore, most of the enrolled participants had TIA or minor stroke, which facilitated timely MRI acquisition during the acute phase. The applicability of this protocol to patients with severe stroke or patients with reduced capacity,

who may be medically unstable or unable to tolerate MRI in the hyperacute setting remains uncertain.

3.5.7 Future research direction

This pilot trial provides a strong foundation for designing and implementation larger, multicentre studies to evaluate the clinical utility and scalability of CCI protocol. Future trials should include more diverse hospital settings, including non-tertiary and resource-limited centres, to better assess generalisability. To establish clinical effectiveness, long-term follow up is required to assess the impact of CCI on stroke recurrences, functional recovery, and mortality. Additionally, studies should incorporate patients-report outcome (e.g. satisfaction, comfort, and perceived burden) to assess acceptability. Cost-effectiveness analysis is also critical to determine the economic viability of implementing CCI protocol at scale. The role of advanced post-processing technology, including AI-assisted ASCO-D classification and automated thrombus detection warrants exploration. Finally, studies should investigate modification of CCI protocol for patients with moderate to severe stroke who may be less able to tolerate MRI in the acute setting.

3.6 Conclusion

The discussion highlighted the feasibility of, diagnostic yield, and clinical promise of a fast-track single-session CCI protocol for acute stroke and TIA. By integrating ECG-gated CCT, CTA, and 3T brain MRI into a structured diagnostic pathway, the protocol achieved greater diagnostic completeness, faster aetiological classification, and improve identification of clinically actionable findings, all while maintaining safety and operational efficiency. This study represents the first randomised pilot trial to evaluate a fully integrated CCI strategy in the acute settings, supporting its novelty and clinical relevance. Despite being conducted in a single centre and involving mostly minor or TIA patients, the findings demonstrate that comprehensive imaging is achievable within a practical timeframe without adverse impact on hospital stay or patients' safety. These results support the continued refinement and evaluation of CCI in broader clinical populations and healthy systems. Its potential to transform stroke care lies in its

capacity to streamline decision-making, reduces diagnostic uncertainty, and deliver timely, mechanism-targeted secondary prevention in real-world practice.

4 Chapter 4-Impact of early imaging on secondary prevention.

4.1 Introduction

Early secondary prevention after ischemic stroke or TIA is essential to reduce the risk of recurrence, which is highest in the days and weeks following the initial event. Studies have shown that up to 25% of patients may experience stroke recurrence within 7 days if left untreated or if secondary prevention is delayed or suboptimal. International guidelines universally recommend the rapid initiation of risk factor modification and mechanism-specific therapy, including antiplatelets, anticoagulation, lipid-lowering, blood pressure control and specific vascular intervention such as carotid endarterectomy or carotid artery stenting where appropriate (Dawson et al., 2022, Powers et al., 2019).

Despite these recommendations, early therapeutic decision-making is frequently empirical in real-world practice. When diagnostic uncertainty persists-particularly in patients with incomplete classification (specified in ASCO-D as codes of 9) or embolic stroke of undetermined source (ESUS)-treatment decisions may be delayed, ambiguous, or based on probabilistic assumptions, and patients are commonly initiated on general secondary prevention measures, such as a single antiplatelet therapy, while awaiting further investigation. However, this empirical approach risks therapeutic mismatch. For example, a covert cardioembolic source (e.g., LAAT) may be later be identified, by which time the opportunity for early anticoagulation may be passed. Conversely, if cardiac imaging is delayed or absent, potential sources of embolism may be missed altogether, resulting in inappropriate or inadequate secondary prevention (Hart et al., 2014, Ntaios et al., 2015).

Evidence from trials as NAVIGATE EUSE and RESPECT ESUS has demonstrated that applying anticoagulation to all ESUS patients fails to improve did not reduce the risk of recurrent ischaemic stroke or systemic embolism compared to aspirin, while producing slightly higher rates of bleedings (Hart et al., 2019, Diener et al., 2019). However, the ESUS construct itself, first defined by Hart et al., describe a heterogenous population in whom embolic mechanisms coexist without a clearly

identified source, underscoring the diagnostic challenge that necessitates precise imaging-based difference (Hart et al., 2014). These findings reinforce the need for mechanism-tailored therapy, rather than empirical treatment based on incomplete aetiological diagnosis.

More recent trials such as ELAN and OPTIMAS have provided additional evidence suggesting that early initiation of direct oral anticoagulants (DOACs) after ischaemic stroke may be safe and effective in carefully selected patients with confirmed cardioembolic mechanisms (Fischer et al., 2023, Ng and Whiteley, 2017). Similarly, the therapeutic approach differs for other mechanism such as large artery atherosclerosis, SVD, or dissection. For example, dual antiplatelet therapy may benefit certain patient groups with non-cardioembolic stroke, as demonstrated in the CHANCE, POINT, and THALES trials, which predominantly enrolled patients with minor stroke or high-risk TIA (Johnston et al., 2018, Wang et al., 2013, Johnston et al., 2020). In contrast, for cervical artery dissection, evidence does not show superiority of anticoagulation over antiplatelet therapy; CADISS trial was underpowered and found no significant difference between treatment class (Markus et al., 2015). This underscores the clinical importance of accurate and timely aetiological classification- not just for risk stratification, but for rationalising treatment choice in the hyperacute and subacute phases.

Recent advances in stroke imaging enable more precise early detection of a broad range of underlying aetiologies. ECG-gated CCT and extended aorta-to-vertex CTA have significantly improved the detection of embolic and vascular sources such as left atria or left ventricle thrombi, aortic arch and vertebral atheroma, carotid or vertebral dissection, and intracranial atherosclerosis even in acute phase of stroke (Ghozy et al., 2024). At the same time, high-resolution MRI of the brain remains indispensable for evaluation of SVD, including white matter changes, lacunar infarction, and microbleeds. As well as acute infarct patterns that distinguish cardioembolic, lacunar, and watershed mechanisms. Together, these complementary imaging modalities offer a robust diagnostic platform to guide early, mechanism-targeted secondary prevention across the spectrum of stroke mechanisms.

Across healthcare settings diagnostic pathways and therapeutic protocols vary substantially depending on imaging availability, operator expertise, and

institutional protocols, further complicating the early management landscape. Moreover, delays in mechanism-specific therapy may reflect a broader phenomenon of diagnostic-treatment inertia, in which clinicians adopt conservative prescribing patterns despite the presence of clinically actionable diagnostic findings (Touzé et al., 2008).

While the diagnostic superiority of early CCI has been demonstrated (as shown in chapter 3, it remains unclear whether this diagnostic clarity translates into clinically meaningful changes in treatment timing, persistence, or aetiological alignment. Few studies have examined the real-world impact of comprehensive imaging on therapeutic behaviour during the first 30 days an interval marked by the highest risk of recurrence.

Ntaios et al., in a post hoc analysis of the NAVIGATE-ESUS trial, highlighted that although comprehensive diagnostic workup revealed multiple potential embolic sources in many ESUS patients, these findings did not consistently influence clinical management. They emphasized that current imaging use has not yet fully translated into improved treatment stratification in early ESUS care, reinforcing the disconnect between diagnostic findings and therapeutic action (Ntaios, 2020). However, this apparent disconnect may also reflect the limited evidence for mechanism-specific treatment strategies, which restrict their confident implementation in routine care.

This chapter builds on the diagnostic findings presented in chapter 3 and shifts focus towards clinical management and treatment outcomes. We assess whether early CCI leads to more timely initiation of therapy, greater treatment stability, and improved alignment between early aetiological classification (ASCO-D) and actual therapeutic decision, including procedure intervention such as carotid endarterectomy where indicated. In addition, the occurrence of new clinical events (stroke, TIA, death) within the follow-up period was analysed to evaluate the potential of an early CCI protocol to influence not only treatment behaviour but also recurrence. Although the present pilot study was not powered to detect differences in clinical outcomes, its findings provide preliminary feasibility data to inform the design and sample-size estimation of a larger trial capable of evaluating outcome effects.

By evaluating these dynamic endpoints in a prospective, randomised design, this chapter aims to determine whether fast-track CCI protocol can translate diagnostic precision into tangible improvement in therapeutic targeting and patients' outcomes during the subacute post-stroke period.

4.2 Methods

This chapter builds upon the diagnostic framework outlined in chapter 3, with a specific focus on treatment-related endpoints. Full details of the study design, setting, patient selection, imaging protocol, and aetiological classification methods are described in chapter 3.

4.2.1 Study population and design

The present analysis includes all patients who completed 30-day follow-up in the prospective, single-centre, randomised controlled pilot study trial comparing early comprehensive cardiovascular imaging (CCI) with routine diagnostic pathway in acute ischaemic stroke and TIA. Patients were allocated in a 1:1 ratio to either the CCI or routine investigation arm and followed for 30 days post-event.

4.2.2 Endpoints and definitions

The primary focus of this chapter is the evaluation of early treatment decisions and their alignment with diagnostic findings. The following endpoints were assessed:

- Initial secondary prevention strategy: defined as the first documented antithrombotic, lipid lowering and blood pressure lowering therapy initiated after admission, and recorded within the 30-day follow-up period
- Treatment persistence: assessed for key therapeutic classes using Kaplan-Meier survival analysis. This analysis examined treatment persistence, defined as the time from initial prescription of each therapy to the first documented modification or discontinuation within the 30-day follow-up period. Events represented any change or cessation of the initial therapy, and patients remaining on their baseline regimen were censored at Day 30.

Time-to-modification was calculated from time of admission to documented treatment change or cessation.

- Aetiological-treatment alignment: assessed based on the relation between ASCO_D classification at Day 7 and the corresponding treatment class. ASCO-D score of 1 were considered actionable for the purposes of treatment alignment analysis. Patients with multiple ASCO-D domains were included in each relevant domain subgroup and analysed descriptively.
- Generic (Empirical) therapy (ASCO-D 9): inferred from presence of ASCO-D 9 classification at Day 7 and modified treatment after Day 7. In the context, generic therapy refers to non-mechanism-specific secondary prevention measures initiated in the absence of a confirmed aetiological diagnosis, consistent with the concept of therapeutic inertia described by Touze` et al.(Touzé et al., 2008). Patients who remained ASCO-D 9 at Day 30 were considered to have unresolved aetiology.
- Final treatment adjustment and Day 30 ASCO-D classification: treatment changes occurring after Day 7 were analysed in relation to final ASCO-D classification at Day 30. Focus was given to patients who transitioned from ASCO-D 9 at Day 7 to defined mechanism by Day 30, as indicator of delayed aetiological clarification. This endpoint assessed whether early CCI reduced the frequency of late diagnostic reclassification and subsequent treatment modifications.
- New clinical events: defined as any new stroke, TIA or death occurring within the 30-day follow-up period, confirmed through clinical records.
- Procedural intervention: the occurrence of carotid endarterectomy or carotid artery stenting was recorded as an exploratory therapeutic decision.

4.2.3 Statistical analysis

All analyses were conducted using IBM SPSS statistics (v24). Descriptive statistics were presented as frequencies and percentage for categorical variables, and

medians with interquartile ranges (IQR) or means with standard deviation (SD) for continuous variables, depending on data distribution. Kaplan-Meier curves were used to evaluate time to treatment modifications for individual therapeutic classes, with group comparisons performed using the log-rank test. Between-group comparisons for categorical outcomes were conducted using Pearson's chi-square or Fisher's exact test as appropriate. A two-sided p-value < 0.05 was considered statistically significant. Where applicable, treatment alignment was further stratified by individual ASCO-D domains (A1, S1, C1, D1), including patients with multi-domain classification.

4.3 Results

To evaluate the clinical impact of early comprehensive cardiovascular imaging this chapter builds upon the diagnostic outcomes established in chapter 3. The baseline demographic and clinical characteristics were well balanced between the CCI and routine groups. There were no statistically significant differences in age, sex distribution, vascular risk factors, or stroke severity at presentation. However, given the modest sample size of the study, the absence of statistical significance does not exclude the possibility of clinically relevant baseline imbalances therefore, comparability should be interpreted with caution.

Patients in the CCI group demonstrated a significantly higher proportion of definite aetiological classification ASCO-D1 compared to those in the routine group at both Day 7 and Day 30, $p = 0.01$. Incomplete diagnostic work-up (ASCO-D 9), particularly in the cardiac and large artery domains, were markedly more frequent in the routine group (C9:70% vs. 4% , $p < 0.001$, A9: 16% vs.2%, $p = 0.01$ at Day 7), and although these improved by Day 30, diagnostic uncertainty persisted (C9: 66% vs. 2%, A9: 12% vs. 0%). Additionally, definite small vessel disease (S1) was significantly more frequently identified in the CCI group at Day 7 (44% vs. 16%, $p = 0.002$), reflecting the added value of early MRI in detecting lacunar infarcts. This diagnostic advantage in the CCI group was further supported by superior infarct detection rates on MRI (68% vs. 40%, $p = 0.005$) and higher completion rates of cardiac and aortic imaging, which identified more embolic substrates including PFO, LAAT, and aortic arch atheroma.

These findings provide the foundation for assessing whether early CCI translated into earlier treatment initiation, greater treatment persistence, and improved alignment between therapy and aetiological classification.

4.3.1 Initial secondary prevention strategies

Of the 100 patients analysed, secondary prevention therapies were initiated at similar frequencies in both groups, with no statistically significant differences observed. Among the CCI group, 24 (48%) patients received antiplatelet (aspirin or clopidogrel) monotherapy versus 22 (44%) in routine group. Dual antiplatelet therapy (DAPT) was prescribed in 15 (30%) patients in each group. Direct oral anticoagulants (DOACs) were commenced in 4 patients (8%) in the CCI group versus 3 patients (6%) in the routine group. Statins and antihypertensive drugs were initiated in 39, 14 patients of the CCI group and 15 patients in the routine group, respectively Figure 4-1. Across both groups, there was a clear preference for clopidogrel over aspirin when monotherapy was chosen, with 38 clopidogrel alone versus only 11 on aspirin alone. Notably, five patients in the CCI group did not reveal any evidence of treatment modifications or changes. This was attributed either to absence of imaging evidence of infarct, or the patients were already on an optimal long-term regimen prior to the admission. By comparison, only three patients in the routine group remained without treatment changes following their initial work-up.

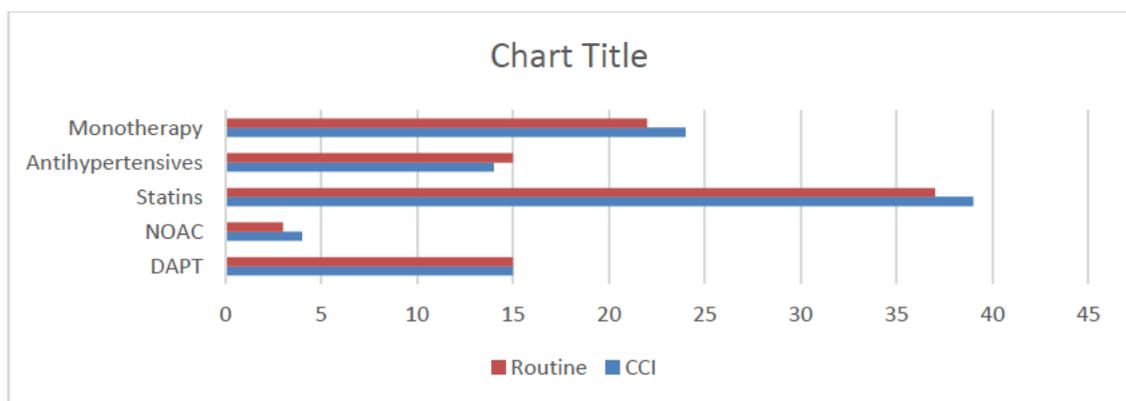


Figure 4-1. Distribution of initial secondary prevention therapies by treatment group (CCI vs Routine) following randomisation (n = 50 per group).

The figure illustrates secondary prevention regimen initiated within the 30-day follow-up period after randomization. Bars represent the number of patients receiving each therapy class, including monotherapy (aspirin or clopidogrel), dual antiplatelet (DAPT), direct oral anticoagulants (DOACs), statins, and antihypertensives.

4.3.2 Treatment persistence (Kaplan-Meier analysis)

4.3.2.1 Antiplatelet monotherapy

A total of 100 patients were analysed with 46 antiplatelet monotherapy (aspirin or clopidogrel) cases were initiated or changed and 54 censored cases. Event distribution was similar between groups (CCI 48%, Routine 44%). The mean time to treatment modification was comparable, and Median survival times could not be calculated owing to the low number of events. No significant difference between groups ($p>0.05$). Most changes occurred within the first 48 hours after admission. Therefore, the curves plateaued, indicated sustained persistence on the initial antiplatelet regimen through the 30-day follow up Figure 4-2.

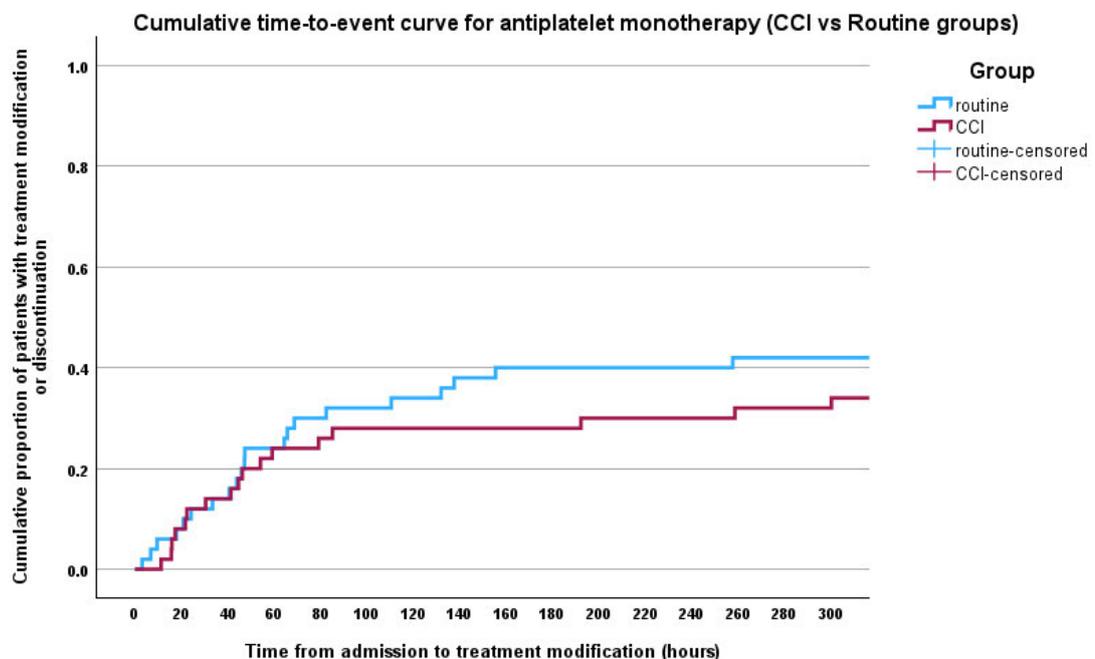


Figure 4-2 Cumulative time-to-event curve for antiplatelet monotherapy (CCI vs Routine).

The plot displays the cumulative proportion of patients who experienced treatment modification or discontinuation within 30 days (one minus survival function).

4.3.2.2 Dual antiplatelet therapy (DAPT)

Among the 100 patients analysed, including 30 DAPT cases were initiated or changed and 70 censored observations. Event distribution was 15(30%) in each group. The mean time to treatment modification was comparable between groups,

$p > 0.05$ most treatment changes occurred within the first 48 hours after admission, after which both curves plateaued, indicating stable persistence Figure 4-3.

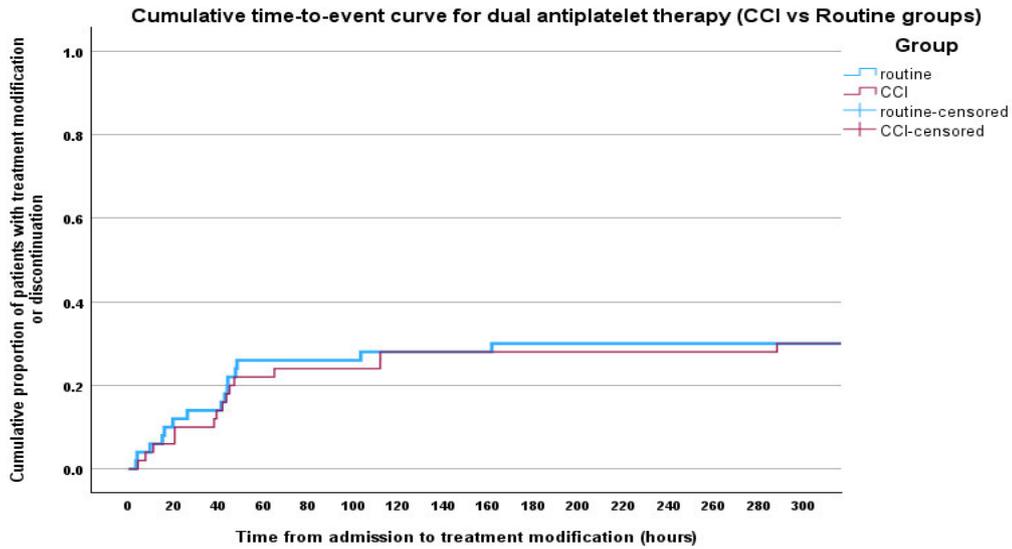


Figure 4-3 Cumulative time-to-event curve for dual antiplatelet therapy (DAPT) (CCI vs Routine).

The plot displays the cumulative proportion of patients who experienced treatment modification or discontinuation within 30 days (one minus survival function)

4.3.2.3 Direct oral anticoagulants (DOACs)

Only 7/100 commenced DOACs therapy during the study period- four in the CCI and three in the routine group. Because of the very small number of events and high censoring, formal time to event estimates such as median or percentile time were not calculated. The curves remained largely stable in both groups Figure 4-4

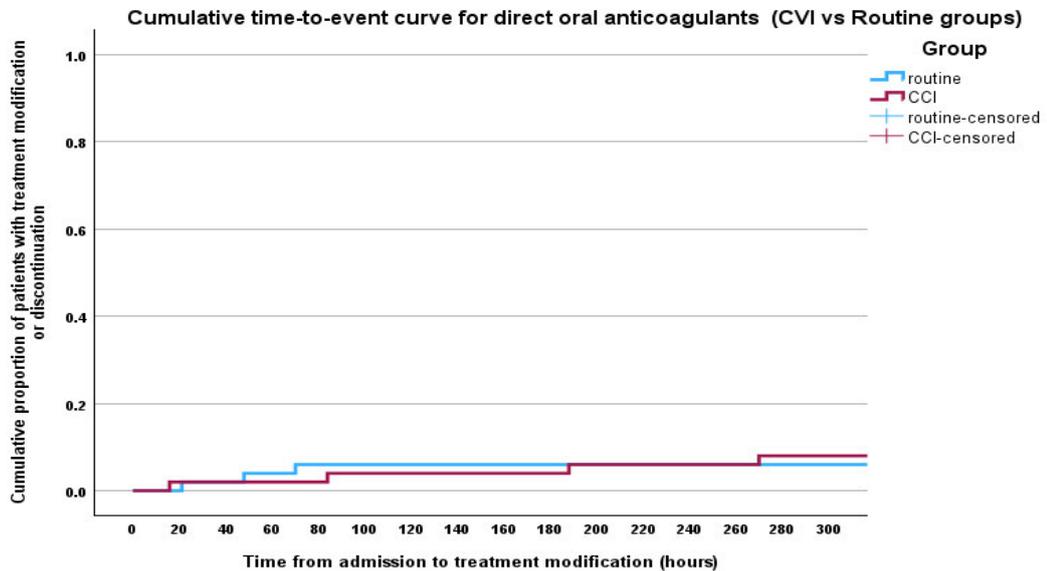


Figure 4-4 Cumulative time-to-event curve for direct oral anticoagulants (DOACS) (CCI vs. Routine).

The plot displays the cumulative proportions who experienced treatment modification or discontinuation within 30 days (one minus survival function).

4.3.2.4 Statin therapy

Statins were commenced or modified in 76/100 patients, 39 (78%) in the CCI arm and 37 (74%) in the routine arm. Event distribution was similar between groups, and no significant difference in treatment modification rates was observed, $p > 0.05$. The Kaplan-Meier curves demonstrated a steep change within the first 24-48 hours, followed by a prolonged plateau extending through the remainder of the 30-day follow-up Figure 4-5

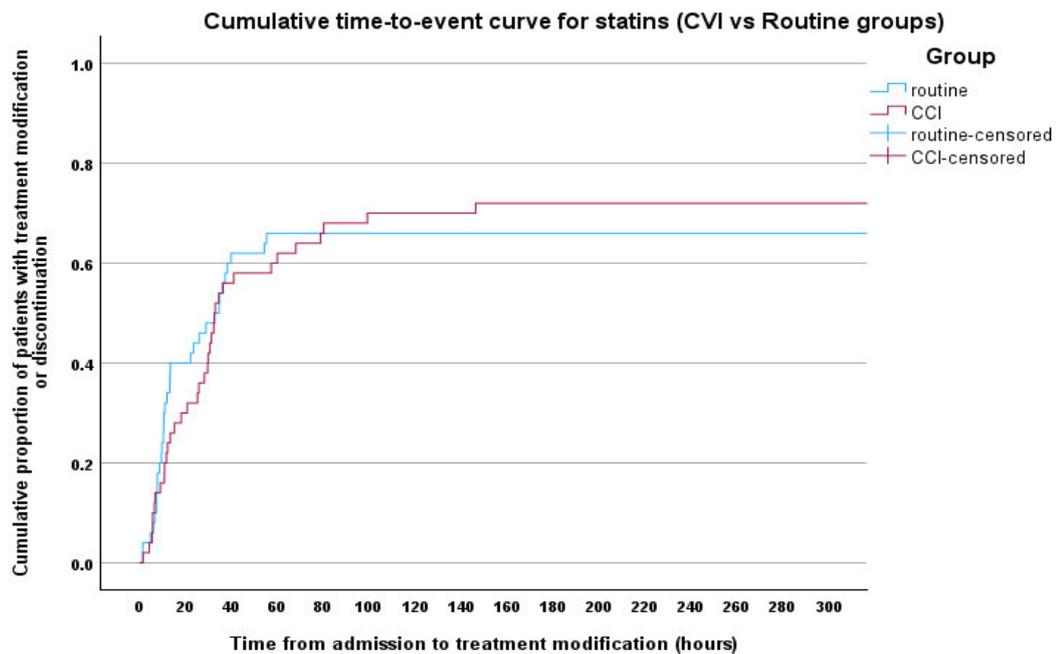


Figure 4-5 Cumulative time-to-event curve for statin therapy (CCI vs Routine).

The plot displays the cumulative proportion of patients who experienced treatment modification or discontinuation within 30 days (one minus survival function)

4.3.2.5 Antihypertensive therapy

Antihypertensive therapy was initiated or modified in 29/100 patients, 15 in the routine group, and 14 in the CCI group. The event distribution was similar between groups, and no significant difference in treatment persistence was observed, $p > 0.05$. Most changes occurred within the first 100 hours after admission, after which both curves remained parallel and stable Figure 4-6.

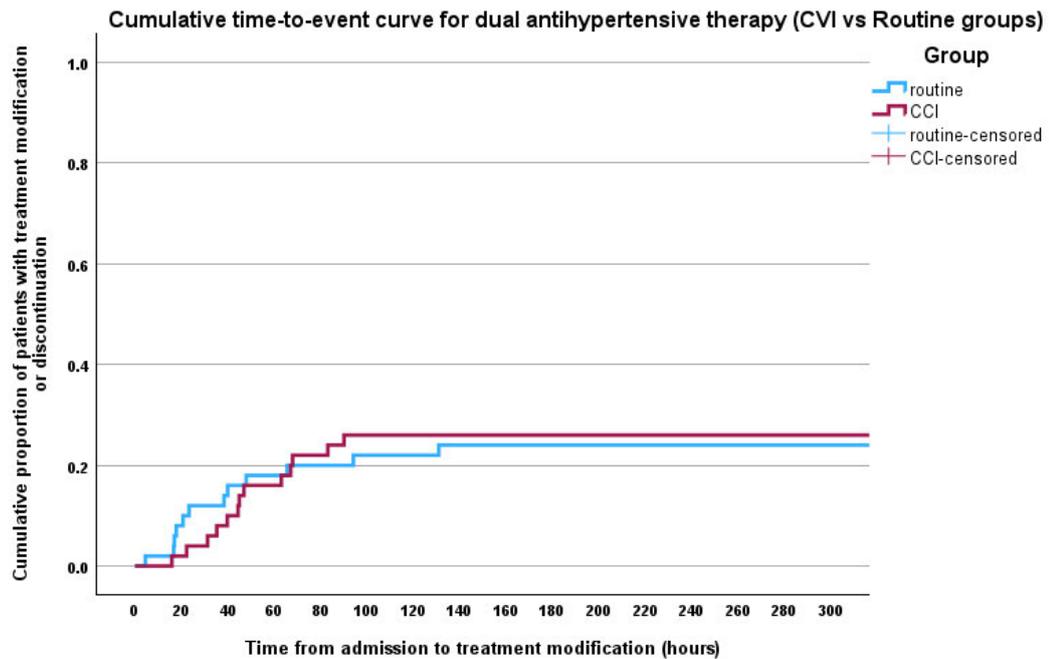


Figure 4-6 Cumulative time-to-event curve for Antihypertensive therapy (CCI vs, Routine).

The plot displays the cumulative proportion of patients who experienced treatment modification or discontinuation within 30 days (one minus survival function)

The following table summarises the Kaplan-Meier persistence estimates for each major secondary prevention therapy. The reported mean and percentile times represent the interval from hospital admission to the first treatment modification or discontinuation. Consistent with the Kaplan-Meier curves, most treatment changes occurred within the first 48-72 hours after admission, while the high mean estimates reflect the predominance of patients who remained on their initial regimen throughout the 30-day follow-up. No statistically significant differences in treatment persistence were observed between groups across any therapy Table 4-1.

Table 4-1 KM survival analysis of treatment persistence

Therapy	Group	Mean(hrs)	95% CI	Median	25 th	75 th	p-value
Monotherapy	CCI	400.1	307.1-493.0	-	-	41.4	0.60
	Routine	428.5	337.1-520.0	-	-	47.4	
DAPT	CCI	517.7	431.8-603.5	-	-	91.9	0.97
	Routine	679.6	635.2-723.9	-	-	48.2	
DOAC	CCI	667.3	617.7-716.9	-	-	-	0.70
	Routine	679.6	635.2-723.9	-	-	-	
statin	CCI	185.1	106.0-264.1	33.0	99.2	15.3	0.85
	Routine	215.2	130.7-299.7	34.9	-	13.2	
Antihypertensive	CCI	533.3	450.2-616.3	-	-	90.2	0.79
	Routine	531.2	449.0-613.4	-	-	131.0	

Values represent time from admission to the first treatment modification or discontinuation. High mean estimates reflect prolonged persistence due to predominance of censored observations at Day 30. For most therapies median could not be calculated due to small number of events.

4.3.3 Empirical therapy

To assess the impact of diagnostic uncertainty on treatment decisions, patients who received secondary prevention therapy up to Day 7 while having incomplete work-up (i.e. ASCO-D category 9 assigned for A, S, C, O, or D) were identified. These cases represent empirical therapeutic decisions made in the context of incomplete imaging work-up.

Among 100 patients, 30 in the routine group (60%) and only 2 in the CCI group (4%) received therapy while having at least one ASCO-D9 classification. This difference was highly significant, $p < 0.001$, indicating that early comprehensive imaging substantially reduced the frequency of provisional treatment based on incomplete work-up.

A more restrictive analysis was conducted to identify “true empirical” treatment - defined as therapy given in the absence of any ASCO_D 1 classification across all domains and the presence of at least one ASCO-D 9 classification. By this definition, 18 (36%) of routine patients (36%) and 1 (2%) of CCI were treated without any confirmed aetiology, $p < 0.001$ (Table 4-2). Among patients meeting this definition, antiplatelet monotherapy was most frequent used in 11 patients and DAPT in 8 patients. This highlights a tendency toward antiplatelet initiation in uncertain cases, particularly in the routine group.

Table 4-2 Empirical therapy Day 7 by group

Group	CCI n (%)	Routine n (%)	P value
Empirical therapy	2 (4)	30 (60)	< 0.001
True empirical therapy	1 (2)	18 (36)	< 0.001

A binary logistic regression was conducted to explore what factors predict the use of empirical therapy, and does early imaging (CCI) or the precedence of acute infarct independently reduce the likelihood of initiating treatment in the absence of a confirmed stroke mechanism? True-empirical was used as dependent variable and defined as initiation of therapy without any confirmed ASCO-D1 classification, in the presence of at least one ASCO-D9 domain at Day 7. Two predictors were entered: group allocation and acute infarct on brain imaging.

The analysis showed that being in the CCI group significantly reduce the odds of empirical treatment ($B = -3.11$, $p = 0.003$, $OR = 0.045$, $95\% CI: 0.006-0.356$). in contrast, acute infarct detection did not reach statistically significant (Table 4-3), despite being more prevalent in the CCI group (68% vs. 40%, $p = 0.005$, see chapter 3). This suggests that CCI protocol's value lies not only on visualising infarcts but in delivering broader diagnostic clarity, thereby reducing reliance on provisional or non-mechanism-based therapy.

Table 4-3. Binary logistic regression predicting empirical therapy at Day 7

Predictor	B	OR	95% CI for OR	P value
Group (CCI vs Routine)	-3.476	0.031	0.003-0.281	0.002
Acute infarct(present)	0.997	2.711	0.440-16.691	0.282

4.3.4 Aetiological confirmation by day 30 and its impact on treatment modifications.

To evaluate whether empirical treated patients subsequently received a definitive aetiological diagnosis that justified their therapy, all individuals who received true empirical therapy at Day 7 were reassessed at Day 30. True empirical therapy was defined as the initiation of secondary prevention in the absence of any confirmed ASCO-D1 classification and the presence of at least one domain classified as ASCO-D9. Among the 19 patients who met the definition (18 routine, and 1 CCI), two patients were reclassified as A1 by day 30 -one from each group - and both had been initiated on DAPT at baseline. This retrospectively validated their empirical treatment decision. Additionally, three other patients (one in the routine group and two in the CCI group) underwent diagnostic reclassification to S1 by Day 30. All had been initiated on SAPT, thus achieving delayed alignment with an ultimately confirmed stroke. While most empirical therapy remained unconfirmed by Day 30, two cases (one per group) were reclassified to ASCO-D1, creating incidental alignment. These few instances highlighted that empirical treatment may not always correspond to the eventual confirmation aetiology. Early CCI protocol therefore remains valuable for refining and personalising secondary prevention once diagnostic clarity is achieved.

4.3.5 New clinical events within 30 Days

To assess short-term safety and potential benefit of early diagnostic imaging, new clinical events occurring within the 30-day follow-up period were evaluated. These events included recurrent ischemic stroke, TIA, or death. Event identification was confirmed through clinical records. Among 100 patients, a total of 6 new events were recorded. The routine group had four events: two ischemic stroke, one TIA, and one death. The death occurred due to myocardial infarction. All three non-fatal events occurred in patients classified as S1 at Day 7, while the patient who died had no confirmed mechanism and remained C9, D9, reflecting unresolved cardiac and dissection domains. The CCI group had two new events, both were TIAs, and both patients had an S1 classification.

No death or recurrent stroke occurred in the CCI group. While the total number of new events was higher in the routine group (8% vs.4%), this difference did not reach statistically significant (Fisher 's exact test $p = 0.67$). All non-fatal recurrent cerebrovascular events occur in patients with definite small vessel disease (S1), underscoring the persistent risk of recurrence even in non-embolic mechanism. The only death occurred in a patient with incomplete imaging work-up (C9 and D9) in the routine group, highlighting the potential risk of adverse outcome in the context of unresolved aetiology. Although limited by event count, these findings support a possible trend towards reducing clinical recurrence in CCI arm, aligning with improve diagnostic precision and earlier tailored treatment.

4.3.6 Procedural intervention

As an exploratory endpoint, the occurrence of procedural intervention specifically carotid endarterectomy (CEA) was examined to assess whether early comprehensive imaging facilitates timely referral for revascularisation in patients with significant large artery disease. Two patients underwent CEA during the 30-day follow-up: one patient in CCI group found to have more than 75% right ICA stenosis based on extended CTA. CEA was performed five days post imaging confirmation, following multidisciplinary evaluation and surgical planning. In the routine group one patient was diagnosed with high grade ICA stenosis and CEA was performed on the sixth day after imaging confirmation. Both patients received DAPT prior to surgery.

4.4 Discussion

4.4.1 Summary of key findings

This study evaluated whether early comprehensive cardiovascular imaging (CCI) meaningfully influenced secondary prevention decision in patients with acute ischemic stroke or TIA. The findings demonstrated that, although the overall selection of secondary prevention therapies was similar between the CCI and routine care groups, the CCI arm achieved greater diagnostic clarity by Day 7. Enabling more mechanism-informed therapeutic decisions. In parallel, empirical treatment based on incomplete imaging work-up (ASCO-D 9) was markedly reduced, reflecting the practical impact of early comprehensive imaging on therapeutic confidence.

Kaplan-Meier analysis showed that early therapeutic decisions were typically stable over the 30-day follow-up. However, in the CCI group, these decisions were more likely to be mechanism-informed rather than empirical, reflecting the clinical utility of earlier aetiological classification. No deaths or recurrent strokes occurred in the CCI group, compared to two stroke and one death in routine group, though the overall event rate was low and did not differ significantly between groups.

Taken together, these findings suggest that early CCI may enable more timely, evidence-aligned treatment decisions during the high-risk early phase of stroke care, without compromising workflow feasibility or safety. Whether this translates into reduction in stroke recurrence, cardiovascular events or deaths is uncertain.

4.4.2 Empirical treatment and diagnostic uncertainty

The analysis revealed a significant difference in the frequency of empirical treatment between groups, with the routine arm demonstrated a higher reliance on initiating secondary prevention in the absence of definite stroke mechanism.

The clinical implications of empirical therapy are well established. Touze et al. noted that delayed or incomplete investigation often contribute to therapeutic inertia, whereby generalised prescribing persists despite opportunities for more appropriate secondary prevention strategies (Touzé et al., 2008) In acute stroke

management, this can result in inadequate protection against early recurrence, particularly in patients with unrecognised cardioembolic sources (Ntaios et al., 2015) .

Large, randomised trials in embolic stroke of undetermined source (ESUS) further highlighted the limitations of empirical therapy. The NAVIGATE ESUS and RESPECT ESUS trials found no benefits of empirical anticoagulant over antiplatelet therapy in the absence of confirmed embolic mechanism and reported an associated increase in bleeding risk as a therapy side effect (Diener et al., 2019, Hart et al., 2019). These findings underscore the importance of establishing a clear aetiological diagnosis before initiating mechanism-tailored therapy.

In this study, logistic regression confirmed that allocation to the CCI group was independently associated with a lower likelihood of empirical treatment, regardless of whether an acute infarct was detected. This indicates that the benefit of CCI extends beyond infarct identification alone and lies in its capacity to provide comprehensive diagnostic information across ASCO-D domains. By clarifying the underlying aetiology early, CCI enable more confident and mechanism-guided treatment decision during the subacute phase of stroke care.

Among patients who received empirical treatment, antiplatelet therapy was the most frequently selected, either single or dual, reflecting current evidence-based practice rather than therapeutic bias. Early aspirin administration within 24-48 hours and short-term dual antiplatelet therapy for minor stroke or high-risk TIA are both supported by international guidelines and randomised trials (Dawson et al., 2021, Kleindorfer et al., 2021) Anticoagulant is appropriately reserved for cardioembolic stroke, which accounts for fewer than 20% of cases, and its optimal timing of initiation remains uncertain.

In the Athens Stroke Registry, Ntaios et al. reported that most patients with embolic stroke of ESUS were initially treated with antiplatelet despite later identification of embolic mechanisms (Ntaios et al., 2015). While such empirical choices may appear pragmatically cautious, they risk misalignment with the patient's eventual aetiology. Yaghi et al. further highlighter that, in absence of early diagnostic clarification, clinicians often default to antiplatelet therapy as an

empirical treatment, despite the recognised heterogeneity of ESUS many of which may warrant anticoagulant rather than antiplatelets (Yaghi et al., 2017).

In this context, the potential added value of early comprehensive cardiovascular imaging lies in identifying structural cardioembolic substrates such as LAT, PFO, or complex aortic atheroma, before AF is detected, therapy refining anticoagulant decisions.

4.4.3 Treatment persistence across therapeutic classes

The analysis of treatment persistence across key secondary prevention strategies showed broadly similar between the CCI and routine groups. Kaplan-Meier survival analysis revealed no statistically significant differences in the timing or durability of single antiplatelet therapy (SAPT), DAPT, statins, antihypertensive agents, or oral anticoagulants.

For SAPT, most treatment modifications occurred within the first 48 hours after admission, after which both groups demonstrated stable persistence through 30 days. This likely reflects SAPT use was comparable across groups and consistent with current guidelines-based practice, where early initiation of SAPT is recommended for most non-cardioembolic stroke and maintained as where early aggressive therapy may be unnecessary and emphasized that its initiation is not time sensitive in acute phase (Johnston et al., 2018, Johnston et al., 2016). The observed stability of SAPT over follow-up therefor reflects persistence on appropriate therapy rather than delay or modification, supporting that both study pathways adherence to established secondary prevention standards.

Across both groups, clopidogrel was the predominant choice when monotherapy was used (38 patients vs. 11 patients on aspirin). The marked preference for clopidogrel reflects current clinical practice, supported by meta-analytic data indicating improved cardiovascular outcome and reduced bleeding compared to aspirin monotherapy in post-stroke patients (Paciaroni et al., 2019).

DAPT was prescribed mainly for large artery diseases or high-risk minor stroke/TIA, consistent with current evidence and guidelines recommendation. The

short-term use of DAPT reflects the risk-benefit crossover, where recurrent ischaemic risk falls as bleeding risk persists (Dawson et al., 2021).

In contrast to SAPT, oral anticoagulants were prescribed to a small subset, typically those with confirmed cardioembolic sources or arterial dissection. Anticoagulants were typically initiated only after definitive diagnosis clarification, reflecting its mechanism-specific use.

Because early anticoagulation is time sensitive and safety dependent, accurate early aetiological clarification is essential. Historically, anticoagulation after cardioembolic stroke has been delayed for up to two weeks due to concerns about haemorrhagic transformation, particularly in patients with large infarct or uncontrolled hypertension (Powers et al., 2019). However, timely initiation is critical to prevent early recurrence once a cardioembolic mechanism is confirmed. The CRYSTAL-AF trial demonstrated that occult AF is frequently detected only after prolonged cardiac monitoring, underscore that delayed identification of embolic sources can postpone anticoagulant therapy and exposed patients to recurrent events (Sanna et al., 2014).

More recent evidence challenges the traditional delay. The ELAN and TIMING trials showed that, in patients with minor or moderate stroke, early DOAC initiation (within 48 hours to 6 days) was safe as later treatment and may reduce recurrent ischaemia events without increasing intracranial bleeding (Oldgren et al., 2022, Fischer et al., 2023). Accordingly, early CCI, by identifying structural Atrial stasis, or complex AA, can provide the diagnostic confidence necessary to start DOACs safely and at earlier, more effective time point.

For Statins and antihypertensive therapy, Kaplan-Meier analysis showed similar time to initiation or modification patterns between groups, with no statistically significant differences observed. Statin were initiated or modified and maintained consistently across both groups, in accordance with international guidelines recommendations advocating early high-intensity therapy for nearly all patients with ischaemic stroke or TIA, irrespective of underlying aetiology (Kernan et al., 2014, Amarenco and Labreuche, 2009). Antihypertensive therapy showed a comparable pattern. These findings suggested that blood pressure control and

statins therapy are largely initiated early based on vascular risk profiles rather than specific diagnostic information (Kleindorfer et al., 2021).

Taken together, the results show that although CCI improve diagnostic precision and facilitated mechanism-specific therapeutic choices, it did not materially alter the timing and persistence of secondary-prevention therapies across drug. The consistent use of SAPT, DAPT, statins, and antihypertensives reflects adherence to standards stroke-unit and international guidelines protocols mandating prompt evidence-based treatment.

The principal impact of CCI lay in what therapy was selected; most notably the more targeted use of anticoagulant and the reduced reliance on empirical prescribing; rather than in when therapy was initiated or modified. This highlights CCI's strengthening the link between accurate diagnosis and tailored secondary prevention in acute stroke care.

4.4.4 Clinical events and early recurrence

Within 30 days, six new clinical events were recorded-two in the CCI group (both TIAs) and four in the routine group (two recurrent stroke, one TIA, and one death). Although the differences between groups was not significant (4% vs. 8%, $p = 0.67$), the distribution of event types was clinically meaningful: no recurrent stroke or death occurred in the CCI group.

The recurrent rate among the whole cohort was 5%. This aligns with findings from large prospective cohorts reporting 30-day recurrence rates between 5-10 % after TIA and minor stroke population (Rothwell et al., 2007, Amarenco et al., 2016). Notably, in our study, all non-fatal recurrent stroke or TIA events occurred in patients classified as S1 (definite SVD), reinforcing the growing recognition that SVD, while often perceived as low risk, may still confer a substantial short-term recurrence risk. Recent studies emphasized its potential association with early recurrence and functional deterioration, especially in patients with a high burden of microangiopathy (Goeldlin et al., 2023, Ren et al., 2018). These findings underscore the importance of including SVD within early secondary prevention strategies and ensuring diagnostic classification accounts for recurrence risk across all aetiological domains.

4.4.5 Clinical impact of early diagnostic clarification

The diagnostic advantages established in chapter 3 particularly earlier and more complete ASCO-D1 classification, had measurable therapeutic consequences. Patients in the CCI group achieved faster and more definitive stroke mechanism identification, which enable more targeted therapy and reduced reliance on empirical prescribing. In the routine care group, SAPT was more commonly used in the context of diagnostic uncertainty. While the timing of initiation did not differ significantly between groups, the rationale for prescribing was more frequently mechanism-guided in CCI arm.

CCI also, supported earlier procedural referral. One patient in the CCI group underwent carotid endarterectomy one day earlier than the matched case in the routine group; while this isolated observation is anecdotal, it illustrates the potential for early imaging to inform procedural planning where appropriate.

Finally, while clinical events were infrequent, recurrent stroke and death cases occurred in the routine group. This pattern reinforces the potential benefit of early aetiological clarification especially in SVD, where early targeted prevention may mitigate short-term recurrence risk.

Taken together, these findings highlight that the CCI protocols diagnostic gains translated into improved treatment precision, reduced therapeutics uncertainty, and earlier clinical action, making a step toward more pro-active mechanism-based stroke care.

4.4.6 Limitations

This pilot study has key limitations. It was conducted at a single tertiary centre with advanced imaging infrastructure, which may limit applicability in broader healthcare settings lacking similar resources.

The modest sample size, though appropriate for feasibility evaluation, limited the power to detect differences in clinical outcomes. Trends observed, such as reduced empirical treatment and earlier targeted intervention, therefore warrant confirmation in larger studies.

Follow up was limited to 30 days, capturing early therapeutic decision but not long-term adherence, recurrence, or outcomes.

Treatment decisions, though linked to diagnostic findings, may have been influenced by clinician preferences or prior outpatient management, factors not fully controlled in this design.

Finally, the study focused on patients with minor stroke or TIA, limiting generalizability to those with more severe presentations who may be less suitable for CCI protocol.

Despite these constraints, the study provides important early evidence supporting the feasibility, safety, and potential clinical value of fast-track cardiovascular imaging in acute stroke care.

4.4.7 Clinical implications and future research

The findings of this pilot study have practical implications for acute stroke care. Early CCI enabled more accurate and timely aetiological classification, facilitating mechanism-informed therapeutic decision. This supports the role of CCI as a decision-making tool, not only for diagnostic clarity, but for initiating appropriate, mechanism-tailored secondary prevention during the critical early phase.

Despite similar treatment persistence across groups, early CCI appeared to shift the rationale behind therapy choice. This highlighted the potential of early imaging to reduce empirical prescribing and improve therapeutic precision without prolonging hospital stay.

Although the trial was not powered for clinical outcome, the lower recurrence and mortality rate observed in the CCI group suggest a possible benefit that warrants further investigation. These findings are consistent with the hypothesis that enhanced diagnostic resolution enables earlier, risk-targeted intervention, and safer initiation of mechanism-appropriate therapy.

Future studies should assess the long-term effect of CCI protocol on recurrence, disability, and mortality. Large, multicentre trials are needed to confirm

generalizability, including in non-tertiary settings. In addition, economic evaluation and patients-reported outcomes should be incorporated to inform implementation. Protocol adaptation for patients with moderate to severe stroke, who may be unable to tolerate MRI, represents another priority for future research.

5 Chapter 5-Mediation Analysis of Imaging Modality and Timing.

5.1 Introduction

In this pilot study, patients were randomised not to a single imaging investigation, but to structured diagnostic protocol comprehensive cardiovascular imaging (CCI) that included expedited brain MRI (3T), cardiac CT, and extended CTA. As such, the randomised group allocation represents a complex diagnostic strategy where multiple diagnostic tests were deployed in a coordinated way. While the CCI strategy resulted in higher rates of definite aetiological classification by Day 30, the mechanism underlying this effect remains uncertain. It is not impossible to state which individual component of the protocol - or whether the speed of access to imaging- accounts for the observed outcome differences. Understanding which components of the CCI pathway contribute most to diagnostic certainty could help streamline future imaging protocols and improve resource allocation.

To explore these aspects, we used mediation analysis to assess whether specific imaging modalities (brain MRI or cardiac CT), or their timing, served as intermediate variables (mediators) in the pathway between group allocation and diagnostic certainty (ASCO-D=1). Mediation analysis may aid understanding of how or why a diagnostic strategy like CCI influences outcomes. It does this by breaking down the total effect into two parts: the direct effect (e.g. CCI leading to better classification on its own), and an indirect effect (where CCI influences something else such as use of imaging or timing which in turn affects the outcome) Figure 5-1.

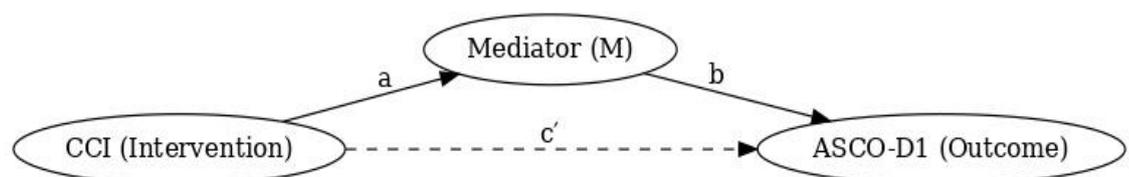


Figure 5-1 Conceptual model of mediation analysis.

Mediation is commonly implemented using a regression-based approach. This involves first modelling the relationship between the exposure and the mediator (path a), followed by a second model estimated the outcome as a function of both the exposure and mediator (paths b and c'). the total effect of exposure on the outcome (path c) can then be partitioned into a direct effect and indirect effect, allowing interpretation of whether-and to what extend- the impact of complex intervention is transmitted through intermediate variables. This approach is especially useful when the intervention involves multiple elements that cannot be isolated via randomisation alone.

Prior studies have shown that imaging can directly influence stroke classification by detecting covert mechanisms such as small vessel disease or left atrial thrombus, which may not be evident on initial clinical evaluation alone (Wardlaw et al., 2019). Therefore, both the type and timing of imaging may plausibly mediate the effect of a structured strategy. By exploring these pathways, we aim to identify which imaging components are most influential in improving aetiological certainty.

Previous research has demonstrated the value of mediation analysis in stroke care by identifying how specific processes or behaviour may explain the effect of a complex intervention on clinical outcomes. Parry-Jones et al. applied causal mediation analysis in a real-world study of patients with intracerebral haemorrhage, showing that improvements in supportive care processes such as delaying early do-not-resuscitate orders and increasing admission to critical care, but not the major components of the protocolised interventions themselves, substantially mediated the reduction in 30-day mortality associated with a standardised care bundle. This study illustrates how mediation analysis can be used to evaluate the mechanisms of complex, multi-component interventions, particularly in situations where randomising individual elements is not feasible (Parry-Jones et al., 2019).

We focus on two hypotheses: 1- whether undergoing MRI or cardiac imaging explains the association between group allocation and ASCO-D1; and 2- whether faster access to imaging after a stroke event mediated the same relationship. These two hypotheses represent different conceptual mechanisms - one based on imaging selection, and the other on imaging timing.

To test these hypotheses, we used both logistic regression with the Sobel test for binary mediators such as MRI or Cardiac imaging, and the PROCESS macro (model 4) in SPSS for continuous mediators such as time to imaging.

This chapter presents the results of four mediation models:

CCI → MRI → ASCO-D1
 CCI → CCT → ASCO-D1
 CCI → time to MRI → ASCO-D1
 CCI → time to CCT → ASCO-D1

We compared the direct and indirect effects across these models to investigate whether imaging utilisation, timing, or both mediated the observed benefit of the CCI protocol.

5.2 Methods

Mediation analysis was conducted using two approaches based on the type of mediator. For binary mediators, such as whether MRI or CCT was performed, we used manual logistic regression modelling combined with Sobel test for indirect effects. This was necessary because the PROCESS macro for SPSS (V 5.0), used within the SPSS statistics version 29, while robust for many mediation scenarios, does not support binary mediators due to its reliance on ordinary least squares (OLS) regression to estimate indirect pathways (Hayes, 2017). PROCESS does, however, support logistic regression for binary outcomes, which enabled us to use it for models where the mediator was continuous e.g. time to MRI or CCT.

Alternative mediation frameworks were considered, including logistic structural equation modelling (SEM) and causal mediation analysis. However, these approaches were deemed not to be feasible due to limitations of sample size, model complexity, and implementation constraints in SPSS (Imai et al., 2010, Muthén, 2002). PROCESS was selected for continuous mediators because it is well-established in behavioural and clinical research, provides clear estimation of direct and indirect effects, and supports bootstrapped confidence intervals for improved accuracy and robustness.

Bootstrapping was selected for estimating indirect effects in PROCESS because it does not rely on the assumption of normality of the sampling distribution, an important consideration given the modest sample size and potential skewness in the time-to-imaging variable (Preacher and Hayes, 2004). This enhances statistical validity and provides more reliable confidence intervals around the indirect effect estimate.

This dual approach allowed us to analyse both types of meditation pathways, categorical and continuous, using valid statistical techniques tailored to each case, while ensuring comparability of direct and indirect effect estimate across the four models tested.

Data obtained from the D-CCIST study described in chapter 3, which investigated the effect of early CCI protocol on stroke classification was used. We applied the Baron and Kenny framework (Baron and Kenny, 1986) and the PROCESS macro (Model 4) in SPSS to evaluate mediation. The independent variable (X) was randomized group allocation. The dependent variable (Y) was achievement of definite aetiological classification (ASCO-D1). Mediators (M) included MRI performed. Cardiac imaging, time from admission to MRI in hours, and time from admission to cardiac imaging in hours.

For binary mediators such as MRI and CCT use, we used manual logistic regression and Sobel testing as PROCESS macro does not support binary mediators. For continuous mediators, such as “time to scan”, we used PROCESS model 4, which applies ordinary least squares regression for continuous mediators and logistic regression for binary outcomes, allowing for bootstrapping confidence intervals.

Full imaging protocols are detailed in chapter 3. Briefly, patients underwent brain MRI though scanner type and timing differed: CCI group received early 3T MRI, while routine group typically involved 1.5T MRI. Cardiac imaging included cardiac CT in CCI group and TTE or TOE in routine care. The CCI protocol also incorporated extended arch-to-vertex CTA to evaluate extracranial and intracranial vasculature. For mediation analysis, imaging use and timing were treated as mediators, without further stratification by imaging technique.

For each mediation model, we evaluated the association between group allocation and the mediator (Path a), followed by relationship between the mediator and ASCO-D1 classification (path b). The indirect effect was tested using Sobel test or bootstrapped confidence intervals. The direct effect (path c) was assessed by including both randomised group allocation and the specific imaging variables (mediators) in the logistic regression model, allowing us to determine whether group assignment predicted diagnostic classification independently of the imaging performance.

5.3 Results

5.3.1 MRI use

5.3.1.1 Total effect: association between group allocation on ASCO-D1 classification (path c)

A binary logistic regression was performed to assess the total effect of randomised group allocation on the likelihood of achieving a definite aetiological classification. The model was statistically significant $p = 0.006$ with $R^2 = .102$, indicating that approximately 10.2% of variance in ASCO-D1 classification was explained by group allocation alone. Patients in the CCI group were significantly more likely to receive a definite score ASCO-D1 compared to the routine group, $B = 1.153$, $SE = 0.417$, $Wald = 7.637$, $p = 0.006$, with an odds ratio $\text{Exp}(B)$ of 3.17. This suggests that participants in CCI arm had more than three times the odds of achieving diagnostic certainty by Day 30.

5.3.1.2 Association between group allocation and brain MRI use (path a)

A binary logistic regression was conducted to examine the association between group allocation and the likelihood of undergoing brain MRI. The model was statistically significant $p < 0.001$, explaining approximately 27% of variance in MRI use ($R^2 = 0.268$). Group allocation significantly predicted MRI use, the odds ratio was 21.00, $B = 3.045$, $SE = 1.56$, $Wald = 8.308$, and $p = 0.004$, indicating that assignment to the CCI arm was associated with 21-fold higher odds of receiving MRI.

5.3.1.3 Association between brain MRI use and ASCO-D1 classification (path b)

A binary logistic regression was conducted to examine the effect of undergoing brain MRI on achieving definite aetiological classification (ASCO-D1). The model was non-significant, $p=0.078$, indicating that MRI use was not significantly associated with ASCO-D1 classification. The odds ratio ($\text{Exp}(B)$) was 2.80, suggesting that patients who underwent MRI were approximately 2.8 times more likely to achieve ASCO-D1 classification, although this effect did not reach statistical significance.

The regression coefficient for MRI use was $B = 1.03$, with $SE = 0.58$, and $Wald = 3.11$. the R^2 was 0.044, indicating a small proportion of variance in ASCO-D1 was explained by brain MRI use.

5.3.1.4 Combined model: independent effects of group allocation and brain MRI use on ASCO-D1 classification (paths b and c')

A binary logistic regression was conducted to assess the combined effects of group allocation and brain MRI use on achieving a definite ASCO-D1 classification. The model was statistically significant $p = 0.013$, indicating that the predictors jointly explained a significant proportion of variance in ASCO-D1 classification. The model explained 11.1% of variance ($R^2 = .111$).

In this model group allocation remained a significant predictor of ASCO-D1 classification, $B = 1.016$, $SE = 0.446$, $Wald = 5.179$, and $p = 0.023$, corresponding to an odds ratio of 2.76, indicating that patients in CCI group had 2.76 times higher odds of receiving a definite aetiological classification compared to the routine group, even when MRI use was included. However, brain MRI use itself was not a statistically significant predictor in this model, $B = 0.518$, $SE = 0.628$, $Wald = 0.680$, $p = 0.412$ and $\text{Exp}(B) = 1.68$, indicating that although brain MRI use was more frequent in the CCI group, it did not independently explain the association between group allocation and diagnostic certainty.

5.3.1.5 Indirect effect of group allocation on ASCO-D1 via MRI use (a x b)

To test the significance of the indirect effect of group allocation on ASCO-D1 classification through MRI use, a Sobel test was conducted using unstandardised

coefficient and standard errors from the logistic regression models for path a and b. The test yielded a Sobel test of 1.31, with a two-tailed p-value of 0.189, indicating that the indirect effect was not statistically significant. This suggests that while brain MRI use was more frequent in CCI group and was associated with higher odds of achieving ASCO-D1 score, it did not significantly mediate the effect of group allocation on diagnostic certainty Figure 5-2.

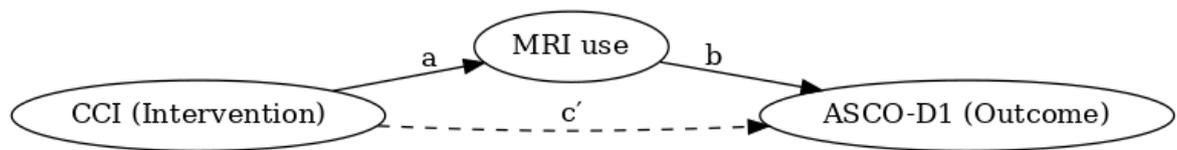


Figure 5-2 Mediation model: CCI → MRI → ASCO-D1

5.3.2 Cardiac imaging use

As previously reported in section 1.3.1.1, group allocation significantly predicted ASCO-D1 score (path c), with patients in the CCI group having over three times the odds of definite classification compared to the routine group.

5.3.2.1 Association between group allocation and cardiac imaging use (path a)

To assess whether group allocation predicted the likelihood of undergoing cardiac imaging (echo or CCT), a binary logistic regression was performed. The model was statistically significant, $p < 0.001$, indicating that group allocation significantly influenced imaging use, $R^2 = 0.615$. Patients in CCI group had significantly higher odds of undergoing cardiac imaging compared to those in routine group, with $B = 2.855$, $SE = 0.609$, $Wald = 40.063$, $p < 0.001$, and odds ratio of 47.25.

5.3.2.2 Association between cardiac imaging and ASCO-D1 classification (path b)

A binary logistic regression model was used to examine the association between receiving cardiac imaging and the likelihood of achieving ASCO-D1 score. The model was statistically significant $p = 0.009$, explaining approximately 8.7% of variance in ASCO-D1, $R^2 = 0.087$. Cardiac imaging was found to be a significant predictor of definite score ASCO-D1, with odds ratio of 2.88, $B = 1.059$, $SE = 0.415$, $Wald = 6.515$, and $p = 0.011$ suggesting that patients who underwent cardiac

imaging were nearly three times more likely to achieve a definite aetiological classification.

5.3.2.3 Combined model: independent effects of group allocation and cardiac imaging on ASCO-D1 (paths b and c')

A binary logistic regression model was used to assess the joint effects of group allocation and cardiac imaging on the likelihood of achieving ASCO=D1. The model was significant, $p = 0.014$, and explained 10.9% of the variance in ASCO-D1 score, $R^2 = 0.109$. In this model, neither cardiac imaging use nor group allocation remained statistically significant predictors. Group allocation had a coefficient of $B = 0.811$, $SE = 0.603$, $Wald = 1.813$, $p = 0.178$. Cardiac imaging use had $B = 0.469$, $SE = 0.603$, $Wald = 0.605$, and $p = 0.437$.

5.3.2.4 Indirect effect of group allocation on ASCO-D1 via cardiac imaging use (paths a and b)

To assess whether the use of cardiac imaging mediated the effect of group allocation on achieving a definite ASCO-D1 score, a Sobel test was performed using the unstandardised regression coefficient (B) and standard errors (SE) obtained from the logistic regression model for path a and b. The test yielded a Sobel test of 2.24, with a two-tailed p-value of 0.025, indicating a statistically significant indirect effect. This suggests that part of the effect of the CCI protocol on diagnostic certainty was mediated through increased use of cardiac imaging.

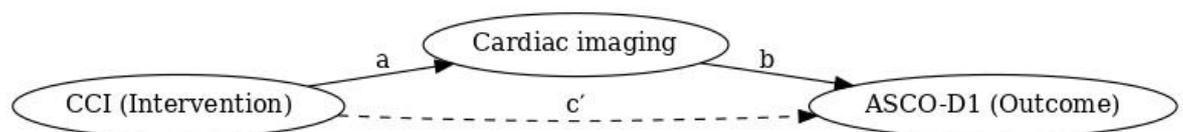


Figure 5-3 Mediation model CCI → cardiac imaging → ASCO-D1

5.3.3 Mediation analysis: Time to brain MRI (MRIhrs)

Mediation analysis was conducted using the PROCESS macro (model 4) in SPSS to test whether the time from administration to brain MRI (MRIhrs) mediated the effect of group allocation on achieving definite diagnostic score ASCO-D1.

5.3.3.1 Effect of group allocation on time to brain MRI (path a)

There was no significant association between group allocation and time to MRI. The regression coefficient was $B = -3.17$, $SE = 6.17$, $t = -0.51$, $p = 0.609$, with 59% CI -15.42 to 9.08. this indicates that time to brain MRI did not significantly differ between groups.

5.3.3.2 Effect of time to MRI on ASCO-D1, controlling for group allocation (path b)

Time to MRI was not a significant predictor of achieving a definite ASCO-D1 score. The coefficient was $B = -0.0011$, $SE = 0.0068$, $Z = -0.17$, $p = 0.868$, with 95% CI -0.0145 to 0.0122.

5.3.3.3 Direct effect of group allocation on ASCO-D1, controlling for time to brain MRI

Group allocation remained a significant predictor of ASCO-D1 when controlling for time to brain MRI, with $B = 1.15$, $SE = 0.42$, $Z = 2.75$, $p = 0.006$, suggesting that participants in the CCI group had approximately 3.16 times higher odds of receiving a definite classification $\text{Exp}(B) = 3.16$.

5.3.3.4 Indirect effect of group allocation on ASCO-D1 via time to MRI (a x b)

The bootstrapped indirect effect of group allocation on ASCO-D1 via time to MRI was not significant, indirect effect = 0.0036, Boot SE = 0.0489, 95% CI -0.0722 to 0.1351.

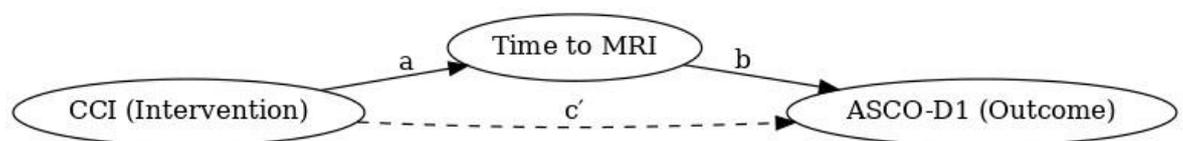


Figure 5-4 Mediation model: CCI → time to MRI → ASCO-D1

5.3.4 Mediation analysis: Time to cardiac imaging (echo or CCT)

Mediation analysis was conducted using the PROCESS macro (model 4) in SPSS to test whether the time from administration to cardiac imaging mediated the effect of group allocation on achieving definite diagnostic score ASCO-D1.

5.3.4.1 Effect of group allocation on time to cardiac imaging (path a)

There was no significant association between group allocation and time to cardiac imaging. The regression coefficient was $B = -10.56$, $SE = 20.30$, $t = -0.52$, $p = 0.604$, with 59% CI -50.85 to 29.73 . This indicates that group allocation did not significantly predict time to cardiac imaging.

5.3.4.2 Effect of time to cardiac imaging on ASCO-D1, controlling for group allocation (path b)

Time to cardiac imaging was not a significant predictor of achieving a definite ASCO-D1 score. The coefficient was $B = 0.0012$, $SE = 0.0020$, $Z = 0.58$, $p = 0.563$, with 95% CI -0.0028 to 0.0051 .

5.3.4.3 Direct effect of group allocation on ASCO-D1, controlling for time to cardiac imaging

Group allocation remained a significant predictor of ASCO-D1 when controlling for time to cardiac imaging, with $B = 1.17$, $SE = 0.42$, $Z = 2.79$, $p = 0.005$, suggesting that participants in the CCI group had approximately 3.21 times higher odds of receiving a definite classification $\text{Exp}(B) = 3.21$

5.3.4.4 Indirect effect of group allocation on ASCO-D1 via time to cardiac imaging (a x b)

The bootstrapped indirect effect of group allocation on ASCO-D1 via time to cardiac imaging was not significant, indirect effect = -0.1023 , Boot SE = 0.1022 , and 95% CI -0.1889 to 0.2244 .

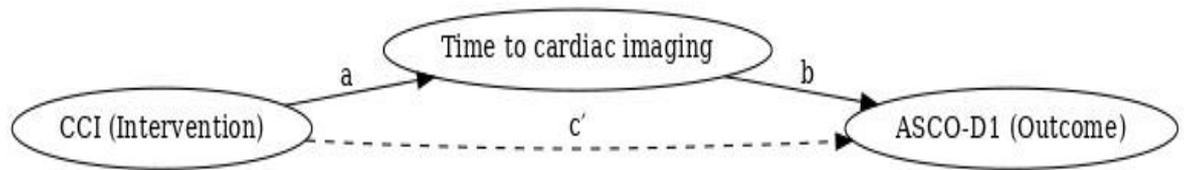


Figure 5-5 Mediation model CCI → time to cardiac imaging → ASCO-D1

5.4 Discussion

5.4.1 Overview of findings

This chapter aimed to evaluate whether specific imaging modalities (brain MRI, cardiac imaging) or their timing acted as a mediator between the randomised diagnostic strategy (CCI vs. routine care) and the likelihood of achieving a definite aetiological classification ASCO-D1. As demonstrated in the total effect model, group allocation alone had a statistically significant effect on ASCO-D1 classification, with patients in CCI group showing over threefold higher odds of achieving a definite diagnosis. This confirms the primary findings reported in chapter X and provides the basis for evaluating potential mediation pathways.

We used a combination of manual logistic regression with Sobel testing for binary variables and the PROCESS macro for SPSS for continuous variables to estimate direct and indirect effects across four models. Of the four models tested, cardiac imaging use was the only mediator to demonstrate a statistically significant indirect effect, suggesting partial mediation. Brain MRI use, although more common in the CCI group, did not significantly predict the outcome. Similarly, time to brain MRI and time to cardiac imaging showed no mediation effect.

These results indicate that the diagnostic benefit of the CCI protocol is likely driven by the type of imaging used, particularly cardiac evaluation, rather than by earlier imaging access.

5.4.2 Interpretation of mediation effect

5.4.2.1 Imaging use as a mediator

Brain MRI and cardiac imaging were both obtained significantly more frequently in the CCI group. However, only cardiac imaging was independently associated with ASCO-D1 classification and showed a statistically significant indirect effect. This indicates that the cardiac imaging component of the CCI protocol exhibited partial mediation and explains part -but not all- of the effect of CCI on diagnostic certainty. The direct effect of group allocation remained significant even after adjusting for cardiac imaging, suggesting that other factors within the CCI protocol may contribute to the outcome.

In contrast, MRI use did not independently predict ASCO-D1 classification, and the indirect effect was not significant, indicating no mediation. However, it worth noting that the odds ratio for the association between MRI use and ASCO-D1 classification was 2.8, suggesting a potential trend despite the lack of statistical significance. While this does not meet conventional thresholds for mediation, the effect size may reflect a clinically relevant signal, particular given the limited sample size and exploratory nature of this analysis.

5.4.2.2 Time to imaging as a mediator

There were no significant differences between groups in time to brain MRI or time to cardiac imaging, and neither timing variable was associated with ASCO-D1 classification. Both indirect and direct effects were insignificant. This indicates that the effect of the CCI protocol is unlikely to be attributable to earlier imaging access, but rather the nature of imaging performed.

5.4.3 Clinical implications

These findings clarify that the diagnostic benefits of the CCI protocol arose from the use of high yield imaging, particularly cardiac imaging, rather than from earlier imaging access. This supports the theoretical view that multi-component strategies like CCI function through integration of diagnostic processes rather than a single dominant element.

Clinically, the results support the broader value of incorporating early cardiovascular imaging as a part of comprehensive diagnostic workup for all patients, not just those with initially unclear mechanisms. The observed improvement in aetiological classification reinforces its relevance even in unselected stroke and TIA patients.

The lack of mediation through imaging timing suggests that timely access alone is insufficient unless the imaging performed is diagnostically informative. These insights reinforce that diagnostic content, not just speed, drives meaningful improvement in stroke aetiological classification.

5.4.4 Strengths and limitations

A key strength of this study is its novel application of mediation analysis to evaluate the mechanisms by which a complex diagnostic strategy influences aetiological classification in stroke. To our knowledge, this is the first analysis to formally examine whether specific imaging components or their timing mediate the diagnostic impact of a structured imaging protocol. Methodologically, the use of both logistic regression with Sobel testing and the PROCESS macro allowed appropriate handling of binary and continuous mediation, enhancing robustness.

However, several limitations should be considered. The relatively small sample size reduced the statistical power of analysis, especially for detecting indirect effects that may exist but are modest in magnitude. It is worth noting that a similar pattern was observed in the main trial analysis (chapter 3) where logistic regression models predicting ASCO-D1 classification also explained only a modest proportion of overall variance. This suggests that aetiological certainty is likely determined by multiple interacting clinical, imaging, and procedural factors beyond those directly captured in the mediation framework.

In addition, the use of binary mediators, such as whether imaging was performed, restricted the analysis to simpler regression-based models. More advanced methods like structural equation modelling or causal mediation analysis could not be applied due to sample size constraints and software limitations. Moreover, the analysis did not account for potentially important factors, such as the quality of

imaging interpretation or how imaging results influenced clinical decision, areas that merit further investigation in future studies.

Finally, it is important to recognize that the variables investigated here explained only a small proportion of the variance in ASCO-D 1 classification. This suggests that other factors, such as clinical stroke features, vascular risk burden, lesion pattern, interpretation quality, and decision-making dynamics, may contribute meaningfully to diagnostic certainty. Some of these may be inferred from the trial dataset and incorporated into future mediation models (e.g., Fazekas score, aortic arch findings, or treatment changes), while others may require prospective data collection. Expanding the mediation framework to include these additional variables could help clarify the broader mechanisms by which structured diagnostic protocols improve classification.

5.4.5 Comparison to existing literature

Mediation analysis is increasingly recognised as a valuable method for exploring how complex interventions exert their effects, yet its application in diagnostic research particularly in stroke is rare. Most diagnostic studies focus on comparative accuracy, yield, or prognostic value, without addressing how multi-step protocols produce benefit. By contrast, the present study used mediation analysis to explore the contribution of individual components within a complex imaging strategy, providing mechanistic insight into diagnostic improvements.

This approach aligns with work in other clinical domains where mediation has been used to explore intermediate processes. In oncology, Kesson et al. demonstrated that improved survival at research-active hospitals was partly mediated by enhanced treatment delivery pathways (Kesson et al., 2012). In neurodegenerative research, Zhao et al. used high-dimensional mediation analysis to demonstrate how regional brain atrophy mediates the relationship between biomarker changes in CSF and cognitive decline in Alzheimer's disease by using quantitative imaging features as mediators (Zhao and Li, 2022).

In paediatric pain research, Leake et al. conducted a systematic review of 53 mediation models across behavioural pain interventions to understand how treatments for paediatric pain exert their effect, instead of just reporting whether

they work. They found that only a minority demonstrated significant indirect effects (Leake et al., 2021). The authors highlighted challenges such as limited sample sizes, confounding, and inconsistent analytic strategy, issues also present in our study. Yet their review reinforced the scientific importance of mediation analysis for identifying causal pathways in complex clinical context and parallels our use of Sobel test and PROCESS macro to explore how imaging components within CCI contribute to diagnostic clarity.

These studies, like ours, shift the focus from whether an intervention works to how it works. This reflects a broader trend in clinical research toward mechanism-based evaluation, particularly when component randomisation is not feasible. By applying this methodology to stroke diagnostics, this study demonstrates how mediation analysis can inform not only the efficacy of but also the design and refinement of diagnostic protocol. This represents an important contribution to evidence-based diagnostic research, extending beyond traditional accuracy studies to address underlying causal mechanisms.

5.4.6 Future directions

This study highlights the value of mediation analysis in understanding the mechanisms through which complex diagnostic strategies influence clinical outcomes. Future research should aim to replicate these findings in larger, prospectively designed studies with adequate power to detect small indirect effects. The use of advanced method such as causal mediation analysis or structural equation modelling may offer greater flexibility and precision, particularly for handling multiple mediators.

Further exploration of mediators beyond imaging completion and timing is warranted. These may include diagnostic yield, imaging interpretation quality, detecting of embolic source, or early treatment modification. Longitudinal designs incorporating repeated measures could also clarify the temporal relationship between diagnostic processes and clinical decision-making.

Incorporating mediation frameworks into diagnostic research will support more transparent evaluation of multi-component interventions and enable optimisation of resources- intensive strategies such as CCI protocol.

6 Chapter 6-Risk factor profiles and imaging biomarkers across ASCO-D aetiological subtypes

6.1 Abstract

6.1.1 Background

Stroke mechanisms often overlap, with hypertension and other cardiovascular risk factors contributing across aetiological subtypes. The ASCO-D classification system provides a structured framework for aetiological subtyping of ischemic stroke, but its validity depends on how well domain certainty aligns with both vascular risk profiles and imaging findings. Most ASCO-D validation studies have focused primarily on clinical and laboratory variables, with relatively few incorporating quantitative imaging biomarkers that reflect small-vessel pathology. In particular, it remains unclear whether white matter hyperintensity (WMH) burden, cerebral microbleed (CMB), and acute lesion patterns independently map onto ASCO-D certainty levels. This study aimed to evaluate the association between vascular risk factors, MRI biomarkers, and ASCO-D domain certainty, to test whether imaging features enhance the construct validity of small-vessel disease classification.

6.1.2 Methods

We conducted a cross-sectional analysis of a prospectively recruited acute stroke or TIA cohort. ASCO-D scoring was applied within the A (large artery atherosclerosis), S (Small vessel disease), and C (cardioembolism) domains. WMH burden was graded using Fazekas scores, CMBs were rated on T2*/SWI MRI, and acute infarct topography was classified on DWI. Association between vascular risk factors, imaging biomarkers, and ASCO-D certainty were examined using ordinal trend test, cross-tabulations, and logistic regression models.

6.1.3 Results

Increasing ASCO-D domain certainty was associated with distinct vascular and imaging patterns. Smoking ($p=0.023$) and prior cardiac illness ($p=0.029$) rose with

higher atherosclerosis certainty. In the small vessel domain, age correlated with SVD certainty ($\rho = 0.27$, $p = 0.007$) and WMH burden ($X^2 = 18.4$, $p = 0.005$). CMBs correlated with age ($\rho = 0.30$, $p = 0.009$) and WMH severity ($p < 0.001$), reflecting shared small vessels pathology. Both WMH and CMBs burden were associated with higher S domain certainty, but only WMH severity independently predicted definite SVD (aOR=3.16, 95% CI 1.73-5.77, $p < 0.001$), whereas the CMB-SVD association attenuated after adjustment. Cardioembolism certainty increase with age ($\rho = 0.43$, $p < 0.001$) and AF ($p = 0.001$). Mixed-mechanism classification was frequent (61%), with 25% of patients showing triple-domains overlap. Lesion patterns mapped coherently onto domains: lacunar infarct with SVD, single territory cortical with infarcts due to atherosclerosis, and multi-territory cortical infarcts with cardioembolism.

6.1.4 Conclusion

ASCO-D certainty scores reflect biologically coherent gradients with vascular risk factors, while neuroimaging biomarkers provided mechanistic specificity, particularly with SVD. These findings support the construct validity of ASCO-D and highlighted the added value of integrating WMH, CMBs and lesion patterns into aetiological classification. The results provide a foundation for thesis' broader focus on comprehensive multimodal imaging to enhance diagnostic certainty and optimise secondary prevention strategies.

6.2 Introduction

6.2.1 Background

Vascular risk factors are unequally distributed across stroke aetiological subtypes, reflecting distinct underlying pathophysiological mechanisms. Large artery atherosclerosis is most consistently linked to smoking, dyslipidaemia, and systemic atherosclerosis, all of which accelerate plaque formation through endothelial dysfunction, oxidative stress, and chronic inflammation. Small vessel disease is strongly associated with age and hypertension, leading to arteriosclerosis, lacunar infarction, and white matter changes. Cardioembolic aetiology is most often caused by AF or other arrhythmias, as well as structural cardiac abnormalities such as prior cardiac infarction or valvular disease (Wolf et al., 1991, Hart et al., 2014).

The ASCO-D phenotyping system captures these mechanisms across five domains: Atherosclerosis (A), Small vessel disease (S), Cardioembolism (C), Other causes (O), and Dissection (D), and grades each according to the certainty that it contributed to the index event (Amarenco et al., 2013). Although imaging findings contribute substantially to ASCO-D classification, they are incorporated subjectively, alongside clinical and laboratory evidence, rather than through quantitative weighting.

Beyond traditional vascular risk factors, neuroimaging biomarkers of small vessel disease provide important mechanistic insight into the brain's microvascular health. Common MRI markers include white matter hyperintensity (WMH), cerebral microbleed (CMB), lacunes, and acute lesion patterns (lacunes vs. cortical). These features have been consistently linked to stroke subtypes, greater odds of cognitive decline, risk of future vascular events, and are now recognised as established imaging marker of SVD (Pantoni, 2010, Wardlaw et al., 2013, Markus and de Leeuw, 2023).

Recent work has emphasized that imaging features such as WMH and CMB represent true markers for microvascular pathology. Meta-analysis confirmed that higher CMBs predicts ischemic stroke, haemorrhages, and cognitive decline (Charidimou et al., 2018). Moreover, CMB prevalence and location can differ across ischemic stroke subtypes (Kim et al., 2016), suggesting their potential to discriminate aetiological mechanisms.

Although ASCO-D and other classification systems already incorporate imaging findings, they do so in a largely qualitative and subjective manner, alongside clinical and laboratory data. Because many vascular risk factors are shared across stroke subtypes, such as Hypertension and DM, this can blur mechanistic distinctions. Integration quantities imaging markers such as WMH and CMBs may therefore help refine these classifications by anchoring clinical phenotypes with objective evidence of underlying vascular pathology.

6.2.2 Rationale

Associations between stroke mechanisms and vascular risk factors are well established at the population level, however their expression within an aetiologic

certainty framework such as ASCO-D has rarely been quantified. Specifically, it is unclear how risk factor prevalence changes across graded certainty levels within each domain. Understanding these gradients can help test the construct validity of ASCO-D system, i.e. do ASCO-D certainty scores behave as mechanistic influences of different risk factor profiles predict, e.g. smoking more strongly associated with atherosclerosis, old age and AF with Cardioembolism, and increasing age and hypertension with small vessel disease.

At the same time, any clinical inferences drawn from ASCO-D depend on the validity of classification itself. Because ASCO-D integrates clinical history alongside test results, it is vulnerable to selective investigation and to assignments that may rely on history rather than purely objective findings. We do not assume ASCO-D is valid; instead, we use observed risk-factor gradients as one way of testing whether its certainty scores reflect real pathophysiology. For this reason, our trend analyses are presented primarily as a construct-validity check and hypothesis-generating, rather than as definitive proof of causation or as a basis for prescriptive triage. Put simply, if the expected gradients are present, that is consistent with validity; if not, it may reflect either biological reality or limitation/biases in classification and work-up.

In addition, stroke mechanisms frequently overlap, with mixed-mechanism presentation reported in 20-60% of patients in cohorts using comprehensive vascular and cardiac evaluation (Sirimarco et al., 2013, Liu et al., 2018). This is often driven by the co-occurrence of multiple vascular and cardiac risk factors in the same patient, but the relationship between total risk-factor burden and the number of concurrent mechanisms has rarely been quantified. Demonstrating such a dose-response relationship would support the concept of a cumulative pathology model, in which vascular ageing, systemic atherosclerosis, and cardiopathy act together to generate complex stroke phenotypes.

However, most ASCO-D validation studies have focused primarily on clinical and laboratory variables, with relatively few incorporating quantitative neuroimaging biomarkers that reflect small vessel pathology. Imaging markers such as WMH, CMB, and lacunes are now recognised as robust indicators of microvascular damage (Wardlaw et al., 2013, Pantoni, 2010, Charidimou et al., 2018), yet their relationship to ASCO-D certainty levels remains possibly underexplored.

Specifically, it is unclear whether WMH burden, CMBs, and acute lesion classification independently map onto definite versus non-definite SVD categories, or whether they add predictive value for stroke mechanism classification beyond conventional risk factors.

Addressing this gap, the present study extends the ASCO-D framework by exploring the relationships between Fazekas WMH scoring, CMB quantification, and lesion pattern into aetiological comparisons. By examining their distribution across domains and testing their predictive role for definite ASCO-D certainty level, we aim to strengthen constructive validity checks and assess whether these imaging markers provide mechanistic specificity within the ASCO-D system.

6.2.3 Aims

6.2.3.1 Primary aim

To describe domain-specific risk-factor gradients and the prevalence of mixed-mechanism stroke in an acute stroke/ TIA cohort using the ASCO-D system, and to assess whether certainty levels correlate with established vascular and cardiac risk factors and with neuroimaging biomarkers, including Fazekas WMH scores, CMB burden, and acute infarct lesion pattern.

6.2.3.2 Secondary aims

To examine the association between total vascular risk-factor burden and the extent of mechanism overlap.

To explore predictors of stroke aetiology (A, S, and C domains) using multivariable analysis.

To investigate the relationship between imaging biomarkers (WMH, CMBs, lesion pattern) and ASCO-D certainty levels within the A, S, and C domains.

To test whether WMH, CMBs, and lesion patterns independently predict definite SVD beyond conventional vascular risk factors.

6.3 Methods

6.3.1 Study design and setting

This was a cross-sectional analysis of prospectively collected data from the pilot acute stroke and TIA cohort described in chapter 3. The study was conducted in a tertiary stroke service with access to comprehensive vascular and cardiac imaging. The analysis focused on the distribution of vascular and cardiac risk factors across ASCO-D domains, prevalence of mixed-mechanism presentation, and the relationship between total risk-factor burden and the number of concurrent mechanisms. Full details of patient's recruitment, imaging protocols, and general data collection are provided in chapter 3.

6.3.2 Inclusion and exclusion criteria

We included all patients with ASCO-D scoring for at least one of the A, S, and C domains. Patients scored only in the other (O) or dissection (D) domains were excluded due to low numbers. Only patients who underwent brain MRI were included in WMH and CMB analyses.

6.3.3 ASCO-D scoring

Certainty scoring within each domain was coded as an ordinal variable (0<3<2<1), and cases with score 9 (incomplete work up) were excluded from domain-specific analysis. Scoring followed published definitions (Amarenco et al., 2013), score 1= definite cause, 2= probable cause, 3= possible cause, 0= the absence of the disease, and 9= incomplete work-up, which were based on all available imaging and clinical data.

6.3.4 Imaging biomarkers

6.3.4.1 White matter hyperintensity (WMH) severity

WMH burden was assessed using Fazekas visual scale, which, separately scores periventricular (PV) and deep white matter (DWM) hyperintensities from 0 (absent) to 3 (severe), and combined into a total score. This scale is widely used in clinical research and recommended in STRIVE consensus criteria for SVD imaging markers. For analysis, we combined PV and DWM into a total score, then severity

was categorised as mild ($PV \leq 1$ and $DWM \leq 1$), moderate ($PV = 2$ or $DWM = 2$), and severe ($PV \geq 3$ or $DWM \geq 3$) (Fazekas et al., 1987, Wardlaw et al., 2013, Staals et al., 2014).

6.3.4.2 Cerebral microbleeds (CMBs)

CMBs were rated on T2* or SWI MRI and classified by burden into four categories: none, 1, 2-4, and ≥ 5 , consistent with consensus recommendations e.g. STRIVE and MARS criteria (Fazekas et al., 1987, Staals et al., 2014, Wardlaw et al., 2013).

6.3.4.3 Acute infarct lesion pattern

Patients with acute infarcts on DWI were classified into categories: single lacunar infarct, single-territory cortical, multi-territory cortical, or mixed lacunar + cortical, based on vascular territory and morphology, in line with prior stroke imaging studies (Jose and James, 2022, Sharobeam et al., 2020).

6.3.5 Statistical analysis

- Domain-specific gradient: linear-by-linear association χ^2 test for categorical variable; Spearman's rank correlation for continuous variable.
- Mixed-mechanism prevalence: Calculated overall, by pairwise overlaps, and for triple-domain overlap (A+S+C).
- Risk-factor burden: Total count of major risk factors correlated with number of concurrent mechanisms using Spearman's rank correlation.
- Predictor models: Binary logistic regression for the A, S, and C domains, coding scores 1-3 as disease present vs 0 as no disease: case with score 9 were excluded from analyses. This approach treats 1-3 as a single presence category to reduce rater across certainty levels and to maintain statistical power in subdomains, adjusting for relevant risk factor variables with $p < 0.10$ in univariable analysis.
- Sensitivity analysis. To assess robustness, we reported key comparisons after collapsing domains to binary categories of definite (score 1) vs less

certain (A2+A3). For each collapsed domain we ran bivariate crosstabs and binary logistic regression.

- WMH severity (Fazekas categories): Crosstabs of WMH categories vs. ASCO-D domains; χ^2 test for association; linear-by-linear trend test. Logistic regression tested whether WMH severity predicted definite SVD (S1 vs. non-S1).
- CMB burden: crosstabs of CMB categories vs. ASCO-D domains; χ^2 test for association; linear-by-linear trend test. Logistic regression tested whether CMBs predicted definite SVD (S1 vs. non-S1).
- Lesion patterns: crosstabs of lesion pattern categories vs. ASCO-D domains; X tests for overall association. Exploratory analyses compared collapsed categories (single lacunar vs. non-lacunar)
- Statistical significance was set at $p < 0.05$ for all tested. All statistical analysis were performed using IBM SPSS Statistics for windows Version 29.0

6.4 Results

6.4.1 Risk factor distribution in the large artery atherosclerosis (A) domain

Across the A subdomains, there were no significant differences in age (mean age 60.5-70.6 years, $p=0.11$) or sex distribution ($p=0.84$). Smoking rose with greater certainty of atherosclerosis, 15/19 (78.9%) in A1, versus 13/29 (44.8%) in A0, p for trend = 0.023. prior cardiac illness followed a similar pattern, 7/19(36.8%) in A1, and 2/29(6.9) in A0, p for trend = 0.029. CAD was most common in the A3 subdomain 9/34(26.5%) compare with only 1/19 (5.3%) in A1, and overall differences were significant ($p= 0.006$), although the pattern was not monotonic. These results should be interpreted cautiously given the very small subdomains size. Other vascular risk factors, including HTN, and DM, were variably represented across subdomain but showed no significant association (all $p > 0.05$). A full breakdown of risk factors by subdomain is presented in Table 6-1.

Table 6-1. Risk factors distribution by A subdomain

Variable	A1 (n=19)	A2 (n=12)	A3 (n=34)	A0 (n=29)	A9 (n=6)	p value	p trend
Age (mean± SD)	60.5±13.38	65.3±13.34	70.6±12.20	63.6±16.89	62.5±11.55	0.111	0.329
Male n (%)	14 (73.7)	8 (66.7)	23 (67.6)	18 (62.1)	5 (83.3)	0.842	0.433
Female n (%)	5 (26.3)	4 (33.3)	11 (32.4)	11 (37.9)	1 (16.7)		
HTN n (%)	9 (47.4)	6 (50.0)	25 (73.5)	16 (55.2)	2 (33.3)	0.188	0.388
DM n (%)	3 (15.8)	1 (8.3)	11 (32.4)	3 (10.3)	0	0.090	0.966
PVD n (%)	3 (15.8)	1 (8.3)	5 (14.7)	0	0	0.201	0.111
H.Choles. n (%)	4 (21.1)	1 (8.3)	7 (20.6)	5 (17.2)	0	0.661	0.961
Smoking n (%)	15 (78.9)	7 (58.3)	19 (55.8)	13 (44.8)	3 (50.0)	0.182	0.023
Alcohol n (%)	4 (21.1)	2 (16.7)	3 (8.8)	5 (17.2)	1 (16.7)	0.788	0.622
CAD n (%)	1 (5.3)	0	9 (26.5)	2 (6.9)	3 (50)	0.006	0.451
AF n (%)	1 (5.3)	1 (8.3)	1 (2.9)	1 (3.4)	1 (16.7)	0.788	0.800
TIA/IS n (%)	5 (26.3)	4 (33.3)	11 (32.4)	6 (20.7)	3 (50.0)	0.341	0.985
cardiac illness* n (%)	7 (36.8)	2 (16.7)	9 (26.5)	2 (6.9)	2 (33.3)	0.119	0.029

*Cardiac illnesses= MI 16 patients, VD two patients, VD with MI or HF 3 patients, and PFO 1 patients

6.4.2 Risk factors by large artery atherosclerosis (LAAs) status

Patients with large artery atherosclerosis (LAAS) (n=65) tended to be slightly older than those without LAAs (n= 29), although the difference was not statistically significant (mean difference = 3.1 years, p = 0.337). HTN and DM were more frequent in group with LAAs, but neither showed a significant association.

PVD occurred exclusively among patients with LAAs, yielding a large odds ratio but with wide confidence intervals due to the low prevalence rate. The prevalence of high cholesterol was similar between groups. Smoking history appeared more common in LAAs group, whereas higher alcohol intake was less frequent, though neither difference reached significance.

Cardiovascular comorbidities, including CAD, history of AF, and prior TIA and IS, were more prevalent in LAAs group, but these association were not statistically significant. The significant association was observed for prior cardiac illness, which was markedly more frequent among patient with atherosclerosis, p = 0.023 Table 6-2.

Table 6-2. Risk factors by LAAs status

Variable	"A" present n= 65	"A" absent n=29	95%CI	OR	P value
Age (mean± SD)	66.7±13.3	63.6±16.9	-9.59 to 3.31		0.337
HTN n (%)	40 (61.5)	16 (55.2)	0.54-3.09	1.29	0.561
DM n (%)	15 (23.1)	1 (10.3)	0.69-9.79	2.60	0.147
PVD n (%)	9 (13.8)	0	0.82-258.6	14.57	0.035
High Cholesterol n (%)	12 (18.5)	5 (17.2)	0.36-3.32	1.09	0.887
Smoking / ex-smoker n (%)	41 (63.1)	13 (44.8)	0.88-5.05	2.11	0.098
Alcohol n (%)	9 (13.8)	5 (17.2)	0.22-2.67	0.77	0.699
CAD n (%)	10 (15.4)	2 (6.9)	0.50-12.01	2.46	0.255

H/O AF n (%)	5 (7.7)	1 (3.4)	0.26-21.66	2.37	0.437
H/O TIA/IS n (%)	17 (26.2)	5 (17.2)	0.55-5.34	1.71	0.346
H/O cardiac illness* n (%)	18 (27.7)	2 (6.9)	1.11-24.01	5.17	0.023

6.4.3 Multivariate analysis for predictors of large artery atherosclerosis (LAAs)

The logistic regression model was statistically significant (Omnibus χ^2 (5) = 14.37, $p = 0.013$), indicating that the set of predictors reliably distinguished between patients with and without LAAs. Model calibration was acceptable (Hosmer-Lemeshow χ^2 (8) = 7.98, $p = 0.436$).

In the adjusted model, smoking was associated with more than twice the odds of having LAAs, although this did not reach statistical significance, $p = 0.121$. Prior cardiac illnesses showed a fourfold increased odd, approaching significance and suggesting a clinically meaningful association, $p = 0.062$. PVD was retained in the model because it showed a strong link in unadjusted analysis; however, the adjusted odds ratio was extremely large and unstable due to very few cases so it should be interpreted with caution. Neither age nor sex were significant independent predictors in this cohort (Table 6-3).

Collapsing the A domains to definite (A1, $n=15$) versus less certain (A2+A3, $n=27$) showed similar results. On bivariable tests smoking was more frequent in A1 (12/15, 80%) than in A2+A3 (14/27, 51.9%), and CAD was less frequent (0/15, 0% vs 5/27, 18.5%), neither difference reached significance ($p > 0.05$). A multivariable model including age per decade, smoking, and sex showed no independent predictors of A1 (all $p > 0.05$), with good fit (Hosmer-Lemeshow $p=0.944$), consistent with subdomain analyses above. All other risk factors were similar between A1 and A2+A3 (all $p > 0.05$).

Table 6-3. Multivariate logistic regression for independent predictors of LAAs

Variable	Adjusted OR (aOR)	95%CI for aOR	P value
Smoking (ex/current)	2.16	0.82-5.71	0.121
PVD	5.44x10 ⁷	0.00- *	0.999
Prior cardiac illness	4.61	0.93-22.86	0.062
Age (per year)	1.01	0.98-104	0.619
Sex (male vs. female)	0.98	0.36-2.70	0.976

*Upper bound omitted in SPSS output due to unstable estimate (sparse data in PVD)

6.4.4 Risk factors distribution in the small vessel disease (S) domain

Across the S subdomain, age differed significantly, mean age 68.4±17.2 years in S1 vs 59.3±13.2 years in S0(p=0.022), with clear positive trend (Spearman $\rho = 0.269$, $p = 0.007$). sex distribution did not differ significantly(p=0.181) although smoking showed a significant difference between subdomains, overall p= 0.022, however, no monotonic trend was observed. These results should be interpreted cautiously given the very small subdomains size. Other vascular risk factors including HTN, DM, and cardiac comorbidities, were variably distributed but did not differ significantly across subdomains (all p >0.05). full distributions are shown in Table 6-4.

Table 6-4. Risk factor distribution across S subdomain

Variable	S1 (n=31)	S2 (n=9)	S3 (n=31)	S0 (n=29)	p value	P trend
Age (mean± SD)	68.4±17.2	62.3±13.0	69.4±10.3	59.3±13.2	0.022	0.007
Male n (%)	19 (61.3)	8 (88.9)	24 (77.4)	17 (58.6)	0.181	0.938
Female n (%)	12 (38.7)	1 (11.1)	7 (22.6)	12 (41.4)		
HTN n (%)	21 (67.7)	3 (33.3)	19 (61.3)	15 (51.7)	0.255	0.344
DM n (%)	4 (12.9)	0	7 (22.6)	7 (24.1)	0.295	0.157
PVD n (%)	3 (9.7)	0	5 (16.1)	1 (3.4)	0.270	0.724
H.Choles n (%)	7 (22.6)	0	5 (16.1)	5 (17.2)	0.467	0.682
Smoking n (%)	13 (41.9)	6 (66.7)	19 (61.3)	19 (65.5)	0.022	0.067
Alcohol n (%)	5 (16.1)	2 (22.2)	3 (9.7)	5 (17.2)	0.752	0.871
CAD n (%)	3 (9.7)	1 (11.1)	8 (25.8)	3 (10.3)	0.248	0.594
AF n (%)	2 (6.4)	2 (22.2)	2 (6.4)	1 (3.4)	0.285	0.504
TIA/IS n (%)	10 (32.3)	6 (66.7)	2 (6.4)	7 (24.1)	0.783	0.389
cardiac illness* n (%)	9 (29.0)	2 (22.1)	8 (25.8)	3 (10.3)	0.276	0.121

*Cardiac illnesses= MI 16 patients, VD two patients, VD with MI or HF 3 patients, and PFO 1 patients

6.4.5 Vascular risk factors by SVD status

Patients with SVD (n = 71) were significantly older than those without SVD (n = 29) (68±14 vs.59± 13.2 years, p =0.005; mean difference = 8.75 years, 95% CI -14.97 to -2.71, Cohen's d = 0.63) indicating a moderate age effect. HTN was more common in the SVD group, whereas DM was slightly less frequent, but neither association reached statistical significance.

PVD was more frequent among patients with SVD, though the wide confidence interval reflected small case numbers. High cholesterol was observed at similar rates in both groups. Smoking and alcohol intake were somewhat more common in patients without SVD, but these differences were not significant.

A history of prior TIA or IS was comparable between groups. CAD, AF, and prior cardiac illness were all more prevalent in the SVD group, but none of these differences reached statistical significance Table 6-5.

Table 6-5. Risk factor by SVD status

Variable	SVD present (n=71)	SVD absent (n=29)	95%CI	OR	P value
Age (mean± SD)	68±14	59±13.2	-14.79 to -2.71		0.005
HTN n (%)	43 (60.6)	15 (51.7)	0.60 - 3.42	1.43	0.416
DM n (%)	11 (15.5)	7 (24.1)	0.20 - 1.67	0.58	0.307
PVD n (%)	8 (11.3)	1 (3.4)	0.42 - 29.80	3.56	0.215
High Cholesterol n (%)	12 (16.9)	5 (17.2)	0.31 - 3.07	0.98	0.967
Smoking / ex-smoker n (%)	38 (53.5)	19 (65.5)	0.25 - 1.49	0.61	0.272
Alcohol n (%)	10 (14.1)	5 (17.2)	0.24 - 2.54	0.79	0.688
CAD n (%)	12 (16.9)	3 (10.3)	0.46 - 6.78	1.79	0.405
H/O AF n (%)	6 (8.5)	1 (3.4)	0.30 - 22.48	2.58	0.374
H/O TIA/IS n (%)	18 (25.4)	7 (24.1)	0.39 - 2.91	1.07	0.899
H/O cardiac illness* n (%)	19 (26.8)	3 (10.3)	0.86 - 11.68	3.17	0.072

6.4.6 Multivariate analysis for independent predictors of SVD

The logistic regression model was statistically significant (Omnibus χ^2 (5) = 14.37, $p = 0.013$) and showed acceptable calibration (Hosmer-Lemeshow χ^2 (8) = 3.92, $p = 0.864$). The model explained between 11.9% (Cox & Snell R^2) and 17.0% (Nagelkerke R^2) of the variance.

After adjustment, age was the only statistically significant independent predictor, with each year increasing the odds of SVD by 4.6% (aOR 1.046, 95%CI 1.01-1.08; $p=0.016$), equivalent to 1.57-fold increase per decade (95%CI 1.10-2.16). Prior history of cardiac illness was associated with more than twice the odds of SVD, though this did not reach statistical significance, $p = 0.164$. Sex, CAD, and history of AF were not independently associated with SVD in this model Table 6-6.

Collapsing the S-domain to definite (S1, $n = 25$) versus less certain (S2+S3, $n = 19$) yielded no independent predictors. On bivariable testing, most factors were null; prior TIA/IS was more common in S1 (8/25, 32.0%) than S2+S3 (2/19, 10.5%), but did not reach significance, $p=0.148$. A multivariable model including age per decade, hypertension, smoking and sex, was not significant overall $p = 0.65$ and no variable independently predicted S1. Although age independently predicted the presence of SVD in the full cohort, it did not differentiate S1 from less-certain categories within S domains.

Table 6-6. Multivariate analysis for independent predictors of SVD

Variable	Adjusted OR (aOR)	95%CI for aOR	P value
CDA	1.15	0.27-4.88	0.849
AF	1.30	0.13-12.27	0.822
Prior cardiac illness	2.64	0.67-10.39	0.164
Age (per decade)	1.57	1.10-2.16	0.016
Sex (male vs. female)	0.49	0.19-1.29	0.150

6.4.7 Risk factors distribution in the cardiac disease (C) domain

Across the C subdomains, age differed significantly (C1:75.8±8.4 years vs C0:59.3 ± 16.2 years; p= 0.003). AF was concentrated in C1 (6/12, 50%) and rare elsewhere (C0 1/37, 2.7%; p<0.001; p for trend = 0.001). Prior cardiac illness increased with higher certainty (C1 5/12, 41.7% vs C0 4/37, 10.8%; p for trend = 0.010). Smoking varied across groups (p=0.017), being lower in C1 and higher in C3, was more frequent in C1 (4/12, 33.3%) than C0 (2/37, 5.4%), p=0.056: p for trend =0.025, should be interpreted cautiously given small counts in C2 and C3. Other vascular risk factors were variably represented without significant differences between groups. Full distributions are shown in .

Table 6-7.**Table 6-7 Risk factors distribution across C subdomains**

Variable	C1 (n=12)	C2 (n=7)	C3 (n=10)	C0 (n=37)	C9 (n=34)	P value	P trend
Age (mean± SD)	75.8± 8.4	65.9±16.1	67±11.3	59.3±16.2	68.0±11.4	0.003	<0.001
Male n (%)	9 (75.0)	5 (71.4)	8 (80.0)	23 (62.2)	23 (67.6)	0.818	0.362
Female n (%)	3 (25.0)	2 (28.6)	2 (20.0)	14 (37.8)	11 (32.4)		
HTN n (%)	8 (66.7)	4 (57.1)	4 (40.0)	19 (51.4)	23 (67.6)	0.452	0.385
DM n (%)	3 (25.0)	1 (14.3)	2 (20.0)	8 (21.6)	4 (11.8)	0.788	0.945
PVD n (%)	2 (16.7)	0	2 (20.0)	2 (5.4)	3 (8.8)	0.460	0.358
H.Choles. n (%)	1 (8.3)	1 (14.3)	3 (30.0)	9 (34.3)	3 (8.8)	0.299	0.230
Smoking n (%)	3 (25.0)	3 (42.8)	7 (70.0)	22 (59.5)	22 (64.7)	0.017	0.014
Alcohol n (%)	3 (25.0)	1 (14.3)	0	7 (18.9)	4 (11.8)	0.488	0.781
CAD n (%)	4 (33.3)	0	1 (10.0)	2 (5.4)	8 (23.5)	0.056	0.025
AF n (%)	6 (50.0)	0	0	1 (2.7)	0	<0.001	0.001
TIA/IS n (%)	4 (33.3)	1 (14.3)	2 (20.0)	9 (24.3)	9 (26.5)	0.939	0.797
cardiac illness* n (%)	5 (41.7)	3 (42.9)	3 (30.0)	4 (10.8)	7 (20.6)	0.051	0.010

*Cardiac illnesses= MI 16 patients, VD two patients, VD with MI or HF 3 patients, and PFO 1 patients

6.4.8 Risk factors by cardioembolic aetiology (CE) status

Patients with CE aetiology (n= 29) were significantly older than those without CE causes (n = 37) with mean difference of 11.2 years, 95% CI 4.11 to 18.52, p = 0.003. Cohen's d = -0.78, representing a moderate to large age effect. HTN was slightly more common in CE group, whereas DM reported as same rates, but neither associated reached statistically significant. PVD was more frequent among

patients with CE, though the wide CI reflect small case numbers. High cholesterol was more frequent in patients without CE, but these differences were not significant.

A history of prior TIA or IS was comparable between groups. CAD was more prevalent in the CE group with no significant reached. In contrast both history of AF and prior cardiac illnesses were more common in CE group and demonstrating statistically significant association $p = 0.019$, and 0.009 respectively Table 6-8.

Table 6-8 Risk factors by cardioembolic aetiology

Variable	CE present (n = 29)	CE absent (n = 37)	95%CI	OR	P value
Age (mean± SD)	70.6±12.1	59.3±16.2	4.11-18.52		0.003
HTN n (%)	16 (55.2)	19 (51.4)	0.44-3.09	1.16	0.758
DM n (%)	6 (20.7)	8 (21.6)	0.29-3.11	0.95	0.927
PVD n (%)	4 (13.8)	2 (5.4)	0.48-16.49	2.81	0.239
High Cholesterol n (%)	5 (17.2)	9 (24.3)	0.19-2.20	0.65	0.485
Smoking / ex-smoker n (%)	13 (44.8)	22 (59.5)	0.82-2.15	1.33	0.237
Alcohol n (%)	4 (13.8)	7 (18.9)	0.44-4.24	1.40	0.579
CAD n (%)	5 (17.2)	2 (5.4)	0.67-20.63	3.71	0.121
H/O AF n (%)	6 (20.7)	1 (2.7)	0.98-58.82	7.63	0.019
H/O TIA/IS n (%)	7 (24.1)	9 (24.3)	0.43-2.38	1.01	0.986
H/O cardiac illness* n (%)	11 (37.9)	4 (10.8)	1.25-9.90	3.51	0.009

6.4.9 Multivariate logistic regression for predictors of cardioembolic causes

The logistic regression model was statistically significant (Omnibus $X^2 (5) = 18.65$, $p = 0.002$) and showed acceptable calibration (Hosmer-Lemeshow $X^2 (7) = 6.40$, $p = 0.494$). The model explained between 24.6% (Cox & Snell R^2) and 33.0% (Nagelkerke R^2) of the variance.

After adjustment, age was only statistically significant independent predictor, with each year increasing the odds of CE by 5% (aOR 1.05, 95%CI 1.00-1.10, $p = 0.042$) equivalent to a 1.6-fold increase per decade. prior cardiac illness and history of AF also show positive association that did not reach statistical significance. Sex and CAD were not independent predictors in this model Table 6-9.

Collapsing the C domain to definite (C1, n= 10) versus less certain (C2+C3, n= 17) gave consistent findings. On bivariable testing, AF occurred in 5/10, 50% of C1 versus 0/17 in C2+C3, $p < 0.001$, smoking was lower in C1 (2/10, 20%) than C2+C3

(10/17, 58.8%), $p=0.044$. in the multivariable model including age per decade, sex, smoking, the regression was significant, $p= 0.023$. only the age independently predicted a definite C1 classification (aOR 2.74, 95% CI 1.01-7.45, $p=0.049$). variables with sparse cells like AF, were not entered to avoid quasi-separation

Table 6-9. Multivariate logistic regression for predictors of CE aetiology

Variable	Adjusted OR (aOR)	95%CI for aOR	P value
CDA	1.10	0.16-7.81	0.923
AF	5.21	0.49-55.78	0.172
Prior cardiac illness	3.34	0.81-13.66	0.094
Age (per decade)	1.6	1.00-2.59	0.042
Sex (male vs. female)	0.42	0.13-1.59	0.214

6.4.10 Cross-domain overlap of ASCO-D aetiology: single vs mixed aetiology and risk factors profile

Aiming to quantify overlap between ASCO-D aetiology and compare the risk factor profile of single versus mixed aetiology, 64 complete cases were analysed. At least one mechanism was present in 90.6% (58/64) (score 9 was excluded). Mixed presentations (≥ 2 mechanisms) were more common than single-mechanism profiles (60.9% vs. 29.7%), and one quarter exhibited all three potential mechanisms. Pairwise associations were not statistically significant, indicating that co-occurrence was frequent but not clustered in specific pairs in this cohort (A+S $p = 0.523$, A+C $p = 0.224$, S+C $p = 0.183$).

pattern	N (%)
Nono	6 (9.4)
C	2 (3.1)
S	9 (14.1)
S + C	5 (7.8)
A	8 (12.5)
A + C	4 (6.3)
A + S	14 (21.9)
A + S + C	16 (25.0)

Patients with potentially mixed aetiology (≥ 2 mechanisms; $n= 39$) were older than those with a single aetiology ($n=19$) 68 ± 12.37 vs. 56.74 ± 19.26 years; mean difference 12.4 years (5%CI 3.87-20.61), $p = 0.005$, and more likely to have a prior cardiac illness (35.9% vs. 5.3% 10.08 ; OR=), $p = 0.012$. AF occurred only in mixed cases (15.4% vs 0%). Other risk factors did not differ significantly between groups, all $p \geq 0.25$ Table 6-10.

The burden of vascular risk factors was positively associated with the multiplicity of aetiological mechanism. Spearman's correlation between risk factor count (0-7) and the number of ASCO_D aetiology (0-3) was $\rho = 0.367$ ($p = 0.003$), indicating a moderate monotonic relationship.

Table 6-10. single and mixed mechanisms and risk factors

Variable	Single (n = 19)	mixed (n = 39)	95%CI	OR	P value
Age (mean± SD)	56.74±19.26	68.97±12.37	3.87-20.61		0.005
HTN n (%)	9 (47.4)	23 (59.0)	0.53-4.82	1.60	0.404
DM n (%)	2 (10.5)	9 (23.1)	0.49-13.19	2.55	0.252
High Cholesterol n (%)	5 (26.3)	8 (20.5)	0.21-2.61	0.72	0.622
Smoking / ex-smoker n (%)	9 (47.4)	21 (53.8)	0.43-3.89	1.30	0.643
Alcohol n (%)	3 (15.8)	6 (15.4)	0.21-4.38	0.97	0.968
CAD n (%)	1 (5.3)	5 (12.8)	0.29-24.42	2.65	0.375
H/O AF n (%)	0	6 (15.4)	-	-	0.071
H/O TIA/IS n (%)	4 (21.1)	9 (23.1)	0.30-4.26	1.23	0.862
H/O cardiac illness* n (%)	1 (5.3)	14 (35.9)	1.21-83.75	10.08	0.012

6.4.11 White matter hyperintensity severity and ASCO-D classification

White matter hyperintensity (WMH) burden was assessed using Fazekas scores. Among 75 patients, 44 (58%) had mild WMH, 24 (32%) had moderate WMH, and 7 (9.3%) had severe WMH, with a mean Fazekas total of 2 (IQR 1-4).

6.4.11.1 Association with ASCO-D domains

Cross-tabulation showed a strong relation between WMH severity and S domain. Fazekas severity increased progressively across S certainty categories ($X^2=18.4$, $p=0.005$). In contrast, no association was observed with either the A domain ($p=0.514$) or C domain ($p=0.203$).

When analyses were restricted to definite aetiologies, findings were consistent: WMH severity remained significantly associated with S domain ($X^2= 9.07$, $p=0.011$; trend $p=0.003$), but not for A domain ($p=0.844$). An association was seen with C domain ($X^2 = 6.70$, $p=0.035$), although this was based on small cell counts and should be interpreted with caution Table 6-11.

Table 6-11 White matter hyperintensity severity and ASCOD 1

Fazekas severity	S1	A1	C1
Mild	11/44 (25.0)	9/44 (20.5)	3/44 (6.8)
Moderate	13/24 (54.2)	5/24 (20.8)	2/24 (8.3)
Severe	5 (71.4)	1/7 (14.3)	3/7 (42.9)

P value	0.011	0.844	0.035
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6.4.11.2 Risk factors profiles across Fazekas categories

Age demonstrated a strong gradient across WMH categories, increasing from 57.4 years in mild to 69.7 years in moderate and 79.4 years in severe WMH (ANOVA $F = 13.96$, $p < 0.001$). Tukey post-hoc tests confirmed significant differences between mild and moderate ($p < 0.001$) and between mild and severe groups ($p < 0.001$). Severe WMH was more common in women (71.4%, $p = 0.039$). Smoking also showed an association, more prevalent in moderate group (75%) compared to mild (47.7%) and severe (28.6%), $p = 0.034$. By contrast other vascular risk factors did not differ significantly across Fazekas categories Table 6-12.

Table 6-12 Risk factor profile across Fazekas categories

Risk factor	Mild (n=44)	Moderate (n=24)	Severe (n=7)	p-value
Age (years, means \pm Sd)	57.41 \pm 12.2	69.67 \pm 9.9	79.43 \pm 6.2	< 0.001
Female sex	11/44(25)	10/24 (41.7)	5/7 (71.4)	0.039
Smoking	21/44 (47.7)	18/24(58.3)	2/7 (28.6)	0.034
HTN	25/44 (56.8)	14/24 (58.3)	6/7(85.7)	0.343
DM	8/44(18.2)	2/24(8.3)	0/7()	0.288
High cholesterol	9/44(20.5)	4/24(16.7)	0/7(0)	0.412
H/O CAD	4/44(4.5)	3/24(12.5)	1/7(14.3)	0.863
H/O AF	2/44(4.5)	1/24(4.2)	1/4(14.3)	0.541
H/O IS /TIA	8/44(18.2)	9/24(37.5)	3/7(42.9)	0.135
H/O cardiac illness	8/44(18.2)	5/24(20.8)	4/7(57.1)	0.071

6.4.11.3 Multivariable regression

Binary logistic regression was performed with SVD presence (S1-3) as the dependent variable and predictors age per decade, HTN, sex, smoking and total Fazekas number (WMH burden). The model was statistically significant (Omnibus $X = 30.99$, $p < 0.001$), explained 34-47% of variance (Cox & Snell $R = 0.34$; Nagelkerke $R = 0.47$), and showed good calibration (Hosmer-Lemeshow $p = 0.75$). Classification accuracy was 83% with high sensitivity (94.1%) but modest specificity (58.3%).

Fazekas total score was the only independent predictor of SVD presence (aOR=3.16, 95% CI 1.73-5.77, $p < 0.001$), reflecting the close relationship between SVD severity and its classification as present in ASCO-D system. Sex, age, HTN, and smoking, were not significant. Thus, while severe WMH was more common in women descriptively, sex did not independently predict SVD once WMH burden and other risk factors were accounted for.

6.4.12 Cerebral microbleed and ASCO-D classification

Cerebral microbleed (CMBs) were absent in 58/75 (77.3%) patients; 17/75 (22.7%) had ≥ 1 CMB, with 8/75 (10.7%) showing a burden of ≥ 5 . CMB count correlated positively with age; Spearman $\rho = 0.30$, $p = 0.009$, and increased across Fazekas categories of WMH ($\chi^2(6) = 23.67$, $p < 0.001$).

6.4.12.1 Association with ASCO-D domains

When examined against ASCO-D certainty levels, higher CMB burden tended to cluster with higher SVD certainty (S1 or S2/3) compared to S0 ($\chi^2(6) = 12.07$, $p = 0.060$; likelihood ratio $\chi^2(6) = 15.05$, p for trend = 0.020). In the A domain, there was no consistent monotonic trend across A0-A3; although moderate-high CMB burden appeared more frequent in A3, the overall association was not significant ($\chi^2(9) = 12.10$, $p = 0.208$). In the C domain, most patients with CMBs remain C0, with no significant association observed ($\chi^2(9) = 7.18$, $p = 0.618$). In the A domain, none of the patients with CMBs were classified as A1, while 5/16 (31.3%) were assigned to A2-A3; however, this distribution did not reach significance. In the C domain, 4/17 (23.5) of patients with CMB were classified as C1 and 1/17 (5.9) as C2-C3, without significant association Table 6-13.

6.4.12.2 Logistic regression for definite SVD

In logistic regression models, CMB burden predicted definite SVD (S1 vs not S1) in univariable analysis; OR= 2.01 per category, 95% CI 1.20-3.36, $p = 0.004$; Hosmer-Lemeshow $p = 0.816$. In multivariable model adjusted for age, HTN, and sex, the association attenuated (aOR = 4.17, 95% CI 0.85-20.45, $p = 0.078$), while age showed a borderline effect (aOR = 1.50 per decade, 95% CI 0.98-3.31, $p = 0.062$).

CMBs were associated with age, WMH severity and S-domain certainty, supporting their role as a marker of cerebral small vessel disease Table 6-14. No meaningful associations were found with A and C domains, consistent with their specificity for SVD rather than larger artery or cardioembolism mechanisms.

Table 6-13 Association cerebral microbleeds with ASCO-D1

	S1	A1	C1
CMB = 0	18/58 (31.0)	14/55 (25.5)	4/41 (9.8)
CMB = 1	1/4 (25.0)	0/4 (0)	1/3 (33.3)
CMB = 2-4	4/5 (80.0)	0/4 (0)	1/4 (25.0)

CMB \geq 5	6/8 (75.0)	0/4 (0)	2/6 (33.3)
P value	0.004	0.169	0.271

Table 6-14 Association of cerebral microbleeds with Fazekas severity

Fazekas category	No CMB	CMB=1	CMB=2-4	CMB \geq 5
Mild	37/44 (84.1)	3/44 (6.8)	2/44 (4.5)	2/44 (4.5)
Moderate	15/24 (62.5)	1/24 (4.2)	2/24 (8.3)	6/24 (25)
Severe	6/7 (85.7)	0/7 (7)	1/7 (14.3)	0/7 (0)

6.4.13 MRI lesion patterns and ASCO-D classification

On brain MRI, 46/75 (61.3%) patients demonstrated an acute infarct. Lesion patterns were 22/46 (47%) single lacunar, 6/46 (13%) lacunar with cortical, 10/46 (21.7%) single territory cortical, and 8/46 (17.4%) multi-territory cortical infarcts.

Definite ASCO-D association showed distinct mapping. S1 was predominant in single lacunar infarcts, 19/22(86.4%), $\chi^2 = 24.87$, $p < 0.001$. A1 score was most frequent in single territory cortical (8/10; 80.0%) and half of multi-territory (2/4; 50%), $\chi^2 = 25.78$, $p < 0.001$. Multi-territory cortical infarcts showed the strongest association with C1 (3/4; 75%) for both multi-territory cortical with lacunar and non-lacunar infarct, $\chi^2 = 26.98$, $p < 0.001$. however, very small counts.

Table 6-15 MRI lesion patterns and ASCO-D classification

Lesion pattern	n/N(%)	S1 n/N (%)	A1 n/N (%)	C1 n/N (%)
Single lacunar	22/46 (47.8)	19/22 (86.4)	0/22 (0.0)	1/22 (4.5)
Lacunar+ single-territory cortical	6/46 (13.0)	4/6 (66.7)	3/6 (50)	0/6 (0.0)
Lacunar+ multi-territory cortical	4/46 (8.7)	4/4 (100)	0/4 (0.0)	3/4 (75.0)
Single territory-cortical	10/46 (21.7)	0/10 (0.0)	8/10 (80.0)	0/10 (0.0)
Muti-territory -cortical	4/46 (8.7)	2/4 (50.0)	2/4 (50)	3/4 (75.0)
P value		< 0.001	< 0.001	< 0.001

6.5 Discussion

6.5.1 Main findings

Across domains, ASCO-D certainty levels showed biologically coherent relationships with both vascular risk factors and imaging biomarkers. Atherosclerosis certainty aligned mainly with smoking and prior cardiac illness, small vessel disease with advancing age; severe WMH; and greater CMB burden, and cardioembolic certainty with age and AF. these domain-specific gradients, together with the high frequency of mixed-mechanism stroke presentation,

support the construct validity of ASCO-D as a tool reflecting the complex interplay of vascular and cardiac pathology in stroke.

Within the atherosclerosis (A) domain, smoking and prior cardiac illness increases with diagnostic certainty, consistent with their role in systemic atherosclerosis. However, no independent predictors emerged in multivariable analysis, reflecting the limited specificity of these risk factors once adjusted for shared vascular comorbidities.

Within the SVD domain, age and WMH severity showed the most consistent association. Age increased progressively across SVD certainty levels(S0-S1), but WMH burden was the only independent predictor of SVD presence in multivariable analysis, while age and other risk factors lost significance. CMBs burden also more frequent in patients with definite or probable SVD, though this association attenuated after adjusting for age, sex, and HTN; these findings should be interpreted cautiously given the small number of cases with high CBMs burden (≥ 5), which limits reliability. Within the cardiac domain, diagnostic certainty rose primarily with aging and history of AF, both of which remained strongly correlated of CE stroke.

Across domains, mixed-mechanism presentation was common (61%), with 25% of them showing triple-domain overlap. These overlapping attributions likely reflect shared risk factors profiles and cumulative vascular pathology rather than truly independent mechanisms.

Imaging biomarkers reinforced these domain pattern. WMH and CMBs clustered with SVD. Acute infarcts were detected in over half the cohort, with single lacunar infarcts being the most common pattern. Lesion distribution mapped closely to ASCO-D domains: single lacunar infarcts were strongly associated with definite SVD, single- territory cortical infarcts with large artery atherosclerosis, and multi-territory cortical infarcts with cardioembolism.

6.5.2 Pathophysiology interpretation

The observed gradients across the A, S, and C domains align with established vascular and cardiac pathophysiology, strengthen the construct validity of ASCO-D framework in this cohort.

Within the atherosclerotic (A) domain, the associations of smoking and prior cardiac illness with large artery atherosclerotic certainty is consistent with smoking-induced endothelial dysfunction and oxidative injury, which accelerates atherosclerosis via nitric oxide depletion and vascular damage (Addissouky et al., 2024). The absence of independent effects in adjusted model likely reflects the limited specificity of vascular risk factors such as smoking, which contribute to systemic atherosclerotic burden rather than distinguishing definite from probable larger artery atherosclerosis. Prior cardiac illness, including MI and heart failure, also reflect systemic atherosclerosis that shares pathways with cerebrovascular atherothrombosis, including endothelial dysfunction, chronic inflammation, and prothrombotic states (Libby et al., 2009).

In the SVD domain, age and WMH severity both increased with diagnostic certainty, reflecting vascular ageing and arteriolosclerosis, and white matter changes (Yaghi et al., 2021, Pantoni, 2010, Power et al., 2015). WMH burden was only independent predictor of SVD presence, reflecting its role as a structural imaging correlate of microangiopathy. CMBs also clustered with higher S-domain certainty, although their predictive value attenuated after adjustment suggesting variance with vascular ageing. Smoking did not follow this linear pattern; this may be because smoking is more strongly linked to atherosclerotic microangiopathy, than with hypertensive arteriopathy or cerebral amyloid angiopathy which are less smoking dependent (Power et al., 2015, Wang et al., 2024). In patients with overlapping macro- and microvascular disease, strong larger-artery signals may down-rank SVD certainty within the ASCO-D classification

In the cardioembolic (C) domain, higher certainty among older patients with history of AF and prior cardiac illness reflects age-related atrial changes including fibrosis, enlargement, and electrical instability, that predisposed to left atrial thrombus formation and embolic stroke (Sajeev et al., 2020, Chen et al., 2018).

The strong association between AF and C1 is therefore biologically coherent and validates ASCO-D's certainty grading for cardioembolic mechanisms.

Cross-domain overlap further supports the concepts of cumulative vascular pathology. The coexistence of atherosclerosis, microangiopathy, and cardiopathy in the same individuals indicates that mixed-mechanism stroke frequently arises from interacting vascular risk factors and systematic atherosclerotic burden predisposing to both arterial and cardiac pathology (Liu et al., 2018, Sirimarco et al., 2013).

It is important to recognise that ASCO-D certainty may be partly influenced by confirmation bias. Clinicians are often more confident in their classification when imaging findings match what they already expect based on clinical features. This can increase diagnostic certainty even when the same information informed both the clinical and imaging judgement.

6.5.3 Comparison with prior literature

Our findings that mixed-mechanism presentations predominated (60.9%), and 25% of patients exhibited triple-domain overlap is broadly consistent with previous ASCO-D based stroke cohorts that applied comprehensive vascular and cardiac evaluation. In Sirimarco et al.'s cohort of 405 patients, 25% of patients had multiple underlying disease when using grades 1 and 2, and this rose to 80% when all grades were included (Sirimarco et al., 2013). In the CHALLENGE ESUS/CS registry, 11.8% of patients had triple pathogenesis and up to 26.2% with two underlying stroke aetiology (Shimizu et al., 2023) and in other prospective stroke cohorts (Liu et al., 2018), confirming the concept of stroke as a multifactorial disease, where shared systemic vascular pathology often contributes to more than one mechanism.

In the atherosclerotic domain, the association of smoking and prior cardiac disease with higher ASCO-D certainty aligned with large population studies showing these factors as determinants of extracranial and intracranial atherosclerosis (Li et al., 2022, Ding et al., 2020). The absence of an independent effect after multivariable adjustment is consistent with prior analysis indicating smoking contribute to overall vascular injury but not distinguish definite from probable large artery

causes (Ahn et al., 2023, Abd El-Hady Abd El-Ghany et al., 2019, Libby et al., 2009).

Within the small vessel domain, the relationship between age, WMH burden, and SVD certainty reflects prior MRI-based studies linking white-matter damage to vascular ageing and hypertension (Pantoni, 2010, Wardlaw et al., 2019, Yaghi et al., 2021). Our findings that total Fazekas score independently predicted definite SVD supports Staals et al. (Staals et al., 2014) and Wardlaw et al. (Wardlaw et al., 2019) who showed that combined WMH, lacunes, and microbleeds form an integrated SVD burden index associated with stroke and cognitive decline. The clustering of CMBs with SVD in our cohort, though attenuated after adjustment, mirrors prior meta-analysis demonstrated that CMBs predict both recurrent ischaemic stroke and intracranial haemorrhage (Schirmer et al., 2019, Kesav et al., 2020, Charidimou et al., 2018). Together, these findings emphasise the specificity of WMH and CMB as imaging biomarkers of cerebral microangiopathy rather than of large-artery or cardioembolic mechanisms.

In the cardioembolic domain, the association of increasing age and AF with higher diagnostic certainty parallels multiple ASCO-D and TOAST-based datasets (Chen et al., 2018, Liu et al., 2018, Yaghi et al., 2021), confirming age-related atrial remodelling as a key driver of CE attribution. The persistence of age as an independent predictor after adjustment supports its biological role, consistent with evidence linking atrial fibrosis and conduction abnormalities to embolic risk (Sajeev et al., 2020, Chen et al., 2018).

Our study also extends previous work by jointly analysing WMH, CMBs, and acute lesion patterns within the same aetiological framework. Few prior ASCO-D studies have incorporated the quantitative imaging markers. Lesion pattern classification mapped coherently to underlying mechanism: single infarct with SVD, single territory cortical with LAA, and multi-territory cortical with CE. These associations are consistent with recent reviews emphasising the value of infarct topography in mechanism attribution (Schirmer et al., 2019, Jose and James, 2022, Sharobeam et al., 2020).

Taken together, these results confirm that integrating structural MRI markers into aetiological classification enhances mechanistic specificity. However, differences

in population age, imaging protocol, and risk-factor profiles between studies highlight the need for standardised imaging definitions in future ASCO-D-based research (Markus and de Leeuw, 2023, Wardlaw et al., 2019, Wardlaw et al., 2013)

6.5.4 Methodological considerations

The analytic approach was designed to align with the ordinal nature of ASCO-D certainty scoring, therapy preserving gradation in diagnostic confidence. Ordinal trend tests were selected accordingly: the linear-by-linear association X^2 test for categorical variables and Spearman's rank correlation for continuous variables. This method enabled detection of monotonic association between risk factors and increasing certainty with each domain without artificially dichotomising outcome, thus retaining the ordinal structure of ASCO-D scores to preserve diagnostic gradation and maximise statistical power.

Logistic regression was used to explore predictors of high-certainty ASCOD classification, with calibration assessed via Hosmer-Lemeshow test to ensure model fit. In domains where some categories were infrequent e.g. C2, C3, sparse data posed a risk of unstable estimates; this was mitigated by applying likelihood-ratio X^2 or Fisher's exact test where appropriate. We performed sensitivity analysis, including restricting to complete cases and collapsing rare certainty categories, to ensure that findings were not driven by missing data or sparse subdomains. These checks reproduced the main gradients, supporting the internal validity of the results. Convergent findings from both ordinal trend test and logistic regression further strengthen confidence in these association, although low event counts in some strata inevitably widen the CI and limit the precision of certain effect estimates.

These methodological choices strengthen construct validity by matching statistical techniques to the underlying scoring framework, but they also underscore the need for larger datasets to confirm subtle gradients and to produce stable multivariable estimates in subdomains.

Grouping 1-3 as present improves clarity and reproducibility and is aligned with our construct-validity aim, but it may obscure differences between definite and possible causes.

To probe whether specific risk factors predict certainty (score 1), we performed sensitivity analyses collapsing each domain to definite vs less certain. Results were concordant with the main ordinal trends: no independent predictor for A1 or S1, and age predicating C1. In the C-domain, AF produce quasi-separation (5/10 vs 0/17), so it was excluded from multivariable model by design; this protects estimation stability but underestimates the clinical impact of AF on certainty. These analyses are constrained by low event-per-variable and missing in some crosstabs, so effect sizes should be interpreted cautiously.

The use of imaging biomarkers (WMH, CMBs, and lesion patterns) is both a strength and a limitation. While these validate visual scales support the constructive validity of ASCO-D, they remain semi-quantitative, and small strata e.g. CMBs ≥ 5 , multi-territory infarcts, limit power and may inflate effect sizes. Biomarkers were assessed cross-sectionally at baseline, whereas ASCO-D score were finalised after one month, linking baseline imaging to final aetiological classification but not longitudinal change. These methodological choices enhance construct validity by aligning analysis with structure of the scoring framework, but they also underscore the need of larger prospective datasets with repeated imaging to confirm gradient, refine multivariable estimates, and establish predictive value.

6.5.5 Clinical implications

Secondary prevention in stroke should proceed on two complementary layers. First, all patients should receive universal risk-factor modification: statin therapy, blood-pressure reduction, optimisation of glycaemic control, smoking cessation, and weight management. In routine practice these measures are not always applied intensively or consistently; many patients could benefit from systematic implementation, particularly tighter BP control and more proactive metabolic and weight intervention. Second, treatment should be adapted to the likely mechanism: anticoagulation for cardioembolism, antiplatelet therapy for non-cardioembolic stroke, and carotid interventions when applied.

Because vascular and cardiac abnormalities frequently co-exist, management usually involves layering mechanism-specific therapy on top of universal measures, rather than replacing them. In this chapter, ASCO-D categories are used descriptively and do not determine access to investigations. When additional tests

are already clinically indicated, ASCO-D patterns may help with the order/sequence of these tests. The goal is to streamline workflow and avoid work-up bias, not restrict care. Multidisciplinary review using ASCO-D certainty can reduce therapeutic mismatch in patients with overlapping aetiologies, but grades should be interpreted cautiously and not as prescriptive rules. Final decisions integrate bleeding risk, comorbidities, imaging details, and other domains findings.

Evidence for combined antithrombotic strategies remains limited; beyond the COMPASS trial (Eikelboom et al., 2017) few trials have evaluated concurrent anticoagulant and antiplatelet therapy in stroke pathways. The recent Japanese Optimal Antithrombotic Trial (Okazaki et al., 2025) found no net clinical benefit of combination therapy compared with anticoagulant monotherapy in patients with stroke, AF, and atherosclerosis, while bleeding risk was significantly higher. Escalation beyond standard care should therefore be individualised, and time-limited to specific indications, with careful consideration of bleeding risk. Practically, the C-domain signal supports earlier cardiac work-up in order patients when CE is plausible, with ASCO-D guiding the sequence of further tests.

In addition, our imaging findings suggested practical opportunities for refining stroke care pathways. WMH severity and CMBs burden consistently clustered with S-domain certainty reinforcing their role as markers of small-vessels pathology and supporting earlier intensive blood pressure management and antiplatelet therapy in patients with these imaging features. Lesion patterns could provide rapid mechanistic cues that mapping onto ASCO-D domains and could be incorporated into early multidisciplinary review to reduce diagnostic delay.

6.5.6 Integration with thesis aims

Findings from this cohort form the foundation for the other chapters of the thesis. The high rate of mixed-mechanism stroke, combined with lack of clustering between specific domains pairs, supports the main thesis argument that accurate aetiological classification requires comprehensive evaluation. The observed domain-specific risk-factor patterns, show that ASCO-D certainty scores reflect plausible biological relationship rather than chance association. This strengthens the basis for analyses on whether early, structured imaging can increase diagnostic

certainty, resolve incomplete classification (ASCO-D 9), and improve treatment decisions.

The positive link between overall vascular risk burden and the number of concurrent mechanisms also suggests that patients with multiple risk factors may benefit most from early, thorough investigation. These baseline results therefore justify the focus in other chapters on the role of early multimodal cardiovascular imaging in shifting ASCO-D certainty profile, guiding secondary prevention, and influencing treatment pathways.

Integration of imaging biomarkers strengthened the construct validity of ASCO-D. WMH severity and CMBs burden aligned with S-domain certainty, while lesion patterns mapped coherently onto S, A, and C domains, supporting their utility as mechanistic imaging signatures. These findings justify the thesis focus on multimodal cardiovascular imaging, showing that MRI markers can enhance aetiological certainty, reduce incomplete classification, and guide targeted secondary prevention.

6.5.7 Limitations

This study has several limitations. The sample size was small, and some subdomains had very few patients. This reduces statistical power and leads to wide confidence intervals. The cross-sectional design shows association but cannot prove cause-and-effect or rule out reverse causation or capture longitudinal evolution of WMH, CMBs, or lesion patterns.

While most patients had a full diagnostic work-up, some had incomplete investigation (ASCO-D 9), which could have affected the prevalence and strength of observed trends. The O and D domains were excluded due to very low case numbers, so results cannot be applied to these less common stroke types. ASCO-D classification also involves some subjective judgment and may be influenced by selective investigation or reliance on prior medical history when objective evidence is incomplete, introducing potential attribution bias, although structured criteria were used to reduce this risk. In addition, our dichotomisation of score 1-3 as present may obscure distinctions between definite and possible categories; this trade-off was chosen to reduce subjectivity and preserve power

in small subdomains. Collapsing scores to definite versus less certain improve clarity but, together with small cell counts in some strata, limited the precision of adjusted estimates.

Finally, this was a single-centre study in tertiary hospital with advanced imaging, which may limit how well the results apply to settings with fewer resources. All in all, the main limitation is the small sample size, with sparse data in some ASCO-D subdomains, which reduces precision and limits the generalisability of findings beyond a single tertiary centre.

6.5.8 Future directions

Future studies should confirm these findings in larger, prospective ASCO-D-based cohorts to improve the precision of risk-factor gradients and overlap estimates. Research should also examine whether the degree of mechanism overlap predicts important outcomes, such as stroke recurrence and mortality. Targeted interventions especially smoking cessation and intensive cardiovascular risk control could be tested to see if they reduce the frequency of mixed-aetiology stroke. Developing a simple “risk-count to overlap” score may help clinicians quickly identify patients who would benefit most from full vascular and cardiac evaluation. Testing these strategies in different healthcare settings will show how well they work beyond tertiary care.

In addition, future research should integrate longitudinal imaging to track the evolution of WMH, CMBs and lesion patterns, and assess whether these biomarkers add predictive value for aetiological certainty and clinical outcomes. Automated or volumetric MRI methods may complement visual scale, offering greater reproducibility and sensitivity to change. Embedding such imaging biomarking into routine ASCO-D classification could enhance its precision and utility in guiding mechanism-specific secondary prevention.

7 Chapter 7-General discussion

7.1 Introduction

The principal aim of this thesis was to evaluate the role of a comprehensive cardiovascular imaging (CCI) protocol in patients with acute ischaemic stroke and transient ischaemic attack, with the objective of improving aetiological classification and guiding early, mechanism-specific secondary prevention. Accurate and timely identification of stroke mechanism is critical for effective treatment, yet conventional diagnostic pathways are often constrained by incomplete investigation, delayed access to cardiac imaging, and reliance on empirical therapeutic decisions. By integration of advanced multimodal imaging within a fast-track protocol, this work sought to address these limitations and to examine both diagnostic and therapeutic consequences.

The thesis approached this aim through several complementary studies. The systematic review provided the foundation by benchmarking the diagnostic performance of cardiovascular imaging modalities, highlighting the strengths of transthoracic echocardiography (TTE), cardiac CT (CCT) and cardiac MR (CMR) compared to transoesophageal echocardiography (TOE), and identifying gaps in the literature, particularly the absence of randomised evaluations of structured multimodal strategies.

The pilot randomised controlled trial (chapter 3) then demonstrated the feasibility of implementing such a protocol in the acute setting, showing that CCI significantly improved diagnostic completeness, reduced the frequency of incomplete classifications (ASCO-D 9), and enabled earlier assignment of definite mechanisms across multiple domains.

Building on these diagnostic findings, the treatment outcomes analysis (chapter 4) examined whether greater diagnostic clarity translated into meaningful clinical impact. This chapter showed that CCI improved alignment between prescribed secondary prevention strategies and underlying stroke mechanisms, reduced reliance on empirical therapy, and supported more targeted clinical decision-making.

The mediation analysis (chapter 5) clarified the mechanisms underlying the observed benefit, demonstrating that the advantage of CCI was mediated primarily through use of cardiac imaging, rather than through brain MRI or shorter time to imaging, emphasizing the importance of diagnostic content over speed alone.

Finally, the analysis of vascular risk-factor gradients and imaging biomarkers (chapter 6) provided validation of ASCO-D classification framework, showing coherent association between vascular risk profile and ASCO-D-domain certainty, and confirming the predictive role of neuroimaging markers such as Fazekas white matter hyperintensity scores, cerebral microbleeds, and infarct patterns in small vessel disease.

Together, these studies offer a multi-layered evaluation of how comprehensive imaging influences stroke diagnosis and management, moving from systematic evidence review, through feasibility testing, to mechanistic explanation and construct validation. This general discussion will integrate these findings, place them in the context of current literature, and consider their implications for clinical practice, stroke classification systems, and future research.

7.2 Main findings in context

7.2.1 Diagnostic yield and completeness

One of the central findings of this thesis is that the comprehensive cardiovascular imaging protocol significantly improved diagnostic completeness compared with routine care. In the pilot randomised trial, patients in the CCI arm achieved more definite aetiological classification (ASCO-D 1) in 68% of cases compared with 42% in the routine arm, while incomplete work-up (ASCO-D 9) was substantially reduced (10% vs. 33%) by day 30. This demonstrates that embedding cardiac CT, extended CTA, and brain MRI within a single, fast-track diagnostic pathway reduces the frequency of incomplete investigations and enhances the certainty of mechanistic attribution in acute stroke and TIA.

These findings build directly upon the systematic review, which identified important limitations in existing diagnostic strategies. Across the literature, TOE

was consistently regarded as the reference standard for detecting cardiac sources of embolism, yet its use was constrained by its semi-invasive nature, and limited feasibility or availability in the acute setting. TTE was widely available but showed limited sensitivity for key embolic substrates such as left atrial appendage thrombus and patent foramen oval. More recent studies of cardiac CT and cardiac MR demonstrated promising diagnostic accuracy compared to TOE, but the review highlighted substantial heterogeneity in study designs, variable protocols, and importantly, the absence of randomised evaluations of structured multimodal strategies.

By testing CCI as an integrated pathway, the pilot trial addressed this gap and provided the first randomised evidence that comprehensive imaging reduces incomplete classification in real-world acute stroke care. In addition to improved completeness, the CCI protocol achieve earlier diagnostic resolution, with significantly more definite classifications already evident by Day 7, confirming that comprehensive imaging accelerates diagnostic clarity during the critical early phase of stroke care.

The improvement in diagnostic yield was not restricted to a single mechanism but extended across multiple ASCO-D domains, reflecting the added value of combining modalities capable of detecting large artery atherosclerosis, small vessel pathology, and cardiac or aortic embolic sources within the same protocol. This broader mechanistic coverage was reflected in greater detection of small vessel disease, occult cardiac embolic sources, and aortic arch atheroma, highlighting the capacity of CCI to delineate overlapping stroke mechanisms within a single integrated pathway.

This suggests that benefit of CCI arises from the cumulative diagnostic reach of the protocol, rather than from any single test in isolation. Radiation exposure for the combined cardiac CT and CTA remained within national diagnostic reference levels, confirming that these diagnostic gains were achieved safely and feasibly within routine workflow.

These findings are consistent with prior evidence showing that multimodal cardiac imaging improve detection of embolic sources compared with echocardiograph alone (Hur et al., 2009c) . Dual-phase cardiac CT achieves diagnostic accuracy

compared to TOE while enabling faster workflow and greater patient tolerability, and more studies confirm its value for early detection of LAAT in acute stroke. In contrast, this thesis provides the first randomised evidence that integrating cardiac CT, CTA, and brain MRI within a unified protocol translates this diagnostic advantage into earlier and more complete aetiological classification.

In summary, the pilot trial provided the first randomised evidence that a multimodal CCI strategy improves diagnostic completeness beyond routine care. By reducing incomplete classification and increasing definite assignments, CCI addresses a key gap in prior literature and supports mechanism-based secondary prevention.

7.2.2 Treatment impact and clinical management

CCI not only improved diagnostic certainty but also shaped subsequent management. At Day 7, treatment was aligned with confirmed mechanism in 54% of CCI patients compared with 30% in routine care ($p=0.008$). Anticoagulation was more consistently prescribed for patients with confirmed cardioembolic sources (e.g. AF, LAAT), while dual antiplatelet therapy was prescribed in a similar proportion of patients in both groups but was more often used for appropriately for large artery atherosclerosis in the CCI arm. In contrast, empirical single antiplatelet therapy remained the default in the routine group (60% vs. 4%, $p<0.001$), reflecting greater reliance on uncertain treatment strategies when mechanisms were unresolved.

Kaplan-Meier survival analyses provided further insight into treatment dynamics. Persistence on monotherapy, dual antiplatelet, and statins was generally high across both groups, indicating stable prescribing once treatment was initiated. The key difference lay in the appropriateness rather than durability: CCI facilitated early, targeted treatments decision that were subsequently maintained, rather than frequent switching or escalation. Dual antiplatelet therapy, for example, was sustained in patients with atherosclerotic stroke, while anticoagulant remained reserved for confirmed cardioembolic mechanisms.

This emphasis on mechanism-specific prescribing addresses an important evidence gap. As highlighted in the systematic review, most prior diagnostic studies focused

on yield or accuracy without linking imaging results to management decisions. By demonstrating that CCI results not only clarify mechanisms but also influence therapeutic choice and persistence, this thesis provides novel evidence of clinical utility. These findings highlight the clinical value of integrating diagnostic improvements with therapeutic outcomes, moving beyond accuracy studies to demonstrate patient-centred impact.

7.2.3 Mechanistic insight from mediation analysis

The mediation analysis helped to explain how the CCI protocol achieved its diagnostic advantage. Of the four models tested, only cardiac imaging use showed a significant indirect effect $B= 0.13$, 95% CI 0.03-0.28, indicating that part of the benefit of CCI was mediated through the inclusion of cardiac CT. In contrast, brain MRI use and timing of either MRI or CCT did not significantly mediate the relationship between group allocation and definite aetiological classification.

These findings suggest that the value of CCI lies in what imaging is performed rather than in how quickly it is delivered. The increased use of high-yield cardiac imaging was the key driver of improved diagnostic certainty, while early access alone was not sufficient to explain the observed differences. This provides new mechanistic insight and emphasises that diagnostic content, particularly comprehensive cardiovascular evaluation, is central to the effectiveness of multimodal imaging strategies.

This represents a valuable application of mediation analysis within the context of stroke diagnostics, offering a methodological contribution to identifying the causal pathway through which complex interventions exert their effect.

In summary, the mediation model confirmed that cardiac imaging, rather than MRI or imaging timing, was the primary pathway through which CCI improved diagnostic certainty, highlighting diagnostic content as the critical determinant of benefits

7.2.4 Risk factor gradients and biomarker validation

Analysis of vascular risk factors and imaging biomarkers provided further support for the construct validity of ASCO-D classification system. Across domains, risk

factors tracked in biologically coherent patterns: smoking and prior cardiac illness were associated with atherosclerosis, AF with cardioembolism, and age with small vessel disease. These gradients confirmed that certainty scores within ASCO-D reflected expected underlying risk profile.

Neuroimaging markers added further specificity. Fazekas white matter hyperintensity scores independently predicted definite SVD (aOR=3.16, 95% CI 1.73-5.77, $p<0.001$), and cerebral microbleed burden showed similar trend although the association did not reach statistical significance. Infarct topography mapped consistently to domains, with lacunar infarcts linked to SVD, single territory cortical infarcts to atherosclerosis, and multi-territory cortical infarcts to cardioembolism. Mixed-mechanism strokes were common (60%), particularly in patients with high vascular risk burden, underscoring the cumulative nature of stroke pathophysiology.

Together, these findings show that ASCO-D not only aligns with established vascular risk profiles but is strengthened by the integration of imaging biomarkers, which provide additional mechanistic precision in classification. This supports its use as both a research framework and a clinically relevant tool for mechanism-based stroke subtyping.

7.3 Clinical and research implications

7.3.1 Clinical implications

The findings of this thesis have several clinical implications. Early comprehensive cardiovascular imaging significantly improved diagnostic completeness and accelerated identification of stroke mechanisms. More patients achieved definite classification in CCI arm compared to routine group, highlighting the protocol's capacity to resolve diagnostic uncertainty early during patient management. By providing early and accurate classification, CCI supports more targeted secondary prevention during the high-risk early phase after stroke or TIA, when recurrence risk is highest. Patients in the CCI group were more likely to receive therapy that matched their underlying cause and less likely to be treated empirically. This suggests that adopting CCI could reduce inappropriate anticoagulation and missed

opportunities for timely anticoagulation, improving both the safety and effectiveness of early treatment.

The protocol also demonstrated feasibility and safety within an acute workflow. All imaging studies were completed without delaying care, or increasing hospitalization time, and radiation exposure for combined cardiac CT and CTA remained within national diagnostic reference levels. These findings support the practicality of integrating comprehensive imaging into modern stroke pathways.

In practice, early CCI could enable more informed multidisciplinary decision-making between stroke physicians, cardiologists, and radiologists, while maintaining efficient patient throughput. Ultimately, by enabling accurate early diagnosis, CCI may contribute to fewer recurrent events and shorter diagnostic delays, translating diagnostic precision into tangible patient benefit.

7.3.2 Research implications

The results also have clear implications for future research. This pilot trial provides the first randomised evidence supporting the diagnostic and therapeutic value of an integrated multimodal imaging pathway. The next step is to confirm these findings in larger, multicentre pragmatic randomised controlled trials powered for clinical outcomes such as recurrence, disability, and cost-effectiveness. Such studies should incorporate health-economic evaluation, workflow feasibility, and replication in diverse healthcare systems to assess generalisability.

Future research should also explore workflow optimization and automation using quantitative imaging analysis or artificial intelligence to further reduce interpretation time and observer variability. The combination of ASCO-D classification with imaging biomarkers could form the basis for more precise, biologically informed stroke subtyping. Longitudinal studies linking these imaging markers with clinical outcomes and treatment response would help establish their prognostic value and integrate them into future precision-medicine frameworks.

7.3.3 Methodological contribution

This work also contributes methodologically. It includes application of mediation analysis to stroke diagnostics, demonstrating how this statistical approach can be used to identify the relevant components through which complex imaging interventions exert their effects. By distinguishing between the impact of imaging use and timing, this analytic framework provides a new way to understand causal mechanisms in diagnostic research. The same approach could be applied to other multimodal diagnostic interventions where workflow or imaging combinations may influence outcomes.

Together, these implications demonstrate how early comprehensive imaging can be translated from diagnostic innovation to practical, mechanism-guided stroke care.

7.4 Strengths and limitations

7.4.1 Strengths

This thesis has several notable strengths. It represents the first randomised evaluation of a comprehensive cardiovascular imaging protocol in acute stroke and TIA, combining cardiac CT, extended CTA, and brain MRI within a single streamline pathway. This design enabled a direct comparison with routine diagnostic practice and provided new evidence on the feasibility, safety and clinical value of multimodal imaging in the acute phase.

Another key strength is the integration of diagnostic, therapeutic, and mechanistic perspectives. Unlike many previous studies that focused only on diagnostic yield, this work examined how imaging findings influenced treatment alignment and persistence, linking diagnostic improvements to clinical management. The inclusion of a mediation analysis provided an additional methodological strength, allowing identification of the causal pathway through which CCI improved diagnostic certainty.

The study benefited from a well-balanced randomised design, ensuring comparability between groups in baseline characteristics, stroke severity, and risk-factor profile. Imaging protocols were standardized and reproducible, with

high completion rates across all modalities. Radiation exposure for the combined cardiac CT and CTA remained within national diagnostic reference levels, confirming safety and protocol efficiency. Together, these factors enhance the internal validity and practical relevance of the findings.

7.4.2 Limitations

Several limitations should also be acknowledged. First, this was a single-centre pilot study with a modest sample size ($n = 100$), which limits generalisability and statistical power for some secondary endpoints such as new vascular events or procedural outcomes. The study was designed to assess feasibility and proof-of-concept rather than definitive clinical benefit; larger multicentre trials are required to confirm these results and evaluate long-term outcomes such as stroke recurrence, mortality, and cost effectiveness.

Second, the follow-up period was relatively short (30 days). While this timeframe captured early diagnostic and therapeutic effects, it did not allow assessment of sustained clinical impact, adherence to secondary prevention, or late recurrences.

Third, the study population mainly included minor stroke and TIA cases who were stable enough to undergo advanced imaging in tertiary stroke centre. Findings may not be generalised to patients with severe stroke, contraindications to MRI, or those managed in lower-resource settings where comprehensive imaging is less available. The feasibility and cost-effectiveness of implementing this protocol in other healthcare environments remain to be established.

Fourth, the statistical analysis performed in later chapters were exploratory and constrained by sample size. Logistic regression and mediation analysis were appropriate methods but underpowered for detecting small or complex effects. The mediation model, although methodologically valid, was limited to measured variables and may have excluded other potential mediators such as clinician interpretation, communication, or workflow efficiency, which could also have contributed to the observed diagnostic improvement.

Fifth, although the cardiac CT component of the CCI protocol provided high diagnostic yield for cardiac and aortic embolism sources, it was not fully dedicated

cardiac CT optimised for comprehensive evaluation of coronary or structural heart disease. As highlighted in recent work such as ENCLOSE study, such protocol may improve embolic sources detection but do not replace the need for subsequent targeted cardiac imaging when detailed morphological or coronary assessment is required (Kauw et al., 2023)

Finally, the analysis of vascular risk factors and imaging biomarkers were cross-sectional and based on data collected for exploratory secondary analyses. These associations demonstrate internal consistency but cannot establish causality, and small numbers within some ASCO-D subdomains produced wide confidence intervals.

7.5 Future directions

Future research should focus on confirming these results in larger, multicentre trials powered for clinical outcomes such as recurrence, functional recovery, and mortality. Extended follow-up will clarify the durability of early diagnostic and therapeutic effects, while concurrent health-economic evaluation will determine cost-effectiveness and system-level feasibility. Studies should also assess how comprehensive imaging can be integrated into diverse healthcare settings, identifying workflow adaptations and training requirements for sustainable implementation.

Further work should explore quantitative and automated imaging approaches. Advances in artificial intelligence, imaging segmentation, and decision-support algorithms may reduce interpretation time and variability, enabling broader adoption of CCI across centres with different expertise levels.

Finally, combining ASCO-D classification with imaging biomarkers offers an opportunity to refine mechanism-based subtyping and improve recurrence prediction. Longitudinal validation of these imaging markers against treatment response and outcomes will help translate comprehensive imaging into a precision-medicine framework for stroke.

7.6 Conclusion

This thesis demonstrates that early comprehensive cardiovascular imaging can meaningfully enhance the diagnostic and therapeutic management of acute ischaemic stroke and TIA. By integration cardiac CT, extended CTA, and brain MRI within a single coordinated protocol, CCI significantly improved aetiological classification, reduced incomplete work-up, and enabled earlier mechanism-specific secondary prevention.

In fulfilling its principal aim, this thesis demonstrates that integrating advanced multimodal imaging into acute stroke evaluation can bridge the gap between diagnostic and mechanism-specific secondary prevention.

These findings provide the first randomised evidence that a structured multimodal imaging approach is both feasible and clinically relevant in real-world acute care. The work also highlights how diagnostic clarity can translate into better treatment alignment, supporting a shift from empirical therapy to evidence-based, mechanism-guided care.

Methodologically, the application of mediation analysis introduced a new framework for exploring causal pathways in stroke diagnostics, while the analysis of imaging biomarkers reinforced that CCI not only improves diagnostic precision but also deepens understanding of the mechanisms linking vascular pathology to clinical decision-making.

In conclusion, early comprehensive imaging offers a practical route toward precision stroke medicine and establishes a strong foundation for multicentre studies to confirm its benefits on long-term outcomes and health-system efficiency.

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Bibliography

Appendices

Appendix A -NHS Greater Glasgow & Clyde Research & Development Management approval letter (GN17ST585)

<p>Coordinator/administrator: M Travers/ R Syed Telephone Number: 0141 222 1813 E-Mail: Maureen.travers@ggc.acad.nhs.uk Website: www.nhs.gov.uk/rhd</p> <p>02/08/2018</p> <p>University of Glasgow Queen Elizabeth University Hospital Office Block, Zone 9.01 1345 Govan Road Glasgow G51 4TF Scotland</p>	 <p>Clinical Research & Development West Glasgow ACH Dahnir Street Glasgow G2 8SJ Scotland, UK</p>
NHS GG&C Board Approval	
<p>Dear Professor Keith Muir</p> <p>Study Title: Diagnosis by Comprehensive Cardiovascular Imaging for Stroke & TIA (D-CCIST): randomized, controlled pilot study. Principal Investigator: Professor Keith Muir GG&C HB site: Queen Elizabeth University Hospital Sponsor: NHS Greater Glasgow & Clyde R&D reference: GN17ST585 REC reference: 18/WS/0066 Protocol no. (including version and date): 1.0 (01/09/2017)</p>	
<p>I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant Approval for the above study.</p>	
<p>Conditions of Approval</p> <ol style="list-style-type: none">1. For Clinical Trials as defined by the Medicines for Human Use Clinical Trial Regulations, 2004<ol style="list-style-type: none">a. During the life span of the study GGHB requires the following information relating to this site:<ol style="list-style-type: none">i. Notification of any potential serious breaches.ii. Notification of any regulatory inspections.	
<p>Page 1 of 2 GN17ST585 Management Approval Letter</p>	
	
<p>It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy (www.nhs.gov.uk/content/default.asp?Page=1411), evidence of such training to be filed in the site file.</p> <ol style="list-style-type: none">2. For all studies the following information is required during their lifespan.<ol style="list-style-type: none">a. Recruitment Numbers on a quarterly basisb. Any change of staff named on the original SS formc. Any amendments – Substantial or Non Substantiald. Notification of Trial/Study end including final recruitment figurese. Final Report & Copies of Publications/Abstracts	
<p>Please add this approval to your study file as this letter may be subject to audit and monitoring. Your personal information will be held on a secure national web-based NHS database. I wish you every success with this research study</p>	
<p>Yours sincerely,</p>	

Appendix B- D-CCIST patient information sheet.



D-CCIST PIS.docx

Appendix C- DCCIST participant consent form



Consent form .docx

Appendix D- D-CCIST data collection form



Amended D-CCIST
Data collection form v

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