Non-coding RNAs versus protein biomarkers to diagnose and differentiate acute stroke

Florijn, Barend W; Leontien van der Bent, M; Nguyen, Truc My T; Quax, Paul H A; Wermer, Marieke J H; Yaël Nossent, A; Kruyt, Nyika D

Published in:
Journal of Stroke & Cerebrovascular Diseases

DOI:
10.1016/j.jstrokecerebrovasdis.2023.107388

Publication date:
2023

Journal of Stroke and Cerebrovascular Diseases 32 (2023) 107388

Contents lists available at ScienceDirect

Journal of Stroke and Cerebrovascular Diseases

journal homepage: www.elsevier.com/locate/jstroke

Non-coding RNAs versus protein biomarkers to diagnose and differentiate acute stroke: Systematic review and meta-analysis

Barend W. Florijn, MD, PhD. [a,c,*], M. Leontien van der Bent, PhD. [b,c], Truc My T. Nguyen, MD, PhD. [a], Paul H.A. Quax, PhD [b,c], Marieke J.H. Wermer, MD, PhD [a,e], A. Yaël Nossent, PhD. [b,c,1], Nyika D. Kruyt, MD, PhD. [a,d,1]

[a] Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands
[b] Department of Surgery, Leiden University Medical Center, Leiden, the Netherlands
[c] Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, the Netherlands
[d] Department of Neurology Institution, Leiden University Medical Center, Leiden/The Hague, Zuid Holland, The Netherlands
[e] Department of Neurology, University Medical Center Groningen, Groningen, the Netherlands

ARTICLE INFO

Keywords:
Stroke
Biomarkers
Non-coding RNAs
Meta-analysis

ABSTRACT

Background: Stroke diagnosis is dependent on lengthy clinical and neuroimaging assessments, while rapid treatment initiation improves clinical outcome. Currently, more sensitive biomarker assays of both non-coding RNA- and protein biomarkers have improved their detectability, which could accelerate stroke diagnosis. This systematic review and meta-analysis compares non-coding RNA- with protein biomarkers for their potential to diagnose and differentiate acute stroke (subtypes) in (pre-)hospital settings.

Methods: We performed a systematic review and meta-analysis of studies evaluating diagnostic performance of non-coding RNA- and protein biomarkers to differentiate acute ischemic and hemorrhagic stroke, stroke mimics, and (healthy) controls. Quality appraisal of individual studies was assessed using the QUADAS-2 tool while the meta-analysis was performed with the sROC approach and by assessing pooled sensitivity and specificity, diagnostic odds ratios, positive- and negative likelihood ratios, and the Youden Index.

Summary of review: 112 studies were included in the systematic review and 42 studies in the meta-analysis containing 11627 patients with ischemic strokes, 2110 patients with hemorrhagic strokes, 1393 patients with a stroke mimic, and 5548 healthy controls. Proteins (IL-6 and S100 calcium-binding protein B (S100B)) and microRNAs (miR-30a) have similar performance in ischemic stroke diagnosis. To differentiate between ischemic- or hemorrhagic strokes, glial fibrillary acidic protein (GFAP) levels and autoantibodies to the NR2 peptide (NR2aAb, a cleavage product of NMDA neuroreceptors) were best performing whereas no investigated protein or non-coding RNA biomarkers differentiated stroke from stroke mimics with high diagnostic potential.

Conclusions: Despite sampling time differences, circulating microRNAs (< 24 h) and proteins (< 4,5 h) perform equally well in ischemic stroke diagnosis. GFAP differentiates stroke subtypes, while a biomarker panel of GFAP and UCH-L1 improved the sensitivity and specificity of UCH-L1 alone to differentiate stroke.

Introduction

Rapid establishment of ischemic stroke diagnosis, preferably in the pre-hospital setting, accelerates treatment and improves clinical outcomes (TIME=brain).[1] Biomarkers that differentiate ischemic- from hemorrhagic stroke and strokes from stroke mimics (i.e., conditions with a clinical stroke presentation but a different underlying pathology) potentially fit this profile. Ideally, stroke biomarkers should have high accuracy across a variety of populations and are rapidly assessed with a point-of-care (POC) device. Various blood biomarker candidates have been investigated so far but it remains unclear whether they can be used as a POC test in prehospital or acute clinical settings.[2]

* Corresponding author at: Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.
E-mail address: b.w.florijn@lumc.nl (B.W. Florijn).
[1] These authors contributed equally.

https://doi.org/10.1016/j.jstrokecerebrovasdis.2023.107388
Received 14 July 2023; Received in revised form 16 September 2023; Accepted 24 September 2023
Available online 29 September 2023

Categories of potential acute stroke biomarkers are plasma non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) or circulatingproteins and -metabolites, although the reviews until now have been restricted to the analysis of single categories of biomarkers. These reviews have demonstrated high accuracy for plasma miRNAs, particularly in differentiating acute from subacute ischemic stroke,[3] while plasma proteins differentiated ischemic stroke from hemorrhagic stroke[4] and ischemic stroke from stroke mimics.[5] However, there has been no study so far, that has compared ncRNAs with established protein biomarkers in stroke. In other cardiovascular disease pathologies, like myocardial injury, a comparative assessment of ncRNAs and protein biomarkers identified that the combination of miRNAs with proteins (cardiac troponins) yielded the highest area under the curve values for myocardial injury.[6] Because ncRNAs,[7] and particularly microRNAs,[8] have shown potential for implementation in (pre-)hospital clinical settings, their potential in comparison to protein biomarkers to diagnose and differentiate acute stroke needs to be analysed.

This systematic review and meta-analysis compares the performance of circulating non-coding RNAs versus proteins to diagnose and differentiate acute stroke (i.e., within 24 h of ictus). We compare single biomarkers and biomarker-panels to assess the effect of time-from-stroke-onset on the diagnostic performance of different biomarkers (Supplemental Results).

Methods

Information sources and study selection

The systematic review was conducted conforming to PRISMA guidelines.[9] The review was not registered. We performed a systematic search of PubMed, EMBASE, Web of Science, and Cochrane and included studies published from 1-1-1974 to 19-8-2022. The full search strategy can be found in Supplemental File 1. Additional studies were identified by screening bibliographies of relevant review studies. Titles and abstracts of the studies that were identified through the systematic search were screened by one of the reviewers (BWF, TTMN, or MLvdB). In case of doubt, studies were included in the full-text screening. All full-text articles were screened independently by three reviewers (BWF, TTMN, MLvdB). Cases of disagreement were discussed or a fourth independent reviewer, blinded from the scoring of the three reviewers (BWF, TTMN and MLvdB), could be consulted to reach consensus.

Criteria for eligibility of studies

Criteria for study eligibility were formulated using the *PICO* framework.[10] The population (*P*) was defined as patients with ischemic stroke, hemorrhagic stroke, or stroke mimic. The intervention (*I*) was defined as the measurement of any stroke-diagnosing blood biomarker within 24 h ictus. The comparison (*C*) had to be with either i.) healthy controls ii.) stroke mimics or iii.) hemorrhagic stroke. When studies described a comparison to a transient ischemic attack (TIA), without any corresponding lesions found on neuroimaging, they were excluded due to diagnostic uncertainty. The outcome (*O*) was a measure of the diagnostic accuracy of biomarker(s). Studies were excluded if i.) the full text was not written in the English language, ii.) they did not include original research, iii.) they described an animal or postmortem study, iv.) they did not concern a stroke population or a comparison as defined above, v.) they did not study a blood biomarker, vi.) the time from stroke onset to sampling was more than 24 h or not reported, vii.) they did not describe a diagnostic study viii) studies were determining prognostic biomarkers, ix.) they reported on a case series (n $<$10), or x.) the blood sample was collected after thrombolysis or thrombectomy given the confounding effect of stroke treatments on circulating biomarker levels.[11]

Data extraction and assessment of the methodological quality of studies in the systematic review

Data were extracted by either one of three reviewers (BWF, TTMN, MLvdB). All data were collected and subsequently entered in RevMan5.4 (Cochrane). The following data items were collected from each study if available: year of publication, digital object identifier (doi), title, journal, country, study setting, inclusion period, study scene, study design, study groups, control groups, sample size, age, sex, baseline NIH Stroke Scale (NIHSS) score, time from stroke onset to admission or venipuncture (Supplemental Results), inclusion criteria, exclusion criteria, control group characteristics, aim of the study, biomarker class, unbiased approach (i.e. unbiased screening efforts to find new biomarkers with novel techniques), specific biomarker(s), clinical outcome measures, neuro-imaging modality, infarct and/or hemorrhage volume, reference test, biofluid type, time from stroke onset to first and any consecutive sample collection, methods of sample collection, methods of sample analysis, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), confidence intervals, cut-off values, area under receiver operating characteristic curve (AUROC), main findings and the risk of bias. In cases where a ROC curve was provided, but not the sensitivity and specificity, we determined sensitivity and specificity manually by selecting the point on the ROC curve where the sensitivity equals the specificity, namely at the intersection of the ROC curve with an overlaid diagonal line from the top left to the bottom right. The square root of the area from the bottom right of the graph to the intersection is then equal to the sensitivity and specificity.[12] In addition, we assessed sample timing to determine which biomarker can be used in preclinical or clinical settings in which the time window of sampling should be less than 4.5 h.

Quality assessment of studies in the systematic review

We used the QUADAS-2 items to assess the risk of bias and the applicability to the general population with the following four methodological points: patient selection, the index test, the reference standard used, and the flow of patients through the study or timing of the index test.[13] Each study was evaluated independently by three reviewers (BWF, TTMN, MLvdB) and a fourth reviewer was consulted in the case of discrepancies. Publication bias and/or selective reporting were not formally assessed.

Statistical analysis and data synthesis of studies in het meta-analysis

Articles investigating biomarkers that were included in at least 2 separate studies (two studies to allow for pooling, fitting the predefined inclusion and exclusion criteria) were included in the meta-analysis. We conducted exploratory analysis by plotting estimates of sensitivity and specificity from included studies in forest plots and in receiver operating characteristic (ROC) curves. Parameters for the summary curve and summary point were determined by bivariate model fitting.[14] The heterogeneity of studies was assessed through visual examination of the forest plot and the SROC plot for each biomarker. The Youden Index (sensitivity + specificity – 1, a common summary measure of the ROC curve that demonstrates the maximum potential of a biomarker) was assessed to investigate the overall performance of biomarkers. A Youden Index $>$ 0.5 was considered clinically relevant.[15] Bivariate model fitting, diagnostic odds ratios (DOR), positive- and negative likelihood ratios, pooled estimates of sensitivity and specificity, and the corresponding 95 % CIs in the meta-analysis were determined using the R version 3.2.3, as described by Partlett and Takwoingi.[16] Data processing and statistical analysis were conducted using Review Manager version 5.4 (Cochrane Collaboration, Copenhagen, Denmark).

Results

Description and characteristics of studies

We identified a total of 4252 studies. After removing duplicates, 2565 studies were screened by title and abstract of which 2074 were excluded because they did not meet our criteria. Following the initial screening, 491 studies were assessed for eligibility based on the full-text, after which a further 379 were excluded. Finally, we included 112 studies in the systematic review. An overview of the study selection is shown in Fig. 1 and a summary of the study characteristics can be found in Supplemental Table 1. After removing the articles investigating a biomarker that was not investigated by any other study, 42 articles were included in the meta-analysis (Table 1). All studies were executed in an in-hospital setting, except for three studies that were (also) done in the pre-hospital setting.[17-19] Most of the studies (n = 58) had a case-control design and 36 studies had a cohort design. Furthermore, 18 studies had a hybrid design, although the analysis also included a comparison of ischemic stroke compared to hemorrhagic stroke. Final diagnosis assessment (i.e. ischemic stroke, hemorrhagic stroke or stroke mimic diagnosis) was validated by a combination of MRI and CT (n = 53), MRI alone (n = 18), CT alone (although not specified whether there should be large vessel occlusion or perfusion deficit) (n = 29), transcranial doppler (n = 1), or was not specified (n = 8). Lastly, 99 studies were single-center studies, and 13 studies were multicenter.

Identified biomarker subtypes

A total of 94 plasma miRNAs were reported when validated (i.e. following validation experiments after their expression was initially identified in test cohorts). 8 miRNA-studies used microarrays and 1 study used RNA-sequencing of identified microRNAs. The most often studied plasma miRNAs were miRNA-16 (n = 6), let-7b (n = 4), miRNA-106b-5p (n = 4), miRNA-126 (n = 4), and miRNA-21-5p (n = 4). Two studies tested plasma microRNA panels, with either 3 (miRNA-125a-5p, miRNA-125b-5p and miRNA-143-3p) or 5 (miRNA-126, miRNA-130a, miRNA-222, miRNA-218 and miRNA-185) plasma microRNAs each, both in which compared ischemic- versus hemorrhagic stroke. One study reported a combination of 10 plasma miRNA molecules, also with the ischemic- versus hemorrhagic stroke. Finally, three studies combined plasma miRNAs with plasma proteins.[20,21,22] Proteins that were most often studied were S100 calcium-binding protein B (S100B) (n = 26), GFAP (n = 18), high-sensitivity C-reactive protein (hs-)CRP (n = 19), matrix metalloproteinase-9 (MMP-9) (n = 15), interleukin-6 (IL-6) (n = 12), brain natriuretic peptide (BNP) (n = 10), and D-dimer (n = 10). Protein panels that were most often studied were GFAP and ubiquitin C-terminal hydrolase L1 (UCH-L1) (n = 2) and the panel s100B, MMP-9, beta-nerve growth factor (BNGF), von Willebrand factor (vWF), and

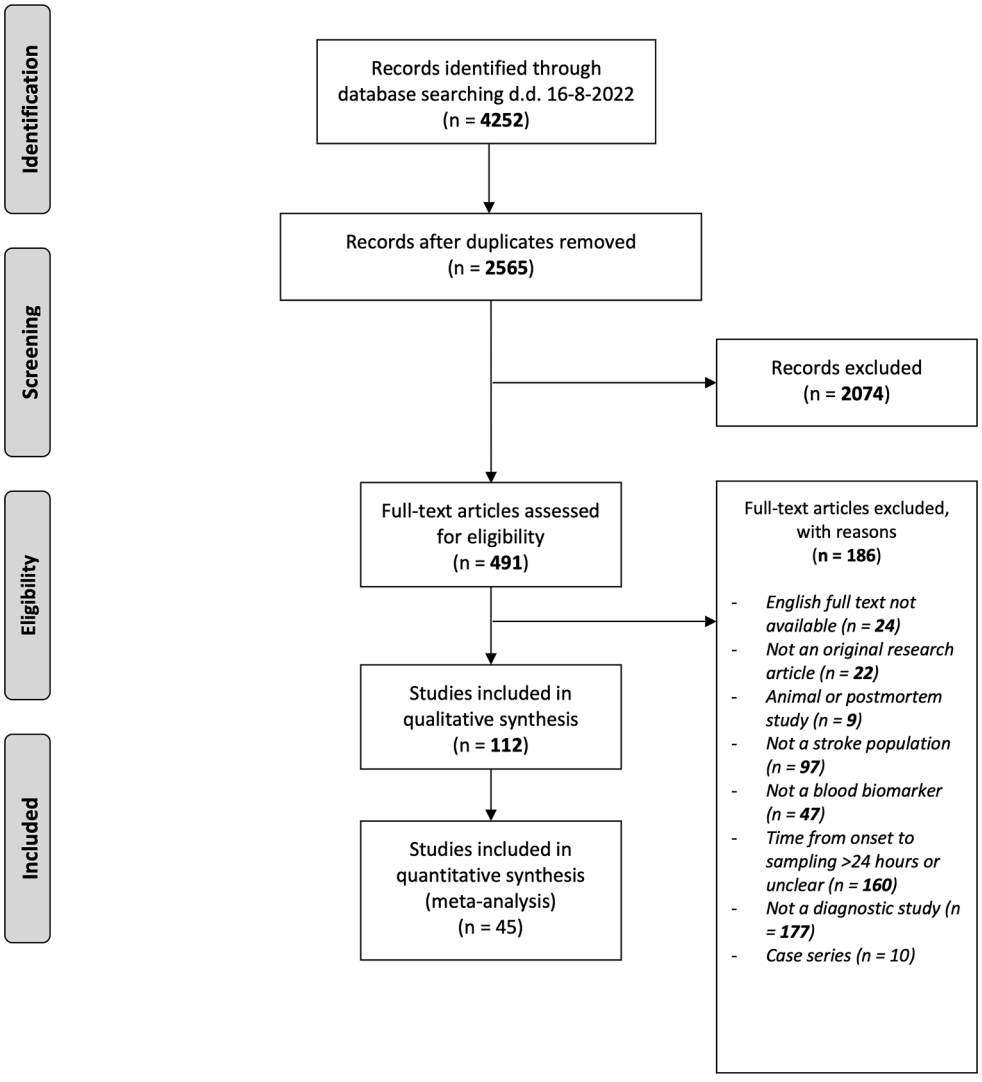

Fig. 1. Flow diagram of the identified, in- and excluded articles at each stage.

Table 1
Summary of characteristics for each biomarker in the meta-analysis.

Biomarker	Number of studies	Number of patients	Number of controls	Timing of sample collection (hrs)	Studies with cut-off
Ischemic stroke compared to controls					
S100B	3	352	407	6-12-24	2
CRP	5	692	684	6-12-24-24-24	1
IMA	2	85	75	6-24	2
Il-6	2	522	585	6-24	0
NSE	2	298	290	24-24	1
miR-30a	2	340	74	6–24	1
Ischemic stroke compared to hemorrhagic stroke					
GFAP	12	1145 438	161	1^2-2-3-4^2-6^3-12^2-24	11
S100B	2	424 77	325	6-24	0
CRP	2	287 67	325	24-24	1
NR2aAb	2	80 41	307	3-24	1
UCH-L1	2	250 109	73	4-4.5	2
UCH-L1 + GFAP	2	250 109	73	4-4.5	2
Stroke compared to stroke mimics					
MMP-9	2	958	127	3-24	2
D-dimer	2	1104	127	6-24	2

For the comparison ischemic stroke versus hemorrhagic stroke the column depicting number of patients was split into ischemic stroke and hemorrhagic stroke. The column timing of sample collection displays sample timing for each study separately.

monocyte chemoattractant protein-1 (MCP-1) (n = 3 studies, although this panel was tested using different comparisons such as ischemic stroke compared to controls, hemorrhagic stroke compared to controls, and strokes compared to controls).

Methodological assessment of studies

64 studies (56.6 %) showed a high risk of bias in the patient selection domain (Fig. 2). A small majority of the studies consisted of case-control studies (n = 58 studies) which can be over- or underrepresented in different groups because of the retrospective nature of the data. Furthermore, a high risk of bias was also seen in the index test domain in 37 studies (33 %). The most common reason for studies to score high risk of bias for the index test was that cut-off values were not pre-specified. A low risk of bias was seen in the reference standard domain in 84 studies (75 %), while an unclear risk of bias was seen in the flow and timing domain in 63 studies (56.3 %). Most studies demonstrated a low risk of bias in the domain of applicability concerns based on reference standards (94 %), patient selection (94 %), and index test (99 %). Given the variation across studies, and since there were insufficient studies in all three comparisons, we did not perform meta-regression by including each potential source of heterogeneity as a covariate in the bivariate model as planned.

Youden's Index of included studies

When we calculated the Youden's Index for each biomarker and each comparison, we observed that most reported miRNAs were assessed for the comparison of ischemic stroke to healthy controls or stroke mimics. These identified miRNAs displayed a higher average Youden's Index (0.77, 95 %CI 0.71–0.83) compared to protein biomarkers (0.56, 95 %CI 0.47–0.64) (Fig. 3A). When hemorrhagic stroke was compared to controls, the mean Youden's Index for protein biomarkers was 0.53 (95 %CI 0.29–0.77) (Fig. 3B). A comparable value was identified for proteins (0.54, 95 %CI 0.44–0.64) when hemorrhagic strokes were compared to ischemic strokes (Fig. 3C). The Youden Index for the comparison of stroke versus stroke mimics for circulating proteins was 0.2 (95 %CI 0.14–0.26) (Fig. 3D).

Biomarkers for the diagnosis of ischemic stroke

The forest plot and SROC curve of biomarkers differentiating ischemic stroke from controls are depicted in Figs. 4 and 7A, respectively. In total, 10 studies were included in the meta-analysis including 1028 patients with ischemic stroke, 46 patients with a stroke mimic, and 915 controls. The pooled diagnostic odds ratios of S100B (21.19, 95 % CI 12.52–29.85), IL-6 (33, 95 % CI –25.44 to 91.46), ischemia-modified albumin (IMA) (15.80, 95 % CI 1.46–30.14) and miRNA-30 (30.67,

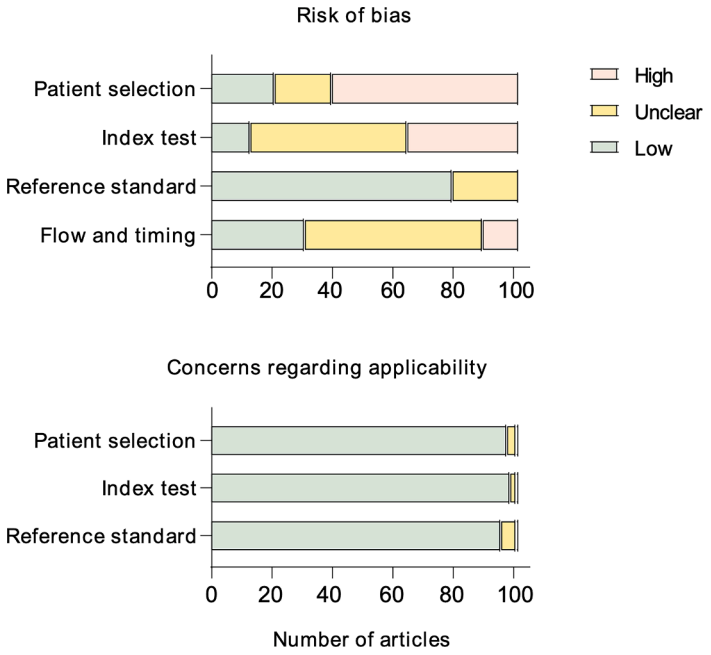

Fig. 2. Risk of bias assessment using the QUADAS-2 tool. The risk of bias and concerns regarding applicability for each individual study were scored as either high, unclear or, low.

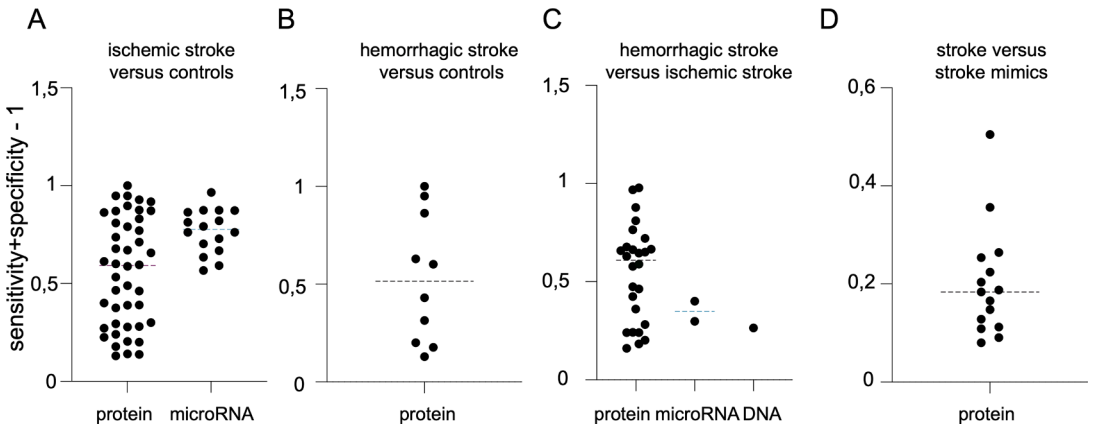

Fig. 3. The Youden Index (a common summary measure of the ROC curve) was assessed to investigate the overall performance of biomarkers. Subgroup analysis of Youden's Indices (Sensitivity + Specificity – 1) for different biomarker classes per comparison was plotted for (A) ischemic stroke versus controls (B) hemorrhagic strokes versus controls (C) hemorrhagic strokes versus ischemic stroke and (D) stroke versus stroke mimics.

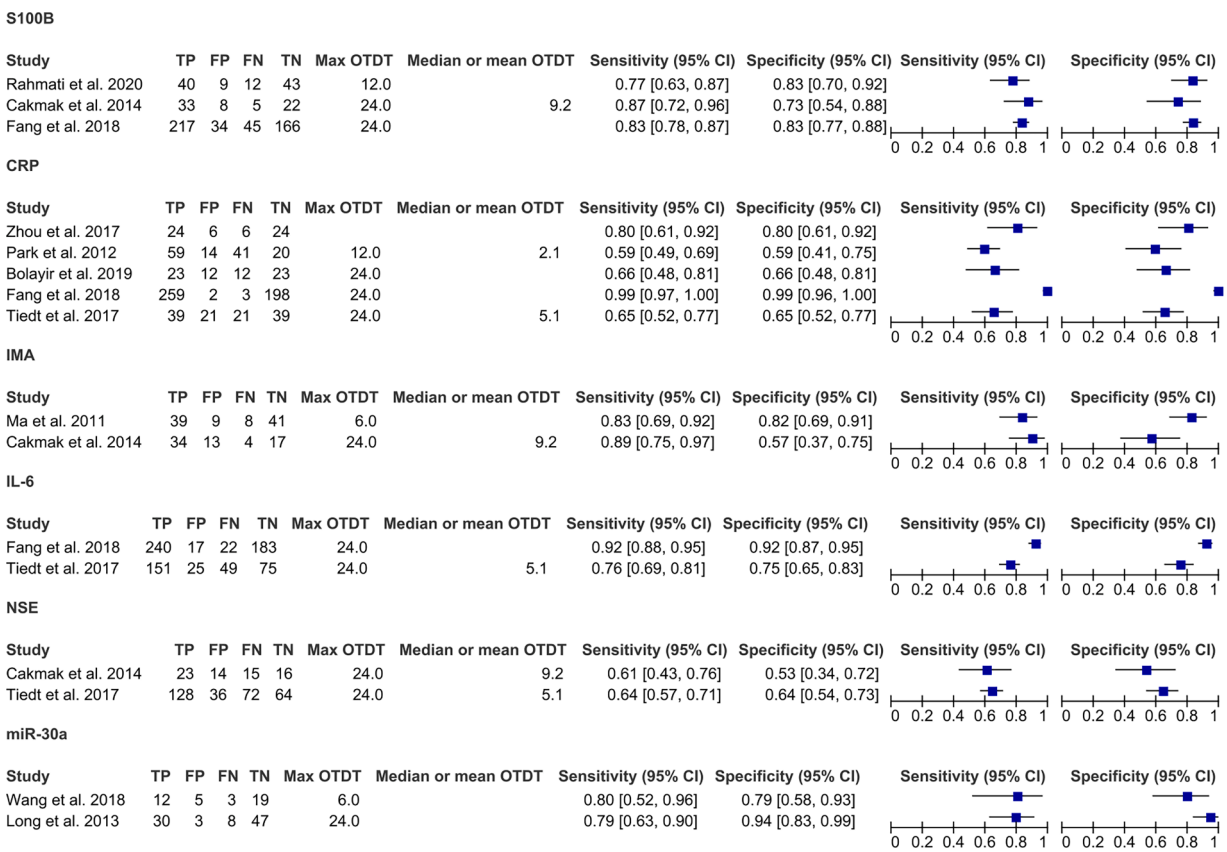

Fig. 4. Forest plot of single biomarkers for which sensitivity and specificity could be retrieved from at least two studies comparing ischemic strokes and healthy controls. For studies reporting subgroups at different times from onset, the subgroup with the indicated OTDT was used. Horizontal lines represent 95 % confidence intervals. TP= true positive; FP= false positive; FN= false negative; TN= true negative.

95 % CI –5.73 to 67.07) demonstrated the potential for differentiating ischemic- from hemorrhagic stroke. Pooled estimates of sensitivity and specificity displayed similar values (Table 3). SROC curve analysis (Fig. 7) pinpoints variability for both CRP and IL-6 while the forest plot (Fig. 4) visualises variability for IL-6 only, particularly in the sensitivity and specificity, as identified in two studies.[21,23] In one of these two studies, biomarkers levels were obtained within 6 h after ischemic stroke onset in a study in which they compared ischemic stroke patients (n = 262) to healthy controls (n = 200) and patient controls (n = 125), yielding significant AUC values, not only for IL-6 (0.96), but also for CRP (0.99), plasminogen activator inhibitor-1 (PAI-1) (0.99), P-selectin (0.91), and tumour necrosis factor alpha (TNF-α) (0.99).[23] Furthermore, regarding miRNA-30a (DOR 30.67, 95 % CI –5.73 to 67.07 and sensitivity 70–80 % and specificity 79–94), both the included studies compared 149 ischemic stroke patients with 74 controls.[24,25] The latter study also identified a significant decrease for miRNA-126 in ischemic stroke patients (n = 197) with AUC values of 0.92, 0.94, 0.93, and 0.92, at 24 h, 1 week, 4 weeks and, 24 weeks respectively.[25] Lastly, the pooled DOR of CRP levels in ischemic stroke (19.99) displayed a 95 % CI of –33.56 to 73.53 and the largest variability.

Ischemic stroke compared to hemorrhagic stroke

The forest plot of biomarkers differentiating ischemic stroke from hemorrhagic stroke is depicted in Fig. 5 comprising of 17 studies that included 1778 ischemic stroke patients, 572 hemorrhagic stroke patients, and 733 controls. GFAP had a strong DOR (43.57, 95 % CI 43.04–44.10) with a positive likelihood ratio of 8.48 (95 % CI 8.41–8.54). Based on the SROC analysis, GFAP levels and NR2aAb (a peptide fragment of synaptic N-Methyl-D-aspartate receptors that passes the blood-brain barrier (BBB) after ischemia) were the best-performing biomarkers to differentiate ischemic stroke from hemorrhagic stroke, although the corresponding confidence intervals of the NR2aAb DOR indicates large variability (24.15, 95 % CI –80.15 to 128.45). This is mostly because only two studies investigated NR2aAb, in a total of 80 ischemic stroke patients and 41 hemorrhagic stroke patients.[26,27] Regarding GFAP, sensitivity and specificity values ranged from 36 to 96 % and 69 to 100 % respectively (Fig. 5). Particular heterogeneity was seen in the study that demonstrated that GFAP differentiated ischemic stroke and hemorrhagic stroke within 4.5 h of symptom onset with a sensitivity of 84.2 % and a specificity of 96.3 % (AUC 0.92).[28] In another study, GFAP generated an AUC of 0.86 within 4.5 h of symptom onset, with a sensitivity of 61 % and a specificity of 96 % using a cut-off of 0.34 ng/ml.[29] Alternatively, GFAP also provided a sensitivity of 77.8 % and a specificity of 94.2 % among 59 hemorrhagic stroke patients and 148 ischemic stroke patients (AUC 0.87, 95 % CI, 0.80– 0.94).[30] Lastly, regarding protein panels, the combination of GFAP and UCH-L1 displayed a DOR of 16.52 (6.0–27.03) (Table 2) and a specificity of 82 % (Table 3).

Table 2
Calculated diagnostic accuracy analysis

	DOR	LR+	LR-
Ischemic stroke compared to controls			
S100B	21.19 (12.52–29.85)	4.55 (3.40–5.71)	0.22 (0.17–0.27)
CRP	19.99 (–33.56 to 73.53)	4.50 (–1.66 to 10.66)	0.23 (–0.07 to 0.52)
IMA	15.80 (1.46–30.14)	3.02 (1.19–4.85)	0.19 (0.08–0.30)
IL-6	33 (–25.44 to 91.46)	5.72 (–0.52 to 10.92)	0.17 (0.02–0.33)
NSE	2.78 (1.55–4.00)	1.65 (1.26–2.04)	0.59 (0.46–0.72)
MiR-30a	30.67 (–5.73 to 67.07)	7.16 (0.82–13.59)	0.23 (0.11–0.36)
ischemic stroke compared to hemorrhagic stroke			
GFAP	43.57 (43.04–44.10)	8.48 (8.41–8.54)	0.194 (0.193–0.196)
S100B	5.23 (-6.38–16.83)	2.03 (–0.23 to 4.29)	0.39 (-0.05–0.83)
CRP	6.19 (2.57–9.80)	2.55 (1.93–3.17)	0.41 (0.26–0.57)
NR2aAb	24.15 (-80.15 to 128.45)	6.05 (–7.93 to 20.03)	0.25 (–0.27 to 0.77)
UCH-L1	18.83 (8.54–29.11)	6.89 (4.40–9.38)	0.37 (0.27–0.46)
GFAP/UCH-L1	16.52 (6.0–27.03)	4.40 (1.18–7.63)	0.26 (0.12–0.42)
stroke compared to stroke mimics			
MMP9	2.30 (1.39–3.20)	1.45 (1.15–1.76)	0.63 (0.51–0.75)
D-dimer	2.90 (2.14–3.67)	1.35 (1.22–1.48)	0.46 (0.38–0.54)

DOR diagnostic odds ratio; LR+ positive likelihood ratio; LR- negative likelihood ratio.

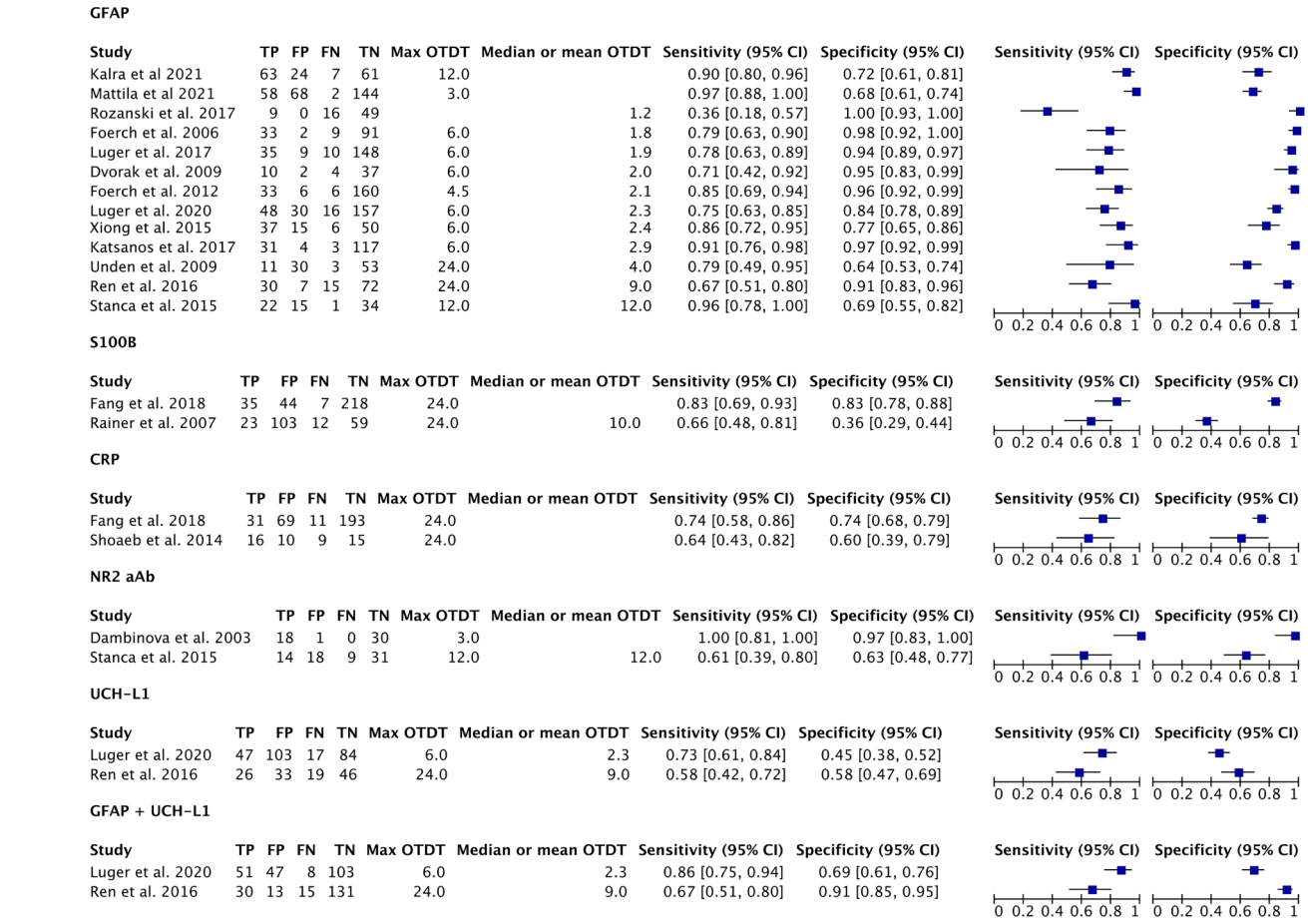

Fig. 5. Forest plot of single biomarkers for which sensitivity and specificity could be retrieved from at least two studies comparing ischemic strokes and hemorrhagic strokes. For studies reporting subgroups at different times from onset, the subgroup with the indicated OTDT was used. Horizontal lines represent 95 % confidence intervals. TP= true positive; FP= false positive; FN= false negative; TN= true negative.

Table 3
Calculated pooled estimates of sensitivity and specificity

	Sensitivity	Specificity
ischemic stroke compared to controls		
S100B	0.82 (0.78–0.86)	0.82 (0.77–0.86)
CRP	0.82 (0.54–0.94)	0.82 (0.53–0.95)
IMA	0.86 (0.76–0.93)	0.71 (0.51–0.86)
IL-6	0.85 (0.70–0.93)	0.85 (0.69–0.94)
NSE	0.63 (0.57–0.69)	0.62 (0.53–0.70)
MiR-30a	0.79 (0.66–0.88)	0.89 (0.75–0.96)
ischemic stroke compared to hemorrhagic stroke		
GFAP	0.824 (0.823–0.826)	0.903 (0.902–0.904)
S100B	0.75 (0.57–0.88)	0.63 (0.27–0.88)
CRP	0.70 (0.58–0.80)	0.73 (0.67–0.77)
NR2aAb	0.78 (0.25–0.98)	0.87 (0.44–0.98)
UCH-L1	0.67 (0.58–0.75)	0.50 (0.41–0.59)
GFAP/UCH-L1	0.78 (0.59–0.90)	0.82 (0.61–0.93)
stroke compared to stroke mimics		
MMP9	0.65 (0.62–0.68)	0.55 (0.46–0.64)
D-dimer	0.82 (0.80–0.83)	0.39 (0.34–0.45)

Stroke compared to stroke mimics

The sensitivity and specificity of biomarkers (Table 3) in the meta-analysis of stroke compared to stroke mimics (Fig. 6) were generally much lower than the other comparisons. As such, no specific biomarkers differentiated strokes from stroke mimics. Two biomarkers (D-dimer and MMP-9) were included and demonstrated moderate diagnostic accuracy (DOR ranging from 2.3 to 2.9, Table 2). One particular study tested a multi-biomarker panel to differentiate ischemic strokes (n = 941) from hemorrhagic strokes (n = 174) and stroke mimics.[31] Logistic regression analysis showed that, when relevant clinical variables (age, sex, alcohol, dyslipidemia, atrial fibrillation, previous stroke, systolic blood pressure, and baseline NIHSS) were included in the regression model, none of the studied biomarkers independently associated with the discrimination between stroke and stroke-mimics. When baseline NIHSS was excluded however, only D-dimer emerged as a significant contributor to the model to differentiate stroke from mimics, while the AUC of the model was reduced from 0.81 to 0.76. The models proposed included several clinical parameters, in addition to several blood biomarkers, and did not show clearly different AUC values for these comparisons in a validation cohort (0.74 for stroke compared to stroke mimics). Nonetheless, from a study among 915 patients with stroke compared to 90 patients with a stroke mimic, results could suggest that D-dimer levels may function as an independent predictor of stroke compared to mimics (OR = 2.97; 95 % CI 1.72–5.16, sensitivity 81 %, specificity 38 %).[32]

Discussion

To the best of our knowledge, this is the first study combining a systematic review and meta-analysis of studies evaluating ncRNA- and protein biomarkers in acute stroke patients. The systematic review demonstrated a higher Youden Index of microRNAs, compared to proteins for ischemic stroke diagnosis regardless of the time window of sampling. In the meta-analysis, we identified that miRNA-30a and several protein biomarkers, like IL-6 and S100B, were the best performing biomarkers to distinguish ischemic stroke from controls. Protein biomarkers like circulating GFAP or UCH-L1, or their combination in a protein panel, provided the best differentiation of stroke subtypes. Future studies should compare plasma miR-30, or a panel combining miR-30 with IL-6 and S100B, to prehospital screening such as the FAST test.

The acronym FAST (facial drooping, arm weakness, speech difficulties and time) describes an emergency screening tool that has been used by the American Heart Association to educate the public on detecting symptoms of a stroke. Currently FAST triage is used in prehospital settings to clinically differentiate stroke from stroke mimics and a meta-analysis of 9 studies determined that it has a combined sensitivity of 0.77 [95 % CI (0.64–0.86)] and specificity of 0.60 [95 % CI (0.38–0.78)].[33] Nonetheless, in practice, about 40 % of stroke code patients triaged with FAST appear to have a stroke mimic,[34] while FAST also has poor diagnostic potential for posterior circulation stroke.[35] Therefore, novel biomarkers for stroke diagnosis should display higher diagnostic accuracy and robust positive likelihood ratios, not affected by disease prevalence. Given the high risk associated with incorrectly diagnosing a hemorrhagic stroke as ischemic, when proceeding with thrombolytics, specific target levels for sensitivity (detect ischemic stroke when truly present) and specificity (rule out hemorrhagic stroke when it is truly absent) should be robust.

From the meta-analysis, one could conclude that the early sampling time of proteins (< 4,5 h) and subacute sampling time of microRNAs (< 24 h) perform similarly. In our results, miRNA-30a has the highest specificity (89 %) compared to other protein biomarkers for ischemic stroke diagnosis. A recently published systematic review of stroke miRNA biomarkers demonstrated that their performance, as a whole category, has a DOR of 16 (95 % CI: 10–26).[36] In line with these results, our group previously published that another class of non-coding RNAs, namely tRNA-derived fragments were promising in their ability to distinguish between acute ischemic stroke, hemorrhagic stroke, and stroke-mimics.[7] Although these findings were exploratory, we could confirm them in an independent database of ischemic stroke and stroke mimic patients.[21]

With regard to the performance of circulating proteins in our study, IL-6 levels in 522 stroke patients (Table 1) displayed high sensitivity (85 %) and specificity (85 %) values for ischemic stroke diagnosis. This is in line with results from the most recently performed meta-analysis of stroke biomarkers in which IL-6 differentiated ischemic stroke from healthy controls, but not from stroke mimics or hemorrhagic stroke.[37] This meta-analysis also identified that brain natriuretic peptide and D-dimer differentiated ischemic stroke from healthy controls and stroke mimics or hemorrhagic stroke, a finding which we could not replicate due to different inclusion criteria of studies.[37] In contrast, in our study we identified other proteins like CRP, S100B and IMA (albeit that the latter was identified among n = 85 patients) with summary sensitivity

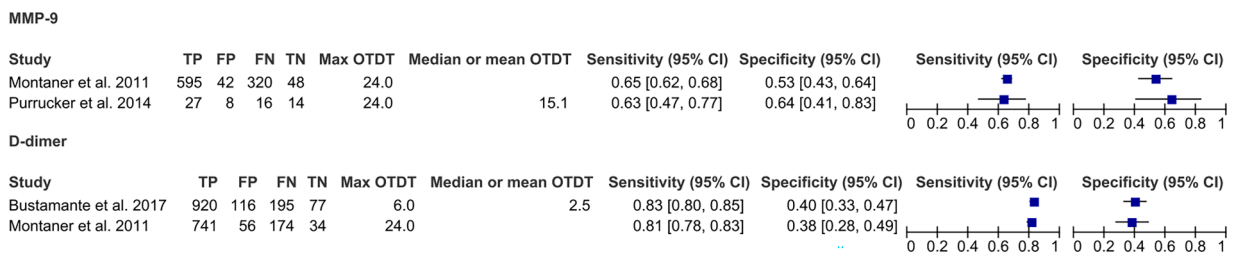

Fig. 6. A forest plot of single biomarkers for which sensitivity and specificity could be retrieved from at least two studies comparing real stroke and stroke mimics. For studies reporting subgroups at different times from onset, the subgroup with the indicated OTDT was used. Horizontal lines represent 95 % confidence intervals. TP= true positive; FP= false positive; FN= false negative; TN= true negative.

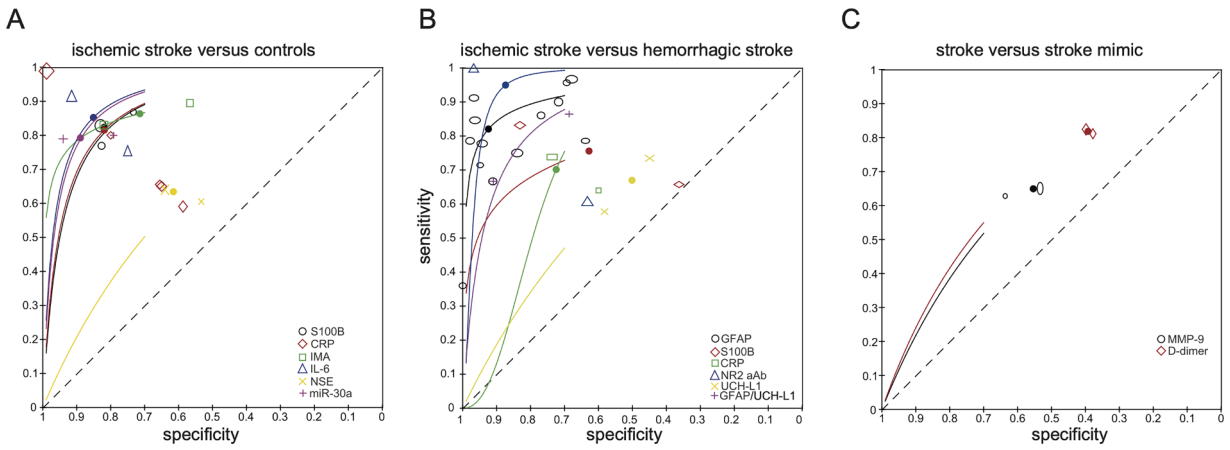

Fig. 7. Summary Receiver Operating Characteristic (sROC) curves of (A) ischemic stroke versus controls, (B) ischemic stroke versus hemorrhagic strokes, and (C) stroke versus stroke mimics. Only biomarkers for which sensitivity and specificity could be retrieved from at least two studies were included in this analysis. The size of the individual study points is proportional to the sample size. Parameters for the summary curve and summary point were determined by bivariate model fitting. Summary points are depicted as solid circles.

values ranging from 82 to 86 %. Moreover, the pooled results from 12 studies including 1145 stroke patients demonstrated that GFAP differentiated stroke patients into ischemic stroke or hemorrhagic stroke within 6 h with a sensitivity of 82 % and specificity of 90 %, a finding previously identified in the study by Misra et al.[37] However, in order for their routine use in clinical practice, these biomarkers also need to be fast and feasible in the (pre-)hospital setting. An important example of the application of stroke biomarkers is a recent study that tested the combination of GFAP with the Prehospital Stroke Score (PreSS) in the pre-hospital setting (< 4.5 h of symptom onset) thereby identifying stroke mimics and differentiating patients with large-vessel occlusion (LVO) from non-LVO patients.[38]

Another important question in our analysis was the performance of single biomarkers versus biomarker panels. Previous research demonstrated that GFAP and NR2aAb should always be included, in whatever possible combination of biomarkers, given they are the most promising brain-specific biomarkers related to stroke.[39] One particular study demonstrated that the combination of GFAP and NR2aAb differentiated stroke with a sensitivity and specificity of 94 % and 91 %, a significant improvement of the performance of each biomarker separately.[26] However, the performance of GFAP differs depending on the severity of hemorrhagic stroke[18] and our analysis shows that GFAP had the best performance when assessed within 6 h. Furthermore, since in stroke TIME=BRAIN, ideal biomarkers should be able to rule out hemorrhagic stroke with POC testing in a short time window.[40] Still, the included studies into NR2aAb investigated different time windows[27] or identified a rather low diagnostic accuracy.[26] Therefore, we think other promising combinations of biomarkers should also be studied, such as the combination of GFAP with levels of ubiquitin C-terminal hydrolase L1 (UCH-L1). Results from our meta-analysis suggest they perform better together compared to both biomarkers separately with a pooled diagnostic odds ratio of 16.52 (95 % CI 6.0–27.03), a positive likelihood ratio of 4.40 (95 % CI 1.18–7.63) (Table 2) and a specificity of 82 % (Table 3).

Our review has several limitations. First, although we excluded TIA's, studies exhibited some degree of heterogeneity regarding the establishment of stroke diagnosis and control groups that were used (either stroke mimics, healthy controls, or risk factor-matched controls). Another limitation when conducting a meta-analysis is the technical variability of immune-based protein assays. These can be a source of bias and inconsistent results across studies. In the meta-analysis however, 29 out of 42 studies have published assay cut-off values (Table 1) while assay cut-offs that maximized the sensitivity and specificity were also published. Furthermore, regarding sample size, studies were often conducted using small sample sizes and the identified biomarkers in these small cohorts were not validated in independent patient cohorts (except for GFAP, S100B, and CRP) nor were results corrected for multiple testing to reduce the risk of false positive (or negative) results. In addition, some studies did not perform ROC analysis, or ROC analysis was performed in discovery- but not in validation or replication cohorts. Lastly, future stroke biomarker studies should include stratification of biomarkers expression values by sex or race.

Conclusion

We conclude that acute sampling time (< 4.5 h) of circulating proteins and late sampling time of circulating microRNAs (< 24 h) has promising performance in diagnosing ischemic stroke. Promising biomarkers (Graphical Abstract) should be validated prospectively and replicated in larger unselected cohorts representing the various clinical subgroups of stroke-code patients. In addition, the statistical significance of biomarker performance should be corrected for multiple testing in both the discovery and validation phases, to reduce the risk of false positive results. This will improve diagnostic accuracy and thereby potential clinical applicability. To be clinically applicable, these studies should also focus on early time windows and biomarkers should be tested against a background of other (pre-hospital) triage tools.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jstrokecerebrovasdis.2023.107388.

References

1 Emberson J, Lees KR, Lyden P, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic

stroke: a meta-analysis of individual patient data from randomised trials. *Lancet*. 2014;384(9958):1929–1935.
2 Dagonnier M, Donnan GA, Davis SM, Dewey HM, Howells DW. Acute stroke biomarkers: are we there yet? *Front Neurol*. 2021;12, 619721.
3 Deng Y, Huang P, Zhang F, Chen T. Association of MicroRNAs with risk of stroke: a meta-analysis. *Frontiers in Neurology*. 2022;13.
4 Misra S, Kumar A, Kumar P, et al. Blood-based protein biomarkers for stroke differentiation: a systematic review. *Proteom Clin Appl*. 2017;11:9–10.
5 Monbailliu T, Goossens J, Hachimi-Idrissi S. Blood protein biomarkers as diagnostic tool for ischemic stroke: a systematic review. *Biomark Med*. 2017;11(6):503–512.
6 Schulte C, Barwari T, Joshi A, et al. Comparative analysis of circulating noncoding RNAs versus protein biomarkers in the detection of myocardial injury. *Circ Res*. 2019; 125(3):328–340.
7 Nguyen TTM, van der Bent ML, Wermer MJH, et al. Circulating tRNA fragments as a novel biomarker class to distinguish acute stroke subtypes. *Int J Mol Sci*. 2020;22(1).
8 Florijn BW, Bijkerk R, Kruyt ND, van Zonneveld AJ, Wermer MJH. Sex-specific MicroRNAs in neurovascular units in ischemic stroke. *Int J Mol Sci*. 2021;22(21).
9 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7), e1000097.
10 Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak*. 2007;7:16.
11 He XW, Shi YH, Liu YS, et al. Increased plasma levels of miR-124-3p, miR-125b-5p and miR-192-5p are associated with outcomes in acute ischaemic stroke patients receiving thrombolysis. *Atherosclerosis*. 2019;289:36–43.
12 Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: the case of tests with continuous results. *Biochem Med (Zagreb)*. 2016;26(3):297–307.
13 Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8): 529–536.
14 Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol*. 2005;58(10):982–990.
15 Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden index to discriminate individuals using pooled blood samples. *Epidemiology*. 2005;16(1):73–81.
16 Partlett C., Takwoingi Y. Meta-analysis of test accuracy studies in R: a summary of user-written programs and step-by-step guide to using glmer. Version 1.0. Available from: http://methodscochraneorg/sdt/. (2016).
17 Luger S, Jæger HS, Dixon J, et al. Diagnostic accuracy of Glial Fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase-L1 serum concentrations for differentiating acute intracerebral hemorrhage from ischemic stroke. *Neurocrit Care*. 2020;33(1):39–48.
18 Rozanski M, Waldschmidt C, Kunz A, et al. Glial Fibrillary acidic protein for prehospital diagnosis of intracerebral hemorrhage. *Cerebrovasc Dis*. 2017;43(1-2): 76–81.
19 Mattila OS, Ashton NJ, Blennow K, et al. Ultra-early differential diagnosis of acute cerebral ischemia and hemorrhagic stroke by measuring the prehospital release rate of GFAP. *Clin Chem*. 2021;67(10):1361–1372.
20 Jia L, Hao F, Wang W, Qu Y. Circulating miR-145 is associated with plasma high-sensitivity C-reactive protein in acute ischemic stroke patients. *Cell Biochem Funct*. 2015;33(5):314–319.
21 Tiedt S, Prestel M, Malik R, et al. RNA-seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as potential biomarkers for acute ischemic stroke. *Circ Res*. 2017;121(8):970–980.
22 Chen Z, Wang K, Huang J, et al. Upregulated serum MiR-146b serves as a biomarker for acute ischemic stroke. *Cell Physiol Biochem*. 2018;45(1):397–405.
23 Fang C, Lou B, Zhou J, et al. Blood biomarkers in ischemic stroke: role of biomarkers in differentiation of clinical phenotype. *European Journal of Inflammation*. 2018:16.
24 Wang W, Li D-B, Li R-Y, et al. Diagnosis of hyperacute and acute ischaemic stroke: the potential utility of exosomal MicroRNA-21-5p and MicroRNA-30a-5p. *Cerebrovascul Dis*. 2018:204–212, 212.
25 Long G, Wang F, Li H, et al. Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. *BMC Neurol*. 2013;13:178.
26 Stanca DM, Marginean IC, Soritau O, et al. GFAP and antibodies against NMDA receptor subunit NR2 as biomarkers for acute cerebrovascular diseases. *J Cell Molecul Med*. 2015;19(9):2253–2261.
27 Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-Methyl-d-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. *Clin Chem*. 2003;49(10):1752–1762.
28 Foerch C, Niessner M, Back T, et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. *Clin Chem*. 2012;58(1):237–245.
29 Ren C, Kobeissy F, Alawieh A, et al. Assessment of serum UCH-L1 and GFAP in acute stroke patients. *Sci Rep*. 2016;6:24588.
30 Luger S, Witsch J, Dietz A, et al. Glial Fibrillary acidic protein serum levels distinguish between intracerebral hemorrhage and cerebral ischemia in the early phase of stroke. *Clin Chem*. 2017;63(1):377–385.
31 Bustamante A, Lopez-Cancio E, Pich S, et al. Blood biomarkers for the early diagnosis of stroke: the stroke-chip study. *Stroke*. 2017;48(9):2419–2425.
32 Montaner J, Mendioroz M, Ribo M, et al. A panel of biomarkers including caspase-3 and D-dimer may differentiate acute stroke from stroke-mimicking conditions in the emergency department. *J Intern Med*. 2011;270(2):166–174.
33 Chen X, Zhao X, Xu F, Guo M, et al. A systematic review and meta-analysis comparing FAST and BEFAST in acute stroke patients. *Front Neurol*. 2021;12, 765069.
34 Nguyen TTM, van den Wijngaard IR, Bosch J, et al. Comparison of prehospital scales for predicting large anterior vessel occlusion in the ambulance setting. *JAMA Neurol*. 2021;78(2):157–164.
35 Hoyer C, Szabo K. Pitfalls in the diagnosis of posterior circulation stroke in the emergency setting. *Front Neurol*. 2021;12, 682827.
36 Zhao J, Wang Q, Zhu R, Yang J. Circulating non-coding RNAs as potential biomarkers for ischemic stroke: a systematic review. *J Mol Neurosci*. 2022;72(8): 1572–1585.
37 Misra S, Montaner J, Ramiro L, et al. Blood biomarkers for the diagnosis and differentiation of stroke: a systematic review and meta-analysis. *Int J Stroke*. 2020;15 (7):704–721.
38 Jæger HS, Tranberg D, Larsen K, et al. Diagnostic performance of Glial Fibrillary acidic protein and prehospital stroke scale for identification of stroke and stroke subtypes in an unselected patient cohort with symptom onset < 4.5 h. *Scand J Trauma Resusc Emerg Med*. 2023;31(1):1.
39 Baez SC, García Del Barco D, Hardy-Sosa A, et al. Scalable bio marker combinations for early stroke diagnosis: a systematic review. *Front Neurol*. 2021;12, 638693.
40 Jickling GC, Wartenberg KE. Can Biomarkers Differentiate Between Ischemic and Hemorrhagic Stroke in the Prehospital Setting? *Neurology*. 2021;96(15):689–690.