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Short Communication

The effect of systemic inflammation on human brain barrier function

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ABSTRACT

The blood-brain barrier (BBB) plays an important role in the clinical expression of neuropsychiatric symptoms during systemic illness in health and neurological disease. Evidence from *in vitro* and preclinical *in vivo* studies indicate that systemic inflammation impairs blood-brain barrier function. In order to investigate this hypothesis, we evaluated the association between systemic inflammatory markers (leucocytes, erythrocyte sedimentation rate and C-reactive protein) and BBB function (cerebrospinal fluid/serum albumin ratio) in 1273 consecutive lumbar punctures. In the absence of cerebrospinal fluid (CSF) abnormality, systemic inflammation did not affect the CSF/serum albumin ratio. When CSF abnormality was present, systemic inflammation significantly predicted the CSF/serum albumin ratio. Amongst the systemic inflammatory markers, C-reactive protein was the predominant driver of this effect. Temporal analysis in this association study suggested causality. In conclusion, the diseased BBB has an increased susceptibility to systemic inflammation.

1. Introduction

The blood-brain barrier (BBB) is a highly regulated interface between the brain and the rest of the body (Abbott et al., 2010). BBB permeability is an important aspect of several important neurological conditions such as multiple sclerosis and Alzheimer's disease (Varatharaj and Galea, 2016). Recent compelling evidence suggests that BBB integrity is an important player in the expression of neuropsychiatric manifestations of circulating anti-NMDA antibodies (Hammer et al., 2014). It is interesting to note that preceding systemic infections are very common in neuronal surface antibody-mediated encephalitis (Irani et al., 2010); the aetiology of these prodromal infections are very varied, arguing against molecular mimicry, and perhaps more supportive of a generic effect of systemic infection on the BBB.

A number of *in vitro* and *in vivo* preclinical studies have shown that inflammatory challenge results in an increase in BBB permeability. This effect appears to be a feature of the BBB, unrelated to the type of inflammatory trigger since it has been observed in a wide variety of experimental settings including lipopolysaccharide, poly I:C, bacteria, viruses, chemically-induced inflammation, anaphylaxis and cytokines (Varatharaj and Galea, 2016). The main mechanisms underlying this phenomenon are tight junction changes and increased vesicular transport, but re-induction of fenestrae, endothelial cell damage, denudation of the glycocalyx, degradation of the glia limitans and astrocyte changes also play a role (Varatharaj and Galea, 2016).

While the effect of inflammatory challenges on human BBB permeability has been demonstrated *in vitro* using human brain microvessel endothelial cells (Varatharaj and Galea, 2016), the relevance of this large body of preclinical literature to the *in vivo* human situation remains to be shown. In this study, we set out to study the effect of systemic inflammation on human BBB permeability *in vivo* by examining the association between a panel of systemic inflammatory markers and the CSF/serum albumin quotient in 1273 consecutive unselected lumbar punctures. The CSF/serum albumin ratio (Q_{alb}) is a widely accepted indicator of blood–CSF barrier function (Thompson, 2005). Since albumin is not synthesised in the brain, the ratio of CSF to serum albumin concentration is a quotient representing the fraction of serum albumin diffusing into the CSF, independent of serum concentration. Changes in serum albumin do not occur rapidly; hence CSF albumin can be assumed to be in constant equilibrium with serum as a function of BBB permeability. Immunoglobulins and cytokines are not suited for this type of study since they may be secreted intrathecally by blood-derived cells transmigrating into the brain. The effectiveness of Q_{alb} for measurement of BBB function has been demonstrated by studies with radiolabelled albumin (Tourtellotte et al., 1980).

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2. Methods and materials

2.1. Data collection

Data was collected by retrospective review of the medical records of 1273 individuals having lumbar puncture with Q_{alb} assessment at Southampton General Hospital, Hampshire, UK in a three year period (2011–2013), during a service evaluation, with institutional approval. The white cell count, neutrophil, lymphocyte, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) measurements within a five day period centred around the lumbar puncture were recorded (Fig. 1).

We additionally collected data on: age, CSF total protein, CSF glucose, CSF red and white blood cells, and oligoclonal bands. Cases were excluded if <16 years of age and/or had a CSF red blood cell (RBC) count >127 cells per microlitre. Since CSF total protein concentration rises by 1 mg/dl for every 100 red blood cells/μL that enter the CSF during traumatic lumbar puncture (Blakeley and Irani, 2009), the CSF RBC count threshold was determined by calculating the maximum CSF RBC count which did not change any of the Q_{alb} values in the dataset.

CSF and blood were collected in sterile polypropylene tubes (Sterilin, Newport, Gwent, UK) and Vacutainers (Becton Dickinson, Plymouth, UK) respectively. CSF volume was not available. Samples were analysed on the same day, except for isoelectric focussing in which case samples were kept at 4 °C and batch analysed within one week. Blood counts were performed on a Sysmex XE-2100 automated hematology system. ESR was performed on a Vitech Starrsed system using the Westergren sedimentation method. CRP, albumin, protein and glucose were assayed on a Beckman Coulter AU5800 automated system. CSF cell counts were performed manually using a modified Fuchs-Rosenthal haemocytometer. Oligoclonal band assessment was performed manually using isoelectric focussing on CSF/serum pairs.

2.2. Data preparation

Data preparation was performed in Excel v14. Cases were identified as having normal findings if the following conditions were met: CSF total protein <500 mg/L, CSF glucose >2/3 serum glucose, white blood cells ⩽5 cells/μL, polymorphs were absent, and there was no evidence of intrathecal synthesis of oligoclonal immunoglobulin G. Single systemic inflammatory variables included total leucocytes, neutrophils, lymphocytes, ESR and CRP; a composite variable (Inf_{Blood}) integrating all these indices was created to reflect systemic inflammation. To reflect central nervous system inflammation, the variable Inf_{CSF} was derived from the CSF white cell count. The derivation of Inf_{Blood} and Inf_{CSF} is detailed in Supplementary Methods.

2.3. Statistical analysis

Statistical analysis was performed in SPSS v22. Since data was non-parametric, Mann-Whitney test was used for group comparisons. General linear model was used for analysis of covariance. Q_{alb}, Inf_{CSF}, and systemic inflammatory variables were logarithmically transformed. Multivariate linear regression was used to examine the association of systemic inflammatory markers with Q_{alb}. A significant difference from the null hypothesis was assumed at $p < 0.05$.

3. Results

3.1. Characteristics of cases

Out of a total of 1273 lumbar punctures, 829 cases were identified with a full CSF analysis including Q_{alb} and at least one systemic inflammatory variable result available within the 5 day period centred around the lumbar puncture. The characteristics of this study population are shown in Tables 1 and 2. Median CRP was 4 mg/L,

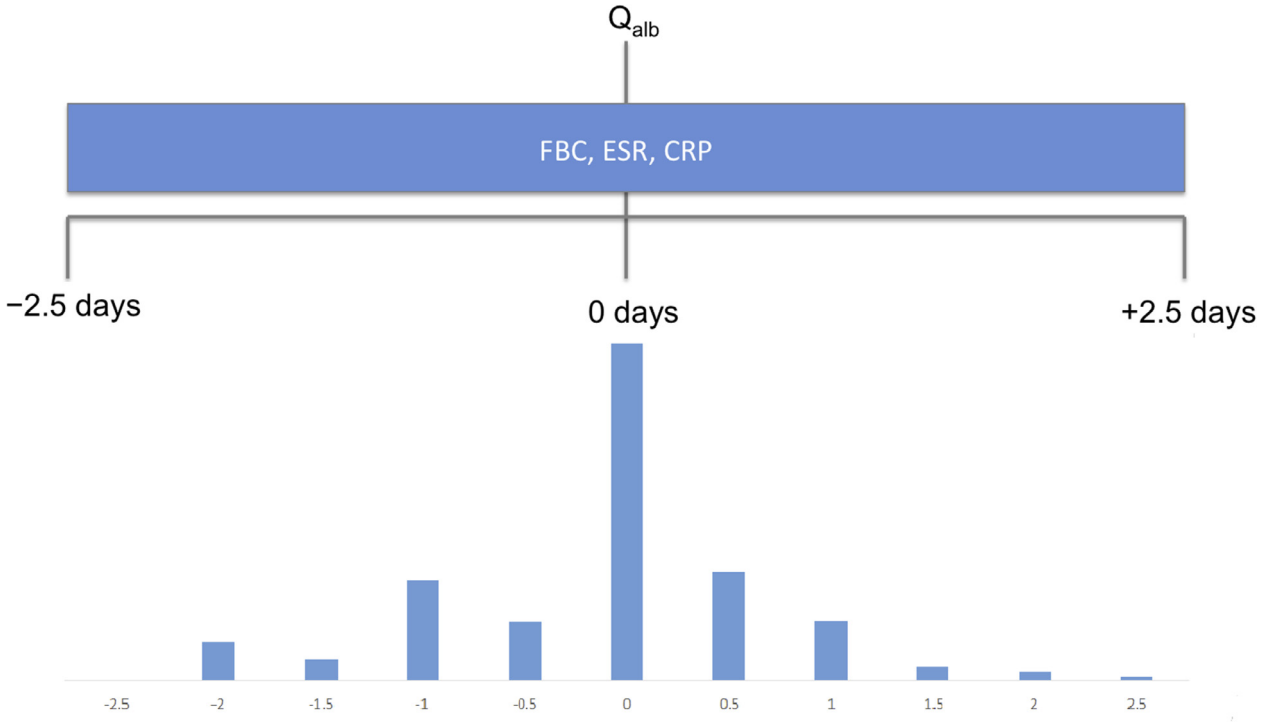

Fig. 1. Study design. FBC: full blood count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.