Supplementary Methods

Inclusion criteria

Embolic stroke of undetermined source (ESUS) was defined, according to standard criteria,[1] as a non-lacunar stroke in the absence of (1) extracranial or intracranial atherosclerosis causing ≥50% luminal stenosis in arteries supplying the area of ischemia; (2) major-risk cardio-embolic sources of embolism (permanent or paroxysmal atrial fibrillation (AF), sustained atrial flutter, intracardiac thrombus, prosthetic cardiac valve, atrial myxoma or other cardiac tumors, mitral stenosis, recent [<4 weeks] myocardial infarction, left ventricular ejection fraction less than 30%, valvular vegetations, or infective endocarditis); and (3) any other specific cause of stroke identified.

All patients underwent a comprehensive minimum diagnostic assessment, as specified in ESUS criteria. This assessment included: brain computed tomography (CT) or magnetic resonance imaging (MRI), 12-lead electrocardiogram (ECG), precordial echocardiography, cardiac monitoring for ≥24 hours with automated rhythm detection, and imaging of both the extracranial and intracranial arteries supplying the area of brain ischemia (catheter, MR or CT angiography, or cervical duplex plus transcranial Doppler ultrasonography).

Exclusion criteria

According to the revised ESUS-construct update,[2] we excluded: (1) patients aged <60 years with high-risk patent foramen ovale (PFO) (clinical and anatomical features), categorized as probably or possibly associated with stroke according to the PFO-Associated Stroke Causal Likelihood (PASCAL) classification system;[3] (2) patients with high-risk (plaque ulceration, endoluminal or mobile thrombus) non-stenosing (<50%) ipsilateral (in an intra- or extracranial artery supplying the ischemic field, including the aortic arch) supra-cardiac atherosclerosis;[4–6] and (3) patients with probable cancer-related hypercoagulability (defined as active cancer with or without other concurrent arterial-venous thrombosis).[7]

Echocardiographic parameters

Transthoracic echocardiography (TTE) was performed on each patient during hospitalization. All tests were conducted, and measurements were acquired in accordance with the American Society of Echocardiography guidelines.[8] All data were reviewed by three cardiologists (AB, BDC, and AM). Parameters such as left atrial (LA) volume, LA volume index (LAVI), left ventricular ejection fraction (LVEF), and LV diastolic function were obtained from previous reports. LV diastolic dysfunction (LVDD) was defined according to the American Society of Echocardiography guidelines,[9] using the mitral valve inflow pattern with pulsed-wave Doppler, e'-wave at tissue Doppler of the lateral and septal mitral annulus, tricuspid regurgitation velocity, and LAVI.

PFO diagnosis

For patients aged <60 years, transcranial Doppler (TCD) was performed, both at rest and during provocative maneuvers using an intravenous injection of agitated saline, to identify the presence of a right-to-left shunt (RLS). Among patients aged ≥60 years, a PFO search was conducted in selected cases. In cases where RLS was detected, patients underwent further evaluation using transesophageal echocardiography (TEE) to confirm the presence of a PFO. TEE was also used to further assess the anatomical characteristics of the shunt, including the presence of an atrial septal aneurysm (ASA). A large shunt was defined as >30 bubbles at rest on TCD[10] and/or >20 bubbles in the left atrium after TEE.[11] In the presence of a PFO, the Risk of Paradoxical Embolism (RoPE)[12] was also calculated. High-risk PFO was defined based on anatomical features (large shunt and/or ASA) and/or clinical features (RoPE score ≥7).[3]

Non-stenosing supra-cardiac atherosclerosis

Head and neck CT angiography images obtained during admission were reviewed for each patient to evaluate the presence of non-stenosing (<50%) supracardiac atherosclerosis in the aortic arch and the intra- or extracranial arteries supplying the ischemic field. The degree of carotid stenosis was determined according to the NASCET criteria (North American Symptomatic Carotid Endarterectomy Trial).[13] High-risk plaque was defined as any ulcerated or "soft" plaque or any plaque with endoluminal thrombus, causing <50% of luminal narrowing in an intra- or extracranial artery supplying the ischemic field, including the aortic arch (ascending aorta or proximal arch).

AF detection after stroke

Atrial fibrillation detected after stroke (AFDAS) was defined as any occurrence of AF detected after a stroke in patients without known AF, excluding AF detected during admission.[14] During admission, each patient underwent a 12-lead ECG and cardiac monitoring for ≥24 hours with automated rhythm detection. Outpatient cardiac monitoring, including Holter monitoring (ranging from 24 hours to 30 days) and/or an implantable loop recorder (ILR), was performed for all patients at the discretion of the treating physician.

Neuroimaging assessment

Brain CT and/or MRI scans were thoroughly reviewed for each patient. Stroke lesions were analyzed based on (1) location (an-

terior circulation, posterior circulation, and multi-territory [both anterior and posterior circulation or bilateral anterior circulation]) and (2) site (cortical [small isolated cortical lesions], cortico-subcortical [lesions located across cortical and subcortical areas], and deep [involving deep white/grey matter such as the corona radiata, basal ganglia, brainstem, and deep cerebellum]). The occlusion site on CT angiography was also recorded, and large vessel occlusions (LVO) were defined as occlusion of the intracranial internal carotid artery, M1, M2-dominant, A1, P1, basilar, and vertebral arteries; meanwhile, medium vessel occlusions (MeVO) were defined as occlusion of the A2, A3, M2 non-dominant, M3, P2, and P3 segments.[15]

Outcomes definition

Stroke severity was evaluated using the National Institutes of Health Stroke Scale (NIHSS) score, considering either a 1-point increase from the baseline score or an NIHSS score >5. Ninety-day functional status was defined based on the modified Rankin Scale (mRS) score of 0–2 and 0–3. For patients with a pre-ischemic stroke mRS >2 or >3, achievement of mRS 0–2 and 0–3, respectively, was considered in cases of return to baseline mRS. Ischemic stroke recurrence and AFDAS from discharge to the last available follow-up were considered as long-term follow-up outcomes.

Standard protocol approval, registration, and patient consent

This study was approved by the local ethics committee (Comitato Etico Milano Area 3, n. 346-18052022). Upon admission, patients were duly apprised that all data obtained during routine clinical practice would be utilized for research endeavors and subsequently granted their written informed consent. This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[16]

Statistical analysis

Baseline characteristics, acute-phase therapy, radiological sites of the ischemic lesions, echocardiographic features, and 3-month outcomes were evaluated in the included patients. Differences in variables between the AC(+)/ESUS and AC(-)/ESUS groups were analyzed through univariate analysis (including χ^2, Fisher's exact test, and Wilcoxon-Mann-Whitney test, as appropriate). The association between AC (considered as continuous [LAVI] and dichotomous variable [AC(+) vs. AC(-); moderate/severe AC vs. mild AC/AC(-)]) and various outcomes, such as stroke severity (measured by baseline NIHSS score), 90-day mRS 0–2 and 0–3, stroke recurrence, and AFDAS, was assessed through univariate and multivariate logistic (or ordered logistic, as appropriate) regression analyses. The latter were adjusted for pre-specified baseline variables. The association between AC and stroke severity was adjusted for site of vessel occlusion (no occlusion/LVO/MeVO) and vascular territory (anterior circulation/posterior circulation/multi-territory); 90-day mRS adjusted for age, sex, baseline NIHSS score, pre-AIS mRS score >2, site of vessel occlusion, vascular territory, arterial hypertension, diabetes, coronary artery disease (CAD), dyslipidemia, and acute-phase treatment; stroke recurrence and AFDAS adjusted for age, sex, arterial hypertension, diabetes, CAD, dyslipidemia, obesity, and ILR. Moreover, the association between time to stroke recurrence was evaluated using Kaplan–Meier survival analysis stratified according to the presence of AC. Subsequently, the significance of the differences was evaluated using the log-rank test. Additionally, a sensitivity Kaplan–Meier survival analysis was performed, excluding patients discharged on anticoagulant therapy. Statistical analyses were performed using Stata statistical software (Version 17; StataCorp., College Station, TX, USA). The significance level was set at $P<0.05$.

Supplementary Results

Among the 2,050 patients with acute ischemic stroke admitted to our stroke unit during the study period (between 2018 and 2022), 21.3% (436 patients) were classified as having ESUS. LAVI measurements were available for 95% (414 patients) of ESUS cases, with 22 patients (5%) having no available LAVI due to a poor echo acoustic window. A total of 116 patients (28%) were reclassified and excluded as per the ESUS construct update, resulting in a final cohort of 298 patients with ESUS. A flowchart of the study is shown in Supplementary Figure 1. In the final ESUS cohort (revised criteria), the median age was 71 years (IQR 61–80), the baseline NIHSS score was 5 (IQR 2–11), and 45.6% of the patients were women. Three-months mRS data were available for 295 patients (1% lost at follow-up), with 215 patients (72.9%) achieving an mRS score of 0–2 at 90 days. No significant differences were observed in the location of infarcts (multi-territorial, anterior, or posterior circulation) or intracranial vessel occlusions between AC(+)/ESUS versus AC(-)/ESUS.

Long-term follow-up data were available for 290 patients (3 patients [1.0%] were lost to follow-up and 5 [1.7%] died during the acute phase) (Supplementary Table 1).

Kaplan–Meier analysis (Supplementary Figure 2) revealed no difference in stroke recurrence among patients stratified according to AC (log-rank test, $P=0.149$). Further, we conducted a sensitivity Kaplan–Meier analysis, excluding patients discharged on anticoagulant therapy (n=9 patients), which led to consistent results.

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