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REVIEW

Blood/Brain Biomarkers of Inflammation After Stroke and Their Association With Outcome: From C-Reactive Protein to Damage-Associated Molecular Patterns

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Abstract Stroke represents one of the most important causes of disability and death in developed countries. However, there is a lack of prognostic tools in clinical practice to monitor the neurological condition and predict the final outcome. Blood biomarkers have been proposed and studied in this indication; however, no biomarker is currently used in clinical practice. The stroke-related neuroinflammatory processes have been associated with a poor outcome in stroke, as well as with poststroke complications. In this review, we focus on the most studied blood biomarkers of this inflammatory processes, cytokines, and C-reactive protein, evaluating its association with outcome and complications in stroke through the literature, and performing a systematic review on the association of C-reactive protein and functional outcome after stroke. Globally, we identified uncertainty with regard to the association of the evaluated biomarkers with stroke outcome, with little added value on top of clinical predictors such as age or stroke severity, which makes its implementation unlikely in clinical practice for global outcome prediction. Regarding poststroke complications, despite being more practical scenarios in which to make medical decisions following a biomarker prediction, not many studies have been performed, although there are now some candidates for prediction of poststroke infections. Finally, as potential new candidates, we reviewed the pathophysiological actions of damage-associated molecular patterns as triggers of the neuroinflammatory cascade of stroke, and their possible use as biomarkers.

Keywords Stroke · Inflammation · Biomarkers · Outcome · Cytokines · C-reactive protein · Alarmins

Introduction

Stroke currently represents one of the most important causes of death and disability worldwide. In fact, stroke causes 1 of every 20 deaths in USA, being the fifth leading cause of death. On average, someone dies of stroke every 4 min [1]. In-hospital mortality rates for ischemic stroke have been estimated to be between 11 % and 15 % [2]. However, beyond its lethality, stroke represents also one of the main causes of disability; approximately half of stroke survivors are disabled some months after stroke, and around 20 % require institutionalization. Stroke is the third most common cause of disability worldwide, with 1.6 % and 2.5 % of disability-adjusted life years for ischemic and hemorrhagic stroke, respectively [3].

The main factors that account for this poor outcome after stroke in terms of disability and mortality, such as age or baseline stroke severity, are not modifiable. However, several circumstances may occur after stroke and may contribute to this poor outcome, representing a unique opportunity for researchers and clinicians for interventions to improve stroke outcome. Examples include neurological complications, such as brain edema or seizures, and systemic complications, such as infections or cardiac events. In fact, the modification of these circumstances, by early detection and treatment or prophylaxis in high-risk patients, might result in an improved stroke outcome [4].

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The Biomarkers Definitions Working Group describes a biomarker as a characteristic that can be objectively measured and evaluated as an indicator of normal or pathologic biological processes, or pharmacologic responses to therapeutic interventions. Molecular biomarkers, detectable from blood, urine, or other biological fluids, may represent measurable indicators to predict the physiological states of a disease. However, despite many years of research in the field, no blood biomarker is currently used in stroke clinical practice, although the use of blood biomarkers to predict stroke outcome seems attractive for both clinicians and researchers, with several reviews published in recent years [5–7].

In this review, we will focus on how the neuroinflammatory processes triggered by stroke can be monitored through the measurement of blood biomarkers, and how these biomarkers might be used from a clinical point of view, both for global outcome prediction and also for prediction of poststroke complications. Specifically, we will focus on 3 different groups of biomarkers: cytokines and C-reactive protein (CRP), as the most studied inflammatory biomarkers in the stroke field and, as potential new candidates, damage-associated molecular patterns (DAMPs).

Neuroinflammatory Cascade in Relation to Outcome and Complications of Stroke

In the acute stroke setting, a lack of cerebral blood flow causing the interruption of oxygen and glucose supply to cerebral neurons and supporting cells results in massive cell necrosis within the infarct core [8]. In the surrounding area, cells are functionally impaired but still structurally intact, which makes that region potentially salvageable. If oxygen and glucose supplies are not restored, neuronal apoptosis processes are initiated, resulting in an increase of the lesion size.

Dying cells from both the ischemic core and the peri-infarcted area release damage signals, known as DAMPs [9]. These signals activate the local microglia and peripheral leukocytes, resulting in a massive release of proinflammatory cytokines, upregulating the expression of leukocyte adhesion molecules and stimulating the synthesis of chemokines. Together with an increased blood–brain barrier (BBB) permeability, this response facilitates leukocyte infiltration into the brain compartment, to clear away the large amount of debris caused by cell death [10,11]. At later stages, the immune system also works to resolve postischemic inflammation, producing anti-inflammatory mediators and removing the remaining inflammatory molecules.

Although the main reason for the activation of both resident and infiltrating immune cells is the facilitation of the clearance of debris in the infarcted area, it also results in deleterious effects on the ischemic brain. If the activation of peripheral leukocytes and microglia persist over time, the excess of inflammatory cytotoxic mediators will prolong the inflammatory response, increasing brain damage and contributing to secondary complications, such as edema or hemorrhagic transformation due to increased BBB permeability [12]. These effects are even worse in severe strokes, where the extension of the brain lesion is highly correlated with the strength of the neuroinflammatory reaction.

The hyperactivation of the peripheral immune cells may lead to exhaustion of mature leukocytes and the subsequent recruitment of immature leukocytes, a subpopulation unable to respond appropriately to brain injury [9]. The recruitment and expansion of this subpopulation causes lymphocytopenia, which significantly contributes to poststroke immunosuppression [13]. In addition, the excessive concentration of proinflammatory mediators can promote the release of glucocorticoids and catecholamines by the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system. This results in the stimulation of anti-inflammatory pathways and the inhibition of proinflammatory mechanisms [14]. The rapid and inappropriate activation of these mechanisms also contributes to stroke-related immunosuppression, which enhances the risk of infection after cerebral ischemia. Moreover, the release of catecholamines by the hypothalamic–pituitary–adrenal axis might contribute to the development of cardiologic complications such as cardiac rhythm disorders or myocardial ischemia, especially when the right insula is affected [15]. Figure 1 summarizes the neuroinflammatory processes that brain ischemia generates, and their relationship with poststroke complications leading to poor outcome.

In addition to those phenomena occurring during the acute phase of stroke, it is known that chronic inflammation represents a crucial factor in the development and progression of atherosclerosis. The release of proinflammatory cytokines that happens in conditions such as rheumatoid arthritis, systemic autoimmune diseases, metabolic syndrome, or diabetes results in oxidative stress, insulin resistance, and endothelial dysfunction, therefore contributing to atherosclerosis [16]. Also, chronic infections are known to be associated with the risk of stroke. These low-grade chronic inflammatory diseases such as periodontal disease or *Chlamydia pneumoniae* infections have been also associated with stroke, as they can increase the likelihood of platelet adhesion [17,18]. Therefore, chronic inflammation might act not just as a risk factor for stroke, but also as a triggering factor [19]. This background should be considered when measuring blood biomarkers during the acute episode, as these biomarkers are difficult to distinguish between acute, stroke-related elevations or chronically altered levels on inflammatory biomarkers.

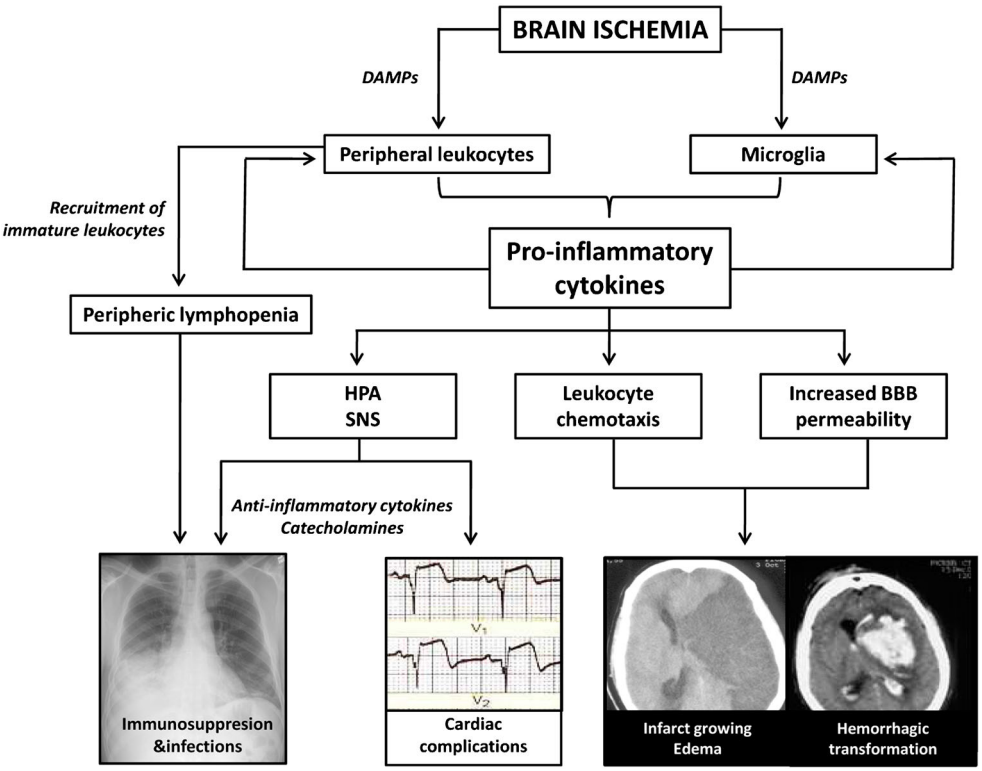

Fig. 1 Neuroinflammatory processes in brain ischemia and their relationship with poststroke complications leading to poor outcome
DAMPs = damage-associated molecular patterns;
HPA = hypothalamic–pituitary–adrenal axis;
SNS = sympathetic nervous system; BBB = blood–brain barrier

Molecular Blood Markers of Inflammation and Stroke Outcome

As shown in the previous section, neuroinflammation is related to some of the main factors related to stroke outcome, such as the extent of the ischemic brain injury (which is related to stroke severity), and poststroke complications. In this sense, associations between the inflammatory markers altered in the ischemic cascade and stroke outcome would be expected. The question remains on how these associations could be used by clinicians for stroke outcome improvement.

Blood stroke biomarkers can be either brain-specific markers, released from damaged tissue, or other more systemic indicators, such as those resulting from the inflammatory response at either local or peripheral level. In any case, levels of inflammatory markers in the peripheral blood usually reflect the peripheral response against stroke. Therefore, all molecular processes described in the previous sections should be taken into consideration for the detection of these candidate biomarkers, although the question of whether this inflammatory pattern is specifically reflecting a brain insult remains unanswered. Comparisons between patients with stroke and other brain-unrelated inflammatory diseases might help to clarify this issue. Inflammatory mediators such as cytokines, acute phase reactants, and some molecules involved in more specific pathways, such as cell adhesion molecules in chemotaxis or matrix metalloproteinases in BBB degradation, represent surrogate candidates to predict stroke outcome or its specific complications. In this review we will focus on cytokines and CRP as the candidates that have been more thoroughly explored in the literature and therefore the information about their association with stroke outcome should be more robust. Also, as a recent area of research and potential source for new biomarkers, we will focus on DAMPs or alarmins, mediators that are supposed to initiate the inflammatory response, being released from the cellular components of the infarct core and penumbra.

Cytokines

Cytokines are a family of pleiotropic polypeptides that regulate cell activation, proliferation, and differentiation [20]. In the normal brain, cytokines are barely detectable, as their receptors are expressed at very low levels. However, after an ischemic insult to the brain, cytokines are quickly and extensively upregulated [21], being responsible for both proinflammatory and anti-inflammatory mechanisms. Proinflammatory cytokines stimulates the inflammatory response, which could result in an amplification of the initial brain injury, as mentioned above. Interleukin (IL)-1β, tumor necrosis factor (TNF), and IL-6 represent the main proinflammatory cytokines in stroke [21–23]. Anti-inflammatory cytokines inhibit the expression of proinflammatory cytokines, therefore reducing inflammation. Transforming growth factor (TGF)-β and IL-10 represent the most studied anti-inflammatory cytokines after ischemic stroke at the experimental level [24]. However,

the different effects of cytokines cannot be exclusively divided into pro- or anti-inflammatory, as some of them may exert neurotoxic, as well as neuroprotective, functions [25,26]. The balance between deleterious and beneficial effects of cytokines will depend on the physiological and biochemical context in the brain.

Cytokine measurement in acute stroke has been associated with stroke outcome, as well as the occurrence of some poststroke complications. Moreover, the time in which the measurement is performed might also result in different associations, as some biomarkers have been shown to be associated with different features at different time points. Table 1 shows an overview of the main studies exploring these associations.

Proinflammatory cytokines

IL-1β After stroke, IL-1β stimulates the expression of other proinflammatory mediators such as other cytokines and adhesion molecules, as well as the activation and proliferation of astrocytes and microglia [27]. Moreover, IL-1β induces edema formation and primes the endothelium for leukocyte adherence [27,28]. Beyond these neurotoxic effects, IL-1β also activates astrocytes to produce survival-promoting factors. Moreover, increases in IL-1β will lead to upregulation of IL-1 receptor antagonist (IL-1Ra), with opposite effects, being finally the balance between IL-1β and IL-1Ra more important for the global effect than IL-1β levels by itself [29].

In this sense, circulating levels of IL-1β and IL-1Ra might be good surrogate markers for outcome prediction. However, few clinical studies have explored their role as stroke biomarkers, resulting in controversial data. IL-1β levels were associated with poor long-term functional outcome in 1 study [30], while IL-1Ra levels have shown to be predictive of the development of poststroke infections, as well as its polymorphisms [31,32]. Moreover, recombinant IL-1Ra has been also explored from a therapeutic point of view, and was shown to reduce systemic inflammation in a placebo-controlled, phase II trial in patients with acute stroke [33].

TNF TNF is a pleiotropic cytokine with both neurotoxic and neuroprotective effects [34]. There is no consensus on the effect of TNF after ischemic stroke, but neurotoxic or neuroprotective effects will depend on several factors such as the extent and timing of microglial activation and the amount of TNF expression [35,36]. The main neurotoxic effects of TNF are potentiation of excitotoxicity by the inhibition of glutamate uptake and microglia activation [37], thereby promoting the production of neurotoxic mediators. TNF also promotes the apoptosis of endothelial cell, contributing to vasogenic edema and infiltration of circulatory inflammatory cells [38]. Regarding its neuroprotective effects, TNF activates the repair of the brain microvasculature and mediates neuronal plasticity [25].

Clinical studies measuring TNF have shown inconsistent results. In fact, higher plasma TNF concentration has been found associated with poor outcome at 3 months when measured in the acute phase [39,40]. However, other studies have shown no differences regarding stroke outcome, despite similar time windows [41,42], nor poststroke infections [43,44]. The role of the circulating levels of TNF receptors 1 and 2 in outcome prediction is unclear so far, although levels of TNF receptor 1 have been described to predict recurrent vascular events after lacunar stroke [45].

IL-6 IL-6 is mainly produced not only by activated microglia, but also by astrocytes, neurons, and peripheral immune cells [46]. It helps to attract T lymphocytes to the brain, contributing to an exacerbation of the inflammatory response. However, IL-6-deficient mice do not show improved outcome after stroke, therefore putting into question its detrimental effects [47].

IL-6 has been one of the most studied inflammatory biomarkers in stroke patients, especially as a prognostic marker, although its role as a predictor of stroke risk has been also described [48]. In this sense, some studies have shown good associations between high IL-6 levels and short-term neurologic outcome [49], long-term functional outcome [50–52], or poststroke infections [53,54], although its additional predictive value over clinical information for outcome prediction has been questioned [41]. These data were confirmed by our group by performing both a literature-based and individual participant data meta-analyses of 20 studies including 4389 patients. The results showed an independent association with long-term functional outcome but a very modest additional predictive value over clinical information over clinical variables such as age, sex, or stroke severity (a modest 1.5 % increase in discrimination), in addition to publication bias [55].

Anti-inflammatory cytokines

TGF-β From the different TGF-β cytokines, only TGF-β1, produced by activated microglia, and TGF-β2, produced by astrocytes and neurons, are prominent after stroke [24]. TGF-β reduces glial activation, decreases the expression of other cytokines, suppresses the release of oxygen- and nitrogen-derived products, promotes angiogenesis, and stimulates the release of IL-1Ra [56]. Its protective effects, however, are limited to the peri-infarcted area, as TGF-β can inhibit apoptosis but not necrosis. Administration of TGF-β before the induction of an ischemic insult has been shown to save neurons from cell death in mice [57]. However, not all studies agree on the beneficial effects of this cytokine, as a recent study reported that TGF-β1 enhanced the expression of

Table 1 Cytokines and outcome in stroke

Cytokines	Role	Association with outcome	Association with complications
IL-1β	Proinflammatory	Poor functional outcome [30]	Infections (not associated) [100]
IL-1Ra	Anti-inflammatory	Favorable functional outcome [33]	Infections [31,32]
IL-2	Proinflammatory	–	Infections (not associated) [44]
IL-4	Anti-inflammatory	–	Infections (not associated) [44]
IL-5	Anti-inflammatory	–	Infections (not associated) [44]
IL-6	Proinflammatory	Neurological deterioration [39,40,101]	Infections [50,51,53,54,64,109,117]
		Poor functional outcome [39–42,49–53,101–116]	Edema (not associated) [40,118]
		Poor functional outcome (not associated) [117]	Hemorrhagic transformation (not associated) [40]
IL-8	Proinflammatory	–	Infections (not associated) [44]
IL-10	Anti-inflammatory	Functional outcome (not associated) [41,59]	Infections: [59,118]
			Infections (not associated) [44]
			Edema (not associated) [119]
IL-12a	Proinflammatory	–	Infections (not associated) [44]
IL-13	Anti-inflammatory	–	Infections [44]
IFN-γ	Proinflammatory	Functional outcome (not associated) [59]	Infections [44]
			Infections (not associated) [59]
TNF	Proinflammatory	Poor functional outcome [39,40]	Infections (not associated) [43,44,118]
		Poor functional outcome (not associated) [41,42,59]	Edema (not associated) [40,119]

IL = interleukin; IL-1Ra = interleukin-1 receptor antagonist; IFN = interferon; TNF = tumor necrosis factor

classical proinflammatory cytokines and enzymes that can disrupt the BBB [58]. To our knowledge, no clinical study has evaluated the role of TGF-β in the prediction of outcome or complications of ischemic stroke.

IL-10 IL-10 is primary produced in activated microglia and astrocytes. It acts by inhibiting cytokine production and the expression of their receptors, as well as attenuating astrocytic activation. Some studies have evaluated the role of IL-10 as a biomarker for stroke outcome, without finding any relevant relationship [41,59]. Regarding poststroke infections, some studies showed higher IL-10 levels in patients who developed infections [43,60], although other studies did not find any association [44]. In this sense, although IL-10 might represent a potential candidate for this indication, more evidence is needed.

CRP

CRP is an acute-phase reactant, being part of the innate immune response. It is mainly produced in the liver, under the stimulation of IL-6, although peripheral lymphocytes and monocytes can also produce small amounts [61,62]. CRP binds to the phosphocholine expressed on the surface of dead or dying cells and some bacteria, activating the complement system and promoting phagocytosis by macrophages [63]. CRP levels rapidly increase as a result of stroke [64], although this acute-phase response occurs in response to a wide range of inflammatory conditions, reflecting the low specificity of CRP elevations. Regrettably, the ultimate role of CRP in acute stroke is not completely understood as it has anti-inflammatory and proinflammatory effects [65]. In addition to a higher stroke risk in population-based studies [66], elevated levels of CRP after stroke have been related to poor functional outcome and mortality [67–70], and also to the occurrence of poststroke infections [71], or brain edema [72]. Moreover, similar to other inflammatory markers, CRP levels were associated with infarct volume and stroke severity [50]. Despite its unspecific character, some studies have suggested that CRP elevations in stroke might reflect different phenomena depending on the time of rising, with early elevations being more related to stroke severity and late elevations with poststroke infections [70].

In order to investigate further the association of blood CRP levels and functional outcome after ischemic stroke, we performed a systematic review by searching the PubMed database up to March 2016, without language or other restrictions, for studies measuring CRP in patients with acute stroke and assessing long-term functional outcome. As search terms, we

used a combination of medical subject heading terms and text words defining "stroke", "CRP", and "outcome". Additional references were obtained from www.stroke-biomarkers.com.

Inclusion criteria for the studies were 1) patients with ischemic stroke patients; 2) CRP blood levels measured during hospital admission, and 3) assessment of long-term (at least 1 month) functional outcome, measured with an accepted disability scale. Exclusion criteria were 1) unknown languages, 2) experimental studies with animal models or cell cultures, 3) nonoriginal studies (reviews, abstracts, letters, editorials, case reports), 4) studies including just hemorrhagic stroke or transient ischemic attacks, 5) stroke outcome reported just as neurological scores or death-survival rates, and 6) interventional studies or clinical trials.

Three different researchers performed the data extraction. The quality of the articles was assessed using a 15-point questionnaire for the evaluation of biomarker studies in stroke (available at www.stroke-biomarkers.com) [73]. All articles fulfilling the inclusion criteria were included in the meta-analysis independently of the quality score.

The initial PubMed search identified a total of 1497 studies. After the first screening, 40 studies met the inclusion criteria. An additional search on the website www.stroke-biomarkers.com identified 6 more studies. Thus, 46 studies were considered for systematic review (Fig. 2).

The quality of the articles was moderate (median 9 points; interquartile range 8–10 points; range 3–12 points). The most frequently missing points were sample size calculation (not reported by any study), reporting of blindness (8/46 for clinical data collection and 10/46 for biomarker measurement), and use of previously established cut-offs for the biomarker (6/46). Quality points of each article are shown in detail in Supplementary material S2.

An overview of the studies reporting association between CRP and long-term functional outcome is given in Table 2. Sample size varied from 11 to 985 [<100 cases in 15 (33 %) studies]. From the included 46 studies, the modified Rankin score was the most used disability scale (40 studies). Only 26 studies reported baseline stroke severity, most of them by the National Institutes of Health Stroke Scale score. Time of long-term functional outcome assessment varied from 3 to 15 months. High-sensitivity assays measuring ultrasensitive CRP were used in 19 studies.

Elevated CRP levels were found to be associated with long-term functional outcome in all but 6 of the included studies (87 %). From the remaining 40, the existence of an independent association by regression analysis was not assessed in 13, while the remaining 27 did further adjustment by clinical covariates associated with stroke outcome. CRP was found to be an independent predictor of long-term functional outcome in 20 studies, while 7 of them found associations that were not further independent when adjusting for clinical covariates and/or other blood biomarkers.

Despite the huge amount of literature on the association of CRP and outcome, we concluded that there is uncertainty regarding whether CRP levels might be an independent predictor of poor functional outcome in ischemic stroke. The wide variability in study design and data reporting represents the main limitation of this systematic review. Moreover, the additional predictive value of CRP over clinical variables, to our knowledge, has not been assessed in any study by using reclassification or discrimination tools [74]. All these data suggest that CRP is not a useful tool in clinical practice with which to predict stroke outcome, at least as a single biomarker. Further research should explore the particular association of CRP with poststroke complications. Moreover, these results show, again, that only using general inflammatory markers for predicting global outcome is not a good strategy, and we suggest dissecting further the different causes of bad outcome in order to identify specific markers for each of those conditions (infections, edema, etc.).

New Candidates: DAMPs

It remains unknown what exactly triggers the inflammatory response in the initial stages of cerebral ischemia. Candidates for this immune activation are DAMPs, or alarmins, such as heat shock proteins (Hsp) or high-mobility group box 1 (HMGB1) protein, which are released from dying cerebral tissue after stroke. The main receptors of DAMPs are located on immune cells, such as Toll-like receptors (TLR) and receptor for advanced glycation end products (RAGE), and their activation leads to the secretion of proinflammatory cytokines, generating an overall inflammatory environment [9,75].

HMGB1

HMGB1 is a highly conserved nonhistone nuclear DNA-binding protein, expressed in most eukaryotic cells including neurons [76]. It has been described as a major autocrine and paracrine inflammatory signal in the context of inflammation [77,78]. In acute stroke, both passive release and active secretion have been described, being passive for neurons and astrocytes and active for activated astrocytes and microglia [79,80]. In the central nervous system, HMGB1 can bind to its putative receptors, including TLR2, TLR4, and RAGE. HMGB1–RAGE binding results in the activation of inflammatory processes, leading to the overexpression of inflammatory mediators such as TNF, IL-1β, intracellular adhesion molecule 1, vascular adhesion molecule 1, or E-selectin. Congruently, the inhibition of the HMGB1–RAGE pathway reduces inflammation and stroke size [77,81]. A recent study found that the cytokine-inducing isoform of HMGB1 was released from the ischemic brain in the hyperacute phase of stroke in mice and patients, and, furthermore, HMGB1–RAGE signaling

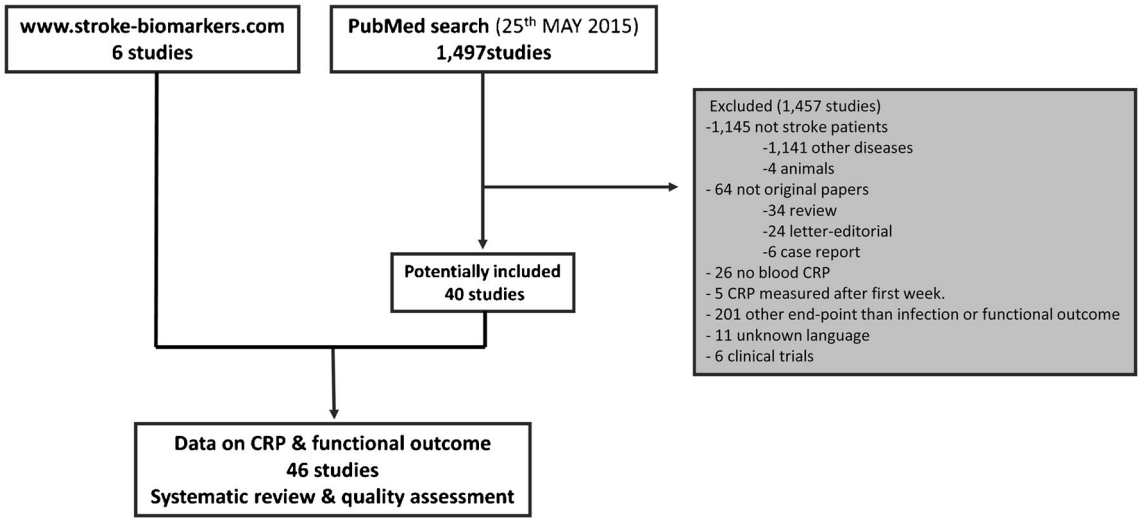

Fig. 2 Flow chart of the systematic review on C-reactive protein (CRP) and outcome after stroke

resulted in functional exhaustion of mature monocytes and lymphopenia, hallmarks of immune suppression after extensive ischemia [9]. These features introduce the HMGB1–RAGE-mediated pathway as a key mechanism explaining the postischemic brain–immune interactions in relation to poststroke immunosuppression.

The association between HMGB1 and outcome after stroke has been assessed in some studies. In a study including 338 patients with stroke, plasma HMGB1 was reported as an independent predictor of 1-year clinical outcome, having a similar prognostic value to National Institutes of Health Stroke Scale score [82]. In an another study of 42 patients with ischemic stroke, increased plasma levels of HMGB1 were associated with a poor functional outcome, in addition to significantly higher levels of HMGB1, when compared with healthy controls [83]. An additional study found no association between HMGB1 and outcome, despite elevated levels that persisted for 30 days [84]. Regarding hemorrhagic stroke, a strong correlation was found between HMGB1 levels in cerebrospinal fluid and clinical outcome in 10 patients with Fisher-4 subarachnoid hemorrhage and acute hydrocephalus [85].

Hsp

Hsp play a crucial role in eukaryotes, acting as chaperones, preventing protein misfolding and aggregation [86]. The best-studied Hsp in the context of ischemia is Hsp70. Following stroke, Hsp70 is upregulated not only in neurons, but also in microglia, astrocytes, and endothelial cells [87]. Several *in vivo* and *in vitro* models have established neuroprotective effects of Hsp70 following stroke [88]. Even though Hsp70 seems to be involved in pathways that are both potentially protective and detrimental, such as apoptosis inhibition or immune system activation in stroke, its overall effects on final outcome seem to be protective. A recent study measured Hsp70 levels in plasma and lymphoid tissue of 46 patients with stroke and 16 healthy controls. Although plasma Hsp70 concentration at day 7 was similar in patients and controls, patients disclosed stronger immunoreactivity to Hsp70 in lymphoid tissue than controls, with most of the $Hsp70^{+}$ cells being antigen presenting cells located in T-cell zones [89]. Beyond Hsp70, it has been reported that serum Hsp27 antibody levels measured 24 h after stroke onset were significantly higher than in controls but did not differ among patients with different stroke types and did not predict 6-month outcome [90]. Also, our group identified Hsp75 as a key protein in the inflammatory response that statins are able to block, in a proteomic study from ischemic rat brains, treated with simvastatin or placebo after embolic middle cerebral artery occlusion [91]. The study found relevantly lower Hsp75 levels in simvastatin-treated ischemic brains, and this reduction was also found in plasma samples from a clinical trial of simvastatin in acute stroke, in which simvastatin-treated patients showed lower levels than those treated with placebo [92].

Other DAMPs

Peroxiredoxin (Prx) family proteins are recently discovered DAMPs, expressed in different intracellular compartments. In neurons, their physiological functions include the maintenance of the redox homeostasis, by regulating levels of intracellular peroxide [93]. Following stroke, released Prx might also function as a DAMP, reversing the physiologically neuroprotective properties of DAMPs into detrimental functions [94]. In fact, the expression of Prx1 was found to be increased

Table 2 Studies identified in the systematic review of C-reactive protein (CRP) and functional outcome in stroke

Study	Material	CRP/hsCRP	Assay	First blood collection	Sample size	Functional scale	Time of outcome assessment	Association CRP outcome
Vila et al. [120]	Serum	CRP	Latex particle-enhanced nephelometry	<48 h	41	mRS	6 months	Associated
Anuk et al. [121]	Plasma	hsCRP	Nephelometer	<24 h	60	mRS	8–12 months	Associated
Winbeck et al. [122]	Serum	CRP	Clinical chemistry analyzer	12–24 h	127	mRS	1 year	Independent predictor
Smith et al. [50]	Plasma	CRP	ELISA	<24 h	25	mRS	3 months	Associated
Christensen et al. [123]	Unknown	CRP	–	<24 h	719	mRS	3 months	Associated
Waje-Andreassen et al. [51]	Serum	CRP (hsCRP < 10)	ELISA	<4 h	11	BI	1 year	Not associated
Hamidon et al. [124]	Serum	CRP	–	<72 h	49	BI	1 month	Independent predictor
Masotti et al. [69]	Plasma	CRP	Nephelometric method	<12 h	196	mRS	1 month	Associated
Montaner et al. [70]	Serum	hsCRP	Nephelometer	<3 h	143	mRS	3 months	Associated
Efstathiou et al. [125]	Plasma	hsCRP	Immunonephelometric	<24 h	211	mRS	5 years	Associated
Vibo et al. [126]	Plasma	CRP	Immunoturbidimetric assay	1 week	52	BI	15 months	Associated
Geiger et al. [127]	Serum	CRP	Chemistry analyzer	<24 h	63	BI	12 months	Not associated
Topakian et al. [128]	Unknown	CRP	Particle-enhanced immunoturbidimetry assay	<6 h	111	mRS	3 months	Associated, not independent predictor
Ryu et al. [129]	Serum	CRP	–	<24 h	28	BI	6 months	Associated
Welsh et al. [107]	Plasma	CRP	–	<24 h	180	mRS	1 month	Independent predictor
Sienkiewicz-Jarosz et al. [130]	Serum	CRP	Immunoprecipitation	<72 h	54	mRS	3 months	Independent predictor
Song et al. [131]	Serum	hsCRP	–	<24 h	417	mRS	3 months	Associated
Varoglu et al. [132]	Serum	hsCRP	–	–	33	mRS	1 month	Not associated
Worthmann et al. [109]	Serum	hsCRP	Clinical chemistry analyzer	6 h	69	mRS	3 months	Associated but not independent predictor
Brouns et al. [133]	Serum	CRP	Autoanalyzer	Admission (median 4.3 h)	149	mRS	3 months	Associated
Song et al. [134]	Serum	hsCRP	–	<24 h	309	mRS	3 months	Associated
Rajeshwar et al. [135]	Serum	hsCRP	ELISA	<24 h	581	mRS	3 months	Independent predictor
Whiteley et al. [41]	Serum	hsCRP	Immunonephelometric	<24 h	270	mRS	3 months	Associated but not independent predictor
Tsai et al. [136]	Serum	hsCRP	ELISA	<48 h	100	mRS	3 months	Independent predictor
Lai et al. [137]	Plasma	hsCRP	ELISA	–	269	mRS	3 months	Independent predictor
Huang et al. [82]	Plasma	–	–	<24 h	338	mRS	1 year	Associated but not independent predictor
Schulze et al. [84]	plasma	CRP	Clinical chemistry analyzer	<72 h	110	mRS	3 months	Independent predictor
Zhang et al. [54]	Unknown	CRP	Chemiluminiscence	<24 h	106	mRS	1 month	Independent predictor

Table 2 (continued)

Study	Material	CRP/hsCRP	Assay	First blood collection	Sample size	Functional scale	Time of outcome assessment	Association CRP outcome
Park et al. [138]	Serum	CRP	Immunonephelometric assay	<12 h	105	mRS	3 months	Associated but not independent predictor
Gensicke et al. [139]	Blood	CRP	–	<4.5 h	257	mRS	3 months	Associated but not independent predictor
Tiainen et al. [140]	Unknown	CRP	–	<4.5 h	985	mRS	3 months	Independent predictor
Zhang et al. [141]	Plasma	CRP	Chemiluminiscence	<72 h	245	mRS	1 year	Independent predictor
Tu et al. [142]	Serum	hsCRP	Enzyme cycling method	<48 h	189	mRS	3 months	Independent predictor
Abubakar et al. [143]	Serum	CRP	Particle-enhanced immunoturbidimetric assay	<1 week	80	mRS	1 month	Independent predictor
Tu et al. [144]	Unknown	–	–	<48 h	189	mRS	3 months	Independent predictor
Kim et al. [145]	Blood	hsCRP	–	–	604	mRS	3 months	Not associated
Gong et al. [146]	Unknown	hsCRP	–	–	977	mRS	1 year	Independent predictor
Men et al. [147]	Serum	CRP	Latex immunoturbidimetric assay	<24 h	308	mRS	1 month	Independent predictor
Ozkan et al. [148]	Serum	hsCRP	Ultrasensitive latex-enhanced immunoassay	<48 h	62	FIM-FAS	3 months	Not associated
Taheraghdan et al. [149]	Serum	hsCRP	Immunoturbidimetric assay	48 h	102	mRS	3 months	Not associated
Karlinski et al. [150]	Plasma	CRP	Immunoturbidimetric assay	<24 h	341	mRS	3 months	Associated but not independent predictor
Potpara et al. [151]	Unknown	CRP	Latex-enhanced nephelometric	<24 h	240	mRS	1 month	Independent predictor
Deng et al. [152]	Serum	hsCRP	Autoanalyzer	<24 h	378	mRS	3 months	Independent predictor
Sezer et al. [153]	Serum	CRP	Autoanalyzer	<24 h	52	mRS	3 months	Associated
Wang et al. [154]	Serum	hsCRP	Autoanalyzer	<24 h	376	mRS	1 year	Independent predictor
Richard et al. [155]	Serum	CRP	ELISA	<36 h	75	mRS	3 months	Independent predictor

hsCRP = ultrasensitive CRP assay; mRS = modified Rankin scale; ELISA = enzyme-linked immunosorbent assay; BI = Barthel index; FIM-FAS = functional independence measure/functional ambulation scale (FAS)

in extracellular fluid from infarct core when compared with penumbra and the contralateral area in microdialysates from patients with ischemic stroke. Moreover, blood Prx1 levels were increased in patients with ischemic stroke compared with healthy controls [95]. In another study [96], circulating Prx5 levels were also measured in 98 patients with acute stroke, being inversely correlated with stroke severity and other inflammatory markers but not associated with outcome [96].

Besides the well-studied HMGB1, Hsp, and Prx, further DAMPs include S100 calcium-binding proteins A8 and A9 [97], myeloid-related proteins 8 and 14 [98], and cold-inducible RNA-binding protein [99]. These have been

reported to be capable of signaling via TLRs and/or RAGE. Nevertheless, the functional relevance of these molecules in stroke is so far unclear.

Conclusions and Implications for Future Research

From a theoretical point of view, given the close pathophysiological relationship between neuroinflammatory mediators and processes related to poor outcome in stroke, a role for inflammatory molecules as prognostic biomarkers could be expected. In this review, however, inconsistent results have been the rule for many of the explored molecules, as is the case for CRP and TNF. Additionally, for the majority of these molecules, their additional predictive value over clinical information, using statistical tools such as the integrated discrimination improvement index, remains unexplored. This step is extremely important, as has been shown for IL-6, probably one the most explored biomarkers, and with more consistent results. Despite the independent association of IL-6 with poor outcome, its additional discrimination over clinical variables was very modest, a fact that limits its use in clinical practice [55]. Regarding CRP, the systematic review showed a huge variability in the results, with some studies showing no further association when adjustment by covariates is performed, or even no association at the univariate level. With this background, an additional predictive value over highly explicative clinical variables such as stroke severity is difficult to expect.

The other key finding of this review is that the prediction of poststroke complications with inflammatory biomarkers has not been sufficiently explored through the literature. This is surprising, as a biomarker related to a specific process leading to poor outcome provides clinicians with more intuitive information on how to act, rather than a global prognostic approach. Future research in biomarkers to predict outcome in patients with stroke should focus on both: exploration of the additional predictive value of the biomarker over clinical information, and exploration of the predictive value for poststroke complications.

This review has focused on blood biomarkers. However, we cannot forget that other biomarkers of inflammation are becoming increasingly popular, such as those based on imaging of atherosclerosis. Although a review on imaging of inflammation is outside the scope of this article, future studies might explore whether a combination of biologic and imaging modalities is able to improve prediction. Beyond this issue, further efforts in the validation of inflammatory blood biomarkers should focus on the realization of large prospective, multicenter, international studies with the support of specific consortia such as the International Biomarker in Cerebrovascular Disease consortium (http://stroke-biomarkers.com/page.php?title=Network), as this type of study remains the best way in which to clarify the usefulness of a biomarker. As measurement of multiple biomarkers in large cohorts is expensive, selection of the best candidates via systematic reviews and meta-analyses could be useful as a preliminary step. Finally, interventional studies should be designed using biomarkers to make real decisions in the field, in order to assess their real impact in clinical practice.

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