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Preoperative protein profiles in cerebrospinal fluid in elderly hip fracture patients at risk for delirium: A proteomics and validation study

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ABSTRACT

Background: A neuroinflammatory response is suggested to play an important role in delirium, a common complication in older hospitalized patients. We examined whether hip fracture patients who develop postoperative delirium have a different proteome in cerebrospinal fluid (CSF) prior to surgery.
Methods: Patients (≥75 years) were admitted for hip fracture surgery. CSF was collected during spinal anaesthesia; proteins were separated using gel electrophoresis and identified with mass spectrometry. We compared the proteome of patients with and without postoperative delirium. Findings were validated in an independent, comparable cohort using immuno-assays.
Results: In the derivation cohort 53 patients were included, 35.8% developed postoperative delirium. We identified differences in levels of eight CSF proteins between patients with and without subsequent delirium: complement factor C3, contactin-1, fibulin-1 and I-beta-1,3-N-acetylglucosaminyltransferase were significantly lower in patients with postoperative delirium, while neural cell adhesion molecule-2, fibrinogen, zinc-α-2-glycoprotein and haptoglobin levels were significantly higher. In the validation cohort 21.2% of 52 patients developed postoperative delirium. Immuno-assays confirmed contactin-1 results although not statistically significant. Complement factor C3 was significantly higher in patients with postoperative delirium.
Conclusion: Our results show the complexity of pathophysiological mechanisms involved in delirium and emphasizes the need of independent validation of findings.
General significance: This study highlights the challenges and inconsistent findings in studies of delirium, a serious complication in older patients. We analysed proteins in CSF, the most proximal fluid to the brain. All patients were free from delirium at the time of sampling.

Abbreviations: 2D-DIGE, Two-dimensional difference gel electrophoresis; b3GNT3, N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase; C3, Third component of the complement system; CSF, Cerebrospinal fluid; ELISA, Enzyme linked immunosorbent assay; IQR, Interquartile range; LC-MS/MS, Liquid chromatography–mass spectrometry; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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1. Introduction

Delirium is the most common complication in hospitalized older patients and is known to be associated with both short- as well as long-term detrimental outcomes [1]. The exact pathophysiological mechanisms in delirium and its associated poor outcomes are still unknown, but a neuroinflammatory response has been suggested to play a role [2]. Neuroinflammation may arise in response to peripheral insults, such as infection, surgery and fracture [3]. The communication

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pathways between periphery and the brain may lead to activation of microglia, the innate immune cells of the central nervous system. In response to activation, microglia release a range of inflammatory mediators, influencing neuronal function and possibly inducing delirium in susceptible individuals [4]. Aging and neurodegenerative disease are well known predisposing risk factors for delirium [3] and are both accompanied by a pro-inflammatory state [4]. Older people are especially vulnerable, since impaired cholinergic inhibition can elicit increased microglia activation and inflammation, which may result in neuronal damage [4].

Previous research revealed that hip fracture patients who develop postoperative delirium show lower preoperative cerebrospinal fluid (CSF) concentrations of anti-inflammatory cytokines compared to patients who do not develop postoperative delirium [5]. This finding is consistent with a role for a dysfunctional neuroinflammatory response in delirium. However, inflammation is a highly complex and dynamic process in which many different effectors interact. The state of inflammation is not likely to be dependent on the net effect of individual cytokines only, but on the balance between numerous pro- and anti-inflammatory mediators in the central nervous system [6].

A recent study assessed the CSF proteome in patients with delirium, and found 16 dysregulated proteins, including acute phase proteins like complement C3, fibrinogen and haptoglobin, providing supplementary evidence that inflammation is involved in delirium [7].

We hypothesized that elderly hip fracture patients who develop postoperative delirium, have specific CSF protein profiles prior to operation, as compared to patients who do not develop postoperative delirium. Differences in the CSF proteome can be assessed using two-dimensional difference gel electrophoresis (2D-DIGE), a technique frequently used in research on potential CSF biomarkers in neurological diseases such as Parkinson's and Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis [8–11]. We used this technique to compare the pre-operative CSF proteome of patients with postoperative delirium with that of patients without delirium after surgery. Proteomic findings were validated in a separate, comparable cohort with immunoassays.

2. Methods

The study was approved by the medical ethical committees of both hospitals where patients were included, and was conducted in accordance with the guidelines of Good Clinical Practice. All patients gave written informed consent.

2.1. Patients

CSF for proteomic analysis (derivation cohort) was collected in a teaching hospital in Alkmaar, The Netherlands between March 2008 and March 2009. Patients participated in a double blind randomized study comparing effectiveness of taurine versus placebo in reducing morbidity and 1-year mortality in elderly hip fracture patients [5]. Patient recruitment and study procedures for the proteomics study have been described in detail elsewhere [5]. In brief: all patients of 75 years or older who were admitted for surgical repair of a hip fracture were assessed for eligibility. Patients were excluded if they had no acute trauma or a pathological fracture, received total hip prosthesis, had contraindications regarding the administration of taurine (that is, renal failure defined as a creatinine clearance <30 ml/min), or did not provide consent. The Informant Questionnaire on Cognitive Decline Short Form (IQCODE-sf) was completed by the primary caregiver. Cognitive impairment was defined as a score of 3.4 or higher on this questionnaire or a record of dementia in the medical history.

The main outcome was postoperative delirium, defined according to the Confusion Assessment Method [12]. The presence of delirium was assessed daily from admission until the fifth postoperative day. To assess risk factors for delirium instead of markers of delirium, all patients with preoperative delirium were subsequently excluded from analysis. Because all participants were at high risk of delirium, they all received routine care with prophylactic treatment of 0.5 mg haloperidol, three times daily from admission until postoperative day three, unless contraindications were present [13].

To check whether preoperative protein differences between groups represent reproducible, clinically relevant changes in patients with postoperative delirium, we performed a validation study in an independent cohort. CSF for validation, using immuno-assays, was collected in a teaching hospital in Amsterdam, The Netherlands between March 2012 and April 2014. Patients were participants in a prospective descriptive cohort study assessing incidence, prevalence and pathophysiology of delirium. All patients of 65 years or older who were admitted for surgical repair of a hip fracture were checked for eligibility. Patients were excluded if they were discharged or died within 48 h after inclusion, did not receive spinal anesthesia or if no communication was possible (e.g. aphasia, language barrier, deafness). In the validation cohort, the IQCODE-sf was also completed by the primary caregiver. To make sure patients included in the validation cohort would be comparable to those in the derivation cohort, we subsequently excluded patients who were younger than 75 years, had no acute trauma or a pathological fracture, received total hip prosthesis, or had preoperative delirium. The main outcome was postoperative delirium, assessed daily by trained nursing staff using the delirium observation screening scale [14]. In case of a score of 3 or higher, a psychiatrist was consulted to assess delirium as defined in the fourth edition of the Diagnostic and Statistical manual of Mental disorders [15]. If necessary, patients received treatment with haloperidol according to local clinical practice.

2.2. CSF samples

CSF samples for the proteomics and validation studies were collected according to the same procedures: during cannulation for the introduction of spinal anaesthesia, always prior to administration of any anaesthetic. Of each patient CSF was collected in polypropylene tubes which were transported to the laboratory within 15 min after withdrawal. Upon arrival at the laboratory the CSF samples were centrifuged, aliquoted into polypropylene tubes, and stored at −80 °C.

2.3. 2D-DIGE

To analyse the CSF proteome, we adapted a protocol previously described in more detail [16]. In brief: of each patient, 2 ml of CSF sample was spun down and the supernatant was precipitated in 80% action. The protein pallet was resuspended and the protein concentration was estimated by the Bradford method using bovine serum albumin as standard (Bio-Rad, Hercules, CA, USA). Protein extracts were labelled using the fluorescent cyanine dyes developed for 2D-DIGE technology (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Protein extracts (40 μg) were labelled with 333 pmol of fluorescent dye (Cy2, Cy3 or Cy5). Samples were randomly labelled with Cy3 or Cy5 to avoid dye related bias. An internal control sample, to avoid inter-gel variations, was created by pooling 20 μg of each protein sample and was labelled with Cy2. Three protein samples (Cy3, Cy5 and Cy2) were pooled and passively loaded onto 24 cm pH 3–10 NL strips (GE Healthcare), followed by isoelectric focussing using a manifold-equipped IPGphor IEF unit (GE Healthcare). Second dimensional SDS-PAGE was performed on hand-cast 12% SDS-PAGE gels using low fluorescence glass plates. Electrophoresis was carried out at 1 W per gel until completion using an Ettan DALT-12 unit (GE Healthcare). Gels were scanned with a Typhoon 9410 imager (GE Healthcare). Spot detection was performed with DeCyder 7.0 software (GE Healthcare): the Cy2, Cy3 and Cy5 images for each gel were merged, spot boundaries are automatically detected and spot volumes are calculated. This analysis was used to calculate