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Association Between Inflammatory Markers and Cognitive Outcome in Patients with Acute Brain Dysfunction Due to Sepsis

Sepsiste Akut Beyin Disfonksiyonu Olan Hastalarda İnflamatuvar Belirteçler ile Kognitif Sonuç İlişkisi

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ABSTRACT

Introduction: Sepsis-induced brain dysfunction (SIBD) has been neglected until recently due to the absence of specific clinical or biological markers. There is increasing evidence that sepsis may pose substantial risks for long term cognitive impairment.

Methods: To find out clinical and inflammatory factors associated with acute SIBD serum levels of cytokines, complement breakdown products and neurodegeneration markers were measured by ELISA in sera of 86 SIBD patients and 33 healthy controls. Association between these biological markers and cognitive test results was investigated.

Results: SIBD patients showed significantly increased IL-6, IL-8, IL-10 and C4 d levels and decreased TNF-α, IL-12, C5a and iC3b levels than healthy controls. No significant alteration was observed in neuronal loss and neurodegeneration marker [neuron specific enolase (NSE), amyloid β, tau] levels. Increased IL-1β, IL-6, IL-8, IL-10, TNF-α and decreased C4 d, C5a and iC3b levels were associated with septic shock, coma and mortality. Transient mild cognitive impairment was observed in 7 of 21 patients who underwent neuropsychological assessment. Cognitive dysfunction and neuronal loss were associated with increased duration of septic shock and delirium but not baseline serum levels of inflammation and neurodegeneration markers.

Conclusion: Increased cytokine levels, decreased complement activity and increased neuronal loss are indicators of poor prognosis and adverse events in SIBD. Cognitive dysfunction and neuronal destruction in SIBD do not seem to be associated with systemic inflammation factors and Alzheimer disease-type neurodegeneration but rather with increased duration of neuronal dysfunction and enhanced exposure of the brain to sepsis-inducing pathogens.

Keywords: sepsis; encephalopathy; cytokine; complement; amyloid beta; neurodegeneration.

ÖZ

Amaç: Sepsiste beyin disfonksiyonu (SBD), spesifik klinik veya biyolojik belirteçlerin bulunmaması nedeniyle son zamanlara kadar ihmal edilmiştir. Sepsisin uzun dönem kognitif bozukluk için önemli bir risk oluşturabileceğine dair kanıtlar artmaya başlamıştır.

Yöntem: Bu çalışmada sepsis ile uyarılan bilişsel işlev bozukluğunun altında yatan klinik ve inflamatuvar faktörleri ortaya koymak için,86 sepsis hastası ile 33 sağlıklı kontrol hastasının serumlarında ELISA yöntemi ile geniş bir inflamasyon ve nörodejenerasyon belirteçleri serisinin düzeyleri ölçüldü. Bu biyolojik belirteçler ve kognitif test sonuçları arasındaki ilişki araştırıldı.

Bulgular: SBD hastalarında sağlıklı kontrollere göre IL-6, IL-8, IL-10 ve C4d seviyelerinde anlamlı derecede artma ve TNF-α, IL-12, C5a ve iC3b seviyelerinde düşme görüldü. Nöronal kayıp ve nörodejenerasyon belirteçlerinin (nöron spesifik enolaz, amiloid β, tau) düzeylerinde anlamlı bir değişiklik gözlenmedi. Artmış IL-1β, IL-6, IL-8, IL-10, TNF-α ve azalmış C4d, C5a ve iC3b düzeyleri septik şok, koma ve mortalite ile ilişkilendirildi. Nöropsikolojik değerlendirmeye alınan 21 hastanın 7'sinde geçici hafif kognitif bozukluk gözlendi. Kognitif disfonksiyon ve nöronal kayıp, artmış septik şok süresi ve deliryum ile ilişkilendirildi ancak başlangıçtaki serum inflamasyon ve nörodejenerasyon belirteçlerinin düzeyleri ile ilişkili değildi.

Sonuç: Artan sitokin seviyeleri, azalan kompleman aktivitesi ve artan nöronal kayıp, kötü prognoz ve olumsuz olayların göstergeleridir. SBD'deki kognitif işlev bozukluğu ve nöronal yıkım, sistemik inflamasyon faktörleri ve Alzheimer hastalığı tipi nörodejenerasyon ile ilişkili görünmemekte, fakat nöronal işlev bozukluğunun süresinin uzamasına ve beyindeki sepsis indükleyici patojenlere artan maruz kalma ile ilişkilendirilmektedir.

Anahtar Kelimeler: sepsis; ensefalopati; sitokin; kompleman; amyloid beta; nörodejenerasyon.

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INTRODUCTION

Recent reports have demonstrated that progressively more sepsis and septic shock patients are admitted to intensive care units. Advances in medical treatment of sepsis have increased the survival ratios and also escalated number of patients with post-critical disease cognitive, functional and emotional disorders (1).

Sepsis induces organ dysfunction due to inflammation and decreased tissue perfusion and is associated with a mortality of around 50% in the intensive care units (2). Brain as an immunologically active organ is open to hazardous effects of systemic inflammatory reactions and is frequently afflicted in sepsis and septic shock patients. Sepsis-associated brain involvement is characterized by enhanced cytokine production, disrupted blood-brain barrier permeability and altered neurotransmitter release (2). While systemic organ dysfunction is easily evaluated with certain routine laboratory tests, there are no well-defined and practical biological markers for central nervous system dysfunction induced by sepsis. Therefore, evaluation of the progression of brain dysfunction and cognitive performance is rather difficult in sepsis patients (2).

Sepsis-induced brain dysfunction (SIBD) is a common term used for a wide range of sepsis-induced clinical conditions including coma, delirium, focal neurological deficits and seizures (2). Delirium, the most common form of acute brain dysfunction, is encountered in up to 80% of critically ill patients (3). SIBD is an independent contributor of increased hospitalization time, treatment cost (4), cognitive dysfunction and mortality (5).

Long-term cognitive dysfunction is diagnosed in 46–70% of sepsis patients in the first year and 25% of the patients in six years following discharge from an intensive care unit (6, 7). Although cognitive functions may return to normal levels in many patients, some SIBD patients develop chronic cognitive dysfunction and dementia in due course (8).

In this study, to disclose critical clinical and inflammatory factors underlying sepsis-induced cognitive dysfunction and to identify biomarkers that would predict development and progression of sepsis-associated cognitive dysfunction, we measured serum levels of a broad panel of inflammation and neurodegeneration markers and investigated their association with clinical and cognitive parameters.

METHODS

Patients

This was a prospective observational study that was conducted in adult medical and surgical intensive care unit (ICU) of a university teaching hospital (Istanbul Faculty of Medicine, Istanbul University, Turkey). Patients were enrolled at our institution between August 2013 and February 2017. All patients had clinical diagnosis of severe sepsis and septic shock as defined by international sepsis definition conference (9). Patients were included if they had been admitted to the ICU for ≥48 hour, were ≥18 years of age and had an acute onset of brain dysfunction. Acute brain dysfunction was defined as the sudden onset of altered mental status (delirium or coma), clinically or electroencephalographic detectable seizures and focal neurological deficits.

Patients with previously diagnosed brain disorder (brain lesions, neurodegenerative, inflammatory, cerebrovascular disease and traumatic brain injury), dementia and hypoxic brain injury were excluded. Patients with central nervous system infection were excluded. Pregnant patients and patients who had undergone by-pass surgery within the last 3 months were also not included. Hearing and vision impaired, illiterate and non-Turkish speaking patients were excluded from the study (Figure 1). Thirty-three healthy controls were also enrolled for biomarker measurement studies. The study was approved by the Institutional Review Board (approval number: 2013/98) and signed consent was obtained from patients or their relatives.

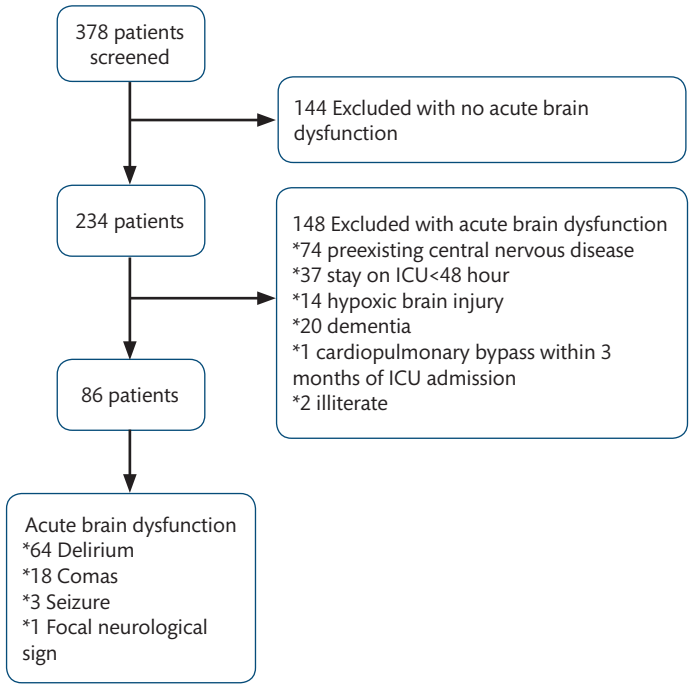

Figure 1. Flow chart of inclusion and exclusion of patients (ICU, intensive care unit).

Neurologic examination

When neurological examination was made, the patients were not sedated. Delirium was evaluated twice daily in the ICU by trained nurses or staff using the Confusion Assessment Method for the Intensive Care Unit (CAM ICU) (10). Daily sedation discontinuation protocol was applied in the ICU and sedation interruption made daily at 7:30 a. m. Level of consciousness was measured by the Richmand Agitation-Sedation Scale (RASS) (11). Patients with RASS score ranging from-1 to +1 were evaluated for delirium. Patients were diagnosed with delirium when they had at least one positive CAM-ICU screening criterion. Coma was diagnosed if patients showed a state of unarousable unresponsiveness with a Glasgow Coma Scale ≤8 in non-sedated patients or after discontinuation of sedation in previously sedated patients (12). Seizures were defined as generalized or focal tonic or clonic movement or an acute neurological disturbance associated with electroencephalographic ictal activity (13). Any lateralized deficit was considered a focal neurological deficit.

Baseline clinical and demographic data were collected at study enrollment. Severity of clinical findings was assessed at enrollment using the Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Simplified Acute Physiology Score 2 (SAPS2) and on a daily basis during the ICU stay using the Sequential Organ Failure Assessment (SOFA) score. Patients were followed with a standard protocol including regular total blood count, blood biochemistry, serum procalcitonin, C-reactive protein and oxygen saturation measurements. Microbiological data were recorded at admission and during the ICU. Bacteremia was defined as the presence of bacteria in the blood which was confirmed by blood culture. From admission to the day of discharge vital signs, use of steroids, duration of septic shock, days of mechanical ventilation and days of sedation were recorded daily. Hypotension was defined as a systolic blood pressure of <90 mmHg or a mean arterial pressure <70 mmHg. Septic shock was defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation in the absence of other causes of hypotension (9). Steroid treatment was administered to 42 patients,

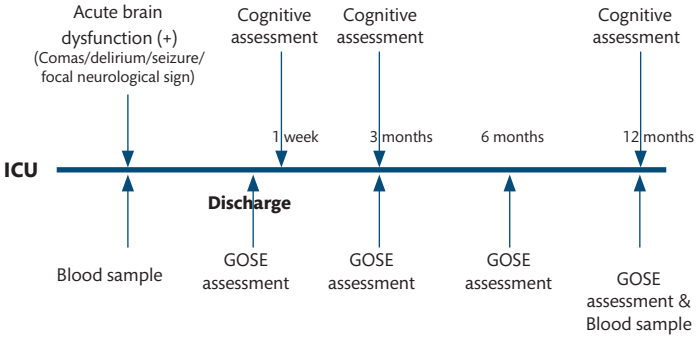

Figure 2. Timeline of study procedures (ICU, intensive care unit; GOSE, Glasgow outcome scale-extended).

who were septic shock patients receiving high dose vasopressors, acute respiratory distress syndrome patients or patients with exacerbation of a comorbid autoimmune disorder. Hemofiltration was performed to 38 patients who were acute renal failure and unstable hemodynamic status. Neurological recovery was assessed during hospitalization (baseline) and follow-up visits at 3, 6 and 12 months thereafter using the extended Glasgow Coma Scale GOSE (14) (Figure 2).

Assessment of neuropsychological functions

All patients underwent a clinical evaluation that included a thorough neurological examination and a detailed neuropsychological evaluation. A neuropsychologist, who was blind to clinical data, performed cognitive assessment at 0, 3 and 12-month follow-up (Figure 2). In addition to mini mental state examination (MMSE), the revised version of Addenbrook cognitive examination (ACE-R) was also used to measure global cognitive functions, including orientation-attention, memory, verbal fluency, language and visuospatial ability. Geriatric depression scale (GDS) was employed to measure self-rated depressive symptoms in patients. Based on MMSE scores, patients were grouped as those with no cognitive impairment [24–30], mild cognitive impairment [18–23] and severe cognitive impairment [0–17], as per previously recommended criteria for MMSE score evaluation (15).

ELISA

Sera were obtained during the first few hours of the onset of acute neurological dysfunction (baseline) and one year after discharge (1st year) and kept at -80°C until use. Patients were not under immunosuppressive drug treatment during serum collection. Serum levels of cytokines (IL-1β, IL-6, IL-8, IL-10, IFN-γ, TNF-α, IL-17 and IL-12) (Abcam, Cambridge, UK), complement breakdown products (Factor Bb, C4 d, iC3b and C5a) (Quidel, San Diego, CA, US) and neurodegeneration and gliosis markers [amyloid β peptides 1-40, 1-42, total tau, phosphorylated tau, S100 calcium-binding protein B (S100b) and neuron specific enolase (NSE)] (Abcam) were measured with ELISA as per manufacturers recommendations. Optical density was measured at 450 nm and concentrations were calculated by referring to a standard curve. Serum levels of cytokines and complement factors were measured only in the baseline sera. To evaluate the long-term impact of SIBD on neuronal death, neurodegeneration and gliosis markers were measured in baseline and 1st year sera.

Statistical analysis

Data were expressed in the form of mean ± standard deviation. Due to uneven distribution of the data, serum levels of biological parameters were compared with non-parametric Mann-Whitney U (cytokines and complement factors) or Kruskal-Wallis tests (neurodegeneration markers). Dunn's post-hoc test was used for two-group comparisons. Demographic, clinical and laboratory features of specific clinical SIBD subgroups were compared with Mann-Whitney U or chi-square tests, as required. Correlation statistics were performed with Spearman's correlation test; $p<0.05$ was considered as statistically significant.

RESULTS

Clinical features

We screened 378 patients admitted to the ICU with severe sepsis and septic shock. A total of 86 sepsis patients (50 men, 36 women; 53.2±10.4 year-old) were enrolled (Figure 1). They displayed delirium (n=64), coma (n=18), seizure (n=3) or focal neurological deficits (n=1). Thirty-three age and gender matched healthy controls (19 men, 14 women; 48.5±8.1 year-old) were also enrolled. Primary diagnosis was acute respiratory failure in 45, circulatory failure in 35 and acute renal failure in 6 patients. Comorbid conditions included diabetes mellitus in 18, cancer in 29, heart disease in 32, lung disease in 11 and kidney disease in 14 patients. Baseline characteristics and outcomes of the study population are presented in Table1.

SIBD patients were followed for an average duration of 39.1±22.2 days. Average duration in the intensive care unit was 21.2±13.5 days. All patients underwent mechanical ventilation (16.6±11.0 days) and sedation (13.5±8.9 days). During their follow-up, 75 patients developed septic shock (with an average duration of 7.4±5.8 days) and bacteremia was shown in 22 patients. Source of infection was pneumonia in 58, intraabdominal infection in 20, soft tissue infection in 4 and urinary tract infection in 2 patients. Blood culture showed gram negative bacteria (n=19), gram positive bacteria (n=2), Candida albicans (n=1) and no pathogens in the remaining patients. Culture obtained from the infection source yielded gram negative bacteria (n=62), gram positive bacteria (n=5), mixed bacteria (n=5) and Candida albicans (n=1). No pathogens were detected in 13 patients. Baseline APACHE II, SOFA and SAPS2 scores were 23.0±4.9, 8.5±3.7, and 48.5±17.6, respectively. Delirium (n=64) and coma (n=18) patients were followed for 4.1±2.4, and 6.8±1.8 days, respectively. GOSE scores for baseline, 3, 6 and 12-month visits were 3.7±2.8, 3.8±3.2, 3.8±3.4, and 3.8±3.4, respectively. Forty patients died during their hospitalization.

Table 1. Baseline characteristics and outcomes of the study population

Variables	n=86
Age (years)	53.2±10.4
Female n (%)	50 (58.1)
APACHE II at admission	23.0±4.9
SAPS II at admission	48.5±17.6
SOFA at admission (from 0 to 24)	8.5±3.7
Admission category-unscheduled surgery n (%)	29 (33.7)
Delay from septic shock to neurologic signs (days)	7.8±5.3
Delay from admission to neurologic signs (days)	5.6±5.0
Steroid treatment n (%)	42 (48.8)
Duration of septic shock (days)	7.4±5.8
Duration of mechanical ventilation (days)	16.6±8.9
Duration of sedation (days)	13.5±8.9
Days in the intensive care unit	21.2±13.5
Duration of hospitalization (days)	39.1±22.2
Mortality n (%)	40 (46.5)
GOSE at discharge	3.7±2.8
GOSE at 3 months	3.8±3.2
GOSE at 6 months	3.8±3.4
GOSE at 12 months	3.8±3.4

Values are denoted as mean ± standard deviation or number of patients (percentage of patients).

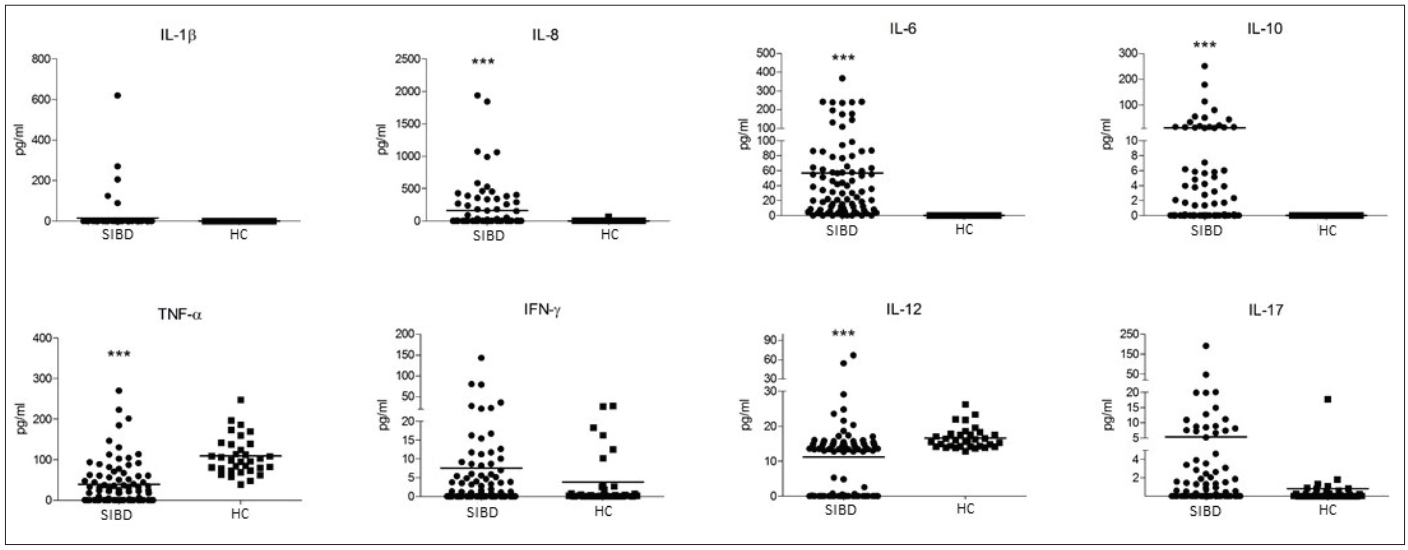

Figure 3. Serum cytokine levels of sepsis-induced brain dysfunction (SIBD) patients and healthy controls (HC) (horizontal lines indicate mean values. ***, p<0.001 by Mann-Whitney U).

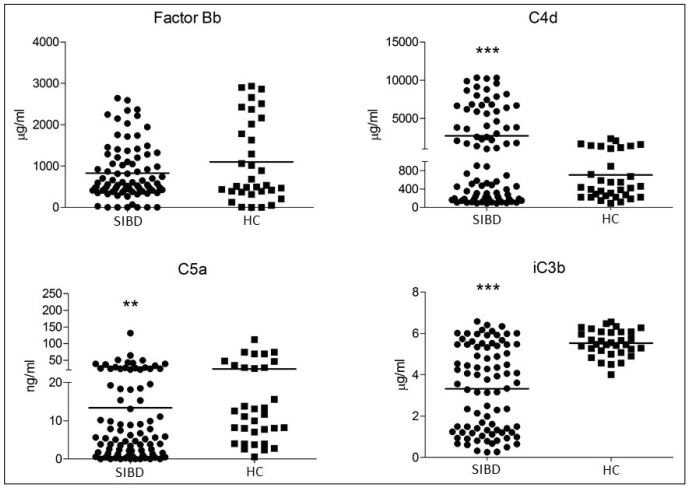

Figure 4. Serum complement breakdown product levels of sepsis-induced brain dysfunction (SIBD) patients and healthy controls (HC) (horizontal lines indicate mean values. **, p<0.01 and ***, p<0.001 by Mann-Whitney U).

Biological parameters in SIBD and association with clinical subgroups

SIBD patients showed significantly increased IL-8 (p=0.0005), IL-6 (p<0.0001) and IL-10 (p<0.0001) and significantly decreased TNF-α (p<0.0001) and IL-12 (p<0.0001) levels as compared to healthy controls. IL-1β (p=0.508), IFN-γ (p=0.474) and IL-17 (p=0.239) levels were identical between patients and controls (Figure 3). As to the complement breakdown and activation products, SIBD patients had significantly increased C4 d (p=0.0006) and decreased C5a (p=0.007) and iC3b (p<0.0001) levels than healthy controls. Factor Bb levels were comparable between two groups (p=0.513) (Figure 4). Baseline and/or 1st year serum levels of amyloid β peptide (1-40) (p=0.165), amyloid β peptide (1-42), total tau (p=0.819) and phosphorylated tau (p=0.063) showed trends toward decreasing in SIBD patients. However, this trend showed statistical significance only for amyloid β peptide (1-42) levels (p=0.037). SIBD patients displayed elevated baseline and 1st year S100b and NSE (p=0.892) levels with only S100b levels attaining statistical significance (p=0.043) (Figure 5). Dunn's post-hoc test did not show significance in two group comparisons of amyloid β peptide, total tau, phosphorylated tau, S100b and NSE levels.

Correlation analysis showed that factor B levels were directly correlated with amyloid β (1-40) (p<0.0001, R=0.787), amyloid β (1-42) (p<0.0001, R=0.802), total tau (p<0.0001, R=0.418) and phosphorylated tau (p<0.0001, R=0.744) levels. C4 d levels were directly correlated with phosphorylated tau (p<0.0001, R=0.486) levels and iC3b levels were inversely correlated with amyloid β (1-40) (p<0.0001, R=-0.423) and phosphorylated tau (p<0.0001, R=-0.538) levels. Moreover, SIBD patients with higher IFN-γ levels displayed higher S100b levels (p<0.0001, R=0.737), whereas other cytokines and complement factors were not significantly correlated with S100b. Likewise, levels of cytokines and C5a were not significantly correlated with levels of neurodegeneration markers, other complement factors and cytokines.

When biological parameters of delirium (n=64) and coma (n=18) patients were compared, coma patients were found out to display significantly higher IL-6, TNF-α, IL-12 and significantly lower C4 d levels than delirium patients (Table 2). Serum levels of investigated parameters were also compared among SIBD patients with and without unfavorable clinical features such as bacteremia, requirement for steroid treatment, septic shock and death during the follow-up. Overall, SIBD patients with unfavorable clinical factors showed trends towards increased cytokine (IL-6, IL-8, IL-10, IL-1β, IL-12, IFN-γ) and NSE levels and reduced common complement pathway activity (iC3b, C5a). Moreover, increased amyloid β peptide levels were observed in patients with requirement for steroid treatment (Table 3).

Potential correlations between cytokine complement breakdown product and neurodegeneration marker levels and clinical-demographic features listed in Table 3 were investigated. An inverse correlation was found between serum C4 d levels and SAPS2 scores (p<0.0001, R=-0.423) indicating that lower classical pathway activity is associated with a more severe clinical course for sepsis. Also, a marginal direct correlation was found between serum C5a levels and baseline (p=0.007, R=0.287), 3-month (p=0.006, R=0.293), 6-month (p=0.005, R=0.297) and 12-month (p=0.003, R=0.310) GOSE scores. Likewise, baseline serum NSE levels were moderately but significantly correlated with duration of mechanical ventilation (p=0.009, R=0.279) and sedation (p=0.001, R=0.339).

Association of cognitive dysfunction with clinical and inflammatory features

Only 21 patients (55.0±10.2 year-old; 12 men, 9 women) with sufficient cooperation could undergo neuropsychological assessments. Number of patients who could attend second and third follow-up visits were 17 (52.3±10.0 year-old; 8 men, 9 women) and 7 (47.0±12.5 year-old; 3

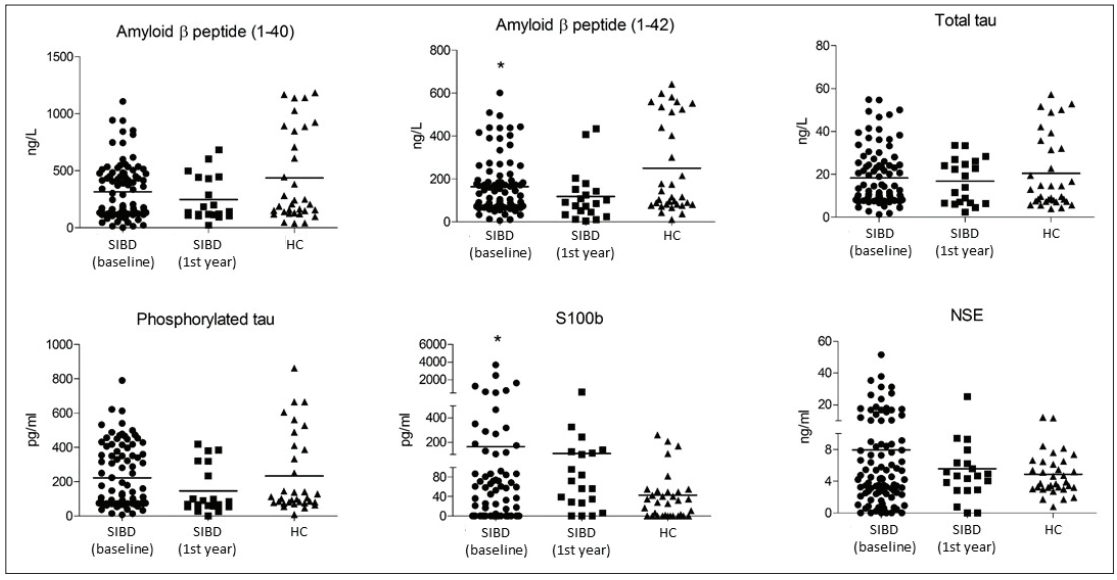

Figure 5. Serum levels of neurodegeneration and gliosis markers in sepsis-induced brain dysfunction (SIBD) patients and healthy controls (HC) (horizontal lines indicate mean values. *p<0.05 by Kruskal-Wallis.

Table 2. Comparison of serum immunological and neurodegenerative parameter levels of sepsis-induced brain dysfunction patients with delirium and coma

Biomarkers	Patients with delirium (n=64)	Patients with coma (n=18)	p value
IL-1β (pg/mL)	17.3±10.9	9.7±6.8	0.277
IL-8 (pg/mL)	120.3±37.3	268.4±100.8	0.090
IL-6 (pg/mL)	43.9±6.8	90.9±21.3	**0.023**
IL-10 (pg/mL)	8.5±4.1	24.9±9.7	0.066
IFN-γ (pg/mL)	6.2±1.8	11.0±6.5	0.243
TNF-α (pg/mL)	32.1±6.0	58.6±14.1	**0.048**
IL-17 (pg/mL)	2.9±0.9	12.6±8.6	0.136
IL-12 (pg/mL)	9.1±1.1	16.6±2.6	**0.007**
Factor Bb (μg/mL)	774.0±73.2	974.3±175.8	0.151
C5a (ng/mL)	13.6±2.5	12.1±3.3	0.362
C4 d (μg/mL)	3277.2±424.3	1208.2±533.1	**0.001**
iC3b (μg/mL)	3.2±0.2	3.4±0.3	0.327
Amyloid β (1–40) (ng/L)	319.3±26.1	364.5±72.5	0.281
Amyloid β (1–42) (ng/L)	161.9±13.5	204.7±38.3	0.151
Total tau (ng/L)	20.4±2.9	20.7±3.5	0.475
Phosphorylated tau (pg/mL)	232.0±22.1	221.3±45.6	0.417
S100b (pg/mL)	101.2±58.7	1604.4±1351.0	0.139
NSE (ng/mL)	6.2±1.0	9.0±2.3	0.138

Data are presented as mean ± standard error and statistical assessment is done by Mann-Whitney U test.
Significant comparisons are denoted with bold characters.
NSE, neuron specific enolase; S100b, S100 calcium-binding protein B.

Table 3. Comparison of serum immunological and neurodegenerative parameter levels of sepsis-induced brain dysfunction patients with and without specific clinical features

Parameters	Mean ± SE values*	p values
Patients with (n=22) vs without (n=64) **bacteremia**		
IL-8 (pg/mL)	**328.3±115.8** vs 99.7±29.8	0.034
IL-6 (pg/mL)	**102.6±20.7** vs 39.9±6.5	0.004
IL-10 (pg/mL)	**30.4±12.2** vs 6.5±2.9	0.035
Patients with (n=42) vs without (n=44) **steroid treatment**		
IL-1β (pg/mL)	**31.5±16.6** vs 0.0±0.0	0.033
IL-8 (pg/mL)	**247.8±72.1** vs 72.6±23.5	0.012
IL-10 (pg/mL)	**20.2±7.4** vs 5.4±2.8	0.034
IFN-γ (pg/mL)	**12.1±4.2** vs 2.9±0.7	0.019
IL-12 (pg/mL)	**13.1±2.1** vs 9.0±1.1	0.040
Amyloid β (1–40) (ng/L)	**379.4±43.4** vs 284.5±30.6	0.039
Amyloid β (1–42) (ng/L)	**202.1±23.6** vs 144.8±14.8	0.021
NSE (ng/mL)	**8.2±1.4** vs 5.6±1.2	0.044
Patients with (n=75) vs without (n=11) **septic shock**		
IL-1β (pg/mL)	**17.6±9.4** vs 0.0±0.0	0.033
IL-8 (pg/mL)	**177.8±43.2** vs 24.3±4.2	0.001
IL-10 (pg/mL)	**14.3±4.5** vs 1.6±1.3	0.004
iC3b (μg/mL)	**3.1±0.2** vs 4.5±0.6	0.022
NSE (ng/mL)	**7.2±1.0** vs 4.5±1.1	0.047
Patients with (n=40) vs without (n=46) **death**		
IL-8 (pg/mL)	**256.8±75.3** vs 72.4±22.8	0.011
IL-6 (pg/mL)	**81.1±13.3** vs 34.1±7.3	0.001
IL-10 (pg/mL)	**24.1±8.1** vs 2.7±0.8	0.006
C5a (ng/mL)	**9.4±1.9** vs 16.5±3.4	0.037
NSE (ng/mL)	**9.5±1.8** vs 4.6±0.6	0.007

Note that only statistically significant comparisons were listed.
*In the middle column, values in bold characters belong to patients with a specific clinical feature and values without bold characters belong to patients without the same clinical feature.
Data are presented as mean ± SE and statistical assessment is done by Mann-Whitney U test.
SE, standard error; NSE, neuron specific enolase.

men, 4 women), respectively. In the first assessment, none of the patients had severe cognitive impairment and 7 patients showed mild cognitive impairment. Mean MMSE score was 25.4±3.9 (range 18–30). In the 3-month visit MMSE scores improved (27.8±2.8, range 19–30) and in the 12-month visit none of the patients had cognitive impairment (28.4±1.4, range 26–30). None of the 21 patients that were available for cognitive assessment developed coma or died during their follow-up periods.

SIBD patients with and without cognitive impairment showed comparable age, gender, education level and cognitive assessment time values. Patients with lower MMSE scores also showed significantly reduced total ACE-R, orientation-attention, memory, verbal fluency and language scores, whereas visuospatial function scores were comparable among patients with no and mild cognitive impairment. Also scores of GDS, sepsis severity scales and GOSE were comparable among two groups. As to the clinical features, patients with mild cognitive impairment had a higher prevalence of bacteremia, increased duration of septic shock, delirium and hospitalization (Table 4). However, there were no significant

Table 4. Comparison of clinical and demographic features of sepsis-induced brain dysfunction patients with and without cognitive impairment

Parameters	MMSE <24 (n=7)	MMSE 24–30 (n=14)	p value*
Age	57.3±3.1	53.2±3.0	0.179
Gender (men/women)	4/3	8/6	>0.999
Duration of education (years)	7.1±1.1	8.7±1.0	0.148
Time of cognitive assessment (days after hospitalization)	6.7±2.5	7.9±1.8	0.349
ACE-R	57.9±5.2	76.1±3.2	**0.006**
Orientation-attention score	12.1±0.7	17.1±0.3	**<0.001**
Memory score	13.6±1.5	16.7±0.9	**0.048**
Verbal fluency score	4.9±0.8	7.6±0.7	**0.012**
Language score	15.6±2.0	20.9±1.3	**0.024**
Visuospatial function score	11.7±1.8	13.7±0.8	0.172
GDS	8.7±0.4	8.1±2.3	0.439
APACHE II	23.7±1.2	21.6±0.9	0.085
SOFA	8.0±0.4	8.0±1.0	0.500
SAPS2	52.4±3.9	41.8±5.2	0.058
GOSE-baseline	6.4±0.5	6.7±0.3	0.327
GOSE-3 months	6.9±0.5	7.2±0.5	0.302
GOSE-6 months	7.3±0.4	7.4±0.5	0.457
GOSE-12 months	7.0±1.0	7.0±0.7	0.500
Bacteremia present/absent	3/4	1/13	**0.049**
Steroid treatment present/absent	3/4	6/8	>0.999
Septic shock present/absent	6/1	11/3	0.694
Delirium present/absent	6/1	12/2	>0.999
Duration of septic shock (days)	10.5±2.8	3.5±0.5	**0.034**
Duration of mechanical ventilation (days)	15.6±3.5	12.9±2.2	0.264
Duration of sedation (days)	15.6±3.4	12.6±1.6	0.229
Duration of delirium (days)	7.7±1.1	3.4±0.4	**0.007**
Duration of hospitalization (days)	52.9±5.1	36.6±3.7	**0.012**

Parametric data are presented as mean ± standard error.
*Statistical assessment of parametric and non-parametric values is done by Mann-Whitney U and chi-square tests, respectively. Significant comparisons are denoted with bold characters.
MMSE, mini mental state examination; ACE-R, Addenbrook cognitive examination; GDS, geriatric depression scale; APACHE II, Acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; SAPS2, simplified acute physiology score 2; GOSE, Glasgow outcome scale-extended.

differences between neuropsychological test scores of SIBD patients with and without bacteremia, septic shock, delirium and requirement for steroid treatment. Also, there were no differences between patients with mild and no cognitive impairment by means of cytokine, complement breakdown product, amyloid beta, tau, NSE and S100b levels (Table 5).

Correlation studies were performed to find a possible correlation between scores of MMSE, ACE-R and other neuropsychological parameters and clinical, demographic, inflammatory features listed in Table 1 and 3. Significant correlations could be found only between visuospatial function scores and 3-month (p=0.0007; R=0.679), 6-month (p=0.0005; R=0.693) and 12-month (p=0.002; R=0.642) GOSE scores. No significant correlations could be found between cognitive test scores and cytokine, complement, neurodegeneration, NSE and S100b values.

Table 5. Comparison of laboratory parameters of sepsis-induced brain dysfunction patients with and without cognitive impairment

Biomarkers	MMSE <24 (n=7)	MMSE 24–30 (n=14)	p value
IL-1β (pg/mL)	38.5±38.5	0.0±0.0	0.178
IL-8 (pg/mL)	66.9±53.5	52.9±36.0	0.416
IL-6 (pg/mL)	39.9±32.8	31.8±11.0	0.411
IL-10 (pg/mL)	5.6±4.8	1.5±0.6	0.213
IFN-γ (pg/mL)	4.1±2.9	4.4±1.8	0.460
TNF-α (pg/mL)	52.6±20.8	39.8±15.0	0.313
IL-17 (pg/mL)	1.5±0.7	1.7±0.7	0.426
IL-12 (pg/mL)	10.9±2.8	10.4±2.4	0.449
Factor Bb (µg/mL)	856.3±214.5	988.9±192.6	0.326
C5a (ng/mL)	19.3±8.8	13.7±3.4	0.282
C4 d (µg/mL)	2932.6±1527.7	2222.2±746.7	0.343
iC3b (µg/mL)	3.0±0.6	3.6±0.5	0.240
Amyloid β (1–40) (ng/L)	297.6±71.7	328.6±51.5	0.366
Amyloid β (1–42) (ng/L)	173.9±44.2	184.3±32.5	0.426
Total tau (ng/L)	16.0±3.5	19.6±3.6	0.245
Phosphorylated tau (pg/mL)	210.4±61.1	232.1±42.9	0.388
S100b (pg/mL)	106.9±54.7	20.0±9.3	0.083
NSE (ng/mL)	6.0±2.0	3.9±0.7	0.172

The values belong to 21 patients with cognitive assessment.
Data are presented as mean ± standard error.
Statistical assessment is done by Mann-Whitney U test.
Significant comparisons are denoted with bold characters.
MMSE, mini mental state examination; NSE, neuron specific enolase; S100b, S100 calcium-binding protein B.

DISCUSSION

Increased serum pro-and anti-inflammatory cytokine levels have been well defined in patients with critically ill and sepsis patients and animal model of SIBD. Moreover, levels of cytokines have been reported to be associated with neurological outcome and sepsis-related complications such as bacteremia and septic shock (16, 17). In line with previous publications, increased IL-6, IL-8 and IL-10 levels were found in patients with bacteremia, septic shock and death in our study. Moreover, in addition to these three cytokines, serum IFN-γ and IL-12 levels were increased in patients that fulfilled the criteria for steroid treatment requirement. Cytokine levels also inclined to increase in encephalopathy patients with more severe clinical presentation with IL-6, TNF-α and IL-12 levels attaining statistical significance.

On the other hand, there was no correlation between serum cytokine levels and cognitive test scores and patients with and without cognitive impairment showed comparable cytokine levels. Cytokine levels also did not correlate with neuronal loss and Alzheimer-type neurodegeneration markers amyloid β and tau. Thus, our results suggest that cytokines are not directly involved in cognitive dysfunction observed in SIBD patients and cognition is presumably afflicted by altered activation of intracellular pathways that regulate neuronal functions and cell survival by other inflammatory mediators, as demonstrated in animal models of sepsis (17,

18). By contrast, IFN-γ levels correlated with S100b, a marker of blood-brain barrier damage suggesting that this cytokine induces leakage of S100b to the peripheral blood, as previously implied (19).

No significant changes were observed in serum levels of IL-17 and IFN-γ in SIBD patients indicating that polarization to Th1-and Th17-type immunity does not play a critical role in SIBD. In contrast with many cytokines, TNF-α and IL-12 levels were reduced in SIBD patients. A potential explanation might be the immunosuppression and reduced cytokine secretion of immune cell subsets in sepsis in early stages of SIBD (20).

SIBD patients showed reduced common pathway (C5a, iC3b levels) and increased classical pathway (C4 d levels) activity, while alternative pathway activity (Factor Bb levels) did not appear to be crucially involved in SIBD pathogenesis. Increased levels of classical complement pathway breakdown product C4 d may be explained by enhanced activation of this pathway by antibodies directed against sepsis-inducing pathogens. Notably, SIBD patients with lower C4 d levels showed worse SAPS2 scores and patients with coma displayed lower C4 d levels. These results suggest that C4 d levels indicate the capacity of the immune system to cope and deal with increased pathogen burden and thus patients with increased C4 d levels have a more favorable clinical course.

Reduced common complement pathway activity might be the end-result of sepsis-induced immunosuppression or alternatively over-utilization of complement factors in the central nervous system and consequent depletion of these factors in peripheral blood. Significance of C5a is well known in sepsis and sepsis-associated brain damage (21) and C5a has been associated with increased blood-brain barrier disruption and neuronal death (22). Moreover, in a recent study carried out with a rat model of sepsis, intravenous immunoglobulin treatment has been shown to ameliorate neuronal death through suppression of C5a-induced apoptosis and inflammation pathways (18). On the other hand, SIBD patients with septic shock and mortality displayed reduced C5a or iC3b levels in our study and higher C5a levels were associated with a better short and long-term neurological outcome (GOSE) score. Also, complement breakdown product levels did not show significant correlation with NSE levels, GDS scores and neuropsychological test scores. These results indicate that enhanced common complement pathway activity might be crucial for amelioration of sepsis-related complications and complement system might have a protective rather than destructive action on neurons in sepsis patients. In line with this suggestion, both C3a and C5a have been shown to exert neuroprotective and anti-inflammatory effects in animal models of sepsis (23, 24). Thus, common complement pathway has a dual and complicated role in sepsis, and SIBD depending on whether its components are increased in brain or peripheral blood. Precise cerebral and serum C3 and C5 breakdown product concentration ranges that are most favorable for sepsis patients need to determined.

An intriguing finding in our study was direct and inverse correlations between amyloid β peptide and complement factor levels. Amyloid β is a well-established activator of both classical and alternative pathways and enhanced complement factor deposition is observed in brain tissues of patients with amyloid β related disorders, Alzheimer disease and cerebral amyloid angiopathy (25–27). Thus a direct correlation between C4 d, factor Bb and amyloid peptides is highly expected. On the other hand, C3 breakdown products and particularly iC3b is known to participate in phagocytosis and clearance of amyloid β peptides by microglia (28) and thus an inverse correlation between iC3b and amyloid peptide levels is also perceivable. A likely hypothesis is that increased release of C3 breakdown products in sepsis facilitates clearance of amyloid and tau proteins leading to diminished activation of alternative and classical complement pathways.

As exciting as they are, these amyloid β-related mechanisms do not appear to play a major role in SIBD pathogenesis. Amyloid β peptide and tau protein levels were reduced especially in the earlier stages of SIBD and no significant correlation could be demonstrated between these neurodegeneration parameters and the intensity of neuronal loss, severity of sepsis or cognitive assessment scores. Although a correlation between increased cerebral amyloid β levels and cognitive impairment has been demonstrated in a recent SIBD animal model study (29), our data suggest that Alzheimer disease-type neuropathological changes do not seem to play a significant role in SIBD patients. As a matter of fact, patients that could be assessed for cognitive functions during hospitalization showed only mild cognitive impairment that vanished in the ensuing follow-up visits further ruling out Alzheimer-type dementia as a common post-morbid complication in SIBD. However, as a limitation of our study, patients that underwent detailed neuropsychological assessment had inadvertently milder disease course and thus our results do not entirely exclude dementia that might conceivably emerge in SIBD patients with a severe disease course.

SIBD patients show increased baseline S100b and NSE levels indicating blood-brain barrier damage, gliosis and neuronal loss during early stages of the disease. S100b and NSE levels then approach to healthy control levels in one year alongside an increase in MMSE scores suggesting that central nervous system changes are mild and transient in most SIBD patients. However, none of the biological markers investigated in our study correlated with NSE levels. Instead, NSE was found to be associated with septic shock, death due to sepsis and increased duration of sepsis and septic shock. These results suggest that sepsis-induced neuronal loss is associated with increased burden of circulating pathogens and presumably pathogen-derived mediators rather than pathogen-reactive mediators of the immune-system. Interestingly, NSE levels did not correlate with MMSE scores and other cognitive test scores as well suggesting that cognitive dysfunction in SIBD is mediated by neuronal dysfunction rather than neuronal loss. In this context, intracellular inflammation and inflammasome systems emerge as potential target pathways in animal models of SIBD (18, 30).

In conclusion, Increased cytokine levels, decreased complement activity and increased neuronal loss stand out as indicators of poor prognosis in SIBD. IL-6, IL-8, IL-10, C4 d and C5a levels might particularly be used as biomarkers for prediction of adverse events and death in SIBD. Cognitive dysfunction and neuronal destruction are mild and transient in most SIBD patients and are not associated with systemic humoral immune system factors or Alzheimer disease-type neurodegeneration. Cognition appears to be affected from increased duration of neuronal dysfunction and enhanced exposure of the brain to sepsis-inducing pathogens. Therefore, to shed further light on the pathogenesis of SIBD, interactions between neuronal survival pathways and pathogen-induced hazardous soluble mediators need to be further investigated.

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