Early-Phase Fluid Diagnostic Biomarkers in Acute Ischemic Stroke: An Umbrella Review

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BACKGROUND

Acute ischemic stroke (AIS) is a time-critical emergency in which rapid diagnosis within established therapeutic windows is essential to optimize outcomes. Fluid biomarkers offer a promising adjunct to clinical and neuroimaging assessment but their temporal dynamics in the acute phases remain incompletely characterized.

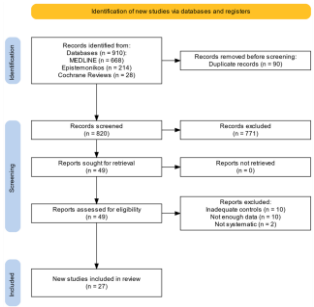

Figure 1. Prisma flowchart of the selected studies.

METHODS

We performed an umbrella review of systematic reviews and meta-analyses evaluating fluid biomarkers in AIS versus controls or stroke mimics (**Figure 1**). Quantitative synthesis of primary studies (random-effects meta-analysis of standardized mean differences [eG]) was stratified by clinically relevant time windows. Heterogeneity (I^2), small-study effects (Egger's test) and excess significance bias were assessed.

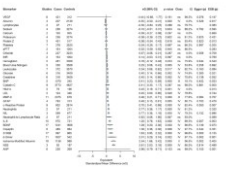

Figure 2. Meta-analyses for each factor, all time-points

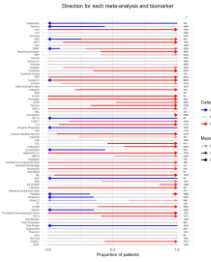

Figure 3. Summary of the systematic review: direction of biochemistry biomarkers (all primary studies combined)

RESULTS

We included 27 publications (18 biochemistry, 1 metabolomic, 10 transcriptomic, 5 cell-free-DNA [cfDNA]). Across all time-points the largest effect sizes were observed for neuron-specific enolase (NSE), ischemia-modified albumin (IMA), d-Dimer, S100B, GFAP, and IL-6 (**Figure 2,3; Table 1,2**). Looking at metabolites, studies revealed early accumulation of lactate, succinate, glutamate and lysophosphatidylcholines, alongside depletion of arginine, citrulline and citrate. A catalogue of 220 micro-RNAs (132 upregulated; 108 downregulated) identified robust markers (miR-16-5p, let-7e-5p, miR-107, miR-451a and miR-126-3p) validated in at least 3 cohorts. 46 circulating-RNAs, and 55 long-non-coding-RNAs were consistently dysregulated. Five studies reported elevated nuclear (B-globin, TERT) and mitochondrial (MT-ND2; 2.5–3.5×) cfDNA within 6 h.

Table 1. Class of evidence* of biochemistry biomarkers based on systematic review

Class I	Class II	Class III
At all time-points		
ALP, aPTT, CRP, IL-6, IMA, Mg^{2+}, NfL, thrombotic microangiopathy score	BUN, creatinine, glucose, S100B, VCAM-1	e-Selectin, p-Selectin, ferritin, fibrinogen, HbA1c, LDL, NLR, Neutrophils
At 4.5 h		
IL-6	S100B, VCAM-1	-
At 6 h		
aPTT, CRP, IL-6	BUN, creatinine, glucose, S100B, VCAM-1	NLR, Neutrophils
At 9 h		
aPTT, CRP, IL-6, NfL	BUN, creatinine, glucose, S100B, VCAM-1	NLR, Neutrophils
At 24 h		
aPTT, CRP, IL-6, IMA, NfL	BUN, creatinine, glucose, S100B, VCAM-1	e-Selectin, p-Selectin, NLR, Neutrophils

Evaluation of evidences based on number of cases, risk of bias and direction of evidence, as follow: Class I: number of cases >1000, low risk of bias, same direction of evidence >75%; Class II: number of cases >1000, low risk of bias, same direction of evidence >50%; Class III: number of cases <1000, low risk of bias, same direction of evidence >50%.

Table 1. Class of evidence* of biochemistry biomarkers based on meta-analysis

Class II Ioannidis	Class III Ioannidis	Class IV Ioannidis
At all time-points		
IMA	MMP-9, BNP	Albumin, BUN, Leucocytes, Glucose, Creatinine, HbA1c, LDL, GFAP, CRP, Neutrophils, NfL, IL-6, BDNF, Copeptin, S100B, d-Dimer, NSE
At 4.5 h		
-	-	GFAP, IL-6
At 6 h		
-	-	Leucocytes, Lymphocytes, d-Dimer, Creatinine, GFAP, CRP, Neutrophils, NLR, IL-6, S100B, NSE
At 9 h		
-	-	Lymphocytes, Leucocytes, d-Dimer, Creatinine, GFAP, CRP, Neutrophils, NLR, IL-6, S100B, NSE
At 24 h		
IMA	BNP, MMP-9	Lymphocytes, Leucocytes, Creatinine, GFAP, CRP, Neutrophils, IL-6, S100B, d-Dimer, NSE

*No biomarker in Class I of Ioannidis.

CONCLUSIONS

Fluid biomarkers exhibit a temporally evolving signature: early coagulopathy (D-dimer), glial activation (GFAP, S100B) and inflammation (IL-6), followed by neuronal necrosis (NSE) and oxidative stress (IMA) within 24 h. Multi-omic integration, including metabolomics, transcriptomics and cfDNA, highlights convergent pathways (PI3K/Akt, NF-κB, immunometabolism) and supports the development of rapid, point-of-care panels. Standardized sampling windows and harmonized assay protocols are essential for clinical translation and prospective validation in prehospital settings.