Biomarkers in the Prediction of Hemorrhagic Transformation in Acute Stroke: A Systematic Review and Meta-Analysis

Soumya Krishnamoorthy^a Gurpreet Singh^b Jithu Jose K^c Biju Soman^b
Christian Foerch^d W. Taylor Kimberly^e Mónica Millán^f Milena Świtońska^g
Ilaria Maestrini^{h,i} Régis Bordetⁱ Konark Malhotra^j Laura Mechtouff^k
P.N. Sylaja^a

^aComprehensive Stroke Care Program, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India; ^bAchutha Menon Centre for Health Science Studies, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India; ^cDepartment of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India; ^dDepartment of Neurology, University Hospital, Goethe University, Frankfurt am Main, Germany; ^eDepartment of Neurology, Massachusetts General Hospital, Boston, MA, USA; ^fStroke Unit, Department of Neurosciences, Hospital Germans Trias i Pujol, Departament de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain; ^gDepartment of Neurosurgery and Neurology, Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum, Bydgoszcz, Poland; ^hDepartment of Systems Medicine, Stroke Center, University of Rome Tor Vergata, Rome, Italy; ⁱDepartment of Medical Pharmacology, Degenerative and Vascular Cognitive Disorders, University Hospital CHU Lille, Inserm U1171, University of Lille, Lille, France; ^jDepartment of Neurology, Allegheny Health Network, Pittsburgh, PA, USA; ^kStroke Department, Pierre Wertheimer Hospital, Hospices Civils de Lyon, Lyon, France

Keywords

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Abstract

Background: Hemorrhagic transformation (HT) is a complication that occurs spontaneously or after thrombolysis in acute ischemic stroke (AIS) and can increase morbidity and mortality. The association of biomarkers with the risk of HT has been variably reported. We conducted a systematic review of the literature and meta-analysis and sought to compare blood biomarkers associated with HT and its subtypes by evaluating its predictability and correlation with outcome in AIS. **Methods:** The study protocol was registered in the PROSPERO database (CRD42020201334) and adhered to the

Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Among 2,230 articles identified from Cochrane Library, PubMed, and Web of Science databases, 30 quality-appraised articles were found eligible. Meta-analysis was conducted for matrix metalloproteinase-9 (MMP-9), cellular fibronectin (c-Fn), ferritin, S100 calcium-binding protein B (S100B), and neutrophil-lymphocyte ratio (NLR). We also reviewed biomarkers for correlation with the functional outcome at 90 days from stroke onset (poor outcome modified Rankin scale >2). **Results:** The pooled diagnostic odds ratio (DOR_{pooled}) was the highest for baseline c-Fn levels (299.253 [95% CI, 20.508–4,366.709]), followed by MMP-9 (DOR_{pooled}, 29.571 [95% CI 17.750–49.267]) and ferritin (DOR_{pooled}, 24.032 [95% CI 2.557–225.871]). However, wide confidence intervals for ferritin and c-Fn suggested lesser reliability of the markers. Patients with MMP-9 levels ≥140 ng/mL

were 29.5 times at higher risk of developing symptomatic HT after AIS (area under the curve = 0.881). S100B (DOR_{pooled}, 6.286 [95% CI, 1.861–21.230]) and NLR (DOR_{pooled}, 5.036 [95% CI, 2.898–8.749]) had lower diagnostic accuracies. Among the markers not included for meta-analysis, caveolin-1, thrombin-activated fibrinolysis inhibitor, plasminogen activator inhibitor-1, and soluble ST2 were highly sensitive. Elevated levels of MMP-9, ferritin, and NLR were found to be associated with poor functional outcomes and mortality. **Conclusion:** Of the 5 biomarkers, there was enough evidence that MMP-9 has higher diagnostic accuracy for predicting the risk of HT before thrombolysis. MMP-9, ferritin, and NLR also predicted poor short-term outcomes.

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Introduction

Hemorrhagic transformation (HT) is a complication following acute ischemic stroke (AIS) leading to poor outcomes and mortality [1]. HT is classified based on imaging and clinical deterioration. The European Cooperative Acute Stroke Study classified the radiological appearance of petechiae as hemorrhagic infarction (HI) and severe forms as parenchymal hematoma (PH) [2]. The National Institutes of Neurological Disorders and Stroke (NINDS), classified HT into asymptomatic HT, which is detected by computed tomography (CT) or magnetic resonance imaging (MRI) of the brain and symptomatic HT (sHT), characterized by clinical deterioration along with the imaging evidence [3]. At least 2–6% of the patients who undergo thrombolytic therapy are at risk of developing sHT, whereas in 20–40% patients HT will be asymptomatic [3]. It is known to be associated with neurological worsening in at least 20% of patients thereby increasing morbidity and mortality [4]. Several studies have shown that older age, hypertension, hyperglycemia, stroke severity, and cardioembolic etiology add up to the risk [5, 6].

The pathophysiology of HT in cerebral infarction involves the disruption of the blood-brain barrier resulting from ischemic reperfusion injury activated by free radicals and proteolytic enzymes [7]. Nevertheless, it is speculated that HI and PH follow slightly different pathophysiological pathways [8]. Although HI type-1 and HI type-2 are usually associated with good outcomes, both parenchymal hemorrhage type-1 and type-2 lead to worse outcomes and increased mortality in at least 3% of patients [9].

Circulating biomarkers have the potential to identify patients at risk of developing HT which would benefit

clinical decision-making or may pave the way for novel therapeutic strategies [10]. Their use may also have implications in the timing of resuming anticoagulation treatment. Several biomarkers have been investigated previously. However, the lack of uniformity in defining HT across studies and the dearth of evidence makes it difficult to appraise them. To date, none of these markers have been validated for clinical use; however, an evidence-based study may determine the diagnostic performance best suited as an adjunct to clinical and imaging variables for predicting HT. Hence, the present systematic review and meta-analysis aimed to assess the relationship between blood biomarkers and HT by evaluating the diagnostic accuracy to predict HT in AIS and its correlation with outcome.

Methods

We evaluated biomarkers according to the following outcome measures: (i) comparison of baseline levels of biomarkers in HT and non-HT patients, (ii) biomarker estimation among HT subtypes, (iii) association of biomarker levels in HT post-intervention, and (iv) functional outcome at 90 days from onset, using the modified Rankin scale. The study protocol was registered in the PROSPERO database (reg. no. CRD42020201334) and adhered to Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement [11].

Search Strategy

Search Criteria and Databases

The Cochrane Library (CENTRAL), MEDLINE using PubMed, and Web of Science were searched for eligible studies using keywords and search strings for articles published in English between 1 October 2000 and 30 November 2020 (online suppl. Appendix 1; see www.karger.com/doi/10.1159/000518570 for all online suppl. material).

Study Selection

Eligible studies were reviewed and data extraction was carried out by 2 authors (S.K. and J.J.K.). The author (P.N.S.) was approached in case of discrepancies and was finalized based on consensus. Title and abstract screening were followed by full-text screening.

Inclusion Criteria

The inclusion criteria were (i) full-text publications which included only original articles, in English, (ii) ≥18 years AIS patients, (iii) HT confirmed by CT or MRI scan of the brain, (iv) biomarkers measured in baseline blood samples, (v) samples collected <24 h from stroke onset, and (vi) availability of baseline CT/MRI and follow-up CT/MRI data.

Exclusion Criteria

Articles were excluded if they were (i) related to gene, genetic variants, gene expression, microRNA, or metabolomic studies, (ii) tissue biopsies, cerebrospinal fluid, or any other body fluids apart

from the blood were investigated, (iii) insufficient information and diagnostic data, and (iv) studies using only cell lines and animal models.

Operational Definitions

We defined sHT as any patient with clinical deterioration within 48 h along with HI or PH on follow-up neuroimaging [12] and asymptomatic HT as neuroimaging-documented bleeding without neurological deterioration [13]. We defined sICH as per the NINDS definition [14].

Risk of Bias in Individual Studies

Risk of bias and applicability concerns were estimated using the Quality Assessment of Diagnostic Accuracy Score-2 tool comprising 4 domains based on the patient selection, index test, reference standard, and flow and timing [15]. Selected studies were assessed using the questions provided under the tool (online suppl. Appendix 2) [16]. We evaluated the methodological quality according to the statement: guidelines for reporting observational studies (STROBE) criteria [17].

Statistical Analysis

Descriptive statistics for the studies included sensitivity, specificity, diagnostic odds ratio (DOR), false-positive rates, and likelihood ratios of individual studies. χ^2 tests were performed for equality of sensitivities and specificities. Univariate analysis for pooled estimates of DORs was carried out using DerSimonian-Laird estimator. Cochran's Q statistic was used to estimate variance parameters. The hierarchical bivariate model (linear mixed model with known variances of the random effects) was used to develop hierarchical summary receiver operator characteristic curves. Variance components are estimated by restricted maximum likelihood. The area under the curve, pooled sensitivity, and false positivity rates were calculated. Deeks' funnel plots were prepared to estimate publication bias. Weighted regression with multiplicative dispersion model using standard error and sample size as predictors were used to determine funnel plot asymmetry statistically. The analysis was carried out using RevMan 5.0.3 and R version 4.0.2 (*mada* and *metafor* packages) software.

Results

Study Selection

Database search identified 2,230 results, of which, 2,175 articles were excluded as duplicates and by screening titles and abstracts not relevant to the review. Of the 55 articles that were selected for full-text review, 30 quality-appraised articles were found eligible. The flow diagram is shown in Figure 1.

Study Characteristics

The total sample size of the eligible studies was 9,826. The baseline characteristics of the studies are given in Table 1.

All studies recruited patients admitted within 24 h from stroke onset. Stroke etiology was determined by the Trial of Org 10,172 in Acute Stroke Treatment classification [18] in eighteen studies [19–36]. One study only used the Oxfordshire Community for Stroke patients classification [37] and one study used both Trial of Org 10,172 in Acute Stroke Treatment and Oxfordshire Community for Stroke patients classification [38]. Ten studies did not mention any specific classification for determining stroke etiology [39–48]. Stroke severity was assessed using the National Institutes of Health Stroke Scale score in all studies except for one [19] which had used the Canadian Stroke Scale.

Apart from for 4 studies [19–22], all patients underwent interventions such as intravenous tissue plasminogen activator, endovascular treatment, or bridging therapy. One study included patients who received intra-arterial thrombolytic therapy [43].

Assessment of HT varied across studies. Twenty-four studies [19–32, 36–39, 42–47] defined HT according to the European Cooperative Acute Stroke Study criteria, whereas 2 studies [33, 34] used the Heidelberg Bleeding Classification. Three studies [35, 39, 40] used the NINDS criteria. One study also used the Safe Implementation of Thrombolysis in Stroke-Monitoring Study criteria to define sICH [39]. One study assessed biomarker values in patients who developed early HT, that is, within 48 h from stroke onset compared to late HT detected 5–7 days from onset [21]. Cutoff values of biomarkers were determined in sixteen studies using ROC curve analysis. Sensitivity, specificity, positive predictive value, and negative predictive value (NPV) were examined in twenty studies. The remaining studies used prespecified thresholds as reference values for the markers.

Risk of Bias

Seventeen studies examined risk of bias, 2 studies included flow diagrams, 16 studies appropriately described their study population including details of missing data and patients lost during follow-up, and 20 studies disclosed funding details. The risk of bias and applicability of studies' summary and graph for the included studies are shown in Figure 2 and online supplementary Appendix 2. The methodological quality of studies following the STROBE guidelines were given in online supplementary Appendix 3.

Biomarkers Included in the Meta-Analysis

Meta-analysis of matrix metalloproteinase-9 (MMP-9), cellular fibronectin (c-Fn), ferritin, S100 calcium-binding protein B (S100B) levels in sHT, and neutrophil-

lymphocyte ratio (NLR) in sICH were conducted, respectively. Summary statistics for diagnostic accuracy parameters of included studies are provided in online supplementary Table 1. Forest plots of univariate analysis using DOR are shown in Figure 3. Forest plots for sensitivity and specificity are shown in Figure 4. SROC plot for 5 biomarkers using calculated hierarchical summary receiver operator characteristic parameters is shown in Figure 5

Funnel plots to estimate publication bias is shown in online supplementary Figure 1. The regression test for

funnel plot asymmetry found a lack of publication bias with standard error and sample size as predictors (p value of 0.5574 and 0.3119, respectively).

Matrix Metalloproteinase-9

Ten studies comprising 1,494 patients reported high baseline MMP-9 in sHT assessed by commercially available enzyme-linked immunosorbent assay kits. Three studies had serially measured MMP-9 levels at various timepoints [21, 28, 32].

Fig. 1. PRISMA flow diagram outlining the process of study screening through final selection of articles. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Fig. 2. Summary of the risk of biases using QUADAS-2 tool. **a** Risk of bias summary presenting the risk of bias items for each included study. **b** Risk of bias graph for all studies presented as percentages. QUADAS-2, Quality Assessment of Diagnostic Accuracy Score-2. (For figure see next page.)

Table 1. Basic characteristics of included studies

Reference	Place	Study design	Sample size, <i>n</i>	Mean age $\pm$ SD, years	Males, <i>n</i>	Intervention(s)	First CT/MRI, h	Follow-up CT/MRI, h	Groups, <i>n</i>	Biomarker(s)
Montaner et al. [21]	Spain	Prospective	39	74 $\pm$ 15	20	None	$\leq$ 12	<36–48	sHT patients = 15; non-sHT patients = 24	MMP-9
Montaner et al. [28]	Spain	Prospective	41	70 $\pm$ 10.6	16	IV tPA	$\leq$ 3	<36–48	sHT = 15; non-sHT = 26	MMP-9
Castellanos et al. [19]	Spain	Prospective	250	72.3 $\pm$ 9.2	134	None	$\leq$ 24	>48	sHT patients = 38; non-sHT patients = 212	MMP-9
Castellanos et al. [25]	Spain	Prospective	87	67 $\pm$ 12	48	IV tPA	$\leq$ 6	<24–36	sHT patients = 26; non-sHT patients = 61	MMP-9, c-Fn
Ribo et al. [31]	Spain	Prospective	77	70 $\pm$ 12	47	IV tPA	$\leq$ 3	<36–48	sHT patients = 17; non-sHT patients = 60	Fibrinogen, PAI-1, TAFI, Lp(a), homocysteine
Castellanos et al. [24]	Spain	Prospective	134	67 $\pm$ 12	88	IV tPA	$\leq$ 3	<24–36	sHT patients = 27; non-sHT patients = 107	MMP-9, c-Fn
Foerch et al. [36]	Spain	Retrospective	275	69 $\pm$ 13	126	IV tPA	$\leq$ 6	<24 h	sHT patients = 80; non-sHT patients = 195	S100B
Millán et al. [27]	Spain	Retrospective	134	67 $\pm$ 11	88	IV tPA	$\leq$ 3	<24–36 h	sHT patients = 27; non-sHT patients = 107	Ferritin, MMP-9, c-Fn, IL-6, glutamate
Hernandez-Guillamon et al. [23]	Spain	Prospective	140	71.9 $\pm$ 10.5	76	IV tPA	$\leq$ 3	<36–48	sHT patients = 48; non-sHT patients = 92	VAP-SSAO activity
Navarro-Sobrinho et al. [29]	Spain	Prospective	109	71 $\pm$ 12.4	59	IV tPA	$\leq$ 3	<24–48	sHT patients = 8; non-sHT patients = 101	PDGF-BB, HGF, FGF, VEGF, HB-EGF, KGF, PDGF-AA, TPO, VEGFR-1, VEGFR-2, TSP-1, TSP-2
Choi et al. [26]	Korea	Retrospective	752	NA	455	IV tPA	$\leq$ 6	>48	HT patients = 90; non-HT patients = 662	Ferritin
Kazmierski et al. [22]	Poland	Prospective	458	68 $\pm$ 12.5	247	None	$\leq$ 3	<24	cdHT = 33; non-cdHT = 425	MMP-9, S100B, OCLN, CLDN5, ZO-1, VEGF, NSE
Matosevic et al. [35]	Austria	Prospective	547	68 $\pm$ 15.1	316	IV tPA	$\leq$ 6	<24–36	sICH patients = 33; non-sICH patients = 514	Fibrinogen
El-Banhawy et al. [40]	Egypt	Prospective, case control	70	60.9 $\pm$ 9.37	30	IV tPA	$\leq$ 24	>48	sHT patients = 8; non-sHT patients = 62	MMP-9
Xing et al. [43]	China	Prospective	216	NA	154	IA tPA, EVT	$\leq$ 6	<24	sHT patients = 41; non-sHT patients = 175	Globulin
Maestrini et al. [44]	France and Finland	Retrospective	846	NA	430	IV tPA	$\leq$ 3	<24–36	sHT patients = 54; non-sHT patients = 792	NLR
Guo et al. [47]	China	Prospective	189	NA	123	IV tPA, EVT, bridging therapy	$\leq$ 4.5	<36–48	PH patients = 28; non-PH patients = 161	NLR
Wolcott et al. [30]	USA	Prospective	646	69 $\pm$ 15	364	IV tPA	$\leq$ 9	>48	sHT patients = 42; non-sHT patients = 604	Soluble ST2

Table 1 (continued)

Reference	Place	Study design	Sample size, n	Mean age $\pm$ SD, years	Males, n	Intervention(s)	First CT/MRI, h	Follow-up CT/MRI, h	Groups, n	Biomarker(s)
Che et al. [37]	China	Prospective	428	NA	316	IV tPA	≤ 4.5	> 48	sHT patients = 24; non-sHT patients = 404	Serum albumin
Yuan et al. [20]	China	Prospective, case control	208	66.7 ± 13.1	90	None	≤ 24	< 24 – 36	sHT patients = 29; non-sHT patients = 139	MMP-9
Molnar et al. [42]	Hungary	Prospective	54	HT = 71 ± 7.8 ; non-HT = 66 ± 10	32	IV tPA, EVT	≤ 6	< 24 – 36	sHT patients = 9; non-sHT patients = 45	CCCK-18
Castellanos et al. [45]	Spain	Prospective	133	72 ± 13	67	IV tPA	≤ 4.5	< 24 – 36	sHT patients = 26; non-sHT patients = 107	Caveolin-1
Duan et al. [33]	China	Retrospective	616	NA	368	EVT, bridging therapy	≤ 4.5	< 24 h	sICH patients = 292; non-sICH patients = 324	NLR
Malhotra et al. [41]	USA	Retrospective	657	64.3 ± 14.4	333	IV tPA, EVT, bridging therapy	≤ 4.5	< 24 h	sICH patients = 21; non-sICH patients = 635	NLR
Pikija et al. [34]	Austria	Retrospective	187	NA	86	IV tPA, EVT, bridging therapy	≤ 6	< 24	sHT patients = 31; non-sHT patients = 156	NLR
Goyal et al. [48]	USA	Prospective	293	62 ± 14	147	EVT	≤ 6	< 24 – 36	sHT patients = 21; non-sHT patients = 272	NLR
Lin et al. [39]	Taiwan	Prospective	1,840	NA	1,174	IV tPA, EVT	≤ 3	< 24 – 36	sHT patients = 38; non-sHT patients = 1,802	Serum cholesterol
Jucevičiūtė et al. [46]	Lithuania	Retrospective	201	NA	81	IV tPA	≤ 4.5	< 24 – 36	sHT patients = 23; non-sHT patients = 178	AEC
Mechtouff et al. [32]	France	Prospective	148	69 ± 15	89	IV tPA, EVT, bridging therapy	≤ 4.5	< 24	sHT patients = 40; non-sHT patients = 108	MMP-9
Świtońska et al. [38]	Poland	Retrospective	51	65 ± 16	22	IV tPA, EVT, bridging therapy	≤ 24	> 24	sHT patients = 10; non-sHT patients = 41	NLR

SD, standard deviation; NA, data not available; HT, hemorrhagic transformation; sHT, symptomatic hemorrhagic transformation; sICH, symptomatic intracranial hemorrhage; cdHT, hemorrhagic transformation with clinical deterioration; PH, parenchymal hematoma; HI, hemorrhagic infarction; IV tPA, intravenous tissue plasminogen activator; IA tPA, intra-arterial tissue plasminogen activator; EVT, endovascular treatment; CT, computed tomography; MRI, magnetic resonance imaging; h, hours; MMP-9, matrix metalloproteinase-9; NLR, neutrophil-lymphocyte ratio; c-Fn, cellular fibronectin; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activated fibrinolysis inhibitor; Lp(a), lipoprotein (a); AEC, absolute eosinophil count; VAP-1-SSAO, vascular adhesion protein-1-semicarbazide-sensitive amine oxidase; PDGF-BB, platelet-derived growth factor BB; HGF, hepatocyte growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; HB-EGF, heparin binding epidermal growth factor like growth factor; KGF, keratinocyte growth factor; PDGF-AA, platelet-derived growth factor AA; TPO, thrombopoietin; VEGFR-1, vascular endothelial growth factor receptor-1; VEGFR-2, vascular endothelial growth factor receptor-2; TSP-1, thrombospondin-1; TSP-2, thrombospondin-2; CCCK-18, caspase-cleaved cytokeratin-18; S100B, S100 calcium-binding protein B; OCLN, occludin; CLDN5, claudin5; ZO-1, zonula occludens-1, NSE, neuron-specific enolase.

Fig. 3. Forest plots of univariate analysis using DORs of meta-analyzed studies: pooled DOR for MMP-9 (**a**), c-Fn (**b**), ferritin (**c**), S100B (**d**), and NLR (**e**). MMP-9, matrix metalloproteinase-9; c-Fn, cellular fibronectin; S100B, S100 calcium-binding protein B; NLR, neutrophil-lymphocyte ratio; DORs, diagnostic odds ratios.

Fig. 4. Forest plots for sensitivities and specificities corresponding to the 5 biomarkers.

Four studies [19, 21, 24, 25] reported a baseline cut-off >140 ng/mL yielding high sensitivity and specificity to predict sHT. Threshold levels >700 ng/mL were reported in 2 studies within 24 h of onset [32, 40]. Two studies reported no significant difference in the mean MMP-9 levels between nonthrombolysed patients with early HI and early PH [19, 21]. However, in patients receiving intravenous tissue plasminogen activator, a higher median baseline MMP-9 was observed in patients with PH. Two studies reported pretreatment levels ≥ 191.3 ng/mL to predict PH [24, 28]. One study reported MMP-9 to be significantly higher in patients with poor functional outcomes ($p = 0.021$) as compared to those with a modified Rankin scale <2 at 90 days from onset [27].

Seven studies comprising 754 patients were included for the meta-analysis and the funnel plots to estimate publication bias. Elevated levels of MMP-9 were found to have a high accuracy for predicting PH (pooled DOR, 29.571 [95% CI 17.750–49.267]). Bivariate analysis

(shown in Fig. 5) showed pooled sensitivity as 0.849 and the pooled false positivity rate to be 0.180.

Cellular Fibronectin

Three studies comprising 355 patients examined the correlation of c-Fn in sHT using commercial enzyme-linked immunosorbent assay [24, 25, 27]. All studies assessed c-Fn levels in patients who underwent thrombolysis.

Significant elevation of c-Fn was reported in post-thrombolysis patients with PH in all studies [25]. A cutoff ≥ 3.6 $\mu\text{g/mL}$ yielded high sensitivity and NPV which when combined with MMP-9 (≥ 140 ng/mL), increased specificity and positive predictive value for predicting PH [25].

Functional outcome positively correlated with c-Fn in one study ($p = 0.010$) [27]. Two studies [24, 25] with 221 patients were meta-analyzed. Pooled DOR was found to be highest for c-Fn (299.253 [95% CI, 20.508–4,366.709]), pooled sensitivity and the false positivity rate were 0.982 and 0.153, respectively.

Fig. 5. SROC plot for the meta-analyzed biomarkers using calculated HSROC parameters. MMP-9, matrix metalloproteinase-9: lambda: 3.985; theta: 1.162; beta: 1.205; sigma2alpha: 0.000; sigma2theta: 0.043; c-Fn, cellular fibronectin: lambda: 34.708; theta: 17.156; beta: 4.316; sigma2alpha: 0.000; sigma2theta: 0.048; NLR, neutrophil-lymphocyte ratio: lambda: 1.709; theta: -0.258; beta: -0.367; sigma2alpha: 0.329; sigma2theta: 0.000; ferritin: lambda: 3.326; theta: 0.487; beta: 0.863; sigma2alpha: 2.067; sigma2theta: 0.000; S100B, S100 calcium-binding protein B: lambda: 1.910; theta: -0.004; beta: -0.592; sigma2alpha: 0.000; sigma2theta: 2.281; HSROC, hierarchical summary receiver operator characteristic.

Ferritin

Two studies comprising 886 patients evaluated ferritin in sHT using immunoassay techniques [26, 27]. Choi et al. [26] evaluated ferritin levels within 48 h from onset, whereas Millán et al. [27] serially measured the marker using 3 timepoints.

Baseline ferritin levels were increased in patients with PH and sHT (cutoff ≥ 144.8 ng/mL). Ferritin levels were the highest in patients with PH at 24 h from intervention [27]. Similarly, Choi et al. [26] reported thresholds of ≥ 164.1 ng/mL to predict PH and ≥ 171.8 ng/mL to predict sHT with good sensitivity and specificity. Baseline ferritin levels were significantly elevated in patients who had poor outcomes ($p < 0.001$) [27].

Both studies were included in the meta-analysis. Pooled DOR was 24.032 (95% CI 2.557–225.871), and pooled sensitivity and the false positivity rate were 0.802 and 0.140, respectively.

S100 Calcium-Binding Protein B

Two studies with 733 patients estimated baseline S100B in sHT using commercial immunoassays [22, 36]. Median S100B levels were elevated in patients with sHT with a threshold ≥ 0.23 $\mu\text{g/L}$ predicting parenchymal hemorrhage type-2 [36]. S100B was significantly elevated in spontaneous HT than in non-HT (0.1002 $\mu\text{g/L}$ vs. 0.01415 $\mu\text{g/L}$; $p = 0.003$) [22]. Meta-analysis revealed pooled DOR of 6.286 (95% CI 1.861–21.230), pooled sensitivity value of 0.782, and the false positivity rate of 0.32.

Neutrophil-Lymphocyte Ratio

Eight studies including 3,040 patients that estimated NLR from the complete blood count. All patients underwent thrombolytic intervention within 6 h from onset. One study [47] additionally included 3 timepoints for NLR estimation.

Baseline NLR values were significantly associated with sICH in 4 studies [33, 34, 44, 48] and with sHT in one study [38], post-revascularization. Thresholds varied across studies from 2.2, being the lowest cutoff value to 10.59, being the highest. No association was reported between baseline NLR and HT subtypes in 3 studies [41, 46, 47]. Guo et al. [47] reported no correlation between baseline NLR and sICH; however, the maximum peak was attained at 12–18 h post-revascularization.

Four studies reported baseline NLR to be significantly associated with poor outcomes [33, 34, 44, 48] with a higher mortality rate in patients with baseline NLR ≥ 7 [33]. Five studies comprising 2,172 patients were included for the meta-analysis (shown in Fig. 3). Pooled DOR for baseline NLR was 5.036 (95% CI 2.898–8.749) predicting sICH in patients who underwent revascularization (pooled sensitivity – 0.672 and pooled false positivity rate – 0.284).

Biomarkers Not Included in the Meta-Analysis

A forest plot could not be synthesized for biomarkers in fourteen studies due to insufficient data or reporting in a single study. Among single studies, caveolin-1 (99% NPV and 86% sensitivity) [45], soluble ST2 (area under the curve = 0.747) [30], and a combination of thrombin-activated fibrinolysis inhibitor and plasminogen activator inhibitor-1 (97.6% specificity, 97% NPV) [31] were found to be independent predictors of sHT.

Discussion

Our meta-analysis included 5 biomarkers, namely, MMP-9, c-Fn, ferritin, and S100B, associated with sHT and NLR with sICH, respectively. MMP-9, which was

commonly investigated and evaluated before thrombolysis, increased within 6–8 h after ischemia and was higher in patients who developed sHT, specifically, the PH subtype [21, 28]. Our meta-analysis showed that patients with MMP-9 levels ≥ 140 ng/mL had 29.5 times higher risk of developing sHT after stroke. SROC plot comparing the performance of all 5 biomarkers showed a high pooled sensitivity and a low false positivity rate for MMP-9, consistent with previous findings [19, 28], signifying MMP-9 with higher discriminating ability for predicting sHT risk. In our analysis, the highest sensitivity was obtained for c-Fn and ferritin. In the studies reviewed, serum levels of both markers correlated with each other as well as with MMP-9 levels at 24 h from onset in sHT patients [24, 27]. However, both c-Fn and ferritin generated wide confidence intervals in our meta-analysis thereby affecting their reliability in sHT prediction.

Our meta-analysis found both S100B and NLR to yield low pooled sensitivities and S100B with the highest false-positive rate. We speculate that the low sensitivity of S100B may be attributed to the heterogeneity in the HT subtypes between the 2 studies.

We also reviewed the correlation of markers with short-term outcome, which is closely linked to HT. Baseline levels of MMP-9, ferritin, and NLR were associated with the poor outcome. We found that NLR weakly correlated with sICH indicating low discriminative ability as compared to other markers. Further evaluation may be required for potential biomarkers, caveolin-1, thrombin-activated fibrinolysis inhibitor, plasminogen activator inhibitor-1, and soluble ST2 reported in single studies.

The strengths of our review were the availability of individual studies with at least 2 well-studied biomarkers such as MMP-9 and NLR. However, some limitations may affect our findings. Heterogeneity in the patient population, timing of blood samples, whether plasma or serum was used, commercial assay kits, and the reporting of HT subtypes may explain the varying thresholds of MMP-9 and NLR across studies. The pooled analysis conducted in the present study was based on random effects model due to the presence of heterogeneity in cutoff values and varied techniques used as shown in Figure 4. Though heterogeneity is a known feature in diagnostic test accuracy studies, large sample studies are recommended to ascertain diagnostic test accuracies of multiple biomarkers. Also, the pooled analysis of c-Fn and ferritin in the present study were limited due to the availability of only 2 studies for each marker among the included studies. Moreover, some studies did not exclude patients with prior infections, anticoagulant, and antithrombotic use

which may influence the severity and frequency of HT and its subtypes. Finally, most of the included studies followed a monocentric design which may limit the generalization of our findings.

In conclusion, MMP-9 was found to be a sensitive and specific marker for predicting the risk of sHT with high diagnostic accuracy, particularly for severe forms of HT in AIS patients. Baseline levels of MMP-9, ferritin, and NLR also predicted poor short-term outcome.

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Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on the published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

P.N.S., S.K., and B.S. conceived and designed the study; S.K. and J.J.K. performed search of the data; S.K. and G.S. conducted the systematic review and meta-analysis; P.N.S. and S.K. drafted and critically revised the manuscript. Final approval of the version to be published was given by C.F., W.T.K., M.M., M.S., I.M., R.B., K.M., and L.M.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary files. Further enquiries can be directed to the corresponding author.

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