

## SUPPLEMENTARY MATERIALS

### Detailed Methodology

This study's dataset was derived from two main sources. Firstly, among patients admitted with IS to Mersey and West Lancashire Teaching Hospitals NHS Trust (MWL, United Kingdom) between July 2021 and July 2022, we randomly selected the first 25 patients with visually evident intracranial arterial calcification (IAC) on head CTA (or otherwise referred to as CTA of the carotid arteries), and the first 20 patients without visually evident IAC. The visual assessment of the calcification was done independently by researchers AHR (stroke consultant) and SE (medical registrar) who were blinded to patients' outcome.

Secondly, as IAC is not readily assessed or reported on CTA, we needed to obtain and calculate calcium scores. All identifiable information were removed from included the scans, which were pseudo-anonymised and given pseudo-ID numbers (the key or link codes were kept at MWL servers and only authorised researchers had access to this information).

Then, the baseline characteristics and outcome events were subsequently retrieved from the Combined Intelligence for Population Health Action (CIPHA) database. Operated by Graphnet Health Limited, CIPHA functions as the unified data platform for the Cheshire and Merseyside Integrated Care System, incorporating deidentified data from multiple sources, such as acute hospitals, community healthcare providers, social services, mental health care, and local government authorities.

The final dataset extracted from Graphnet was anonymised and consisted of calcium scores, patients' baseline characteristics, and relevant outcome data. Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) [REC reference: 23/NW/0310] and from the Confidentiality Advisory Group (CAG) [CAG reference:24/CAG/0023].

### Study subjects and outcomes

A total of 45 patients were randomly selected by selecting consecutive scans (CTA of the carotid arteries) of patients admitted to MWL with IS. As only adults aged 18 and above were included, one scan was excluded. Patients who had opted out through the National Data Opt-Out (NDOO), a service that enables individuals to prevent their confidential data from being used for purposes such as research [10], were identified and excluded.

To give all patients the chance to opt out of the study, posters and leaflets were displayed throughout various hospital sites. These materials informed individuals about the study, provided contact details for those with questions or wishing to opt out, and directed them to resources for learning more about the National Opt-Out system. However, no patients in our study opted out using this method.

One patient was from outside the region; hence, we had no accurate record of their outcomes or baseline characteristics. This patient was excluded leaving a final cohort of 37 subjects. Patients were then divided into two groups according to the presence or absence of IAC. The outcomes of this study were recurrent IS and all-cause mortality. The follow-up period was calculated from the date of their scans until recurrent IS, all-cause death or the end of follow-up (i.e. date of outcome data extraction from CIPHA- 28<sup>th</sup> January 2025), and those without events were censored data.

### Covariates extraction

Graphnet Health Limited [11] facilitated the integration of the obtained calcium scores with corresponding patient data within CIPHA, creating an anonymised dataset that included baseline characteristics and outcomes. Structured Query Language (SQL) was employed to extract relevant information from CIPHA records. To define the clinical conditions of interest, we relied on SNOMED CT [12] concepts. Initially, we attempted to use existing SNOMED concepts, such as those found in the Quality and Outcomes Framework (QOF), to streamline the process and enhance validity. However, this approach proved insufficient, as QOF is primarily tailored to Primary Care, whereas our dataset encompassed non-Primary Care data as well.

To address this limitation, we adopted an alternative strategy. For each clinical category (e.g., diabetes mellitus), we selected the most suitable SNOMED code at the Parent level to minimise ambiguity. The corresponding concept lists for each category were then expanded to include all Child codes, utilising the SNOMED hierarchy for both inferred and stated relationships.

A similar methodology was applied to medications. Using locally developed SQL queries with SNOMED Hierarchy tables, we generated concept lists for Actual Medical Product (AMP) and Virtual Medical Product (VMP) classifications, aligning them with the specified generic medication groups. Where applicable, the British National Formulary (BNF) was also referenced, particularly when medications were categorised within distinct chapters.

This approach enabled the thorough construction of SNOMED CT concepts across all relevant hierarchical levels. However, there was a potential risk of including concepts beyond the intended scope, necessitating additional manual refinement. SNOMED CT UK Browser was used as a validation reference [13].

Besides calcium scores, we collected other variables including demographic variables (such as age and sex), lifestyle factors such as smoking status (categorised as current, never, or former smoker) and body mass index (BMI), and medical history and comorbidities including; hypertension, diabetes mellitus (DM) (both type 1 and type 2), history of AF, history of IS, history of transient ischaemic attack (TIA), congestive heart failure (CHF), chronic kidney disease (CKD), valvular heart disease (VHD), dyslipidaemia, and coronary artery disease (CAD) (including angina, myocardial infarction, ischaemic heart disease, coronary artery disease, and coronary artery bypass graft). Peripheral artery disease (PAD) was also included, covering conditions such as peripheral arterial disease, intermittent claudication, percutaneous intervention for the abdominal aorta, atherosclerosis of the aorta, and femoro-femoral bypass. Information on the use of antithrombotic therapy (direct oral anticoagulants, warfarin) or antiplatelet agents were also collected.

## IAC segmentation

The Insight Segmentation and Registration Toolkit (ITK-SNAP, version 4.2.2) [14] was used by researcher SE to manually annotate intracranial arteries. A total of 21 brain CTA volumes containing intracranial artery calcifications (IAC) were annotated by clinicians. These annotated datasets were then combined with an additional 16 brain CTA volumes without IAC, forming a comprehensive dataset for training the segmentation model.

For the IAC segmentation task, nnU-Net [15] was selected and trained. Unlike traditional deep learning models that require extensive manual tuning, nnU-Net is a self-configuring pipeline that dynamically optimizes model training. This adaptability enables nnU-Net to generalize effectively across diverse imaging datasets without requiring task-specific modifications, making it highly suitable for clinical applications.

As shown in Figure 1, the nnU-Net was trained directly on 3D CTA volumes, utilizing a loss function that combines weighted Dice loss and cross-entropy loss. This combination enhances segmentation accuracy by addressing class imbalance and improving model convergence.

Our model framework is implemented in PyTorch and leverages ‘batchgenerators’ for data augmentation, ‘SimpleITK/nibabel’ for medical image I/O, and ‘medpy’ for evaluation metrics. It uses ‘numpy’, ‘scipy’, and ‘joblib’ for numerical processing and parallelism, with system-level tasks managed via standard Python libraries and environment variables. Given the limited number of patients with IAC ( $n = 21$ ) in our cohort, we employed a simplified, exploratory approach to quantify IAC burden. Specifically, the total volume of all calcified lesions ( $\text{mm}^3$ ) was first calculated, followed by the computation of the mean Hounsfield Unit (HU) density across these lesions. The final IAC burden was estimated by multiplying the total calcified volume by the mean density. This method provided a continuous metric for IAC severity; however, it remains exploratory and has not been independently validated.

## Statistical analysis

The data extracted in this analysis had only 4 (10.8%) missing values for BMI, so we ran the ‘mice’ package in R for interpolation based on Multivariate Imputation by Chained Equation [16]. Continuous variables were presented as mean with standard deviation (SD) and compared between groups using the Student’s t-test. Categorical variables were reported as counts and percentages, with differences between groups evaluated using the Fisher’s exact test.

To assess the relationship between IAC and survival in IS patients with recurrent stroke and all-cause mortality, Kaplan-Meier survival analysis was used, and a log-rank test was performed to compare the survival curves of the groups with or without IAC for significant differences. To further assess the association between the presence or absence of IAC and the risk of IS recurrence and death, we constructed univariate and multivariate Cox proportional hazards models. IAC status (with vs. without calcification) was used as the main exposure variable, and

Cox regression analyses were performed in two steps: (1) univariate Cox proportional risk model: only IAC status was included to assess its crude association with study outcomes, and (2) multivariate Cox proportional risk model: age and sex were adjusted for to control for potential confounders. Hazard ratios (HR) and their 95% confidence intervals (CI) were used to quantify risk.

In this analysis, STATA (version 18, USA), R (version 4.4.1, Austria) software, and Python (version 3.10.4, USA) were used, with a two-sided  $p < 0.05$  as the threshold for statistical significance.

### Ethical approval and informed consent statement

Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) [REC reference: 23/NW/0310] and from the Confidentiality Advisory Group (CAG) [CAG reference:24/CAG/0023].

### Declaration of conflicting interests

None directly relevant to this work.

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No funds, grants, or other support was received.

### Authors contribution

SE, YZ, GYHL and AHAR conceived the research and designed the study. SE, JX and YW performed the methodology and AI analysis. SE completed the statistical analysis. GJI, AMH, YZ, GYHL and AHAR handled the supervision and verified the analysis results. SE drafted the initial manuscript. All authors read and made critical revision of the manuscript for key intellectual content and approved the final version.

### Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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are grateful for their contribution to participant engagement and for helping us reach a wider audience.

**Supplementary Table SI. Baseline characteristics of individuals with and without calcification.**

|                        | Overall         | No Calcification | With Calcification | P      |
|------------------------|-----------------|------------------|--------------------|--------|
| N                      | 37              | 16               | 21                 |        |
| Age, years             | 58.08 ± 18.20   | 42.31 ± 13.07    | 70.10 ± 10.84      | <0.001 |
| Female, n (%)          | 19 (51.4)       | 10 (62.5)        | 9 (42.9)           | 0.484  |
| BMI, kg/m <sup>2</sup> | 28.74 ± 6.83    | 27.30 ± 6.94     | 29.83 ± 6.71       | 0.270  |
| Calcification score    | 526.68 ± 774.34 | 0.0 ± 0.0        | 927.95 ± 829.64    | 0.394  |
| Smoking status, n (%)  |                 |                  |                    | 0.440  |
| Current smoker         | 9 (24.3)        | 5 (31.2)         | 4 (19.0)           |        |
| Past smoker            | 13 (35.1)       | 4 (25.0)         | 9 (42.9)           |        |
| Never smoker           | 15 (40.5)       | 7 (43.8)         | 8 (38.1)           |        |
| Comorbidities, n (%)   |                 |                  |                    |        |
| Hypertension           | 14 (37.8)       | 2 (12.5)         | 12 (57.1)          | 0.015  |
| Diabetes mellitus      | 7 (18.9)        | 3 (18.8)         | 4 (19.0)           | 1.000  |
| History of AF          | 7 (18.9)        | 0 (0.0)          | 7 (33.3)           | 0.032  |
| History of IS          | 13 (35.1)       | 4 (25.0)         | 9 (42.9)           | 0.436  |
| History of TIA         | 4 (10.8)        | 1 (6.2)          | 3 (14.3)           | 0.806  |
| CAD                    | 9 (24.3)        | 0 (0.0)          | 9 (42.9)           | 0.009  |
| PAD                    | 2 (5.4)         | 0 (0.0)          | 2 (9.5)            | 0.592  |
| CHF                    | 5 (13.5)        | 1 (6.2)          | 4 (19.0)           | 0.520  |
| CKD                    | 5 (13.5)        | 0 (0.0)          | 5 (23.8)           | 0.107  |
| VHD                    | 7 (18.9)        | 1 (6.2)          | 6 (28.6)           | 0.196  |
| Dyslipidaemia          | 3 (8.1)         | 0 (0.0)          | 3 (14.3)           | 0.332  |
| Medications, n (%)     |                 |                  |                    |        |
| DOAC                   | 6 (16.2)        | 0 (0.0)          | 6 (28.6)           | 0.059  |
| Warfarin               | 4 (10.8)        | 0 (0.0)          | 4 (19.0)           | 0.189  |
| Antiplatelet agents    | 17 (45.9)       | 4 (25.0)         | 13 (61.9)          | 0.059  |

Note: continuous variables are presented as mean ± standard deviation and are compared between groups using Student's t-test. Categorical variables are expressed as counts (n) and percentages (%) and are analysed using the chi-square test or Fisher's exact test.

Abbreviations: *AF*: atrial fibrillation; *BMI*: body mass index; *CAD*: coronary artery disease; *CHF*: chronic heart failure; *CKD*: chronic kidney disease; *DOAC*: direct oral anticoagulant; *IS*: ischaemic stroke; *PAD*: peripheral arterial disease; *SD*: standard deviation; *TIA*: transient ischaemic attack; *VHD*: valvular heart disease.

**Supplementary Table SII. Cox proportional hazards models based on the different CAC DRS categories.**

(a) Ischaemic stroke

| Group                                     | HR        | 95% CI      | P     |
|-------------------------------------------|-----------|-------------|-------|
| Unadjusted Cox proportional hazards model |           |             |       |
| Without calcification                     | Reference |             |       |
| With calcification                        | 3.17      | 1.13-8.88   | 0.029 |
| Adjusted Cox proportional hazards model   |           |             |       |
| Without calcification                     | Reference |             |       |
| With calcification                        | 16.52     | 2.50-109.30 | 0.004 |

(b) All cause mortality

| Group                                     | HR        | 95% CI     | P     |
|-------------------------------------------|-----------|------------|-------|
| Unadjusted Cox proportional hazards model |           |            |       |
| Without calcification                     | Reference |            |       |
| With calcification                        | 3.30      | 0.37-29.53 | 0.286 |
| Adjusted Cox proportional hazards model   |           |            |       |
| Without calcification                     | Reference |            |       |
| With calcification                        | 0.28      | 0.01-6.76  | 0.430 |

Adjusted Cox proportional hazards model for age and sex;

Abbreviations: *CI*, confidence interval; *HR*, hazard ratio.

**Supplementary Table SIII. Quantitative scoring of intracranial arterial calcification burden for participants with detected calcification.**

| Participants | Volume (m <sup>3</sup> ) | Mean Density (HU) | Score      |
|--------------|--------------------------|-------------------|------------|
| 1            | 76.28                    | 1725.89           | 131658.60  |
| 2            | 744.79                   | 1736.54           | 1293354.00 |
| 3            | 172.11                   | 1653.03           | 284500.10  |
| 5            | 1052.37                  | 1747.10           | 1838603.00 |
| 7            | 95.52                    | 1693.20           | 161728.30  |
| 10           | 90.58                    | 1818.33           | 164697.40  |
| 11           | 606.06                   | 1774.12           | 1075224.00 |
| 12           | 352.05                   | 1752.87           | 617099.30  |
| 13           | 86.43                    | 1593.79           | 137745.90  |
| 14           | 322.16                   | 1731.69           | 557886.10  |
| 15           | 232.17                   | 1620.42           | 376220.60  |
| 16           | 164.31                   | 1752.35           | 287926.30  |
| 17           | 29.07                    | 1543.34           | 44858.90   |
| 18           | 636.23                   | 1773.95           | 1128641.00 |
| 19           | 195.91                   | 1612.36           | 315870.90  |
| 20           | 413.83                   | 1743.75           | 721609.30  |
| 21           | 33.96                    | 1529.28           | 51938.19   |
| 22           | 88.10                    | 1593.11           | 140360.30  |
| 23           | 84.72                    | 1715.40           | 145323.20  |
| 24           | 154.70                   | 1743.00           | 269643.30  |
| 25           | 469.48                   | 1714.94           | 805134.20  |