Section: General Medicine

Original Research Article

DIAGNOSTIC EFFICACY OF BLOOD-BASED PROTEIN BIOMARKERS IN ISCHEMIC STROKE: A SYSTEMATIC REVIEW ACCORDING TO PRISMA GUIDELINES

Sridhar Amalakanti[1], Shruthika K[2], Sai Teja Gadde[3], Rithish Nimmagadda[4], Vijaya Chandra Reddy Avula[5]

[1]*Assistant Professor, Department of General Medicine, AIIMS Mangalagiri, India.*
[2]*Junior Resident, Department of General Medicine, AIIMS Mangalagiri, India.*
[3]*Junior Resident, Department of General Medicine, AIIMS Mangalagiri, India.*
[4]*Junior Resident, Department of General Medicine, Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, India.*
[5]*Additional Professor, Department of Psychiatry, AIIMS Mangalagiri, India.*

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Corresponding Author:
Dr. Sridhar Amalakanti,
Assistant Professor, Department of General Medicine, AIIMS Mangalagiri, India.
Email: sridhar@aiimsmangalagiri.edu.in

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ABSTRACT

Background: Stroke, the second leading cause of death globally, predominantly manifests as ischemic stroke. This review synthesizes current evidence on blood-based protein biomarkers for diagnosing ischemic stroke, aiming to enhance early detection and treatment strategies.

Materials and Methods: Adhering to PRISMA guidelines, we systematically searched PubMed, Cochrane, Embase, and Web of Science databases for studies published up to 2024, focusing on blood-based protein biomarkers in ischemic stroke diagnosis. Quality assessment and data extraction were meticulously performed, emphasizing biomarker sensitivity, specificity, and diagnostic value.

Results: Our review included 190 studies, highlighting several promising biomarkers such as GFAP, and S100B for their diagnostic accuracy in distinguishing ischemic stroke from other stroke types and healthy controls.

Conclusion: Blood-based protein biomarkers demonstrate significant promise for early and accurate ischemic stroke diagnosis. Their integration into clinical practice could revolutionize stroke management, offering a non-invasive, rapid diagnostic tool. However, further large-scale studies are necessary to validate these findings and establish standardized protocols for their clinical application.

Keywords: Ischemic stroke, Biomarkers, Protein biomarkers, Stroke diagnosis, Acute Stroke, Neurological Biomarkers, Predictive value of tests, early diagnosis.

INTRODUCTION

Stroke has emerged as the second most prevalent cause of death and the third most prevalent cause of disability adjusted life years (DALY) worldwide.[1] Ischemic stroke accounts for 87% of all stroke cases in western countries, while the remaining 13% are caused by hemorrhagic stroke.[2] The proportion fluctuates according to the Indian population, with the ischemic stroke representing 68-80% and the hemorrhagic stroke representing 20-32% of the total stroke type.[3]

The management of ischemic stroke entails the administration of thrombolytic medications, which must be given within a certain time window of 3 – 4.5 hours.[4] Currently, a non-contrast Computed Tomography (CT) brain scan is frequently used to verify the diagnosis of stroke and distinguish between ischemic stroke and hemorrhagic stroke.[5] CT scans, while reliable, have limited accessibility, especially in remote areas of low-middle income countries.[6] Its primary application is for the purpose of diagnosing or excluding an hemorrhagic stroke, however its diagnostic sensitivity for an ischemic stroke is restricted.[7] In addition, it has the disadvantage of exposing patients to radiation.[8]

Diffusion tensor Magnetic Resonance Imaging (MRI) is a more dependable modality.[7] than a CT scan for immediately diagnosing strokes, however it is expensive and not readily available. Despite the

advancements in neuroimaging, there are various obstacles that have impeded its use in stroke diagnosis and differentiation. These include the lengthy process, expensive equipment, restricted availability, and inconsistencies in the processing of radiological images.[9]

Contrarily, blood tests have the capacity to reduce the cost of diagnostic procedures and may easily be employed in primary healthcare settings. Blood biomarkers offer a reliable, rapid, and cost-effective approach for diagnosing ischemic stroke.[10] Biomarker signatures can assist in the selection of appropriate treatment protocols for individuals with acute stroke. Hence, it is imperative to utilize a blood-based biomarker approach that has both heightened sensitivity and specificity in order to efficiently and promptly detect ischemic stroke in acute environments, thereby facilitating enhanced therapeutic methods.[11]

Multiple blood biomarkers associated with different pathophysiological processes of stroke have been identified as potentially beneficial for its therapy. The field of biomarker research is rapidly evolving, with new potential markers being discovered and validated regularly.[12] An updated systematic review can synthesize the latest evidence, providing clinicians and researchers with a comprehensive overview of the current state of knowledge in this area. The aim of this study was to conduct a thorough analysis of all the diagnostic test studies published so far in order to find prospective blood-based biomarkers that can be utilized for the diagnosis of ischemic stroke.

MATERIAL AND METHODS

Search Criteria

A thorough literature search was performed until January 5, 2024, using the PubMed, Cochrane, Embase, and Web of Science databases. The search included prominent trial registries such as clinicaltrial.gov (www.clinicaltrials registry.gov), Stroke Trial Registry (www.strokecenter.org/trials), and Indian Clinical Trial Registry (www.ctri.nic.in). The search criteria used were: ('Blood biomarkers') AND ('Ischemic Stroke'). The precise search criteria are described in the supplementary appendix. In addition, we thoroughly analyze and evaluate any secondary materials that are available. The search was restricted to studies conducted on human subjects and published solely in the English language.

Selection Criteria

To be eligible for inclusion in the systematic review, qualifying studies had to meet the following criteria: (a) The diagnostic studies enrolled patients diagnosed with ischemic stroke. (b) The diagnosis of stroke must be validated by CT/MRI scans that demonstrate the presence of a recent blockage of blood supply to the brain, in addition to the clinical diagnosis. (c) The research must have identified blood-based biomarkers within the initial week following the onset of the stroke. (d) The studies must have included data on either the biomarker levels or the sensitivity and specificity values for the diagnostic biomarker. The main rationales for eliminating the research were: (a) The study did not function as a diagnostic tool. (b) There existed other articles that addressed the same participants from the identical study. (c) The study involved patients who did not have ischemic stroke.[5] Insufficient information was available to assess the quality of their approach. The systematic evaluation did not include conference presentations since there was not enough material available to assess their methodological quality. The systematic review did not impose any stipulation regarding the inclusion of a minimum sample size.

Population

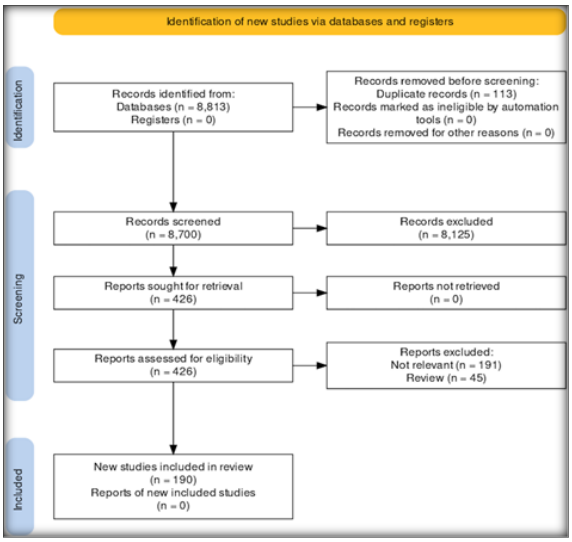
Two authors independently collected data from all available sources within each article, including details such as the sample size, analyzed protein profiles, employed technique, findings, study limitations, and the sensitivity and specificity of the biomarkers. All authors reached a compromise to address any conflict.

Evaluation of Quality

We evaluated the methodological rigor of each study included in our systematic review using the QUADAS approach that was modified by Whiteley et al.[13] Two authors independently assessed the quality of the investigations. The disparity in the quality scores was resolved through a collaborative conversation involving all the authors.

RESULTS

We examined 8944 records and evaluated 526 complete texts to determine their eligibility. Ultimately, a total of 190 studies from 40 nations were incorporated, as shown in PRISMA(14) Figure 1.



According to the QUADAS quality evaluation of the 190 studies analyzed in this systematic review, the overall risk of bias and concerns about applicability can be stated as follows:

Bias Risk

- Patient Selection: The level of bias in patient selection was predominantly uncertain throughout the research, with 111 studies (58.8%) having an uncertain risk and 67 studies (35.2%) having a low risk. Merely 12 papers, accounting for 6.0% of the total, were classified as high risk.
- Index Test: A significant majority of studies (182 studies, 96.2%) shown a low likelihood of bias in the index test domain. Out of the total number of studies, only 7 (3.8%) were found to have a significant risk of bias for the index test.
- Regarding the reference standard, the risk of bias was low in 136 studies (71.4%), unclear in 46 studies (24.2%), and high in 8 studies (4.4%).
- Flow and Timing: The majority of studies (153 studies, 80.8%) exhibited a low risk of bias in terms of flow and timing. The risk was ambiguous in 29 studies (15.4%) and elevated in 7 studies (3.8%) for this particular area.

Concerns Regarding Relevance

- Patient Selection: The majority of research (88.5%) demonstrated minimal problems regarding the applicability of their patient selection (1618 studies). Merely 17 research (8.8%) exhibited ambiguous problems, while 5 studies (2.7%) displayed significant worries.
- Index Test: The vast majority of studies (189 studies, 99.5%) had few reservations with the applicability of the index test. Out of all the studies, only one (0.5%) raised significant issues.
- Reference Standard: The applicability of the reference standard was deemed low in 183 research (96.7%), unclear in 5 studies (2.7%), and high in 1 study (0.5%).

Important findings are

- The research covers a broad variety of publication years, ranging from 1997 to 2022. The latest research includes 6 studies from 2022, 11 studies from 2021, 14 studies from 2020, and 12 studies from 2019. There has been a noticeable rise in stroke biomarker research in recent years.
- The studies originate from various countries across the globe. The countries that have conducted the most number of studies include China (63 studies), USA (29 studies), Germany (16 studies), Turkey (15 studies), and Spain (10 studies).
- China has produced the highest number of studies in both overall and recent years, with a notable example being 22 studies from China between 2019 and 2022. China's present leadership in stroke biomarker research is indicated by this.
- Additional nations with much research encompass Poland, Iran, Japan, Korea, India, and Egypt. This highlights the worldwide research focus on identifying stroke biomarkers.
- This systematic review encompassed a variety of study designs to investigate potential biomarkers for ischemic stroke. Prospective studies were the most common (39%, 74/190), Case-control studies made up 29% (55/190). Cross-sectional (15/190, 8%) and retrospective (11/190, 6%) designs were less common.

Just over one-third of studies (67/190, 35%) compared ischemic stroke to healthy controls only. The remaining studies made comparisons to hemorrhagic strokes (31/190, 16%), transient ischemic attacks (10/190, 5%), and stroke mimics (11/190, 6%).

Blood-based biosamples were heavily predominant, including whole blood (19%, 36/190), plasma (49%, 93/190), and serum (51%, 97/190). Other sample types like microvesicles (10/190, 5%) and PBMCs (3/190, 2%) were rarer. Interestingly, 10% of studies (19/190) analyzed platelets.

The included studies investigated a wide range of potential biomarkers for ischemic stroke. Proteins were by far the most commonly analyzed, examined in 59% (111/190) of studies. Frequently measured proteins included S100B (18%, 34/190), GFAP (18%, 31/172), NSE (5%, 10/190), and NfL (3%, 5/190). These proteins are biomarkers of neuronal and glial injury, making them logical targets in ischemic stroke. Other protein classes like metalloproteinases, acute phase proteins, cell adhesion molecules, and growth factors were also commonly studied.

MicroRNAs (miRNAs) emerged as another major biomarker category, investigated in 16% (31/190) of included studies. Top studied miRNAs were miR-124, miR-9, and miR-21. Metabolites and metabolomics approaches were utilized in 15% (28/190) of the studies. Similarly, transcriptomics and a focus on genes/transcripts was seen in 15% (28/190) of the included research. Proteomics techniques like mass spectrometry were applied in 13% (24/190) of studies.

The 190 studies varied considerably in their sample sizes, settings, diagnostic reference standards, and sampling times after stroke onset. Half of the studies (50%, 95/190) had sample sizes less than 100 participants. The largest studies had sample sizes over 300. Out of the total number of research papers, 19 (which accounts for 10%) had validation or replication. The setting was often not reported (45%, 85/190). For studies that did specify, the emergency department (15%, 29/190), neurology ward (14%, 27/190), and inpatient stroke units (6%, 7/190) were most common. Diagnosis was confirmed via CT (61%, 116/190) and/or MRI (48%, 92/190) in most studies. A total of 29% (55

out of 190) of studies failed to mention the diagnostic reference standard that was utilized. Finally, sampling times varied from 1 to 24 hours after symptom onset, with 24 hours (51%, 97/190 and 6 hours (17%, 32/190) as the most frequent single time points. However, 26 studies (15%) analyzed biomarkers longitudinally across multiple time points.

Table 1: Presents comprehensive findings from our quality evaluation

Study	Risk of Bias				Concerns of Applicability		
	Patient Selection	Index test	Reference Standard	Flow and timing	Patient Selection	Index test	Reference Standard
Zhou 2021	Unclear	Low	Low	Low	Low	Low	Low
Zhang 2022	Unclear	High	Unclear	Unclear	Low	Low	Unclear
Yang 2022	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear
"Turek-Jakubowska 2022	Unclear	Low	Low	Low	Low	Low	Low
Tian 2022	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Rahmati 2021	Low	Low	Low	Low	Low	Low	Low
Li 2021	Unclear	Low	Low	Low	Low	Low	Low
Induruwa 2022	Low	Low	Unclear	Unclear	Low	Low	Unclear
Gawryś 2022 (9)	Low	Low	Low	Low	Low	Low	Low
Cho 2022 (10)	Unclear	Low	Unclear	Unclear	Low	Low	Low
Intiso 2004 (11)	Low	Low	Low	Low	Low	Low	Low
Perini 2001(12)	Low	Low	Low	Low	Low	Low	Low
Pedersen 2004 (13)	Unclear	Low	Low	Low	Low	Low	Low
Nayak 2011 (14)	Unclear	Low	Low	Low	Low	Low	Low
Senes 2007 (15)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Feng 2019 (16)	High	Low	Low	Low	Low	Low	Low
Blann 1999 (17)	Low	Low	Unclear	Low	Low	Low	Unclear
Shyu 1997 (18)	Low	Low	Low	Low	Low	Low	Low
Liu 2015 (19)	Low	Low	Low	Low	Low	Low	Low
Supanc 2011 (20)	Low	Low	Low	Low	Low	Low	Low
Wunderlich 2005(21)	Low	Low	Low	Low	Low	Low	Low
Hu 2016 (22)	High	Low	Unclear	Unclear	Unclear	Low	Unclear
Uno 2003 (23)	Low	Low	Low	Low	Low	Low	Low
Sun 2019 (24)	Unclear	Low	Low	Low	Low	Low	Low
Song 2006 (25)	Low	Low	Low	Low	Low	Low	Low
Zitnanova 2016 (26)	Low	Low	Low	Low	Low	Low	Low
Can 2015 (27)	Low	Low	Low	Low	Low	Low	Low
Kimberly 2013 (28)	Low	Low	Low	Low	Low	Low	Low
Abboud 2007 (29)	Low	Low	Low	Low	Low	Low	Low
Tang 2006 (30)	Unclear	Low	High	Low	Low	Low	Low
Rainer 2007 (31)	Low	Low	Low	Low	Low	Low	Low
Tiedt 2018 (32)	Low	Low	Low	Low	Low	Low	Low
Zhu 2019 (33)	Low	Low	Low	Low	Low	Low	Low
Zhu 2018 (34)	Unclear	Low	Low	Low	Low	Low	Low
Zhou 2016 (35)	Unclear	Low	High	Low	Low	Low	Unclear
Zhou 2018 (36)	Unclear	Low	Low	Low	Low	Low	Low
Zhou 2022 (37)	Low	Low	Low	Low	Low	Low	Low
Zhao 2016 (38)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Zhao 2017 (39)	High	Low	Low	Low	Low	Low	Low
Zhao 2016 (40)	Unclear	Low	Low	Low	Low	Low	Low
Zhang 2017 (41)	Unclear	Low	Low	Low	Low	Low	Low
Zhang 2020 (42)	Low	Low	Low	Low	Low	Low	Low
Zaremba 2006 (43)	Unclear	Low	Low	Low	Low	Low	Low
Yuan 2020 (44)	Unclear	Low	Low	Low	Low	Low	Low
Yigit 2017 (45)	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Yang 2016 (46)	Unclear	Low	Low	Low	Low	Low	Low
Xiong 2015(47)	Low	Low	Low	Low	Unclear	Low	Low
Wu 2020 (48)	Unclear	Low	Low	Low	Low	Low	Low
Williams 2007 (49)	Low	Low	Low	Low	Low	Low	Low
Wang 2017 (50)	Low	Low	Low	Low	Low	Low	Low
Wang 2014 (51)	Low	Low	Low	Low	Low	Low	Low
Wang 2018 (52)	Unclear	Low	Low	Low	Low	Low	Low
Wang 2017 (53)	Unclear	Low	Low	Low	Low	Low	Low
Walsh 2016 (54)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Vukasovic 2006 (55)	Unclear	Low	Low	Low	Low	Low	Low
von Recum 2015 (56)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Unden 2009 (57)	Low	Low	Low	Low	Unclear	Low	Low
Tunç 2018 (58)	Unclear	Low	Low	Low	Low	Low	Low
Tiedt 2017 (59)	Low	Low	Low	Low	Low	Low	Low
Tiedt 2020 (60)	Low	Low	Low	Low	Low	Low	Low
Tian 2015 (61)	Low	Low	Low	Low	Low	Low	Low
Tian 2016 (62)	Low	Low	Unclear	Unclear	Low	Low	Unclear

Taema 2014 (63)	Unclear	Low	High	Low	Low	Low	High
Stejskal 2011 (64)	High	Low	Low	Low	Low	Low	Low
Stanca 2015 (65)	Low	Low	Low	Low	Low	Low	Low
Stamova 2010 (66)	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear
Stamova 2019 (67)	Unclear	Low	Low	Low	Low	Low	Low
Song 2019 (68)	Unclear	Low	Low	Low	Low	Low	Low
Simats 2020 (69)	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Simats 2018 (70)	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear
Simats 2018 (71)	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Sharma 2014 (72)	Low	Low	Low	Low	Low	Low	Low
Sharma 2015 (73)	Unclear	Low	Low	Low	Low	Low	Low
Shaker 2020 (74)	Unclear	Low	Low	Low	Low	Low	Low
Sepramaniam 2014 (75)	Unclear	Low	Low	Low	Low	Low	Low
Sayan 2016 (76)	Low	Low	Low	Low	Low	Low	Low
Rozanski 2017 (77)	Low	Low	Low	Low	Low	Low	Low
Roudbary 2011 (78)	High	Low	Low	Low	Low	Low	Low
Rico Santana 2014 (79)	Unclear	Low	Low	Low	Low	Low	Low
Richard 2016 (80)	Unclear	Low	Low	Low	Low	Low	Low
Reynolds 2003 (81)	Low	Low	Low	Low	Low	Low	Low
Ren 2016 (82)	High	Low	Low	Low	Low	Low	Low
Ranga 2016 (83)	High	Low	Low	Low	High	Low	Low
Rahmati 2020 (84)	Unclear	Low	Low	Low	Low	Low	Low
Qi 2021 (85)	Unclear	Low	Low	Low	Low	Low	Low
Peycheva 2021 (86)	Low	Low	Low	Low	Low	Low	Low
Perovic 2017 (87)	Low	Low	Low	Low	Low	Low	Low
Penn 2018 (88)	Unclear	Low	Low	Low	Low	Low	Low
Park 2013 (89)	Low	Low	Low	Low	Low	Low	Low
Park 2018 (90)	Low	Low	Low	Low	Low	Low	Low
Pan 2020 (91)	High	Low	Unclear	Low	High	Low	Unclear
Palm 2018 (92)	Low	Low	Low	High	Low	Low	Low
Oraby 2019 (93)	High	Low	Low	Low	Low	Low	Low
O'Connell 2019 (94)	Unclear	Low	Low	Low	Low	Low	Low
O'Connell 2019 (95)	Unclear	Low	Low	Low	Low	Low	Low
O'Connell 2016 (96)	Unclear	Low	Low	Low	Low	Low	Low
O'Connell 2017 (97)	Unclear	Low	Low	Low	Low	Low	Low
O'Connell 2017 (98)	High	Low	Unclear	Unclear	Low	Low	Unclear
O'Connell 2020 (99)	Unclear	Low	Low	Low	Low	Low	Low
Nielsen 2020 (100)	Low	Low	Low	Low	Low	Low	Low
Nguyen 2020 (101)	Low	Low	Low	Low	Low	Low	Low
Nahan 2017 (102)	High	Low	Unclear	Unclear	Low	Low	Unclear
Montaner 2012 (103)	Low	Low	Low	Low	Low	Low	Low
Menon 2018 (104)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Mattila 2021(105)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Matsuo 2013 (106)	Low	Low	Low	Low	Low	Low	Low
Matsumori 2002 (107)	Low	Low	Low	Unclear	Low	Low	Low
Maly 2021 (108)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Mahovic 2013 (109)	High	Low	Low	Low	Low	Low	Low
Ma 2019 (110)	Unclear	Low	Low	Low	Low	Low	Low
Luger 2017(111)	Low	Low	Low	Low	Low	Low	Low
Lu 2020 (112)	High	Low	Low	Low	Low	Low	Low
Long 2013 (113)	High	Low	Unclear	Unclear	Low	Low	Unclear
Llombart 2016 (114)	High	Low	Low	Low	Low	Low	Low
Liu 2015 (115)	Low	Low	Low	Low	Low	Low	Low
Liu 2017 (116)	High	Low	Low	Low	Low	Low	Low
Liu 2020 (117)	High	Low	Low	Low	Low	Low	Low
Liswati 2009 (118)	High	Low	Low	Low	Low	Low	Low
Li 2021 (119)	Unclear	Low	Low	Low	Low	Low	Low
Li 2015 (120)	High	Low	Low	Low	Low	Low	Low
Li 2018 (121)	Low	Low	Low	Low	Low	Low	Low
Leung 2014 (122)	Low	Low	Low	Low	Low	Low	Low
Laterza 2006 (123)	High	Low	Unclear	Unclear	Unclear	Low	Unclear
Laskowitz 2009 (124)	Low	Low	Low	Low	Low	Low	Low
Kokocinska 2007 (125)	High	Low	Low	Low	Low	Low	Low
Kokocinska 2005 (126)	High	Low	Low	Low	Low	Low	Low
Kodali 2013 (127)	High	Low	Unclear	Unclear	Unclear	Low	Unclear
Kodali 2012 (128)	High	Low	Unclear	Unclear	Unclear	Low	Unclear
"Kochanowski 2012 (129)"	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Kavalci 2011 (130)	High	Low	Low	Low	High	Low	Low
Katsanos 2017 (131)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Kashyap 2009 (132)	High	Low	Unclear	Unclear	Low	Low	Unclear
	High	Low	Low	Low	Low	Low	Low

Kara 2014 (133)	Low	Low	Low	Low	Low	Low	Low
Kalra 2021 (134)	Low	Low	Low	Low	Low	Low	Low
Kalani 2020 (135)	Low	Low	Low	Low	Low	Low	Low
Jin 2017 (136)	High	Low	Low	Low	Low	Low	Low
Jiang 2011 (137)	High	Low	Low	Low	Low	Low	Low
Jia 2015 (138)	Low	Low	Low	Low	Low	Low	Low
Ji 2016 (139)	Unclear	Low	Low	Low	Low	Low	Low
Inoue 2019 (140)	Low	Low	Low	Low	Low	Low	Low
Iltumur 2006 (141)	High	Low	Low	Low	Low	Low	Low
Herisson 2010 (142)	Low	Low	Low	Low	Low	Low	Low
Han 2012 (143)	High	Low	Low	Low	Low	Low	Low
Gunduz 2008 (144)	High	Low	Low	Low	Low	Low	Low
Gunaydin 2014 (145)	Low	Low	Low	Low	Low	Low	Low
Glickman 2011 (146)	High	Low	Low	Low	Low	Low	Low
"Giannopoulos 2008	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
(147)"	High	Low	Low	Low	Low	Low	Low
Garlichs 2003 (148)	High	Low	Low	Low	Low	Low	Low
Foerch 2012 (149)	Low	Low	Low	Low	Low	Low	Low
Foerch 2006 (150)	Low	Low	Low	Low	Low	Low	Low
Fiszer 1998 (151)	High	Low	Low	Low	Low	Low	Low
Fassbender 1997 (152)	Low	Low	Low	Low	Low	Low	Low
Fang 2018 (153)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Ewida 2021 (154)	Low	Low	Low	Low	Low	Low	Low
Ekingen 2017 (155)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Dvorak 2009 (156)	Low	Low	Low	Low	Unclear	Low	Low
Duan 2015 (157)	Low	Low	Low	Low	Low	Low	Low
De Marchis 2018 (158)	Low	Low	Low	Low	Low	Low	Low
Dassan 2012 (159)	Low	Low	Low	Low	Low	Low	Low
Dambinova 2003 (160)	High	Low	Low	Low	Low	Low	Low
Dambinova 2012 (161)	Low	Low	Low	Low	Low	Low	Low
Cheng 2018 (162)	Low	Low	Low	Low	Low	Low	Low
Chen 2018 (163)	Low	Low	Low	Low	Unclear	Low	Low
Cavrak 2021 (164)	High	High	Low	Low	Low	Low	Low
Cano 2003 (165)	Low	Low	Low	Low	Low	Low	Low
Cakmak 2014 (166)	Low	Low	Low	Low	Low	Low	Low
Büttner 1997 (167)	High	Low	Low	Low	High	Low	Low
Bustamante 2021 (168)	Low	Low	Low	Low	Low	Low	Low
Bustamante 2017 (169)	Low	Low	Low	Low	Low	Low	Low
Bolayir 2019 (170)	Low	Low	Low	Low	Low	Low	Low
Bibl 2012 (171)	Unclear	Low	Low	Low	Low	Low	Low
Barr 2010 (172)	High	Low	Low	Low	Low	Low	Low
"Azarpazhooh 2010	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
(173)"	High	Low	Low	Low	Low	Low	Low
Atik 2016 (174)	Low	Low	Low	Low	Low	Low	Low
"Alvarez-Perez 2011	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
(175)"	Low	Low	Unclear	Unclear	Low	Low	Unclear
Allard 2004 (176)	Low	Low	Low	Low	Low	Low	Low
Allard 2005 (177)	Low	Low	Low	High	Low	Low	Low
Algin 2019 (178)	Low	Low	Low	Low	Low	Low	Low
Algawwam 2021 (179)	Low	Low	Unclear	Unclear	Low	Low	Unclear
Ahn 2011 (180)	Low	Low	Low	Low	Low	Low	Low
Sadik 2021 (181)	Low	Low	Low	Low	Low	Low	Low
Abe 2020 (182)	Low	Low	Low	Unclear	Low	Low	Unclear
catana 2023 (183)	low	low	low	low	low	low	low
Ding 2023(184)	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Xie 2023 (185)	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Jiang 2022 (186)	unclear	unclear	unclear	unclear	unclear	unclear	unclear
Wu j 2022 (187)	unclear	unclear	unclear	unclear	unclear	unclear	unclear

Table 2: The attributes of the 190 studies that were considered are displayed in the Supplementary

Supplementary Table 2: Concise characteristics of 190 included studies									
Study ID	Country	Design	Comparison	Setting	Reference	Specimen	Sampling time	Omics	Biomarkers
Zhou 2021 (1)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	transcripts	miR-124
Zhang 2022 (2)	China	Case control	IS vs. Controls	Not stated	Not stated	serum	9h	proteins	JKAP

		study							
Yang 2022 (3)	China	Case control study	IS vs. Controls	Not stated	Not stated	plasma; exosome	12h	Transcripto mics	circ_0112036, circ_0066867, circ_0093708, circ_0041685
Turek-Jakubowska 2022 (4)	Poland	Case control study	IS vs. Controls	Neuro lo gy ward	CT	plasma	24h	Proteomics	Alpha-1B-glycoprotein
Tian 2022 (5)	China	Case control study	IS vs. Controls	Neuro - vascul a r centre	Not stated	whole blood	6h	Transcripto mics	lncRNA NR_120420
Rahmati 2021 (6)	Iran	Case control study	IS vs. Controls	Not stated	MRI	serum	24h	transcripts ; proteins	miR-210, HIF-1a
Li 2021 (7)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins; metabolites	uric acid, CRP, NT-proBNP
Induruwa 2022 (8)	UK	Prospective study	IS vs. Controls; HS vs. Controls; Total stroke vs. controls	ED	Not stated	whole blood; platelet	8h	proteins; platelet	IS vs. control: GPVI-dimer, HS vs. control: GPVI-dimer, total stroke vs. control: GPVI, GPVI-dimer, platelet P-selectin
Gawryś 2022 (9)	Poland	Case control study	IS vs. Controls	Neuro lo gy ward	CT	platelet	24h	Proteomics; platelet	Beta-amyloid protein A4, Amyloid-like protein 2, coactosin-like protein, thymidine phosphorylase 4 (TYMP-4), interferon regulatory factor 7 (IRF7), vitamin K-dependent protein S, histone proteins (H2A type 1 and 1-A, H2A types 2B and J, H2Av, -z, and -x), platelet basic protein
Cho 2022 (10)	not stated	Case control study	IS vs. Controls	ED	Not stated	PBMCs	24h	PBMC number	NK cells, CD14+ monocytes
Intiso 2004 (11)	Italy	Prospective study	IS vs. Controls	Neuro lo gy ward	CT; MRI	serum	24h	proteins	TNFa
Perini 2001 (12)	Italy	Prospective study	IS vs. Controls	Stroke Unit	CT	serum	12h	proteins	IL-6, IL-10
Pedersen 2004 (13)	Norway	Case control study	IS vs. Controls	Not stated	CT; MRI	plasma	24h	proteins	CRP
Nayak 2011 (14)	India	Case control study	IS vs. Controls	Not stated	CT	serum	24h	proteins	IMA
Senes 2007 (15)	Turkey	Prospective study	IS vs. Controls	Not stated	Not stated	serum	24h	metabolites	nitrite, nitrate, IMA, TBARS
Feng 2019 (16)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	plasma	24h	transcripts	lncRNA ANRIL
Blann 1999 (17)	UK	Prospective study	IS vs. Controls	Not stated	Not stated	serum; plasma	12h	proteins	ICAM-1, E-selectin, VCAM-1, vWF
Shyu 1997 (18)	China (Taiwan)	Prospective study	IS vs. Controls	ED	CT	serum	24h	proteins	ICAM-1, E-selectin
Liu 2015 (19)	China	Prospective study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins	CXCL12
Supanc 2011 (20)	Croatia	Prospective study	IS vs. Controls	Neuro lo gy ward	CT	serum	24h	proteins	ICAM-1, VCAM-1
Wunderlich 2005 (21)	Germany	Prospective study	IS vs. Controls	Neuro lo gy ward	CT	serum	24h; 18h; 12h; 6h; 3h; 2h; 1h	proteins	B-FABP, H-FABP
Hu 2016 (22)	China	Case control study	IS vs. HS	Not stated	Not stated	whole blood	12h	metabolites	Asn, C5:1, Arg/Orn, Val/Phe, (C0 +C2 + C3 + C16 + C18:1)/Cit
Uno 2003 (23)	Japan	Prospective study	IS vs. Controls	depart m ent of neurol o gical surger y	CT; MRI	plasma	24h	proteins	OxLDL
Sun 2019 (24)	Germany	Prospective study	IS vs. Controls	ED	CT; MRI	serum	24h	Metabolomi cs	tetradecanedioate, hexadecanedioate
Song 2006 (25)	Korea	Prospective study	IS vs. Controls	Not stated	CT; MRI	plasma	24h	proteins	IL-6,PAI-1,PAP
Zitnanova 2016 (26)	Slovakia	Case control	IS vs. Controls	Neuro lo gy	CT	plasma	24h	proteins	lipid peroxides, superoxide dismutase activity, catalase activity,

		study		ward					paraoxonase activity, glutathione peroxide activity
Can 2015 (27)	Turkey	Prospective study	IS vs. Controls	ED	MRI	serum	12h	proteins	MBP, IMA
Kimberly 2013 (28)	USA	Prospective study +animal experiment study	IS vs. Controls	ED	MRI	plasma	2h; 9h	Metabolomi cs; targeted	BCAA (leucine, isoleucine, valine), carnitine, threonine, histidine, glucose, methionine, glycine, proline, lysine, cysteamine, uridine, 5'-adenosylhomocysteine, creatinine, N-carbamoyl- beta-alanine, cis/trans hydroxyproline, asparagine
Abboud 2007 (29)	France	Prospective study	IS vs. HS; Total stroke vs. TIA	ED	CT; MRI	serum	3h	proteins	IMA
Tang 2006 (30)	USA	Case control study	IS vs. Controls	Not stated	CT	whole blood	24h; 5h; 3h	Genomics	Hox 1.11 gene, CKAP4 gene, S100A9 gene, MMP9 gene, S100P gene, F5 gene, FPR1 gene, S100A12 gene, RNASE2 gene, ARG1 gene, CA4 gene, LY96 gene, SLC16A6 gene, HIST2H2AA gene, ets-2 gene, BCL6 gene, PYGL gene, NPL gene
Rainer 2007 (31)	China	Prospective study	IS vs. HS	ED	CT; MRI	plasma	24h	proteins; cfDNA	cfDNA, S100
Tiedt 2018 (32)	Germany	Prospective study	IS vs. Controls	ED	MRI	serum	24h	proteins	NfL
Zhu 2019 (33)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	PBMC	24h	transcripts	PBMC circ-DLGAP4
Zhu 2018 (34)	China	Case control study	IS vs. Controls	Not stated	MRI	leukocytes	24h	transcripts	lncRNA MIAT
Zhou 2016 (35)	China	Prospective study	IS vs. HS	ED	CT	plasma	6h	proteins	S100B
Zhou 2018 (36)	China	Case control study	IS vs. Controls	Not stated	MRI	serum; exosome	24h	transcripts	miR-134
Zhou 2022 (37)	China	Case control +animal study	IS vs. Controls	Neurology department	CT; MRI	Serum (small extracellular vesicles)	24h	Transcriptomics	miR-9-3p, miR-124-3p, miR-143-3p, miR-93-5p
Zhao 2016 (38)	China	Prospective study	IS vs. Controls	Neurology ward	Not stated	serum	24h	proteins	Apolipoprotein A1-Unique Peptide (APOA1-UP)
Zhao 2017 (39)	China	Case control study	IS vs. Controls; HS vs. Controls	ED, neurology department	CT; MRI	plasma; neutrophils, lymphocytes	6h	transcripts	miR-99a-5p
Zhao 2016 (40)	China	Prospective study	IS vs. Controls	Neurology ward	MRI	plasma	24h	transcripts	miR-335
Zhang 2017 (41)	China	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	Not stated	CT; MRI	dried blood spot	12h	Metabolomi cs	IS vs. control: C22, C5, C3DC, C4, C5DC/C5-OH, C3DC/C10, C14:2, C10:2, (0+2+3+16+18:1)/Cit, Arg, Pro HS vs. control: C16-OH/C16, C16:1-OH, C10, C5/C3, C12, C18, C18:1, C4DC, Val/Phe, C16, Arg, Thr IS vs. HS: C4-OH, C5DC, C14, C16-OH, Tyr/Cit, Val/Phe, C5DC,/C5-OH, C5DC/C16, C18-OH, (0+2+3016+18:1)/Cit, C3/Met
Zhang 2020 (42)	China	Prospective study	IS vs. Controls	Not stated	CT; MRI	plasma; endothelial micro-vesicles	24h	transcripts; endothelial microvesicle s	EMVs, EMVs-miR-155
Zaremba 2006 (43)	Poland	Case control study	IS vs. Controls	Not stated	CT	serum	24h	proteins	IL-12
Yuan 2020 (44)	China	Case control study + animal	IS vs. Controls	Neurology ward	CT; MRI	plasma	24h	proteins	GMFB

		experiment study							
Yigit 2017 (45)	Turkey	Case control study	IS vs. Controls; HS vs. Controls	ED	Not stated	serum	24h	proteins	UCH-L1
Yang 2016 (46)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	plasma	24h	transcripts	miR-107, miR-128b, miR-153
Xiong 2015 (47)	China	Prospective study	IS vs. HS	Not stated	CT; MRI	serum	6h	proteins	GFAP
Wu 2020 (48)	China	Case control study	IS vs. Controls	Neurology ward	CT; MRI	plasma	6h	transcripts	miR-99b
Williams 2007 (49)	USA	Prospective study	IS vs. SM	ED	MRI	plasma	24h	Endothelial microparticles (EMPs)	number of Endothelial microparticles
Wang 2017 (50)	China	Case control study	IS vs. Controls	ED	CT; MRI	serum	6h	transcripts	miR-221-3p, miR-382-5p
Wang 2014 (51)	China	Prospective study	IS vs. Controls	Not stated	MRI	plasma	24h	transcripts	miR-106b-5P, miR-4306, miR-320e, miR-320d
Wang 2018 (52)	China	Case control study	IS vs. Controls	Neurology ward	CT; MRI	plasma; exosome	6h	transcripts	miR-21-5p, miR-30a-5p
Wang 2017 (53)	China	Case control study + animal experiment study	IS vs. Controls	Not stated	MRI	plasma; lymphocytes	3h	transcripts	lncRNA H19
Walsh 2016 (54)	USA	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	ED, neurology depart m	Not stated	plasma	12h	proteins	Apo A-I, Apo C-I, Apo C-III, MMP-3, MMP-9, paraoxonase-1
Vukasovic 2006 (55)	Croatia	Case control study	IS vs. Controls	Not stated	CT	serum	24h	proteins	MMP-2, TIMP-2
von Recum 2015 (56)	Germany	Prospective study	IS vs. TIA	ED	Not stated	serum	4.5h	proteins	copeptin
Unden 2009 (57)	Sweden	Prospective study	IS vs. HS	Not stated	CT	not stated	24h	proteins	S100B, NSE, GFAP, APC-PCI
Tunç 2018 (58)	Turkey	Prospective study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins	SPA
Tiedt 2017 (59)	Germany	Prospective study	IS vs. TIA; IS vs. Controls	ED	CT; MRI	plasma	24h	Transcriptomics	miR-125a-5p, miR-125b-5p, miR-143-3p
Tiedt 2020 (60)	Germany	Prospective study	IS vs. SM; IS vs. Controls	ED	CT; MRI	serum	24h	Metabolomi cs	asymmetrical dimethylarginine (ADMA), symmetrical dimethylarginine (SDMA), pregnenolone sulphate, adenosine
Tian 2015 (61)	China	Prospective study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins	PCT, hsCRP, HCY
Tian 2016 (62)	China	Prospective study	IS vs. Controls	Cerebrovascular Diseases Centre	Not stated	plasma	6h	Transcripto mics	miR-16
Taema 2014 (63)	Egypt	Prospective study	IS vs. HS	Not stated	CT	serum	24h	proteins	CRP
Stejskal 2011 (64)	Czech Republic	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	3h	proteins	VILIP-1
Stanca 2015 (65)	Romania	Prospective study	IS vs. HS	ED	CT	serum	24h; 12h	proteins	GFAP, antibodies against NMDA receptor subunit NR2
Stamova 2010 (66)	USA	Case control study	IS vs. Controls	Not stated	Not stated	whole blood	24h; 5h; 3h	Genomics	GENES (ABCA1, PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, DKFZP434L187, CXADR, OVOL2, RNF141, CLEC4E, ELL2, SPIB, BXDC5, UNC5B, TIMP2, ASTN2, FLJ35934, ANKRD28,

									CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 ///PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, OSBPL1A, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1,LTB4R,F5,IFRD1,KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5,VAPA,THBD,LOC283027, LOC344595,RPL22, LOC100129488, RPL22, MCTP1, SH3GL3)
Stamova 2019 (67)	USA	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	Not stated	CT; MRI	not stated	24h	Transcripto mics	HS vs. control: 489 transcripts; IS vs. control: 396 transcripts; IS vs. HS: 256 transcripts
Song 2019 (68)	USA	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	Proteomics	clusterin, cystatin C (CST3)
Simats 2020 (69)	Spain	Case control study + animal	IS vs. SM	ED	Not stated	plasma	6h	Transcripto mics; Proteomics	CTNND2
		experiment study							
Simats 2018 (70)	Spain	Case control study+ animal experiment study	IS vs. Controls	Not stated	Not stated	plasma	6h	Proteomics; proteins	CKB, CMPK
Simats 2018 (71)	Spain	Prospective study + animal experiment study	IS vs. Controls	ED	Not stated	serum	6h; 4.5h	proteins	CCL23, CCL9
Sharma 2014 (72)	USA	Prospective study	IS vs. HS; IS vs. SM; Total stroke vs. SM	ED	CT; MRI	plasma	24h	proteins	eotaxin, epidermal growth factor receptor, S100A12, metalloproteinase inhibitor-4 (TIMP-4), prolactin
Sharma 2015 (73)	India	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	Proteomics	vWF, ADAMTS13, S100A7
Shaker 2020 (74)	Iraq	Case control study	IS vs. Controls	Not stated	CT	plasma	24h	proteins	GPBB
Sepramaniam 2014 (75)	Singapore	Prospective study+ animal experiment study	IS vs. Controls	Not stated	CT; MRI	whole blood	24h	Transcripto mics	miR-125b-2*, miR-27a*, miR-422a, miR-488, miR-627
Sayan 2016 (76)	Turkey	Prospective study	IS vs. Controls	Neurology ward	CT	plasma	24h	proteins	BNP
Rozanski 2017 (77)	Germany	Prospective study	IS vs. HS	Stroke Emergency Mobile	CT	plasma	3h; 1h	proteins	GFAP
Roudbary 2011 (78)	Iran	Cross sectional study	IS vs. HS	Neurology ward	CT	serum	24h	proteins	hsCRP
Rico Santana 2014 (79)	Spain	Case control study	IS vs. Controls	ED, neurology department	CT; MRI	serum	6h	Proteomics	2155-Da peptide
Richard 2016 (80)	France	Case control study	IS vs. Controls	Not stated	CT; MRI	plasma	24h; 6h; 3h	proteins	PRDX1
Reynolds 2003 (81)	USA	Case control study	IS vs. HS; IS vs. TIA;	ED	CT; MRI	serum	24h; 12h; 6h; 3h	proteins	S100B, BNGF, vWF, MMP9, MCP-1

			IS vs. Controls; HS vs. TIA; HS vs. Controls; Total stroke vs. controls						
Ren 2016 (82)	China	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	ED	CT; MRI	serum	24h; 4.5h	proteins	UCH-L1, GFAP
Ranga 2016 (83)	India	Cross sectional study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins	CEA
Rahmati 2020 (84)	Iran	Case control study	IS vs. Controls	Neuro lo gy ward	CT; MRI	serum	12h	transcripts ; proteins	S100B, miR-602
Qi 2021 (85)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum; extracellular vesicle (EV)-derived	6h	transcripts	miR-124-3p
Peycheva 2021 (86)	Bulgaria	Cross-sectional study	IS vs. Controls	Neuro lo gy ward	CT	serum	24h	proteins	fibrinogen
Perovic 2017 (87)	Croatia	Case control study	IS vs. Controls	Neuro lo gy ward	CT	serum	24h	proteins	resistin, copeptin
Penn 2018 (88)	Canada	Prospective study	IS vs. Controls	ED	CT; MRI	plasma	24h	Proteomics	E-selectin, Apolipoprotein C-I, Calponin, Coagulation factor XII, Clusterin, CRP, IGF-1, Complement component 4b (C4b and C4a), Serum paraoxonase/aryl esterase 1(Paraoxonase- PON1), Prothrombin/thrombin, Plasminogen/plasmin/angiostatin, Vitamin K-dependent protein S (Protein S), Serum paraoxonase/lactonase 3 (Paraoxonase- PON3), Vitamin K-dependent protein C (Protein C), Antithrombin III, Vitamin K-dependent protein Z (Protein Z), Coagulation factor V, Apolipoprotein D, Coagulation factor XI, Insulin-like growth factor-binding protein 3 (IBP 3), L-selectin, Plasma protease C1 inhibitor (C1 inhibitor), Plasma serine protease inhibitor (Protein C inhibitor), IL-6, S100A12, Fatty acid binding protein 3 (FABP3), Guanylate cyclase A(NPR1) (ANPR1), Epidermal growth factor receptor (EGFR), Platelet endothelial cell adhesion molecule (PECAM 1), Prolactin
Park 2013 (89)	Korea	Prospective study	IS vs. SM	Not stated	CT; MRI	plasma	24h	proteins	H-FABP, S100B
Park 2018 (90)	USA	Prospective study	IS vs. Controls	Not stated	CT; MRI	plasma	12h	proteins	GPBB
Pan 2020 (91)	China	Case control study	IS vs. Controls	Not stated	Not stated	platelet	7.5h	Genomics	EGR2 gene
Palm 2018 (92)	Germany	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	proteins	MMP-8, MPO, TIMP-1
Oraby 2019 (93)	Egypt	Case control study	IS vs. Controls	Neuro lo gy ward	CT; MRI	serum	24h	proteins	thioredoxin
O'Connell 2019 (94)	USA	Case control study	IS vs. SM	ED	CT; MRI	whole blood	24h	genes; leukocyte count	PLXDC2 gene, STK3 gene, ANTXR2 gene, KIF1B gene, CD163 gene, PDK4 gene, CTSZ gene, GRAP gene, MAL gene, ID3 gene
O'Connell 2019 (95)	USA	Case control study	IS vs. HS; Total stroke vs. SM ; Other: HS vs. IS+SM	ED	CT; MRI	whole blood	12h	Proteomics	NVAVAQDENLAG, NNYWANVASGLG, QSLKPKGVALSG, GASVHDGVALSG, GEYFRWNWDSVA,APFGQKDV ALGL, GDRRPLGVALSG,KGQRGYHL

									KHDA, AEQREFNKHLSA,PEFRELSKHDVA, PKPHGFPGQEYV,KPEKLNGVALSG, NSLKENGVALSG,VLGPRHEPDSGA, EKLYYHDSQEKH,AWQKSKGVALSG, QRPDPKDGQAKD
O'Connell 2016 (96)	USA	Case control study	IS vs. SM; IS vs. Controls	ED	MRI	whole blood	4.5h; 5.3h	Genomics	ANTXR2 gene, STK3 gene, PDK4 gene, CD163 gene, MAL gene, GRAP gene, ID3 gene, CTSZ gene, KIF1B gene, PLXDC2 gene
O'Connell 2017 (97)	USA	Case control study	IS vs. SM	ED	CT; MRI	plasma	4.5h	cell free DNA	cfDNA
O'Connell 2017 (98)	USA	Case control study	IS vs. Controls	public platform	Not stated	whole blood	24h; 5h; 3h	genes	ANTXR2 gene, STK3 gene, PDK4 gene, CD163 gene, MAL gene, GRAP gene, ID3 gene, CTSZ gene, KIF1B gene, PLXDC2 gene
O'Connell 2020 (99)	USA	Case control study	IS vs. Controls	ED	CT; MRI	plasma	24h	proteins	NfL, Tau
Nielsen 2020 (100)	Denmark	Prospective study	IS vs. TIA; IS vs. Controls	Neurology ward	CT; MRI	plasma	8h	proteins	NfL, VEGF-A, VCAM-1, ICAM-1, IL-6, S100B, E-selectin
Nguyen 2020 (101)	Netherland	Prospective study	IS vs. HS; IS vs. SM; IS vs. Controls; HS vs. SM	ED	CT	plasma	6h	Transcriptomics	tRNA-TyrGTA, tRNA-ThrCGT, tRNA-ValCAC
Nahan 2017 (102)	USA	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	ED	Not stated	plasma	12h	Proteomics	IS vs. control (As, Co, Fe, Li, Sr, U, Se, Cd), HS vs. control (Ag, Al, As , Co, Ni, U , Zn, Fe, Sr, Cd, Pb, Se); HS vs. IS (Ag, Co, Fe, Al, As, Li, Ni, U, W), IS special markers (calpain-15, titin Isoform 3, tropomyosin alpha-4 chain); HS special markers (bestrophin-3, GIRK-1, TTBK1, CAB3)
Montaner 2012 (103)	Spain	Prospective study	IS vs. HS	ED	CT	plasma	6h	proteins	S100B, sRAGE
Menon 2018 (104)	India	Prospective study	IS vs. Controls	Not stated	Not stated	serum	24h; 1h	proteins	IMA
Mattila 2021 (105)	Finland	Prospective study	IS vs. HS	ED	Not stated	plasma	3h; 1h	proteins	GFAP
Matsuo 2013 (106)	Japan	Prospective study	IS vs. Controls	Not stated	CT; MRI	plasma	24h	proteins	VEGF
Matsumori 2002 (107)	Japan	Case control study	IS vs. Controls	Not stated	CT	serum	24h	proteins	HGF
Maly 2021 (108)	Czech Republic	Cross-sectional study	IS vs. Controls	Not stated	Not stated	plasma	4.5h	Metabolomics; lipidomics	FA (20:2), FA (20:3), FA (20:4), FA (20:5), FA (22:4), FA (22:5), FA (22:6), FA (16:1), FA (17:1), AHFA (14:0/16:2), FAHFA (16:1/18:3), FAHFA (18:1/20:3), FAHFA (18:2/20:4), FAHFA (20:4/18:3), LPC (20:5), LPC (22:5), LPC (22:6), LPE (18:2), LPE (20:4), LPE (22:6), LPI (18:1), LPI (18:2), TG (14.0_16.1_20.3), TG (16:0_16:1_18:0), TG (16:0_18:2_18:3), TG (16:0_18:2_22:6), TG (17:1_17:2_19:0)
Mahovic 2013 (109)	Croatia	Prospective study	IS vs. Controls	Not stated	CT	serum	24h	proteins	soluble Fas/APO 1 (sFas/APO 1)
Ma 2019 (110)	China	Case control study	IS vs. Controls	ED + Neurology department	CT; MRI	plasma	6h	transcripts	miR-93
Luger 2017 (111)	Germany	Retrospective study	IS vs. HS; Other: HS vs. IS+SM	ED + Neurology depart	CT; MRI	serum	6h	proteins	GFAP

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Lu 2020 (112)	China	Case control study +	IS vs. Controls	Not stated	MRI	whole blood	24h; 3h	Transcript o mics	circ-PHKA2, circ-BBS2
Long 2013 (113)	China	Cross-sectional study	IS vs. Controls	Not stated	Not stated	plasma	24h	transcripts	miR-30a, miR-126, let-7b
Llombart 2016 (114)	Spain	Retrospecti ve study	IS vs. HS	ED	CT	plasma	6h	proteins	RBP4, GFAP
Liu 2015 (115)	China	Prospectiv e study	IS vs. Controls	Neuro lo gy ward	MRI	serum	24h	transcripts ; proteins	miR-124, miR-9, miR-219, MMP9
Liu 2017 (116)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	9h	Metabolo mi cs	serine, isoleucine, betaine, PC (5:0/5:0), LysoPE (18:2)
Liu 2020 (117)	China	Case control study	IS vs. HS; IS vs. Controls	Neuro lo gy ward	CT; MRI	serum	24h	proteins	Sphingosine 1-phosphate (S1P)
Liswati 2009 (118)	Indonesia n	Case control study	IS vs. HS	Not stated	CT	plasma	24h	proteins	S100B, MBP
Li 2021 (119)	China	Case control study	IS vs. Controls	Neuro lo gy ward	MRI	serum	24h	proteins	Lp-PLA2
Li 2015 (120)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	24h	Transcript o mics	miR-32-3p, miR-106-5p, miR-532-5p, miR-1246
Li 2018 (121)	China	Prospectiv e study	IS vs. Controls	ED	CT; MRI	plasma; lymphocyt es , neutrophil s	6h	transcripts ; proteins	miR-424, TNFa, IGF1
Leung 2014 (122)	China	Prospectiv e study	IS vs. HS; IS vs. Controls; HS vs. Controls	ED	CT; MRI	plasma	24h; 6h	transcripts	miR-124-3p, miR-16
Laterza 2006 (123)	USA	Case control study + animal experiment study	IS vs. Controls	Not stated	Not stated	plasma	24h	Genomics ; proteins	VLP-1 gene, VLP-1
Laskowitz 2009 (124)	USA	Prospectiv e study	IS vs. HS; Total stroke vs. SM; Total stroke vs. TIA	Not stated	CT; MRI	serum	24h; 12h; 6h; 3h	proteins	MMP9, BNP, D-dimer, S100B
Kokocinska 2007 (125)	Poland	Case control study	IS vs. Controls	Neuro lo gy ward	CT	plasma	24h	proteins	S100B, Tissue Polypeptide Antigen (TPA)
Kokocinska 2005 (126)	Poland	Case control study	IS vs. Controls	Neuro lo gy ward	CT	serum	12h	proteins	S100B
Kodali 2013 (127)	USA	Case control study	IS vs. HS; IS vs. SM; HS vs. SM	ED	Not stated	plasma	12h	Proteomic s	Fibrinogen gamma chain, Protein kinase C eta type, Fibrinogen beta chain, Fibrinogen alpha chain, Complement C3, Methylenetetrahydrofolate reductase, Antithrombin-III, Collagen alpha-1(IV) chain
Kodali 2012 (128)	USA	Case control study	IS vs. HS; IS vs. SM; HS vs. SM	ED	Not stated	plasma	12h	Proteomic s	metalloproteins: Mg, Mn, Cu, Se, Pb, Mo
Kochanows ki 2012 (129)	Poland	Case control study	IS vs. Controls	Neuro lo gy ward	CT	plasma	24h	proteins	resistin, TNFa
Kavalci 2011 (130)	Turkey	Prospectiv e study	IS vs. HS	ED	Not stated	serum	24h	proteins	BNP, D-dimer, MMP-9, S100B
Katsanos 2017 (131)	Greece	Prospectiv e study	IS vs. HS; HS vs. SM; HS vs. Controls	ED	Not stated	plasma	6h	proteins	GFAP
Kashyap 2009 (132)	India	Case control study	IS vs. Controls	Neuro lo gy ward	CT	serum	24h	proteins	ITIH4
Kara 2014	Turkey	Prospectiv	IS vs.	ED	MRI	serum	24h	proteins	hsCRP, Lp-PLA2

(133)		e study	Controls						
Kalra 2021 (134)	Germany	Prospectiv e study	IS vs. HS; HS vs. IS+SM	Neuro lo gy ward	CT; MRI	serum	12h	proteins	GFAP
Kalani 2020 (135)	USA	Prospectiv e study	IS vs. HS	ED	CT; MRI	plasma	24h	Transcript o mics; extracellul ar vesicles	miR-150-3p, miR-4286, miR-132-3p, miR-30e-3p, miR-21-3p, miR-27b-3p, miR-342-3p, miR-186-5p, miR-338-3p, miR-5010-5p, miR-134-5p, miR-7c-5p, miR-485-5p
Jin 2017 (136)	China	Case control study	IS vs. Controls	Neuro lo gy ward	CT; MRI	plasma	24h	transcripts	miR-126, miR-130a, miR-222, miR-218, miR-185
Jiang 2011 (137)	China	Case control study	IS vs. Controls	Not stated	CT; MRI	serum	6h	Metabolo mi cs	folic acid, cysteine, S-adenosyl-homocysteine, oxidized glutathione, Tetrahydrofolate, Hydroxy eicosatetraenoic acid, Adenosine, Aldosterone, Hydroxy octadecadienoic acid, Deoxocathasterone, Sucrose 6-phosphate, Betanin
Jia 2015 (138)	China	Prospectiv e study	IS vs. Controls	Neuro lo gy ward	MRI	serum	24h	transcripts ; proteins	miR-145, miR-23a, miR-221, hsCRP, IL-6
Ji 2016 (139)	China	Case control study	IS vs. Controls	Neuro lo gy ward	CT; MRI	serum; exosome	24h	transcripts	miR-9, miR-124
Inoue 2019 (140)	Japan	Prospectiv e study	IS vs. HS	Not stated	CT; MRI	serum	24h	proteins	LOX-1
Iltumur 2006 (141)	Turkey	Case control study	IS vs. Controls	NICU	CT; MRI	plasma	24h	proteins	NT-proBNP, troponin I, CK-MB
Herisson 2010 (142)	France	Prospectiv e study	IS vs. HS; Total stroke vs. controls	stroke depart m ent	CT; MRI	serum	4.5h	proteins	IMA, HFABP
Han 2012 (143)	China	Case control study	IS vs. Controls; HS vs. Controls	Not stated	CT; MRI	serum	3h	proteins	IMA
Gunduz 2008 (144)	Turkey	Case control study	IS vs. HS; IS vs. Controls; HS vs. Controls	ED	CT; MRI	serum	24h	proteins	IMA
Gunaydin 2014 (145)	Turkey	Prospectiv e study	IS vs. Controls	ED	CT; MRI	plasma	12h; 6h	proteins	SCUBE1
Glickman 2011 (146)	USA	Prospectiv e study	IS vs. SM	ED	CT	plasma	5h	proteins	BNP, MMP-9, D-dimer, S100B, CRP
Giannopoul os 2008 (147)	Greece	Case control study	IS vs. Controls	Not stated	CT	plasma	24h	proteins	Endothelin-1, CRP, fibrinogen
Garlichs 2003 (148)	Germany	Case control study	IS vs. TIA; IS vs. Controls; TIA vs. Controls	Neuro lo gy ward	CT	serum; plasma	24h	proteins	platelet CD154, platelet P-selectin, soluble CD154, monocyte CD40, MCP- 1
Foerch 2012 (149)	Germany	Prospectiv e study	IS vs. HS; IS vs. SM; HS vs. SM	Stroke centre	CT; MRI	plasma	4.5h	proteins	GFAP
Foerch 2006 (150)	Germany	Prospectiv e study	IS vs. HS	Stroke unit or NICU	CT; MRI	serum	6h	proteins	GFAP
Fiszer 1998 (151)	Poland	Case control study	IS vs. Controls	Not stated	CT	whole blood	12h	proteins	CD54, CD11a, CD11b, CD18
Fassbender 1997 (152)	Germany	Case control study	IS vs. Controls	Not stated	CT	serum	24h; 10h, 8h, 4h	proteins	S100B, NSE
Fang 2018 (153)	China	Prospectiv e study	IS vs. HS; IS vs. SM; IS vs. Controls; HS vs. SM; HS vs. Controls; Total stroke vs.	ED	Not stated	plasma	24h	proteins	S100B, CRP, IL-6, PAI-1, MMP-9, P-selectin, ICAM-1, TNFa, LDL, IL- 10, NO, GFAP

			controls						
Ewida 2021 (154)	Egypt	Prospective study	IS vs. HS; IS vs. Controls; HS vs. Controls; Total stroke vs. controls	Neurology ward	CT; MRI	serum	24h	transcripts; proteins	IS vs. HS(lncRNAs HIF1A-AS2, lncRNAs LINK-A, mRNA HIF1-α, MDA, VEGF), IS vs. control (PI3K, p?Akt, VEGFR2, TIE2), HS vs. control (PI3K, p?Akt, VEGFR2, TIE2), total stroke vs. control(lncRNAs HIF1A-AS2, lncRNAs LINK-A, mRNA HIF1-?, TAC, VEGF, ANG1, BDNF, PI3K, p?Akt, VEGFR2, TIE2)
Ekingen 2017 (155)	Turkey	Prospective study	IS vs. Controls	ED	CT	serum	24h	proteins	Galectin-3, GFAP
Dvorak 2009 (156)	Germany	Prospective study	IS vs. HS	Not stated	CT; MRI	serum	6h; 4h; 3h; 2h	proteins	GFAP
Duan 2015 (157)	China	Prospective study	IS vs. Controls	ED	CT; MRI	serum	24h	proteins	CXCL12
De Marchis 2018 (158)	Switzerland	Prospective study	IS vs. TIA; IS+TIA vs. control	ED	MRI	serum	24h	proteins	NfL
Dassan 2012 (159)	UK	Prospective study	IS vs. SM	ED	MRI	serum	24h	proteins	VEGF
Dambinova 2003 (160)	Russia	Prospective study	IS vs. HS; IS vs. TIA; IS vs. Controls; TIA vs. Controls	Neurology and Neurosurgery Department	CT; MRI	serum	3h	proteins	NR2A/2B aAb
Dambinova 2012 (161)	USA	Prospective study	IS vs. Controls	ED	CT; MRI	plasma	12h	proteins	NR2 peptide
Cheng 2018 (162)	China	Prospective study	IS vs. Controls	ED	CT; MRI	serum	24h	transcripts	miR-148b-3p, miR-151b, miR-27b-3p
Chen 2018 (163)	China	Prospective study	IS vs. Controls	Not stated	MRI	serum; plasma	24h	transcripts; proteins	miR-146b, hsCRP, IL-6
Cavrak 2021 (164)	USA	Case control study	IS vs. TIA; IS vs. SM; TIA vs. SM	ED	MRI	whole blood	24h	cell count and percentage	neutrophil percentage > 60
Cano 2003 (165)	Venezuela	Prospective study	IS vs. Controls	ED	CT	serum	24h	metabolites	malondialdehyde, nitric oxide
Cakmak 2014 (166)	Turkey	Prospective study	IS vs. Controls	ED	CT; MRI	serum	24h	proteins	IMA, S100B, NSE
Büttner 1997 (167)	Germany	Prospective study	IS vs. Controls	Not stated	CT	serum	24h; 12h	proteins	S100B
Bustamante 2021 (168)	Spain	Prospective study	IS vs. HS	ED	CT; MRI	plasma	4.5h	proteins	GFAP, RBP-4, NT-proBNP, endostatin
Bustamante 2017 (169)	Spain	Prospective study	IS vs. HS; Total stroke vs. SM	ED	CT; MRI	plasma	6h	proteins	NT-proBNP, IGFBP-3, TNF-R1, GroA, FasL, IL-6, D-dimer, vWF, VAP-1, Endostatin, S100B, Hsc70, Apo CIII, NCAM, MMP-9, bNGF, Caspase-3, NSE, cFn, IL-2RG, IL-17A
Bolayir 2019 (170)	Turkey	Prospective study	IS vs. Controls	Neurology ward	CT; MRI	serum	24h	proteins	SCUBE1, hsCRP
Bibl 2012 (171)	Germany	Case control study	IS vs. Controls	Not stated	CT; MRI	plasma	12h	proteins	Abeta1-37, Abeta1-38
Barr 2010 (172)	UK	Case control study	IS vs. Controls	Not stated	MRI	whole blood	24h	Genomics	ARG1 gene, CA4 gene, CCR7 gene, CSPG2 gene, IQGAP1 gene, LY96 gene, MMP9 gene, ORM1 gene, S100A12 gene
Azarpazhooh 2010 (173)	Iran	Case control study	IS vs. HS; Total stroke vs. controls	Not stated	CT; MRI	serum	24h	proteins	anti-HSP27, hsCRP
Atik 2016 (174)	Turkey	Prospective study	IS vs. Controls; HS vs. Controls	ED	CT; MRI	serum	12h; 3h	proteins	albumin, ischemic modified albumin (IMA), IMA/albumin ratio (IMAR), total antioxidant status, total oxidant status (TOS), oxidative stress index (OSI)
Alvarez-Perez 2011 (175)	Portugal	Prospective study	IS vs. Controls	ED	Not stated	plasma	24h	proteins	fibrinogen, CRP

Allard 2004 (176)	Switzerland	Prospective study	IS vs. HS; IS vs. Controls; HS vs. Controls; Total stroke vs. controls	ED	CT; MRI	plasma	6h	Proteomics	ApoC-I, ApoC-III
Allard 2005 (177)	Switzerland	Retrospective study	IS vs. Controls; HS vs. Controls; TIA vs. Controls; Total stroke vs. controls	ED	CT; MRI	plasma	24h	proteins	PARK7, NDKA
Algin 2019 (178)	Turkey	Prospective study	IS vs. Controls	ED	MRI	serum	4h	proteins	BDNF, VILIP-1
Algawwam 2021 (179)	Iraq	Prospective study	IS vs. Controls	Not stated	Not stated	serum	24h	proteins	GPBB
Ahn 2011 (180)	Korea	Prospective study	IS vs. SM	ED	CT; MRI	serum	6h	proteins	IMA index, IMA
Sadik 2021 (181)	Egypt	Prospective study	IS vs. Controls	Not stated	CT	serum	12h	transcripts	miR-155, JAK2 mRNA, STAT3 mRNA
Abe 2020 (183)	Japan	Prospective study	IS vs. Controls	Not stated	Not stated	whole blood	12h	Transcripto mics	miR-505-5p, miR-1255b-5p, miR-550b-2-5p, miR-4523, miR-6795-3p
2023 (184)	China		Ischemic stroke	Not stated	Not stated	Plasma	Not stated	genes	CDK-10; ERCC3; CHEK2
Xie y 2023 (185)	China	Prospective study	Ischemic stroke	Not stated	Not stated	Blood	Not stated	proteins	Lipocalcin-2
Jian 2022 (186)	China	Retrospective study	Acute ischemic stroke with carotid artery plaque	Not stated	Not stated	serum	Not stated	proteins	IgE; LP-PLA2; SAA; LDL-Cholesterol; Total cholesterol; triglycerides; D-dimer
Wu J 2022 (187)	China		Acute ischemic stroke vs controls	Not stated	Not stated	Plasma	Not stated	proteins	Plasma neurofilament light chain
Wang G 2022 (188)	China	Prospective study	Acute ischemic stroke vs controls	Not stated	Not stated	Blood	Day1 ,2,3	genes	Long noncoding RNA intersectin 1-2
Qian M 2022 (189)	China	Retrospective study	Acute ischemic stroke	Not stated	Not stated		1		Red cell index

DISCUSSION

The study provides a comprehensive updated perspective on diagnostic circulating biomarkers in ischemic stroke. We examined a comprehensive set of 190 publications and combined 518 biomarkers, encompassing genes, transcripts, proteins, and metabolites.

In general, quality assessment suggests a relatively low likelihood of bias and worries about the relevance of the findings in the studies that were included. Nevertheless, there are certain constraints to consider, such as a significant number of ambiguous assessments regarding patient selection and potential bias in the reference standard. Additionally, the relevance of the findings may be questionable for certain research. Due to a significant amount of research lacking sufficient documentation of their methods, it is advisable to approach the results with caution. Subsequent research should prioritize the enhancement of study quality assessment by emphasizing transparent and thorough reporting, in accordance with established quality evaluation tools like QUADAS. Standardized reporting is crucial for assessing the robustness of evidence when combining diagnostic accuracy studies in systematic reviews. The included studies span publication years from 1997 to 2022, indicating sustained and increasing research interest in this field over the past 25 years. The most recent years have seen a notable rise, with 43 studies published from 2019-2022. This growth likely reflects advances in omics technologies and analytical techniques that enable more comprehensive biomarker discovery and validation.[15] The studies originated from 30 different countries across 5 continents, demonstrating the global prevalence of stroke and widespread research efforts to develop stroke biomarkers. China has been especially prolific in recent years, contributing 22 of the 43 studies (51%) published from 2019-2022. This reflects the high stroke burden in China and the nation's increasing investment and focus on stroke biomarker research. The included studies represent a diverse set of publication years and countries of origin. The

growing number of studies from China in particular, along with steadily increasing publications overall, exemplifies the intensifying global efforts to find clinically useful stroke biomarkers. Continued multinational research across different populations remains crucial.

The studies encompass a diverse range of prospective, retrospective, case-control, and cross-sectional designs. The largest group consists of 67 prospective studies. This diversity increases confidence that findings are not simply an artifact of particular study methodologies. Prospective studies were the most common allowing biomarkers to be measured early after stroke onset and correlated with diagnosis and outcomes over time. Case-control studies made up 29%, enabling comparisons between stroke patients and healthy controls. Other designs were less common but still provided useful data. The majority of research conducts comparisons between patients with ischemic stroke and individuals who are in good health. While useful for identifying potential biomarkers, such studies lack the context of comparing ischemic to hemorrhagic strokes. Additional comparisons encompass ischemic versus hemorrhagic stroke, stroke versus transient ischemic attack, and so forth. These more clinically relevant comparisons aid in distinguishing stroke subtypes and ruling out mimics, both of which are vital for optimal triage and treatment. More studies directly comparing ischemic and hemorrhagic strokes would be valuable.

The most prevalent biosamples utilized are blood, serum, and plasma. This reflects the minimally-invasive nature and ease of obtaining blood samples in clinical settings. Some studies additionally utilize whole blood, platelets (potentially leveraging their role in thrombosis and inflammation in stroke), PBMCs (peripheral blood mononuclear cells), and microvesicles. The biomarkers commonly examined encompass proteins (such as S100B, GFAP, NSE, etc.), microRNAs, metabolites (This allowed unbiased discovery of small molecules altered in ischemic stroke, generating hypotheses for further mechanistic investigation), and genes (This enabled examination of differential gene expression related to stroke pathways). Circulating miRNAs have potential as minimally invasive biomarkers given their regulatory roles and relative stability. Proteomics approaches are commonly utilized as well. These untargeted proteomics analyses can identify novel protein biomarker candidates complementary to targeted assays.

Proteins emerged as the predominant biomarker class, but miRNAs, metabolites, genes/transcripts, and proteomics data also contributed substantially. Each biomarker type offers unique advantages and insights into stroke pathobiology. Integrating data across multiple omics levels represents a promising strategy going forward. S100B and GFAP were the most consistent biomarkers in the diagnosis and management of stroke, particularly ischemic stroke. S100B, originating from astrocytes, is involved in the regulation of calcium balance in neurons and plays a critical role in neuroprotection and neuroplasticity. Its elevated levels in serum have been associated with brain damage, making it a potential marker for the severity and outcome of ischemic stroke.[16] GFAP, on the other hand, is a filament protein found in astrocytes, critical for maintaining the structural integrity of the central nervous system. It becomes elevated in response to astrocytic damage or activation, serving as a sensitive marker for brain injury. Studies have shown GFAP to be more sensitive than S100B in detecting small lesions and minor strokes, with its concentration rise indicating the extent of brain injury.[17,18] Both biomarkers have shown promise in improving the acute diagnosis and management of stroke, offering insights into the severity and potential outcomes of ischemic events.

The frequently employed comparator group consisted of healthy controls, which did not correctly depict the situation in the medical setting. Merely 9.3% of the studies incorporated a SAH group, which is an important differential diagnosis of ischemic stroke. 50% of the studies had a sample size smaller than 100 individuals. Smaller sample sizes increase the risk of false positive/negative findings and reduce statistical power. Ongoing collaborative efforts to pool samples and data across centers would help increase statistical robustness. The location was unspecified in most of the investigations (45%, 85/190), limiting assessment of generalizability. The studies that included information on the setting reported that the emergency department (ED) was the most frequent (15%, 29/190), followed by the neurology ward (14%, 26/190), and inpatient stroke units (6%, 11/190). Enrollment from stroke centers or off-site through mobile stroke units could help improve representation.

About one third of the studies, 29% (55/190) did not report the reference standard used, potentially bringing diagnoses into question. Clear reporting of rigorous diagnostic criteria is critical. The sample times varied extensively, ranging from 1 hour to 24 hours after the onset of stroke. The predominant sampling intervals were 24 hours (51%, 97/190), 6 hours (17%, 32/190), and 12 hours (16%, 31/190). The practice of collecting data at various time intervals was observed in 15% (29/190) of the investigations. This provides valuable information about biomarker kinetics. Early time points and repeated sampling are crucial for biomarkers intended to guide acute triage and clinical decision-making.

Overall, there was some lack of reporting on the setting and reference standards. The most often reported details were the emergency department (ED), inpatient wards, CT scans, and MRI scans. Sampling during the first 24 hours was common, with 24 hours and 6 hours being the most commonly used time intervals. Several research utilized

multiple time points to monitor the fluctuations of biomarkers over a period of time.

This review highlights opportunities to increase sample sizes through multi-center collaborations, enroll subjects from a wider range of clinical settings, enforce stricter diagnostic criteria, and measure biomarkers repeatedly at early time points after stroke onset. These strategies could optimize the development of accurate and clinically useful ischemic stroke biomarkers.

Biomarker research for ischemic stroke (ischemic stroke) is extensive and lacks specificity. The biomarkers lack adequate independent validation cohorts, resulting in a lack of depth. While research teams often discover new possible biomarkers, there is a shortage of sufficient validation. The adoption of these methods in clinical settings is impeded by the process of validation. There is a need to promote the involvement of research teams in the validation process to enhance the reliability of their findings and the ability to replicate results. One problem revolved around the difficulty of evaluating the diagnostic accuracy of the suggested biomarkers. Approximately 46.7% of the studies solely reported the biomarker concentration, neglecting to report sensitivity.

For future research, we suggest utilizing prospective study designs and recruiting a sample of individuals with SAH. When researching new biomarkers, it is important to concurrently validate or compare them with existing ones. Previously documented ideal biomarkers facilitate the synthesis and comparison of data to find highly valuable candidates. Researchers should strive to augment the sample size via collaborations across several centers to discover biomarkers that distinguish between ischemic stroke (ischemic stroke) and hemorrhagic stroke, subarachnoid hemorrhage (SAH), transient ischemic attack (TIA), and healthy individuals and control subjects. In order to expedite the diagnosing process, blood biomarkers may be employed. For reperfusion therapy, it is preferable to focus on sampling time within 6 hours of the onset of symptoms. Moreover, it is advisable to choose compounds as potential biomarkers that demonstrate substantial alterations during a specific period and possess adequate levels of blood concentration to be identified.

CONCLUSION

Our review emphasizes the crucial necessity of having easily available and precise diagnostic instruments for ischemic stroke in order to enhance clinical results. Protein biomarkers found in blood show potential for quickly and non-invasively diagnosing ischemic stroke. The biomarkers could transform stroke management by allowing early detection and distinction from hemorrhagic stroke, therefore aiding in prompt therapeutic approaches. Future research should prioritize verifying these biomarkers in broader, diverse populations and incorporating them into clinical practice to improve stroke diagnosis, treatment, and patient prognosis.

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