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Questioning the role of actinfree Gc-Globulin as actin scavenger in neurodegenerative central nervous system disease: Relationship to S-100B levels and blood–brain barrier function

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ABSTRACT

Introduction: Preliminary studies report on significantly higher levels of the major cytoskeleton protein actin in CSF of patients with neurodegenerative conditions and that the dynamics of these levels obviously correlates with disease progression and clinical disability. One of the primary functions of actinfree Gc-Globulin is to bind and neutralize extracellular monomeric actin, released into the circulation by necrotic or ruptured cells, and thus ameliorating the clinical outcome in situations of severe organ damage.
Aim and methods: This is the first study to investigate actinfree Gc-Globulin and S100-B levels (as reliable marker of neurodegeneration) in paired CSF and serum samples of patients with multietiological CNS diseases.
Results: 42% of all patients with CNS disease displayed serum concentrations of actinfree Gc-Globulin above the established reference range. CSF concentrations of actinfree Gc-Globulin and S100-B were positively correlated with the severity of blood–brain barrier (BBB) dysfunction. Furthermore, patients with severe BBB dysfunction presented a higher percentage of intrathecal synthesis of actinfree Gc-Globulin compared to patients with mild to moderate dysfunction and to patients with normal BBB function. Representative longitudinal data from selected patients demonstrated an inverse behaviour of actinfree Gc-Globulin and S100-B CSF concentrations, suggesting a consumption of the actin scavenger capacity of Gc-Globulin in times of increased neuronal damage. This presumption was supported by the fact that those conditions associated with a severe neuronal damage, in particular CNS trauma, and highest S100-B concentrations simultaneously displayed lowest actinfree Gc-Globulin levels, and thus residual actin binding capacity of Gc-Globulin.
Conclusion: In summary, our data propose a function of actinfree Gc-Globulin also in the clearance of actin filaments from CSF of patients with neuronal damage. However, active recruitment of hepatic derived actinfree Gc-Globulin to the site of CNS injury is not observed. Much more, BBB leakage enables extraneuronally synthesized actinfree Gc-Globulin to extent its scavenger capacity for actin also to the subarachnoidal space. Furthermore, intrathecal synthesis of actinfree Gc-Globulin seems to be increased in patients with severe neurodegeneration.

1. Introduction

The axonal cytoskeleton of the neuron is a highly regulated system that plays a central role in maintaining the integrity of axons. The physiological functions of axonal cytoskeleton are dependent upon several interconnected filaments that primarily consist of actin microfilaments (6 nm in diameter), L-neurofilaments (10 nm) and microtubules (23 nm) [1]. Collectively, these proteins control axonal shape and caliber, maintain axonal transport of nutrients and organelles, define specialized membrane domains and regulate growth and focal adhesions [2–4]. Actin, one of the main proteins of the cytoskeleton, also plays an important role in axonal growth and guidance, in the formation and elongation of neuritis and in a variety of other biological responses [5].

Preliminary reports suggest that some cytoskeletal proteins may be detected in the cerebrospinal fluid (CSF) from patients with neurodegenerative conditions and that these proteins may serve as useful confirmatory markers of neurodegeneration or progression of CNS disorders [6]. For example, high levels of actin or its regulatory proteins have been detected in the CSF form patients with Alzheimer's disease [7,8]. Furthermore, significantly higher levels of the three major cytoskeleton proteins described above have recently been found in the CSF of MS patients compared to healthy and neurological controls and were obviously associated with progressive disease and clinical disability [9].

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Gc-Globulin is known as a multifunctional plasma protein of a molecular mass of 51–58 kD, which belongs to the albumin gene family predominantly synthesized in the liver. Its primary function is to serve as a carrier protein for vitamin D and its plasma metabolites. Furthermore, it is able to scavenge and neutralize extracellular monomeric actin (G-actin) released by necrotic or ruptured cells [10–15]. Thus, low total and actinfree Gc-Globulin concentrations, the latter being an index of residual actin-scavenging capacity, have been demonstrated to be prognostic markers in situations of severe organ damage, such as fulminant hepatic failure [16–19], acetaminophen (paracetamol) overdose [20], multiple trauma [21], and multiple organ failure [22–24].

This is the first study to investigate actinfree Gc-Globulin levels in CSF as well as in paired serum samples of patients with multi-etiological CNS diseases. The investigation is based on the hypothesis that the actin scavenging function of actinfree Gc-Globulin may be of considerable importance in ameliorating the overall clinical outcome of CNS pathologies, possibly contributing to reduce the onset of secondary brain damage.

2. Materials and methods

2.1. Study group

A total of 155 patients, 91 male, 64 female, age 52±21 years (mean±SD, range 1–86) with multietiological CNS diseases, presenting at the Neurological Emergency Unit, RWTH University Hospital Aachen, Germany, were included in this study.

At admission, advanced surgical residents under consultant supervision carried out a brief neurological examination (cranial nerves, as well as strength and sensation in the arms and legs) and a neuropsychological assessment according to the Glasgow Coma Scale requirements [25]. Those patients with additional non-CNS disorders or under current medication use were excluded from the study.

2.2. Cerebrospinal fluid and serum sample collection

Written informed consent was obtained from each participant or his/her spouses, and the study was approved by the local ethics committee. Upon admission, lumbar puncture was performed between the fourth and fifth lumbar vertebrae with the patient in the lateral decubitus position. The first 1 ml of CSF was discarded and the subsequently obtained CSF collected and aliquotted into polystyrene tubes closed with screw-caps (Sarstedt AG, Nümbrecht, Germany). Peripheral venous blood samples were taken at the time of lumbar puncture. Serum was separated at 4000 *g* after clot-retraction and, such as CSF, stored at −80 °C until further analyses were conducted.

Analysis of the immunoglobulin subclasses and albumin in CSF and serum was performed nephelometrically by a Siemens Healthcare BN2 Nephelometer Analyzer using commercially available kits provided by the company (Siemens Healthcare, Erlangen, Germany). Total protein, glucose and lactate were measured using the Roche Modular Analytics System (Roche, Mannheim, Germany).

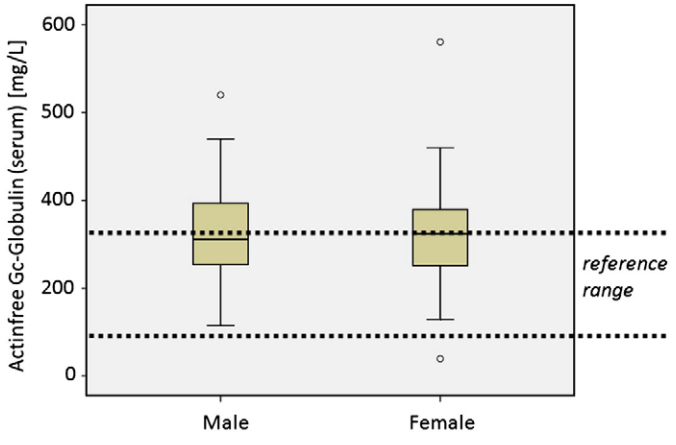

Fig. 1. Comparative demonstration of actinfree Gc-Globulin serum concentrations in male and female patients with multietiological CNS pathologies: No significant difference between the genders was observed, but 41% of the patients with neurodegenerative conditions displayed serum concentrations above the established reference range. Box plots are displayed, where the central line indicates the median per group. The box represents 50% of the values and horizontal lines show minimum and maximum. The dotted line depicts the reference range as provided by the respective manufacturer given in Materials and methods.

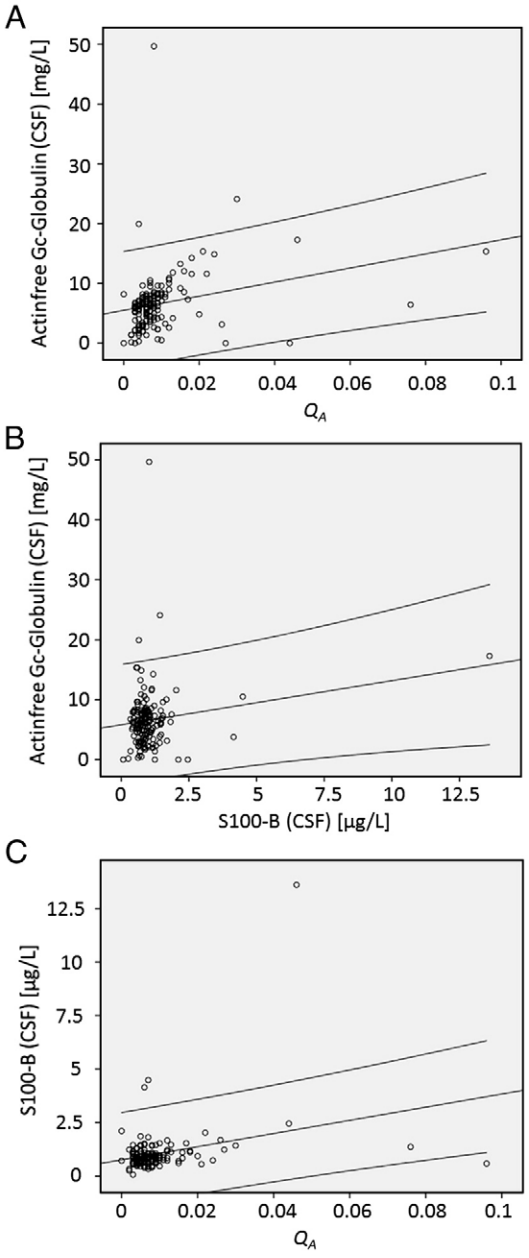

Fig. 2. Association of concentrations of actinfree Gc-Globulin and S100-B in CSF and their relation to the integrity of the blood–brain barrier: Overall, CSF concentrations of actinfree Gc-Globulin (A) and S100-B (C) are positively correlated with the severity of BBB dysfunction [as determined by the ratio of CSF/serum albumin (Q_A)], as well as to each other (B). Shown are range diagrams, regression lines and individual 95% confidence intervals.

2.3. Quantification of S-100B in serum and cerebrospinal fluid

S-100B levels in CSF and serum were analyzed by means of a fully automated electrochemiluminescence assay (ECLIA, Roche Elecsys) with reference values for serum set at <11 µg/L, according to the manufacturer's instructions.

2.4. Quantification of actinfree Gc-Globulin in serum and cerebrospinal fluid

Actinfree Gc-Globulin levels in serum were determined by ELISA (Kit 034, AntibodyShop, Gentofte, Denmark). Reference values for serum ranged from 92 to 332 mg/L, as determined by the manufacturer. Intra-assay (inter-assay) coefficient of variation (CV) ranged from 3.5–3.6% [*n*=6] (3.1–7.9% [*n*=8]).