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RESEARCH ARTICLE

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A comparison of HMGB1 concentrations between cerebrospinal fluid and blood in patients with neurological disease

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ABSTRACT

Aims: To determine whether a correlation exists between paired cerebrospinal fluid (CSF) and serum levels of a novel inflammatory biomarker, high-mobility group box 1 (HMGB1), in different neurological conditions.

Methods: HMGB1 was measured in the serum and CSF of 46 neurological patients (18 idiopathic intracranial hypertension [IIH], 18 neurological infection/inflammation [NII] and 10 Rasmussen's encephalitis [RE]).

Results: Mean serum (\pm SD) HMGB1 levels were 1.43 ± 0.54 , 25.28 ± 27.9 and 1.89 ± 1.49 ng/ml for the patients with IIH, NII and RE, respectively. Corresponding mean (\pm SD) CSF levels were 0.35 ± 0.22 , 4.48 ± 6.56 and 2.24 ± 2.35 ng/ml. Both CSF and serum HMGB1 was elevated in NII. Elevated CSF HMGB1 was demonstrated in RE. There was no direct correlation between CSF and serum levels of HMGB1.

Conclusion: Serum HMGB1 cannot be used as a surrogate measure for CSF levels. CSF HMGB1 was elevated in NII and RE, its role as a prognostic/stratification biomarker needs further study.

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KEYWORDS

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Introduction

High-mobility group box 1 (HMGB1) is a small, highly conserved ubiquitous protein that shuttles between the nucleus and cytoplasm. In response to infection and sterile inflammation, it is released into the extracellular milieu to function either as a chemoattractant or a pro-inflammatory cytokine (Schiraldi *et al.* 2012). Several studies have shown that serum HMGB1 may be a sensitive and specific biomarker for patient stratification in a number of diseases, ranging from drug-induced liver injury (Antoine *et al.* 2013) to acute coronary syndrome (Goldstein *et al.* 2006, Cirillo *et al.* 2009, Hashimoto *et al.* 2012) to many types of cancer (Chen *et al.* 2013, Fahmueller *et al.* 2013, Tabata *et al.* 2013a, 2013b).

In neurological conditions such as meningitis (Tang *et al.* 2008, Asano *et al.* 2011, Hohne *et al.* 2013), multiple sclerosis and neuromyelitis optica (Wang *et al.* 2013, Uzawa *et al.* 2013), cerebrospinal fluid (CSF) levels of HMGB1 may have prognostic value. However, limited data are available to assess the relationship between peripheral blood and CSF levels of HMGB1, particularly in conditions where the blood-brain barrier (BBB) can be disrupted, for example in neurological infection or seizures. Clearly in conditions

where there is disruption of the BBB, a conduit for transfer of biomarkers exists between the different compartments. CSF bathes the central nervous system (CNS) and potentially reflects the most dynamic changes that can occur in disease. However, obtaining CSF by lumbar puncture is invasive and is sometimes contraindicated in certain neurological conditions. Blood is less invasive to obtain and hence blood biomarkers would be particularly advantageous for diagnosing neurological disease, determining response to treatment and for determining prognosis.

Therefore, in this study, we have compared HMGB1 levels between CSF and blood in different neurological conditions to determine whether there is a predictable relationship between the two compartments. If so, this would ultimately allow the physician to use blood biomarker levels as a surrogate for CSF levels. The conditions investigated include idiopathic intracranial hypertension (IIH), neurological infection/inflammation (NII) with fever (including viral and bacterial) and Rasmussen's encephalitis (RE). The selected conditions allow an examination of HMGB1 from paired CSF and blood samples in neurological conditions of both inflammatory (CNS infection and RE) and non-inflammatory (IIH) aetiology in which the BBB is either disrupted (CNS infection and RE) or intact (IIH).

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Methods

IIH cohort

Otherwise healthy male and female patients ($n=18$, aged 19–58 years) with suspected or confirmed IIH were recruited from The Walton Centre NHS Foundation Trust (WCFT). Ethical approval was granted by the NRES Committee Liverpool East (11/NW/0761). Patients with both confirmed IIH attending for drainage via lumbar puncture or suspected IIH confirmed post-procedure were included.

Neurological infection/inflammation (NII) cohort

CSF samples from adult patients with proven neurological infection were obtained via the UK Meningitis Study cohort ($n=3$) and from paediatric patients with suspected acute encephalitis syndrome (AES), a constellation of fever, impaired consciousness \pm seizure ($n=15$). Ethical approval was granted for the UK meningitis study from the Wales research ethics committee 5 (11/WA/0218) and for the encephalitis study from the Liverpool School of Tropical Medicine ethics committee. Samples were taken for diagnostic purposes but patients also provided written, informed consent for the use of their samples in research.

RE cohort

Patients with confirmed RE under the specialist care of the neuroimmunology group at WCFT donated paired serum and CSF samples into a research study prior to 2006. Irreversibly anonymised paired samples were accessed under the UK Human Tissue Act; ethical approval was granted by the NRES proportionate review service of Greater Manchester West (13/NW/0879).

We recorded the age, sex, comorbidities, medications, CSF variables, admission Glasgow coma score (GCS), seizure history and diagnosis for patients with IIH and CNS infection. No clinical details were available about the stored RE samples because all samples had been irreversibly anonymised.

Sample collection

CSF was collected by lumbar puncture. Patients were positioned either in the left lateral decubitus position or sitting upright for the procedure. A sample volume of between 2 and 5 ml was obtained. Venepuncture was performed concurrently to collect paired serum. Blood and CSF were centrifuged at 2000g for 20 min immediately following collection. Serum was removed and cellular debris discarded from CSF. Samples were stored at -80°C until analysis.

CSF and serum HMGB1 measurement

The CSF and serum HMGB1 concentrations were determined according to the manufacturer's guidelines using an HMGB1 ELISA kit (Shino-Test corp., Tokyo, Japan). In brief, thawed samples were centrifuged at 2000g for 1 min. The 96-well plate was coated with sample diluent to which 10 μl of sample (serum or CSF) was added in duplicate. Samples were incubated overnight at 37°C for 20 h. Plates were washed

five times in wash buffer and air-dried. Detection antibody solution (100 μl /well) was added for 2 h at room temperature. The plate was washed again, substrate solutions were added in equal parts (100 μl /well) and incubated at room temperature protected from light with a foil seal for 30 min. Stop solution (100 μl /well) was added and after 5 min, the optical density was measured at 450 nm. Results were fitted to the standard curve. The HMGB1 detection range was 0.2–80 ng/ml.

Calculation of the CSF: serum albumin quotient

The degree of permeability of the BBB in patients with IIH was estimated from the CSF/serum quotient for albumin (QALB). Analysis was not possible in the RE and NII cohorts due to restricted sample volume. Analysis was undertaken by the Neuroimmunology and CSF Laboratory, University College London Hospitals NHS Trust.

Statistical analysis

The mean and standard deviation for both compartments in each condition was calculated. Correlation between compartments was determined by the Kendall's tau correlation coefficient (r). Correlation between blood and CSF biomarker levels and clinical variables (where available) was undertaken by Kendall's tau correlation coefficient for continuous variables and Mann–Whitney test for binary variables. The false discovery rate (FDR) (<0.05) was used to correct for multiple testing.

Results

Eighteen patients with IIH were recruited and analysed. A further 18 CSF samples and 15 paired serum samples (3/18 patients did not have paired serum taken) from patients with NII (viral or bacterial) were analysed. The mean age of the patients was 38.3 years (range 19–58) in the IIH cohort and 15.5 years (range 1.5–68) in the NII cohort, with female to male ratios of 10:8 and 11:9, respectively. Ten paired samples from patients with RE were analysed. The demographic details for the patients are detailed in [Tables 1](#) and [2](#).

The mean (\pm SD) serum HMGB1 levels were: IIH 1.43 ± 0.54 , NII 25.28 ± 27.9 and RE 1.89 ± 1.49 ng/ml. The corresponding mean (\pm SD) CSF HMGB1 levels were: IIH 0.35 ± 0.22 , NII 4.48 ± 6.56 and RE 2.24 ± 2.35 ng/ml. Sub-categorisation of the NII group into bacterial vs. viral pathogen/AES did not reveal any significant difference in total CSF HMGB1 concentration. There was no significant correlation between the serum and CSF HMGB1 concentrations in any of the conditions examined ([Figure 1](#)). However, there were significant positive correlations between CSF HMGB1 levels and CSF white cell count and history of seizures ([Figure 2](#), [Table 3](#)) and both associations survived correction for multiple testing with FDR. Calculation of the QALB confirmed that the BBB was intact in all of the IIH individuals examined,

Discussion

This study demonstrates, in conditions involving both normal and abnormally high HMGB1 concentrations, that there is no

Table 1. Demographics of patients with idiopathic intracranial hypertension.

Sex	Age (years)	Cigarettes/day	Alcohol units/week	Brain imaging (CT/MRI)	Symptoms/diagnosis	Medical history	Medications
M	49	0	2	Normal	Painless visual loss right eye	Nil	Paracetamol 1 g QDS
F	29	0	0	Normal	Headache, papilloedema	Nil	Acetazolamide 250 mg TDS
M	32	0	0.5	Normal	Back pain	Pseudomeningocele following microdiscectomy	Tramadol 50 mg PRN
F	26	0	0	Normal	Headache, papilloedema	Nil	Nil
F	44	20	0.5	Normal	Headaches, diplopia, vomiting	Osteoporosis, depression	Citalopram 40 mg OD
F	44	0	0	Normal	Headache	Hysterectomy (fibroids)	Nil
M	58	0	2	Normal	Headache	Nil	Nil
F	52	0	0.5	Normal	IIH confirmed	IIH 2011	Nil
M	48	0	2	Normal	Headache	Nil	Nil
F	41	0	0	Normal	Headache	Nil	Nil
F	58	0	0	Normal	Headache	Nil	Nil
M	32	0	4	Normal	Headache	Nil	Nil
M	20	0	0	Normal	IIH confirmed	IIH 2010	Nil
M	19	0	16	Normal	Headache	Nil	Nil
F	44	20	0	Normal	IIH confirmed	Benign breast lump, IIH 1998	Nil
F	21	15	0	Normal	IIH confirmed	IIH 2010	Nil
F	41	0	0	Normal	Headache	Nil	Nil
M	31	0	20	Normal	Headache, nausea	Hypertension	Lisinopril 20 mg OD

Demographics of subjects with idiopathic intracranial hypertension recruited to the “healthy control substitute” arm. M: Male; F: Female; CT; Computed Tomography; MRI: Magnetic Resonance Imaging; IIH: Idiopathic Intracranial Hypertension; g: gram; QDS: Four-times daily; mg: milligrams; TDS: Three-times daily; PRN: As required; OD: once-daily.

Table 2. Demographics of patients with neurological infection.

Category	Pathogen (if known)	Age	Gender	Ethnicity	Fever within 14/7	Seizure	Admission GCS (3–15)	CSF WCC
Viral encephalitis		11	Male	SE Asian	Yes	Yes	9	100
Viral encephalitis		2.0	Male	SE Asian	Yes	Yes	10	60
Viral encephalitis		9.0	Female	SE Asian	Yes	Yes	10	4
Transverse myelitis		10.0	Female	SE Asian	Yes	No	14	97
Viral encephalitis	Japanese encephalitis	12.0	Female	SE Asian	Yes	Yes	15	Unknown
Viral encephalitis		5.0	Male	SE Asian	Yes	Yes	15	200
Viral encephalitis	Dengue	9.0	Female	SE Asian	Yes	Yes	14	Unknown
Viral encephalitis		6.0	Female	SE Asian	Yes	No	15	3
Acute encephalitis syndrome		12.0	Female	SE Asian	Yes	No	9	Unknown
Viral encephalitis		6.0	Female	SE Asian	Yes	No	14	5
Acute encephalitis syndrome		7.5	Male	SE Asian	Yes	Unknown	15	Unknown
Bacterial meningitis		11.0	Female	SE Asian	Yes	No	15	Unknown
Unknown		8.0	Male	SE Asian	Yes	No	Unknown	Unknown
Viral encephalitis		13.0	Male	SE Asian	Yes	Unknown	Unknown	40
Viral encephalitis		1.5	Male	SE Asian	Yes	Yes	7	100
Fungal meningitis	Cryptococcus	41.0	Male	Caucasian	Yes	No	15	440
Bacterial meningitis	Pneumococcus	68.0	Female	Caucasian	Yes	No	8	3426
Bacterial meningitis	Pneumococcus	35.0	Female	Caucasian	Yes	No	15	1415

The study subjects were combined from a paediatric study in South East (SE) Asia and a nationwide study across the United Kingdom of confirmed bacterial meningitis. GCS: Glasgow Coma Score, a numerical assessment of consciousness, minimum 3 and maximum 15. Sub-categories include (1) viral encephalitis: raised white cell count in CSF ± virus antibody positive, (2) acute encephalitis syndrome (fever, impaired consciousness ± seizures) and (3) bacterial/fungal meningitis.

correlation between CSF and serum concentrations of HMGB1. Therefore, it is not possible to use peripheral blood HMGB1 as a surrogate measure for CSF. The present study is the first report to our knowledge to examine HMGB1 levels in both CSF and paired peripheral blood in IIH, which were found to be consistent with the normal healthy range (Fukami *et al.* 2009).

IIH affects mainly obese women of childbearing age and is characterised by headaches and visual loss resulting from elevated intracranial pressure (Corbett and Mehta 1983). Treatment includes drainage of CSF. The pathophysiology is incompletely understood but importantly, inflammation is

not considered to be a component and CSF cell count and biochemistry must be normal to satisfy the diagnostic criteria (Corbett and Mehta 1983). As such the condition was selected as an “unhealthy control”. Recently, speculation about the contribution of some immunological factors to the pathogenesis of IIH have been cited (Sinclair *et al.* 2008, Edwards *et al.* 2013, Altiokka-Uzun *et al.* 2015). Varying levels of cytokine expression in the CSF in IIH have been shown (Dhungana *et al.* 2009a, 2009b) but the evidence is inconsistent. Some studies have shown that IIH patients show higher CSF IL-17, IL-4, IL-2, IL-10 and IFN- γ levels than comparator patients with the inflammatory neurological condition

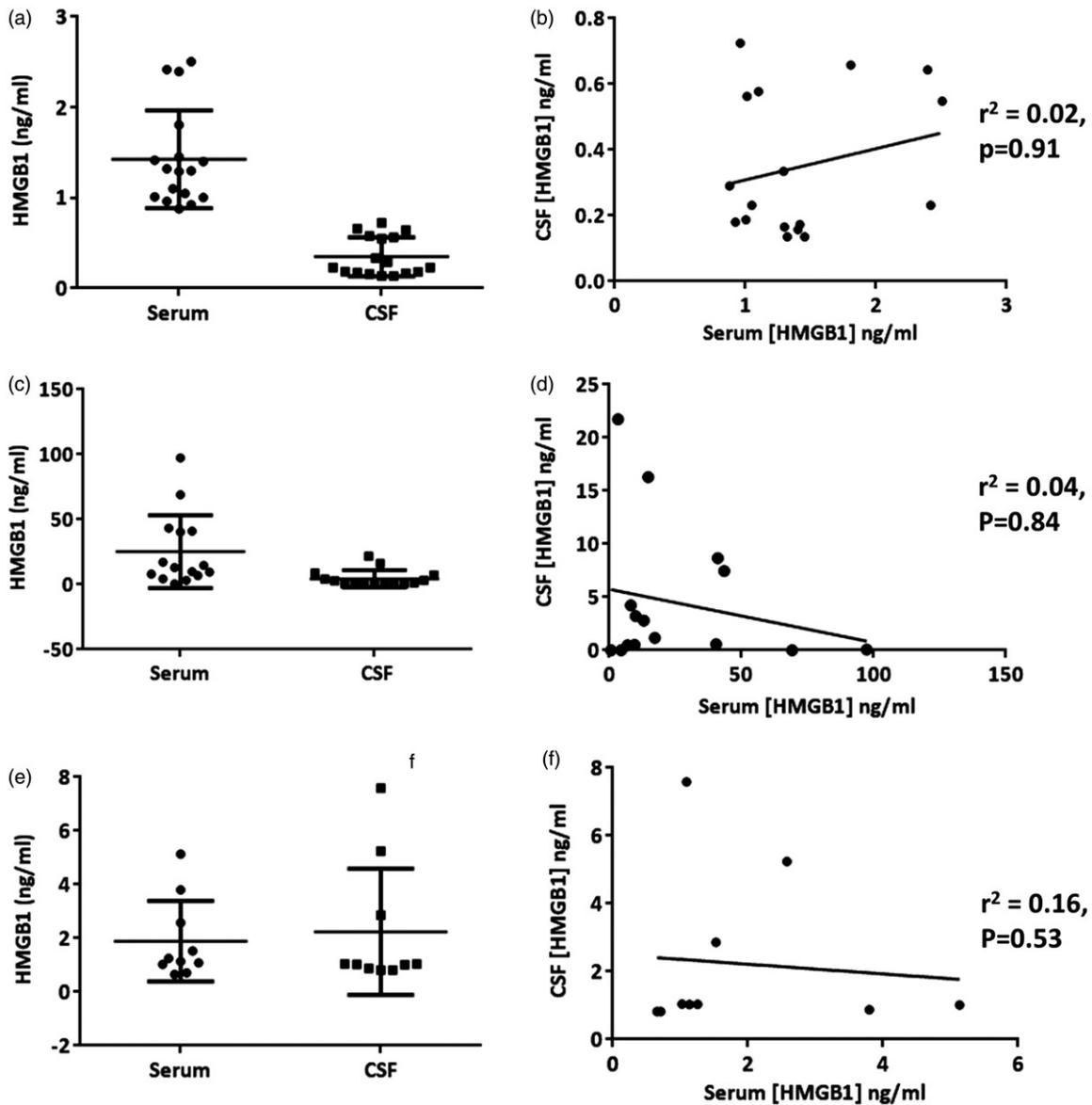


Figure 1. High-mobility group box 1 (HMGB1) concentrations across serum and cerebrospinal fluid (CSF) compartments in (a) idiopathic intracranial hypertension (IIH), (c) neurological infection/inflammation (NII) and (e) Rasmussen's encephalitis (RE). Box and whisker plots represent mean \pm SD, $n = 18$, 15 and 10, respectively. Regression analysis performed using Kendall's Tau correlation coefficient between compartments was not significant in any condition tested: (b) IIH, (d) NII, (f) RE.

multiple sclerosis (Edwards *et al.* 2013, Altiokka-Uzun *et al.* 2015). However, it is unknown whether this is a cause or effect of the disease. No correlation was found between serum and CSF cytokine levels, which is consistent with our finding for HMGB1.

We have also identified for the first time, albeit in a small cohort, that CSF HMGB1 is elevated in patients with RE when compared to IIH patients, who served as "controls". RE is a rare CNS inflammatory disorder characterised by intractable seizures and progressive hemiparesis. The aetiology remains unclear, although lymphocyte infiltration and microglial nodules are characteristic (Rasmussen *et al.* 1958). The numbers studied were small and require further exploration in a larger cohort to evaluate any diagnostic or prognostic value. Our findings are, however, consistent with the proposed immune pathogenesis of RE where cytotoxic T cells have been implicated. Spectral analysis of individual T cells from brain lesions has shown clonal expansion of CD8+ cells, suggesting an

antigen-driven CD8+ T cell-mediated autoimmune process (Bauer *et al.* 2002, Bien *et al.* 2002). In combination with CSF white cell counts, HMGB1 may represent a possible marker of white cell infiltration of brain parenchyma (inflammation) and contribute to greater understanding of the disease process.

Neurological infection is known to be associated with raised CSF and serum HMGB1 (Tang *et al.* 2008) but paired compartment analysis has never been undertaken to our knowledge. In patients with NII, a wide range of CSF HMGB1 values was demonstrated. This likely reflects the varied timing of sampling following the onset of symptoms/infection which was not standardised. Regardless of aetiology (viral or bacterial), serum HMGB1 was significantly higher in neurological infection compared to the IIH group (Figure 3). No significant difference in total CSF values was observed when the patients were sub-categorised into bacterial vs. viral/AES (Figure 4). Therefore, although the mechanisms responsible for the increase in CSF HMGB1 concentration in neurological

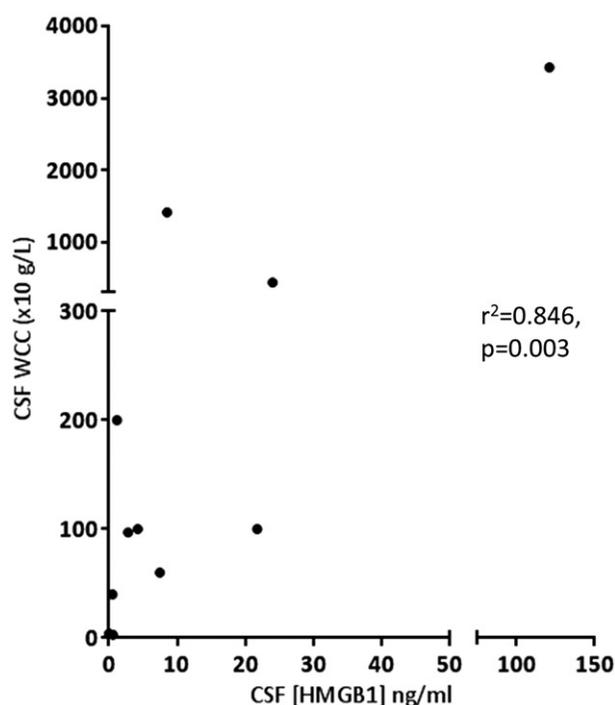


Figure 2. Cerebrospinal fluid (CSF) High-mobility group box 1 (HMGB1) was significantly associated with CSF white cell count in patients with neurological infection/inflammation. Regression analysis was performed by Kendall's Tau correlation coefficient.

Table 3. Associations between clinical variables and serum and cerebrospinal fluid High Mobility Group Box-1.

Variable	Serum HMGB1 <i>p</i> value	CSF HMGB1 <i>p</i> value
CSF white cell count ($\times 10^6/L$)	0.53	0.003
CSF Protein (g/L)	0.47	0.21
CSF Glucose (mmol/L)	0.79	0.11
Glasgow Coma Score	0.32	0.45
Serum white cell count (\times/L)	0.09	0.93
Serum platelets ($\times 10^9/L$)	0.54	0.84
Serum haemoglobin (g/dL)	0.53	0.96
Seizure (Yes/No)	0.64	0.004

infection are unknown, and indeed, much of the pathology of these neurological diseases is unknown, it could possibly reflect intracerebral synthesis, leakage from blood across the disrupted BBB or a combination of these mechanisms. Unfortunately, this study was limited by sample volume and therefore the QALB could not be calculated in order to ascertain the contribution of the disrupted BBB to transport between compartments. What is more, certainly in the case of meningitis, the epithelial cells of the choroid plexus that form the blood-CSF barrier may play their own independent role (Schwerk *et al.* 2012). CSF HMGB1 may also undergo proteolytic degradation in blood, and the level therefore affected by clearance from blood via the liver or kidney. Failure to correlate HMGB1 levels between compartments in neurological infection and RE, both conditions in which the BBB is impaired, may reflect the dynamic nature of the barrier, and the change in permeability over time. The isolated samples were obtained at varying time points following symptom onset and serial studies are not possible in this patient group. Furthermore, in neurological infection wherein

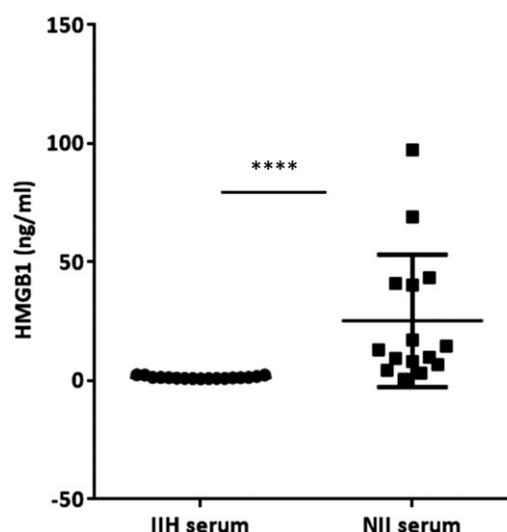


Figure 3. Blood, but not cerebrospinal fluid (CSF), high-mobility group box 1 (HMGB1) was significantly higher in neurological infection ($n = 15$) as compared to the healthy-control substitute (IIH, $n = 18$). Data presented as mean \pm standard deviation. Association was determined by Mann-Whitney *U*-test, **** $p < 0.0001$.

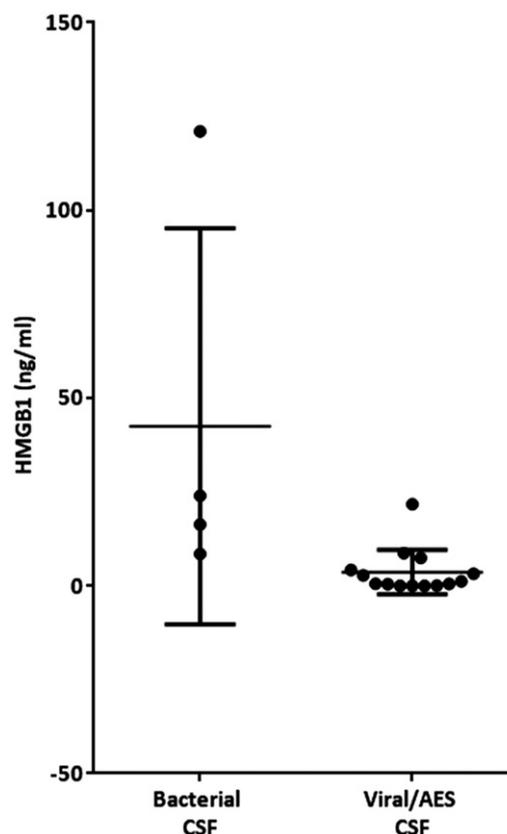


Figure 4. Cerebrospinal fluid (CSF) High-mobility group box 1 (HMGB1) in patients with neurological infection/inflammation sub-categorised into those with a known bacterial or viral/unknown pathogen. Individual values and the mean value (\pm SD) in each group are presented.

both blood and CSF HMGB1 is elevated above the normal range (1.65 ± 0.04 ng/ml; (Fukami *et al.* 2009)), it is unclear whether the source of serum HMGB1 is purely CNS, CNS and CSF or whether it reflects concurrent peripheral production.

In this study, CSF HMGB1 was significantly associated with elevated CSF white cell count in patients with neurological

infection. CSF pleocytosis is associated with excessive host immune response in human meningitis and contributes to brain injury (Woodbury and Davenport 1952, Gekakis *et al.* 1998, Redzic 2011, Guidelines 2012). Furthermore, release of inflammatory mediators activates inflammatory cells and promotes vascular permeability, both of which are injurious to tissue, and are associated with poor neurological prognosis (Mustafa *et al.* 1989, Ohga *et al.* 1994, Ichiyama *et al.* 1997). Invading pathogens trigger recognition of pathogen-associated molecular patterns (PAMPs) by Toll-like receptors 2 and 4 (Klein *et al.* 2008) leading to assembly of the Nod-like Receptor family, Pyrin Domain Containing-3 (NLRP3) inflammasome. Consequently, large numbers of blood-borne leukocytes are recruited into the leptomeninges. Leukocytes recruited into the CSF space may be a source of inflammatory mediators (Griffiths *et al.* 2012), including HMGB1. Release of HMGB1 from inflammatory and/or necrotic cells is thought to be central to persistent inflammation in pneumococcal meningitis through its chemoattractant function (Hohne *et al.* 2013). Fully reduced HMGB1 recruits inflammatory cells to compartments undergoing necrosis (Andersson *et al.* 2014). Injection of HMGB1 into the CSF of mice induces a significant increase in CSF leukocyte counts, an effect blocked by pre-treatment with neutralising anti-HMGB1 antibodies (Hohne *et al.* 2013). In a mouse model of pneumococcal meningitis, treatment with ceftriaxone plus HMGB1 antagonist therapy was associated with a significant amelioration of brain pathology compared to ceftriaxone alone (Hohne *et al.* 2013).

CSF biomarkers may become increasingly useful diagnostic markers for other CNS disorders, in particular Alzheimer's disease (AD) (Andreasen *et al.* 2001, Hansson *et al.* 2006, Landau *et al.* 2010, Johansson *et al.* 2011), where they can act as a surrogate for inaccessible brain tissue. CSF is mainly produced by the epithelium of the choroid plexus and acts both as a mechanical cushion and as a circulation system for the brain, carrying peptides secreted in one region to another and eliminating waste from the brain and spinal cord into the blood circulation (Sakka *et al.* 2011). The physiology, structure and function of the choroid plexus, that secretes and purifies the CSF, have recently been updated (Spector *et al.* 2015a). The functions are manifold including generating intracranial pressure, maintaining CSF ion homeostasis, and providing micronutrients, proteins and hormones for neuronal and glial development, maintenance and function. The choroid plexus and CSF have been termed "the third circulation" and perform many functions comparable to the kidney including nourishing the brain and maintaining homeostasis (Spector *et al.* 2015b). Ultimately CSF is re-absorbed back into the blood via the venous system. There are small but consistent differences between blood and CSF. CSF has higher concentrations of sodium and chloride, but lower concentrations of potassium, magnesium, bicarbonate, glucose, amino acids and uric acid (Segal 1993). CSF is also virtually depleted of proteins, reflecting the tight intracellular junctions of the BBB. It may be reasonable to assume that the CSF can act as a surrogate for brain tissue as CSF is in direct contact with nervous tissue and as such, its composition is affected by biochemical changes occurring in the brain (Anoop *et al.* 2010).

However, it is unclear whether peripheral blood can act as a surrogate for CSF sampling in some neurological conditions. Indeed, in the case of AD, the potential of blood-based AD markers has yet to be further evaluated. Conflicting data regarding blood A β levels, which are decreased in the CSF of AD patients, have been reported; levels that are elevated, reduced or sometimes even unchanged have been observed (Borroni *et al.* 2010). One example where this has been studied extensively is neurological infection of the human immunodeficiency virus (HIV), an event that occurs almost universally following systemic HIV infection and can impact upon neurological function. CSF biomarkers have proven effective in the diagnosis and assessment of treatment effects in this disorder however, the dilution or degradation of CNS metabolic traces in the blood have largely prevented blood from providing useful or direct information about brain pathobiology in HIV (Price *et al.* 2013). In the demyelinating inflammatory disorder multiple sclerosis, exploratory studies have reported increased IL-17 levels in CSF but the timing of detection in serum is very variable, depending on disease stage and treatment modality/effect (Balasa *et al.* 2013, Huber *et al.* 2014, Luchtman *et al.* 2014, Babaloo *et al.* 2015). Successful correlation between compartments has not been demonstrated.

Conclusions

In summary, the findings of this study comparing CSF and serum show that no correlation in HMGB1 levels exists between bio-compartments in any condition examined, regardless of the integrity of the BBB. It also identifies for the first time that HMGB1 levels are normal in IIH, despite recent reports suggesting a possible inflammatory pathogenesis. Elevated serum and CSF HMGB1 concentrations were identified in RE, where it may be contributing to the pathogenesis. Lastly, elevated HMGB1 in both serum and CSF in neurological infection support the growing literature that excessive host inflammation is relevant to the pathogenesis of meningitis and encephalitis, contributing to delayed resolution of inflammation and a vicious cycle of inflammation-induced cell injury. HMGB1 may represent a potential therapeutic target for novel adjuvant therapies in specific patients with neurological infection and inflammatory conditions, but further work which allows for biomarker-based stratification of patients into those likely to benefit or not from such therapies is needed.

Summary points

- No correlation in HMGB1 levels exists between bio-compartments, regardless of neuropathology or BBB integrity.
- CSF HMGB1 is normal in IIH.
- HMGB1 is elevated in both peripheral blood and CSF in RE and NII.

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Disclosure statement

None of the authors declare any conflict of interest.

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