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NONCONVULSIVE SEIZURES IN SUBARACHNOID HEMORRHAGE LINK INFLAMMATION AND OUTCOME

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Abstract

Objective—Nonconvulsive seizures (NCSz) are frequent following acute brain injury and have been implicated as a cause of secondary brain injury but mechanisms that cause NCSz are controversial. Pro-inflammatory states are common after many brain injuries and inflammatory mediated changes in blood-brain-barrier permeability have experimentally been linked to seizures.

Methods—In this prospective observational study of aneurysmal subarachnoid hemorrhage (SAH) patients we explored the link between the inflammatory response following SAH and in-hospital NCSz studying clinical (systemic inflammatory response syndrome, SIRS) and laboratory markers of inflammation (tumor necrosis factor receptor 1, TNF-R1; high sensitivity C-reactive protein, hsCRP). Logistic regression, cox proportional hazards regression, and mediation analyses were performed to investigate temporal and causal relationships.

Results—Among 479 SAH patients, 53(11%) had in-hospital NCSz. Patients with in-hospital NCSz had a more pronounced SIRS response (OR1.9 per point increase in SIRS; 95%-CI1.3-2.9), inflammatory surges were more likely immediately preceding NCSz onset, and the negative impact of SIRS on functional outcome at 3 months was mediated in part through in-hospital NCSz. In a subset with inflammatory serum biomarkers we confirmed these findings linking higher serum TNF-R1 and hsCRP to in-hospital NCSz (OR1.2 per 20 point hsCRP increase [95%-CI1.1-1.4]; OR2.5 per 100 point TNF-R1 increase [95%-CI2.1-2.9]). The association of inflammatory biomarkers with poor outcome was mediated in part through NCSz.

Interpretation—In-hospital NCSz were independently associated with a pro-inflammatory state following SAH reflected in clinical symptoms and serum biomarkers of inflammation. Our findings suggest that inflammation following SAH is associated with poor outcome and this effect is at least in part mediated through in-hospital NCSz.

Keywords

inflammation; electrographic seizures; nonconvulsive seizures; subarachnoid hemorrhage; continuous EEG monitoring; brain metabolism

Introduction

Nonconvulsive seizures (NCSz) occur in 10-25% of patients with acute brain injury and are associated with worse outcome.¹ Most experts agree that NCSz should be treated^{2, 3} but choice, duration, and aggressiveness of treatment as well as seizure prophylaxis are controversial.⁴ Some argue that side effects of treatment outweigh the benefits and that NCSz are surrogates of the extent of brain injury.⁵ Randomized controlled trials (RCT) are needed,^{2, 3} but despite promising animal data, treatments often fail in acute brain injury due to limited insights into human disease mechanisms.^{6, 7} This lack of mechanistic insight prevents upstream interventions for acute brain injury seizures targeting underlying causes.⁸ Experimental^{9, 10} and human temporal lobe epilepsy data¹¹ support models of inflammatory induced modification of blood brain barrier permeability resulting in seizures. Pro-inflammatory states are prevalent after acute brain injury and particularly amongst acute brain injuries that frequently have seizures, such as brain hemorrhages and CNS infections.¹²⁻¹⁵ Inflammatory acute phase proteins correlate with treatment refractoriness and mortality in patients with SE.¹⁶

All algorithms for prophylaxis and treatment of seizures in this setting use antiepileptics typically used in epilepsy.^{2, 3} Emerging experimental data suggest that immune modulation may have a role in seizure treatment, which may address the underlying cause rather than downstream effects.^{10, 17, 18} The central hypothesis of this study is that pro-inflammatory states following SAH, characterized by clinical variables (systemic inflammatory response syndrome, SIRS) and serum markers (tumor necrosis factor receptor 1, high sensitivity C reactive protein, and transthyretin), are independently associated with the development of NCSz and that NCSz are possibly one mechanism by which inflammation negatively impacts outcome after acute brain injury.

Patients and Methods

Study Population

We enrolled 479 patients into a prospective observational cohort study of aneurysmal SAH patients admitted to the neurological ICU at Columbia University Medical Center between March 2006 and April 2012. The diagnosis of SAH was established by computed tomography (CT) or xanthochromia of the cerebrospinal fluid if the CT was negative.^{1, 19} Patients were not enrolled if any of the following were met (1) age <18 years old, (2) pregnant, or (3) patients or families did not want to participate in the study. Data were collected as part of an ongoing prospective database approved by the local Institutional Review Board.

General Management

Medical and surgical treatment followed guidelines by the American Heart Association³⁴ and existing management protocols at most large medical centers.²⁰ Patients received mechanical ventilation, external ventricular drainage, daily interruption of sedation, prophylactic oral nimodipine, and intravenous hydration according to a standardized management protocol. Deviating from AHA guidelines all patients were on prophylactic phenytoin for one week after SAH which was then discontinued unless seizures had been recorded on scalp EEG.^{1, 19} Isolated scalp seizures were initially treated with levetiracetam and status epilepticus with midazolam infusions. Periodic epileptiform discharges (PEDs) were not considered seizures and there was no attempt made to eliminate them with medications; however, patients with PEDs were maintained on anticonvulsants to prevent seizures.¹⁹

Data collection

Physiologic data was acquired using a high-resolution acquisition system (BedmasterEXTM; Excel Medical Electronics Inc) from General Electric Solar 8000i monitors and inserted into a Microsoft SQL database. EEG was recorded using a digital video EEG bedside monitoring system (XLTEKTM, Oakville, ON; low-pass filter 70 Hz, high-pass 0.1 Hz, sampling rate 200 Hz). Additionally we collected clinical variables including demographics, disease specific variables (i.e., aneurysm size), laboratory values, neurological examination findings, medications, hospital complications, and three months functional outcome data (modified Rankin Scale) as part of an ongoing, prospective observational SAH outcomes study.^{1, 19}

EEG monitoring

At our institution, patients are typically not connected to continuous EEG monitoring before post bleed day 3 at which time removal of tight bandages for scalp incisions occurs. Rarely EEG monitoring is continued beyond post bleed day 10 unless ongoing seizure activity is present. Seizures were defined as any spikes, sharp-waves, or sharp-and-slow wave complexes lasting for 10 seconds or more at either a frequency of at least 3 per second or a frequency of at least 1 per second with clear evolution in frequency, morphology, or location.¹⁹

SIRS definition

Systemic inflammatory response syndrome (SIRS) score was calculated, following previously described methodology,²¹ by assigning a value of one for each of the following: heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute, body temperature >38°C or <36°C, and serum white blood cell count <4,000 or >12,000/mm³. We calculated the SIRS burden defined as the mean of daily SIRS scores as well as the slope of the SIRS curve.

Serum markers of inflammation

As part of a separate prospective study, we collected serum measures of inflammation at pre-specified time points for SAH patients enrolled between May 2008 and June 2010.¹³ Serum samples were assayed using enzyme-linked immunoassays for high sensitivity C-reactive protein (hsCRP, BioCheck; normal range, <3.0 mg/L) and tumor necrosis factor TNF receptor 1 (TNF-R1; R&D Systems; Minneapolis, MN), and an immunoturbidimetric assay for transthyretin levels (TTR, also known as prealbumin; Cobas-Integra 400 Plus Chemistry Analyzer; normal range, 17-40 mg/dL). Given the time frame of cEEG monitoring we restricted the comparison between inflammatory serum markers and cEEG findings for the purposes of this study to measurements taken between days 4 to 7 (phase 2) and days 8 to 10 (phase 3). In the primary analysis we compared serum biomarkers to findings on EEGs obtained within 24 hours of measuring the serum markers. In secondary analyses we compared the same serum markers to EEGs obtained on the day following the serum marker measurement.

Statistical Analysis

(1) Association—For univariate comparisons Mann-Whitney-U, Student-t-Test, Chi-square and Fishers exact tests were performed as appropriate. We ran a series of logistic regression models to identify inflammatory variables that were independently associated with in-hospital seizures after controlling for demographic, radiographic, and clinical variables associated with in-hospital seizures. For each inflammatory variable including SIRS, hsCRP, TTR, and TNF-R1 we ran a separate logistic regression model. Next, we identified if these inflammatory variables and in-hospital seizures were independently associated with poor 3 month functional outcome after controlling for established demographic, radiographic, and clinical predictors of poor outcome. Differences were considered significant at $P < 0.05$. Follow-up bias analysis comparing those with to those without 3-month functional outcome did not reveal any differences in demographics, admission clinical findings, or hospital complications. Logistic regression analysis was repeated on ten complete datasets generated by multiple imputation using Bayesian methods as outcome data was missing in 28% of patients.

(2) Temporal relationship—Cox proportional hazards regression was used to calculate hazard ratios and their 95 % confidence intervals of predictors on the onset of NCSz and the onset of inflammation measured as a rise in SIRS. Time-dependent covariates within the Cox proportional hazards framework were used to describe temporal relationships between

NCSz and markers of inflammation, while controlling the analysis for patient age, Hunt Hess grade, aneurysm size, and aneurysm treatment modality.

(3) Causality—It is difficult to determine causal relationships in an observational dataset;²² however, mediation analysis identifies variables that linearly predict other variables given the presence of potentially related variables. This provides an estimate of whether a mediation variable (i.e., NCSz) governs the relationship between an independent (i.e., inflammatory variables: SIRS, TNF-R1, or hsCRP) and a dependent variable (i.e., functional outcome). Mediation analysis follows a three step-approach: (1) identify a significant relationship between the independent and dependent variable, (2) identify a significant relationship between the independent and mediator variable, thereby establishing a co-existing linear relationship between mediating and independent variables, and (3) determine the amount by which the prediction of the dependent from the independent variable is decreased in the presence of the mediating variable when regressing all three together compared to regressing only the independent on the dependent variable alone. We chose a least squares optimization approach to determine the magnitude of the predictive power for individual variables. Significance was determined by calculating both p-values applying the F-test²³ as well as 95%-CIs based on a covariance matrix generated by bootstrapping.²⁴ Building on these simplified models we then performed subgroup analyses to account for confounders including age, clinical exam (Hunt Hess grade), and infections (including pneumonia, urinary tract infections, ventriculitis, and sepsis).

All analyses were made using commercially available software (R, Institute for Statistics and Mathematics, Vienna; SPSS 18, SPSS Inc., Chicago, IL; MATLAB, The MathWorks Inc, Natick, MA).

Results

SIRS and Seizures

Mean SIRS burden over the first 4 days of admission was 2.1+/-0.8 (IQR 1.6; 2.2; 2.8; Table 1). cEEG monitoring was started on median SAH day 4 (IQR 2, 8; and continued for a median total of 5 days [IQR 2, 11]). The median SAH day of the first NCSz was 8.5 (IQR 4.8, 17). Patients with in-hospital NCSz (N=53, 11%) had consistently higher SIRS scores throughout their hospital course starting on post SAH day 3 (Figure 1). SIRS burden over the first four days after hemorrhage was an independent predictor of in-hospital NCSz (OR 1.9 for each point increase in SIRS score; 95%-CI 1.3-2.9) after controlling for known predictors and other variables identified in this dataset including age, gender, Hunt Hess grade, aneurysm size, and aneurysm clipping (see Table 2). SIRS burden was further associated with later in-hospital infections, including sepsis (19% [N=10] vs. 6% [N=27]; OR 3.5, 95%-CI 1.6-7.7), pneumonia (54% [N=28] vs. 18% [N=77]; OR 5.2, 95%-CI 2.8-9.5), urinary tract infections (39% [N=20] vs. 19% [N=81]; OR 2.6, 95%-CI 1.4-4.8), and ventriculitis (31% [N=16] vs. 10% [N=40]; OR 4.2, 95%-CI 2.2-8.3). Not associated with in-hospital NCSz were a history of epilepsy, Hunt Hess grade, modified Fisher score, and delayed cerebral ischemia. The mean SIRS burden over the first 10 days after bleed was also independently associated with in-hospital NCSz (OR 3.3, 95%-CI 1.9-5.6 for each point

increase in SIRS score) but the slopes of the SIRS curve over the first 4 and 10 days were not associated with in-hospital NCSz. After controlling for age, admission Hunt–Hess grade, aneurysm size, and aneurysm treatment modality, Cox regression analysis using time-lagged covariates revealed that SIRS of 3 or above was more likely in the 24 hours preceding the onset of NCSz (Table 3). The risk of elevated SIRS remained for 72 hours after NCSz onset before it started to decrease.

Inflammatory biomarkers

Patients with serum measurements of inflammation and metabolism had similar baseline characteristics to those that did not, except for being less likely white and more commonly being treated with aneurysm clipping (Table 1). Seizures were seen in 15% of SAH patients in whom inflammatory biomarkers were available. hsCRP was higher for those with than those without moderate to severe EEG background attenuation at both post SAH days 4 to 7 (87 [IQR 47, 160] vs 29 [IQR 9, 56] mg/dL; OR of NCSz increased by 1.2 [95%-CI 1.1-1.4] per 20 point increase in hsCRP) and 8 to 10 (98 [IQR 48, 185] vs 41 [IQR 9, 75] mg/dL; OR 1.2 [95%-CI 1.1-1.4] per 20 point increase in hsCRP). At both time points, hsCRP was higher for those with compared to those without NCSz as seen on EEG recorded within 24 hours of the serum measurements after controlling for age, aneurysm size, radiological findings, clipping, and Hunt Hess grade (Table 4). Subgroup analysis revealed that higher hsCRP measurements with NCSz were only seen for patients with no or mild EEG background attenuation at post SAH days 4 to 7 (99 [IQR 38, 132] vs 17 [IQR 6, 40] mg/dL; the OR of NCSz increased by 2.3 [95%-CI 1.4-3.7] for each 20 point increase of hsCRP) and days 8 to 10 (129 [IQR 65, 224] vs 27 [IQR 9, 49] mg/dL; OR of NCSz increased by 1.7 [95%-CI 1.2-2.4] for each 20 point increase of hsCRP; Figure 2).

TTR was lower for those with than those without moderate to severe EEG background attenuation at both post SAH days 4 to 7 (15 [IQR 11, 19] vs 21 [IQR 15, 25] mg/dL; OR 0.9 [95%-CI 0.8-1.0]) and 8 to 10 (18 [IQR 12, 22] vs 20 [IQR 15, 25] mg/dL; OR 0.9 [95%-CI 0.8-1.0]). At both time points, TTR was lower for those with compared to those without NCSz after controlling for age, aneurysm size, radiological findings, clipping, and Hunt Hess grade (Table 4). The hsCRP/TTR ratio was higher for those with NCSz at post SAH day 4-7 (Table 4) and for both time points when limited to those with no or mild background attenuation (days 4 to 7: 6 [IQR 2, 11] vs 1 [IQR 0, 2]; OR 1.6 [95%-CI 1.2-2.3]; days 8 to 10: 8 [IQR 3, 17] vs 1 [IQR 0, 3]; OR 1.5 [95%-CI 1.1-2.1]; Figure 2). Logistic regression analysis found higher serum TNF-R1 measurements to be independently associated with occurrence of NCSz within post SAH days 4 to 7 and 8 to 10 after controlling for age, aneurysm size, radiological findings, clipping, and Hunt Hess grade (Table 4 and Figure 3). In secondary analyses we substituted the EEG variable with EEG obtained on the day following the serum marker measurement and our results did not change (data not shown).

Inflammation and seizures vs. outcome

SIRS burden over the first 4 days as well as in-hospital NCSz were independently of each other associated with poor outcome (OR 2.1, 95%-CI 1.5-2.9 and OR 4.8, 95%-CI 2.1-11.0, for SIRS and in-hospital NCSz respectively). After controlling for other known predictors of

poor outcome including age, Hunt Hess grade, modified Fisher score, aneurysm size, and delayed cerebral ischemia, in-hospital NCSz remained independently associated with poor outcome but SIRS did not (Table 5). These observations were confirmed on the imputed dataset (data not shown). Mediation analysis indicated that NCSz mediate the effect of SIRS, hsCRP, and TNF-R1 on poor outcome based on P-values (see Figure 4), but that the analysis may be underpowered based on the 95%-CI. Only for the subgroup of patients 57 years or younger and those with a Hunt Hess grade of 4 or 5 we were not able to support this hypothesis. Bootstrapping confirmed our results. Additionally to NCSz, DCI and any infections were mediators for SIRS on poor 3 month functional outcome (data not shown) but NCSz did not mediate the effect of infection or DCI on poor outcome (data not shown). All of the above mediation regressions were linear.

Discussion

We found that the pro-inflammatory state following SAH as reflected both clinically by SIRS criteria and serum biomarkers of inflammation (hsCRP, TNF-R1, and TTR) was independently associated with in-hospital NCSz. We further confirmed the association between post brain injury inflammation and poor outcome, and observed that this effect was likely in part mediated through in-hospital NCSz.

Inflammation after acute brain injury

Neuronal injury such as that from SAH triggers a pro-inflammatory cascade involving up-regulation of damage-associated molecular patterns (DAMPs) leading to microglial toll-like receptor (TLR) linked expression of pro-inflammatory cytokines (IL1 β , IL6, and TNFalpha).²⁵⁻²⁷ Biomarkers that best capture the inflammatory state are controversial as many are influenced by the metabolic state. Following SAH elevations of serum hsCRP¹³ and cytokines¹⁵, and brain interstitial TNFalpha¹² have been reported. The IL-6 and TNFalpha mediated decrease of TTR and the increase in hsCRP in SIRS are proportional to the severity of the illness²⁸ and the hsCRP/TTR ratio is influenced more by inflammation than metabolism.²⁹ TNF alpha elicits much of the damaging effects of the pro-inflammatory response involving TNF-R1 signaling.³⁰ In the present study we decided to study hsCRP, TTR, and TNF-R1 to characterize the inflammatory response following SAH as these are widely available and relatively stable in serum.³¹ TNF-R1 as the natural homeostatic regulator of TNF- α activity is thought to more accurately reflect the biological activity of TNFalpha than direct serum TNFalpha measurements.³² Pro-inflammatory states may lead to additional neuronal injury setting the stage for a vicious cycle^{33, 34} but may also play a role in recovery as they may promote removal of dead cells and tissue debris.^{25, 33}

Inflammation and seizures

NCSz are frequent after acute brain injury particularly in patients with brain hemorrhages and meningitis or encephalitis.^{1, 35, 36} Underlying mechanisms of these seizures are not clear and the post injury inflammatory response may be a link. Inflammation alters blood brain barrier permeability via TNF-R1 mediated TNF alpha signaling,³⁷ which has been linked to seizures in patients with temporal lobe epilepsy.^{9-11, 38, 39} It is not entirely clear yet how a damaged blood brain barrier results in a pro-seizure state, but extravasation of serum

albumin may contribute.^{38, 39} Interestingly, emerging experimental evidence implicates systemic inflammatory states in TNF α mediated increases of CNS excitability¹⁶⁻¹⁸ and septic patients without brain injury have a particularly high risk of NCSz.⁴⁰ Our data suggest that NCSz after SAH are associated with an inflammatory response as SIRS, TNF-R1, and hsCRP were positively and TTR anti-correlated with the risk of NCSz. Seizures in turn may also induce cerebral inflammation⁹ setting the stage for a viscous cycle between inflammation and NCSz. The relationship between seizures and inflammation is complex and likely bidirectional but the Cox regression and mediation analyses suggest that at least in part inflammation sets the stage for NCSz after acute brain injury.

Seizures and outcome

The present study confirms prior reports associating NCSz with secondary brain injury^{35, 41, 42} and poor outcome.^{1, 35, 36, 42, 43} Invasive brain monitoring shows elevations in intracranial pressure and metabolically challenged brain tissue (elevations in cerebral lactate-pyruvate ratio and glutamate) with NCSz^{43, 44} raising the concern for secondary brain injury from inadequately matched hypermetabolism.¹⁹ The potential for NCSz to cause secondary damage may depend on the pathophysiological state of the injured brain. Sample size considerations precluded extensive subgroup analyses in the present study but the association between inflammation and NCSz was more pronounced for patients with less background attenuation, a crude measure of the extent of underlying brain tissue damage.¹⁹

Inflammation and outcome

We confirmed that pro-inflammatory states captured by clinical syndromes such as SIRS⁴⁵ or inflammatory biomarkers such as interleukines or TNF α are associated with poor outcome after SAH.^{14, 15} In acute brain injury, it is not entirely clear if the inflammatory response is a surrogate marker for the extent of injury, directly damages brain cells, or impairs the recovery processes, or promotes secondary complications that frequently follow acute brain injury. Prior studies linked inflammation and delayed cerebral ischemia a common complication of SAH.^{12, 13} In patients with SE inflammatory markers are associated with difficulty to control seizures and poor outcome.¹⁶ The current study revealed that in-hospital seizures mediate the effect of inflammation on poor outcome but the established relationship between infection and delayed cerebral ischemia with poor outcome was not mediated through NCSz. Further, it appears from the presented data that the inflammation-seizures complex primarily impacts patients that are young and have less brain injury (Hunt Hess 1 to 3). Likely in poor grade and elderly patients other factors such as the primary effects of the bleed and in-hospital complications overwhelm the contribution from the seizure-inflammation complex.

Inflammation and intervention

Successful treatment of seizures with TNF α blockers in an epilepsy model and benefits from IL1 receptor antagonists (IL1-RA) in an SAH model illustrate the therapeutic potential of immune modulation.^{17, 46} Some early evidence shows that modulation of systemic inflammation may even modulate the seizure threshold.¹⁸ Intervening in the inflammatory response following acute brain injury needs to be done with caution,³³ realizing the complex and at times opposing role that it plays, both, promoting repair and inflicting further brain

injury.²⁵ Failed immunosuppressive trials in stroke⁴⁷ highlight the challenges and modulation of the immune response may hold more promise^{48, 49}.

Limitations

True causality cannot be established in observational studies but mediation analysis more closely investigates the potential causal structure of variables. Mediation analyses are simplified due to sample size limitations and findings need to be treated as preliminary data, illustrated by the 95%-CI results. However, we suspect that the results will hold up in a larger dataset as cox regression analyses and P-values confirmed the relationships. Functional outcome and biomarker data were only available in 72% and 29% of the cohort, respectively, raising concerns of generalizability, however, follow-up bias analyses revealed similar characteristics of these patients when compared to the overall cohort (Table 1 for biomarker). Further, logistic regression on complete datasets generated by multiple imputation using Bayesian methods confirmed our observations.

The link of seizures between inflammation and poor outcome cannot directly be translated to other disease processes, but prominent inflammatory reactions are known from other acute brain injuries and these deserve further study. This investigation did not comprehensively test different inflammatory pathways, which would allow identifying the most promising target for immunomodulation. Further, the study did not differentiate between the complex interplay between peripheral immunosuppression and central immune activation that has been well described after ischemic stroke.²⁵ More mechanistic insights linking peripheral and central inflammation with seizures and outcome after acute brain injury will be needed before exploring immunomodulation for the treatment of SAH patients. Therapeutic regimens of acutely brain injured patients potentially affect the prevalence of in-hospital seizures and the presented dataset does not allow differentiation between the impact of seizures and treatment of seizure. Seizure prevalence may be lower in the setting of seizure prophylaxis and avoidance of daily interruption of sedation accompanied by deeper sedation regimens, only the former of these being practiced in the presented study.

Conclusions

These findings suggests that post SAH inflammation and in-hospital seizures may be linked and that both independently as well as seizures as a mediator for inflammation affect outcome. While this does not translate into the conclusion that treating seizures or inflammation leads to better outcome the findings do suggest that inflammatory modulation may be considered as a potential treatment target that would address the underlying cause (i.e., inflammation) rather than the downstream effect (i.e., seizures).

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ABREVIATIONS

APACHE	Acute Physiology and Chronic Health Evaluation
EEG	electroencephalogram
hsCRP	high sensitivity C reactive protein
ICU	intensive care unit
IVH	intraventricular hemorrhage
NCSE	nonconvulsive status epilepticus
NCSz	nonconvulsive seizure
SAH	subarachnoid hemorrhage
SIRS	systemic inflammatory response syndrome
TNF-R1	tumor necrosis factor TNF receptor 1
TTR	transthyretin (also known as prealbumin)

Figure 1.
Daily mean SIRS score for SAH patients with and without in-hospital seizures.

Figure 2.

Measures of inflammation of those with (grey boxes) and without NCSz (white boxes) stratified by EEG background attenuation (none-mild vs moderate-severe background attenuation). Higher hsCRP measurements and hsCRP/TTR ratios are seen for those with moderate to severe EEG background attenuation and those with NCSz. Interestingly, there was no clear difference between patients with and without NCSz amongst those with moderate to severe EEG background attenuation. This observation was true at post SAH days 4 to 7 (phase 2) as well as 8 to 10 (phase 3). hsCRP, high sensitivity C reactive protein; SAH, subarachnoid hemorrhage; TTR, transthyretin (also known as prealbumin). * P<0.05; ** P<0.001

Figure 3.

TNF-R1 measurements for those with (grey boxes) and without NCSz (white boxes) during days 4 to 7 (left panel) and days 8 to 10 after SAH (right panel). TNF-R1, tumor necrosis factor TNF receptor 1 * $P < 0.05$; ** $P < 0.001$

Figure 4.

Mediation analyses indicating that SIRS, hsCRP and TNF-R1 significantly predict 3 months functional outcome independently and via NCSz. Moreover, we found that NCSz significantly predict outcome while controlling for SIRS, hsCRP and TNF-R1 (bolded in figure), suggesting that NCSz mediate the relationship between inflammatory markers and poor functional outcome. Data reported as b; P-values (95%-CI). The strongest effect is bolded.

Table 1

Baseline characteristics of SAH patients

	SAH patients (N=479)	SAH patients with serum markers (N=140)
Demographics		
Age [years]	56+/-15	54+/-14
Female	315 (67)	81 (72)
White	180 (38)	38 (27)*
Admission findings		
Hunt Hess score	3 (2, 4)	3 (2, 4)
APACHE 2 score	16+/-9	14+/-8
Modified Fisher Score	3 (1, 4)	3 (1, 4)
IVH	270 (60)	77 (59)
Global cerebral edema	166 (35)	40 (29)
Aneurysm clipping	243 (51)	96 (69)*
Hospital course		
Any in-hospital seizure	53 (11)	21 (15)
SIRS	2.1+/-0.8	2.2+/-0.7
Fever (>102.5°F)	115 (24)	37 (26)
Sepsis	37 (8)	14 (10)
Pneumonia	105 (22)	41 (29)
Urinary tract infections	101 (21)	30 (21)
Delayed cerebral ischemia	93 (20)	32 (23)
Functional outcome at 3 months		
Modified Rankin score	2 (1, 5)	2 (1, 3)

Data is reported as Mean +/- standard deviation, N (%), or median (IQR).

* indicates significant differences between those with and those without serum biomarker measurements P<0.05;

APACHE, Acute Physiology and Chronic Health Evaluation; IVH, intraventricular hemorrhage; SIRS, systemic inflammatory response syndrome

Table 2

In-hospital nonconvulsive seizures after SAH.

	In-hospital seizures (N=53)	No in-hospital seizures (N=426)	Univariate analysis		Multivariate analysis	
			OR	95%-CI	OR	95%-CI
Demographics						
Age [years]	61+/-16	55+/-15	1.0	1.0-1.1	1.0	1.0-1.1
Female	44 (83)	275 (65)	2.7	1.3-5.7	N.S.	N.S.
History of epilepsy	0 (0)	2 (1)	-	-	-	-
Admission findings						
Hunt Hess score 4-5	29 (55)	143 (34)	2.4	1.3-4.3	N.S.	N.S.
Modified Fisher Score 3-4	38 (73)	260 (61)	1.7	0.9-3.3	-	-
Aneurysm size > 10 mm	14 (28)	49 (14)	2.4	1.2-4.8	2.4	1.2-4.8
Hospital course						
Aneurysm clipping	38 (72)	208 (49)	2.7	1.4-5.0	2.3	1.1-4.6
Delayed cerebral ischemia	15 (28)	79 (19)	1.7	0.9-3.3	-	-
SIRS burden in first 4 days	2.5+/-0.6	2.1+/-0.8	1.8	1.3-2.7	1.9	1.3-2.9

Table 3

SIRS of 3 or above in relation to the onset of the first NCSz.

Hours in relation to NCSz onset	Hazard	95%-CI	P
120 hrs pre	2.0	0.9-4.5	0.079
96 hrs pre	2.3	1.1-5.0	0.038
72 hrs pre	2.6	1.2-5.7	0.013
48 hrs pre	2.3	1.1-4.6	0.020
24 hrs pre	2.6	1.3-5.1	0.005
NCSz onset	2.1	1.1-4.0	0.022
24 hrs post	3.9	2.0-7.6	0.001
48 hrs post	3.9	1.8-8.4	0.001
72 hrs post	2.9	1.3-6.4	0.007
96 hrs post	2.5	1.1-5.4	0.023
120 hrs post	2.6	1.1-6.0	0.023

hrs, hours

Table 4

Association of laboratory biomarkers of inflammation and metabolism in relation to NCSz.

	Percent affected	With NCSz	Without NCSz	OR (95%-CI)
Post SAH day 4-7				
hsCRP [mg/dL]	17% (18/109)	94 (38, 140)	34 (14, 72)	3.0 (1.4-4.6)*
TTR [mg/dL]	16% (17/107)	15 (10, 20)	19 (15, 24)	0.9 (0.8-1.0)
hsCRP/TTR ratio	16% (17/107)	6 (2, 18)	2 (1, 5)	1.1 (1.0-1.2)
TNF-R1 [pg/mL]	12% (9/74)	1556 (1335, 1891)	977 (661, 1436)	2.5 (2.1-2.9)**
Post SAH day 8-10				
hsCRP [mg/dL]	15% (15/101)	120 (75, 207)	41 (10, 86)	2.5 (1.3-3.7)*
TTR [mg/dL]	15% (15/101)	16 (13, 19)	20 (16, 25)	0.9 (0.8, 1.0)
hsCRP/TTR ratio	15% (15/101)	8 (4, 17)	2 (0, 4)	1.0 (1.0-1.0)
TNF-R1 [pg/mL]	11% (8/75)	2438 (1518, 2680)	1023 (730, 1414)	1.4 (1.0-1.8)**

hsCRP, high sensitivity C reactive protein; TTR, transthyretin (also known as prealbumin); TNF-R1, tumor necrosis factor TNF receptor 1. Data reported as: percent and N measurements with seizures of total; Median (IQR); OR (95%-CI); the OR indicated with

* refers to OR per 20 and

** per 100 point increase in biomarker;

those bolded were significant in multivariate analysis after controlling for predictors of in-hospital NCSz identified above excluding SIRS burden in first 4 days (see Table 2)

Table 5

Functional outcome and in-hospital seizures (N=345).

	Poor outcome (N=123)	Good outcome (N=222)	Univariate analysis		Multivariate Analysis	
			OR	95%-CI	OR	95%-CI
Demographics						
Age [years]	60+/-17	52+/-13	1.1	1.0-1.1	1.1	1.0-1.1
Female	89 (72)	142 (64)	1.5	0.9-2.4	-	-
Admission findings						
Hunt Hess score 4-5	94 (76)	32 (14)	19.3	11.0-33.7	17.0	8.3-34.8
Modified Fisher Score 3-4	86 (71)	121 (55)	2.0	1.2-3.2	N.S.	N.S.
Aneurysm size > 10 mm	26 (27)	20 (10)	3.3	1.7-6.3	2.5	1.1-6.0
Hospital course						
SIRS burden in first 4 days	2.5+/-0.7	2.0+/-0.8	2.2	1.6-3.0	N.S.	N.S.
In-hospital seizures	24 (20)	9 (4)	5.7	2.6-12.8	3.5	1.2-9.8
Aneurysm clipping	53 (43)	123 (55)	0.6	0.4-1.0	-	-
Delayed cerebral ischemia	32 (26)	32 (14)	2.1	1.2-3.6	2.7	1.1-6.0

Poor outcome was defined as moderate to severe disability or death (modified Rankin Score of 4-6) at 3 months after SAH.