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Assessment of systemic cellular inflammatory response after spontaneous intracerebral hemorrhage

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ABSTRACT

Objective: After spontaneous intracerebral hemorrhage (ICH) a local and systemic inflammatory response is activated. Interleukin-6 (IL) is one of most relevant orchestrators of inflammatory responses in the brain and is released from multiple immune cells, including neutrophils. Herby we assessed the relevance of systemic inflammation in patients suffering ICH.
Methods: From October 2010 to October 2011 we included in our routine of laboratory investigations besides to C-reactive protein (CRP), the addition of IL-6 and an analysis of the subpopulation of circulating blood cells. Values at admission, at 3rd and 7th day after admission were evaluated. We analyzed 43 patients with non-traumatic ICH; stroke-related ICH or tumor associated hemorrhage were excluded. Outcome variables were 30 and 90-day mortality and NIHSS at discharge. A natural logarithmic transformation of IL-6, lymphocytes, and monocytes was used.
Results: 8.6% died within 30-days and mortality increased to 39.5% at 90th day. Total leukocytes and neutrophils as well as IL-6 at admission were statistically significant increased among patients who died within 30 days after ICH onset ($p = 0.002$). IL-6 and CRP in follow-up (3rd and 7th day) were higher among patients with poor outcome (NIHSS >15). The number of circulating lymphocytes and monocytes was not different in measurement. Leukocytes and neutrophils at 3rd day after admission were augmented in patients with respiratory infection and CRP in follow-up increased if some kind of infection was clinically or microbiologically detected. IL-6 at admission and in follow-up and monocytes at 7th day were related to ICH volume. CRP-values at 3rd or 7th day but not at admission were associated to bigger ICH-volume. The values of IL-6 were highly correlated to 30-day mortality and volume of ICH as CRP only with ICH volume.
Conclusion: After ICH onset a systemic activation of immune system seems to be induced and may be influencing outcome. Peripheral recruitment of leukocytes, especially neutrophils could be a target for future therapeutic interventions. Because of the tighter correlation of IL-6 at admission, it might be more accurate for prognostic issues than CRP.

1. Introduction

Spontaneous intracerebral hemorrhage (ICH) represents a major devastating event despite maximal therapeutic efforts. It accounts for 15% of all strokes with mortality rates reaching as high as 30% [1]. Several potential treatment options failed to reduce mortality or to improve the outcome in patients with ICH. Trials on drug treatment as well as on surgical approaches have failed to demonstrate a benefit towards any of those options [2,3].

The mechanical damage generated by hematoma formation and expansion with the consecutive loss of brain substance clearly represents the main primary cause of injury [4]. A second mechanism of injury mediated by inflammatory cells and inflammatory-induced cascades may play an additional role affecting disease progression and therefore its outcome [5]. Neutrophils have been found to be the first leukocyte subtype to infiltrate the hemorrhage [6,7]. The main functions of neutrophils are to locate, attack, and destroy potential threats. In this way neutrophils infiltrate into the damage site and by their activation a more specific response is started [8]. The burden of neutrophils is not only detrimental for threats like microbes, but also harmful to host cells [8,9]. On the other hand, interleukin-6 (IL) is one of the most relevant

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cytokines of inflammatory responses in the brain, together with TNF-alpha and IL-1beta [10]. Interleukin-6 is released from leukocytes, including neutrophils and it modulates a vast array of genes and molecules. Both neurotoxic and neuroprotective effects of IL-6 have been evocated [10–12]. In this way, regulation of leukocytic recruitment and activation as well as cytokine regulation could represent a target for therapeutic approaches. In our study we aim to investigate the effect of systemic inflammation on the outcome of patients with ICH by assessing the level of circulating white blood cells types (neutrophils, lymphocytes, and monocytes) as well as the plasma levels of IL-6 after ICH.

2. Materials and methods

2.1. Origen of the data

The prospective series contains patients admitted in a German university hospital from December 2010 to October 2011; these patients were treated in the specialized neurosurgical intensive care unit (ICU) of the department of Neurosurgery, Otto von Guericke University Magdeburg, Germany. The local ethics committee approved the study.

2.2. Design and patient data

We excluded patients with history of cerebral tumor or with hemorrhage after ischemic stroke (fibrinolysis). The patient demographics included the presence of hypertension, diabetes mellitus, prior stroke or ICH, use of anticoagulant or anti-platelet drugs or statins. Clinical evaluation at admission included NIHSS (National Institutes Stroke Scale), Glasgow coma score, systolic and diastolic arterial blood pressure. NIHSS was classified in two categories for analysis: NIHSS 0–15 and >15 [26]. Topography of the bleeding, ventricular extension, hydrocephalus, and potential sources of bleeding were recorded as radiological findings. The volumetric measure (ABC2 method) of the bleeding was calculated; the highest measurement within 24 h was used for analysis. The type of antihypertensive drug administered during the first 24 h after ICH, use of mannitol or glycerol and kind of surgical management (decompression, evacuation, external ventricular drainage [EVD]) were compiled. Incidence of infections was registered too.

2.3. Objectives

Our main objective was to evaluate the impact of the systemic inflammatory response on the outcome of patients suffering an ICH. Thus we assessed the levels of IL-6 and the leukocytes subpopulations at admission, after 72 h, and 7 days after ICH-onset in comparison to mortality and functionality. The venous blood samples were acquired at admission, at day 3 and 7 (05:00 h am). Plasma levels of IL-6 were determined by the method of electrochemiluminescence and CRP levels by latex agglutination. Leukocytes counts were performed by flow cytometry. All measurements were performed by the Institute of clinical chemistry and pathobiochemistry, Otto von Guericke University Magdeburg, Germany.

2.4. Statistical analysis

We used the software SPSS v. 21 for Windows. The missing data of patients who died prior 7th day were imputed means a last observation carried forward method. After analysis of the data distribution with Shapiro-Wilk test, a logarithmic-data transformation for volume and IL-6 with the function *In* (y) was used and for monocytes and lymphocytes counts the expression *In* ($y+1$) was applied. Then we compared continuous variables with t-Student test or one-way ANOVA as needed. Ranges of reference for maximum sensitivity and specificity of the significant variables were obtained by constructing receiver operating characteristic (ROC) curves. Cross tabs in different estimated ranges were generated and odds ratios (OR) were calculated for the estimated ranges predicting mortality at 30th – and 90th – day after ICH onset as well as for NIHSS score at discharge. Kaplan-Meier curves were constructed in order to assess the difference of survival considering our generated ranges of references. A Log Rank (Mantel-Cox) test was therefore applied. Sub-analysis regarding the presence of infection, use of statin, performance of surgical therapy, and volume were performed. Finally Pearson correlations were achieved as exploratory analysis of the association between clinical (NIHSS at admission and discharge), radiological (volume) and laboratorial findings (cell counts). P values lower than 0.05 were assumed to be statistically significant.

Table 1
Comparison of baseline characteristics regarding 30-day mortality. *p < 0.05. † ln(y). ‡ ln(y + 1) with numbers on table provided after re-transformation. [a]Mean with standard deviation. [b]Median with min. and max. values. CRP = C-reactive protein. ICH = intracerebral hemorrhage. IL = interleukin.

	30 days mortality			
	Died (n = 8)		Survivor (n = 35)	
Demography				
Age (years) [a]	66.7 ± 10.4		72.09 ± 10.57	
Sex: male	5	62.5%	16	45.7%
Prior ischemic Stroke	3	37.5%	4	11.4%
Hypertension	4	50%	28	80.0%
Myocardial infarction	2	25%	3	8.6%
Diabetes mellitus	3	37.5%	10	28.6%
Prior ICH	0	–	3	8.6%
Anti-platelet drugs	3	37.5%	14	40.0%
Anticoagulation	3	37.5%	11	31.4%
Statin medication	2	25%	7	20.0%
Clinical findings				
Initial GCS <9	7	87.5%	15	42.9%
Bilaterally fixed pupils	2	25%	3	8.6%
NIHSS basal (median)				
≤ 15	0	–	18*	51.4%
> 15	8	100%	17*	48.6%
Arterial Blood pressure				
Systolic (mmHg) [a]	174 ± 36.1		167.8 ± 32.4	
Diastolic (mmHg) [a]	90.2 ± 22.9		84.5 ± 19.01	
Laboratories				
Glucose (mmol/L) [a]	10.74 ± 2.68		9.66 ± 2.9	
Platelet count (Gptl/L) [a]	284.2 ± 82.7		226.5 ± 74.7	
Leukocytes (Gptl/L) [a]	13.0 ± 4.04		10.53 ± 3.48	
Neutrophils (Gptl/L) [a]	10.83 ± 3.7		8.66 ± 3.32	
Lymphocytes (Gptl/L) ‡	0.99 ± 0.30		1.11 ± 0.33	
Monocytes (Gptl/L) ‡	0.42 ± 0.25		0.50 ± 0.18	
IL-6 (pg/l) †	137.19 ± 10.62		23.07 ± 2.83*	
CRP (mg/l) [b]	2.7 (0.6–11.9)		3.1 (0.6–277)	
Hematocrit (%) [a]	0.39 ± 0.05		0.39 ± 0.05	
Radiological findings				
Volume of ICH (ml) †	97.85 ± 2.76		44.14 ± 3.29	
Disruption into ventricle	7	87.5%	18	51.4%
Hydrocephalus	5	62.5%	12	34.3%
Supratentorial localization	7	87.5%	25	71.4%

3. Results

Forty-nine patients were screened and 43 fulfilled the criteria and were analyzed; the demographics and clinical characteristics of these patients at admission in hospital with respect to 30-day and 90-day mortality as well as NIHISS at discharge are presented in Tables 1–3, respectively. Thirty-two hemorrhages were due to hypertension, 10 related to anticoagulation, and one aneurysm. Forty-four percent of the patients were admitted within 24 h after symptoms' onset and only around five percent after 24 h.