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ORIGINAL ARTICLE

Low ADAMTS-13 predicts adverse outcomes in hospitalized patients with suspected heparin-induced thrombocytopenia

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Abstract

Background: Heparin-induced thrombocytopenia (HIT) is a life-threatening thrombotic complication after heparin exposure. However, the role of ADAMTS-13 and von Willebrand factor (VWF) in the disease process and outcomes of HIT is not known.

Objective: To determine the potential role of ADAMTS-13 and VWF in hospitalized patients suspected with HIT.

Methods: Associations of the HIT tests, ADAMTS-13 activity, and VWF antigen or activity with other clinical parameters and outcomes in the patients suspected with HIT were determined.

Results: Of 261 patients, 87 (33.3%) were positive and 174 (66.7%) were negative for a HIT antibody determined by an enzyme immunoassay (EIA). Of these 87 EIA+ patients, 31 (35.6%) were also positive but 56 (64.4%) were negative for serotonin-releasing assay (SRA). There was no statistically significant difference among all three groups (i.e., EIA–, EIA+/SRA+, and EIA+/SRA–) as to their demographic features, reasons for admission to the hospital, type of procedures performed, and in-hospital mortality. Compared to those in the healthy controls, plasma ADAMTS-13 activity in patients suspected with HIT was significantly lower but plasma VWF antigen (VWFAg) and activity (VWFAc) in these patients were significantly higher. While there was no statistically significant difference among all three groups regarding plasma levels of ADAMTS-13 activity, VWFAg, and VWFAc, plasma levels of ADAMTS-13 activity <50% or the low ratios of ADAMTS-13 activity to VWFAg (or VWFAc) are highly predictive for a 90-day mortality rate, particularly in the EIA+SRA+ group.

Conclusions: These results demonstrate that relative deficiency of plasma ADAMTS-13 activity in hospitalized patients suspected with HIT is common, which may contribute at least in part to the adverse outcomes in this patient population, particularly in those with true HIT.

KEYWORDS
ADATMS-13, heparin-induced thrombocytopenia, mortality, thromboembolism, von Willebrand factor

Essentials

- Heparin-induced thrombocytopenia (HIT) is a life-threatening thrombotic complication.
- The role of ADAMTS-13 and von Willebrand factor (VWF) in patients with suspected HIT is not known.
- We show that low ADAMTS-13 and high VWF are prevalent in hospitalized patients with suspected HIT.
- We conclude that relative deficiency of ADAMTS-13 activity may be a risk factor for mortality in these patients.

1 | INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a potentially life-threatening complication after exposure to heparin, characterized by thrombocytopenia and potential catastrophic thrombosis.[1,2] HIT is primarily caused by the formation of antibodies that target platelet factor 4 (PF4)/heparin complexes (e.g., the HIT antibodies), which may activate platelets and coagulation cascade, resulting in arterial and venous thromboses.[3-7]

Approximately 30% to 50% of patients with HIT may develop thrombosis[8-12] with an estimated mortality rate of 5% to 10%.[12-14] Venous thromboembolism is 2 to 5 times more prevalent than arterial thrombosis in patients with HIT.[9,11,13] This includes deep vein thrombosis (DVT) and pulmonary embolism (PE). Arterial thrombosis, including ischemic stroke, myocardial infarction, and peripheral arterial occlusion, may also occur.[9,11,15-17] Rarely, bilateral adrenal hemorrhage,[18] skin necrosis,[19,20] venous gangrene,[21] and thrombosis in a grafted vessel[22] may develop.

Clinical diagnosis of HIT requires a high index of suspicion based on clinical information, followed by laboratory tests, such as enzyme immunoassay (EIA) and serotonin-releasing assay (SRA). The 4T score (i.e., thrombocytopenia, time to platelet falls, thrombosis, and no other causes of thrombocytopenia) has been the widely used to predict the pretest probability of HIT.[23] The higher the scores are, the more likely the patient may have a HIT.[24] A more complex HIT expert probability (HEP) score was also developed on the basis of broad expert opinions, which was considered to have a higher of interobserver's agreement and correlated better with the results of the laboratory tests for HIT.[25]

Regardless of what tests are used for diagnosis of HIT, clinical presentations and outcomes are not always correlated with the HIT test results, suggesting that additional factors may contribute to the pathogenesis of thrombotic events, organ damage, and outcomes.

ADAMTS-13, a plasma metalloprotease, plays a critical role in regulating hemostasis and preventing unwanted thrombosis.[26] It does so through proteolytic cleavage of von Willebrand factor (VWF), which is synthesized and released from activated or injured endothelium.[27] Severe deficiency of plasma ADAMTS-13, primarily resulting from acquired autoantibodies against ADAMTS-13, leads to thrombotic thrombocytopenic purpura (TTP), a potentially fatal blood disorder.[28] Relative deficiency of plasma ADAMTS-13 has been observed in patients with acute cerebral infarction,[29,30] myocardial infarction,[31,32] malignant malaria,[33,34] preeclampsia,[35,36] acute traumatic brain injury, or multiple site injury,[37,38] as well as in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[39-41] However, the role of ADAMTS-13 in pathogenesis, disease progression, and outcome in patients with suspected HIT has not been systematically determined. The present study aims to determine the associations among plasma levels of ADAMTS-13 activity, VWF antigen, VWF activity, and other clinical factors or laboratory parameters, and in particular the role of ADAMTS-13 and VWF in predicting in-hospital mortality in patients with suspected HIT.

2 | METHODS

2.1 | Patients, and sample and data collection

Institutional Review Boards of the University of Kansas Medical Center and the University of Alabama at Birmingham approved the study protocol. The subjects were identified from the list of patients who were tested for HIT antibody (ie, the anti-PF4/heparin complex IgG) in the special coagulation laboratory. Residual plasma after HIT tests was stored at −80°C as part of biorepository for quality assurance and research. Informed consent was waived for the use of retrospective samples and other clinical and laboratory information extracted from electronic medical record. Control samples were collected from local volunteers who did not have any acute illness, with age, sex, and race background matched. Whole blood was collected and anticoagulated with sodium citrate the same way as did the samples for all other coagulation tests.

2.2 | Patient demographic and clinical data

The demographic information including age, sex, race, reasons for admission to hospital (e.g., infection, surgery, nonsurgery, and trauma, etc.), death in hospital, length of stay, and other clinical conditions (e.g., acute or exacerbated renal failure, heart failure, liver failure, and respiratory failure; sepsis and bleeding; etc.), comorbidities (e.g., hypertension, diabetes, hyperlipidemia, coronary heart disease, valvular heart disease, congestive heart failure, chronic obstructive pulmonary disease (COPD), institutional lung disease, connective tissue disease, cirrhosis, stroke, malignancy, etc.), and procedures performed during hospitalization (e.g., intra-aortic balloon pump/ventricular assistant device, extracorporeal membrane oxygenation [ECMO], etc.). The data related to heparin usage and changing of platelet count, as well as form of heparins (unfractionated vs low-molecular-weight heparin), route of heparin administration (e.g.,

intravenous or subcutaneous), timing of thrombocytopenia occurred in relation to heparin exposure, nadir of platelet count, percentage of platelet count falling, and venous or arterial embolic events that were discovered or progressed after exposure to heparin that were considered the results of HIT, and alternative anticoagulants (e.g., direct thrombin inhibitor, factor X inhibitors, etc.). The 4T[23] and HEP scores[25] were determined for each patient. Additionally, plasma samples from healthy individuals were collected for negative controls.

2.3 | HIT antibody tests

An EIA (i.e., the Asserachrom HPIA IgG, #00624) was used to determine the IgG antibodies against PF4/heparin complexes according to manufacturer's recommendations (Stago, Parsippany, NJ, USA). An optical density (OD) value >0.5 was defined as positive, and an OD <0.5 was negative. The HIT EIA test was performed in the Clinical Laboratory Improvement Amendments–certified special coagulation laboratory that serves patient care. All EIA+ samples were sent to a reference lab (Versiti, Milwaukee, WI, USA) for the SRA.

2.4 | Assays for plasma ADAMTS-13 activity, antigen, and anti–ADAMTS-13 IgG

Plasma ADATMS-13 activity was determined using our in-house rFRETS-VWF73 assay as previously described.[42,43] Plasma ADAMTS-13 antigen was determined using ELISA[44] according to manufacturer's recommendation (R&D Systems, Minneapolis, MN, USA). Plasma anti–ADAMTS-13 IgG was determined by ELISA[45] (Diapharma, West Chester, OH, USA) according to the manufacturer's recommendations.

2.5 | Assays for plasma VWF antigen and collagen-binding activity

Plasma VWF antigen (VWFAg) and collagen-binding activity (VWFAc) were determined with the in-house ELISA-based assays as previously described.[44,45]

2.6 | Statistical analysis

The categorical variables of sex, race, mortality, reasons for admission, comorbidity, procedures, special clinical conditions, administered heparin products, administration route, alternative anticoagulants, time for heparin exposure, thromboembolic events before and after heparin exposure, and 4T score graded by risk were expressed as number (%) and compared by chi-square. The normally distributed quantitative data of age, 4T score, and HEP score were expressed as mean with standard deviation (SD) and compared using one-way analysis of variance (ANOVA) for more than three groups and Student *t* test for two groups, while nonnormally contributed data of length of stay, percentage of platelet falling, time of platelet falling, nadir value of platelet, and HIT antibody OD were expressed as median and interquartile range (IQR) and were compared by Kruskal-Wallis one-way ANOVA test for more than three groups or Mann-Whitney U test for two groups. Analysis of consistency between 4T score and HEP score was carried out by linear regression. A $P < 0.05$ and <0.01 was considered to be statistically significant and highly significant, respectively. All the statistical analyses were performed using SPSS version 26 IBM, Armonk, NY, USA) or Prism version 8 (GraphPad Software, San Diego, CA, USA). Multiple variant logistic regression models were performed using SPSS Statistics version 26 (IBM).

3 | RESULTS

3.1 | Patient demographic, clinical, and laboratory characteristics in patients with suspected HIT

Of 261 consecutive patients who were screened for HIT antibodies, 87 (33.3%) patients were EIA+ and 174 (67.7%) patients were EIA–. Of 87 EIA+ patients, 31 (35.6%) were also SRA+, and 56 (64%) were SRA– (Table 1). As also shown in Table 1, there was no statistically significant difference in the demographic features (e.g., age, sex, and race), the reasons for admission, in-hospital mortality, and the type of procedure performed among all three patient groups (ie, EIA+/SRA–, EIA+/SRA+, and EIA–). Additionally, there was no statistically significant difference in term of acute events occurring after admission including acute respiratory failure, liver failure, sepsis, bleeding, and so on, except for acute heart failure ($P = 0.01$) and renal failure ($P = 0.002$). In terms of comorbidities, all three groups of patients exhibited a similar incidence rate. These include hypertension, diabetes mellitus, cardiovascular disease, valvular heart disease, arrhythmia, peripheral vascular disease, cerebral vascular disease, COPD/bronchitis, obesity, liver disease/cirrhosis, malignancy, connective tissue disease, and recent/chronic infections. Again, fewer patients had congestive heart failure in the EIA+/SRA+ group than in the EIA+/SRA– and EIA– groups ($P = 0.03$). While in-hospital mortality rate was similar in all three patient groups, more time in the hospital ($P = 0.005$) or fewer percentage of patients staying in hospital for <30 days ($P = 0.002$) in the EIA+ group, regardless of the SRA results, than in the EIA– group ($P = 0.005$) was observed (Table 1).

3.2 | HIT-specific clinical and laboratory data

Clinical diagnosis of HIT relies on a high index of suspicion based on thrombocytopenia, timing of platelet count fall, thrombosis or other sequalae, and no other causes for thrombocytopenia (i.e., the 4T score).[23,46] In our cohort of patients, the nadir of platelet count (median, IQR) was lower in the EIA+/SRA+ group (37.3; 20.0-51.1) than that in the EIA– groups (66.0; 38.2-90.2) ($P = 0.001$), but not statistically significant different from that in the EIA+/SRA– group (55.0; 27.4-86.5) ($P > 0.05$). There was a higher 4T score (median;

TABLE 1 Demographic and clinical characteristics of 261 patients with a suspected HIT

	EIA+/SRA+ (n = 31)	EIA+/ SRA− (n = 56)	EIA− (n = 174)	*P* values
Demography				
Age (y), mean ±SD	59.1 ± 12.4	58.8 ± 15.6	60.4 ± 14.2	0.74
Sex, female, n (%)	14 (45.2)	21 (37.5)	70 (40.2)	0.78
Black, n (%)	10 (32.3)	21 (37.5)	62 (35.6)	0.85
White, n (%)	19 (61.3)	34 (60.7)	104 (59.8)	
Others, n (%)	2 (6.5)	1 (1.8)	8 (4.6)	
Reasons for admission				
Cardiac surgery, n (%)	9 (29.0)	9 (16.1)	30 (17.2)	
Noncardiac surgery, n (%)	4 (12.9)	5 (8.9)	34 (19.5)	
No surgery, n (%)	16 (51.6)	39 (69.6)	101 (58.0)	
Trauma, n (%)	2 (6.5)	3 (5.4)	9 (5.2)	
Acute events during admission				
Acute respiratory failure, n (%)	12 (38.7)	30 (53.6)	92 (52.9)	0.32
Heart failure, n (%)	9 (29.0)[a]	35 (62.5)[b]	87 (50.0)[b]	**0.01**
Liver failure, n (%)	3 (9.7)	17 (30.4)	52 (29.9)	0.06
Renal failure, n (%)	10 (32.3)[a]	27 (48.2)[a]	111 (63.8)[b]	**0.002**
Sepsis, n (%)	11 (35.5)	24 (42.9)	72 (41.4)	0.79
Bleeding, n (%)	4 (12.9)	13 (23.2)	26 (14.9)	0.30
Comorbidities				
Hypertension, n (%)	17 (54.8)	28 (50.0)	116 (66.7)	0.06
Diabetes mellitus, n (%)	11 (35.5)	23 (41.1)	58 (33.3)	0.57
Cardiovascular diseases, n (%)	8 (25.8)	15 (26.8)	67 (38.5)	0.15
Valvular heart disease, n (%)	3 (9.7)	10 (17.9)	30 (17.2)	0.56
Congestive heart failure, n (%)	5 (16.1)[a]	25 (44.6)[b]	59 (33.9)[b]	**0.03**
Arrhythmia, n (%)	10 (32.3)	21 (37.5)	53 (30.5)	0.62
Peripheral vascular disease, n (%)	0 (0)	10 (17.9)	26 (15.0)	0.05
Cerebral vascular disease, n (%)	7 (22.6)	4 (7.1)	24 (13.8)	0.13
COPD/bronchitis, n (%)	3 (9.7)	12 (21.4)	21 (12.1)	0.16
Chronic kidney disease, n (%)	6 (19.4)	16 (28.6)	43 (24.7)	0.63
Obesity, n (%)	7 (22.6)	7 (12.5)	29 (16.7)	0.48
Cirrhosis/liver disease, n (%)	3 (9.7)	7 (12.5)	23 (13.2)	0.86
Malignancy, n (%)	6 (19.4)	9 (16.1)	41 (23.6)	0.47
Connective tissue disease, n (%)	1 (3.2)	5 (8.9)	15 (8.6)	0.55
Recent/chronic infection, n (%)	1 (3.2)	2 (3.6)	19 (11.0)	0.12
Procedures performed				
IABP/VAD. n (%)	8 (14.3)	1 (3.2)	26 (14.9)	0.21
ECMO, n (%)	5 (16.1)	8 (14.3)	21 (12.1)	0.79
Outcomes				
In-hospital death, n (%)	7(22.6)	18(32.1)	45(25.9)	0.56
Length of stay, d, median (IQR)	25 (10-39)[a]	28 (17-46)[c]	16.5 (9-30)[b]	**0.002**
Length of stay ≤30 days, n (%)	17 (54.8)[a]	32 (57.1)[a]	132 (75.9)[b]	**0.005**

Note: a-b indicates that the difference was significant ($P < 0.05$). However, the differences between a-a, b-b, a-c, and b-c were not statistically significant ($P > 0.05$).

Abbreviations: COPD, chronic obstructive pulmonary disease; CTD, connective tissue disease; ECMO, extracorporeal membrane oxygenation. EIA, enzyme immunoassay; IABP, intra-aortic balloon pump; IQR, interquartile range; SD, standard deviation; SRA, serotonin-releasing assay; VAD, ventricular assistant device.

IQR) in the EIA+/SRA+ group (5; 4.5-6.0) than in the EIA+/SRA− (4.0; 2.3-4.0) and EIA− (3.0; 2.0-4.0) groups ($P < 0.001$). When the 4T score was stratified as low (0-3), medium (4-5), and high (6-8), the percentage of patients with a high-risk score in the EIA+/SRA+ group (35.5%) was significantly higher than that in the EIA+/SRA− (17.9%) and EIA− (2.9%) groups, respectively ($P < 0.001$; Table 2 and Figure 1A). A similar result was obtained with the HEP score. The HEP score (mean ±SD) in EIA+/SRA+ group (5.6 ± 3.1) was significantly higher than that in the EIA+/SRA− (3.6 ± 3.2) and EIA− (2.2 ± 3.3) groups ($P < 0.001$). There was no statistically significant difference in both 4T and HEP scores between the EIA+/SRA+ group and the EIA+/SRA− group (Table 2 and Figure 1B). Spearman correlation analysis demonstrated a strong correlation between 4T score and HEP score with a Spearman correlation coefficient of 0.71 for all patients assessed (Figure S1), suggesting that both scoring systems have similar predictive value for the probability of HIT.

In practice, once HIT diagnosis is suspected, most clinicians may halt heparin and/or consider an alternative anticoagulant. In this cohort of patients, 87.1% of EIA+/SRA+ patients, 48.2% of EIA+/SRA− patients, and 12% of EIA− patients were treated with one of the following alternative anticoagulants: bivalirudin, argatroban, foundaparinux, apixaban, or the like. The incidences of thromboembolic events were significantly higher in the EIA+/SRA+ group (71% before or 67.7% after heparin exposure) than in the EIA+/SRA− group (25% before or 28.6% after heparin exposure) and in the EIA− group (28.7% before or 17.8% after heparin exposure). DVT ($P < 0.001$), acute myocardial infarction ($P = 0.02$), new stroke ($P = 0.02$), and peripheral arterial embolism ($P = 0.02$) were also

TABLE 2 HIT-associated clinical and laboratory characteristics in 261 patients with a suspected HIT

Events	EIA+/SRA+ (n = 31)	EIA+/ SRA− (n = 56)	EIA− (n = 174)	*P* value
Nadir of platelet count ($\times 10^9$/L), median (IQR)	37.3 (20.0-51.1)[a]	55.0 (27.4-86.5)[ab]	66.0 (38.2-90.2)[b]	0.001
Percentage of platelet count fall (IQR)	78.4 (62.3-86.2)[a]	67.3 (51.5-82.7)[ab]	61.9 (48.2-76.1)[b]	0.010
4T score, median (IQR)	5.0 (4.5-6.0)[a]	4.0 (2.3-4.0)[b]	3.0 (2.0-4.0)[c]	<0.001
Relative risk based on 4T score				
Low risk (0-3), n (%)	0 (0)[a]	21 (37.5)[b]	107 (61.5)[c]	<0.001
Medium risk (4-5), n (%)	5 (16.1)[a]	25 (44.6)[a]	53(30.5)[b]	0.05
High risk (6-8), n (%)	11 (35.5)[a]	10 (17.9)[b]	5 (2.9)[c]	<0.001
HEP score, mean ±SD	5.6 ± 3.1[a]	3.6 ± 3.2[b]	2.2 ± 3.3[b]	<0.001
HIT Ab EIA (OD), median (IQR)	1.77 (1.06-3.05)[a]	0.96 (0.69-1.58)[b]	0.12 (0.07-0.20)[c]	<0.001
UFH, n (%)	27 (87.1)	49 (87.5)	136 (78.2)	0.20
Route IV, n (%)	22 (71.0)	38 (57.6)	90 (51.7)	0.13
History of heparin exposure <100 d	12 (38.7)	26 (46.4)	66(37.9)	0.52
Alternative anticoagulants				
Bivalirudin, n (%)	14 (45.2)[a]	23 (41.1)[a]	17 (9.8)[b]	<0.001
Argatroban, n (%)	10 (32.3)[a]	3 (5.4)[b]	0 (0)[c]	<0.001
Foundaparinux, n (%)	3 (9.7)[a]	1 (1.8)[ab]	2 (1.1)[b]	0.01
Apixaban, n (%)	0(0)	0(0)	2(1.1)	0.60
None, n (%)	4(12.9)[a]	29(51.8)[b]	153(87.9)[c]	<0.001
Thrombotic events before the latest heparin exposure, n (%)	22 (71.0)[a]	14 (25.0)[b]	50 (28.7)[b]	<0.001
Thrombotic events after the latest heparin exposure, n (%)	21 (67.7)[a]	16 (28.6)[b]	31 (17.8)[b]	<0.001
DVT, n (%)	14 (45.2)[a]	6 (10.7)[b]	11 (6.3)[b]	<0.001
PE, n (%)	4 (12.9)[a]	5 (8.9)[ab]	6 (3.4)[b]	0.06
New stroke, n (%)	5 (16.1)[a]	4 (7.1)[ab]	6 (3.4)[b]	0.02
AMI, n (%)	0 (0)	2 (3.6)	6 (3.4)	0.57
Peripheral arterial, n (%)	5 (16.1)[a]	3 (5.4)[ab]	6 (3.4)[b]	0.02
Ventricular or aortic, n (%)	1 (3.2)	0 (0)	2 (1.1)	0.40

Note: Here, the differences between a-b, b-c, and a-c were statistically significant ($P < 0.05$) while the differences between a-a, b-b, a-ab, and b-ab were not statistically significant ($P > 0.05$).

Abbreviations: AMI, acute myocardial infarction; DVT, deep vein thrombosis; HEP, HIT expert probability; HIT Ab, heparin-induced thrombocytopenia anti-PF4/heparin IgG; IQR, interquartile range; IV, intravenous; OD, optical density; PE, pulmonary embolism; UFH, unfractionated heparin.

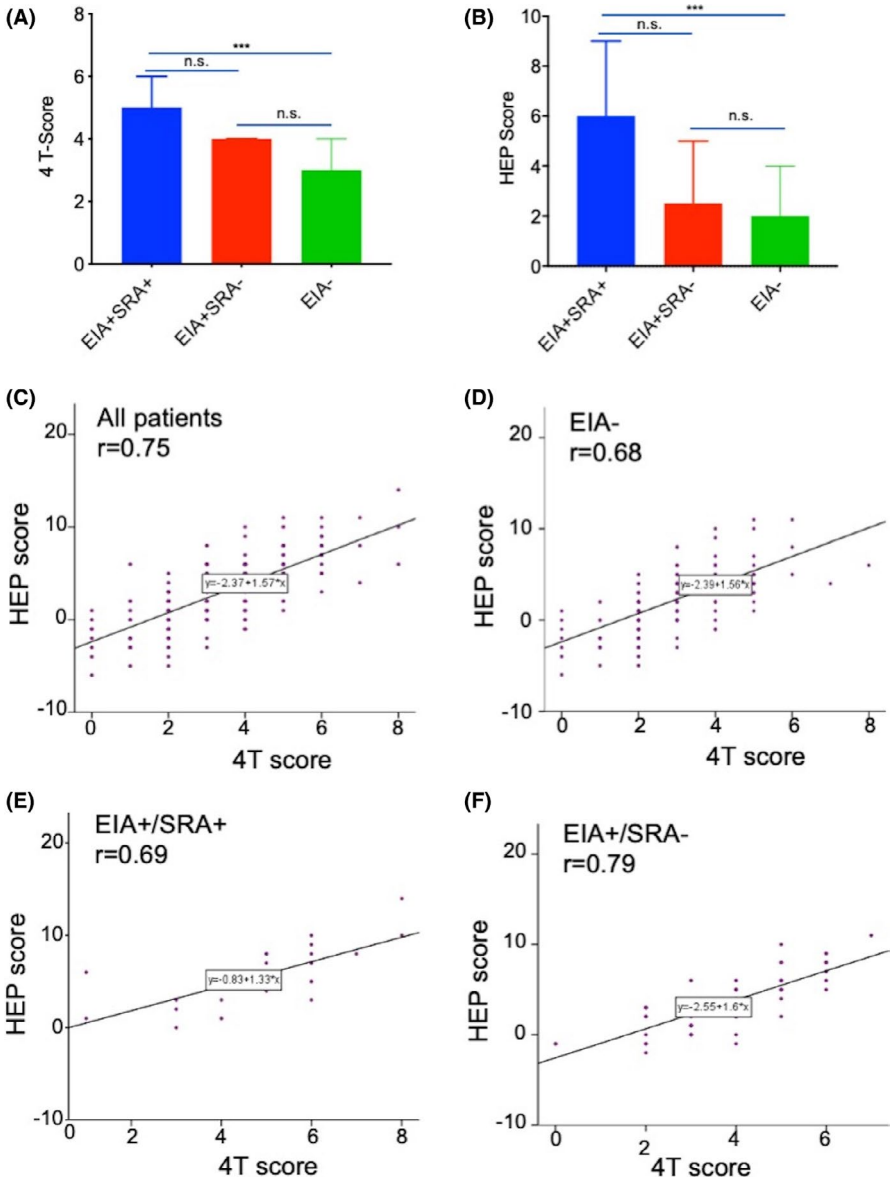

FIGURE 1 4T and HEP scores in patients with a suspected HIT. A and B show 4T and HEP score, respectively, in hospitalized patients with EIA+/SRA+, EIA+/SRA–, and EIA–, as indicated. *** $P < 0.005$. C, D, E, and F show the linear correlation between 4T score and HEP score in all patients ($r = 0.75$), EIA– ($r = 0.68$), EIA+/SRA+ ($r = 0.69$), and EIA+/SRA– ($r = 0.79$) patients as indicated. All r values are statistically significant from zero with $P < 0.001$. 4T, thrombocytopenia, time to platelet falls, thrombosis, and no other causes of thrombocytopenia; EIA, enzyme immunoassay; HEP, HIT expert probability; HIT, heparin-induced thrombocytopenia; SRA, serotonin-releasing assay

more frequently seen in the EIA+/SRA+ group than in the EIA+/SRA– and EIA– patients. However, PE ($P = 0.06$) and ventricular or aortic thrombosis ($P = 0.40$), although rare, in all three groups had a similar incidence rate (Table 2).

3.3 | Plasma ADAMTS-13 activity, VWFAg, and VWFAc in patients with suspected HIT

A delicate balance between ADAMTS-13 and VWF is crucial for normal hemostasis.[47,48] To assess if an imbalance of ADAMTS-13 and VWF in patients with suspected HIT may contribute to the disease process and outcome, we determined plasma levels of ADAMTS-13 activity, VWFAg, and VWFAc in 261 patients with suspected HIT and their correlations with HIT test results, underlying disease or procedure types, thromboembolic complications, and mortality rate. As shown, the median (IQR) plasma level of ADAMTS-13 activity in EIA+/SRA+, EIA+/SRA–, and EIA– groups was 51.6% (33.6%-79.4%) ($P < 0.0001$), 47.2% (28.2%-68.5%) ($P < 0.0001$), and 53.2% (36.0%-74.3%) ($P < 0.0001$), respectively, significantly <104.5% (83.8%-125.5%) in the healthy controls There was no statistically significant difference in the plasma ADAMTS-13 activity among all three patient groups ($P > 0.05$) (Table 3 and Figure 2A). Conversely, plasma levels of VWFAg (Figure 2B) and VWFAc (Figure 2C) in all patients with clinically suspected HIT were dramatically increased compared with those in the healthy controls. Again, there was no statistically significant difference in plasma levels of VWFAg and VWFAc among three patient groups ($P > 0.05$) (Table 3). As a result, the ratios of ADAMTS-13 to VWFAg (median; IQR) in the EIA+/SRA+ (0.1; 0.08-0.24) ($P < 0.0001$), EIA+/SRA– (0.11; 0.06-0.19) ($P < 0.0001$), and EIA– group (0.13; 0.05-0.23) ($P < 0.0001$) were significantly lower than that in the healthy control (0.70; 0.54-1.23) (Table 3 and Figure 2D). Similarly, lower ratios of ADAMTS-13 activity to VWF activity were observed among three patient groups compared with that in the healthy controls (Table 3 and Figure 2E).

TABLE 3 Plasma ADAMTS13 activity, VWF antigen, and VWF activity in 261 patients and healthy controls

	EIA+/SRA+	EIA+/SRA−	EIA−	Healthy control	
	(*N* = 31)	(*N* = 56)	(*N* = 174)	(*N* = 23)	*P*-values
ADAMTS13 ac (%)*	51.60[a]	47.20[a]	53.20[a]	104.50[b]	<0.001
	[33.6, 79.4]	[28.2, 68.5]	[36.0, 74.3]	[83.8, 125.5]	
VWFAg (%)	393.10[ab]	386.80[b]	322.80[a]	136.10[c]	<0.001
	[283.8, 505.2]	[251.2, 607.8]	[221.7, 492.2]	[93.5, 200.2]	
VWFAc (%)	343.40[a]	319.00[a]	269.90[a]	124.20[b]	<0.001
	[228.4, 426.5]	[206.9, 420.8]	[187.6, 366.9]	[94.5, 152.1]	
Ratio of ADAMTS13 ac/VWFAg	0.115[ab]	0.110[b]	0.168[a]	0.700[c]	<0.001
	[0.084, 0.245]	[0.060, 0.199]	[0.086, 0.292]	[0.538, 1.227]	
Ratio of ADAMTS13 ac/VWFAg	0.154[a]	0.160[a]	0.213[a]	0.976[b]	<0.001
	[0.114, 0.286]	[0.083, 0.303]	[0.123, 0.376]	[0.644, 1.214]	
Ratio of VWFAc/VWFAg	0.879	0.887	0.838	0.946	0.239
	[0.522, 1.116]	[0.477, 1.142]	[0.577, 1.207]	[0.816, 1.207]	

Note: All data are presented as the median (interquartile range). Here, the differences between a-b, b-c, a-c, or ab-c were statistically significant ($P < 0.05$), while the differences between a-ab and b-ab were not statistically significant ($P \geq 0.05$).

Abbreviations: EIA, enzyme immunoassay; SRA, serotonin-releasing assay; VWF, von Willebrand factor; VWFAc, von Willebrand factor activity; VWFAg, von Willebrand factor antigen.

There was no statistically significant difference in the ratios of VWFAc to VWFAg among patient groups or between patients and healthy controls (Table 3 and Figure 2F). These results demonstrate for the first time a high prevalence of relative deficiency of plasma ADAMTS-13 activity in all hospitalized patients with suspected HIT.

3.4 | Low plasma ADAMTS-13 activity, but not HIT EIA/SRA positivity, predicts mortality in patients with suspected HIT

To assess the role of ADAMTS-13 and VWF in the disease process and outcomes in patients with suspected HIT, the associations between plasma levels of ADAMTS-13 or VWF or the ratio of ADAMTS-13 to VWF and other clinical events or a 90-day cumulative survival probability were determined. Kaplan-Meier survival analyses demonstrated that the 90-day cumulative survival probability rates were significantly lower in all patients (Figure 3A), EIA+/SRA+ (Figure 3B), EIA− (Figure 3C), and EIA+/SRA− patients whose plasma ADAMTS-13 activity was <50% than in those who had plasma ADAMTS-13 activity ≥50%, suggesting that low ADAMTS-13 activity is predictive for an increased mortality rate in all patients with suspected HIT regardless of their HIT test results.

In contrast, the 90-day survival probability was not different among all three groups of patients based on the HIT test results ($P = 0.30$) (Figure 3E), although the EIA+/SRA+ or EIA+/SRA− patients appeared to have a longer hospital stay, and fewer patients who had stayed in hospital for <30 days compared with the EIA− patients (Table 1). These results suggest that having a positive HIT test may require change in the management strategy, resulting in an increased usage of hospital resource.

3.5 | The ratio of ADAMTS-13 activity to VWFAg or VWFAc also predicts mortality

To determine if the ratio of ADAMTS-13 to VWF would be more sensitive for prediction of mortality, the Kaplan-Meier analysis was performed in each patient group. As shown, the ratio of plasma ADAMTS-13 activity to VWFAg at less than the 50th percentile was associated with low probability of survival in all patients ($P < 0.0001$) (Figure 4A), EIA+/SRA+ ($P = 0.03$) (Figure 4B), EIA- ($P = 0.01$) (Figure 4C), and EIA+/SRA− ($P = 0.03$) (Figure 4D) patients. Similarly, the ratio of ADAMTS-13 activity to VWFAc at less than the 50th percentile was also associated with low probability of survival in all patients ($P = 0.003$) (Figure 4E), EIA+/SRA+ ($P = 0.02$) (Figure 4F), and EIA− ($P = 0.03$) (Figure 4G), but not EIA+/SRA− ($P = 0.29$) (Figure 4H) patients. These results indicate that the abnormality in ADAMTS-13/VWF axis is associated with worse outcome in patients with suspected HIT, regardless of their HIT test results.

3.6 | Plasma ADAMTS-13 activity is correlated with its antigen in patients with suspected HIT

To determine the mechanism underlying low ADAMTS-13 activity in patients with suspected HIT, we determined plasma ADAMTS-13 antigen by ELISA in the same patient samples. Spearman correlation coefficient (r) demonstrated that plasma ADAMTS-13 activity was positively

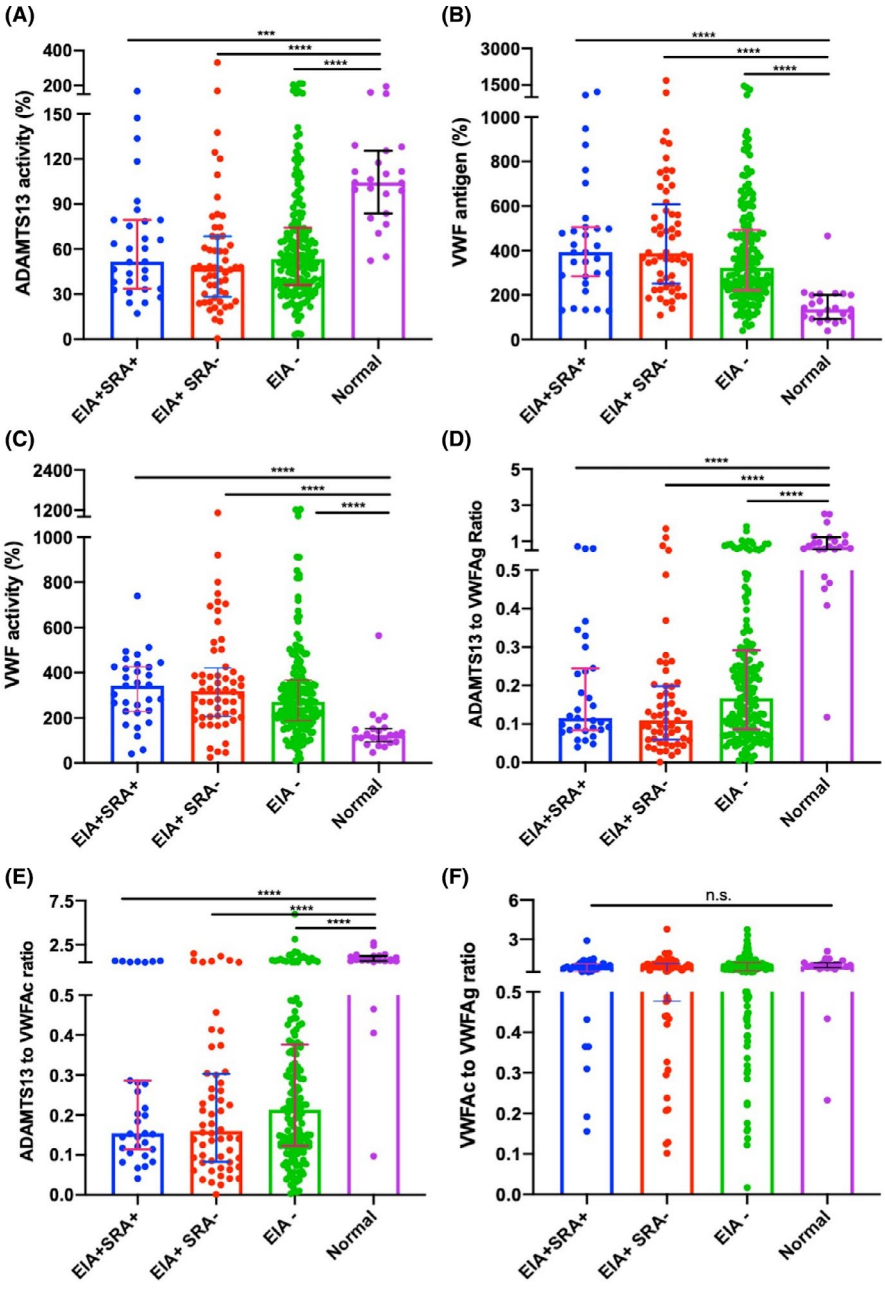

FIGURE 2 Plasma ADAMTS-13 activity, VWF antigen and collagen-binding activity in patients with suspected HIT. Plasma ADAMTS-13 activity (A), VWF antigen (B), VWF collagen-binding activity (C), the ratio of plasma ADAMTS-13 activity to VWF antigen (D), the ratio of plasma ADAMTS-13 to VWF activity (E), and the ratio of plasma VWF activity to VWF antigen (F) in EIA+/SRA+, EIA+/SRA–, EIA–, and healthy controls. Each dot represents a value from each patient or healthy control; Each dot represents each patient value. The median and interquartile range (IQR) are shown. Kruskal-Wallis test determined the statistical significance among all four groups. The symbols **, ***, and **** indicate the *P* values of <0.01, <0.005, and <0.001, respectively. EIA, enzyme immunoassay; SRA, serotonin-releasing assay; VWF, von Willebrand factor

correlated with ADAMTS-13 antigen in all patients ($r = 0.70$; $P < 0.0001$) (not shown), in EIA+/SRA+ ($r = 0.67$; $P < 0.0001$) (Figure 5A), EIA+/SRA– ($r = 0.68$; $P < 0.0001$) (Figure 5B), and EIA– ($r = 0.74$; $P < 0.0001$) groups (Figure 5C), as well as in the healthy controls ($r = 0.71$; $P < 0.0001$) (Figure 5D). These results indicate that the reduction of plasma ADAMTS-13 activity may primarily result from either a decrease in ADAMTS-13 synthesis or an increase in ADAMTS-13 consumption.

However, in patients with acquired TTP, anti–ADAMTS-13 IgG is the primary cause of low ADAMTS-13 activity. To rule out the possibility of antibody-mediated inhibition or clearance of ADAMTS-13, we determined the anti–ADAMTS-13 IgG in patients with ADAMTS-13 activity <30% of normal. Of 261 patients, 9 (≈3.5%) had plasma ADAMTS-13 activity <30%, and their levels of anti–ADAMTS-13 IgG were borderline elevated in 3, but significantly elevated (>15 U/mL) in 6 patients (Table 4). These results suggest that in rare situations the reduction of plasma ADAMTS-13 activity may also result from autoantibodies against ADAMTS-13.

3.7 | Logistic regression analyses to identify other potential clinical factors that may contribute to low ADAMTS-13 activity and mortality in patients with suspected HIT

To determine the potential cause of low plasma ADAMTS-13 activity and other clinical factors contributing to mortality, we performed the binary logistic regression analyses for ADAMTS-13 activity <50% of normal and death. The results showed that factors associated with plasma ADAMTS-13 activity <50% of normal

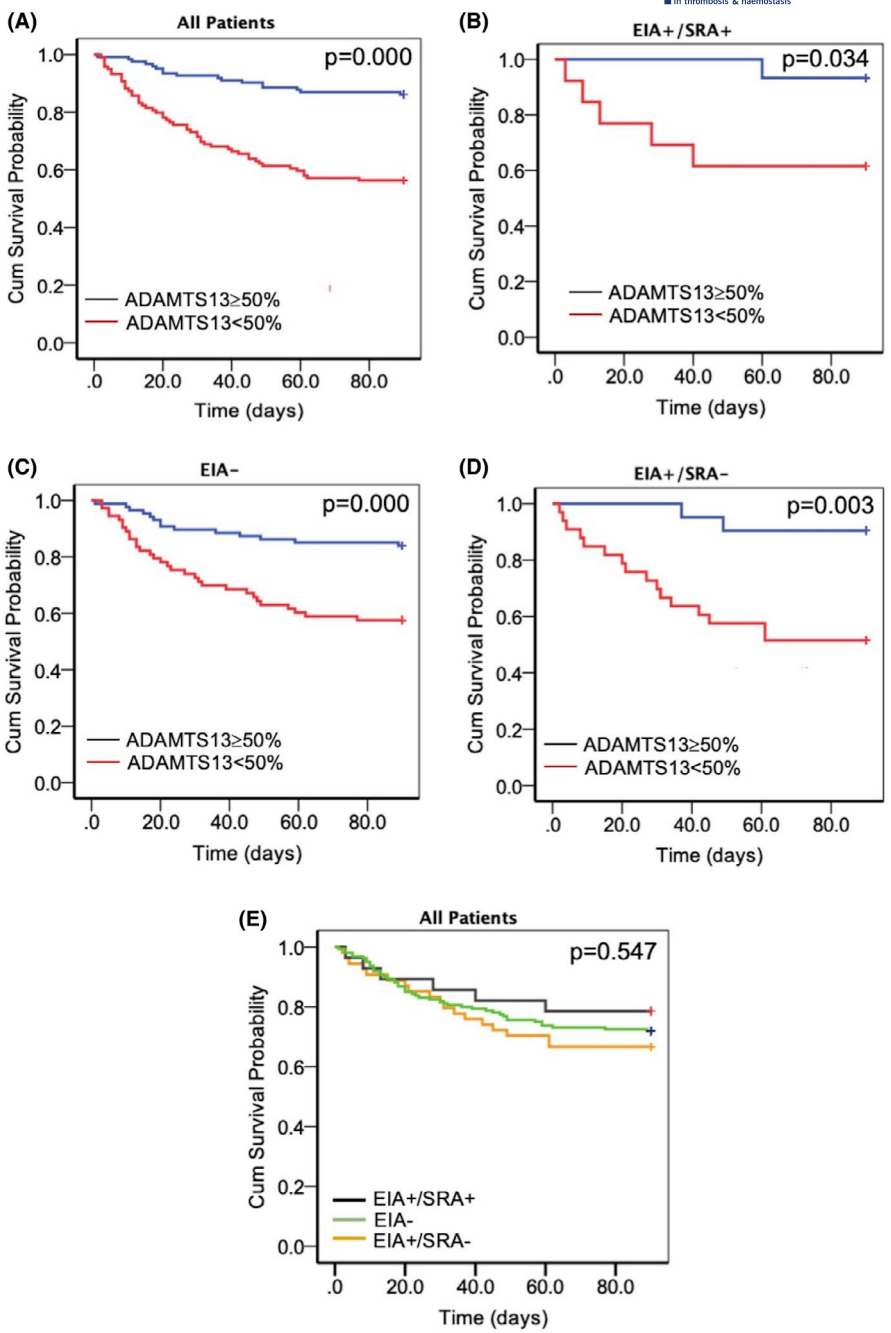

FIGURE 3 Kaplan-Meier survival analyses in patients with suspected HIT. The cumulative survival probability over 90 days after admission in all patients (A), EIA+/SRA+ (B), EIA− (C), and EIA+/SRA− (D) groups who had plasma ADAMTS-13 activity <50% of normal (red line) vs. ≥50% of normal (blue line). Additionally, the cumulative survival probabilities over 90 days after admission in EIA+/SRA+, EIA, and EIA+/SRA− groups are shown in panel E. Here, *P* values >.05, <0.05 and <0.01 are considered to be statistically not significant, significant, and highly significant, respectively. EIA, enzyme immunoassay; HIT, heparin-induced thrombocytopenia; SRA, serotonin-releasing assay

include platelet count fall >50%, pulmonary embolism, sepsis, liver failure, and heart failure. Patients with these clinical conditions had odds ratios (ORs) of 3.4, 18.6, 2.3, and 2.0, respectively, with a sensitivity of 70.2% and a specificity of 69.7% (Table 5). These results indicate that low ADAMTS-13 activity may be associated with an excessive consumption of ADAMTS-13 during various disease processes.

Additionally, logistic regression analysis demonstrated that the factors contributing to the in-hospital mortality were ADAMTS13 activity less than 50% of normal (OR 3.3, 95% CI 1.6-6.8, $P = 0.001$), intra-aortic embolism (OR 19.6, 95% CI 1.8-217.1, $P = 0.015$), bleeding (OR 2.9, 95% CI 1.2-6.7, $P = 0.015$), infections (OR 2.3, 95% CI 1.1-5.0, $P = 0.037$), respiratory failure (OR 2.2, 95% CI 1.0-4.8, $P = 0.042$), arrhythmia (OR 3.6, 95% CI 1.8-7.3, $P < 0.001$), and history of stroke (OR 3.2, 95% CI 1.3-7.9, $P = 0.011$) (Table 6). These results suggest that the causes of death in these patients may be multifactorial, but each may contribute to the mortality independently.

Finally, logistic regression analysis revealed that EIA+/SRA- or EIA+/SRA+, thrombosis before heparin use, respiratory failure, and chronic cardiovascular disease were associated with an increased probability of thrombotic events after heparin exposure (Table 7).

4 | DISCUSSION

HIT is a life-threatening thromboembolic complication after heparin exposure. Clinical and laboratory diagnosis, as well as management of HIT remain to be challenging. Over- and underdiagnosis

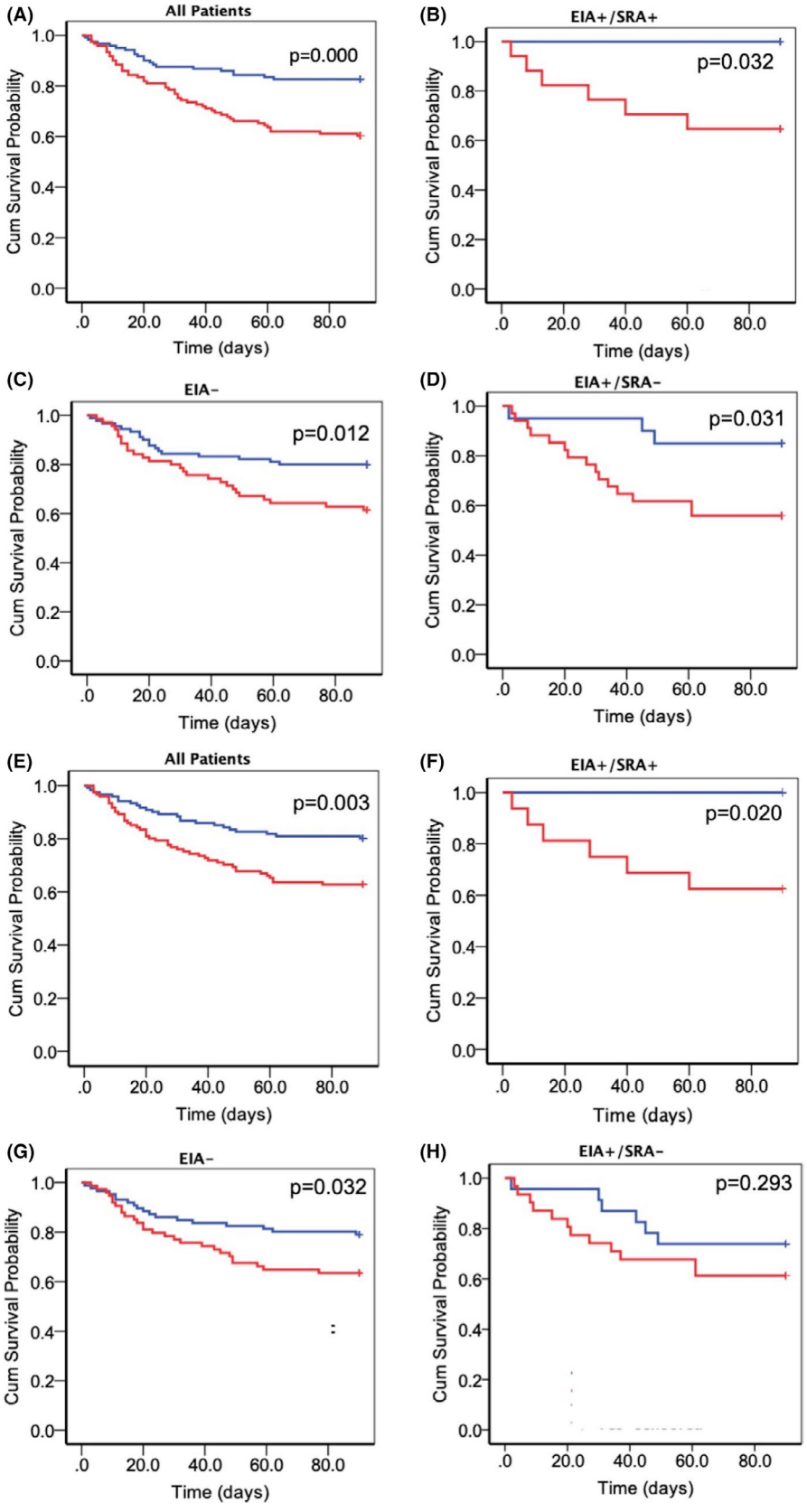

FIGURE 4 Kaplan-Meier survival analyses in patients with suspected HIT. The cumulative survival probability over 90 days after admission in all patients (A), EIA+/SRA+ (B), EIA− (C), and EIA+/SRA- (D) groups who had the ratio of plasma ADAMTS-13 activity to VWF antigen <50th percentile (red line) versus ≥50th percentile (blue line). Also, the cumulative survival probabilities over 90 days after admission in all patients (E), EIA+/SRA+ (F), EIA− (G), and EIA+/SRA− (H) groups who had the ratio of plasma ADAMTS-13 activity to VWF activity at <50th percentile (red line) versus ≥50th percentile (blue line). Here, *P* values >0.05, <0.05 and <0.01 are considered to be statistically not significant, significant, and highly significant, respectively. EIA, enzyme immunoassay; HIT, heparin-induced thrombocytopenia; SRA, serotonin-releasing assay; VWF, von Willebrand factor

of HIT can be potentially catastrophic, resulting in either the formation of thrombosis after exposure to heparin or bleeding complications due to the use of an alternative anticoagulant. Switching to a more expensive anticoagulant after cessation of heparin may also incur an economic burden to patients and health system. In our cohort study, only one-third (35.6%) of the patients with a positive EIA test showed the SRA positivity, confirming the diagnosis of clinically suspected HIT.[46] However, many patients

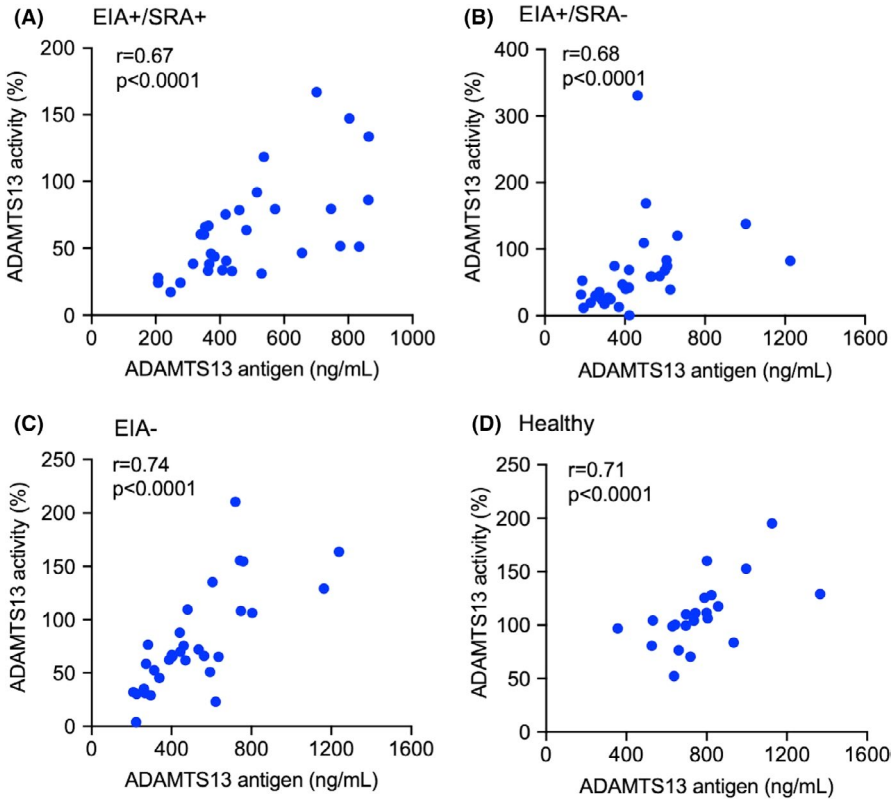

FIGURE 5 Spearman correlation between ADAMTS13 activity and antigen in patients with suspected HIT. Plasma ADAMTS13 activity is strongly correlated with plasma ADAMTS13 antigen in patients with EIA+/SRA+ (A), EIA+/SRA− (B), EIA− (C), and healthy controls (D)

TABLE 4 The clinical and laboratory characteristic of patients with borderline to positive anti–ADAMTS-13 IgG among patients with plasma ADAMTS-13 activity <30%

No.	Age, y	Sex	PLT ($\times 10^3$/μl)	HIT Ab	A13 activity (%)	Anti-A13 IgG (U/mL)	Schistocytes	Cre (mg/dL)	Diagnosis	Procedure
1	70	M	97	EIA+/SRA−	13.2	13.5[a]	Rare	2.5	Severe MR	MVR, ECMO
2	59	M	80	EIA+/SRA−	25.0	16.3[b]	None	1.3	Sepsis, PSC	None
3	76	F	38	EIA+/SRA−	17.8	18.3[b]	None	2.0	HE, cirrhosis	None
4	78	F	31	EIA+/SRA−	24.5	18[b]	None	1.2	Cardiogenic shock	IABP
5	70	M	23	EIA+/SRA−	0.6	17.4[b]	Rare	0.9	CAD, CHF, AS, MR	CABG, AVR, MVR
6	58	M	206	EIA−	23.1	15.2[b]	None	3.6	Cellulitis, CKD	None
7	82	M	376	EIA−	24.4	13.0[a]	None	1.0	Pneumonia	None
8	71	F	80	EIA−	24.1	114[b]	Rare	4.5	Cardiogenic shock, pneumonia; ESRD on PD	None
9	62	F	94	EIA−	18.4	12.0[a]	Rare	5.8	Chest pain, ESRD on dialysis	None

Note: a and b denote the values that are within the borderline (12–15 U/mL) and elevated (>15 U/mL), respectively.

Abbreviations: AS, aortic valve stenosis; AVR, aortic valve replacement; CABG, coronary artery bypass grafting; CAD, coronary arterial disease; CHF, congestive heart failure; CKD, chronic kidney disease; CR, creatinine; ECMO, extracorporeal membrane oxygenation; EIA, enzyme immunoassay; ESRD, end-stage renal disease; HE, hepatic encephalopathy; IABP, intra-aortic balloon pump; MR, mitral regurgitation; MVR, mitral valve replacement; PD: peritoneal dialysis; PLT, platelet count; PSC, primary sclerosing cholangitis; SRA, serotonin-releasing assay.

TABLE 5 Logistic regression for ADAMTS-13 activity <50% of normal

	OR	95% CI	
		Lower	Upper
All patients (χ^2=53.773)			
VWF Ag	1.00	1.00	1.00
PLT count fall >50%	2.58	1.27	5.19
Pulmonary embolism	9.72	1.98	47.77
Sepsis	2.53	1.43	4.49
Liver failure	2.00	1.07	3.75
Heart failure	1.82	1.03	3.21

Note: ORs of all the binary variances were compared by "yes" to "no."
Abbreviations: CI, confidence interval; OR, odds ratio; PLT, platelet.

TABLE 6 Logistic regression for death after admission

	OR	95% CI	
		Lower	Upper
All patients (χ^2 = 84.0)			
ADAMTS13 activity <50%	3.3	1.6	6.8
Intra-aortic embolism	19.6	1.8	217.1
Bleeding	2.9	1.2	6.7
Infection	2.3	1.1	5.0
Respiratory failure	2.2	1.0	4.8
Arrhythmia	3.6	1.8	7.3
Stroke history	3.2	1.3	7.9

Note: Odds ratio (OR)s of all the binary variances were compared by "yes" to "no".
Abbreviation: CI, confidence interval.

TABLE 7 Logistic regression for thrombotic events after heparin use

	OR	95% CI	
		Lower	Upper
All patient (χ^2=49.8)			
EIA+/SRA−	2.1	1.0	4.6
EIA+/SRA+	8.9	3.3	23.8
Thrombosis before heparin use	2.9	1.5	5.6
Respiratory failure	2.2	1.1	4.3
Coronary heart disease	2.3	1.2	4.5

Note: ORs of all the binary variances were compared by "yes" to "no."
Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; OR, odds ratio; SRA, serotonin-releasing assay.

with clinically suspected HIT or an EIA positivity with or without SRA positivity have already been switched to an alternative anticoagulant. The most commonly used anticoagulants in patients with suspected or confirmed HIT include argatroban, apixaban, rivaroxaban, and bivalirudin, as well as fondaparinux. There is still insufficient data to demonstrate the efficacy of these treatments for HIT.[49]

In comparison with EIA- or EIA+/SRA- group, patients with EIA+SRA+ had a longer length of hospital stay, although the mortality rate (22.6%-30.4%) in all three groups of patients was not statistically different. The prolonged hospitalization may be related to an additional time needed to obtain an expert consultation and to achieve stable alternative anticoagulation for treating thromboembolic complications (e.g., inferior vena cava filter placement, percutaneous coronary intervention, and transition to warfarin, etc.). Many patients with suspected HIT had organ failure, infections, and active supportive therapies including ECMO, intra-aortic balloon pump, ventricular assistant device, and so on (Table 1). Therefore, the severity of primary diseases might have caused the fatality. In fact, the EIA+/SRA+ group had fewer acute heart failure (29.0%) and chronic heart failure (16.1%) cases than the EIA+/SRA− (62.5% and 44.6%, respectively) and the EIA− patients (50.0% and 33.9%, respectively). Interestingly, both EIA+/SRA+ and EIA+/SRA− groups had fewer cases of acute renal failure than the EIA− group. These results demonstrate the complexity of diagnosis of HIT in the context of severe diseases and medical conditions that may result in thrombocytopenia and thromboembolism after heparin exposure. A previous study demonstrated that patients who had undergone cardiac surgery had a >50% EIA positivity rate.[50] In our cohort, the number of patients with cardiac surgery was not statistically significantly different among all three groups of patients.

The proportion of patients with thromboembolic events in the EIA+/SRA+ group was significantly higher than that in other two groups, but there was no difference observed between the EIA+/SRA− and the EIA− groups. These results are in agreement with the results of Warkentin et al.[2] DVT, PE, stroke, and peripheral arterial embolus were predominant in our patients. DVT is the most common embolic event but arterial embolism was the major thrombotic event in patients following cardiac surgery.[11,51] Interestingly, 71% of EIA+/SRA+ patients had prior thrombotic events, compared to 25% of EIA+/SRA− patients and 28.7% of EIA− groups, respectively. These results are consistent with the importance of detecting platelet-activating antibodies against anti-PF4/heparin complexes with SRA, although its low sensitivity[52] demands a better functional assay. For instance, using a high concentration of PF4 instead of heparin for detecting platelet activating anti-PF4/heparin antibodies,[53] or anti-FP4/polyanion antibodies.[54] Alternatively, a P-selectin expression, instead of SRA may be used for detecting platelet activation,[55] which may allow the identification of anti-PF4/heparin antibodies in 11 of 16 of EIA+/SRA− patients.[55,56]

Severe deficiency of plasma ADAMTS-13 may result in TTP, a potentially fatal thrombotic disorder.[47,57] However, mild to moderate deficiency of plasma ADAMTS-13 activity or relative deficiency is common in patients with several acute and chronic disorders including myocardial infarction,[29,31,32] cerebral ischemia,[38,58,59]

preeclampsia,[35,60-62] and sepsis,[63-65] as well as severe SARS-CoV-2 infection.[39-41,66,67] Consistent with such a notion, low plasma ADAMTS-13 activity or reduced ratio of ADAMTS-13 activity to VWF antigen or activity is prevalent in our patients with suspected HIT; this may be primarily caused by congestive heart failure, pulmonary embolism, and sepsis, resulting in reduced synthesis and increased consumption of ADAMTS-13. In some cases, low ADAMTS-13 activity may be the result of IgG autoantibodies against ADAMTS-13 that binds ADAMTS-13 and accelerates clearance of immune complexes from circulation.

Most importantly, our Kaplan-Meier survival analysis demonstrates that patients with low ADAMTS-13 activity (<50th percentile) or low ratio of ADAMTS-13 activity to VWF (antigen or activity) exhibits a dramatically reduced survival probability within 90 days of follow-up, regardless of HIT test results, although the predictive value in the EIA+/SRA+ patients is clearly better. This is further supported by the multivariant analysis demonstrating that low ADAMTS-13 activity is an independent factor contributing to the 90-day mortality rate.

There may be certain limitations related to this study. While the control and patient samples were collected and stored at −80°C without repeated freezing and thawing before this analysis, the patient samples were stored longer than the control samples. Fortunately, both VWF and ADAMTS-13 in plasma are stable under such a storage condition. The other limitations include the retrospective nature of patient data collection and potential interassay variability of the HIT EIA test.

Nevertheless, our results demonstrate that while other clinical factors may contribute to the mortality, low levels of plasma ADAMTS-13 activity and high levels of plasma VWF, resulting in relative deficiency of ADAMTS-13 function, appear to be highly predictive for adverse outcomes in hospitalized patients with suspected HIT. Our findings suggest a possible interventional strategy with ADAMTS-13 supplementation to improve the survival rate in this patient population should future prospective studies confirms these findings.

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RELATIONSHIP DISCLOSURE

XLZ is a consultant for Alexion, Sanofi-Genzyme, and Takeda. XLZ is also a speaker for Alexion and Sanofi-Genzyme and cofounder of Clotsolution. MC has no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

MC, XZ, and XLZ designed the research, collected and analyzed the data, and wrote and finalized the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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