

# Soluble ST2 predicts outcome and hemorrhagic transformation after acute stroke

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## Abstract

**Objective:** ST2 is a member of the toll-like receptor superfamily that can alter inflammatory signaling of helper T-cells. We investigated whether soluble ST2 (sST2) could independently predict outcome and hemorrhagic transformation (HT) in the setting of stroke. **Methods:** We measured sST2 in patients enrolled in the Specialized Program of Translational Research in Acute Stroke (SPOTRIAS) network biomarker study. 646 patients had plasma samples collected at the time of hospital admission and 210 patients had a second sample collected 48 h after stroke onset. Functional outcome was assessed using the modified Rankin Scale (mRS), with good and poor outcomes defined as mRS 0–2 and 3–6, respectively. HT was classified using ECASS criteria. The relationships between sST2, outcome, and HT were evaluated using multivariable logistic regression, Kaplan–Meier survival analysis and receiver operating characteristic curves. **Results:** 646 patients were included in the analysis (mean age 69 years; 44% women), with a median NIHSS of 5 [IQR: 2–12]. The median sST2 level on hospital admission was 35.0 ng/mL [IQR: 25.7–49.8 ng/mL] and at 48 h it was 37.4 ng/mL [IQR: 27.9–55.6 ng/mL]. sST2 was independently associated with poor outcome (OR: 2.77, 95% CI: 1.54–5.06;  $P = 0.003$ ) and mortality (OR: 3.56, 95% CI: 1.58–8.38,  $P = 0.001$ ) after multivariable adjustment. Plasma sST2 was also associated with hemorrhagic transformation after adjustment for traditional risk factors (OR: 5.58, 95% CI: 1.40–37.44,  $P = 0.039$ ). **Interpretation:** Soluble ST2 may serve as a prognostic biomarker for outcome and hemorrhagic transformation in patients with acute stroke. ST2 may link neuroinflammation and secondary injury after stroke.

## Introduction

Stroke is a leading cause of long-term disability, yet accurately predicting functional outcome after stroke remains challenging.<sup>1,2</sup> Although clinical factors such as age, sex and stroke severity can stratify risk, a reliable blood-based biomarker at the time of stroke onset would help identify high-risk individuals and potentially inform treatment decisions.<sup>3</sup> Furthermore, a stroke biomarker that could identify patients at greater risk for secondary ischemic

injury, poor functional outcome and mortality would aid in prognosis.

ST2 is a member of the Toll-like/Interleukin (IL)-1 receptor family.<sup>4,5</sup> It serves as the receptor for IL-33 and integrates inflammation, tissue fibrosis and cardiac stress.<sup>5</sup> A soluble form (sST2) is secreted into the circulation and functions as a decoy receptor for IL-33, inhibiting its signaling.<sup>6</sup> Circulating levels of sST2 are associated with adverse outcome and mortality in patients with chronic heart failure (CHF) and myocardial infarction (MI).<sup>7–9</sup>

Recently, higher sST2 has been associated with an elevated risk of incident stroke.<sup>10</sup>

In murine models of ischemic stroke, the administration of interleukin-33 (IL-33) can ameliorate the proinflammatory response, reduce ischemic damage, and improve neurological function.<sup>11</sup> Accordingly, sST2 is highly expressed in astrocytes and microglia, two cell types that may participate in the postischemic inflammatory response.<sup>12</sup> However, no clinical study to date has evaluated the role of sST2 after ischemic stroke in patients. As a result, the relationship between circulating levels of sST2, functional outcome, and its potential for association with intraparenchymal brain pathology is unknown.

In this study, we first sought to determine whether admission sST2 concentration independently predicted outcome and mortality in the setting of ischemic stroke. We also assessed for an association between sST2 and hemorrhagic transformation (HT).

## Methods

### Patient characteristics

The subjects for the study were enrolled in the Specialized Programs of Translational Research in Acute Stroke (SPOTRIAS) network biomarker repository. The SPOTRIAS network repository enrolled patients age 18 years or greater who presented with symptoms consistent with ischemic stroke within 9 (median 3.62, IQR: [2.02–4.87]) hours of last seen well between January 2007 and April 2010. Subjects were eligible if the NIH stroke scale (NIHSS) score was  $\geq 1$ . Clinical data and plasma samples were provided by six academic stroke centers that participated in the SPOTRIAS biorepository. For the current analysis, patients who did not have baseline plasma samples ( $N = 216$ ) were excluded.

Modified Rankin Scale (mRS) assessments were prospectively gathered 90 days after the initial presentation through telephone interview with patients or family members. Poor outcome was defined as mRS of 3–6. Mortality was collected from the medical record and by accessing the Death Master File from the Social Security Administration.<sup>13</sup> For cause of death analysis, the medical records of patients who died and were enrolled at the Partners Healthcare SPOTRIAS sites (Massachusetts General and Brigham and Women's Hospitals) were reviewed ( $N = 314$ ). The primary cause of death was classified based on consensus and categorized into neurological (including stroke, recurrent stroke, hemorrhage, or brain herniation), cardiac (sudden cardiac death), other, or unknown. All subjects or surrogates provided informed consent, and the study was approved by participating institutional review boards.

### Soluble ST2 analysis

Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing blood collection tubes. Within 1 h, EDTA plasma was separated from cellular material via centrifugation, 2000 *g* for 15 min, and the supernatant was stored at  $-80^{\circ}\text{C}$  until the time of analysis. Soluble ST2 was measured from stored plasma samples using a commercially available enzyme-linked immunosorbent assay (Presage ST2 Assay Kit, Critical Diagnostics, San Diego, CA). The mean coefficient of variation for this assay is  $<5\%$ . The lower and upper limits of detection of soluble ST2 were 3.1 ng/mL and 200.0 ng/mL, respectively.

### Hemorrhagic transformation analysis

Imaging data was available for patients enrolled at the Partners Healthcare SPOTRIAS sites (Massachusetts General Hospital and Brigham & Women's Hospital;  $N = 246$ ). Hemorrhagic transformation was previously assessed<sup>14</sup> using the European Cooperative Acute Stroke Study (ECASS) III criteria.<sup>15</sup> All head CTs obtained through day 7 of the initial hospitalization were analyzed (median time to CT 1.2 days; IQR: [1.0–2.1]), discrepancies were adjudicated by consensus, and those performing the imaging analysis were blinded to all clinical data. HT was dichotomized into the presence and absence of HT, and the presence or absence of hemorrhagic infarction type 2 (HI2) or greater. The latter classification was selected because hemorrhagic infarction type 2 (HI2), or parenchymal hemorrhage (PH1 and PH2) have been associated with worse long term functional outcome.<sup>16</sup> Moreover, small petechial hemorrhagic infarction (HI1) may be benign and potentially serves as a marker of early reperfusion.<sup>17</sup> Since symptomatic intracerebral hemorrhages (sICH), as defined by ECASS III criteria, were rare in this cohort ( $n = 7$ ), they were not separately studied.

### Statistical analysis

Baseline characteristics are expressed as mean  $\pm$  standard deviation (SD) for normally distributed continuous variables, or as median with interquartile range [IQR] for ordinal variables or continuous variables showing deviation from normality. Binary variables were represented as frequency and percentage. Skewed variables, such as NIHSS and glucose, were log-transformed to obtain normality prior to analysis. Odds ratios (OR) corresponded to a unit increase in the explanatory variable. Subjects were divided into tertiles based on sST2 concentration to quantify the effect size of the association between cohort characteristics and biomarker data. Differences between

binary variables were analyzed using the Fisher's exact or chi-squared testing as appropriate. Continuous variables were compared between groups using analysis of variance (ANOVA) for parametric and Kruskal–Wallis for non-parametric testing.

Multivariable logistic regression was used to assess the independent association between plasma sST2 levels and functional outcome and mortality. To establish whether the associations between sST2 and functional outcome were independent of underlying cardiovascular disease, we designed a series of multivariable models to sequentially include risk factors for cardiovascular disease (i.e., atrial fibrillation, cardioembolic stroke subtype, and history of congestive heart failure). To avoid model overfitting for the logistic regression model predicting hemorrhagic transformation, only variables with a univariate *P* value <0.10 were included.

The discriminatory value of sST2 was analyzed using receiver operating characteristic (ROC) curve analysis. The incremental discriminatory value of sST2 was estimated by comparing the area under the ROC curve (AUC) for logistic regression models with and without sST2 included in the analysis. We evaluated the ability of sST2 to reclassify risk by comparing the prognostic accuracy of traditional clinical risk factors and calculating the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) for models enriched with sST2. We calculated continuous (category-free) NRI values in groups, since there were no prespecified, externally validated risk categories.<sup>18,19</sup> Kaplan–Meier survival curves were implemented to further investigate the ability of sST2 to predict mortality. Patients were stratified by sST2 tertile and cumulative event rates were compared with the log-rank test. All tests were two-sided and performed with the threshold for significance set at *P* < 0.05. Statistical analysis was performed using JMP Pro 12.0 (SAS Institute, Cary, NC, USA).

## Results

### Clinical characteristics

The initial study population consisted of 862 patients, however, 216 patients did not have baseline plasma samples available for analysis. A total of 646 patients comprised the primary study population. The mean age ( $\pm$  standard deviation) was  $69 \pm 15$  years, and 44% were female. The median admission NIHSS score was 5 [IQR: 2–12], and the 90-day mRS score was 2 [IQR: 1–4]. Plasma samples were collected at  $7.1 \pm 3.3$  h after stroke onset, and the median sST2 concentration for the entire cohort was 35.0 ng/mL [IQR: 25.7–49.8 ng/mL]. The median sST2 concentration for each tertile was

21.86 ng/mL [IQR: 17.76–25.71 ng/mL], 34.98 ng/mL [IQR: 31.62–39.40 ng/mL], and 60.24 ng/mL [IQR: 49.71–76.89 ng/mL], respectively.

The clinical characteristics of the study population are presented in Table 1. Statistically significant differences between sST2 tertiles were observed for age, cardioembolic stroke subtype, history of atrial fibrillation, history of CHF, NIHSS score, and baseline glucose level. In healthy individuals, reported reference values for sST2 differ by sex.<sup>20</sup> Compared to the reported 95% upper reference limit for sST2 in females, the median sST2 for stroke cohort females was higher (38.9 ng/mL vs. 33.5 ng/mL). In contrast, the median sST2 level in the stroke cohort males was lower than the reported 95% upper reference limit of sST2 for that sex (42.7 ng/mL vs. 49.3 ng/mL). Consistent with these findings, 49% of females with stroke had elevated sST2 compared to 38% of males (*P* < 0.001).

### sST2 predicts outcome after stroke

Univariate associations with poor outcome were assessed. Baseline NIHSS, glucose level, age, sex, history of atrial fibrillation, IV tPA use, history of CHF, and baseline sST2 tertile (OR 2.29; 95% CI: 1.53–3.45; *P* = 0.0003) were all associated with poor outcome (see Table 2). Univariate box plot distributions of sST2 level by outcome are shown in Figure 1. In multivariable logistic regression, baseline sST2 remained an independent predictor of poor outcome (OR: 2.77; 95% CI: 1.54–5.06; *P* = 0.003; see Table 2). Additional significant predictors were age, baseline NIHSS, cardioembolic stroke subtype, IV tPA use, and glucose level.

We next assessed predictors of mortality. Age, history of atrial fibrillation, history of congestive heart failure, IV tPA use, cardioembolic stroke subtype, baseline NIHSS, and baseline sST2 tertile (OR: 3.86; 95% CI: 2.15–7.26; *P* < 0.0001) predicted 90-day mortality (Table 2). In multivariable analysis, baseline sST2 (OR: 3.56; 95% CI: 1.58–8.38; *P* = 0.001) remained an independent predictor of death within 90 days after stroke (Table 2). Additional independent predictors included age and baseline NIHSS. The time to death was analyzed using Kaplan–Meier survival curves. Patients in the lowest and second tertiles of sST2 had a minimal risk of death. In contrast, patients with an sST2 level >44.6 ng/mL had the greatest risk of death (Fig. 2), which most often occurred within the first 30 days after the index stroke.

A subgroup of 314 patients had accessible medical records available for analysis to further investigate the cause of death. There were 51 deaths in this subgroup, out of a total of 86 in the entire cohort. Thirty-nine of 51 deaths were due to neurological causes (76%), two were due to cardiac causes (4%), five were attributed to non-neurological, noncardiac causes (10%), and five were due

**Table 1.** Cohort characteristics according to sST2 tertile ( $n = 646$ )

Characteristic	Entire cohort ( $n = 646$ )	sST2 Tertile			<i>P</i> value
		First tertile sST2 $\leq 28.7$ ng/mL ( $n = 215$ )	Second tertile $< sST2 \leq 44.2$ ng/mL ( $n = 216$ )	Third tertile sST2 $> 44.6$ ng/mL ( $n = 215$ )	
Age (Y), mean (SD)	69 (15)	67.01 (15)	68.70 (15.26)	71.13 (14.47)	0.029
Female, $n$ (%)	282 (44%)	97 (45%)	103 (47%)	82 (38%)	0.136
Race, $n$ (%)					
White	578 (89%)	186 (87%)	196 (91%)	196 (91%)	0.222
Black	50 (8%)	23 (11%)	15 (7%)	12 (6%)	0.121
Asian	15 (2%)	6 (3%)	4 (2%)	5 (2%)	0.722
Other	3 (1%)	0 (0%)	1 (0%)	2 (1%)	0.337
Cardioembolic stroke subtype	210 (33%)	46 (21%)	65 (30%)	99 (46%)	$<0.0001$
Past medical history					
Hypertension	464 (72%)	143 (67%)	163 (75%)	158 (73%)	0.085
Diabetes	141 (22%)	37 (17%)	48 (22%)	56 (26%)	0.093
Hyperlipidemia	55 (14%)	14 (14%)	20 (14%)	21 (12%)	0.749
Afib	173 (27%)	34 (16%)	50 (23%)	89 (41%)	$<0.0001$
CHF	69 (11%)	12 (6%)	24 (11%)	33 (16%)	0.004
NIHSS, median [IQR]	5 [2, 12]	5 [2, 10]	5 [2, 13]	7 [3, 14]	0.009
IV tPA, $n$ (%)	277 (43%)	79 (37%)	94 (44%)	104 (49%)	0.040
Glucose, median [IQR]	119 [102, 140]	113 [100, 134]	118 [101, 140]	124 [106, 196]	0.002

sST2 indicates soluble ST2; SD, standard deviation; Afib, Atrial Fibrillation; CHF, Congestive Heart Failure; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; IV tPA, intravenous tissue plasminogen activator.

to unknown causes (10%). The number of neurological deaths was significantly greater in the highest sST2 tertile compared with the lower sST2 tertiles ( $P = 0.033$ ; Fig. S1).

Evaluating the discriminatory capacity of sST2, ROC curves demonstrated better accuracy for the prediction of poor outcome and mortality when sST2 was added to major clinical risk factors.<sup>21</sup> After addition of sST2 to baseline clinical characteristics, the AUC was 0.854 for poor outcome, and the AUC was 0.895 for mortality (see Table S1). Using net reclassification analysis, this was consistent with a small degree<sup>18,22</sup> of reclassification for poor outcome (NRI = 0.164) and a moderate degree of reclassification for mortality (NRI = 0.478, see Table S1).

We next performed a series of sensitivity analyses. We repeated analyses using sST2 quartiles and quintiles, and found that sST2 remained associated with outcome (all  $P < 0.001$ ; Fig. S2). Lastly, in order to exclude a potential effect of differing sample storage time, we including sample storage time as a covariate in the multivariable model, and found that sST2 remained an independent predictor of outcome (Table S2).

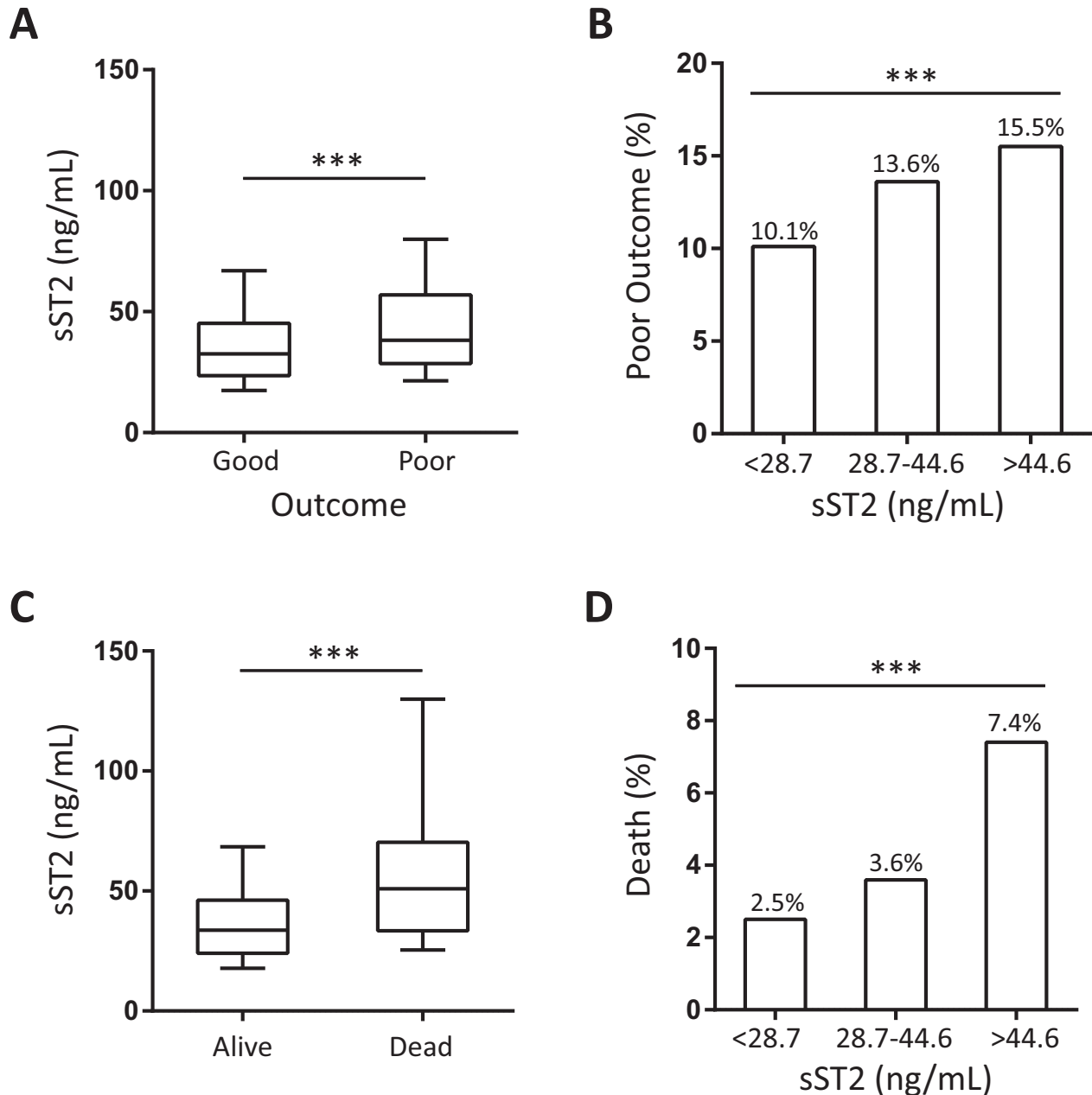
### sST2 predicts outcome and mortality independent from cardiovascular risk factors

Previous studies have identified sST2 as a prognostic biomarker in cardiovascular disease.<sup>7–9</sup> In order to exclude

the possibility that the association between sST2 and outcome was related to underlying cardiovascular disease, we developed sequential multivariable models that included cardiovascular disease risk factors. In all models developed, baseline sST2 remained an independent predictor of poor outcome ( $P < 0.009$ ; Table 3). Likewise, sST2 remained an independent predictor for 90-day mortality ( $P < 0.002$ ) in all models tested (Table 3). Admission values for ejection fraction and troponin were available in a subgroup of 314 patients. When these markers were included in the multivariable analysis, sST2 remained an independent predictor ( $P < 0.049$ ; Table S3).

### sST2 predicts hemorrhagic transformation after stroke

The relationship between sST2 and hemorrhagic transformation (HT) was analyzed. Of 246 patients with available scans, HT occurred in 42 (17%) patients; 23 (55%) with hemorrhagic infarction type 1 (HI1), 11 (26%) with hemorrhagic infarction type 2 (HI2), 4 (10%) with parenchymal hemorrhage type 1 (PH1) and 4 (10%) with parenchymal hemorrhage type 2 (PH2). Soluble ST2 level was significantly higher in patients with HT compared to those without (49.7 ng/mL vs. 42.1 ng/mL,  $P = 0.03$ ; Fig. 3). The HT rate by sST2 tertile was 3%, 6% and 9%, respectively ( $P = 0.049$ ). In some studies, HI2 or greater has been proposed as a marker for poststroke injury,<sup>16</sup> whereas HI1 may be a beneficial marker of reperfusion.<sup>17</sup>



**Figure 1.** Elevated sST2 is associated with poor outcome (mRS 3–6) and mortality. (A) Median sST2 levels are higher in patients with poor outcome (38.2 ng/mL [IQR 28.6–80.0 ng/mL]) as compared to good outcome (32.6 ng/mL [IQR 23.6–45.2 ng/mL]). Wilcoxon Test, \*\*\*,  $P < 0.0001$ . Box plot represents the median and IQR, whiskers the 10th and 90th percentile. (B) The proportion of patients with poor outcome is greater by sST2 tertile. The percentage is shown above each bar, which represents the event rate (%). Chi-squared test, \*\*\*,  $P = 0.0003$ . (C) Median sST2 levels are higher in patients who died (51.0 ng/mL [IQR: 33.4–70.3 ng/mL]) relative to survivors (33.7 ng/mL [24.1–46.23 ng/mL]). Wilcoxon Test, \*\*\*,  $P < 0.0001$ . (D) The number of patients who died increase by sST2 tertile. The percentage is shown above each bar. Chi-squared test, \*\*\*,  $P < 0.0001$ .

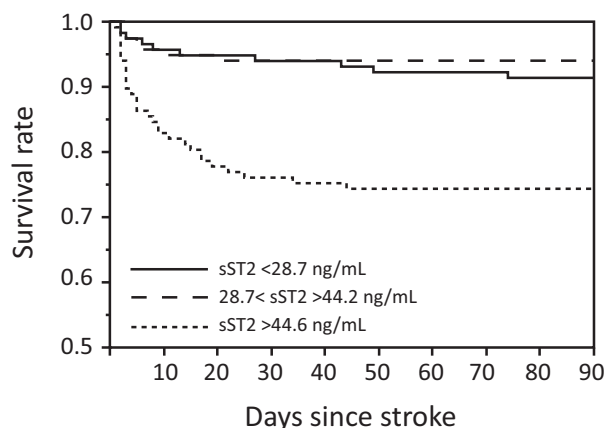
Accordingly, sST2 tertile predicted the risk of HI2 or greater ( $P = 0.003$ ). Table 4 shows the univariate associations between sST2 and HI2 or greater along with additional risks factors of HT including age, NIHSS, sex,

baseline blood glucose, IV tPA, MMP-9 level, DWI volume, antiplatelet use, smoking history and anticoagulant use. Soluble ST2 remained an independent predictor of HI2 or greater (OR: 5.40; 95% CI: 1.40–35.61,  $P = 0.039$ )

**Table 2.** sST2 predicts poor outcome and mortality after ischemic stroke

	Univariate analysis				Multivariate analysis			
	OR	Confidence interval		P Value	OR	Confidence interval		P Value
		Lower 95%	Upper 95%			Lower 95%	Upper 95%	
Univariate and multivariate predictors of poor outcome after stroke (mRS 3-6).								
Age (increase per year)	1.04	1.02	1.05	<0.0001	1.04	1.02	1.06	<0.0001
Sex (F)	1.50	1.08	2.08	0.015	1.41	0.89	2.24	0.139
Glucose (increase per log unit)	2.08	1.27	3.49	0.003	2.18	1.19	3.87	0.012
CE	1.15	0.81	1.63	0.425	0.52	0.31	0.87	0.014
Afib	2.14	1.49	3.10	<0.0001	1.31	0.74	2.31	0.032
CHF	2.07	1.24	3.50	0.006	0.70	0.35	1.40	0.044
IV tPA	2.26	1.62	3.16	<0.0001	0.56	0.34	0.90	0.017
NIHSS (increase per log unit)	4.70	3.67	6.13	<0.0001	5.79	4.25	8.09	<0.0001
<b>sST2 (increase per tertile)</b>	<b>2.29</b>	<b>1.53</b>	<b>3.45</b>	<b>0.0003</b>	<b>2.77</b>	<b>1.54</b>	<b>5.06</b>	<b>0.003</b>
Univariate and multivariate predictors of death after stroke								
Age (increase per year)	1.08	1.05	1.10	<0.0001	1.07	1.04	1.10	<0.0001
Sex (F)	1.31	0.83	2.07	0.241	1.02	0.54	1.90	0.963
Glucose (increase per log unit)	2.17	1.15	4.03	0.017	1.93	0.95	4.31	0.068
CE	1.74	1.09	2.76	0.021	0.66	0.33	1.28	0.220
Afib	3.36	2.10	5.41	<0.0001	1.27	0.63	2.53	0.772
CHF	2.31	1.23	4.17	0.010	0.85	0.37	1.87	0.672
IV tPA	2.35	1.48	3.78	0.0003	0.79	0.42	1.49	0.469
NIHSS (increase per log unit)	1.19	1.15	1.24	<0.0001	7.23	4.53	12.35	<0.0001
<b>sST2 (increase per tertile)</b>	<b>3.86</b>	<b>2.15</b>	<b>7.26</b>	<b>&lt;0.0001</b>	<b>3.56</b>	<b>1.58</b>	<b>8.38</b>	<b>0.001</b>

Afib indicates Atrial Fibrillation; CHF, Congestive Heart Failure; NIHSS, National Institutes of Health Stroke Scale; CE, cardioembolic stroke subtype; IV tPA, intravenous tissue plasminogen activator; sST2, soluble ST2; OR, odds ratio.



**Figure 2.** Kaplan–Meier survival curve for sST2 tertiles. The solid line represents the first tertile (sST2 < 28.7 ng/mL), the dashed line represents the second tertile (sST2 28.7–44.2 ng/mL) and the dotted line represents the third tertile (sST2 > 44.6 ng/mL),  $P < 0.0001$ .

when adjusted for NIHSS, baseline DWI volume, and anticoagulant use. When age, sex, baseline glucose level, IV tPA use, smoking history, antiplatelet use, or MMP-9 level were forced into the model, sST2 remained an independent predictor of HI2 or greater ( $P < 0.050$ ) in all

models tested (Table S4). To further assess any potential impact of IV tPA on the ability of sST2 to predict HT, we divided the cohort into tPA-treated and non-tPA-treated subgroups. We found that sST2 remained an independent predictor of HT in both groups (Figs. S3–S4). Finally, the inclusion of sST2 in models for predicting HI2 or greater had an AUC of 0.747 and was associated with a modest improvement in reclassification (NRI > 0.268; Table S1).

### sST2 concentration at 48 h is similar to admission sST2

210 patients of the original 646 had a second serial plasma sample collected 48 h after stroke onset available for analysis. The median 48 h sST2 concentration for these subjects was 37.4 ng/mL [IQR: 27.9–55.6 ng/mL], which was also associated with poor outcome (OR: 5.84; 95% CI: 2.98–12.52;  $P < 0.0001$ ) and mortality (OR: 9.18; 95% CI: 3.99–24.53;  $P < 0.0001$ ). sST2 measured at 48 h remained an independent predictor of poor outcome and mortality, after adjusting for the same previously described clinical risk factors (see Table S5). Lastly, we examined the relationship between 48 h sST2 and hemorrhagic transformation. Elevated sST2 levels 48 h after

**Table 3.** sST2 predicts outcome and mortality independent from cardiovascular risk factors

Multivariate models for predictors of poor outcome after stroke				Multivariate models for predictors of death after stroke					
OR	Confidence interval		P Value	OR	Confidence interval		P Value		
	Lower 95%	Upper 95%			Lower 95%	Upper 95%			
Model 1	2.29	1.53	3.45	0.001	Model 1	3.83	2.13	7.21	<0.0001
Model 2	2.32	1.35	4.04	0.009	Model 2	2.98	1.41	6.56	0.002
Model 3	2.76	1.53	5.05	0.003	Model 3	3.51	1.52	8.56	0.002
Model 4	2.88	1.59	5.30	0.002	Model 4	3.68	1.55	9.24	0.003
Model 5	2.97	1.62	5.52	0.002	Model 5	3.69	1.54	9.32	0.003
Model 1: Unadjusted					Model 1: Unadjusted				
Model 2: Adjusted for age, sex, NIHSS, glucose, IV tPA, site					Model 2: Adjusted for age, sex, NIHSS, glucose, IV tPA, site				
Model 3: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib					Model 3: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib				
Model 4: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib, CHF					Model 4: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib, CHF				
Model 5: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib, CHF, CE					Model 5: Adjusted for age, sex, NIHSS, glucose, IV tPA, site, Afib, CHF, CE				

NIHSS indicates National Institutes of Health Stroke Scale; IV tPA, intravenous tissue plasminogen activator; site, SPOTRIAS stroke site; Afib, Atrial Fibrillation; CHF, Congestive Heart Failure; CE, cardioembolic stroke subtype.

stroke onset was significantly associated with HT (OR: 2.86; 95% CI: 1.37–6.18;  $P = 0.005$ ) and HI2 or greater (OR: 2.95; 95% CI: 1.25–7.03;  $P = 0.014$ ) (Fig. 3).

## Discussion

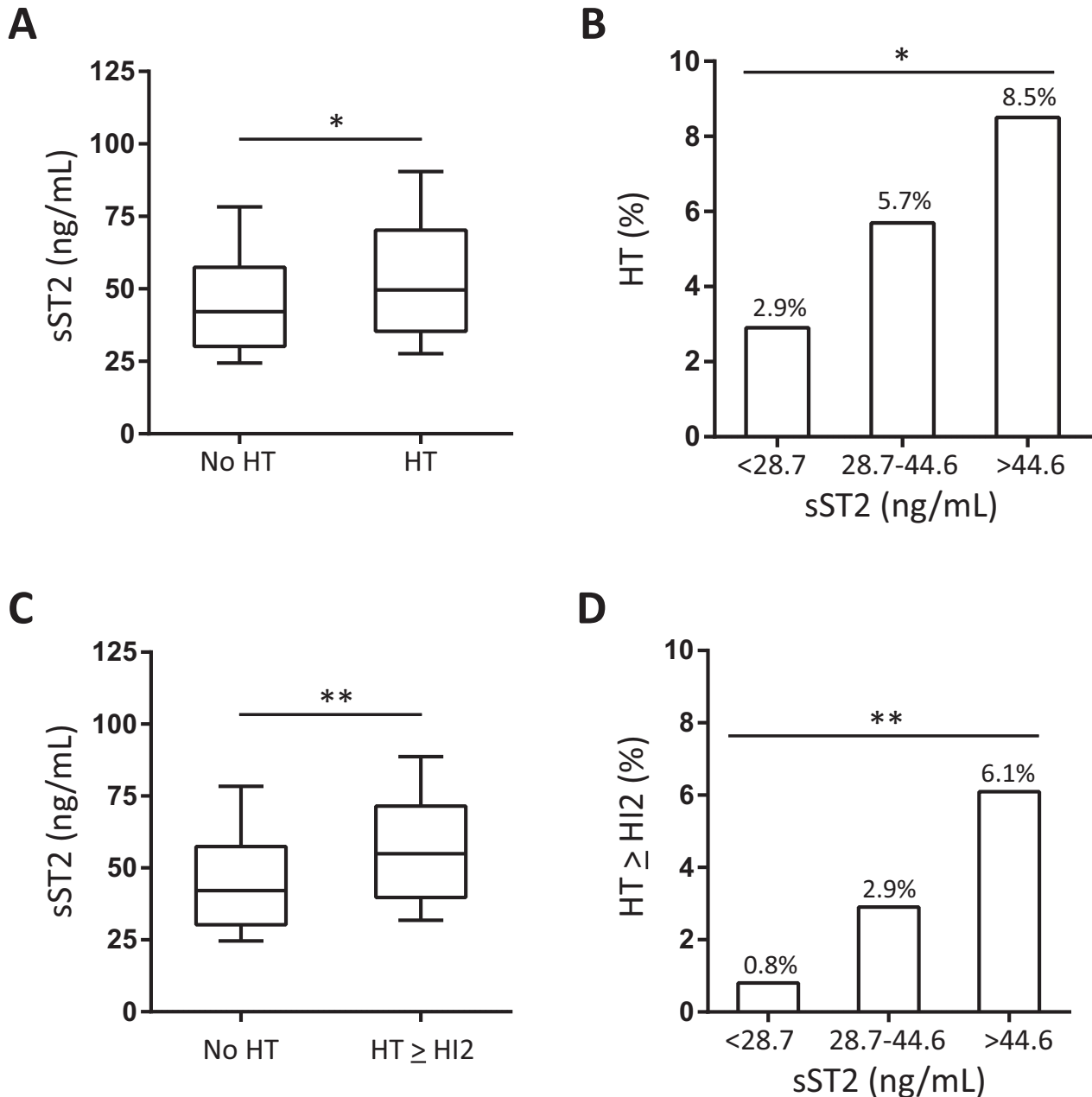
In this study, we found that baseline plasma levels of sST2 independently predicted poor outcome, mortality and HT in patients who present with acute ischemic stroke. Importantly, sST2 was measured in blood samples obtained shortly after presentation to the emergency department, and predicts subsequent clinical events that can aid in risk stratification.<sup>23</sup> Moreover, the association with outcome remained significant after adjusting for age, sex, NIHSS score, as well as a history of cardiovascular disease (e.g., atrial fibrillation and CHF). Furthermore, sST2 predicted the subsequent development of HT, which was independent of other factors known to be associated with HT (age, sex, NIHSS, admission glucose, anticoagulant use, smoking history, antiplatelet use, DWI volume, MMP-9, and IV tPA use). In the subset of subjects with serial samples available for analysis, sST2 level 48 h after stroke onset was also associated with outcome, mortality, and HT.

Circulating sST2 was previously identified as a prognostic marker in heart failure and myocardial infarction.<sup>7–9,24</sup> Together with other cardiac biomarkers such as troponin<sup>25</sup> and B-type natriuretic peptide (BNP),<sup>26,27</sup> our data highlight overlapping injury responses that occur following both cerebrovascular and cardiovascular injury. There are several possible interpretations of our findings in this context. sST2 may be a marker for stroke-induced cardiac injury, which in turn, influences subsequent neurological outcome after stroke. Alternatively, it is possible that sST2 may integrate both stroke severity and cardiovascular disease, each of which is strongly associated with

outcome after stroke.<sup>28–30</sup> Although our current data cannot exclude these possibilities, our multivariable models adjusting for cardiovascular risk factors and stroke severity suggest that sST2 is independent from these factors. Alternatively, it is possible that sST2 may reflect a separate and specific response to cerebral infarction, for example serving as a marker for neuroinflammation.

In this regard, we also found that elevated sST2 circulating levels were associated with HT. Several preclinical studies and biomarker analyses have suggested that neuroinflammatory markers are linked to blood-brain barrier (BBB) breakdown and HT risk.<sup>11,31–33</sup> Disruption of the BBB has been shown to be associated with neuroinflammation following ischemic injury,<sup>32</sup> and hemorrhagic transformation has been reported as a maker of BBB breakdown.<sup>33</sup> The ligand for ST2, IL-33, is hypothesized to serve as an acute inflammatory signaling molecule that participates in maintaining barrier function and integrity.<sup>34</sup> Isoforms of ST2 and IL-33 are highly expressed in the brain and spinal cord, suggesting that IL-33/ST2 may function directly in the central nervous system.<sup>12</sup> In a murine model of stroke, IL-33 signaling through the membrane-bound form of ST2 has been shown to be neuroprotective through anti-inflammatory effects.<sup>35</sup> In contrast, the circulating soluble form (sST2), which was measured in our study, is thought to antagonize IL-33 signaling<sup>6</sup> and augment neuroinflammation by operating as a decoy receptor. While our finding that sST2 is associated with HT is consistent with this hypothesis, the data remains circumstantial.

This study has several limitations. This was a retrospective analysis of a multicenter sample biorepository with samples that were stored for ~5 years at  $-80^{\circ}\text{C}$ . While sST2 stability studies have demonstrated excellent recovery from plasma samples stored long term at this temperature, the maximum time studied was 1.5 years.<sup>36</sup> As the study cohort



**Figure 3.** Elevated sST2 is associated with hemorrhagic transformation after stroke. (A) Median sST2 levels in patients without HT (42.1 ng/mL [IQR: 31.2–57.4 ng/mL]) and patients with HT (49.7 ng/mL [IQR 35.3–70.3 ng/mL]). Wilcoxon Test, \*,  $P = 0.030$ . Box plot represents the median and IQR, whiskers the 10th and 90th percentile. (B) Rate of HT by sST2 tertiles. The percentage is shown above each bar, which represents the event rate (%). Chi-squared test, \*,  $P = 0.049$ . (C) Median sST2 levels in patients with HT  $\geq$  HI2 (54.9 ng/mL [IQR: 39.8–71.5 ng/mL]) versus those without (42.1 ng/mL [30.3–57.5 ng/mL]). Wilcoxon Test, \*\*,  $P = 0.008$ . (D) Rate of HT  $\geq$  HI2 by sST2 tertiles. The percentage is shown above each bar. Chi-squared test, \*\*,  $P = 0.007$ .

contains mostly mild to moderate strokes, our findings may also not be generalizable to all acute stroke patients who present in the emergency department. Additional prospective studies, with prompt measurements of sST2 following collection are needed to validate these preliminary findings

and validate effect sizes.<sup>37</sup> The study also has strengths. The timing of the blood sampling (within the first 9 h of stroke onset) coincides with a time point that can aid in risk stratification.<sup>23</sup> In patients who had serial blood sampling 48 h later, the findings were consistent with the baseline sample.



**Table 4.** sST2 predicts hemorrhagic transformation after ischemic stroke.

	Univariate analysis				Multivariate analysis			
	OR	Confidence interval		P Value	OR	Confidence interval		P value
		Lower 95%	Upper 95%			Lower 95%	Upper 95%	
Age	1.02	0.99	1.05	0.174				
NIHSS	2.33	1.34	4.40	0.002	2.46	1.29	55.11	0.005
Sex (F)	0.47	0.17	1.17	0.108				
Glucose	1.97	0.54	6.21	0.288				
IV tPA	1.02	0.42	2.62	0.971				
MMP-9	0.95	0.61	1.54	0.827				
BL DWI	1.30	1.00	1.74	0.052	1.00	0.99	1.01	0.738
Antiplatelet use	1.11	0.46	2.59	0.814				
Smoking history	1.24	0.52	3.18	0.632				
Anticoagulant use	3.40	1.21	8.78	0.022	3.80	1.22	11.41	0.022
sST2	7.90	2.15	51.02	0.003	5.40	1.40	35.61	0.039

NIHSS indicates National Institutes of Health Stroke Scale; sST2, soluble ST2; IV tPA, intravenous tissue plasminogen activator; MMP-9, matrix metalloproteinase-9; BL DWI, baseline DWI volume.

The reasonably large sample size also included patients who were enrolled from multiple centers.

## Summary

We provide evidence that elevated sST2 is associated with worse 90-day outcome, higher mortality, and increased risk of HT after acute ischemic stroke. These findings highlight the ST2 pathway as a candidate link to neuroinflammation-induced secondary injury. Additional study is needed to validate its role as a clinical biomarker and further investigate its potential connection to outcome.

## Author Contributions

Study concept and design: Z.W., W.T.K. Data acquisition and analysis: Z.W., A.B., M.B.B., C.S., J.K., M.S., W.T.K. Drafting the manuscript and Figures: Z.W., A.B., M.B.B., C.S., J.K., M.S., B.C.M., K.B.W., O.A., J.P.B., W.T.K. All authors edited and approved the final version of the manuscript.

## Conflict of Interest

ZW, AB, MBB, CS, MS, KBW: none. JK: NIH P50NS044283 (Biostatistical Core). BCM: NIH P50NS044148; Speakers Bureau, Genentech. OA: NIH P50NS044283. JPB: NIH P50NS044283; Executive Committee of PRISMS trial, Genentech. WTK: NIH K23NS076597; AHA 14GRNT1 9060044; Remedy Pharmaceuticals, Inc.

## References

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics-2015 Update: a report from the

American Heart Association. *Circulation* 2014;131:e29–e322.

2. Hankey GJ. Long-term outcome after ischaemic stroke/transient ischaemic attack. *Cerebrovasc Dis* 2003;16 Suppl 1(Suppl. 1):14–19.
3. Jickling GC, Sharp FR. Biomarker panels in ischemic stroke. *Stroke* 2015;46:915–920.
4. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479–490.
5. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov* 2008;7:827–840.
6. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007;282:26369–26380.
7. Shimpo M, Morrow DA, Weinberg EO, et al. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation* 2004;109:2186–2190.
8. Ky B, French B, McCloskey K, et al. High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ Hear Fail* 2011;4:180–187.
9. Sabatine MS, Morrow DA, Higgins LJ, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation* 2008;117:1936–1944.
10. Andersson C, Preis SR, Beiser A, et al. Associations of circulating growth differentiation factor-15 and ST2 Concentrations with Subclinical Vascular Brain Injury and Incident Stroke. *Stroke* 2015;46:2568–2575.
11. Korhonen P, Kanninen KM, Lehtonen Š, et al. Immunomodulation by interleukin-33 is protective in

- stroke through modulation of inflammation. *Brain Behav Immun* 2015;2016:322–336.
12. Yasuoka S, Kawanokuchi J, Parajuli B, et al. Production and functions of IL-33 in the central nervous system. *Brain Res* 2011;1385:8–17.
  13. Nalichowski R, Keogh D, Chueh HC, Murphy SN. Calculating the benefits of a research patient data repository. *AMIA Annu Symp Proc* 2006;2006:1044.
  14. Jha R, Battey TWK, Pham L, et al. Fluid-attenuated inversion recovery hyperintensity correlates with matrix metalloproteinase-9 level and hemorrhagic transformation in acute ischemic stroke. *Stroke* 2014;45:1040–1045.
  15. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 h after acute ischemic stroke. *N Engl J Med* 2008;359:1317–1329.
  16. Dzialowski I, Pexman JHW, Barber PA, et al. Asymptomatic hemorrhage after thrombolysis may not be benign: prognosis by hemorrhage type in the Canadian alteplase for stroke effectiveness study registry. *Stroke* 2007;38:75–79.
  17. Molina CA, Alvarez-Sabin J, Montaner J, et al. Thrombolysis-related hemorrhagic infarction: a marker of early reperfusion, reduced infarct size, and improved outcome in patients with proximal middle cerebral artery occlusion. *Stroke* 2002;33:1551–1556.
  18. Pencina MJ, D'Agostino RB, Pencina KM, et al. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol* 2012;176:473–481.
  19. Pencina MJ, D'Agostino RB, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;30:11–21.
  20. Lu J, Snider JV, Grenache DG. Establishment of reference intervals for soluble ST2 from a United States population. *Clin Chim Acta* 2010;411:1825–1826.
  21. Feigin VL, Lawes CMM, Bennett DA, Anderson CS. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol* 2003;2:43–53.
  22. Cook NR. Methods for evaluating novel biomarkers - a new paradigm. *Int J Clin Pract* 2010;64:1723–1727.
  23. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx* 2004;1:182–188.
  24. Mueller T, Dieplinger B, Gegenhuber A, et al. Increased plasma concentrations of soluble ST2 are predictive for 1-year mortality in patients with acute destabilized heart failure. *Clin Chem* 2008;54:752–756.
  25. Ay H, Arsava EM, Saribaş O. Creatine kinase-MB elevation after stroke is not cardiac in origin: comparison with troponin T levels. *Stroke* 2002;33:286–289.
  26. Rost NS, Biffi A, Cloonan L, et al. Brain natriuretic peptide predicts functional outcome in ischemic stroke. *Stroke* 2012;43:441–445.
  27. Chen X, Zhan X, Chen M, et al. The prognostic value of combined NT-pro-BNP Levels and NIHSS scores in patients with acute ischemic stroke. *Intern Med* 2012;51:2887–2892.
  28. Muir KW, Weir CJ, Murray GD, et al. Comparison of neurological scales and scoring systems for acute stroke prognosis. *Stroke* 1996;27:1817–1820.
  29. Adams HP, Davis PH, Leira EC, et al. Baseline NIH Stroke Scale score strongly predicts outcome after stroke: a report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). *Neurology* 1999;53:126–131.
  30. Appelros P, Nydevik I, Viitanen M. Poor outcome after first-ever stroke: predictors for death, dependency, and recurrent stroke within the first year. *Stroke* 2003;34:122–126.
  31. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011;17:796–808.
  32. Yang Y, Rosenberg GA. Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. *Stroke* 2011;42:3323–3328.
  33. Hamann GF, Okada Y, delZoppo GJ, del Zoppo GJ. Hemorrhagic transformation and microvascular integrity during focal cerebral ischemia/reperfusion. *J Cereb Blood Flow Metab* 1996;16:1373–1378.
  34. Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* 2016;17:122–131.
  35. Luo Y, Zhou Y, Xiao W, et al. Interleukin-33 ameliorates ischemic brain injury in experimental stroke through promoting Th2 response and suppressing Th17 response. *Brain Res* 2015;1597:86–94.
  36. Dieplinger B, Egger M, Poelz W, et al. Long-term stability of soluble ST2 in frozen plasma samples. *Clin Biochem* 2010;43:1169–1170.
  37. Ioannidis JPA, Panagiotou OA. Comparison of effect sizes associated with biomarkers reported in highly cited individual articles and in subsequent meta-analyses. *JAMA* 2011;305:2200–2210.

## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Elevated sST2 is associated with neurological deaths. Neuro indicates the number of neurological deaths in each sST2 tertile. Other indicates the number of cardiac, non-neurological/non-cardiac, and unknown deaths in each sST2 tertile. The number of neurological deaths was significantly higher in the highest sST2 tertile compared with the lower sST2 tertiles,  $P = 0.033$ .

**Table S1.** Discrimination analysis of sST2 for predicting poor outcome, mortality, and hemorrhagic transformation **Figure S2.** sST2 tertiles, quartiles and quintiles were significantly associated with poor outcome and mortality. The highest tertile, quartile and quintile were associated with

poor outcome (A,  $P = 0.0003$ ; C,  $P < 0.0001$ ; and E,  $P = 0.0005$ , respectively) and mortality (B,  $P < 0.0001$ ; D,  $P < 0.0001$ ; and F  $P < 0.0001$ , respectively).

**Table S2.** sST2 independently predicts outcome and mortality when adjusted for sample storage time.

**Table S3.** sST2 predicts mortality independent from acute and chronic cardiovascular risk factors.

**Table S4.** sST2 predicts hemorrhagic transformation after ischemic stroke – detailed.

**Figure S3.** sST2 is associated with poor outcome and mortality regardless of tPA treatment status. Box plot represents the median and IQR, whiskers the 10<sup>th</sup> and 90<sup>th</sup> percentile. **A)** Median sST2 levels are higher in patients with poor outcome (37.4 ng/mL [IQR 27.9 – 54.5 ng/mL]) as compared to good outcome (33.9 ng/mL [IQR 23.5 – 45.1 ng/mL]) in patients that did not receive tPA. Wilcoxon Test ( $P = 0.041$ ). **B)** Median sST2 levels are higher in patients with poor outcome (39.8 ng/mL [IQR 31.0 – 59.3 ng/mL]) as compared to good outcome (31.1 ng/mL [IQR 24.0 – 45.7 ng/mL]) in patients that received tPA. Wilcoxon Test ( $P = 0.0001$ ). **C)** Median sST2 levels are higher in patients

who died (41.6 ng/mL [IQR 27.7 – 70.1 ng/mL]) as compared who lived (34.3 ng/mL [IQR 23.8 – 45.7 ng/mL]) in patients that did not receive tPA. Wilcoxon Test ( $P = 0.012$ ). **D)** Median sST2 levels are higher in patients who died (55.0 ng/mL [IQR 35.7 – 78.6 ng/mL]) as compared who lived (33.2 ng/mL [IQR 25.7 – 46.4 ng/mL]) in patients that received tPA. Wilcoxon Test ( $P < 0.0001$ ).

**Figure S4.** sST2 is associated with hemorrhagic transformation regardless of tPA treatment status. Box plot represents the median and IQR, whiskers the 10<sup>th</sup> and 90<sup>th</sup> percentile. **A)** Median sST2 levels are significantly higher in patients who developed hemorrhagic transformation compared to those that did not develop hemorrhagic transformation in patients that did not receive tPA. Wilcoxon Test ( $P = 0.041$ ). **B)** Median sST2 levels are significantly higher in patients who developed hemorrhagic transformation compared to those that did not develop hemorrhagic transformation in patients that received tPA. Wilcoxon Test ( $P = 0.014$ ).

**Table S5.** 48h sST2 predicts poor outcome and mortality after ischemic stroke.