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C-reactive protein in intracerebral hemorrhage

Time course, tissue localization, and prognosis

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ABSTRACT

Objectives: We examined the C-reactive protein (CRP) response after spontaneous intracerebral hemorrhage (sICH) and its relationship to outcome. We additionally characterized early brain localization of CRP.

Methods: In this prospective, multicenter, international, collaborative, longitudinal study with cross-sectional immunohistochemical analysis of brain tissue, 223 patients (M/F: 132/91) were recruited during the 2010 calendar year. CRP was evaluated at admission (median 93 minutes from symptom onset), 24 hours, 48 hours, and 72 hours after sICH. Brains of 5 subjects with sICH were compared to brains of 2 aged controls without evidence of brain pathology and 7 patients with ischemic stroke. Plasma CRP was measured over 72 hours following sICH and its relationship to 30-day mortality and functional outcome at 30 days (Glasgow Outcome Scale) was determined. CRP immunostaining patterns were analyzed in samples of sICH autopsy brains.

Results: Plasma CRP increased over the 48 hours from admission and was significantly ($p < 0.001$) related to hematoma volume at later time points. The predictive utility of CRP for morbidity and mortality were maintained when adjusted for other risk factors and improved at 48 hours and 72 hours when compared with admission values. Although an early CRP localization was present in both ischemic and hemorrhagic lesions, an intense and diffuse neuropil staining was only present in sICH patients and particularly evident proximal to the hemorrhagic areas.

Conclusions: Plasma CRP production increases markedly over the 48 hours to 72 hours period following sICH and is related to outcome. CRP is also present in large amounts around the hemorrhagic lesion and within neurons and glia of patients who died within 12 hours of sICH. *Neurology*® 2012;79:690-699

GLOSSARY

AUC = area under receiver operator characteristic curve; **CI** = confidence interval; **CRP** = C-reactive protein; **GCS** = Glasgow Coma Scale; **GOS** = Glasgow Outcome Scale; **HR** = hazard ratio; **IL-6** = interleukin-6; **IVH** = intraventricular extension; **LR** = likelihood ratio; **oICH** = Hemphill's original ICH score; **sICH** = spontaneous intracerebral hemorrhage.

Experimental and clinical studies indicate that inflammation is involved in the progression of brain injury after spontaneous intracerebral hemorrhage (sICH).^{1,2} These pathologic mechanisms include immunologic, endothelial dysfunction, and coagulopathy, contributing to morbidity and mortality.³

Increased C-reactive protein (CRP) has been shown previously to relate to poor outcome after sICH.⁴⁻⁶ However, direct pathophysiologic evidence that it participates in local inflammatory response is lacking.⁷ Potential limitations of previous studies of inflammatory markers in sICH include the possibility that the systemic acute-phase response may have been attributable to accompanying infection in some cases, and that the inflammatory and CRP response

Supplemental data at www.neurology.org

Supplemental Data

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could still be evolving. Additionally, changes in CRP levels beyond the initial 24 hours after sICH have not been analyzed in detail, yet these may be more reflective of the inflammatory response to sICH. Therefore, the primary aim of our study was to evaluate the kinetics of plasma CRP concentrations after acute sICH, up to 72 hours, and the relationship with clinical outcomes. To investigate a possible role of CRP in local inflammatory responses, we examined CRP localization by performing immunohistochemical studies on brain specimens obtained from patients who died within 12 hours after sICH.

METHODS Participants were included in a prospective, multicenter international observational collaborative project, the aims of which are to study the pathophysiology of the inflammatory response and to determine the prognostic value of inflammatory biomarkers after sICH. Details of the study methodology are given in the supplemental Subjects and Methods on the *Neurology*[®] Web site at www.neurology.org.

Briefly, patients admitted with a diagnosis of sICH within 24 hours of symptom onset had baseline clinical data recorded on an electronic form, including demographic data, medical history, risk factors, presence of comorbidities, physical examination findings, Glasgow Coma Scale (GCS)⁸ after resuscitation, Hemphill's original ICH (oICH) score,⁹ standard routine laboratory panels, and CT scan findings.

Of the participants recruited during 2010 ($n = 384$), only patients with serial blood samples within the first 72 hours and complete clinical and neuroradiologic variables were considered eligible for the current analysis (figure e-1). To avoid confounding effects, we excluded patients with a history of acute or chronic infections in the 4 weeks before sICH or those with clinical evidence of acute infection at admission, as well as those with other concurrent inflammatory comorbidities ($n = 62$). A further 99 patients were also excluded for other reasons (figure e-1). Following this screening procedure, 223 patients were included.

Surgical treatment was performed according to local protocols at participating institutions based on the guidelines of the Stroke Council of the American Heart Association¹⁰ and European Stroke Initiative.¹¹

CRP measurements. In all patients the first plasma CRP concentration (CRP_{Adm}) was determined immediately after admission. The second CRP measurement was done at 24 hours ($\text{CRP}_{24 \text{ hours}}$), the third at 48 hours ($\text{CRP}_{48 \text{ hours}}$), and the fourth at 72 hours ($\text{CRP}_{72 \text{ hours}}$) after symptom onset. CRP analysis was performed locally using high-sensitivity immunoturbidimetric assays with similar performance characteristics.¹²

Neuroradiologic analysis. The initial CT brain scan after admission was reviewed and classified according to site of sICH (basal ganglia, thalamic, lobar, pontine, cerebellar, or other), volume of hematoma measured using the ABC/2 method,¹³ midline shift (by measuring the displacement of the septum pellucidum from the midline),¹⁴ intraventricular extension (IVH), and

presence of hydrocephalus. Investigators who read CT scans were blinded to clinical information.

Neuropathology: processing of tissue specimens and immunohistochemistry. To analyze early CRP brain localization after sICH, formalin-fixed, paraffin-embedded archived brain tissue blocks containing both lesional and perilesional areas were selected from 5 sICH patients who died within 12 hours (mean 8; range 4–12 hours). Seven ischemic stroke patients who died within 12 hours (mean 7; range 4–12 hours) and 2 subjects without brain pathology were used as controls. All brain tissue samples were obtained from patients recruited in the Clinic of Neurology (University of Medicine and Pharmacy, Craiova, Romania).

Immunohistochemistry was performed using anti-CRP monoclonal antibody (mouse antihuman, Clone 1, IgG2b, Genetex, 1:100) recognizing human CRP.¹⁵ Negative controls showed no abnormal crossreactivity. Double immunofluorescence was performed for anti-CRP and anticollagen IV (rabbit antihuman, Novus Biologicals, 1:1,000).

Outcome measurements. The primary outcome was 30-day mortality. The secondary outcome was 30-day functional outcome assessed using Glasgow Outcome Scale (GOS)¹⁶ dichotomized into “poor” (GOS scores 2–3, or death) or “good” (GOS 4–5). For patients in whom 30-day outcome was not available from medical records ($n = 21$), follow-up data were obtained from visits using a standardized questionnaire. The cause of death was confirmed by available medical records and no patients were lost to follow-up. Information regarding inflammatory markers was not used to modify treatment during in-hospital and follow-up periods.

Standard protocol approvals, registrations, and patient consent. Informed consent was obtained from all participants or legal representatives and the protocol was approved by local Institutional Review Boards.

Statistical analyses. All values are given as mean and SD or median and interquartile range, according to manner of distribution. Differences between 2 groups were assessed with independent t tests, the Fisher exact test, or the Wilcoxon test, as appropriate. The differences between CRP_{Adm} , $\text{CRP}_{24 \text{ hours}}$, $\text{CRP}_{48 \text{ hours}}$, and $\text{CRP}_{72 \text{ hours}}$ were analyzed using a 1-way analysis of variance with Bonferroni correction if the overall test was significant. p Values were corrected for sphericity violation when appropriate.

For the calculation of Pearson correlation coefficients, we logarithmically transformed positively skewed CRP data to obtain a normal distribution. CRP increase was defined as 1 log_e unit/l increase between 2 measurements.

CRP concentrations were divided into tertiles to identify nonlinear effects of the CRP and provide more stable risk estimates. We used Kaplan-Meier survival curves to compare event-free survival between groups of patients defined by tertiles of CRP and compared curves with log-rank trend tests.

We used Cox regression analysis to calculate unadjusted hazard ratios (HR) and 95% confidence intervals (95% CI) per log_e unit increase in CRP levels. Logarithmic transformation of the CRP measurement was entered into the Cox regression analysis. To evaluate the impact of study variables and the different time-dependent CRP concentrations on 30-day mortality and poor outcome, we built 2 multivariate Cox regression models, adding variables sequentially that were associated in univariable analysis, keeping those variables that significantly improved the fit of the model (likelihood ratio [LR] test $p < 0.05$). When the model

was complete, we tested the proportional hazards assumption and its goodness of fit. We looked for first order interactions of CRP levels with other variables in the final model by adding multiplicative terms.

C-statistics (area under receiver operator characteristic curves [AUC]) were calculated to estimate predictive discriminatory ability by a nonparametric method for each CRP time point.¹⁷ The 95% CIs were constructed using DeLong variance estimate. Sensitivity and specificity to predict the primary and secondary endpoints were calculated at various cutoff points to identify the best Youden index (J) of diagnostic test for a comparison among the different CRP time points. The CRP time point performance was computed using the cutoff values that generated the best J. A calculated difference of $p < 0.05$ was considered significant.

RESULTS Patient characteristics. A total of 132 men and 91 women (M/F ratio: 1.45; mean age, 67.4 ± 11.8 years) were included in this study (figure e-1 and table 1). Plasma samples were available from all 223 patients on admission, but from only 126 at 72 hours. Forty-four (19.7%) patients underwent surgical hematoma evacuation: 22 (50%) of them at admission, a further 16 within 24 hours, and the others within the first 52 hours after sICH onset. At 30 days, 68 (30.5%) patients were dead. Deaths were attributable to the initial sICH or rebleeding in 55 cases (80.9%) and systemic complications in 13 cases (19.1%). After 30-day follow-up, 49% ($n = 109$) of the patients showed a good functional outcome (GOS 4 and 5).

CRP kinetics. The median time from symptom onset to the admission CRP measurement was 93 minutes (48–275 minutes). CRP concentration increased significantly ($p < 0.0001$, analysis of variance) from the median value of 7.9 mg/L (4–12 mg/L) at admission to 88.3 mg/L (22–216.4 mg/L) after 72 hours (figure e-2). Post hoc analysis revealed a significant difference between CRP_{Adm} and CRP_{24 hours}, CRP_{48 hours}, or CRP_{72 hours} ($p < 0.0001$, for all comparisons). CRP concentration increased from 24 to 48 hours ($p = 0.0016$) and from 24 to 72 hours ($p = 0.0004$), but not from 48 to 72 hours ($p = 0.424$). Relative to CRP_{Adm}, CRP_{24 hours} decreased in 12 patients (5.4%), increased in 190 (85.2%), and remained stable in 13 (5.8%). CRP peak was reached in 52 patients (23.3%) at 24 hours, 60 patients (26.9%) at 48 hours, and 46 patients (20.6%) at 72 hours.

Neuroradiologic findings. The median hematoma volume was 18 mL (8–37). The hematoma volume was larger in patients with an unfavorable outcome (44.5 mL [20.5–82.5] vs 12 mL [8–23]; $p < 0.0001$) and with a worse functional outcome (30 mL [15–60] vs 10 mL [6–18]; $p < 0.0001$). Increased CRP concentration significantly correlated with a larger initial hematoma volume only for the later measurements. The Pearson correlation coeffi-

cients for the relationship between hematoma volume and the natural logarithm of CRP at the different time points were CRP_{Adm}, $r = 0.1$ ($p = 0.1416$); CRP_{24 hours}, $r = 0.43$ ($p < 0.0001$); CRP_{48 hours}, $r = 0.51$ ($p < 0.0001$); CRP_{72 hours}, $r = 0.45$ ($p < 0.0001$).

Neuropathologic findings. General neuropathologic evaluation in sICH patients confirmed petechial or large hemorrhages surrounded by softened cerebral discolored, edematous brain tissue, ventricular blood inundation, and asymmetric enlargement. All cases presented fresh hemorrhages, without any gliotic surrounding reactions. Ischemic lesions were characterized by cavitation and surrounding gliosis.

CRP was detected to different extents and patterns in sICH and ischemic stroke patients (table e-1). Thus, for the immediate perihemorrhagic areas, a diffuse neuropil staining was present, together with almost all cells' silhouettes taking up the stain (figure 1A). Further away from the hemorrhagic core, the diffuse staining pattern diminished, but with some neurons clearly retaining an affinity for the antibody. Although distant from the hemorrhage, white matter fiber tracts showed relatively intense staining for CRP, especially those surrounding intracallosal vessels (figure 1B). Focal intravascular staining sometimes was observed (figure 1C), and only on occasion were microglia-like cells immunoreactive for CRP (figure 1D).

In the region immediately surrounding an ischemic liquefaction area, a high number of gemistocytic astrocytes was noted (figure 1E), while further away from the lesion core numerous neuronal silhouettes were observed (figure 1, F–H). On occasion, diffuse staining could be noted along the white matter tracts and blood vessels. Vascular walls and sometimes plasma inside the vessel were again stained, and this observation seemed to be constant for the respective area of the lobe.

In the control brain, and in the hemispheres contralateral to the lesion, only occasional neuronal silhouettes were stained, with their respective densities being clearly lower compared to the lesioned (ipsilateral) hemispheres (figure 1, I and J). By immunofluorescence, CRP could be clearly localized inside blood vessels (figure 1K), and in the cytoplasm of activated astrocytes (figure 1L) and neurons (figure 1M).

Associations between CRP at different time points with death and poor outcome. Survival of patients according to CRP_{Adm}, CRP_{24 hours}, CRP_{48 hours}, and CRP_{72 hours} tertiles is shown in figure 2. The relationship between CRP and mortality was determined to be nonlinear by the use of tertile analysis. The

Table 1 Baseline characteristics of sICH patients

Characteristics	Total (n = 223)	30-day mortality (n = 66)	HR	95% CI	p Value	30-day poor outcome (n = 115)	HR	95% CI	p Value
Clinical									
Age, y, mean (SD) ^a	67.4 (11.8)	68.9 (12.5)	1.01	0.99-1.04	0.1572	69.0 (12.5)	1.02	1.01-1.03	0.0457
Male sex, n (%)	132 (59.2)	41 (60.3)	1.02	0.61-1.66	0.9304	72 (62.6)	1.14	0.79-1.66	0.4414
GCS score, median (IQR) ^a	13 (9-15)	7 (4-10)	0.71	0.67-0.76	<0.0001	10 (6-13)	0.75	0.71-0.79	<0.0001
sICH score, median (IQR) ^a	1 (0-3)	3 (2-4)	3.19	2.55-3.99	<0.0001	3 (2-4)	2.44	2.09-2.85	<0.0001
Vascular risk factors, n (%)									
Arterial hypertension	183 (82.1)	54 (79.4)	1.22	0.68-2.20	0.5042	94 (81.7)	1.07	0.67-1.73	0.7646
Diabetes mellitus	61 (27.4)	25 (36.8)	1.66	1.02-2.72	0.0434	35 (30.4)	1.32	0.89-1.97	0.1642
Alcohol abuse	58 (27.1)	23 (29.6)	1.51	0.91-2.50	0.1078	34 (29.6)	1.32	0.88-1.97	0.1787
Cigarette smoking	65 (29.2)	18 (26.5)	0.85	0.49-1.45	0.5406	36 (31.3)	1.06	0.72-1.57	0.7702
Hypercholesterolemia	62 (27.8)	21 (30.9)	1.18	0.70-1.97	0.5335	31 (27.0)	0.99	0.66-1.50	0.9770
Use of antiplatelet/oral anticoagulant therapy	37 (16.6)	14 (20.6)	1.41	0.78-2.54	0.2498	19 (16.5)	1.10	0.67-1.80	0.7014
Biochemistry and vital signs on hospital arrival									
CRP _{Adm} , mg/L, median (IQR) ^a	7.9 (4-12)	10.0 (5.9-19.1)				8.7 (4.7-12.0)			
CRP _{24 hours} , mg/L, median (IQR) ^a	46 (12-99)	103.1 (50.0-384.0)				96.0 (39.7-195.0)			
CRP _{48 hours} , mg/L, median (IQR) ^a	70 (18-192)	331.0 (189.0-726.0)				192.0 (72.0-384.0)			
CRP _{72 hours} , mg/L, median (IQR) ^a	88.3 (22-216.4)	384.0 (150-768.0)				152.5 (52.4-409.0)			
BG, mmol/L, median (IQR) ^a	7.6 (6.1-10.6)	10.6 (8.2-13.3)	1.13	1.09-1.18	<0.0001	8.6 (6.8-12.1)	1.04	1.02-1.06	<0.0001
WBC, $\times 10^9/L$, median (IQR) ^a	8.1 (6.5-9.9)	9.9 (8.7-15.6)	1.19	1.14-1.24	<0.0001	9.0 (7.3-13.0)	1.17	1.12-1.21	<0.0001
SBP, mm Hg (IQR) ^a	170 (150-200)	170 (130-200)	0.99	0.98-1.00	0.0778	170 (140-200)	1.00	0.99-1.01	0.0807
DBP, mm Hg (IQR) ^a	100 (80-110)	100 (80-110)	0.99	0.98-1.01	0.2565	100 (80-110)	0.99	0.98-1.01	0.3342
PP, mm Hg (IQR) ^a	80 (60-90)	70 (50-90)	0.99	0.98-1.00	0.0997	70 (50-90)	0.99	0.98-1.00	0.0683
Radiologic variables									
sICH localization, n (%) ^{b,c}			0.79	0.38-1.66	0.5408		0.56	0.29-1.07	0.0786
Basal ganglia	74 (33.2)	25 (36.7)				46 (40.0)			
Thalamic	61 (27.4)	18 (26.5)				34 (29.6)			
Lobar	57 (25.6)	17 (25.0)				25 (21.7)			
Pontine	11 (4.9)	4 (5.9)				4 (3.5)			
Cerebellar	19 (8.5)	3 (4.4)				5 (4.3)			
Other	1 (0.4)	1 (1.5)				1 (0.9)			

—Continued

Table 1 Continued

Characteristics	Total (n = 223)	30-day mortality (n = 68)	HR	95% CI	p Value	30-day poor outcome (n = 115)	HR	95% CI	p Value
IVH, n (%)	107 (47.9)	57 (83.8)	7.12	3.79-13.60	<0.0001	81 (70.4)	3.92	2.62-5.86	<0.0001
Hematoma volume, cc, median (IQR) ^a	18 (8-37)	45 (21-83)	1.02	1.01-1.03	<0.0001	30 (15-60)	1.02	1.01-1.03	<0.0001
Midline shift, mm ^d			3.88	2.18-6.90	<0.0001		3.33	2.00-5.54	
0-5	179 (80.3)	30 (44.1)				73 (63.5)			
6-9	20 (9.1)	15 (22.1)				18 (15.6)			
10-14	18 (7.6)	17 (25.0)				18 (15.6)			
>15	6 (3.3)	6 (8.8)				6 (5.3)			
Hydrocephalus, n (%)	69 (30.9)	23 (33.8)	6.30	3.80-10.44	<0.0001	57 (49.6)	4.05	2.80-5.86	<0.0001
Therapy									
Surgery, n (%)	44 (19.7)	24 (35.3)	0.42	0.25-0.69	0.0007	34 (29.6)	0.46	0.31-0.69	0.0002

Abbreviations: BG = blood glucose; CI = confidence interval; CRP = C-reactive protein; DBP = diastolic blood pressure; GCS = Glasgow Coma Scale; HR = hazard ratio; IQR = interquartile range; oICH = Hemphill's original ICH score; PP = pulse pressure; SBP = systolic blood pressure; sICH = spontaneous intracerebral hemorrhage; WBC = white blood cells.

^a Per unit increase.

^b One missing value.

^c Supratentorial vs infratentorial origin.

^d Midline shift ≤10 mm vs >10 mm.

estimated risk ratios for 30-day mortality and 30-day poor outcome rose sharply between second and third tertile of CRP (table 2).

Table 2 shows the association between CRP with mortality and poor outcome at different time points. In univariate analyses, CRP concentration at any time was associated with 30-day mortality and poor outcome. After adjusting the final model for confounders (demographic data [age and sex], risk factors [arterial hypertension, diabetes mellitus, alcohol abuse, hypercholesterolemia], markers of sICH severity [oICH score, GCS], neuroradiologic findings [ICH volume, IVH, hydrocephalus, midline shift], acute phase biomarkers [blood glucose and leukocytes at admission], therapy), there was still a significant association between mortality or poor outcome and increasing levels of CRP, although attenuated (table 2). Higher CRP concentrations were more strongly associated with death than with poor outcome (table 2).

In this cohort, patients in the highest tertile of CRP_{72 hours} had a 4.74-fold increase in mortality risk compared with those with the lowest tertile, as well as an increased risk of poor outcome (HR 1.74). We added CRP concentration at different time points as continuous variables (in order of the strength of their association with mortality and poor outcome) to the model including demographic data, risk factors, markers of sICH severity, neuroradiologic findings, acute phase biomarkers, and therapy. This significantly improved the original model (LR test $\chi^2 = 160.43$): CRP_{Adm}, LR test $\chi^2 = 164.98$; CRP_{24 hours}, LR test $\chi^2 = 162.77$; CRP_{48 hours}, LR test $\chi^2 = 176.29$; and CRP_{72 hours}, LR test $\chi^2 = 172.93$. All final models, including CRP at different time points, fulfilled the proportional hazards assumption and fitted the data well.

Prediction of primary and secondary endpoints. AUCs of CRP concentration and the related measures of performance at different time points for 30-day mortality and 30-day poor outcome are given in table e-2. CRP_{24 hours} (AUC 0.818), CRP_{48 hours} (AUC 0.894), and CRP_{72 hours} (AUC 0.910) showed a better prediction of 30-day mortality when compared with CRP_{Adm} (AUC 0.601; all $p < 0.0001$; DeLong variance estimate). Similarly, later CRP measurements showed a better prediction of 30-day functional outcome, although were less reliable in poor outcome prediction (table e-2). Different cutoff values of CRP concentrations at different time points were tested to generate the highest J value for the diagnostic test; the best results were obtained at different cutoff values for the different time points. For both mortality and poor outcome, the best prediction was obtained with a CRP_{72 hours} concentration of 94.5 mg/L. The J value indicates that the

Figure 1 C-reactive protein (CRP) expression pattern following spontaneous intracerebral hemorrhage or ischemic stroke and in normal aged brain

In the perihemorrhagic areas, a diffuse neuropil staining was present, with almost all cells stained (A). White matter fiber tracts showed relatively intense staining for CRP, especially those surrounding intracallosal vessels (B). Focal intravascular staining was sometimes observed (C), and only on occasion were microglia-like cells immunoreactive for CRP (D). Numerous gemistocytic astrocytes (E) along numerous neuronal silhouettes (F–H) were detected in the vicinity of the ischemic lesion. In the control brain, only occasional neuronal silhouettes were stained, but at a density lower than the lesioned hemispheres (I, J). By immunofluorescence, CRP could be localized inside blood vessels (K), and in the cytoplasm of activated astrocytes (L, arrows) and neurons (M, arrows). Scale bars, 20 μ m.

CRP_{72 hours} concentration was a reliable predictor for mortality but less reliable for predicting poor outcome.

DISCUSSION There are 3 major findings in the current study. First, sICH results in a rapid increase in plasma concentration of CRP, that evolves from within a few hours of symptom onset, and the magnitude of the response is related to hematoma volume. Secondly, higher levels of CRP are associated with a higher mortality and poor functional outcome at 30 days. This association is stronger for later CRP measurements than for CRP obtained at admission and is stronger for mortality than for poor functional outcome prediction. Finally, there is very early CRP localization in the brain tissue immediately surrounding the hematoma, suggesting the potential for an active role in the extent of tissue damage after sICH. At the cellular level, CRP seemed to be expressed in the cytoplasm of both neurons and glial cells.

Experimental data demonstrate that an acute inflammatory response to the hematoma can occur within 1 hour.² Although a minority of patients ($\approx 6\%$) showed an absent or minor inflammatory re-

sponse after sICH, and a similar percentage of patients showed a reduction of CRP concentration within the first 24 hours (data not shown), the peak CRP concentration occurred mainly within 48 hours. It is plausible that this reflects an evolving response induced by those with greater tissue injury, and this may provide an explanation for the stronger relationship between hematoma size and the later CRP response.

sICH precipitates a complex cascade of both cerebral and systemic events.¹ Not only does a local inflammatory response propagate from blood breakdown, but a systemic state of inflammation is also triggered.¹ The acutely injured brain releases a great amount of interleukin-6 (IL-6), the extent of which correlates with severity of brain injury and prognosis.^{18,19} Furthermore, IL-6 is a major stimulus for hepatic production of acute-phase proteins,²⁰ which is reflected by the elevated levels of CRP found in the systemic circulation of our sICH patients. The CRP immunostaining in brain tissue from patients who died acutely following sICH suggests another potential CRP interaction that may be important during the early phase. It would appear

Figure 2 Survival curves for C-reactive protein (CRP) tertiles at time points within 72 hours of symptom onset

Kaplan-Meier analysis for risk of 30-day mortality depending on CRP tertile levels at admission (A), within 24 (B), 48 (C), and 72 hours (D) after symptom onset. CRP_{Adm} tertiles: bottom third, <4 mg/L, middle third, 4–12 mg/L, top third, >12 mg/L; CRP_{24 hours} tertiles: bottom third, <12 mg/L, middle third, 12–99 mg/L, top third, >99 mg/L; CRP_{48 hours} tertiles: bottom third, <18 mg/L, middle third, 18–192 mg/L, top third, >192 mg/L; CRP_{72 hours} tertiles: bottom third, <22 mg/L, middle third, 22–216.4 mg/L, top third, >216 mg/L.

that CRP is deposited or synthesized in situ within hours, while CRP plasma concentrations usually start to rise after an inflammatory stimulus within 8 to 10 hours.²⁰ Similarly, in acute ischemic stroke the CRP response appears to be triggered quite rapidly.²¹ The presence of brain tissue CRP in early sICH could suggest rapid local synthesis stimulated by the influence of the hematoma or a conversion of the circulating, soluble, pentameric form of CRP to its insoluble, monomeric form.²²

The overall pattern of anti-CRP immunostaining in sICH was dominated by a diffuse staining throughout the neuropil and the cell bodies immediately surrounding the hematoma. With increasing distance, the diffuse neuropil staining diminished in intensity, but the expression remained elevated in some neurons, glial cells, along white matter tracts, and inside some blood vessels. Therefore we hypoth-

esize that there are 2 sources of CRP in the brain after sICH: a constitutive CRP expression in neurons and astrocytes near the hematoma or damaged tissue and a diffusable CRP from plasma, which would seem likely to be largely independent of synthesis induced following sICH.^{19,22} While native CRP in the plasma is produced predominantly by hepatocytes, extrahepatic CRP production has been reported in neurons, atherosclerotic plaques, monocytes, lymphocytes, and adipocytes.²³ CRP is also expressed in the CNS associated with amyloid plaques and neurofibrillary tangles in affected brain regions in AD,^{24,25} and associated with angiogenic microvessels in peri-infarcted regions of patients with acute ischemic stroke.²² At the cellular level, CRP seemed to be expressed in the cytoplasm of both neurons and glial cells. Some intensely positive reactive astrocytes, including those around the ischemic liquefaction cores, showed de-

Table 2 The association between C-reactive protein level and 30-day mortality or poor outcome assuming a linear association between marker level and log hazards

	Hazard ratio per unit increase (95% CI)						Hazard ratio comparing 75th to 25th centile					
	30-day mortality (n = 68), unadjusted			30-day poor outcome (n = 115), unadjusted			30-day mortality (n = 68), unadjusted			30-day poor outcome (n = 115), unadjusted		
	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
CRP _{Adm} , mg/L, median (IQR)	1.29	1.02-1.64	0.0330	1.16	0.96-1.40	0.1270	1.66	1.12-2.45	0.0117	1.40	1.06-1.87	0.0199
CRP _{24 hours} , mg/L, median (IQR)	1.98	1.67-2.35	<0.0001	1.72	1.50-1.97	<0.0001	4.44	2.62-7.53	<0.0001	2.41	1.84-3.16	<0.0001
CRP _{48 hours} , mg/L, median (IQR)	2.21	1.85-2.65	<0.0001	1.28	1.11-1.47	<0.0001	12.86	4.99-33.09	<0.0001	3.35	2.43-4.62	<0.0001
CRP _{72 hours} , mg/L, median (IQR)	2.21	1.86-2.63	<0.0001	1.76	1.57-1.98	<0.0001	12.01	4.77-30.29	<0.0001	3.20	1.36-4.34	<0.0001
	Adjusted ^a			Adjusted			Adjusted			Adjusted		
CRP _{Adm} , mg/L, median (IQR)	1.36	1.03-1.80	0.0328	1.13	0.92-1.39	0.2440	1.19	0.98-2.25	0.0596	1.30	0.96-1.76	0.0941
CRP _{24 hours} , mg/L, median (IQR)	1.24	0.94-1.63	0.1275	1.19	0.99-1.43	0.0586	1.53	0.85-2.76	0.1533	1.33	0.96-1.82	0.0823
CRP _{48 hours} , mg/L, median (IQR)	1.75	1.32-2.33	<0.0001	1.36	1.15-1.61	0.0003	4.28	1.54-11.94	0.0055	1.79	1.22-2.62	0.0028
CRP _{72 hours} , mg/L, median (IQR)	1.59	1.22-2.07	0.0005	1.33	1.13-1.57	0.0007	4.74	1.73-13.07	0.0026	1.74	1.21-2.51	0.0031

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; IQR = interquartile range.

^a Final model adjusted for demographic data (age and sex), risk factors (arterial hypertension, diabetes mellitus, alcohol abuse, hypercholesterolemia), markers of spontaneous intracerebral hemorrhage severity (Hemphill's original ICH score, Glasgow Coma Scale score), neuroradiologic findings (ICH volume, intraventricular extension, hydrocephalus, midline shift), acute phase biomarkers (blood glucose and white blood cells at admission), and therapy.

tailed staining of their processes. Although serum trapped inside blood vessels was sometimes highly reactive, only rarely did endothelial cells themselves express this pentraxin. Our staining results would therefore support the possibility of local production of CRP. As recently demonstrated, damaged cell membranes that are abundant in local inflammation loci can dissociate pentameric CRP into monomeric CRP,²⁶⁻²⁸ and monomeric CRP is highly active in activating complement.²⁹ If this cellular staining represents local production of monomeric CRP, this could potentially amplify inflammation via its strong proinflammatory effects.²⁵ Hence, future immunohistologic studies, using highly selective antibodies against pentameric CRP and monomeric CRP, would provide additional insight, while in situ hybridization could help to confirm the proposed local source of CRP.

From a clinical perspective, it is interesting to consider the different predictive impacts of the serial CRP measurements during the acute stages of sICH. We found that the initial CRP_{Adm} value within 5 hours of symptom onset showed a weak association with mortality and did not predict functional outcome after 30 days of follow-up. The present study indicates that CRP_{24hours} is a better predictor of mortality and unfavorable outcome than CRP measured in plasma at admission, and that this is improved further with determinations at 48 or 72 hours after sICH onset. On the basis of our results, we propose that measurement of CRP levels between 48 and 72 hours after sICH onset may provide a useful

marker to estimate individual inflammatory responses and outcomes.

There are several methodologic strengths of our study. First, consecutive sICH patients with few exclusion criteria and with early baseline sampling and serial measurements of CRP were included. Second, attempts were made to avoid the potential confounding effects of preceding infection or previous inflammatory conditions on interpretation of CRP concentrations. Third, we used several overlapping methods to determine survival status and functional outcome at the end of the follow-up period for the whole cohort, and regular monitoring of data quality, either directly or by review of the medical and imaging records.

However, we also acknowledge that our study has limitations. The association between CRP concentration at the later timepoints and outcome excludes those patients not surviving beyond the initial 24-48 hours, limiting the generalizability of our findings. This, and the fact that we excluded patients with preceding or definite infection at baseline, has implications for the clinical utility of CRP measurements in predicting outcome in clinical practice, where early mortality (within first 48 hours) after sICH is significant, and infection not uncommon. To determine generalizability to other related settings requires external validation using a dataset of sufficient size. We measured plasma CRP as a representative inflammatory marker in this study. Measurement of multiple inflammatory biomarkers, such as uric acid, D-dimer, matrix metalloproteinases,

platelets, fibrinogen, and IL-6, may provide a more detailed measure of the inflammatory response after sICH. Similarly, inspecting the colocalization of CRP with complement in brain staining would also furnish further data in support of a direct pathophysiologic role of CRP in sICH and these studies are in progress. Finally, because of the few available brain autopsies and the few related blood samples, we were unable to correlate anatomopathologic CRP data with clinical data in a quantitative way.

We have demonstrated an association between higher levels of CRP mortality and poor outcome in patients after sICH together with a very early CRP localization in the brain tissue, immediately surrounding the hematoma. The CRP response evolves over the initial 72 hours and the association with mortality or poor outcome is stronger using later CRP measurements.

AUTHOR CONTRIBUTIONS

Agreed with the manuscript's results and conclusions: M.D.N., D.A.G., V.C., L. Masotti, C.J.S., A.R.P.-J., S.J.H., M.S., F.P., L. Mogoanta, D.P., A.P.-W. Designed the experiments/study: M.D.N., D.A.G., A.P.-W. Analyzed the data: M.D.N., D.A.G., M.S., F.P., L. Mogoanta, D.P., A.P.-W. Collected data/did experiments for the study: M.D.N., D.A.G., V.C., M.S., L. Masotti, F.P., L. Mogoanta, D.P., A.P.-W. Enrolled patients: M.D.N., D.A.G., V.C., L. Masotti, F.P., L. Mogoanta, D.P., A.P.-W. Wrote the first draft of the paper: M.D.N. Contributed to the writing of the paper: M.D.N., D.A.G., V.C., L. Masotti, C.J.S., A.R.P.-J., S.J.H., M.S., F.P., L. Mogoanta, D.P., A.P.-W. Provided statistical advice: M.D.N. Contributed to the design of the analyses and the interpretation of the data: M.D.N., C.J.S., A.R.P.-J., S.J.H., M.S., A.P.-W. Contributed to patient characterization: M.D.N., D.A.G., V.C., L. Masotti, M.S., F.P., L. Mogoanta, D.P., A.P.-W. Principal investigator for this study: M.D.N.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. **Go to [Neurology.org](#) for full disclosures.**

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AAN Publishes Guideline Update on Infantile Spasms

The AAN has published evidence-based recommendations for the treatment of infantile spasms that update a 2004 guideline. “Evidence-based Guideline Update: Medical Treatment of Infantile Spasms,” published in the June 12, 2012, issue of *Neurology*®, suggests that the therapy adrenocorticotrophic hormone, also known as ACTH, and the antiepileptic drug vigabatrin (VGB) may be effective in the treatment of infantile spasms in children.

To read the guideline and access PDF summaries for clinicians and patients, a slide presentation, and a clinical example, visit www.aan.com/go/practice/guidelines. For more information, contact Julie Cox at jcox@aan.com or (612) 928-6069.

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C-reactive protein in intracerebral hemorrhage : Time course, tissue localization, and prognosis

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