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Authors

Misra, Shubham
Khan, Erum
Lam, TuKiet
et al.

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RESEARCH ARTICLE

Common Pathways of Epileptogenesis in Patients With Epilepsy Post–Brain Injury

Findings From a Systematic Review and Meta-analysis

Shubham Misra, MTech, PhD,* Erum I. Khan, MBBS,* TuKiet T. Lam, PhD, Rajarshi Mazumder, MD, Kapil Gururangan, MD, L. Brian Hickman, MD, MSc, Vaibhav Goswami, MD, Melissa C. Funaro, MLS, MS, Ece Eldem, MSc, Lauren H. Sansing, MD, MS, Jason J. Sico, MD, MHS, Terence J. Quinn, MD, David S. Liebeskind, MD, Joan Montaner, MD, PhD, Patrick Kwan, MD, PhD, and Nishant K. Mishra, MD, PhD

Correspondence
Prof. Mishra
nishant.mishra@yale.edu

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Abstract

Background and Objectives

Epilepsy may result from various brain injuries, including stroke (ischemic and hemorrhagic), traumatic brain injury, and infections. Identifying shared common biological pathways and biomarkers of the epileptogenic process initiated by the different injuries may lead to novel targets for preventing the development of epilepsy. We systematically reviewed biofluid biomarkers to test their association with the risk of post–brain injury epilepsy.

Methods

We searched articles until January 25, 2022, in MEDLINE, Embase, PsycInfo, Web of Science, and Cochrane. The primary outcome was the difference in mean biomarker levels in patients with and without post–brain injury epilepsy. We used the modified quality score on prognostic studies for risk of bias assessment. We calculated each biomarker's pooled standardized mean difference (SMD) and 95% CI. Molecular interaction network and enrichment analyses were conducted in Cytoscape (PROSPERO CRD42021297110).

Results

We included 22 studies with 1,499 cases with post–brain injury epilepsy and 7,929 controls without post–brain injury epilepsy. Forty-five biomarkers in the blood or CSF were investigated with samples collected at disparate time points. Of 22 studies, 21 had a moderate-to-high risk of bias. Most of the biomarkers (28/45) were investigated in single studies; only 9 provided validation data, and studies used variable definitions for early-onset and late-onset seizures. A meta-analysis was possible for 19 biomarkers. Blood glucose levels in 4 studies were significantly higher in patients with poststroke epilepsy (PSE) than those without PSE (SMD 0.44; CI 0.19–0.69). From individual studies, 15 biomarkers in the blood and 7 in the CSF were significantly associated with post–brain injury epilepsy. Enrichment analysis identified that the significant biomarkers (interleukin [IL]–6, IL-1β]) were predominantly inflammation related.

Discussion

We cannot yet recommend using the reported biomarkers for designing antiepileptogenesis trials or use in the clinical setting because of methodological heterogeneity, bias in the included studies, and insufficient validation studies. Although our analyses indicate the plausible role of inflammation in epileptogenesis, this is likely not the only mechanism. For example, an

*These authors contributed equally to this work and share the first authorship.

From the Department of Neurology (S.M., E.E., L.H.S., J.J.S., N.K.M.), Yale University School of Medicine, New Haven, CT; Medical School (E.I.K.), B.J. Medical College and Civil Hospital, Ahmedabad, India; Keck MS & Proteomics Resource (T.T.L.), Yale University School of Medicine, New Haven, CT; Department of Neurology (R.M., K.G., L.B.H., D.S.L.), University of California, Los Angeles; Department of Neurology (K.G.), Icahn School of Medicine at Mount Sinai, New York, NY; Department of Neurology (V.G.), Tower Health, Philadelphia, PA; Harvey Cushing/John Hay Whitney Medical Library (M.C.F.), Yale University, New Haven, CT; Department of Immunobiology (L.H.S.), Yale University School of Medicine, New Haven, CT; Institute of Cardiovascular and Medical Sciences (T.J.Q.), University of Glasgow, Scotland, UK; Institute de Biomedicine of Seville (J.M.), IBiS/Hospital Universitario Virgen del Rocío/CSIC/University of Seville; Department of Neurology (J.M.), Hospital Universitario Virgen Macarena, Seville; Neurovascular Research Laboratory Vall d'Hebron Institute of Research (VHIR) (J.M.), Barcelona, Spain; and Department of Neuroscience (P.K.), Central Clinical School, Monash University, Melbourne, Australia.

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Glossary

FDA = Federal Drug Administration; **IL** = interleukin; **PRISMA** = Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **PSE** = poststroke epilepsy; **PTE** = Posttraumatic Epilepsy; **SMD** = standardized mean difference; **SOD** = superoxide dismutase; **sST2** = soluble suppression of tumorigenesis–2; **TBI** = traumatic brain injury; **TLC** = total leukocyte count.

individual's genetic susceptibilities might contribute to his/her risk of epileptogenesis after brain injury. Rigorously designed biomarker studies with methods acceptable to the regulatory bodies should be conducted.

Introduction

Persons with brain injuries such as stroke, traumatic brain injury (TBI), and infections carry a 2–7 times greater risk of developing epilepsy and are associated with poor outcomes.[1–3] After an acute injury to the brain, there is often a latent period when the brain undergoes epileptogenic changes in some patients.[4,5] Cerebral repair after a brain injury is mediated through the expression of inflammatory pathways, and prolonged or abnormal activation of the inflammatory pathways can result in maladaptive neuronal plasticity, leading to an increased risk of seizures. Some molecular mediators of inflammation released during cerebral injury and during subsequent repair have been tested as possible biomarkers of epileptogenesis; however, none of these biomarkers have been approved by the Federal Drug Administration (FDA) or other similar national regulatory bodies for use in clinical trials or are used in clinical settings. It is unknown whether there are shared biological pathways in the epileptogenic process among these cerebral injuries. A better understanding of the biological pathways that drive epileptogenesis will allow the discovery of biomarkers for human clinical trials and regulatory approval, for instance, through the FDA Biomarker Qualification framework.[6] Therefore, we first undertook a systematic review of biofluid biomarkers to test their association with humans' risk of epileptic seizures following a cerebral injury. Second, we created an interaction network of significant biomarkers to identify the common biological pathways involved in post–brain injury epilepsy.

Methods

Literature Search

We used a comprehensive search strategy designed with the help of information specialists and librarians. We searched articles from inception until January 25, 2022, in the following databases: MEDLINE, Embase, PsycInfo, Web of Science, and Cochrane. We reviewed the relevant references included in the studies and review articles. Both English and foreign language articles were eligible for inclusion. No date limit was applied. The following Medical Subject Headings (MeSH) or free-text terms were used to search for the concepts of biomarkers, seizure, brain injury, and prediction: (1) "biomarkers," "biological markers," "blood," "plasma," "serum," "CSF," "saliva," and "urine"; (2) "seizure," "epilepsy," "convulsions," "epileptogenesis," "late-onset," and "early-onset"; (3)"CNS," "acute injury," "stroke," "ischemic stroke," "cerebral ischemia," "hemorrhagic stroke," "intracerebral hemorrhage," "TBI," "brain infection," "meningitis," "encephalitis," and "brain abscess"; and (4) "prognosis" and "prediction." A detailed search strategy is provided in the eAppendix, links.lww.com/WNL/D32.

Eligibility Criteria

We included studies that consisted of individuals (aged 18 years or older) with no history of seizure/epilepsy who have experienced 1 of the 3 acute brain injuries (stroke [ischemic and hemorrhagic], TBI, or brain infections) with the development of epilepsy post–brain injury and have potential biomarkers measured in either the blood (plasma/serum), CSF, urine, or saliva. We applied no restrictions based on the date or language of publication, sex, or ethnicity. We included only published studies conducted on humans.

We excluded duplicate publications, narrative or systematic reviews, conference proceedings, dissertations, preprints, ongoing/unpublished studies, and studies without available full texts.

Standard Protocol Approvals, Registrations, and Patient Consents

We registered the protocol of this systematic review on PROSPERO (registration CRD42021297110).[7] Because this was a systematic review and meta-analysis of published studies, no ethics committee approval or protocol approvals were required. No informed consent or authorization for disclosure was required to conduct this systematic review.

Outcomes

The primary outcome of this systematic review was the difference in the mean biomarker levels in patients with postacute brain injury epilepsy compared with patients with postacute brain injury without epilepsy.

Secondary outcomes included differences in the mean biomarker levels in patients with (1) early-onset seizures and late-onset seizures postacute brain injuries; (2) differences in the mean biomarker levels in ischemic and hemorrhagic stroke patients with the development of epilepsy poststroke;

and (3) interaction network and enrichment analyses of the significant biomarkers to identify associated underlying pathways. Considering the lack of uniformity in defining early-onset and late-onset seizures poststroke, post-TBI, and post–brain infections, we decided to accept study definitions as reported (listed in Table 1).

Data Extraction

We report our systematic review findings based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (checklist provided in the supplemental material).[8] We applied the inclusion criteria using the software Covidence. Six review authors (S.M., L.B.H., E.I.K., K.G., V.G., and E.E.) were blinded to each other's work and independently assessed the titles and abstracts of the retrieved articles for eligibility. Subsequently, we screened the full-text articles for inclusion. We documented this in the PRISMA format and resolved conflicts through discussion and consultation with the corresponding author (N.K.M.). We extracted the following information from each eligible study: first author, year of publication, sample size, the mean or median age of individuals, sex, study design, type of acute brain injury considered, type and number of seizures/epilepsies, the definition of early-onset and late-onset seizures, biomarker levels, type of biofluid considered, and follow-up duration. If required, we emailed the original study authors twice to acquire the missing data.

Risk of Bias (Quality) Assessment

Two independent review authors (S.M. and E.I.K.) assessed the methodological quality of the studies included in this systematic review. We used the modified quality score based on the Reporting Recommendations For Tumor Marker Prognostic Studies guidelines and as guided by the systematic review by Montellano et al.[9] Each item in this 18-item quality scale was marked as no–0 point, unclear–1 point, and yes–2 points. The scores ranged from 0 (minimum) to 36 (maximum), and the studies were divided into high risk of bias (score 0–12), some concerns/moderate risk of bias (score 13–24), and low risk of bias (score 25–36) (details in eAppendix, links.lww.com/WNL/D32).

Statistical Analysis

We collected aggregated biomarker data from each study as mean values and SDs. If biomarker levels in the studies were reported as median, ranges, and interquartile ranges, we converted their values to mean and SD using a conversion formula.[10]

For each biomarker, the level difference was calculated using the pooled standardized mean difference (SMD) and 95% CI between the patients who had post–brain injury seizures and those who did not.

A meta-analysis was performed for a given biomarker if 2 or more studies were reporting it, allowing for the pooling of results. We estimated the level of heterogeneity in the meta-analysis using Cochran Q statistic. We also reviewed heterogeneity using I^2 and categorized heterogeneity as low: $I^2 <$ 25%, moderate: $I^2 =$ 25%–75%, and high: $I^2 >$ 75%.[11] If significant heterogeneity was present, then results were combined using a random-effect model. Otherwise, a fixed-effect model was used.

We conducted interaction network and enrichment analyses of the biomarkers significantly associated with post–brain injury epilepsy. We used Cytoscape 3.9.1 software for this analysis. The interaction network and enrichment analysis are the bioinformatic analytical approaches to investigate the protein complexes and functional pathways linked to biological processes, e.g., epileptogenesis. The interaction network is made of nodes and edges. In our analyses, the nodes represent the biomarkers, and the edges represent the interaction between the biomarkers in the network. The strength of association between the proteins is reported as an interaction score of 0–1 and is represented by the thickness of the line connecting the nodes in the network. In the enrichment analysis, we report the strength of interaction of the common pathways associated with post–brain injury epilepsy using the p value.

Data Availability

The full dataset and statistical codes will be available on reasonable request from any qualified investigator.

Results

The final search retrieved a total of 5,684 references, which were pooled in EndNote and de-duplicated.[12] This set was uploaded to Covidence[13] for screening, which identified additional duplicates, leaving 3,810 for screening. After screening 57 full-text articles for eligibility, we included 22 studies in our systematic review and meta-analysis. The study flow diagram is shown in Figure 1. The included studies comprised 1,499 cases with postacute brain injury epilepsy and 7,929 controls without post–brain injury epilepsy. They investigated 45 biomarkers in the blood or CSF of patients who experienced epilepsy poststroke, post-TBI, or post–brain infections. We could not find any studies on post–brain insult epilepsy assessing biomarkers in the saliva or urine of patients who developed epilepsy post–brain injuries. The studies represent populations from 11 distinct countries: 8 from China,[14–21] 3 from the United States,[22–24] 2 each from India,[25,26] Italy,[27,28] and Spain,[29,30] 1 each from Japan,[31] Nigeria,[32] and Russia,[33] and 2 multicentric studies each from Ghana and Nigeria,[34] and Uganda and South Africa.[35] The publication years ranged from 2010 to 2022. The summary characteristics are listed in Table 1.

Risk of Bias (Quality) Assessment

Eight studies (36.4%) had a high risk of bias,[20–22,24,31–34] 13 studies (59.1%) had a moderate risk of bias,[14–19,23,25–29,35] and only 1 study (4.5%) had a low risk of bias[30] (eFigure 1, links.lww.com/WNL/D32). Only 8 of 22 studies prospectively collected data.[15,16,23,25,26,30,32,35]

None of the studies included in our systematic review used a preestablished biomarker cut-off, provided a rationale for sample size calculation, or externally validated their results.

Table 1 Summary Characteristics of Studies Included in the Systematic Review and Meta-analysis

S. No	Study author, year	Country	Study design	Type of brain injury	Early-onset/late-onset seizure (seizure definition)	Cases/ controls	Male individuals (cases/ controls)	Age (cases/ controls)	Type of body fluid	Biomarker(s) assayed	Assay methodology	Blood sample collection time	Time to seizure onset	Epilepsy definition	Follow-up duration
1	Aiwansoba et al. 2022[32]	Nigeria	Prospective cohort study	Stroke	Early (≤7d)	25/226	19/113	62.68 ± 15.70/ 59.67 ± 13.04	Blood	Glucose	NA	<24 h	Within 7 d	NA	42 d
2	Mele et al. 2022[27]	Italy	Retrospective cohort	Trauma	Late (>7 d) + early (≤7 d)	48/195	NA	NA	Blood (serum)	T3, T4, TSH	Automated chemiluminescence assay	14 ± 3 d	Average–6 mo	NA	NA
3	Zhang et al. 2022[14]	China	Case-control	Stroke	Early (NA)	31/49	10/26	83 (74–87)/76 (64.5–86.5)	Blood	HbA1c, Urea, Uric Acid, NSE	NA	<24 h	NA	NA	NA
4	Choudhary et al. 2021[25]	India	Prospective cohort	Trauma	Late (NA)	35/30	32/28	NA	Blood (serum)	IL-6, TNF-alpha, IFN-gamma	Immunoassays	Upon admission	NA	Spontaneous and unprovoked seizures >1 wk after TBI	1 y
5	Sarfo et al. 2021[34]	Ghana and Nigeria	Case-control	Stroke	Late (>7 d) + early (≤7 d)	499/ 2,845	292/1,578	58.3 ± 15.3/60.2 ± 14.1	Blood	TLC	NA	<8 d	NA	ILAE recommendation (2014)[50]	NA
6	Shen et al. 2021[15]	China	Prospective cohort	Stroke	Late (>7 d)	53/862	36/504	68.92 ± 8.19/66.93 ± 8.98	Blood (serum)	IL-1β	Sandwich ELISA	<24 h	NA	ILAE recommendation (2006)[51]	1 y
7	Tian et al. 2021[16]	China	Prospective cohort	Stroke	NA	25/118	24/46	60 (58–66)/60 (58–66)	CSF	TLC, IL-6, PCT, Protein, Glucose	NA	<14 d	NA	NA	NA
8	Wang et al. 2021[17]	China	Retrospective cohort	Stroke	Late (>14 d)	78/86	55/62	67.91 ± 9.60/69.14 ± 10.30	Blood (serum)	Neuropeptide Y	ELISA	<24 h	63.41 ± 18.34 d	ILAE recommendation (2017)[52]	1 y
9	Wang et al. 2021[18]	China	Retrospective cohort	Stroke	Late (>7 d) + early (≤7 d)	47/555	28/315	60 (42–73)/65 (52–71)	Blood	Glucose, PT, APTT	NA	<14 d	7 mo (3–13)	ILAE recommendation (2017)[52]	24 mo (IQR 16–36)
10	Abraira et al. 2020a[30]	Spain	Prospective cohort	Stroke	Late (>7 d)	51/844	30/487	68.4 ± 15.5/72.3 ± 12.9	Blood (plasma)	D-dimer, Endostatin, FasL, S100B, Hsc70, APO-CIII, GroA, IL-6, NT-proBNP, VAP-1, vWF, IGFBP-3, TNF-R1, NCAM	Immunoassays	<6 h	232 d (IQR 86–491)	ILAE recommendation (2014)[50]	4.8 y (IQR 1.6–5.2)

Continued

Table 1 Summary Characteristics of Studies Included in the Systematic Review and Meta-analysis *(continued)*

S. No	Study author, year	Country	Study design	Type of brain injury	Early-onset/late-onset seizure (seizure definition)	Cases/ controls	Male individuals (cases/ controls)	Age (cases/ controls)	Type of body fluid	Biomarker(s) assayed	Assay methodology	Blood sample collection time	Time to seizure onset	Epilepsy definition	Follow-up duration
11	Abraira et al. 2020b[29]	Spain	Retrospective cohort	Stroke	Early (≤7 d)	38/926	20/528	68.8 ± 14.7/72.4 ± 13.1	Blood (plasma)	D-dimer, Endostatin, FasL, S100B, Hsc70, APO-CIII, GroA, IL-6, NT-proBNP, VAP-1, vWF, IGFBP-3, TNF-R1, NCAM	Immunoassays	<6 h	1 d (0–4)	ILAE recommendation (2014)[50]	NA
12	Brigo et al. 2020[28]	Italy	Case-control	Stroke	Early (≤7 d)	79/158	44/88	72.94 ± 9/ 72.557 ± 13	Blood	Glucose	NA	NA	Within 7 d	ILAE recommendation (2017)[52]	NA
13	Lissak et al. 2020[22]	United States	Retrospective cohort	Stroke	Early (NA)	17/29	NA	NA	Blood (plasma)	ST2, IL6, CRP	ST2 Assay kit and ELISA kit	<5 d	average–8 d	NA	NA
14	Takase et al. 2020[31]	Japan	Case-control	Stroke	Late (>14 d)	43/50	21/26	79.0 (71–86)/ 78.5 (74–82)	Blood	CRP, D-dimer, Glucose	NA	NA	14 d	ILAE recommendation (2017)[52]	NA
15	Gazaryan LM, 2019[33]	Russia	Cross-sectional	Trauma	Late (NA)	29/28	25/25	49.55 ± 14.02/ 35.96 ± 7.40	Blood (serum)	NRG1	ELISA	On admission postseizure	6 mo–5 y	Recurrent seizures at intervals of >24 h	NA
16	Misra et al. 2019[26]	India	Prospective cohort	Infection	Late (>120 d) + early (<30 d)	29/20	14/9	28.67 ± 46.78/ 39.33 ± 49.47	Blood (serum)	MDA, Protein Carbonyl, SOD, Serum Catalase, GSH	Different methodologies used	On admission	NA	ILAE recommendation (2017)[52]	6 mo
17	Pastick et al. 2019[35]	Uganda and South Africa	Prospective cohort	Infection	NA	104/590	58/357	36 (IQR 29–42)/35 (IQR 30–40)	Blood and CSF	CD4, Glucose, CSF protein	NA	NA	9 d (IQR 4–36)	NA	46 wk
18	Wang et al. 2019[19]	China	Case-control	Stroke	Late (>14 d)	202/100	65/33	61.62 ± 0.80/61.19 ± 0.74	Blood (serum)	Uric acid	COBAS 8000 analyzer	<24 h postseizure	NA	ILAE recommendation (2017)[52]	NA
19	Xie et al. 2016[20]	China	Case-control	Stroke	NA	20/64	NA	NA	Blood (plasma)	GABA, Ca, Glutamate	HPLC	<72 h postseizure	NA	ILAE recommendation (1989)	NA
20	Claassen et al. 2014[23]	United States	Prospective cohort	Stroke	Early (NA)	18/91	NA	NA	Blood (serum)	hsCRP, TTR, TNF-R1	ELISA, immunoturbidimetric assay	<10 d	8.5 d (IQR 4.8–17)	NA	3 mo

Continued

Table 1 Summary Characteristics of Studies Included in the Systematic Review and Meta-analysis *(continued)*

S. No	Study author, year	Country	Study design	Type of brain injury	Early-onset/late-onset seizure (seizure definition)	Cases/ controls	Male individuals (cases/ controls)	Age (cases/ controls)	Type of body fluid	Biomarker(s) assayed	Assay methodology	Blood sample collection time	Time to seizure onset	Epilepsy definition	Follow-up duration
21	Diamond et al. 2014[24]	United States	Retrospective cohort	Trauma	Late (>7 d)	12/47	10/42	38.5 ± 4.80/37.2 ± 2.49	CSF and blood (serum)	IL-1β	Luminex bead array assay	<6 d postseizure	8 d–3 y	First seizure >7 d postinjury	3 y
22	Xiao et al. 2010[21]	China	Case-control	Infection	NA	16/16	9/11	32.94 ± 14.42/ 32.19 ± 14.49	CSF and blood (serum)	Talin 2	ELISA	NA	NA	ILAE recommendation (1989)	NA

Abbreviations: ApoC = apolipoprotein C; APTT = activated partial thromboplastin time; CD4 = cluster of differentiation 4; CRP = C-reactive protein; FasL = Fas ligand; GABA = gamma-aminobutyric acid; GroA = growth-related oncogene α; GSH = glutathione catalase; Hsc70 = heat shock cognate protein 70; IGFBP3 = insulin-like growth factor–binding protein 3; IL = interleukin; ILAE = International League Against Epilepsy; IQR = interquartile range; NA = not available; NCAM = neural cell adhesion molecule; NRG-1 = neuregulin-1; NSE = neuron-specific enolase; NTproBNP = N-terminal prohormone brain natriuretic peptide; PIE = postinfection epilepsy; PSE = poststroke epilepsy; PT = prothrombin time; PTE = posttraumatic epilepsy; SOD = superoxide dismutase; sST2 = soluble suppression of tumorigenesis-2; T3 = triiodothyronine; T4 = thyroxine; TLC = total leukocyte count; TNF-R1 = tumor necrosis factor–receptor 1; TSH = thyroid-stimulating hormone; VAP-1 = vascular adhesion protein-1; vWF = von Willebrand factor.

Only 2 studies[29,30] conducted blinded biomarker measurement, and only 1 study reported the Net Reclassification Index.[30] The quality assessment of each study is summarized in eTable 1, links.lww.com/WNL/D32.

Biomarkers of Poststroke Epilepsy

We included 15 studies of 1,226 cases with poststroke epilepsy (PSE) and 7,003 controls without PSE. Fourteen studies assessed 30 biomarkers in the blood, while 1 assessed 5 biomarkers in CSF. A meta-analysis was conducted for 18 blood biomarkers listed in Table 2. Only blood glucose levels in 4 studies were significantly higher in patients with PSE than in those without PSE (SMD 0.44; 95% CI 0.19–0.69) (Figure 2). No significant difference was observed for the remaining 17 biomarkers (eTable 2, links.lww.com/WNL/D32). A meta-analysis was not possible for 12 blood biomarkers due to an insufficient number of investigations. From individual studies, 4 biomarkers (soluble suppression of tumorigenesis–2 [sST2], high-sensitivity C-reactive protein [hsCRP], interleukin-1 beta [IL-1β], and neuron-specific enolase) had significantly higher levels in cases with PSE. By contrast, 4 biomarkers (transthyretin, neuropeptide Y, gamma-aminobutyric acid, and calcium) had substantially higher levels in controls who did not develop PSE. Significant biomarkers are listed in Table 2.

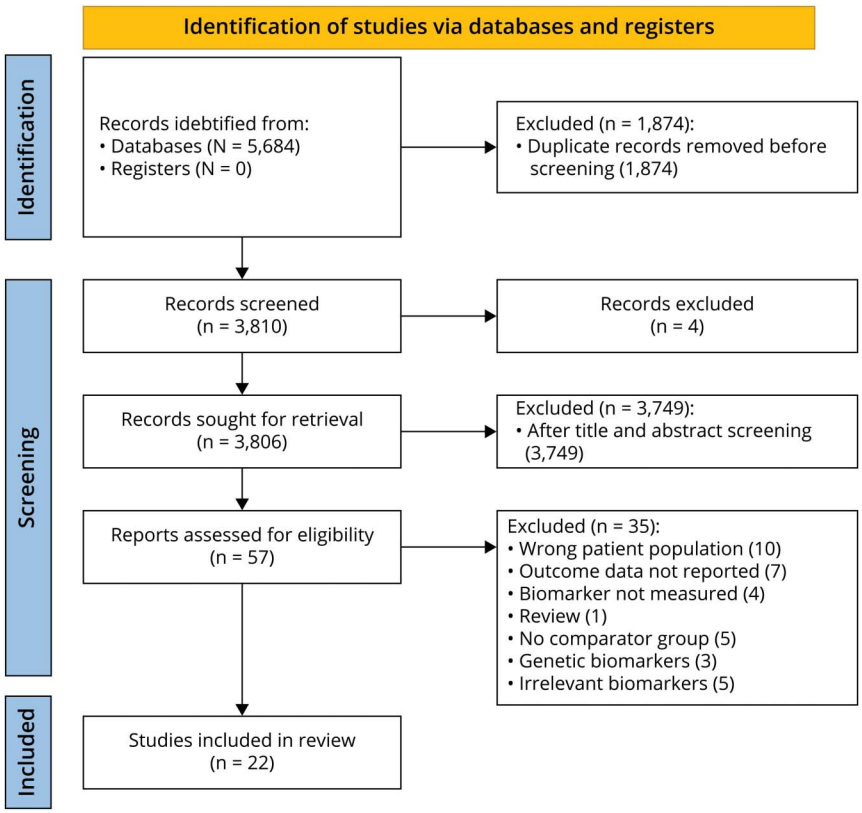
In a subgroup analysis based on the time to seizure, higher blood glucose levels were significantly associated with both early-onset (SMD 0.27; 95% CI 0.05–0.50) and late-onset epileptic seizures (SMD 0.61; 95% CI 0.17–1.05) compared with poststroke patients without epilepsy. Higher uric acid levels were significantly associated with late-onset epileptic seizures (SMD 3.01; 95% CI 2.67–3.35) (eTable 3, links.lww.com/WNL/D32). In another subgroup analysis based on stroke subtypes, no biomarker was associated with epilepsy after particular stroke subtypes in the meta-analysis (eTable 4). From individual studies, IL-1β and neuropeptide Y were significantly associated with postischemic stroke epilepsy, total leukocyte count (TLC) and blood glucose were significantly associated with postintracerebral hemorrhage epilepsy, and tumor necrosis factor–receptor 1, sST2, hsCRP, and transthyretin were significantly associated with postsubarachnoid hemorrhage epilepsy (Table 3).

From individual studies, all 5 biomarkers assessed in the CSF were significantly associated with PSE. Four biomarkers (TLC, IL-6, procalcitonin, and CSF protein) had significantly higher levels in cases with PSE. Unlike in the serum, glucose levels in the CSF were significantly higher in controls with no PSE (Table 3).

Biomarkers of Posttraumatic Epilepsy

We included 4 studies of 124 cases with posttraumatic epilepsy (PTE) and 300 controls without PTE. Four studies assessed 8 biomarkers in the blood, while 1 only evaluated 1 biomarker in the CSF. A meta-analysis was not possible for any biomarker because 2 or more studies could not be pooled. From individual studies, the levels of neuregulin-1 in the

Figure 1 PRISMA Study Flow Diagram



PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

blood were significantly higher in cases with PTE. In the blood, the levels of IL-1β were significantly higher in controls with no PTE. By contrast, in the CSF, the levels of IL-1β were significantly higher in cases with PTE (Table 2).

Biomarkers of Postbrain Infection Epilepsy

We included 3 studies of 149 cases with post–brain infection epilepsy and 626 controls without post–brain infection epilepsy. Brain infections comprised tuberculous meningitis, HIV-infected cryptococcal meningitis, and encephalitis. Three studies assessed 8 biomarkers in the blood, while 2 assessed 2 biomarkers in the CSF. A meta-analysis was not conducted for any biomarker assessed in the blood because of insufficient number of studies. From individual studies, 5 blood biomarkers (malondialdehyde, protein carbonyl, superoxide dismutase [SOD], glutathione catalase, and talin-2) were significantly associated with post–brain infection epilepsy (Table 2).

In the CSF, a meta-analysis was conducted for only CSF protein; however, the association was statistically nonsignificant. In an individual study, talin-2 levels in the CSF were significantly higher in cases with post–brain infection epilepsy (Table 2).

Search for the Common Molecular Pathways Associated With Postbrain Injury Epilepsy

Our systematic review identified 20 biomarkers significantly associated with either PSE, PTE, or post–brain infection epilepsy. However, we could not identify any common biomarker significantly associated with all 3 different major causes of postacute brain injury epilepsy (stroke, TBI, and brain infections). IL-1β levels in the blood were significantly associated in patients with PSE and PTE in individual studies. IL-6 levels were significantly associated in patients with PSE in the CSF and with PTE in the blood.

We analyzed the proteins corresponding to the 20 distinct biomarkers identified in this systematic review associated with epilepsy poststroke, post-TBI, or post–brain infections. The interaction network consisted of 18 biomarkers (nodes) having 29 interactions (edges) with 13 highly connected biomarkers (Figure 3A). IL-6 interacted with 11 proteins in the network and had the highest degree of interaction, followed by IL-1β, CRP, enolase 2 (ENO2), SOD1, etc (eTable 5, links.lww.com/WNL/D32). In our network, the interaction score of 9 of 29 interactions was higher than 0.70, with the most robust interaction score of 0.997 for interaction between IL-6 and IL-1β (eTable 6).

The significant pathways included acute inflammatory response, IL-6–mediated pathways, IL-10 signaling, extracellular space receptor, positive anion generation, endopeptidase-involved apoptotic pathway, and negative nucleocytoplasmic transport (Figure 3B and eTable 7, links.lww.com/WNL/D32).

Table 2 Significant Biomarkers Assessed in the Blood and CSF With Epilepsy Poststroke, TBI, and Brain Infections

Biomarkers and outcomes	No. of cases	No. of controls	No. of studies	SMD (95% CI); I^2	Study reference
Biomarkers in blood					
Glucose					
PSE vs no PSE[a]	194	989	4	0.44 (0.19 to 0.69); I^2 = 52%	18,28,31,32
IL-6					
PTE vs no PTE	35	30	1	0.67 (0.17 to 1.17)	25
sST2					
PSE vs no PSE	17	29	1	0.77 (0.15 to 1.39)	22
hsCRP					
PSE vs no PSE	18	91	1	0.97 (0.45 to 1.5)	23
Transthyretin					
PSE vs no PSE	17	90	1	−0.61 (−1.14 to −0.09)	23
IL-1β					
PSE vs no PSE	53	862	1	1.95 (1.66 to 2.24)	15
PTE vs no PTE	12	47	1	−1.61 (−2.31 to −0.9)	24
Neuropeptide Y					
PSE vs no PSE	78	86	1	−1.71 (−2.07 to −1.35)	17
Glutamate					
PSE vs no PSE	20	64	1	0.67 (0.15 to 1.18)	20
GABA					
PSE vs no PSE	20	64	1	−0.57 (−1.07 to −0.06)	20
Calcium					
PSE vs no PSE	20	64	1	−0.82 (−1.34 to −0.30)	20
NSE					
PSE vs no PSE	31	49	1	0.47 (0.02 to 0.93)	14
Neuregulin-1					
PTE vs no PTE	29	28	1	1.19 (0.62 to 1.76)	33
Malondialdehyde					
PIE vs no PIE	29	20	1	0.75 (0.16 to 1.34)	26
Protein carbonyl					
PIE vs no PIE	29	20	1	1.43 (0.79 to 2.08)	26
SOD					
PIE vs no PIE	29	20	1	-0.74 (−1.34 to −0.15)	26
GSH					
PIE vs no PIE	29	20	1	−2.25 (−2.98 to −1.51)	26
Talin 2					
PIE vs no PIE	16	16	1	−2.31 (−3.23 to −1.39)	21

Continued

Table 2 Significant Biomarkers Assessed in the Blood and CSF With Epilepsy Poststroke, TBI, and Brain Infections *(continued)*

Biomarkers and outcomes	No. of cases	No. of controls	No. of studies	SMD (95% CI); I^2	Study reference
Biomarkers in the CSF					
Glucose					
PSE vs. no PSE	25	118	1	−2.85 (−3.40 to −2.31)	16
Talin 2					
PIE vs no PIE	16	16	1	0.97 (0.23 to 1.71)	21
IL-6					
PSE vs no PSE	25	118	1	5.25 (4.50 to 6)	16
TLC					
PSE vs no PSE	25	115	1	4.61 (3.92 to 5.30)	16
IL-1β					
PTE vs no PTE	12	47	1	2.5 (1.71 to 3.29)	24
Procalcitonin					
PSE vs no PSE	25	118	1	0.7 (0.26 to 1.13)	16
Total protein					
PSE vs no PSE	25	118	1	3.71 (3.10 to 4.32)	16

Abbreviations: CRP = C-reactive protein; GABA = gamma-aminobutyric acid; GSH = glutathione catalase; IL = interleukin; NSE = neuron-specific enolase; PIE = postinfection epilepsy; PSE = poststroke epilepsy; PTE = posttraumatic epilepsy; SMD = standardized mean difference; SOD = superoxide dismutase; sST2 = soluble suppression of tumorigenesis–2; TBI = traumatic brain injury; TLC = total leukocyte count.
SMD >0: higher levels in epilepsy group; SMD <0: lower levels in epilepsy group.
[a] Random-effect model; I^2 was 52%.

Discussion

This systematic review and meta-analysis identified 45 biomarkers, predominantly investigated in the blood, which were tested for their association with the risk of post–brain injury epilepsy. Meta-analysis was possible for only 19 biomarkers because other biomarkers were reported in only a single study. The meta-analysis showed only 1 biomarker, blood glucose, to be significantly associated with early-onset and late-onset epileptic seizures in stroke patients. The remaining 19 biomarkers only showed association in single isolated studies (Table 2).

We report a comprehensive systematic review and meta-analysis that provides evidence on the association of biomarkers measured in biological fluids with the risk of epilepsy secondary to acute brain injuries (stroke, TBI, and brain infections). Dev et al.[36] (2022) systematically reviewed 8 studies that reported the association of 9 biomarkers (genetic/microRNA/protein) with PSE. In comparison, our

Figure 2 Forest Plot for the Association Between Blood Glucose Levels and Patients With Poststroke Epilepsy

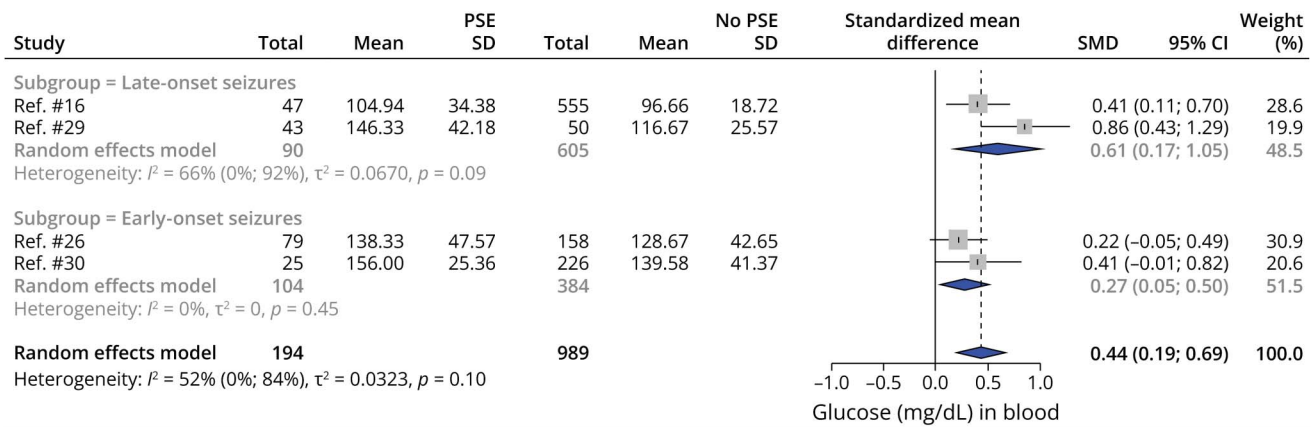


Table 3 Association of Significant Blood Biomarkers With Epilepsy Postischemic Stroke and Intracerebral Hemorrhage

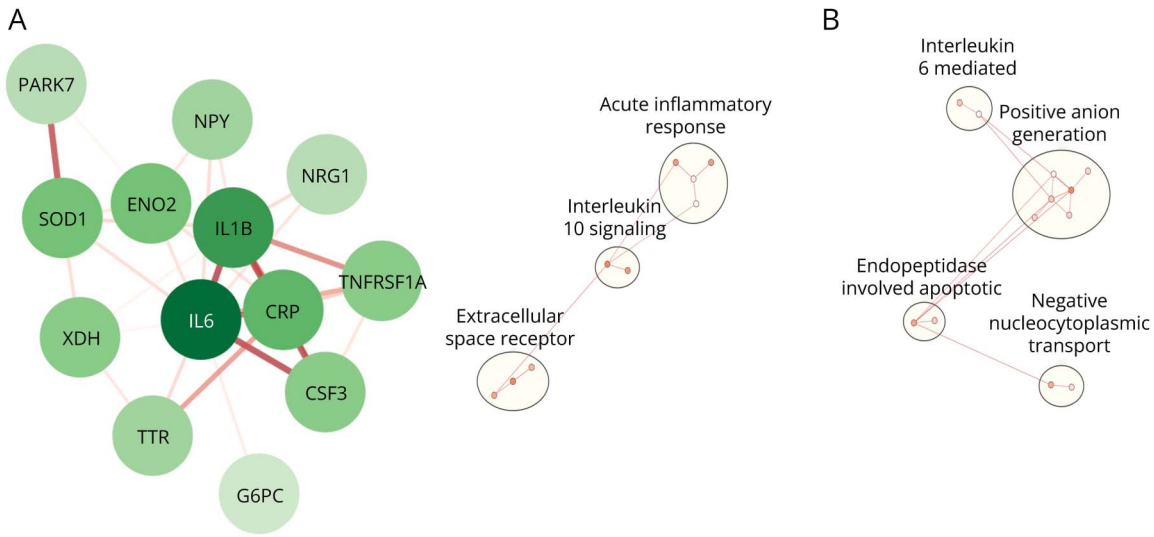
Biomarkers (in blood) and outcomes	No. of cases	No. of controls	No. of studies	SMD (95% CI)	Study reference
Glucose					
PICHE vs no PICHE	47	555	1	0.41 (0.11 to 0.70)	18
TLC					
PICHE vs no PICHE	159	738	1	2.53 (2.32 to 2.73)	34
IL-1β					
PISE vs no PISE	53	862	1	1.95 (1.66 to 2.24)	15
Neuropeptide Y					
PISE vs no PISE	78	86	1	−1.71 (−2.07 to −1.35)	17
NSE					
PISE vs no PISE	31	49	1	0.47 (0.02 to 0.93)	14
APTT					
PICHE vs no PICHE	47	555	1	0.07 (0.22 to 0.37)	18
TNF-R1					
PSAHE vs no PSAHE	9	65	1	0.98 (0.26 to 1.69)	23
sST2					
PSAHE vs no PSAHE	17	29	1	0.77 (0.15 to 1.39)	22
hsCRP					
PSAHE vs no PSAHE	18	91	1	0.97 (0.45 to 1.50)	23
Transthyretin					
PSAHE vs no PSAHE	17	90	1	−0.61 (−1.14 to −0.09)	23

Abbreviations: APTT = activated partial thromboplastin time; CRP = C-reactive protein; IL = interleukin; NSE = neuron-specific enolase; PICHE = post-intracerebral hemorrhage epilepsy; PISE = postischemic stroke epilepsy; PSAHE = postsubarachnoid hemorrhage epilepsy; SMD = standardized mean difference; sST2 = soluble suppression of tumorigenesis–2; TLC = total leukocyte count; TNF-R1 = tumor necrosis factor–receptor 1.
SMD >0: higher levels in epilepsy group; SMD <0: lower levels in epilepsy group.

comprehensive evaluation of the biomarkers in patients with PSE yielded 45 biomarkers, 20 more than that observed by Dev et al. This difference is most likely due to the use of comprehensive search terms detailed in the eAppendix, links.lww.com/WNL/D32, and stringent inclusion and exclusion criteria. Furthermore, Dev et al.[36] observed that endostatin levels were higher and S100B, Hsc70, and neuropeptide Y levels were lower in patients with PSE. Using pooled data across multiple studies, we did not find a significant association between endostatin, S100B, or Hsc70 levels with PSE.

Our meta-analysis found that elevated serum glucose levels were associated with an increased risk of early-onset and late-onset epileptic seizures. Hyperglycemia interacts with ischemic brain tissue, increasing the risk of metabolic stress, neuroinflammation, alpha-synuclein expression, and blood-brain barrier disruptions, which modulate epileptogenesis.[37,38] Hypoglycorrachia is commonly seen in patients with brain infections and other systemic conditions and may be associated with greater seizure risk.[39,40] Because blood glucose levels can fluctuate throughout the day, it is important to analyze the trajectory of glucose levels. The trajectory of glucose level data can be obtained from continuous glucose monitoring. Unfortunately, none of the studies in our meta-analysis provided the trajectory data or the time of blood sample collection for glucose measurement. We also found elevated serum uric acid was associated with only late-onset epileptic seizures. Increased uric acid levels are associated with greater cardiovascular risk and increased risk of epileptic seizures.[41,42] Wang et al. (2019)[19] observed that uric acid levels within 24 hours of seizures were significantly higher than baseline uric acid levels in patients with epilepsy postischemic stroke. Animal studies support these findings wherein a mouse seizure model associated higher uric acid levels with severe seizures.[43] High uric acid levels can lead to oxidative stress and inflammation in the brain, which are linked to seizures. This is because uric acid can activate the Nod-Like Receptor family pyrin domain containing 3 (NLRP3) inflammasome, which releases proinflammatory cytokines that cause tissue damage, inflammation, and impaired immune system function.[43] Altered inflammatory processes can lead to abnormal neural connectivity and hyperexcitability of neuronal networks, which may contribute to the development of epilepsy.[44] The risk of cardiovascular diseases such as stroke,

Figure 3 Protein-Protein Interaction Network and Enrichment Analyses



(A) Protein-protein interaction network analysis of biomarkers associated with postbrain injury epilepsy. The nodes represent the biomarkers, and the edges represent the interaction between the biomarkers in the network. The nodes' color represents the level of interaction between the proteins ranging from 0 to 11, with dark green representing a high degree of interaction (toward 11) and light green representing a low degree of interaction (toward 0). The color of edges represents the interaction score ranging from 0 to 1, with thick dark red edges representing an interaction score with high confidence (toward 1) and light red edges representing an interaction score with low confidence (toward 0). (B) Enrichment analysis identifying the pathways associated with postbrain injury epilepsy.

diabetes, ischemic heart disease, and hypertension increases in patients with gout and epilepsy. Whether the elevated uric acid is an epiphenomenon of increased epilepsy risk or cardiovascular disease is currently unclear and needs further investigation.

For the hypothesis generation purpose, we conducted an interaction network and enrichment analyses of the biomarkers significantly associated with post–brain injury epilepsy. We found that the IL-6, IL-1β, and networks associated with redox reactions were strongly associated with post–brain injury epileptogenesis. This finding hints toward a common pathway associated with epileptogenesis despite variability in the type of brain injury. If supported by robustly designed longitudinal biomarker studies, we may be able to argue in favor of repurposing the anti-inflammatory vasculoprotective neuroprotective agents such as 3K3A-activated protein C or losartan for antiepileptogenesis drug development in randomized controlled trials.[45,46]

The current literature on the biofluid biomarkers of post–brain injury epilepsy has significant limitations. The primary limitations include the following: (1) most of the biomarkers (28/45) were investigated in single studies; (2) only 9 provided validation data; (3) 95% of studies had moderate-to-high risk of bias; (4) studies used variable definitions for early-onset and late-onset seizures poststroke; (5) the follow-up duration varied from 42 days to 5 years, limiting the comparison between biomarkers identified in different studies; (6) temporal profile of the biomarkers were not reported; and (7) and small patient population in individual studies. We could not conduct a subgroup analysis based on time to seizure for PTE and post–brain infection epilepsy due to a lack of consistent definition for early-onset and late-onset seizures. Furthermore, regarding the network analyses, 6 of the 22 studies focused on inflammation-associated biomarkers. Thus, the candidate biomarker approach in the included studies biases our molecular network analysis findings. Although our analyses lend support to the role of inflammation in epileptogenesis, this is likely not the only mechanism of epileptogenesis. There is, therefore, a need for unbiased -omics studies to investigate epileptogenesis mechanisms. Variability in patients' susceptibility to epileptogenesis may be linked to their genetic susceptibilities.[47,48] Whereas a common pathway linked to epileptogenesis, regardless of the type of initial brain insult, would be desirable, the biomarker discovery efforts could currently focus on patient subgroups with distinct mechanisms of brain injury, namely, stroke, trauma, and brain infections. Because of the limitations, we cannot yet recommend using these biomarkers in clinical settings or for designing antiepileptogenesis trials. We could not assess the presence of publication bias to prevent underestimation of the small study effect because less than 10 studies were pooled in our meta-analysis. However, we cannot rule out the possibility of significant publication bias contributing to the reported findings.

A significant challenge with biomarker discovery is the uncertain latent period after brain injury and the overall low incidence of epilepsy after cerebral insults. Biomarker discovery requires a robust longitudinal design and methods acceptable to regulatory bodies such as the US FDA. A collaborative effort[49] toward biomarker discovery is therefore warranted.[50–52]

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Appendix Authors

Name	Location	Contribution
Shubham Misra, MTech, PhD	Department of Neurology, Yale University School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; study concept or design; analysis or interpretation of data; and additional contributions (in addition to 1 or more of the abovementioned criteria)
Erum I. Khan, MBBS	Medical School, B.J. Medical College and Civil Hospital, Ahmedabad, India	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data; and additional contributions (in addition to 1 or more of the abovementioned criteria)
TuKiet T. Lam, PhD	Keck MS & Proteomics Resource, Yale University School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data; and additional contributions (in addition to 1 or more of the abovementioned criteria)
Rajarshi Mazumder, MD	Department of Neurology, University of California, Los Angeles	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to one or more of the abovementioned criteria)
Kapil Gururangan, MD	Department of Neurology, University of California, Los Angeles; Department of Neurology, Icahn School of Medicine at Mount Sinai, New York	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
L. Brian Hickman, MD, MSc	Department of Neurology, University of California, Los Angeles	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Vaibhav Goswami, MD	Department of Neurology, Tower Health, Philadelphia	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Melissa C. Funaro, MLS, MS	Harvey Cushing/John Hay Whitney Medical Library, Yale University, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Ece Eldem, MSc	Department of Neurology, Yale University School of Medicine, New Haven, CT	Major role in the acquisition of data; additional contributions (in addition to 1 or more of the abovementioned criteria)
Lauren H. Sansing, MD, MS	Department of Neurology, Yale University School of Medicine, New Haven, CT; Department of Immunobiology, Yale University School of Medicine, New Haven	Major role in the acquisition of data; additional contributions (in addition to 1 or more of the abovementioned criteria)
Jason J. Sico, MD, MHS	Department of Neurology, Yale University School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Terence J. Quinn, MD	Institute of Cardiovascular and Medical Sciences, University of Glasgow, Scotland, UK	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
David S. Liebeskind, MD	Department of Neurology, University of California, Los Angeles	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Joan Montaner, MD, PhD	Institute de Biomedicine of Seville, IBiS/Hospital Universitario Virgen del Rocío/CSIC/University of Seville, Department of Neurology, Hospital Universitario Virgen Macarena, Seville, Spain; Neurovascular Research Laboratory Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)

Appendix *(continued)*

Name	Location	Contribution
Patrick Kwan, MD, PhD	Department of Neuroscience, Central Clinical School, Monash University, Melbourne, Australia	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)
Nishant K. Mishra, MD, PhD	Department of Neurology, Yale University School of Medicine, New Haven, CT	Drafting/revision of the article for content, including medical writing for content; additional contributions (in addition to 1 or more of the abovementioned criteria)

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