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Cisterno-lumbar gradient of complement fractions in geriatric patients with suspected normal pressure hydrocephalus

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ABSTRACT

Background: The complement system is a functional link between the innate and adaptive immune system and present in all compartments of the body. The composition of the cerebrospinal fluid (CSF) differs between the ventricular, cisternal and lumbar space. Usually, concentrations of blood-derived CSF proteins increase from ventricular to lumbar fractions.

Methods: In 20 geriatric patients with suspected normal pressure hydrocephalus (NPH) [13 women, 7 men, age 80.5 (75/85) years; median (25th/75th percentile)] a lumbar spinal tap of 40 ml was performed, and 10 ml of serum was drawn. CSF, sequentially collected in 8 fractions of 5 ml (1st fraction: lumbar CSF; 8th fraction: cisterna magna-near CSF), was analyzed for complement protein C3, and the activation products C3a and sC5b-9 by enzyme immunoassay.

Results: The concentrations of the complement factors measured in fractions 1 and 8 of each individual patient were strongly correlated: C3 (Spearman's rank correlation coefficient $r_S = 0.75$, $p = 0.0002$); C3a ($r_S = 0.93$, $p < 0.0001$); sC5b-9 ($r_S = 0.64$, $p = 0.002$). CSF complement concentrations were lower in the cistern-near fraction 8 than in the lumbar fraction 1 (C3: $p = 0.005$; C3a: $p = 0.0009$; sC5b-9: $p = 0.0003$, Wilcoxon signed rank test). The concentrations of complement factors in CSF were two orders of magnitude lower than those in serum. C3 levels in the lumbar CSF strongly correlated with the lumbar CSF/serum albumin concentration quotient (Q_{Alb}) as a measure of the functionability of the blood-CSF barrier and the velocity of CSF flow ($r_S = 0.84$, $p < 0.0001$) suggesting diffusion of C3 from blood to CSF. The lumbar and cistern-near concentrations of C3a did not significantly correlate with Q_{Alb} ($r_S = 0.26$) pointing to a local conversion of C3 to C3a. The lumbar concentrations of sC5b-9 moderately correlated with Q_{Alb} ($r_S = 0.62$, $p = 0.004$). Plotting the CSF/serum quotient of C3 and sC5b-9 versus the Q_{Alb} revealed an approx. 50% local synthesis of C3, but a strong production of sC5b-9 in the CNS.

Conclusions: The increase of the complement concentrations from cisternal to lumbar CSF and the strong correlation of C3 with Q_{Alb} suggest that (1) a substantial portion of complement C3 in CSF originates from blood and (2) the complement system is mildly activated in the CSF of NPH patients.

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1. Introduction

The complement system consists of several soluble and membrane-bound proteins. The bulk of most components of the complement system, including the factors C3 and C4, are produced in the liver [1]. C3 is also synthesized/secreted by neutrophilic granulocytes, mast cells, monocytes, macrophages, dendritic cells, T lymphocytes, neurons and microglial cells [1–5]. The complement cascade is activated by the classical, lectin or alternative path leading to the generation of effector molecules that 'complement' the ability of antibodies and phagocytes to eliminate microbial intruders (via opsonization: C3b, iC3b), promote inflammation (via anaphylatoxins: C3a and C5a) and lyse susceptible pathogens (via the C5b-9 membrane attack complex) [6]. As a functional bridge between the innate and adaptive immune response complement enables an integrated host defense to pathogenic challenges and is involved in the removal of debris [1,7]. Some complement activation products are involved in brain development and synapse formation [6,8]. C3 is a central molecule of all three activation pathways. C3a and C3b are produced by proteolytic cleavage of C3 after activation of the complement system [9]. C3a causes histamine release and attracts granulocytes. The terminal complement complex or membrane attack complex (C5b-9) is generated as a consequence of activation of the complement system by either the classical, lectin, or alternative pathways. It mediates an irreversible cell membrane damage by forming pores [10].

Complement activation drives chronic, non-resolving inflammation in neurodegenerative and age-related disorders [6]. Different complement factors including C3 are elevated in the CSF in inflammatory diseases of the CNS including multiple sclerosis [5]. Moreover, complement parameters can be used as biomarkers of disease activity and treatment response in multiple sclerosis [5]. Complement-mediated inflammation also plays an important role in neurodegenerative brain diseases, including Alzheimer's and Parkinson's disease [11]. Alzheimer's disease is associated with a reduction of the density of synapses, and the complement system appears to be involved in synaptic elimination [12]. In the CSF of patients with Alzheimer's disease elevated levels of C3 and C4 were found compared to patients with mild cognitive impairment without progression to Alzheimer's disease [12]. C3 concentrations in CSF of Alzheimer patients were correlated with the severity of cognitive impairment as quantified by Mini-Mental State Examination scores [13]. Moreover, non-fibrillar amyloidβ(1–42) induced dose-dependent activation of C4 [14].

Normal pressure hydrocephalus (NPH) is a syndrome characterized by an impaired flow of CSF from the subarachnoid space into blood. Most of the time, CSF pressure is normal, but often an increased CSF outflow resistance or/and abnormal intracranial pulsatility parameters can be recorded [15]. Clinically, this syndrome is characterized by the triad gait disturbance, bladder incontinence, and dementia. Cerebral imaging shows ventricular dilation, whereas the subarachnoid space on both sides of the superior sagittal sinus is comparatively small [16]. As a consequence of the enlargement of the ventricles and tightness of the sulci in the vicinity of the superior sagittal sinus, the callosal angle is decreased [17]. Response to surgical shunt treatment is difficult to predict [18]. At autopsy or in brain biopsy specimens, many NPH patients show pathological findings suggesting neurodegenerative comorbidities. Morphological findings of Alzheimer's dementia (AD) were seen in 30 to 75% of patients with clinically diagnosed NPH at autopsy or after a biopsy, i.e., a large subgroup of patients had overlapping clinical features of AD and NPH [16,18–21]. A spinal tap of 40 ml is a relatively insensitive, but minimally-invasive and well-tolerated procedure to assess whether patients may benefit from ventriculoperitoneal shunting. Sensitivity of CSF removal is increased by lumbar CSF drainage for approx. 3 days, and gait improvement immediately following lumbar CSF drainage is considered the best prognostic indicator of a positive shunt outcome [22].

The composition of the cerebrospinal fluid (CSF) diverges between ventricular, cisternal and lumbar fractions. Usually, concentrations of blood-derived proteins increase and of brain-derived proteins decrease from ventricular to lumbar fractions [23–25]. Tau protein (Tau), Tau protein phosphorylated at position 181 (pTau), neuron-specific enolase and S-100 protein in general have higher ventricular than lumbar CSF concentrations. Conversely, compounds originating from the blood or the meninges (e.g., albumin, β-trace protein and cystatin C) exhibit higher lumbar than ventricular concentrations [23,24,26,27]. The distribution of leukocytes and proteins in the CSF space can be particularly inhomogeneous, when the CSF spaces are obstructed by pus or when the CSF production is decreased for other reasons. Compounds with a low molecular mass including lactate are distributed more equally among the different regions of the CSF space even in this condition [28].

In normal CSF, complement concentrations usually are low, and it is unknown whether there are ventriculo-cisterno-lumbar concentration differences. When NPH is suspected, 40 ml of CSF are removed, and the gait and cognitive functions are monitored before and after the spinal tap. These 40 ml were collected in fractions of 5 ml [24]. This enabled us to assay the first and the 8th fraction to determine whether complement factors are evenly distributed in the CSF, or whether a gradient exists between cistern-near and lumbar fractions.

2. Methods

2.1. Patients

A diagnostic or therapeutic lumbar spinal tap of 40 ml was performed in 20 patients [13 women, 7 men, age 80.5 (75/85) years; median (25th/75th percentile)] with suspected or documented NPH as part of differential diagnosis or therapy. 18 patients suffered from cognitive impairment (Mini Mental Status Examination ≤ 26; normal: 27–30). In 19 of the 20 patients an abnormal gait, and in 15 patients bladder incontinence were documented. All patients had an abnormal cranial computer tomography (CCT) scan: 20 had wide ventricles, 18 patients had crowding of the gyri at the vertex with small sulci close to the superior sagittal sinus, and 17 patients had a corpus callosum angle below 90°. 6 patients (including the patient who received two lumbar punctures) clearly improved after the spinal taps, and in 3 patients a small clinically non-significant improvement of either gait or cognition was noted. None of the patients studied had a ventriculoperitoneal shunt or external ventriculostomy. The treating physicians recommended ventriculoperitoneal shunting to the 6 patients with clear clinical improvement after the spinal tap. Because of their advanced age, all patients and/or their relatives refused surgery as a consequence of the possible risks of this procedure.

The study was approved by the Ethics Committee of the Medical Faculty of the Georg-August University Göttingen, Germany, and each participant gave written informed consent to participate in this study.

2.2. CSF sampling and analysis

The CSF was sequentially collected in 8 fractions of 5 ml in polypropylene tubes and transported to the laboratory within 1 h for analysis. 10 ml of blood was taken immediately before or after the lumbar puncture, was allowed to clot and centrifuged, and serum was collected. Patients with intracranial hemorrhage or artificial blood contamination of the CSF were excluded in order to avoid samples with unphysiological entry of complement factors from blood into the CSF sample and activation of the complement cascade. One set of CSF fractions was available from 19 patients. Two sets of CSF fractions drawn at an interval of 2 months were available from one patient with documented normal pressure hydrocephalus, who underwent repeated lumbar punctures as part of his therapy. From this patient, only the measurements of the first spinal tap were used for statistical comparisons.