Coagulation Functions Are Associated With Hemorrhagic Transformation in Non-Atrial Fibrillation Patients: A Case Control Study

Hao-Ran Cheng
The First Affiliated Hospital of Wenzhou Medical University

Yun-Bin Chen
The First Affiliated Hospital of Wenzhou Medical University

Ya-Ying Zeng
The First Affiliated Hospital of Wenzhou Medical University

Yi-Ting Ruan
The First Affiliated Hospital of Wenzhou Medical University

Cheng-Xiang Yuan
The First Affiliated Hospital of Wenzhou Medical University

Qian-Qian Cheng
Wenzhou Medical University

Hui-Jun Chen
The First Affiliated Hospital of Wenzhou Medical University

Xiao-Qian Luan
The First Affiliated Hospital of Wenzhou Medical University

Gui-Qian Huang
The First Affiliated Hospital of Wenzhou Medical University

Jincai He (✉ hjc@wmu.edu.cn)
The First Affiliated Hospital of Wenzhou Medical University  https://orcid.org/0000-0002-5493-4357

Research article

Keywords: hemorrhagic transformation, coagulation function, atrial fibrillation, acute ischemic stroke

DOI: https://doi.org/10.21203/rs.3.rs-104422/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Hemorrhagic transformation (HT) is a serious neurological complication of acute ischemic stroke (AIS) after revascularization. The majority of AIS patients do not have atrial fibrillation (AF) which could also develop to HT. In this study, we aimed to explore whether coagulation parameters are risk factors of HT in non-AF patients.

Methods: We consecutively enrolled 285 AIS patients with HT. Meanwhile, age- and sex-matched 285 AIS patients without HT were included. The diagnosis of HT was determined by brain CT or MRI during hospitalization. All patients were divided into two subgroups based on the presence of AF and explore the differences between the two subgroups. Blood samples were obtained within 24 hours of admission, and all patients were evenly classified into three tertiles according to platelet counts (PLT) levels.

Results: In this study, we found the first PLT tertile (OR = 3.509, 95%CI = 1.268-9.711, P = 0.016) was independently associated with HT in non-AF patients, taking the third tertile as a reference. Meanwhile, we also found mean platelet volume (MPV) (OR = 0.605, 95%CI = 0.455-0.805, P = 0.001) and fibrinogen (FIB) (OR = 1.928, 95%CI = 1.346-2.760, P <0.001) were significantly associated with HT in non-AF patients. But in AF patients, coagulation parameters showed no significant difference. Meanwhile, we found the MPV (OR = 1.314, 95%CI = 1.032-1.675, P = 0.027) and FIB (OR = 1.298, 95%CI = 1.047-1.610, P = 0.018) were significantly associated with long-term outcomes in non-AF HT patients.

Conclusions: Low PLT, low MPV and high FIB levels were independently associated with HT in non-AF patients. Additionally, MPV and FIB levels were significantly associated with unfavorable long-term outcomes in non-AF HT patients. Our study showed that coagulation functions at admission may be beneficial for clinicians to recognize patients with high risk of HT at early stage and improve unfavorable long-term outcomes in non-AF patients.

1. Introduction

Hemorrhagic transformation (HT), one of the most common neurological complications after acute ischemic stroke (AIS), is associated with early mortality and poor outcomes after stroke [1–6]. Currently, many risk factors related to HT have been identified including old age, the severity of a stroke, dyslipidemia, hyperglycemia, atrial fibrillation (AF), and thrombolytic therapy [7, 8].

It has been reported that AF and some medication used for its treatment such as Warfarin could cause HT [9–12]. In 2019, Jiao et al. found that AF independently correlated with HT and was a risk factor of HT [8]. In a prospective trial of 101 AIS patients, Tu et al. found that AF patients had significant hypoperfusion that may damage vascular integrity leading to a more frequent HT [11]. Meanwhile, Altavilla et al. found that patients with AF who received low-molecular-weight heparin have a higher risk of HT than non-bridged patients [13]. Besides, an animal experiment found that the incidence of HT in rats treated with Warfarin was increased [12].

However, HT has been underexplored in non-AF patients; the majority of AIS patients are non-AF patients [14, 15] and they could also develop HT. Meanwhile, AF and medication for AF would influence coagulation functions [16, 17]. Thus, the risk factors of non-AF patients to develop HT after AIS needs to be further explored, especially coagulation functions.

In this research, we mainly explored the association between coagulation functions and HT in patients with AF and without AF and aimed to identify coagulation parameters as risk factors in non-AF HT patients.

2. Materials And Methods

2.1 Subjects

This was a retrospective study of patients with and without hemorrhagic transformation after stroke. The study protocol obtained the approval of the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. We didn't have informed consent for it was a retrospective study and the patient profile was anonymous. The cohort was made up of the First Affiliated Hospital of Wenzhou Medical University's clinical database of HT; the same number of stroke patients without HT from our stroke center was matched by age and sex using the same inclusion and exclusion criteria. All patients who were diagnosed with HT in the First Affiliated Hospital of Wenzhou Medical University were included in this study from December 2013 to December 2015. The inclusion criteria were as follows: (1) age between 18 and 99 years; (2) patient was included within 7 days of stroke; (3) the diagnosis of
stroke was confirmed by computerized tomography (CT) or magnetic resonance imaging (MRI) at the time of admission. The exclusion criteria were as follows: (1) hemorrhagic stroke or transient ischemic attack (TIA); (2) patient received intravenous thrombolytic therapy; (3) patients with any severe liver or kidney dysfunction; (4) patients failed to receive a repeat CT/MRI scan; (5) patients’ medical record was incomplete.

2.2 Diagnosis of HT

All patients received a brain CT scan or MRI including diffusion-weighted imaging (DWI) and T2-weighted gradient-echo within 24 h after stroke onset. HT was diagnosed in a subsequent CT/MRI performed 7 ± 2 days after stroke onset or whenever a worsening clinical condition. Two neurologists evaluated the CT/MRI scans independently and diagnosed HT, who were blinded to the results of clinical and laboratory measurements.

2.3 Data collection

Patients’ demographic data and personal hobbies included gender, age, current smoking, and drinking. Past medical history included the previous history of stroke, hypertension, diabetes mellitus, coronary artery disease (CAD), atrial fibrillation, and hyperlipidemia. Meanwhile, the National Institutes of Health Stroke Scale (NIHSS) was used to evaluate the severity of stroke within 24 h after admission. All patients identified the stroke subtypes according to the TOAST criteria [18]. Blood samples and blood pressure were obtained within 24 h of hospital admission and sent blood samples for tests as soon as possible. Laboratory tests included leukocyte counts, erythrocyte counts, platelet counts (PLT), mean platelet volume (MPV), prothrombin time (PT), international normalized ratio (INR) and fibrinogen (FIB). Also, drug applications in the hospital including anticoagulant, antiplatelet, and lipid-lowering drugs were collected and documented. All patients were evenly classified into three tertiles according to the PLT levels (tertile 1, < 175; tertile 2, 175–222; and tertile 3, > 222).

In December 2019, HT patients were followed up by telephone to collect their up-to-date functional conditions. We used the modified Rankin Scale (mRS) to assess long-term functional outcomes, and an unfavorable functional outcome was defined as an mRS score of ≥ 2 [19–21].

2.4 Statistical analyses

Statistical descriptions of continuous variables were mean ± SD or medians (quartiles), while categorical variables were percentages. The comparison of continuous variables used the Student's t-test or Mann-Whitney test, while categorical variables used the Chi-square test or Fisher’s exact test. The comparison of the differences between three PLT tertiles in continuous variables used the Kruskal-Wallis test or one-way analysis of variance (ANOVA), while categorical variables used the Pearson's chi-square or Fisher's exact tests. Multiple logistic regression analysis was performed to identify significant independent related factors of HT and adjust potential confounding factors. Cox proportional hazards model was performed to identify the significant independent related factors of unfavorable long-term outcomes in non-AF HT patients and adjust potential confounding factors. All statistical analyses used IBM SPSS Statistics for Windows, Version: 19.0.0 (Chicago, IL). Two-tailed P-values < 0.05 were considered statistically significant.

3. Results

3.1 Baseline characteristics of patients stratified by HT

In this study, 285 AIS patients with HT and age- and sex-matched 285 AIS patients without HT were included. The baseline demographic, clinical, and laboratory characteristics of the study patients were presented in Table 1. The mean age of the enrolled patients was 68.9 ± 12.4 years. 388 (68.1%) patients were male and 182 (31.9%) were female. Also, 137 (24.0%) patients had a history of AF. As shown in Table 1, patients with HT had lower baseline systolic blood pressure (SBP), PLT, and MPV; higher leukocyte counts, PT, INR, and FIB compared with patients without HT. They also had higher NIHSS scores at admission. AF was more frequently found in patients with HT and the proportion of AF among HT and non-HT patients was 108 (37.9%) and 29 (10.2%) respectively. Less HT patients received anticoagulant therapy and more received antiplatelet therapy. Besides, patients with HT were more likely to smoke and drink.
Table 1
Baseline characteristics of AIS patients without HT and with HT

| Variables                        | Total (n = 570) | Non-HT (n = 285) | HT (n = 285) | P-value |
|----------------------------------|----------------|------------------|--------------|---------|
| **Demographic characteristics**  |                |                  |              |         |
| Age (years)                      | 68.9 ± 12.4    | 68.9 ± 12.3      | 68.9 ± 12.6  | 0.970   |
| Male, n (%)                      | 388(68.1%)     | 191 (67.0%)      | 197 (69.1%)  | 0.590   |
| Baseline SBP (mmHg)              | 153.3 ± 23.1   | 158.0 ± 23.0     | 148.6 ± 22.3 | < 0.001 |
| Baseline DBP (mmHg)              | 82.3 ± 13.8    | 82.1 ± 13.3      | 82.5 ± 14.3  | 0.732   |
| NIHSS on admission, median (IQR)| 5.0(2.0–10.0)  | 3.0(1.0–5.0)     | 9.0(5.0–13.0)| < 0.001 |
| **Vascular risk factors, n (%)**|                |                  |              |         |
| Current smoking                  | 210 (37.1%)    | 118 (41.5%)      | 92 (32.6%)   | 0.028   |
| Current drinking                 | 228 (40.4%)    | 141 (49.8%)      | 87 (30.9%)   | < 0.001 |
| Previous Stroke                  | 72 (12.6%)     | 31 (10.9%)       | 41 (14.4%)   | 0.207   |
| Hypertension                     | 376 (66.0%)    | 197 (69.1%)      | 179 (62.8%)  | 0.112   |
| Diabetes                         | 149 (26.1%)    | 80 (28.1%)       | 69 (24.2%)   | 0.294   |
| CAD                              | 47 (8.3%)      | 15 (5.3%)        | 32 (11.3%)   | 0.010   |
| Dyslipidemia                     | 37 (6.5%)      | 17 (6.0%)        | 20 (7.0%)    | 0.610   |
| AF                               | 137 (24.0%)    | 29 (10.2%)       | 108 (37.9%)  | < 0.001 |
| **Hematological variables**      |                |                  |              |         |
| Leukocyte counts (× 10^9/L)      | 7.7 ± 2.8      | 6.8 ± 1.9        | 8.5 ± 3.3    | < 0.001 |
| Erythrocyte counts (× 10^12/L)   | 4.4 ± 0.6      | 4.4 ± 0.6        | 4.4 ± 0.6    | 0.836   |
| PLT (× 10^9/L), median (IQR)     | 197.0(162.0-235.0) | 205.0(175.0-238.5) | 187.0(148.5–231.0) | < 0.001 |
| MPV (fl)                         | 11.0 ± 1.4     | 11.2 ± 1.2       | 10.8 ± 1.4   | 0.011   |
| PT (s)                           | 13.7 ± 1.0     | 13.5 ± 1.0       | 13.9 ± 1.0   | < 0.001 |
| INR                              | 1.1 ± 0.1      | 1.0 ± 0.1        | 1.1 ± 0.1    | < 0.001 |
| FIB (g/L)                        | 3.8 ± 1.6      | 3.5 ± 1.0        | 4.1 ± 2.0    | < 0.001 |
| **Stroke etiology, n (%)**       |                |                  |              | < 0.001 |
| Atherosclerosis                  | 405 (75.6%)    | 214 (84.9%)      | 191 (67.3%)  |         |
| Cardioembolism                   | 109 (20.3%)    | 20 (7.9%)        | 89 (31.3%)   |         |
| Small vessel occlusion           | 4 (0.7%)       | 3 (1.2%)         | 1 (0.4%)     |         |
| Other causes                     | 18 (3.3%)      | 15(6.0%)         | 3 (1.1%)     |         |
| **Treatment, n (%)**             |                |                  |              |         |
| Anticoagulant therapy            | 114 (20%)      | 86 (30.2%)       | 28 (9.8%)    | < 0.001 |
| Antiplatelet therapy             | 414 (72.6%)    | 158 (55.4%)      | 256 (89.8%)  | < 0.001 |

Abbreviations: HT, hemorrhagic transformation; SBP, systolic blood pressure; DBP, diastolic blood pressure; NIHSS, National Institute of Health Stroke Scale; CAD, coronary artery disease; AF, atrial fibrillation; PLT, platelet counts; MPV, mean platelet volume; PT, prothrombin time; INR, International Normalized Ratio; FIB, fibrinogen.

3.2 Baseline characteristics of AIS patients according to PLT tertiles
Demographic and laboratory variables according to PLT tertiles were presented in Table 2. The incidence of HT was significantly higher in the first PLT tertile than the second and third MLR tertiles (63.7% versus 42.8% and 43.2%, respectively; P < 0.001). As shown in Table 2, patients with lower PLT levels were more likely to be drinker and CAD patients; had lower leukocyte counts and FIB levels, and had higher MPV, PT and INR levels; and were more likely to receive anticoagulant therapy, less likely to receive antiplatelet therapy.
### Table 2
Baseline characteristics of AIS patients according to PLT tertiles

| Variables                        | PLT tertiles |       |       |       | P-value |
|----------------------------------|--------------|-------|-------|-------|---------|
|                                 | Tertile1 (n = 193) | Tertile2 (n = 187) | Tertile3 (n = 190) |       |
| PLT (× 10^9/L)                   | < 175        | 175–222 | > 222 |       | < 0.001 |
| HT, n (%)                        | 123 (63.7%)  | 80 (42.8%) | 82 (43.2%) |       |         |
| Demographic characteristics      |              |       |       |       |         |
| Age (years)                      | 70.2 ± 11.6  | 68.9 ± 12.7 | 67.5 ± 12.8 | 0.970  |
| Male, n (%)                      | 147 (76.2%)  | 130 (69.5%) | 111 (58.4%) | 0.590  |
| Baseline SBP (mmHg)              | 150.0 ± 22.2 | 157.8 ± 21.8 | 152.3 ± 24.5 | < 0.001 |
| Baseline DBP (mmHg)              | 81.6 ± 14.0  | 83.2 ± 14.2 | 82.2 ± 13.2 | 0.732  |
| NIHSS on admission, median (IQR) | 5.0 (3.0–11.0) | 4.5 (2.0–9.0) | 5.0 (2.0–9.0) | 0.028  |
| Vascular risk factors, n (%)     |              |       |       |       |         |
| Current smoking                  | 64 (33.2%)   | 64 (34.2%) | 82 (43.2%) | 0.028  |
| Current drinking                 | 80 (41.5%)   | 74 (39.6%) | 74 (38.9%) | < 0.001 |
| Previous Stroke                  | 26 (13.5%)   | 20 (10.7%) | 26 (13.7%) | 0.207  |
| Hypertension                     | 115 (59.6%)  | 132 (70.6%) | 129 (67.9%) | 0.112  |
| Diabetes                         | 45 (23.3%)   | 56 (29.9%) | 48 (25.3%) | 0.294  |
| CAD                              | 23 (11.9%)   | 14 (7.5%)  | 10 (5.3%)  | 0.010  |
| Dyslipidemia                     | 12 (6.2%)    | 13 (7.0%)  | 12 (6.3%)  | 0.610  |
| AF                               | 63 (32.6%)   | 41 (21.9%) | 33 (17.4%) | < 0.001 |
| Hematological variables          |              |       |       |       |         |
| Leukocyte counts (× 10^9/L)      | 7.4 ± 2.8    | 7.8 ± 3.0  | 7.8 ± 2.6  | < 0.001 |
| Erythrocyte counts (× 10^9/L)    | 4.4 ± 0.6    | 4.5 ± 0.6  | 4.4 ± 0.5  | 0.836  |
| MPV ()                           | 11.3 ± 1.5   | 10.9 ± 1.4 | 10.6 ± 1.2 | 0.007  |
| PT (s)                           | 14.0 ± 1.1   | 13.6 ± 1.0 | 13.5 ± 1.0 | < 0.001 |
| INR                              | 1.1 ± 0.1    | 1.0 ± 0.1  | 1.0 ± 0.1  | < 0.001 |
| FIB (g/L)                        | 3.7 ± 1.2    | 3.7 ± 1.1  | 4.0 ± 2.3  | < 0.001 |
| Stroke etiology, n (%)           |              |       |       |       |         |
| Atherosclerosis                  | 126 (68.4%)  | 137 (73.3%) | 142 (74.7%) | < 0.001 |
| Cardioembolism                   | 51 (26.4%)   | 29 (15.5%) | 29 (15.3%) |         |
| Small vessel occlusion           | 2 (1.0%)     | 1 (0.5%)  | 1 (0.5%)  |         |
| Other causes                     | 6 (3.1%)     | 8 (4.2%)  | 4 (2.1%)  |         |
| Treatment, n (%)                 |              |       |       |       |         |
| Anticoagulant therapy            | 61 (31.6%)   | 26 (13.9%) | 27 (14.2%) | < 0.001 |
| Antiplatelet therapy             | 122 (63.2%)  | 150 (80.2%) | 142 (74.7%) | < 0.001 |
### 3.3 Characteristics of patients with HT in subcategorized groups of AF

The incidence of HT was higher in the AF subgroup than non-AF (78.8% vs. 40.9%, P < 0.001). In the subgroup of non-AF patients, the significant parameters between HT and non-HT (Supplemental Table) were generally consistent with parameters in Table 1. But in AF patients, patients with HT had higher levels of leukocyte counts, lower SBP; higher NIHSS scores at admission; and more smoker and drinker. In AF patients, there were no significant differences in coagulation parameters among HT and non-HT patients.

### 3.4 Association between coagulation parameters and HT

The occurrence of HT was used as a dependent variable and the third PLT tertile was used as a reference in the multivariate regression in all patients. After adjusting for confounding and risk factors, multivariate regression analysis showed that the first PLT tertile (OR = 3.517, 95%CI = 1.526–8.106, P = 0.003; Table 3) was independently associated with HT in all patients. Meanwhile, MPV (OR = 0.698, 95%CI = 0.557–0.875, P = 0.002; Table 3) and FIB (OR = 1.613, 95%CI = 1.199–2.169, P = 0.002; Table 3) were also significantly associated with HT in all patients.

| Variables | PLT tertiles |   |   |
|-----------|--------------|---|---|
|           | Tertile1 (n = 193) | Tertile2 (n = 187) | Tertile3 (n = 190) | P-value |

### Table 3
Multivariate logistic regression analysis of predictive factors for HT in all patients

|   | Model 1 | Model 2 | Model 3 |
|---|---------|---------|---------|
|   | adjust OR(95%CI) | P-value | adjust OR(95%CI) | P-value | adjust OR(95%CI) | P-value |
| PLT T1 | 2.002 (1.160–3.454) | 0.013 | 3.887 (1.762–8.575) | 0.001 | 3.517 (1.526–8.106) | 0.003 |
| T2 | 1.055 (0.617–1.803) | 0.845 | 1.425 (0.710–2.861) | 0.319 | 1.525 (0.738–3.149) | 0.254 |
| T3 | Ref | Ref | Ref | |
| MPV | - | - | 0.714 (0.573–0.890) | 0.003 | 0.698 (0.557–0.875) | 0.002 |
| PT | - | - | 3.624 (1.015–12.939) | 0.047 | 3.599 (0.941–13.767) | 0.061 |
| INR | - | - | 0.904 (0.796–1.027) | 0.121 | 0.904 (0.791–1.035) | 0.143 |
| FIB | - | - | 1.570 (1.191–2.069) | 0.001 | 1.613 (1.199–2.169) | 0.002 |

**Notes:** Model 1: adjusted sex, age, smoking, drinking, CAD, AF, baseline SBP, NIHSS on admission; Model 2: adjusted for covariates from Model 1 and further adjusted for MPV, PT, INR, FIB and leukocyte counts; Model 3: adjusted for covariates from Model 2 and further adjusted for anticoagulant therapy and antiplatelet therapy.

**Abbreviations:** CI, confidence interval; OR, odds ratio; HT, hemorrhagic transformation; PLT, platelet counts; MPV, mean platelet volume; PT, prothrombin time; INR, International Normalized Ratio; FIB, fibrinogen.

### Notes
Considering the potential relationship between AF and coagulation function, we divided all patients into AF and non-AF subgroups. In non-AF subgroup, we found the first PLT tertile (OR = 3.509, 95%CI = 1.268–9.711, P = 0.016; Table 4), MPV (OR = 0.605, 95%CI = 0.455–0.805, P = 0.001; Table 4) and FIB (OR = 1.928, 95%CI = 1.346–2.760, P < 0.001; Table 4) were significantly associated with HT after adjusting for confounding factors. In the AF subgroup, coagulation parameters showed no significant association with HT.
### Table 4

Multivariate logistic regression analysis of predictive factors for HT after subcategorized by AF

|                | Non-AF | AF |
|----------------|--------|----|
|                | Mode 1 | Mode 2 | Mode 3 | Mode 1 | Mode 2 | Mode 3 |
|                | adjust OR (95%CI) | P-value | adjust OR (95%CI) | P-value | adjust OR (95%CI) | P-value | adjust OR (95%CI) | P-value | adjust OR (95%CI) | P-value |
| PLT            |        |        |        |        |        |        |        |        |        |        |
| T1             | 2.236  | (1.220–4.095) | 0.009 | 4.908  | (1.930–12.481) | 0.001 | 3.509  | (1.268–9.711) | 0.016 | 1.894  | (0.467–7.685) | 0.371 | 3.084  | (0.448–21.206) | 0.252 | 4.822  | (0.561–41.468) | 0.001 |
| T2             | 0.920  | (0.508–1.666) | 0.784 | 1.383  | (0.626–3.058) | 0.423 | 1.344  | (0.575–3.143) | 0.496 | 1.985  | (0.440–8.947) | 0.372 | 1.885  | (0.289–12.320) | 0.508 | 1.873  | (0.262–13.378) | 0.532 |
| T3             | Ref    | Ref    | Ref    | Ref    | Ref    | Ref    | Ref    | Ref    | Ref    | Ref    |
| MPV            | -      | -      | 0.620  | (0.479–0.804) | <0.001 | 0.605  | (0.455–0.805) | 0.001 | -      | -      | 1.118  | (0.635–1.968) | 0.700 | 1.134  | (0.648–1.985) | 0.659 |
| PT             | -      | -      | 3.774  | (0.886–16.074) | 0.072 | 3.500  | (0.721–16.997) | 0.120 | -      | -      | 2.865  | (0.093–88.653) | 0.548 | 3.861  | (0.102–146.521) | 0.466 |
| INR            | -      | -      | 0.901  | (0.778–1.042) | 0.159 | 0.908  | (0.774–1.065) | 0.237 | -      | -      | 0.927  | (0.660–1.304) | 0.665 | 0.902  | (0.627–1.298) | 0.580 |
| FIB            | -      | -      | 1.838  | (1.324–2.551) | <0.001 | 1.928  | (1.346–2.760) | <0.001 | -      | -      | 1.023  | (0.726–1.441) | 0.897 | 1.061  | (0.735–1.533) | 0.752 |

**Notes:** Model 1: adjusted sex, age, smoking, drinking, CAD, AF, baseline SBP, NIHSS on admission; Model 2: adjusted for covariates from Model 1 and further adjusted for MPV, PT, INR, FIB and leukocyte counts; Model 3: adjusted for covariates from Model 2 and further adjusted for anticoagulant therapy and antiplatelet therapy.

**Abbreviations:** CI, confidence interval; OR, odds ratio; HT, hemorrhagic transformation; AF, atrial fibrillation; PLT, platelet counts; MPV, mean platelet volume; PT, prothrombin time; INR, International Normalized Ratio; FIB, fibrinogen.

### 3.5 Results of long-term functional outcomes after HT

Of 285 HT patients, 143 (50.2%) were available for follow-up in December 2019 through phone calls and the median follow-up time was 4.75 years (IQR 2.82–6.14). Among 143 follow-up patients, 85 were non-AF patients. In the long term, 58 (68.2%) of the non-AF HT patients had unfavorable functional outcomes (mRS ≥ 2). After adjusting for confounding factors, Cox proportional hazards regression analysis showed that MPV (OR = 1.314, 95%CI = 1.032–1.675, P = 0.027; Table 5) and FIB (OR = 1.298, 95%CI = 1.047–1.610, P = 0.018; Table 5) was independently and significantly associated with long-term outcomes in non-AF HT patients.
Table 5

|                  | HR   | 95% CI          | P-value |
|------------------|------|-----------------|---------|
| Sex              | 0.618 | 0.304–1.258     | 0.184   |
| Age              | 1.020 | 0.985–1.057     | 0.256   |
| NIHSS on admission | 1.065 | 0.989–1.146     | 0.094   |
| Hypertension     | 0.810 | 0.410–1.602     | 0.545   |
| Diabetes         | 2.706 | 1.170–6.254     | 0.020   |
| CAD              | 0.977 | 0.421–2.264     | 0.956   |
| PLT T1           | 0.674 | 0.317–1.433     | 0.306   |
| T2               | 1.399 | 0.632–3.098     | 0.408   |
| T3               | Ref   |                 |         |
| MPV              | 1.314 | 1.032–1.675     | 0.027   |
| FIB              | 1.298 | 1.047–1.610     | 0.018   |
| Anticoagulant therapy | 0.875 | 0.427–1.794 | 0.716   |
| Antiplatelet therapy | 0.838 | 0.429–1.636 | 0.604   |

**NOTE.** CI, confidence interval; HR, hazard ratio; AF, atrial fibrillation; HT, hemorrhagic transformation; NIHSS, National Institute of Health Stroke Scale; CAD, coronary artery disease; PLT, platelet counts; MPV, mean platelet volume; FIB, fibrinogen.

4. Discussion

To the best of our knowledge, this is the first study to explore the association of coagulation parameters and HT in non-AF patients; and to explore the effect of coagulation parameters on long-term outcomes after HT in non-AF patients. Our present study showed that three coagulation parameters including low PLT, low MPV, and high FIB were significant and dependent risk factors with HT in non-AF patients, instead of AF patients. Furthermore, we found that MPV and FIB levels were independently and significantly associated with unfavorable long-term functional outcomes in non-AF HT patients.

Consistent with many studies, we identified that AF was a reliable risk factor of HT, and the incidence of HT among AF patients was higher than non-AF patients [7, 8, 22, 23]. Considering the complex interaction between AF and HT [24], we did a subgroup analysis of our patients to explore the association between coagulation functions and HT. Interestingly, we found none of the coagulation parameters was associated with HT among AF patients. This may be that most AF patients received anticoagulant therapy before AIS which may influence coagulation parameters.

However, our study suggested that PLT, MPV, and FIB were associated with HT significantly in the non-AF patients. PLT is a key factor in the coagulation process. According to clinical guidelines from the American Heart Association/American Stroke Association published in 2018, low PLT levels (<100,000 counts/µl) would increase the risk of HT [25] and do not recommend these patients to receive reperfusion therapies. Several studies also pointed out that PLT was associated with the occurrence of HT [8, 26]. Furthermore, a previous study identified that lower coated-platelets counts increased the likelihood of early HT in patients with non-lacunar ischemic stroke [27]. Platelet-endothelial interactions could maintain the structural integrity of blood vessels when stroke occurs [28]. The patients with low PLT levels are weak in maintaining the blood vessels’ integrity and are more likely to develop HT after AIS. Salas-Perdomo et al. found in the experiment that using an anti-platelet serum in mice would lead to larger intraparenchymal hematomas after stroke [29], thus stressed the vital hemostatic function of platelets in AIS. This may because that platelets can physically cover the damaged vascular endothelium or release protective factors to protect the endothelial barrier function. These all indicated the importance of PLT in HT.
MPV describes the sizes of PLT, which is a marker of PLT activity and influences bleeding [30, 31]. In our study, MPV among HT patients was lower than non-HT. Besides, logistic regression analysis showed low MPV was a risk factor of HT. Early study found that larger PLT had more granules and would produce more vascular activity as well as pre-clotting factors, which may raise the hemostatic efficiency [31, 32]. A retrospective study suggested that baseline MPV was associated with unfavorable stroke outcomes but its relationship with HT was still uncertain [33].

Previous studies briefly mentioned the relationship between FIB and HT [26, 34, 35]. And we found that higher FIB may be associated with a higher incidence of HT among AIS patients, which is congruent with some reports [34, 35]. However, Wang et al. found that FIB < 1.50 g/L was a risk factor for HT [26]. We hypothesize that high levels of FIB may be associated with HT by participating in the inflammatory process. When HT occurs, endothelial dysfunction of capillaries would further lead to abnormal blood-brain barrier permeability within the infarcted area [36]. The dysfunction of blood-brain barrier in the infarcted area was proved to be the main causation of HT. Meanwhile, a review proposed that FIB was a ligand of cell surface receptors and this could promote the intercellular adhesion between inflammatory cells and endothelial [37]. In another study, an anti-inflammatory thrombolytic drug called SMTP-7 could decrease the incidence of HT, which suggested the relationship between HT and inflammation [38]. Thus, the relationship between FIB and HT remains unknown and this needs to be further investigated.

In our study, we found that coagulation functions were associated with unfavorable long-term outcomes in non-AF HT patients. Its exact mechanism remains unknown; however, the mechanism by which coagulation function is related to unfavorable outcomes in intracerebral hemorrhage (ICH) is unclear. Unfavorable outcomes after hemorrhage were reported to be conducted by the inflammatory effects of intraparenchymal blood [39]. Recently, Krenzlin H et al. found that the activated cerebral thrombin system was related to poor outcome after ICH in mice. They suspected that the activated cerebral thrombin system may contribute to secondary brain damage [40]. Meanwhile, a previous study suggested that coagulation function may influence neurovascular injury and neuroprotection [41].

Our study has some limitations. First, this was a single-center and retrospective study, so it is necessary to conduct multi-center, prospective studies to establish causality and provide long-term prognostic information. Second, our study did not discuss the association between different subtypes of HT (hemorrhagic infarction or parenchymal hematoma) and coagulation functions. In the further study, we could explore the association between the severity of HT and coagulation functions. Third, owing to the infarction size were not documented, the associations between infarction size and HT was not described in detail.

5. Conclusions

In summary, we found that three coagulation parameters including low PLT, low MPV, and high FIB were risk factors among the non-AF patients, while these parameters were not associated with HT in AF patients. Meanwhile, MPV and FIB levels were independently and significantly associated with unfavorable long-term outcomes in non-AF HT patients. Our study demonstrated that the coagulation functions may be useful hematological markers for clinicians to recognize patients with high risk of HT at early stage and improve unfavorable long-term outcomes in non-AF patients.

List Of Abbreviations

HT: Hemorrhagic transformation; AIS: acute ischemic stroke; AF: atrial fibrillation; PLT: platelet counts; MPV : mean platelet volume; FIB: fibrinogen; CI: confidence interval; OR: odds ratio; CT: computerized tomography; MRI: magnetic resonance imaging; TIA: transient ischemic attack; DWI: diffusion-weighted imaging; CAD: coronary artery disease; NIHSS: National Institutes of Health Stroke Scale; PT: prothrombin time; INR: international normalized ratio; mRS: modified Rankin Scale; ICH: intracerebral hemorrhage.

Declarations

Ethical standard

This study was approved by the Institutional Review Board (IRB), the First Affiliated Hospital of Wenzhou Medical University, and was following the Declaration of Helsinki promulgated by the National Institute of Health. We didn’t have informed consent for it was a retrospective study and the patient profile was anonymous.
Data Sharing Statement

The data supporting this study are available from the corresponding author for reasonable request.

Competing interests

The authors declare no competing interests.

Funding

This work was funded by grants from the Projects of National Natural Science Foundation of China (No.81873799).

Authors' contributions

Hao-Ran Cheng: Conceptualization, Data Curation, Formal analysis and Writing original draft. Yun-Bin Chen: Data Curation and Formal analysis. Ya-Ying Zeng: Data Curation and Formal analysis. Yi-Ting Ruan: Data Curation and Formal analysis. Cheng-Xiang Yuan: Data Curation and Formal analysis. Qian-Qian Cheng: Data Curation and Formal analysis. Hui-Jun Chen: Data Curation and Formal analysis. Xiao-Qian Luan: Data Curation and Formal analysis. Gui-Qian Huang: Conceptualization, Project administration and Resources. Jin-Cai He: Funding acquisition, Resources and Writing-review & editing.

Acknowledgments

We thank the study participants and the clinical staffs for their support and contribution to this project.

References

1. Li J, Zhang P, Wu S, Wang Y, Zhou J, Yi X, Wang C: Stroke-related complications in large hemisphere infarction: incidence and influence on unfavorable outcome. Therapeutic advances in neurological disorders 2019, 12:1756286419873264.
2. Gumbinger C, Gruschka P, Bottinger M, Heerlein K, Barrows R, Hacke W, Ringleb P: Improved prediction of poor outcome after thrombolysis using conservative definitions of symptomatic hemorrhage. Stroke 2012, 43(1):240-242.
3. Guenego A, Lecler A, Raymond J, Sabben C, Khoury N, Premat K, Botta D, Boisseau W, Maier B, Ciccio G et al: Hemorrhagic transformation after stroke: inter- and intrarater agreement. 2019, 26(3):476-482.
4. Rao NM, Levine SR, Gombein JA, Saver JL: Defining clinically relevant cerebral hemorrhage after thrombolytic therapy for stroke: analysis of the National Institute of Neurological Disorders and Stroke tissue-type plasminogen activator trials. Stroke 2014, 45(9):2728-2733.
5. Kaesmacher J, Kaesmacher M, Maegerlein C, Zimmer C, Gersing AS, Wunderlich S, Friedrich B, Boeckh-Behrens T, Kleine JF: Hemorrhagic Transformations after Thrombectomy: Risk Factors and Clinical Relevance. Cerebrovascular diseases (Basel, Switzerland) 2017, 43(5-6):294-304.
6. Demirtas BS, Ocek L, Zorlu Y, Oztekin O: Factors Associated with Hemorrhagic Transformation in Infarctions Involving the Posterior Circulation System. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association 2019, 28(8):2193-2200.
7. Ge WQ, Chen J, Pan H, Chen F, Zhou CY: Analysis of Risk Factors Increased Hemorrhagic Transformation after Acute Ischemic Stroke. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association 2018, 27(12):3587-3590.
8. Jiao Y, Li G, Xing Y, Nie D, Liu X: Influencing factors of hemorrhagic transformation in non-thrombolysis patients with cerebral infarction. Clinical neurology and neurosurgery 2019, 181:68-72.
9. Nakano Y, Kondo T, Osanai H, Murase Y, Nakashima Y, Asano H, Ajioka M, Sakai K, Inden Y, Murohara T: Clinical usefulness of measuring prothrombin time and soluble fibrin levels in Japanese patients with atrial fibrillation receiving rivaroxaban. Journal of cardiology 2015, 65(3):185-190.
10. Nguyen TN, Morel-Kopp MC, Pepperell D, Cumming RG, Hilmer SN, Ward CM: The impact of frailty on coagulation and responses to warfarin in acute older hospitalised patients with atrial fibrillation: a pilot study. Aging clinical and experimental research 2017, 29(6):1129-1138.
11. Tu HT, Campbell BC, Christensen S, Desmond PM, De Silva DA, Parsons MW, Churilov L, Lansberg MG, Mlynash M, Olivot JM et al: Worse stroke outcome in atrial fibrillation is explained by more severe hypoperfusion, infarct growth, and hemorrhagic transformation. *International journal of stroke : official journal of the International Stroke Society* 2015, 10(4):534-540.

12. Pfeilschifter W, Spitzer D, Pfeilschifter J, Steinmetz H, Foerch C: Warfarin anticoagulation exacerbates the risk of hemorrhagic transformation after rt-PA treatment in experimental stroke: therapeutic potential of PCC. *PloS one* 2011, 6(10):e26087.

13. Altavilla R, Caso V, Bandini F, Agnelli G, Tsivgoulis G, Yaghi S, Furie KL, Tadi P, Becattini C, Zedde M et al: Anticoagulation After Stroke in Patients With Atrial Fibrillation. *Stroke* 2019, 50(8):2093-2100.

14. Nam KW, Kim TJ, Lee JS, Kwon HM, Lee YS, Ko SB, Yoon BW: High Neutrophil-to-Lymphocyte Ratio Predicts Stroke-Associated Pneumonia. *Stroke* 2018, 49(8):1886-1892.

15. Lang C, Seyfang L, Ferrari J, Gatteringer T, Greisenegger S, Willett K, Toell T, Krebs S, Brainin M, Kiechl S et al: Do Women With Atrial Fibrillation Experience More Severe Strokes? Results From the Austrian Stroke Unit Registry. *Stroke* 2017, 48(3):778-780.

16. Christersson C, Wallentin L, Andersson U, Alexander JH, Alings M, De Caterina R, Gersh BJ, Granger CB, Halvorsen S, Hanna M et al: Effect of apixaban compared with warfarin on coagulation markers in atrial fibrillation. *Heart (British Cardiac Society)* 2019, 105(3):235-242.

17. Testa S, Paoletti O, Legnani C, Dellanoce C, Antonucci E, Cosmi B, Pengo V, Poli D, Morandini R, Testa R et al: Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. *Journal of thrombosis and haemostasis : JTH* 2018, 16(5):842-848.

18. Ornello R, Degan D, Tiseo C, Di Carmine C, Perciballi L, Pistoia F, Carolei A, Sacco S: Distribution and Temporal Trends From 1993 to 2015 of Ischemic Stroke Types: A Systematic Review and Meta-Analysis. *Stroke* 2018, 49(4):814-819.

19. Uyttenboogaart M, Stewart RE, Vroomen PC, De Keyser J, Luijckx GJ: Optimizing cutoff scores for the Barthel index and the modified Rankin scale for defining outcome in acute stroke trials. *Stroke* 2005, 36(9):1984-1987.

20. Zhang N, Wang CX, Wang AX, Bai Y, Zhou Y, Wang YL, Zhang T, Zhou J, Yu X, Sun XY et al: Time course of depression and one-year prognosis of patients with stroke in mainland China. *CNS neuroscience & therapeutics* 2012, 18(6):475-481.

21. Shi YZ, Xiang YT, Yang Y, Zhang N, Wang S, Ungvari GS, Chiu HF, Tang WK, Wang YL, Zhao XQ et al: Depression after minor stroke: the association with disability and quality of life—a 1-year follow-up study. *International journal of geriatric psychiatry* 2016, 31(4):421-427.

22. Liu MS, Liao Y, Li GQ: Glomerular Filtration Rate is Associated with Hemorrhagic Transformation in Acute Ischemic Stroke Patients without Thrombolytic Therapy. *Chinese medical journal* 2018, 131(14):1639-1644.

23. Tan S, Wang D, Liu M, Zhang S, Wu B, Liu B: Frequency and predictors of spontaneous hemorrhagic transformation in ischemic stroke and its association with prognosis. *Journal of neurology* 2014, 261(5):905-912.

24. Vilanilam GK, Badi MK, Yarlagadda B, Okromelidze L: Hemorrhagic Transformation After Acute Ischemic Stroke in Atrial Fibrillation Patients. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association* 2019, 28(1):234.

25. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B et al: 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke* 2018, 49(3):e46-e110.

26. Wang R, Zeng J, Wang F, Zhuang X, Chen X, Miao J: Risk factors of hemorrhagic transformation after intravenous thrombolysis with rt-PA in acute cerebral infarction. *QJM : monthly journal of the Association of Physicians* 2019, 112(5):323-326.

27. Prodan CI, Stoner JA, Cowan LD, Dale GL: Lower coated-platelet levels are associated with early hemorrhagic transformation in patients with non-lacunar brain infarction. *Journal of thrombosis and haemostasis : JTH* 2010, 8(6):1185-1190.

28. Ho-Tin-Noé B, Demers M, Wagner D: How platelets safeguard vascular integrity. *Journal of thrombosis and haemostasis : JTH* 2011:56-65.

29. Salas-Perdomo A, Miro-Mur F, Gallizioli M: Role of the S1P pathway and inhibition by fingolimod in preventing hemorrhagic transformation after stroke. 2019, 9(1):8309.

30. Martin JF, Trowbridge EA, Salmon G, Plumb J: The biological significance of platelet volume: its relationship to bleeding time, platelet thromboxane B2 production and megakaryocyte nuclear DNA concentration. *Thrombosis research* 1983, 32(5):443-460.
31. Vizioli L, Muscari S, Muscari A: The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. *International journal of clinical practice* 2009, 63(10):1509-1515.

32. Bath PM, Butterworth RJ: Platelet size: measurement, physiology and vascular disease. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis* 1996, 7(2):157-161.

33. Staszewski J, Pogoda A, Data K, Walczak K, Nowocien M, Frankowska E, Stepien A: The mean platelet volume on admission predicts unfavorable stroke outcomes in patients treated with IV thrombolysis. *Clinical interventions in aging* 2019, 14:493-503.

34. Xu X, Li C, Wan T, Gu X, Zhu W, Hao J, Bao H, Zuo L, Hu H, Li G: Risk Factors for Hemorrhagic Transformation After Intravenous Thrombolysis in Acute Cerebral Infarction: A Retrospective Single-Center Study. *World neurosurgery* 2017, 101:155-160.

35. Huang GQ, Zeng YY, Cheng QQ, Cheng HR, Ruan YT, Yuan CX, Chen YB, He WL, Chen HJ, He JC: Low triiodothyronine syndrome is associated with hemorrhagic transformation in patients with acute ischaemic stroke. *Aging* 2019, 11(16):6385-6397.

36. Alvarez-Sabin J, Maisterra O, Santamarina E, Kase CS: Factors influencing haemorrhagic transformation in ischaemic stroke. *The Lancet Neurology* 2013, 12(7):689-705.

37. Luyendyk JP, Schoenecker JG, Flick MJ: The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood* 2019, 133(6):511-520.

38. Ito A, Niizuma K, Shimizu H, Fujimura M, Hasumi K, Tominaga T: SMTP-7, a new thrombolytic agent, decreases hemorrhagic transformation after transient middle cerebral artery occlusion under warfarin anticoagulation in mice. *Brain research* 2014, 1578:38-48.

39. Babu R, Bagley JH, Di C, Friedman AH, Adamson C: Thrombin and hemin as central factors in the mechanisms of intracerebral hemorrhage-induced secondary brain injury and as potential targets for intervention. *Neurosurgical focus* 2012, 32(4):E8.

40. H K, E G, D J, N R, L T, CF V, F R, O K, B A: The Cerebral Thrombin System Is Activated after Intracerebral Hemorrhage and Contributes to Secondary Lesion Growth and Poor Neurological Outcome in C57Bl/6 Mice. *Journal of neurotrauma* 2020.

41. B C, B F, MA W, JA W, IF L, ES O, Q C, B P, L Z, RY T et al: Thrombin activity associated with neuronal damage during acute focal ischemia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2012, 32(22):7622-7631.