Clin. Lab. 2025;71:XXX-XXX

ORIGINAL ARTICLE

Thrombelastography and Serum Homer1 to Assess Hemorrhagic Transformation After Thrombolysis in Acute Ischemic Stroke

ZhangRong Liang [1], WeiWei Liang [1], Miao Zhou [1], ShangMing Huang [1], JunNa Lei [1], YaLi Liu [2], KuangYi Li [1], YingJian Zhang [1]

[1] *Department of Emergency, Foshan Hospital of Traditional Chinese Medicine, Foshan City, Guangdong Province, China*
[2] *Department of Neurology, Foshan Hospital of Traditional Chinese Medicine, Foshan City, Guangdong Province, China*

SUMMARY

Background: **This study aimed to investigate the correlation between early thrombelastography (TEG) and serum Homer1 with hemorrhagic transformation (HT) after intravenous thrombolysis (IVH) in acute ischemic stroke (AIS).**

Methods: **This prospective cohort study was conducted from January 2021 to December 2023. TEG parameters and serum Homer1 levels were measured after IVH treatment. Baseline clinical factors were constructed using multifactor logistic regression analysis (Model 1). Subsequently, TEG parameters and serum Homer1 were incorporated into Model 1 to construct Models 2 and 3, respectively, for predicting HT after AIS. The predictive value of the three models was evaluated by using ROC curves.**

Results: **A total of 221 patients with AIS (40 cases with HT and 181 cases without HT) and 40 controls were included in this study. Reaction time of blood coagulation (R) was significantly higher in the HT group (6.65 vs. 5.50) than in the non-HT group ($p < 0.001$). Maximal amplitude (MA) was significantly lower in the HT group (61.28 vs. 64.94) than in the non-HT group ($p < 0.001$). Serum levels were significantly higher in AIS patients (20.73 vs. 38.43) than in controls ($p < 0.001$). Serum Homer1 levels were higher in patients in the HT group (54.35 vs. 37.43) than in non-HT patients ($p < 0.001$). Baseline NIHSS, prolonged coagulation reaction time R, and increased serum Homer1 levels were risk factors for post-thrombolytic HT in patients with AIS, whereas elevated Hgb was a protective factor. Both the construction of a model to predict the risk of post-thrombolytic HT in patients with AIS using clinical factors and the combination of clinical factors with TEG parameters (or serum Homer1) had similar predictive value ($p < 0.05$).**

Conclusions: **Measurement of TEG parameters and Homer1 levels in patients with AIS early after IVH may be a potentially useful, relatively rapid, and minimally invasive method for predicting the risk of HT in patients with AIS.**

(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.240740)

Correspondence:
ZhangRong Liang
Department of Emergency
Foshan Hospital of Traditional Chinese Medicine
No. 6, Qinren Road, Chancheng District
Foshan City
Guangdong Province, 528000
China
Email: LZR_zhangrong@hotmail.com

Manuscript accepted August 5, 2024

KEYWORDS

thrombelastography, Homer1, acute ischemic stroke, intravenous thrombolysis, hemorrhagic transformation

INTRODUCTION

Acute ischemic stroke (AIS) is a group of disorders in which ischemic necrosis of brain tissues due to insufficient cerebral blood flow leads to neurological dysfunction [1]. The optimal treatment of AIS is early revascu-

larization by thrombolysis to restore cerebral blood flow and to protect cerebral tissues and functions [2]. Hemorrhagic transformation (HT) is a common complication after intravenous thrombolysis (IVH), which is caused by the restoration of blood perfusion in the ischemic area, leading to deterioration of neurological function and increased morbidity and mortality [3]. The ECASS-3 trial confirmed that recombinant tissue plasminogen activator was used for the acute treatment of cerebral infarction within 4.5 hours of onset of the disease [4]. Its prevalence has been reported to be as high as 27 - 37% [5]. HT is associated with a poor prognosis, especially symptomatic HT, which has a mortality rate close to 50% and significant survival morbidity [6]. Therefore, it is necessary to predict the risk of HT based on relevant indicators to prognosticate the patient's condition in advance.

The coagulation status of patients is closely related to the occurrence of HT after thrombolysis [7], and the routine coagulation indices currently used in clinical practice include prothrombin time (PT), activated partial thromboplastin time, thrombin time, fibrinogen (FIB), and international normalized ratio (INR) [8]. These indices do not provide a comprehensive and accurate reflection of the overall bleeding and coagulation condition, so there is an urgent need for a tool that can comprehensively and accurately assess the coagulation status of patients with AIS.

Thrombelastography (TEG) can dynamically monitor the entire process starting from platelet-fibrin interactions, including platelet aggregation, fibrin cross-linking, and clot dissolution [9]. Compared with conventional coagulation function testing, TEG testing monitors the dynamic changes of blood coagulation process and reflects the interaction between coagulation factors and platelets. It acts in real-time and is efficient, and it is not affected by heparin analogs [10]. However, most studies have focused on TEG and cardiovascular diseases, especially on the analysis of the correlation between the characteristics of TEG parameters and the occurrence of adverse events in patients after percutaneous coronary intervention [11]. Stroke patients are often associated with coagulation disorders, and it has been previously shown that thrombus intensity measured by TEG correlates with platelet reactivity in stroke patients [12]. In addition, the parameters measured by TEG, such as maximal amplitude (MA), are informative in the assessment of acute cerebral hemorrhage volume [13]. However, studies on the role of TEG in predicting HT after IVH in AIS do not seem to be reported.

Homer1 is an important component of the postsynaptic density [14]. Homer1 is expressed at the highest level in brain tissue, while at relatively low levels in other peripheral tissues, such as the heart and skeletal muscle [15]. Homer1 is associated with oxidative stress injury and inflammatory responses. For example, Homer1 inhibits phenotypic transformation of astrocytes, exerts anti-inflammatory effects, and attenuates cerebral hemorrhage [16]. Homer1 can ameliorate ischemic stroke-induced brain injury in mice by modulating neuronal damage and neuroinflammation [17]. Elevated Homer1 expression has been reported to be associated with the risk of AIS in patients with large artery atherosclerosis and is considered a new biomarker for diagnosing stroke [18]. Serum Homer1 concentration has a high predictive value for neurobehavioral outcome after AIS [19]. Several studies have shown a significant correlation between oxidative stress and post-thrombolytic HT [20,21].

Based on the relationship of HT with coagulation function, inflammation, and oxidative stress, we hypothesized that Homer1 can be released into the cerebrovascular fluid after an episode of AIS, that Homer1 is released to a lesser extent in peripheral serum due to the damage of the blood-brain barrier as a result of oxidative stress, and that abnormalities in Homer1 levels and coagulation function may be associated with the risk of developing HT after IVH in patients. In this study, we aimed to investigate the relationship between Homer1 levels in peripheral serum, TEG parameters, and the occurrence of HT after IVH in patients.

MATERIALS AND METHODS

Patients

This was a prospective cross-sectional cohort study. Patients with AIS admitted to Foshan Hospital of Traditional Chinese Medicine from January 2021 to December 2023 were examined. Patients who used alteplase for IVH within 4.5 hours after stroke onset were recruited. Inclusion criteria: 1) patients met the criteria of the Chinese guidelines for diagnosis and treatment of acute ischemic stroke, and the diagnosis of AIS was confirmed by magnetic resonance imaging or computed tomography; 2) patients eligible for thrombolytic therapy, with an onset time of 4.5 hours or less; 3) patients with a history of major head trauma or stroke, with no history of previous cerebral hemorrhage; and 4) patients with NIHSS scores of 4 or more. Exclusion criteria: 1) patients with intracranial hemorrhage, subarachnoid hemorrhage, aneurysm, or intracranial tumor on admission CT; 2) patients with comorbid hematologic disorders, severe hepatic and renal dysfunction, and malignant tumors; 3) patients with hepatitis, cirrhosis, hepatocellular carcinoma, biliary obstruction, renal insufficiency, and hemolysis; 4) patients who had received anticoagulant therapy within 48 hours; and 5) patients with low platelet count ($< 100 \times 10^9$/L). Forty age- (62.5 ± 4.2 years) and gender-matched (23 males and 17 females) subjects were included as controls during the same period. The study was approved by the Foshan Hospital of Traditional Chinese Medicine Ethics Committee and all patients or family members gave informed consent.

General data collection

Basic data of patients were obtained including gender, age, height, weight, risk factors related to cerebrovascular disease (including hypertension, diabetes, dyslipidemia, acute coronary syndrome, atrial fibrillation, smoking previous stroke), and whether antithrombotic or antiplatelet therapy was being administered before AIS. Clinical data included onset-to-thrombolysis time (OTT), TOAST classification, systolic blood pressure (SBP), diastolic blood pressure (DBP), NIHSS score on admission, and NIHSS score 36 hours after IVH. The NIHSS score was performed by highly trained physicians who had no knowledge of the subsequent laboratory measurements and data analysis process.

Laboratory indices

Coagulation and hematology-related indices, including platelet count (PLT), prothrombin time (PT), plasma FIB, and D-dimer (D-D), were determined using a coagulation analyzer (C3100, PRECIL, Beijing, China) and a fully automated biochemistry (Olympus). PT was normalized by standard plasma and expressed as INR. Glycosylated hemoglobin (HbA1c) was measured using a high-pressure liquid chromatography on an automated analyzer (Tosoh, Tokyo, Japan). Hemoglobin (Hgb) values were measured on a hematology analyzer (CoulterAct, USA).

Treatment regimen and endpoint indicators

For the enrolled patients, intravenous alteplase thrombolytic therapy was given to those with symptom duration < 4.5 hours. The total amount of thrombolytic drug administered was calculated according to 0.9 mg/kg [22], and the maximum dose was not more than 90 mg, of which 10% of the total amount was injected intravenously within 1 minute and the remaining 90% was added to physiological saline and injected intravenously at a uniform rate within 60 min. During the process of IVH, blood pressure changes were closely monitored. Thrombolytic drugs should be stopped immediately when the SBP reaches > 180 mmHg or DBP reaches > 100 mmHg. Urapidil hydrochloride injection or sodium nitroprusside injection was intravenously pumped according to the blood pressure level. When the blood pressure was less than 180/100 mmHg and no longer rose, the infusion of antihypertensive drugs was stopped, and the infusion of thrombolytic drugs was continued. During the IVH period and within 24 hours after IVH, cardiac monitors were applied to closely monitor patients' vital signs in the intensive care unit, and neurological examinations were regularly performed on the patients, during which antiplatelet aggregation, anticoagulation, and other medications were not given. Cranial CT was performed within 36 hours of drug administration to clarify the presence of HT, and the patients were divided into the HT group and the non-HT group.

Any type of intracranial hemorrhage detected by CT or MRI was followed up within 22 - 36 hours after IVH according to the criteria of the European Cooperative Acute Stroke Study [23].

Sample collection

Serum samples were collected within 4.5 hours of admission and 2 - 4 hours after IVH. Blood samples (10 mL) were withdrawn from the anterior elbow vein of each patient and collected into serum separator tubes, which were next allowed to stand vertically overnight at 4°C or room temperature for up to 60 minutes and then centrifuged at approximately 1,000 g for 20 minutes. The serum was then transferred to sterile polypropylene tubes and stored at -80°C.

TEG measurements

Elbow vein blood (6 mL) was drawn within 2 - 4 hours after IVH treatment and anticoagulated by sodium citrate solution (0.109 mol/L). TEG indexes were measured using American TEG®5000 thrombelastography (AEMONETICS), including coagulation reaction time (R), coagulation time (K), angle (reflecting the formation rate of blood clot and the function of FIB), maximum amplitude (MA), and coagulation index (CI) (overall blood coagulation status based on R, K, angle, and MA values), and estimated percent lysis (EPL) at 30 minutes after MA.

ELISA

The protein standard was subjected to gradient dilution, and then the absorbance of the standard at each dilution gradient was further measured at 450 nm, and a standard curve was established. The absorbance of each serum sample was further measured to obtain Homer1 concentration. These measurements were performed by independent laboratory technicians without them knowing the clinical results. Human Homer Homolog1 ELISA kit (Abbexa, UK) was used.

Data analysis

The sample size of the study was estimated by using G*Power software version 3.1.9.2 with a significance level of $\alpha = 0.05$, power of $1-\beta = 0.8$, effect size of $d = 0.5$, and two-sided tests. Statistical analyses were performed using SPSS 20.0 software. The Shapiro-Wilk test was used to determine the normality of the data. For the normal distribution, measurement data were shown as mean ± standard deviation, and Student's *t*-test was used for between-group comparisons; for the skewed distribution, continuous variables were shown as median (M1, M3), and Mann-Whitney U-test was used for between-group comparisons. Count data were expressed as frequencies (n) and ratios (%) and were tested by chi-squared test or Fisher's exact test. Spearman's test was used to analyze the correlation between serum Homer and TEG parameters. The predictive model was constructed using stepwise backward logistic regression to analyze the variables that were statistically significant ($p < 0.05$) in the univariate analysis, and the continuous variables were entered directly into the multifactor lo-

gistic regression with their original values to screen out the independent influences. The predictive value of the model was evaluated by receiver operating characteristic curve (ROC) and area under the curve (AUC). Optimized calibration curve and column line were plotted using the R language package 4.0.5. The Hosmer-Lemeshow test (HL) assessed model calibration performance, with p-value greater than 0.05 indicating no significant difference between the predicted and true values. Column line plots assessed the contribution of each of the independent variables of interest to the outcome event. Data were plotted using GraphPad Prism 8. $p < 0.05$ was considered statistically significant.

RESULTS

Clinical baseline

A total of 278 patients with AIS were treated with alteplase thrombolysis during the study period, out of which 57 were excluded: 1) no pre-thrombolysis TEG measurements were available (n = 46); 2) continuation of IVH was not possible due to severe allergy or arrhythmia (n = 2); and 3) secondary HT was present at 36 hours after IVH (n = 9). A total of 221 cases were finally included in the study, including 126 males and 95 females, with patients aged between 43 and 81 years. The median value of OTT was 196.3 minutes, out of which 41 (18.1%, 41/221) patients with AIS developed HT within 36 hours after IVH and 181 (81.90%, 181/221) did not develop HT. The information on clinical baseline are shown in Table 1. Among the cerebrovascular-related factors, diabetes mellitus had a higher rate in the HT group (43.90%, 18/41) than in the non-HT (22.10%, 40/181) group ($p = 0.012$), and atrial fibrillation had a higher rate in the HT group (19.51%, 8/41) than in the non-HT group (8.29%, 15/181), although not statistically significant ($p = 0.090$). Patients' NIHSS scores on admission and NIHSS scores after 36 hours of IVH were higher in the HT group ($p < 0.001$). In coagulation-related indices, PLT and INR were higher in the HT group than in the non-HT group, and plasma FIB was lower than that in the non-HT group (both $p < 0.05$). Due to the higher proportion of diabetic patients in the HT group, HbA1c (%) was significantly higher in this group than in the non-HT group. In addition, patients in the HT group had lower Hgb levels ($p = 0.005$).

TEG parameters in patients

As shown in Table 2, the TEG parameters of patients in the two groups were compared. The coagulation reaction time R in the HT group (6.65 [5.36, 7.81] vs. 5.50 [4.08, 6.51]) was significantly higher than that in the non-HT group ($p < 0.001$); MA in the HT group (61.28 ± 5.00 vs. 64.94 ± 4.91) was significantly lower than that in the non-HT group ($p < 0.001$). The coagulation time K, coagulation angle α, coagulation coefficient, and EPL were not significantly different (all $p > 0.05$).

Correlation of serum Homer1 levels with TEG parameters

As shown in Figure 1A, the serum levels of AIS patients (20.73 [14.71, 25.06] vs. 38.43 [32.19, 43.97]) were significantly higher than those of controls ($p < 0.001$). In addition, the serum Homer1 levels of patients in the HT group (54.35 [37.91, 62.66] vs. 37.43 [31.84, 42.63]) were significantly higher than those in the non-HT group ($p < 0.001$) (Figure 1B). Subsequently, Spearman's analysis showed a moderate-strength positive correlation between serum Homer1 level and coagulation reaction time R ($r = 0.402$, $p < 0.001$) (Figure 2A) and a weak negative correlation with MA ($r = -0.201$, $p = 0.003$) (Figure 2B).

Modeling of post-thrombolytic HT in AIS patients

Further, we included the variables with significant differences ($p < 0.05$) in the univariate analysis in the logistic regression for analysis. Before that, based on PLT, plasma FIB, and INR jointly characterizing coagulation function, we used variance inflation factor (VIF) to test the covariance of the three, and the results showed that VIF was greater than 10, indicating that there was a covariance relationship among the three indexes. Therefore, we excluded PLT and plasma FIB, which had greater covariance, from the regression. In addition, based on the co-interaction between diabetes mellitus and HbA1c, we chose HbA1c to enter the regression. The final clinical factors entered into the regression included NIHSS (on admission), INR, HbA1c, and Hgb. Predictive models 2 and 3 incorporated the TEG parameters (R and MA) and serum Homer1, respectively, on the basis of the clinical factors. In addition, age and gender were entered into the models as calibration variables. As shown in Table 3, among the clinical factors (Model 1), higher baseline NIHSS and HbA1c were independent risk factors for post-thrombolytic HT in patients with AIS, whereas elevated Hgb was a protective factor. After incorporating TEG parameters (R and MA) into the regression, baseline NIHSS and Hgb remained independent correlates, and prolonged R was also an independent risk factor for post-thrombolytic HT in patients with AIS. After incorporating serum Homer1 into the regression, only baseline NIHSS and elevated Homer1 levels were observed to be independent risk factors for post-thrombolytic HT in patients with AIS. Based on the fact that the models were all statistically significant (all $p < 0.05$), these models were plotted by ROC curves, as shown in Figure 3A, and all of these models had good predictive value. The accuracy of the model predictions was assessed by plotting calibration curves to evaluate the relationship between the probabilities, as shown in Figure 3B. The p-values of the HL tests for these models were all greater than 0.05, indicating a good model fit. Finally, column-line plots were utilized to show the contribution of each variable in the model, as shown in Figure 3C. All of these results indicate that either the clinical factors were used to construct a model for predicting the

Table 1. General clinical data of HT and non-HT patients after thrombolysis.

Characteristics	HT (n = 40)	Non-HT (n = 181)	p-value
Age (years)	65 [59 - 69]	63 [59 - 67]	0.097
Gender			0.439
Male	25 (60.98)	101 (55.80)	
Female	15 (36.59)	80 (44.20)	
BMI (kg/m^2)	22.9 ± 4.6	24.3 ± 4.1	0.562
OTT (min)	192.5 [119.3, 203.3]	198.5 [129.3, 210.0]	0.135
Risk factors for cerebrovascular disease			
Hypertension	10 (24.36)	25 (13.81)	0.079
Diabetes	18 (43.90)	40 (22.10)	0.012
Dyslipidemia	8 (19.51)	30 (16.57)	0.846
ACS	4 (9.76)	15 (8.29)	1
Atrial fibrillation	8 (19.51)	15 (8.29)	0.091
Smoking	7 (17.07)	33 (18.23)	0.671
Previous stroke	4 (9.76)	21 (11.60)	0.791
Ongoing antithrombotic therapy	4 (9.76)	19 (10.50)	1
TOAST classification			0.324
Cardioembolism	15 (36.59)	46 (25.41)	
Atherosclerosis	11 (26.83)	62 (34.25)	
Small vessel occlusion	4 (9.76)	12 (6.63)	
Undetermined etiology	10 (24.39)	61 (33.70)	
SBP (mmHg)	164 ± 18	158 ± 21	0.085
DBP (mmHg)	89.7 ± 8.3	89.4 ± 8.9	0.782
NIHSS (on admission)	10 [8, 13]	7 [6, 9]	< 0.001
NIHSS	3 [2 - 4]	2 [1 - 3]	0.001
PLT, 10^9/L	210.5 ± 63.3	221.5 ± 56.3	0.044
Plasma fibrinogen (g/L)	3.52 [3.12, 4.32]	3.62 [3.15, 4.62]	0.023
INR	1.03 [1.00, 1.06]	1.02 [0.98, 1.04]	0.048
HbA1c (%)	6.10 [5.64, 6.43]	5.61 [5.28, 5.94]	< 0.001
Hgb (g/L)	140.99 ± 19.89	152.11 ± 19.35	0.005
D-D (ng/mL)	210.3 [203.3 - 221.3]	207.4 [200.7 - 218.3]	0.152

ACS - Acute coronary syndrome, SBP - systolic blood pressure, DBP - diastolic blood pressure, NHISS - The National Institutes of Health Stroke Scale, Δ NIHSS = NIHSS score (36 hours after thrombolysis) - NIHSS on admission, PLT - Platelet count, INR - international normalized ratio, D-D - D-dimer. Continuous data are shown as median (interquartile range, IQR) or mean ± SD, and categorical data are expressed as n (%). Categorical values were compared by using the chi-squared test or Fishers exact test. Student's *t*-test or Mann-Whitney test was used to assess the differences between the two groups. p-value < 0.05 is statistically significant.

risk of HT after IVH in patients with AIS, or the clinical factors were combined with TEG parameters (or serum Homer1), which had similar predictive value.

DISCUSSION

HT is one of the most serious complications after IVH in patients with AIS, leading to severe disability and high mortality. There is a close correlation between coagulation and oxidative stress status before IVH and the risk of HT after IVH in patients with AIS. This study found that among the TEG parameters, patients in the HT group had higher coagulation reaction time R, lower MA, and higher serum Homer1 levels relative to the non-HT group. The model for predicting the risk of HT after IVH in AIS patients constructed by using the clinical characteristics, TEG parameters after IVH, and serum Homer1 had good predictive value. Prolonged R and increased serum Homer1 levels were risk factors for

Table 2. Patient TEG parameters.

TEG parameters	HT (n = 40)	Non-HT (n = 181)	p-value
R (min)	6.65 [5.36, 7.81]	5.50 [4.08, 6.51]	< 0.001
K (min)	1.83 ± 0.57	1.65 ± 0.54	0.057
α, angle (°)	65.08 ± 4.20	66.04 ± 5.41	0.291
MA (mm)	61.28 ± 5.00	64.94 ± 4.91	< 0.001
CI	1.15 [0.58, 1.5]	0.90 [0.40, 1.3]	0.107
EPL (%)	0.72 [0.51, 0.86]	0.73 [0.55, 0.94]	0.425

R - coagulation reaction time, K-value - coagulation time, angle - angle of coagulation, MA - maximal amplitude, CI - coagulation index, EPL - estimated percent lysis. Student's *t*-test or Mann-Whitney test was used to assess the difference between the two groups. p-value < 0.05 is statistically significant.

Table 3. Stepwise backward logistic regression analysis of risk factors for HT after thrombolysis.

	Model 1		Model 2		Model3	
Variables	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Intercept	0.00 (0.00 - 0.96)	0.049	0.04 (0.00 - 0.96)	0.04	0.00 (0.00 - 0.72)	0.04
NIHSS on admission	1.46 (1.20 - 1.77)	< 0.001	1.31 (1.07 - 1.61)	0.011	1.37 (1.11 - 1.70)	0.004
HbA1c	3.03 (0.99 - 9.49)	0.05				
Hgb	0.97 (0.94 - 0.99)	0.028	0.98 (0.95 - 0.99)	0.046	0.97 (0.95 - 1.00)	0.052
R			1.34 (1.04 - 1.78)	0.038		
MA						
Homer1					1.08 (1.01 - 1.16)	0.023

Model 1 - clinical factors prediction model, Model 2 - clinical factors with TEG parameters (R and MA) prediction model, Model 3 - clinical factors with Homer1 prediction model. p-value < 0.05 is statistically significant.

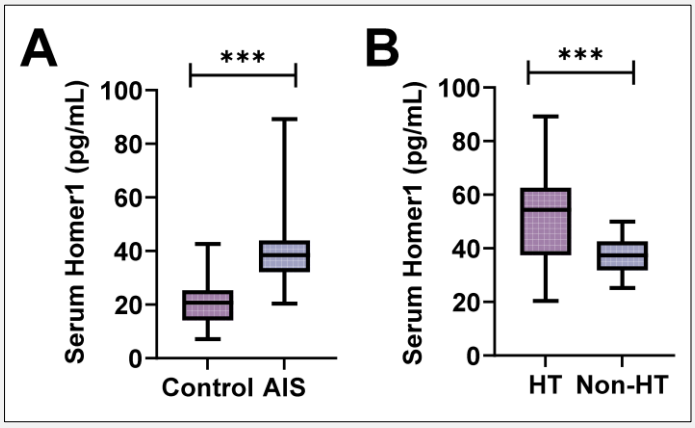

Figure 1. Serum Homer1 levels.

A) Controls versus patients with AIS, B) post-thrombolytic HT and non-HT AIS. *** p < 0.001.

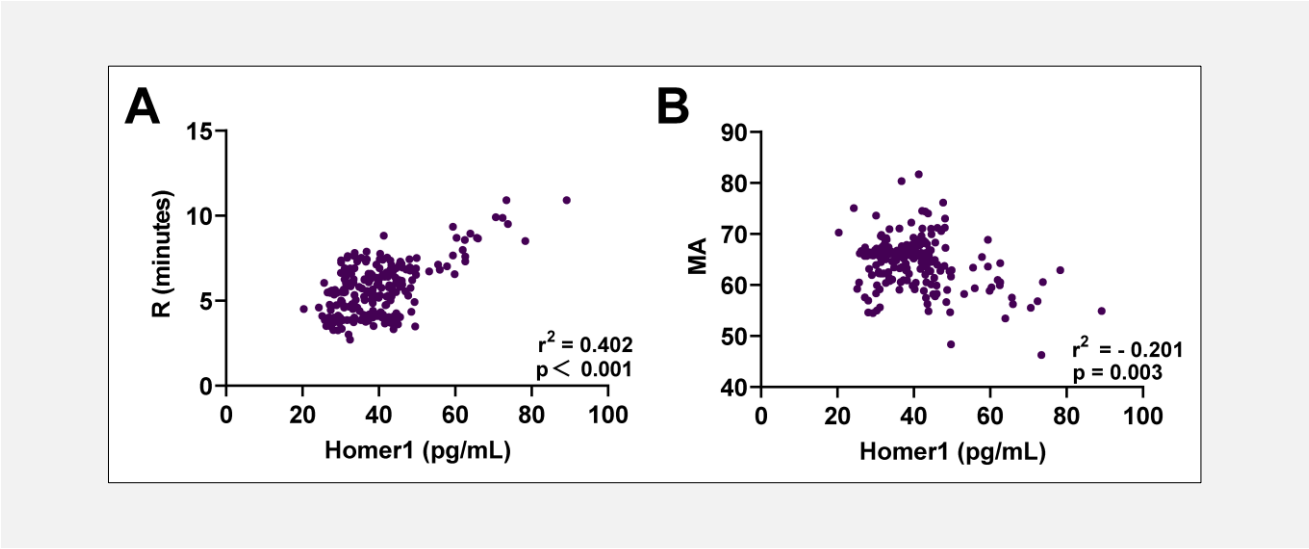

Figure 2. Correlation between serum Homer1 levels and TEG parameters.

A) Correlation of serum Homer1 with R, B) correlation of serum Homer1 with MA. $p < 0.05$.

HT after IVH in patients with AIS, while elevated Hgb was a protective factor.

IVH is an effective treatment in the acute phase of AIS. Clinical studies have shown that an increased risk of HT is a predictor of poor prognosis in AIS. Alteplase is an internationally available IVH drug that converts FIB into active fibrinolytic enzymes. China's guidelines for the diagnosis and treatment of AIS stipulate that alteplase should be used at the onset of the disease at 3 - 4.5 hours. Therefore, in this study, patients who could receive this treatment at 4.5 hours were selected, and there was no significant difference in the OTT between the HT group and the non-HT group. Clinically characterized, HT after IVH is influenced by many factors, including hypertension, atrial fibrillation, diabetes mellitus, advanced age, and severe stroke, which may increase the risk of post-thrombolytic hemorrhage. A previous study has confirmed that in patients with AIS, the proportions of atrial fibrillation, baseline diastolic blood pressure, and baseline NIHSS after IVH are higher among patients who develop HT [24]. Similar results were found in the present study, where we also found a higher proportion of patients with diabetes mellitus associated with cerebrovascular risk factors in HT (43.90%, 18/40), a higher proportion of atrial fibrillation (albeit non-significant), and higher NIHSS on admission. In addition, this study also showed that HbA1c was higher in the HT group relative to the non-HT group. Hyperglycemia or HbA1c on admission better predicts symptomatic HT in AIS after IVH [25,26]. In AIS, hyperglycemia exacerbates cerebrovascular basement membrane damage and disrupts vascular integrity, thereby increasing the risk of post-reperfusion hemorrhage; moreover, hyperglycemia promotes the synthesis of FIB activator inhibitors and antagonizes the fibrinolytic activity of thrombolytic drugs, thereby impeding revascularization, which further increases the risk of hemorrhage [27]. High levels of glycated protein reflect long-term hyperglycemia, which may lead to long-term damage to vascular structure and function. This study found higher HbA1c levels in the HT group. The nutritional status of IVH patients on admission can be a better representation of baseline nutritional status, whereas low hemoglobin Hgb levels reflect host malnutrition [28,29]. In the present study, Hgb levels were lower in the HT group than in the non-HT group.

Assessment of coagulation function is essential to predict the risk of HT after thrombolysis. Abnormalities in coagulation may lead to an increased risk of bleeding during or after thrombolysis, which in turn affects the patient's prognosis. However, fewer studies have reported on coagulation function after thrombolysis, and all of these reports used conventional coagulation indices. Post-thrombolytic coagulation tests predict the risk of intracerebral hematoma, with a 12.82-fold increase in the odds of early intracerebral hematoma when the reduction in FIB is less than 2 g/L [30]. Furthermore, coagulation indices after IVH treatment, including FIB, FIB degradation products, and D-D, are only found to be increased in the 48-hour post-IVH period, and D-D is an independent risk factor for poor prognosis in patients with AIS [31]. Therefore, we felt the need to conduct a study on the early coagulation status after IVH treatment in AIS patients. Our results showed prolonged R and decreased MA in HT patients. The reaction time R reflects the time until the formation of the first fibrin clot and reflects the functional status of coagulation factors. MA reflects FIB and platelet quality

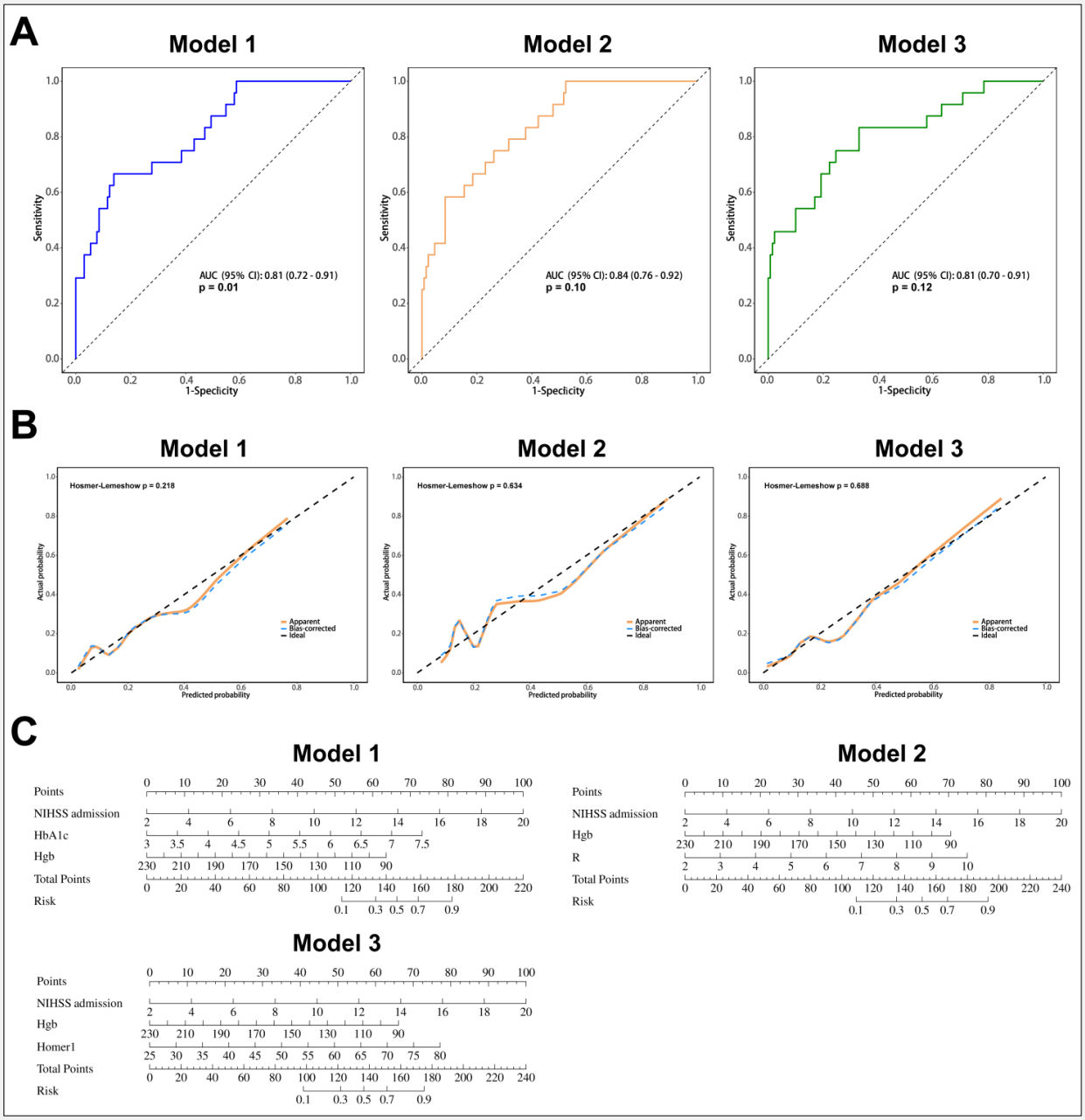

Figure 3. Construction of a multifactorial logistic regression analysis model influencing HT after IVH.

A) ROC plot, (B) calibration curve plot, (C) column lines.
HL test is an indicator of model fit; if the p-value is greater than 0.05, it means that there is no obvious difference between the predicted value and the true value; the line-column plot mainly consists of the names of the variables on the left side and the corresponding line segments with scale on the right side, and the lengths of the line segments reflect the magnitude of the factor's contribution to the outcome.

and quantity. Our results suggest that the patients had abnormal coagulation factor function, FIB, and platelet quality and quantity, and were at risk of bleeding. In addition, our results also confirmed that plasma FIB was decreased in the HT group.

In AIS, oxidative stress can damage the tight junction proteins and basement membranes of the blood-brain barrier, increasing their permeability, which can lead to HT. Normally, Homer1 is expressed in the brain. However, due to ischemia and hypoxia, all neurons in the

neurovascular unit experience endoplasmic reticulum and mitochondrial stress [32,33], and ionic imbalances within and outside the cell membrane lead to cytotoxic edema. Vascular endothelial cells are destroyed and vasogenic edema occurs. Hypoxia leads to oxidative stress. Peroxidation products damage the tight junction proteins and basement membranes of the blood-brain barrier, increase their permeability, and release inflammatory factors and blood components, leading to aggravated damage [34]. A series of pathological injuries ultimately lead to neuronal cell disintegration and death, disruption of the blood-brain barrier, and are ultimately accompanied by elevated levels of Homer1 in the peripheral blood. Consistently, we measured elevated Homer1 levels in the serum of the HT group.

To assess the correlation between TEG and Homer1 and bleeding risk after IVH in patients with AIS, we first analyzed the indicators with close clinical correlations for covariance and excluded the indicators with large covariance. We first constructed three models: clinical factor model (model 1), clinical factor + TEG (model 2), and clinical factor + Homer1 (model 3), all of which included age and gender as calibration variables. All three models showed that baseline NIHSS was a risk factor for HT after IVH in AIS patients. Glucose is a risk factor for post-thrombolytic HT in patients with AIS [35]. Patients with preexisting chronic diabetes and poor glycemic control have a significantly increased risk of post-AIS HT. This is due to chronic damage to vascular endothelial cells caused by long-term chronic hyperglycemia, which leads to atherosclerosis of cerebral arteries and reduced elasticity of the arterial wall [36]. When AIS develops, combined with a series of cascade reactions caused by ischemia and hypoxia, acute severe damage to cerebral vessels, and excessive lactic acid produced by anaerobic digestion caused by high glucose, the vascular wall is vulnerable to damage and permeability increases, thereby increasing the risk of bleeding [37]. The present study showed that higher HbA1c was an independent risk factor for HT after AIS. Hgb is a specialized protein that transports oxygen within erythrocytes, and its synthesis and functioning are influenced by a variety of nutrients [38]. In the present study, elevated Hgb was a protective factor for HT after IVH in AIS patients. In addition, coagulation reaction time R and Homer1 were also risk factors for post-thrombolytic HT in AIS patients in models 2 and 3. However, we did not find that TEG parameters or Homer1 increased the predictive efficacy of the clinical models.

The drawback of our clinical study was the relatively small sample size of patients included in this study. Second, we did not collect consecutive peripheral blood from patients before and continuously after IVH to detect temporal expression trends of TEG and serum Homer1. Neuroimaging data, such as cerebral infarct area on DWI, were also not collected from patients with AIS. In addition, we were unable to characterize in detail the relationship between patient outcome and TEG parameters and Homer1 after IVH as we examined patients' neurological recovery 3 months and longer after stroke. Finally, we measured serum Homer1 levels by ELISA. Although we performed at least three assays on peripheral serum from each patient to obtain relatively accurate concentration results, the ELISA assay results were semiquantitative for Homer1 based on absorbance.

CONCLUSION

This study states that TEG and serum Homer1 levels after IVH in AIS patients are strongly associated with HT in AIS patients. The results suggest that TEG measures and Homer1 levels in patients' peripheral serum early after IVH may be an alternative predictive model of clinical factors for predicting the risk of HT in patients with AIS.

Source of Funds:
Self-funded Science and Technology Innovation Project of Foshan City in 2022, Science and Technology Bureau of Foshan City (No. 2220001005317).

Data Availability Statement:
Data is available from the corresponding author on request.

Ethical Approval Statement:
All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All subjects were approved by Foshan Hospital of Traditional Chinese Medicine (No. 201912FS05).

Declaration of Interest:
The authors have no conflicts of interest to declare.

References:

1. Virani SS, Alonso A, Aparicio HJ, et al. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. Circulation 2021;143(8):e254-743. (PMID: 33501848)

2. Wang L, Zhang B, Yang X, et al. Targeted alleviation of ischemic stroke reperfusion via atorvastatin-ferritin Gd-layered double hydroxide. Bioact Mater 2023;20:126-36. (PMID: 35663341)

3. Lee S-H, Jang MU, Kim Y, et al. High glycemic albumin representing prestroke glycemic variability is associated with hemorrhagic transformation in patients receiving intravenous thrombolysis. Sci Rep 2022;12(1):615. (PMID: 35022517)

4. Muchada M, Rubiera M, Rodriguez-Luna D, et al. Baseline National Institutes of Health stroke scale-adjusted time window for intravenous tissue-type plasminogen activator in acute ischemic stroke. Stroke 2014;45(4):1059-63. (PMID: 24603070)

5. Honig A, Percy J, Sepehry AA, Gomez AG, Field TS, Benavente OR. Hemorrhagic Transformation in Acute Ischemic Stroke: A Quantitative Systematic Review. J Clin Med 2022;11(5):1162. (PMID: 35268253)

6. Liu J, Tao W, Wang Z, Chen X, Wu B, Liu M. Radiomics-based prediction of hemorrhage expansion among patients with thrombolysis/thrombectomy related-hemorrhagic transformation using machine learning. Ther Adv Neurol Disord 2021;14:1756286421 1060029. (PMID: 35173809)

7. Ruan Y, Yuan C, Liu Y, et al. High fibrinogen-to-albumin ratio is associated with hemorrhagic transformation in acute ischemic stroke patients. Brain Behav 2021;11(1):e01855. (PMID: 33314645)

8. Ye C, Wang Y, Song Q, Liu J, Wei C, Liu M. Association Between Coagulation Function and Spontaneous Hemorrhagic Transformation in Acute Ischemic Stroke. Curr Neurovasc Res 2020;17(4):344-53. (PMID: 32407276)

9. Shin KH, Kim IS, Lee HJ, et al. Thromboelastographic Evaluation of Coagulation in Patients With Liver Disease. Ann Lab Med 2017;37(3):204-12. (PMID: 28224766)

10. Tang N, Jin X, Sun Z, Jian C. Effects of hemolysis and lipemia interference on kaolin-activated thromboelastography, and comparison with conventional coagulation tests. Scand J Clin Lab Invest 2017;77(2):98-103. (PMID: 28218013)

11. Gjermeni D, Vetter H, Szabó S, et al. Thrombelastography Compared with Multiple Impedance Aggregometry to Assess High On-Clopidogrel Reactivity in Patients with Atrial Fibrillation Undergoing Percutaneous Coronary Intervention. J Clin Med 2022;11(14):4237. (PMID: 35888001)

12. Huang RSP, McDonald MM, Wetzel JS, et al. Clot Strength as Measured by Thrombelastography Correlates with Platelet Reactivity in Stroke Patients. Ann Clin Lab Sci 2015;45(3):301-7. (PMID: 26116594)

13. Liu Z, Chai E, Chen H, Huo H, Tian F. Comparison of Thrombelastography (TEG) in Patients with Acute Cerebral Hemorrhage and Cerebral Infarction. Med Sci Monit 2018;24:6466-71. (PMID: 30217969)

14. Luo P, Li X, Fei Z, Poon W. Scaffold protein Homer 1: implications for neurological diseases. Neurochem Int 2012;61(5):731-8. (PMID: 22749857)

15. Kaastrup Müller H, Orlowski D, Reidies Bjarkam C, Wegener G, Elfving B. Potential roles for Homer1 and Spinophilin in the preventive effect of electroconvulsive seizures on stress-induced CA3c dendritic retraction in the hippocampus. Eur Neuropsychopharmacol 2015;25(8):1324-31. (PMID: 25935093)

16. Fei X, Dou Y-N, Wang L, et al. Homer1 promotes the conversion of A1 astrocytes to A2 astrocytes and improves the recovery of transgenic mice after intracerebral hemorrhage. J Neuroinflammation 2022;19(1):67. (PMID: 35287697)

17. Lv W, Zhang Q, Li Y, et al. Homer1 ameliorates ischemic stroke by inhibiting necroptosis-induced neuronal damage and neuroinflammation. Inflamm Res 2024;73(1):131-44. (PMID: 38091015)

18. Zhu M, Zuo J, Shen J, et al. Diagnostic Potential of Differentially Expressed Homer1 and Homer2 in Ischemic Stroke. Cell Physiol Biochem 2016;39(6):2353-63. (PMID: 27832625)

19. Lv W, Ruan Z, Zhang Q, et al. Serum Homer1 is a Novel Biomarker for Predicting the Clinical Outcomes of Acute Ischemic Stroke Patients. J Inflamm Res 2024;17:1337-47. (PMID: 38434583)

20. Zhu X, Wang J, Wang H, Cai S. Study on the Relationship between sLOX-1, S100A12, D-dimer, and Cerebral Hemorrhage Transformation after Intravenous Thrombolysis in Stroke. Altern Ther Health Med 2024:AT10672. (PMID: 38870507)

21. Chen R, Jiang G, Liu Y, et al. Predictive effects of S100β and CRP levels on hemorrhagic transformation in patients with AIS after intravenous thrombolysis: A concise review based on our center experience. Medicine (Baltimore) 2023;102(38):e35149. (PMID: 37747023)

22. IST-3 collaborative group. Effect of thrombolysis with alteplase within 6 h of acute ischaemic stroke on long-term outcomes (the third International Stroke Trial [IST-3]): 18-month follow-up of a randomised controlled trial. Lancet Neurol 2013;12(8):768-76. (PMID: 23791822)

23. Neuberger U, Möhlenbruch MA, Herweh C, Ulfert C, Bendszus M, Pfaff J. Classification of Bleeding Events: Comparison of ECASS III (European Cooperative Acute Stroke Study) and the New Heidelberg Bleeding Classification. Stroke 2017;48(7): 1983-5. (PMID: 28455322)

24. Hao Y, Zhou H, Pan C, et al.; CASE II investigators. Prediction factors and clinical significance of different types of hemorrhagic transformation after intravenous thrombolysis. Eur J Med Res 2023;28(1):509. (PMID: 37951926)

25. Rocco A, Heuschmann PU, Schellinger PD, et al. Glycosylated hemoglobin A1 predicts risk for symptomatic hemorrhage after thrombolysis for acute stroke. Stroke 2013;44(8):2134-8. (PMID: 23715962)

26. Huang P, Yi X-Y. Predictive role of admission serum glucose, baseline NIHSS score, and fibrinogen on hemorrhagic transformation after intravenous thrombolysis with alteplase in acute ischemic stroke. Eur Rev Med Pharmacol Sci 2023;27(20):9710-20. (PMID: 37916334)

27. Poppe AY, Hill MD. Hyperglycemia in thrombolysed acute ischemic stroke patients. Int J Stroke 2011;6(3):278; author reply -9. (PMID: 21557817)

28. Xiang W, Chen X, Ye W, Li J, Zhang X, Xie D. Prognostic Nutritional Index for Predicting 3-Month Outcomes in Ischemic Stroke Patients Undergoing Thrombolysis. Front Neurol 2020;11: 599. (PMID: 32670192)

29. Zhao W, Shi M, Zhang J. Preoperative hemoglobin-to-red cell distribution width ratio as a prognostic factor in pulmonary large cell neuroendocrine carcinoma: a retrospective cohort study. Ann Transl Med 2022;10(2):42. (PMID: 35282116)

30. Sun X, Berthiller J, Trouillas P, Derex L, Diallo L, Hanss M. Early fibrinogen degradation coagulopathy: a predictive factor of parenchymal hematomas in cerebral rt-PA thrombolysis. J Neurol Sci 2015;351(1-2):109-14. (PMID: 25783009)

31. Li G, Wang C, Wang S, Hao Y, Xiong Y, Zhao X. Clinical Significance and Dynamic Change of Coagulation Parameters in Ischemic Stroke Patients Treated with Intravenous Thrombolysis. Clin Appl Thromb Hemost 2022;28:10760296221121287. (PMID: 36040718)

32. Louessard M, Bardou I, Lemarchand E, et al. Activation of cell surface GRP78 decreases endoplasmic reticulum stress and neuronal death. Cell Death Differ 2017;24(9):1518-29. (PMID: 28644439)

33. Petrovic-Djergovic D, Goonewardena SN, Pinsky DJ. Inflammatory Disequilibrium in Stroke. Circ Res 2016;119(1):142-58. (PMID: 27340273)

34. Zhang J, Bhuiyan MIH, Zhang T, et al. Modulation of brain cation-Cl(-) cotransport via the SPAK kinase inhibitor ZT-1a. Nat Commun 2020;11(1):78. (PMID: 31911626)

35. Muscari A, Falcone R, Recinella G, et al. Prognostic significance of diabetes and stress hyperglycemia in acute stroke patients. Diabetol Metab Syndr 2022;14(1):126. (PMID: 36038896)

36. Da Silva DCP, Carneiro FD, Almeida KC, Fernandes-Santos C. Role of miRNAs on the Pathophysiology of Cardiovascular Diseases. Arq Bras Cardiol 2018;111(5):738-46. (PMID: 30484515)

37. Shafa MA, Ebrahimi H, Iranmanesh F, Sasaie M. Prognostic value of hemoglobin A1c in nondiabetic and diabetic patients with acute ischemic stroke. Iran J Neurol 2016;15(4):209-13. (PMID: 28435629)

38. Moorthy D, Merrill R, Namaste S, Iannotti L. The Impact of Nutrition-Specific and Nutrition-Sensitive Interventions on Hemoglobin Concentrations and Anemia: A Meta-review of Systematic Reviews. Adv Nutr 2020;11(6):1631-45. (PMID: 32845972)