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Early Brain Injury Associated with Systemic Inflammation After Subarachnoid Hemorrhage

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Abstract

Background Early brain injury (EBI) after aneurysmal subarachnoid hemorrhage (aSAH) is defined as brain injury occurring within 72 h of aneurysmal rupture. Although EBI is the most significant predictor of outcomes after aSAH, its underlying pathophysiology is not well understood. We hypothesize that EBI after aSAH is associated with an increase in peripheral inflammation measured by cytokine expression levels and changes in associations between cytokines.

Methods aSAH patients were enrolled into a prospective observational study and were assessed for markers of EBI: global cerebral edema (GCE), subarachnoid hemorrhage early brain edema score (SEBES), and Hunt–Hess grade. Serum samples collected at ≤ 48 h of admission were analyzed using multiplex bead-based assays to determine levels of 13 pro- and anti-inflammatory cytokines. Pairwise correlation coefficients between cytokines were represented as networks. Cytokine levels and differences in correlation networks were compared between EBI groups.

Results Of the 71 patients enrolled in the study, 17 (24%) subjects had GCE, 31 (44%) subjects had SEBES ≥ 3, and 21 (29%) had HH ≥ 4. IL-6 was elevated in groups with GCE, SEBES ≥ 3, and HH ≥ 4. MIP1β was independently associated with high-grade SEBES. Correlation network analysis suggests higher systematic inflammation in subjects with SEBES ≥ 3.

Conclusions EBI after SAH is associated with increased levels of specific cytokines. Peripheral levels of IL-10, IL-6, and MIP1β may be important markers of EBI. Investigating systematic correlations in addition to expression levels of individual cytokines may offer deeper insight into the underlying mechanisms related to EBI.

Keywords Subarachnoid hemorrhage · Early brain injury · Inflammation · Network modeling

Introduction

Despite improvements in clinical management and a decline in mortality, morbidity after aneurysmal subarachnoid hemorrhage (aSAH) remains high [1]. aSAH research has focused on the prevention of vasospasm and delayed cerebral ischemia (DCI) which typically occur 4–14 days after onset of aSAH [2]. However, the failure of recent trials addressing delayed complications [3] has shifted research focus to addressing mechanisms of brain injury occurring < 72 h after rupture, also known as early brain injury (EBI) [4, 5].

Initial clinical severity after aSAH has been shown to be the most predictive of clinical outcomes [6, 7]. Similarly, initial radiographic markers of EBI have been shown to be important predictors of secondary complications and outcomes [8–10]. The earliest and the most commonly used radiographic marker of EBI is global cerebral edema (GCE) which quantifies the effacement of hemispheric sulci [8]. However, due to its dichotomous nature, low sensitivity, small proportion of patients presenting with GCE, and the qualitative adjudication, its ability to characterize EBI is limited. Recently, we have described a new grading system called the "subarachnoid hemorrhage early brain edema

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score (SEBES)" [11]. The SEBES grade has a higher sensitivity than GCE and provides a finer resolution of EBI as it accounts for the effacement of the sulci at the level of the insular cortex and at the level of the lateral ventricle as well. SEBES is also sensitive to the presence of subarachnoid blood in the sulci near the insular cortex in the Virchow–Robin, thus accounting for the multiple pathophysiological components of EBI. SEBES was found to be a better predictor of DCI and outcomes than clinical status in our previous study [11]. Despite the importance of EBI, the pathophysiological mechanisms underlying the radiographic observations have not been studied. Several processes such as uncontrolled inflammatory reaction to extravascular blood [12], disruption in cerebral autoregulation [13], microvascular disruptions [14], release of products from brain tissue degradation, ischemia/reperfusion injury from transient circulatory arrest at ictus are posited to occur during EBI [8]. In particular, early unregulated inflammation during EBI is posited to be an important pathophysiological cause of secondary complications after SAH [15].

The peripheral immune response, particularly via the dysregulation of the autonomic nervous system interactions with the immune system [16], has shown to be activated after experimental ischemic and hemorrhagic stroke models [17–19]. Although purported to be an important factor in clinical outcomes, the effect of the peripheral immune response has not been adequately studied in humans. Additionally, studies investigating inflammatory response after aSAH typically use reductionist approaches, focusing on one or a few markers of inflammation at a time, without accounting for the interactions between the markers. This limits the understanding of complex systematic associations underlying the inflammatory response.

In this study, we address these limitations by examining the association between clinical and radiographic markers of EBI and the peripheral immune response after aSAH. Two radiographic markers of EBI, GCE and the SEBES [11], and a clinical severity scale (Hunt–Hess) were used. In addition to traditional statistical methods, informatics tools based on network theory were used to examine associations between inflammatory markers. We hypothesize that clinical severity and radiographic markers of EBI will be associated with an increased expression of cytokines and higher systematic associations between cytokines in correlational networks [20].

Methods

Study Population

This is a prospective, observational, single-center study of aSAH patients admitted to the Neuroscience Intensive Care Unit in the Memorial Herman Hospital at the Texas Medical Center at Houston, Texas. The inclusion criteria are the presence of aSAH on initial computed tomography (CT) or the presence of xanthochromia in cerebrospinal fluid, age above 18 years, and presentation to hospital < 72 h of ictus. Exclusion criteria were SAH associated with trauma, arteriovenous malformation, mycotic aneurysms, and subarachnoid hemorrhage without a source of bleeding identified on cerebral angiography. Patients with autoimmune diseases and conditions that affect inflammation including history of malignancy and pregnancy were also excluded. Serum samples were collected within 48 h of hospitalization. This study was approved by the university's *institutional review board*.

Cytokine Analysis

The collected blood samples were centrifuged within an hour of collection at $1460 \times g$ for 10-min generating plasma which was centrifuged a second time at $1460 \times g$ for 10 min at 4 °C generating platelet-poor supernatant. The supernatant was collected and stored at – 80 °C until ready for use. A MAGPIX magnetic bead-based ELISA (Millipore) was used to test for the following cytokines: type II interferon family—IFN-γ; IL-10 family—interleukin (IL-10); IL-17 family—IL-17a; interleukin (IL) 1 superfamily—IL-1, IL-1α, IL-1Ra, IL-4, IL-5; IL-6 (gp130) family—IL-6; CC chemokines—monocyte chemoattractant protein (MCP1), macrophage inflammatory proteins (MIP1α and MIP1β), and the tumor necrosis factor (TNF-α). The cytokine expression levels are measured in pg/ml.

Radiographic Markers

EBI was quantified using the GCE and SEBES based on the admission CT scan. GCE is a qualitative and dichotomous score (either 0 or 1) based on the presence of (1) the complete or near-complete effacement of hemispheric sulci and basal cisterns or (2) bilateral and extensive disruption of the hemispheric gray–white matter junction at the level of the central semiovale, which was due to either blurring or diffuse peripheral "fingerlike" extension of the normal demarcation between gray and white matter [8]. The SEBES is a CT-based 0–4-point grading scale of EBI. One point was assigned for the absence of visible sulci caused by either (1) effacement of sulci or basal cisterns or (2) sulci filled with blood at 2 predetermined levels in each hemisphere: (a) at the level of the insular cortex showing the thalamus and basal ganglion, and (b) at the level of the centrum semiovale above the level of the lateral ventricle (Fig. 1). Each section was scored as 0 for no effacement or 1 for effacement of sulci. Two slices with two sides result in a maximum score of 4. SEBES of ≤ 2 were considered low grade, and SEBES ≥ 3 were considered high grade.

Fig. 1 Expression levels of IL-10, IL-6, MIP1β, and TNF-α across SEBES, GCE, and Hunt–Hess grades. IL-6 was significantly elevated across poor-grade SEBES and Hunt–Hess grades and GCE subjects
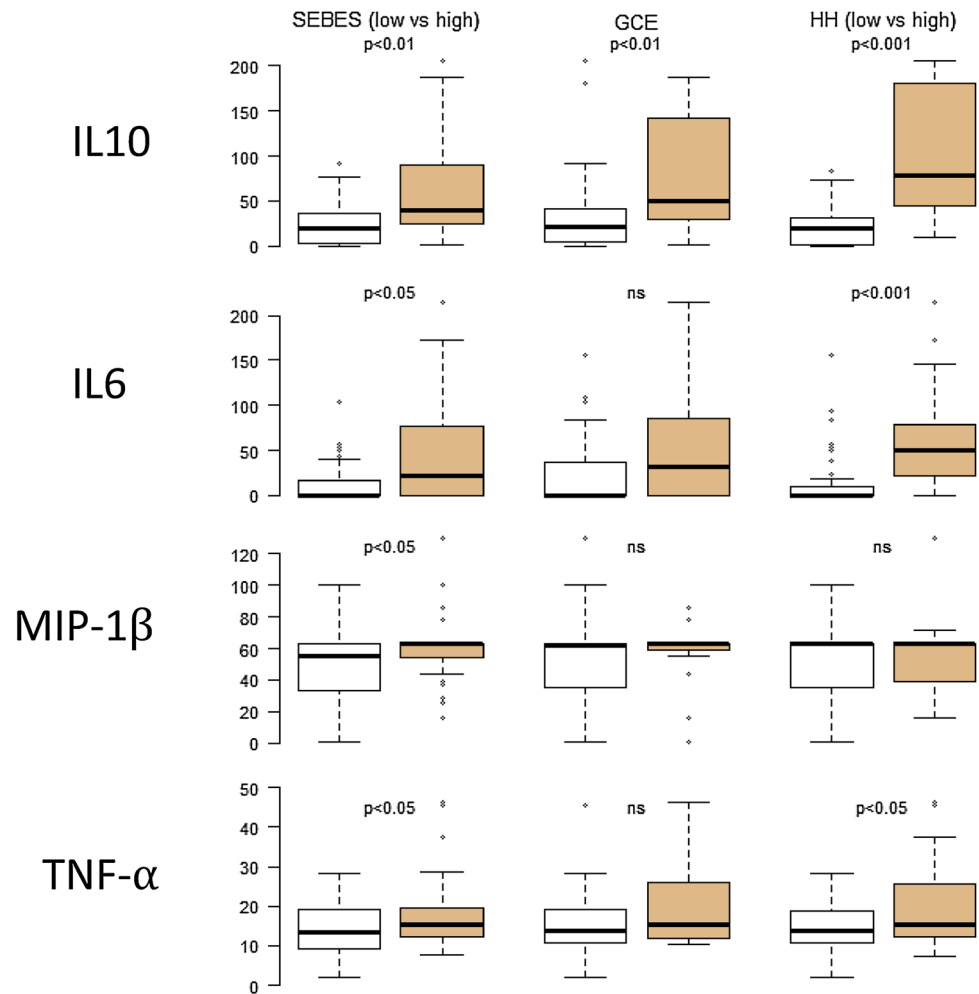
Both GCE and SEBES were adjudicated by at least two independent observers, at least one being an attending neurointensivist.

Clinical Data

Clinical variables including demographics, history of tobacco and alcohol use, past medical history, and loss of consciousness (LOC) at ictus were collected. The clinical severity at admission was quantified using the Hunt–Hess (HH) score [21]. Subjects were dichotomized based on clinical severity (HH ≤ 3 vs HH ≥ 4). All patients were monitored for the occurrence of DCI defined as the decline in the clinical status attributed to the vasospasm or new infarct on follow-up CT which was not evident in the CT or magnetic resonance image scan between 24 and 48 h after aneurysm occlusion and not attributed to surgical clipping or endovascular treatment [2]. Functional outcomes at discharge were quantified by the modified Rankin score (mRS). Good clinical outcomes were defined as mRS ≤ 3, and poor clinical outcomes were defined as mRS ≥ 4.

Hemorrhage volume was calculated using the modified Fisher score (mFS) and the intraventricular hemorrhage (IVH) score [22].

Statistical Methods

To describe demographic differences across groups, χ^2 test, Fisher's exact test, Student's t test, and Mann–Whitney U test were used where appropriate. The Mann–Whitney test was used to test for cytokine expression levels across groups. A multivariate logistic regression model was used to find independent associations after controlling for all variables with a significance level of $p < 0.1$. Due to high collinearity among the cytokine variables, cytokine variables were added sequentially to the multivariate model that included clinical and demographic variables with a significance level of $p < 0.1$. Cytokines which were significantly associated with dichotomized SEBES were examined using the full range of SEBES from 0 to 4. Ordinal regression was used to examine the associations between cytokine expression levels across individual

SEBES and HH grades. Ordinal regression was performed only for cytokines that were independently associated with EBI markers. R (v3.1.3) and SPSS (v) were used for the statistical analysis.

Network Model

An informatics tool based on correlation coefficients between all possible cytokine pairs was used to systematically characterize the immune response [20]. Networks were constructed with cytokines as nodes and Pearson's correlations between them as the edges. The network includes all possible pairwise correlation coefficients between the analyzed cytokines. Prior to computing the correlation coefficient, the Box-Cox transformation [23] was used to normalize each cytokine distribution. The Kolmogorov–Smirnov test was used to test the normality of the transformed variables at a 5% significance level. Transformed variables that failed to reject the null hypothesis were excluded from the analysis. All pairwise correlations were computed, and significant correlations greater than 0.4 were used to construct the network. The open-source statistical packages in R (v3.1.3) and cytoscape (v3.4.0) were used for data transformation and network visualization.

Results

During the study period, 72 patients met the inclusion criteria and were included in the study. Demographics and clinical outcomes between subjects with and without GCE as well as low-grade and high-grade SEBES are presented (Table 1). The presence of GCE was associated with the occurrence of DCI (47 vs 11.3%, $p < 0.01$) and poor clinical outcomes (78 vs 11%, $p < 0.01$). High-grade SEBES subjects had worse HH (51 vs 12.5%, $p < 0.01$), were more likely to experience LOC at ictus (48 vs 17%, $p < 0.05$), had a higher instance of DCI (38 vs 5%, $p < 0.01$), had longer hospital length of stay (LOS) ($p < 0.05$), and intensive care unit LOS ($p < 0.05$). HH ≥ 4 subjects were more likely to have poor outcome (71 vs 20%, $p < 0.01$). The mRS ≥ 4 group had higher HH grade (60 vs 13%, $p < 0.01$) and were more likely to have an IVH (84 vs 58%, $p < 0.05$).

Cytokines and Radiographic Markers of EBI

Expression levels of IL-1α, IL-I1β, IL-4, and IL-5 were undetectable for the majority of samples and were excluded from the analysis. Only IFN-γ, IL-10, IL-17A, IL-1RA, IL-6, MCP1, MIP1α, MIP1β, and TNF-α were included in the analysis. GCE: IL-6 levels were higher in GCE group when

compared with no-GCE [median, IQR 40.2(28–92) vs 21(3.8–42), $p < 0.01$]; however, it was not independently associated with GCE when controlled for confounders: age, Hunt–Hess, mFS, and LOC in the multivariate model (Table 1). SEBES: In the SEBES ≥ 3 group, IL-10, IL-6, MIP1β, and TNF-α were significantly higher when compared to the SEBES ≤ 2 group. After adjusting for Hunt–Hess score, MIP1β was found to be independently associated with high-grade SEBES and had an ordinal association with MIP1β ($p < 0.01$) (Fig. 1). IL-10 was nearly significant ($p = 0.1$) in its association with SEBES.

Association with Hunt–Hess Score at Admission and Outcomes

In total, 21 (29%) of the subjects had a Hunt–Hess score of either 4 or a 5. The HH ≥ 4 subjects were more likely to experience LOC at ictus ($p < 0.01$), had a higher mFS ($p = 0.05$), and had a trend for higher incidence of smoking ($p = 0.07$). As expected, they also had an increased length of stay at the hospital ($p < 0.01$) and poor mRS at discharge ($p < 0.01$). The HH ≥ 4 group had elevated levels of IL-10, IL-1RA, IL-6, MCP1, MIP1α, and TNF-α (Table 2) in comparison with the HH ≤ 3 group. After adjusting for possible confounders such as age, mFS, smoking history, and LOC at ictus in the multivariate analysis, we found that IL-10, IL-6, TNF-α, IL-1RA, MCP1, and MIP1α were independently associated with HH ≥ 4. We also observed a stepwise increase in expression levels of IL-6 ($p < 0.01$), IL-10 ($p < 0.01$), TNF-α ($p < 0.01$), and MIP1α ($p < 0.01$) for each HH grade. IL-6, IL-10, MCP1, and MIP1α were elevated in subjects with poor outcomes. IL-6 was independently associated with outcomes at discharge.

Correlation Networks

Network analysis revealed that both the GCE group and SEBES ≥ 3 group had higher number of correlations and stronger correlation strength between the cytokines (Fig. 2a, b). SEBES ≥ 3 group had almost twice the number of cytokine correlations compared to SEBES ≤ 2 group (15 vs 6), suggesting an increase in interactions among cytokines. In the GCE group, there were several overlapping associations between the cytokines (Fig. 2a). However, the SEBES ≥ 3 group had several distinct associations particularly pertaining to IL-6, TNF-α, and MIP1α. IL-6 and TNF-α were correlated with six other cytokines followed by MCP1, MIP1α, and IL-1RA which were correlated with five cytokines.

The HH ≥ 4 group had a higher number of correlations compared to HH ≤ 3 group (15 vs 3), and the cytokines IL-6 and IL-10 had the most number of correlations with other

Table 1 Differences in cytokine levels measured after aSAH

Variables	Total N = 71	GCE (−) N = 54	GCE (+) N = 17	P	SEBES (−) N = 40	SEBES (+) N = 31	P
Demographics and clinical							
Age (mean, SD)	52 ± 14	53 ± 14	48 ± 12	0.21	54 ± 14	49 ± 13	0.16
Gender (female) n, %	47(66)	35(65)	12(70)	0.77	25(62.5)	22(71)	0.62
Modified Fisher score (3,4) n, %	62(87)	46(85)	16(94)	0.67	33(82.5)	29(93)	0.28
HH (4,5) n, %	21(29.5)	14(25)	7(41)	0.37	5(12.5)	16(51)	**<0.01**
Smoking, n, %	37(52)	29(53)	8(47)	0.8	22(55)	15(48)	0.7
Alcohol, n, %	28(39)	22(40)	6(35)	0.9	19(47)	8(25)	0.18
Hypertension, n, %	41(57)	31(57)	10(58)	1	22(55)	19(61)	0.77
IVH (n, %)	48(67)	37(68)	11(64)	0.4	25(62.5)	23(74)	0.43
LOC (n, %)	22(31)	15(27)	7(42)	0.45	7(17)	15(48)	**0.01**
Outcomes							
Hospital LOS, median, IQR (25–75%)	15(10–21)	14(9.25–21)	17(11–25)	0.16	12(9–18)	18(12–24.5)	**0.03**
DCI	14(20)	6(11)	8(47)	**<0.01**	2(5)	12(38)	**<0.001**
mRS (> 3, %)	25(35)	5(12)	11(18)	**<0.01**	11(27)	14(45)	0.19
Cytokines (pg/ml)							
IFN-γ	0.5(0.5–0.5)	0.5(0.5–0.5)	0.5(0.5–3.48)	0.09	0.5(.5–.5)	0.5(.5–1.55)	0.16
IL-10	0.05(0.5–42.67)	0.5(0.5–39)	0.5(0.5–59.7)	0.54	0.5(.5–17)	21(.5–76)	**0.03**
IL-17a	0.5(.5–.5)	0.5(.5–.5)	0.5(.5–.66)	0.4	0.5(.5–.5)	0.5(.5–.9)	0.08
IL-1RA	37.4(0.5–56.89)	37.4(5.11–56.9)	28.6(0.5–49.22)	0.4	19.45(.5–57)	37(22–60)	0.14
IL-6	28(3.81–51.29)	20.5(2.2–42.6)	35.3(24.2–85)	**0.03**	20(3–35)	39.2(24–90)	**0.001**
MCP1	163.2(115.2–210)	163.2(114.5–210.7)	161.4(125–216)	0.91	154(112–209)	169(142–265)	0.18
MIP1α	0.5(0.5–0.5)	0.5(0.5–0.5)	0.5(0.5–2.9)	0.53	0.5(.5–.5)	0.5(.5–5.4)	0.14
MIP1β	44(25.4–66.7)	39.3(25.4–70.4)	58.5(21.5–65)	0.67	55(34–63)	63(54–63)	**0.01***
TNF-α	14.3(10.74–19.34)	14.3(10.7–19.2)	14.1(11.4–19.4)	0.62	13.4(9.2–19)	15.1(12.4–19)	**0.04**

* Significant after controlling for dichotomized Hunt–Hess score

All p < 0.05 significance levels are highlighted in bold

aSAH aneurysmal subarachnoid hemorrhage, *DCI* delayed cerebral ischemia, *GCE* global cerebral edema, *HH* Hunt–Hess, *IFN* interferon, *IL* interleukin, *IQR* interquartile range, *IVH* intraventricular hemorrhage, *LOC* loss of consciousness, *LOS* length of stay, *MCP* monocyte chemoattractant protein, *MIP* macrophage inflammatory proteins, *mRS* modified Rankin score, *SEBES* subarachnoid hemorrhage early brain edema score, *TNF* tumor necrosis factor,

cytokines (Fig. 2c). However, cytokine associations between the mRS ≤ 3 and mRS ≥ 4 groups appear similar (Fig. 2d).

Discussion

The main finding of our study is that EBI is associated with elevations in inflammatory markers and differential activation of the inflammatory system characterized by correlational networks. IL-6 was elevated in subjects with GCE. MIP1β was independently associated with the SEBES. Higher Hunt–Hess grade patients had elevated levels of IL-10, IL-1Ra, IL-6, MCP1, MIP1α, and TNF-α, and these cytokines were independently associated with clinical severity. Examination of correlations between

cytokines through network analysis showed a significant difference between subjects with EBI characterized by a greater number and increased strength of correlations in subjects with high-grade SEBES.

Radiographic Markers of EBI

Comparison Between GCE and SEBES

A significant challenge in studying the pathophysiology of EBI after SAH has been the lack of a radiographic correlate of EBI that is both reliable and precise to evaluate grades of EBI. GCE has been used most commonly, but the qualitative and small proportion of patients with the finding (only ∼ 10–15%), limits its use. The SEBES is semi-quantitative and provides a range of EBI which is a more

Table 2 Cytokine differences across Hunt–Hess grades and outcomes

	HH (1,2,3) N = 50	HH (4,5) N = 21	P	mRS (0,1,2,3) N = 46	mRS (4,5) N = 25	P
Demographics						
Age (mean, SD)	50.7 ± 12	55 ± 16.7	0.3	49 ± 12	57 ± 15	0.29
Gender (female) n, %	30(60)	17(81)	0.1	29(59)	18(72)	0.61
Smoking, n, %	30(60)	7(33)	0.07	26(56)	11(44)	0.44
Alcohol, n, %	23(46)	5(23)	0.11	19(41)	9(36)	0.85
Hypertension, n, %	27(54)	14(66)	0.46	27(58)	14(56)	1
HH (≥ 4, %)	NA	NA	NA	6(13)	15(60)	**<0.01**
Modified Fisher score (3,4) n, %	41(82)	21(100)	0.05	38(82)	24(96)	0.14
IVH, n, %	31(62)	17(81)	0.16	27(58)	21(84)	**0.03**
Outcomes						
DCI, n, %	8(16)	6(28)	0.37	6(13)	8(32)	0.1
Hospital LOS, median, IQR	12(9–18)	22(16–27)	0.002	12.5(9–19)	18(14–27)	**0.02**
mRS (> 3, %)	10(20)	15(71)	**<0.01**			
Cytokines (pg/ml)						
IFN-γ	0.5(0.5–0.5)	0.5(0.5–0.5)	0.44	0.5(.5–.5)	0.5(.5–.5)	0.7
IL-10	0.5(0.5–8.6)	50.7(0.5–79)	**<0.001***	0.5(0.5–10.2)	42(0.8–79)	**<0.01**
IL-17a	0.5(0.5–0.5)	0.5(0.5–0.88)	1	0.5(.5–.5)	0.5(.5–.5)	0.68
IL-1RA	23(.5–54.5)	47(28–102)	**<0.01**	33(33–57)	38(.5–56)	0.4
IL-6	20(.5–31)	78(44–181)	**<0.001***	20(.5–33)	73(28–92)	**<0.01***
MCP1	156(115–184)	263(142–401)	**<0.01***	159(117–195)	169(61–401)	**0.09**
MIP1α	0.5(0.5–0.5)	0.5(0.5–20.7)	**<0.01***	0.5(.5–.5)	0.5(.5–10.29)	**0.04**
MIP1β	63(35.6–63)	63(39–63)	0.79	63(38–63)	63(35–63)	0.65
TNF-α	13(10.6–18.6)	15(12.3–25)	**0.02***	14(11–18)	15(12–23)	0.2

* Significant in the multivariate model after adjusting for demographic variables that were near significant ($p < 0.1$)

All $p < 0.05$ significance levels are highlighted in bold

DCI delayed cerebral ischemia, *GCE* global cerebral edema, *HH* Hunt–Hess, *IFN* interferon, *IL* interleukin, *IQR* interquartile range, *IVH* intraventricular hemorrhage, *LOC* loss of consciousness, *LOS* length of stay, *MCP* monocyte chemoattractant protein, *MIP* macrophage inflammatory proteins, *mRS* modified Rankin score, *SEBES* subarachnoid hemorrhage early brain edema score, *TNF* tumor necrosis factor,

accurate reflection of the distribution of severity that is seen clinically. In our population, we confirmed previous findings that both GCE and SEBES are predictive of DCI and clinical outcomes. In addition, we found that SEBES was significantly associated with other clinical variables of EBI; high HH and LOC. Similarly, our findings show that the SEBES is associated with markers of inflammation.
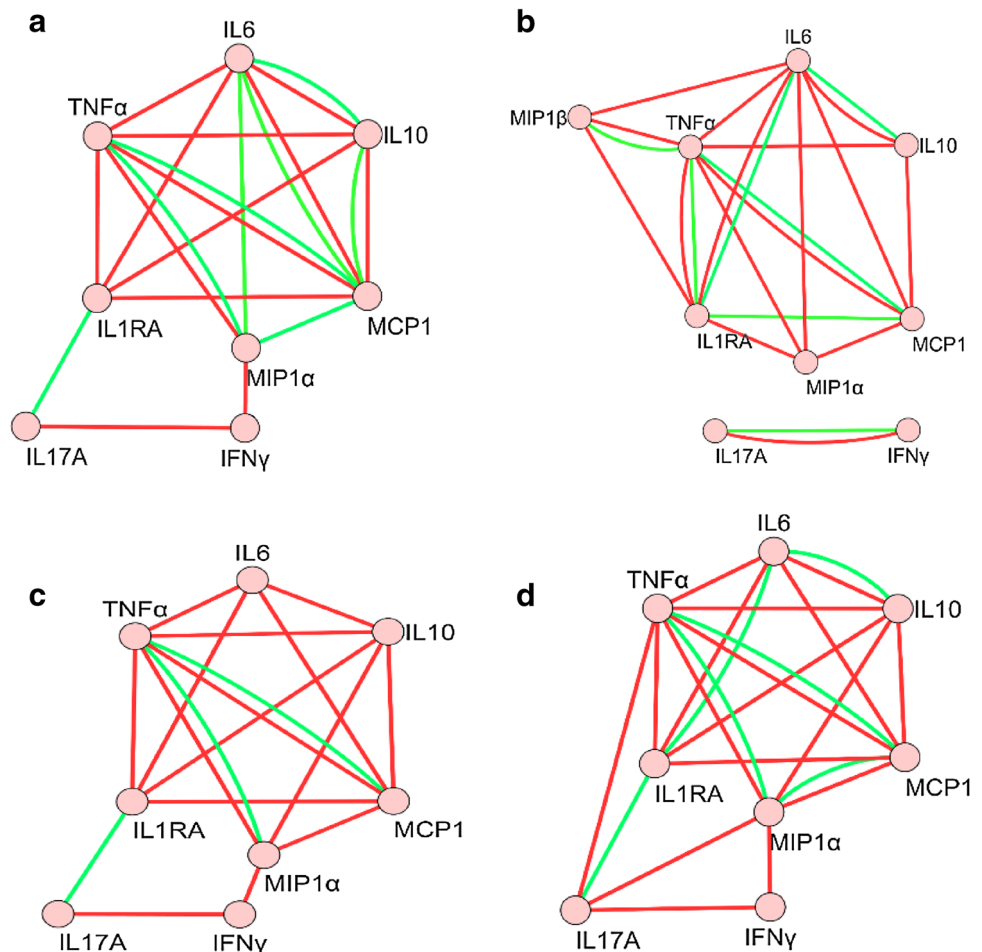
GCE subjects had elevated levels of IL-6. IL-6 is an interleukin that acts primarily as pro-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate the immune response. It has been previously studied in SAH as well as other diseases and has consistently been associated with worse clinical outcomes [24–26]. Although generally considered a pro-inflammatory cytokine because of its actions of inhibiting TNF-α and IL-1, some have stressed it's anti-inflammatory properties [27].

IL-6, IL-10, TNF-α, and MIP1β were significantly elevated in patients with a high-grade SEBES. MIP1β was

found to be independently associated with high-grade SEBES after controlling for confounders HH. Additionally, MIP1β had an ordinal association with SEBES. It is a CC chemokine and is a chemoattractant for natural killer cells and monocytes among many. It is predominantly secreted by peripheral blood lymphocytes as a reaction to lipopolysaccharide [28]. Upregulation of MIP1β in lungs after experimental SAH has been reported and is purported to be a risk factor for neurogenic pulmonary edema after SAH [29].

Also, only 17 patients had GCE, but 31 patients had a high-grade SEBES, indicating that SEBES is a more sensitive radiographic scale than GCE. IL-10, MIP1β, and TNF-α were only elevated in the high-grade SEBES subjects or high-grade HH subjects, but not across GCE status. This suggests that SEBES characterizes clinical status better than GCE. This association between radiographic markers and elevation of systemic inflammatory molecules

Fig. 2 Correlation networks based on **a** the presence or absence of GCE from 17 and 55 patients, respectively, **b** high- and low-grade SEBES from 40 and 31 patients, **c** high Hunt–Hess grade at admission from 21 and 51 patients, respectively, and **d** poor and good mRS at discharge from 25 and 47 patients. Each line joining any two cytokines denotes a Pearson's correlation coefficient greater than 0.4. The green lines represent correlations in subjects who did not have GCE, low SEBES grade, low clinical severity at admission, and good outcomes at discharge. The red lines are correlations from subjects who had GCE, higher SEBES, higher clinical severity at admission, and poor outcomes at discharge. The raw expression levels of cytokines were normalized using Box-Cox transformation prior to the computation of the correlations. All correlations were *p < 0.05. IL-6, IL-10, TNF-α, and MCP1 shown increased systematic correlation (Color figure online)

Clinical Severity and Outcomes

Our finding that HH ≥ 4 subjects had higher levels of IL-10, IL-6, and MIP1β is novel. IL-10 is Th2 anti-inflammatory cytokine and a potent inhibitor of Th1 cells. It is secreted by monocytes and lymphocytes and has pleiotropic effects including immune regulation and inflammation. It is also known to deactivate monocyte/macrophage pro-inflammatory cytokine synthesis. The finding that IL-10 is elevated can have different implications. It is possible that IL-10 elevation is simply in response to elevations in pro-inflammatory processes. In conjunction with elevated pro-inflammatory markers, IL-10 may be simply a marker of injury. On the other hand, some have suggested that immune suppression after acute brain injury is an important driver of outcomes. In ischemic stroke, and more recently in SAH, immune suppression has been associated with worse clinical outcomes. These findings, in addition to our findings of an elevation in IL-

suggests that early brain injury could have an adverse impact on systemic peripheral inflammation.

10, support the idea that early immune suppression may play a detrimental role in brain injury. Perhaps a dysregulation of the immune process after brain injury rather than overwhelming inflammation is the driver of worsening brain injury [26]. Preclinical studies in animal models have shown an increase in IL-10 to be associated with worse hemorrhage [30]. Previous studies have detected elevated IL-10 levels after SAH but have not examined the significance of elevated levels with clinical severity [31]. Our study is the first to describe elevated levels of IL-10 after SAH. A cerebral microdialysate study in SAH and TBI patients have found that in majority of patients IL-10 could not be detected at all in brain interstitial fluid [32]. Network analysis also reveals that IL-6 and IL-10 were highly correlated for all subject subgroups with the exception of the HH ≤ 3 group. There is some evidence that IL-6 induces an strong anti-inflammatory response at the cellular level through regulatory mechanisms [33]. The role of these mechanisms pertaining to aSAH requires investigation.

Networks

Since most cytokine levels did not significantly differ in the presence or absence of GCE, and the associations between cytokines across the subject groups suggest that the presence of GCE does not perturb the inflammatory response captured by the cytokines examined. However, in the SEBES ≥ 3 group, IL-6, MIP1α, MIP1β, and MCP1 had more correlations compared to SEBES ≥ 2. MIP1 proteins activate granulocytes leading to inflammation [34]. They also stimulate the macrophages and fibroblasts to release IL-6 and TNF-α. In high SEBES grade, high intercorrelations between TNF-α, IL-6, MIP1α, and MIP1β indicate an increased macrophage activity. MCP1 had many correlations in the poor-grade SEBES group [35]. MCP1 is a chemoattractant cytokine that directs leukocyte migration to sites of inflammation. It has previously been shown to be associated with poor outcomes but not delayed cerebral ischemia after SAH [36]. There is in vivo and in vitro evidence that increases in MCP1 could compromise endothelial tight junctions and increases blood–brain barrier permeability and consequently vasogenic edema [37–39]. Both the increase in the number of correlations and the clusters between cytokines indicate that such approaches, in addition to the examination of individual markers, offer a deeper insight into the inflammatory process associated with EBI. Higher clinical severity at admission was associated with an increased inflammatory response, characterized by both an increase in the expression level of several cytokines and a higher degree of associations between cytokines. However, whether the inflammatory reaction is a response to an ongoing physiological event associated with worsening clinical severity or vice versa is not clear.

Limitations

Firstly, being a single-center study it is bound by inherent biases. Secondly, due to the high variance in biomarker variables and large number of possible correlations, the complete elimination of spurious correlations is not possible. Though we have used appropriate data transformation methods to meet assumptions for computing the correlational network, the results should be interpreted cautiously. The number of spurious correlations could be further reduced by comparison with other acute pathological conditions as controls. Thirdly, the study design only permits correlational relationships and causality should not be inferred. Fourth, only peripheral inflammation is examined in this study. The study of central inflammation through analysis of cerebrospinal fluid or cerebral microdialysate, in addition to peripheral inflammation, may offer a deeper insight into the injury mechanisms.

Conclusion

Early brain injury after SAH quantified by GCE and SEBES is associated with elevated levels of IL-6, IL-10, and MIP1β. Higher clinical severity at admission is associated with elevated levels of IL-10, IL-6, MCP1, and TNF-α. The correlations between inflammatory biomarkers were proportional to clinical severity and severity of brain injury, indicating an elevation on overall systematic inflammatory activity.

Author Contributions JS, HC, and DK were involved in the conception and design of the study. JS, KP, GH, and SA assisted in the acquisition and analysis of data. JS, HC, and TC contributed substantially in drafting the manuscript and figures.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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