Supplementary methods

Subjects and study design

In this single-center retrospective cohort study, we used a prospective registry of patients with acute ischemic stroke (AIS) who were admitted to our government-initiated comprehensive stroke center between January 2010 and May 2019. Patients were enrolled consecutively (1) if they had AIS and were admitted within 24 hours of onset of symptoms and (2) if the diagnosis of embolic stroke of undetermined source (ESUS) could be confirmed based on brain magnetic resonance imaging (MRI), 12-lead electrocardiography, cardiac monitoring for ≥24 hours, transthoracic echocardiography, and computed tomography (CT) or magnetic resonance angiography for the evaluation of extracranial and intracranial arteries. ESUS was defined as non-lacunar embolic ischemic stroke without evidence of a major-risk cardioembolic source, presence of ≥50% stenosis of the lumen of an extracranial or intracranial artery supplying the area of brain ischemia, or stroke without other specific causes, such as arteritis, dissection, migraine/vasospasm, or drug misuse. Cardiac troponin (cTn) levels were measured immediately after admission. The exclusion criteria were as follows: (1) patients who did not undergo baseline serum troponin level assessment on admission or (2) patients lost to follow-up whose clinical outcomes could not be investigated (Supplementary Figure 1). Data on baseline characteristics, underlying risk factors of stroke, and laboratory findings were collected from all subjects.

Subjects were categorized into normal or high troponin groups according to their baseline cTn levels (Supplementary Figure 1). We used sex-specific 99th percentile upper reference limits (URL) of cTn I (cTnI) and high-sensitivity cTn T (hs-cTnT) as thresholds for classifying groups. cTnI levels were measured using a sensitive assay on an automated platform (Dimension Vista, Siemens, Berlin, Germany), with a reported 99th percentile value of ≤30 ng/L for healthy males and ≤15 ng/L for healthy females. hs-cTnT levels were measured using a highly sensitive assay on an automated platform (Elecsys, Roche Diagnostics, Basel, Switzerland), with a reported 99th percentile value of ≤20 ng/L for healthy males and ≤13 ng/L for healthy females (Supplementary Figure 1). The limits of detection of the cTnI and hs-cTnT assays were 10 and 5 ng/L, respectively. At our center, hs-cTnT testing was performed since January 2015. Prior to January 2015, cTnI levels alone were tested. From January 2015, testing for a specific type of troponin (cTnI or hs-cTnT) depended on the physician's clinical judgment for each patient.

Clinical assessment and outcome measurements

The primary outcome measure was the first occurrence of major adverse cerebrovascular and cardiovascular events (MACCE; a composite of stroke, acute myocardial infarction [AMI], or death from a vascular cause) according to the baseline cTn levels over a 1-year period after ESUS. The key secondary outcomes included the incidence of recurrent ischemic stroke, intracerebral hemorrhage, AMI, and death from a vascular cause. Recurrent stroke was defined as a sudden development of a new focal neurologic deficit or worsening of an existing focal neurologic deficit after the index stroke event, with evidence of attributable new stroke lesions (ischemic or hemorrhagic stroke) on brain imaging (CT or MRI). The severity of the neurological deficit was assessed using the National Institutes of Health Stroke Scale (NIHSS). Paradoxical embolism suggesting right-to-left shunt was defined as the presence of microembolic signals on the transcranial Doppler bubble test conducted during hospitalization for all patients. Information regarding clinical outcomes was obtained from all patients during hospitalization, during routine clinic visits, or via telephone interviews with patients or their caregivers. The information was assessed by trained stroke physicians or nurses.

Statistical analysis

Differences between the groups were analyzed using one-way analysis of variance, the Kruskal-Wallis test, or the Mann–Whitney U test for continuous variables. The chi-square test or Fisher's exact test was used for non-continuous variables. Crude associations between cTn levels and the risk for clinical outcomes were analyzed over the study period using Kaplan-Meier curves with log-rank tests. Time-to-first event methods were used for the primary endpoint and for each secondary endpoint. Cox proportional hazard regression models were constructed to calculate the adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for clinical outcomes, according to the baseline cTn levels. Adjustments were performed for age; sex; presence of hypertension, dyslipidemia, diabetes mellitus, coronary heart disease, and cancer; current smoking; prior history of stroke or transient ischemic attack; initial NIHSS score; initial blood pressure; leukocyte counts; hemoglobin, creatinine, and C-reactive protein levels; and administration of reperfusion therapy (intravenous alteplase or mechanical thrombectomy) based on their clinical significance. We conducted additional sensitivity analyses using the overall 99th percentile URL cutoff levels of 21 ng/L for cTnI and 15 ng/L for hs-cTnT without a sex-specific difference. Statistical significance was set at $P<0.05$ in all analyses. Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA), SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and R software version 3.3.1 (R Foundation, Vienna, Austria).

Data availability and ethics statement

All supporting study data can be obtained from the corresponding author on reasonable request from a qualified investigator. Our Institutional Review Board approved the present study. Written informed consent was obtained from all patients or their legal representatives for inclusion in the prospective stroke registry. We used the acronym "TOP-ESUS" to define our research, which stands for cTn levels and outcomes in patients with ESUS. All clinical and laboratory investigations described in this study were conducted in accordance with the principles outlined in the Declaration of Helsinki.

Supplementary results

Patient characteristics

In this study, we included 1,838 consecutive patients with ESUS (Supplementary Figure 1). A total of 1,037 (56.4%) patients underwent cTnI testing and 801 (43.6%) patients underwent hs-cTnT testing. The mean time from symptom onset to cTn testing was 11.9 hours. The mean follow-up duration was 10.6 months. The clinical and biochemical characteristics of patients according to cTn levels are presented in Supplementary Tables 1 and 2. Elevated cTnI levels were detected in 209 (20.2%) of 1,037 patients tested for cTnI and elevated hs-cTnT levels were detected in 170 (21.2%) of 801 patients tested for hs-cTnT. Patients who underwent cTnI testing were more likely to have hypertension, while those who underwent hs-cTnT testing were more likely to have dyslipidemia and old myocardial infarction (Supplementary Table 3). Detailed information on the incidence of ESUS and cTn assays performed each year at our center is provided in Supplementary Table 4.

Clinical outcomes

Among patients who underwent cTnI and hs-cTnT testing, 69 and 58 patients experienced MACCE during follow-up. The annual rates of MACCE were higher in the high cTnI and hs-cTnT groups than in the respective normal groups (14.62% vs. 6.00% and 17.99% vs. 6.01%, respectively) (Supplementary Table 5). Kaplan-Meier analyses showed that the risk of MACCE was significantly higher in the high troponin groups than in the respective normal groups, regardless of the type of troponin test performed (log-rank $P<0.001$) (Figures 1 and 2). In multivariate Cox regression analyses, patients in the high cTnI group had a significantly increased risk of MACCE compared to those in the normal cTnI group (HR, 1.97; 95% CI, 1.13 to 3.44; $P=0.016$) (Figure 3 and Supplementary Table 5) after adjustment for confounders. Similarly, patients with high hs-cTnT levels had a significantly increased risk of MACCE compared to those with normal hs-cTnT levels (HR, 2.69; 95% CI, 1.44 to 5.01; $P=0.002$) (Figure 3 and Supplementary Table 5).

Regarding secondary outcomes, high cTnI and hs-cTnT levels were significantly associated with the risk of vascular death in patients with ESUS (HR, 3.76; 95% CI, 1.40 to 10.1; $P=0.008$ and HR, 4.86; 95% CI, 1.34 to 17.6; $P=0.016$, respectively) (Figure 3 and Supplementary Table 5). High hs-cTnT levels were significantly associated with the risk of recurrent ischemic stroke (HR, 2.62; 95% CI, 1.05 to 6.57; $P=0.039$); this association was not observed for cTnI levels (HR, 1.40; 95% CI, 0.57 to 3.45; $P=0.454$) (Figure 3 and Supplementary Table 5). No significant differences were observed in the risk of hemorrhagic stroke and AMI between the normal and high troponin groups (Figure 2 and Supplementary Table 5).

In the sensitivity analysis, we evaluated the association between elevated cTn levels and clinical outcomes using the overall cTn cutoff levels without sex-specific differences. The prognostic values of both cTnI and hs-cTnT for predicting the risk of MACCE were similar (Supplementary Table 6). High cTnI and hs-cTnT levels were significantly associated with a higher risk of MACCE (HR, 2.40; 95% CI, 1.39 to 4.14; $P=0.002$ and HR, 3.58; 95% CI, 1.87 to 6.86; $P<0.001$, respectively) and vascular death in patients with ESUS (Supplementary Table 6). The high hs-cTnT group, identified using the overall hs-cTnT cutoff levels, had a higher risk of recurrent ischemic stroke than the normal hs-cTnT group (HR, 2.96; 95% CI, 1.22 to 7.16; $P=0.016$) (Supplementary Table 6).

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