CEREBROSPINAL FLUID CONCENTRATION OF NEUROGRANIN IN HIP FRACTURE PATIENTS WITH DELIRIUM

Nathalie Bodd **Halaas**[a,b,c]; Henrik **Zetterberg**[d,e,f,g]; Ane-Victoria **Idland**[a,c]; Anne-Brita **Knapskog**[h]; Leiv Otto **Watne**[a]; Kaj **Blennow**[d,e]

RUNNING TITLE: Cerebrospinal fluid neurogranin in delirium

AFFILIATIONS

Nathalie Bodd Halaas

[a] Oslo Delirium Research Group, Department of Geriatric Medicine, Oslo University Hospital, Oslo, Norway

[b] Research Group for Lifespan Changes in Brain and Cognition, Department of Psychology, University of Oslo, Oslo, Norway

[c] Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[d] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[e] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[f] Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, United Kingdom

[g] UK Dementia Research Institute at UCL, London, United Kingdom

[h] Department of Geriatric Medicine, Oslo University Hospital, Oslo, Norway

Corresponding author:

Nathalie Bodd Halaas, University of Oslo, Campus Ullevål, Department of Geriatric Medicine
PB 4956 Nydalen
Telephone: + 47 98640857
E-mail: nathaliebodd@gmail.com

Email co-authors

Leiv Otto Watne: l.o.watne@medisin.uio.no
Anne-Brita Knapskog: anne-brita@knapskog.net
Ane-Victoria Idland: a.v.idland@medisin.uio.no
Henrik Zetterberg: henrik.zetterberg@clinchem.gu.se
Kaj Blennow: kaj.blennow@neuro.gu.se

WORD COUNT:

Title: 11 (87 characters including spaces)
Running title: 5 words, 43 characters (with spaces)
Main text (introduction, method, results, discussion):
Abstract: 247
Number of references:
Figures:; Tables: 2.

KEY WORDS: delirium; biomarkers; cerebrospinal fluid proteins; Alzheimer's disease; neurotransmitter agents.

ABSTRACT

Background: Delirium is associated with an increased risk of incident dementia and accelerated progression of existing cognitive symptoms. Reciprocally, dementia increases the risk of delirium. Cerebrospinal fluid (CSF) concentration of the dendritic protein neurogranin has been shown to increase in early Alzheimer's disease (AD), likely reflecting synaptic dysfunction and/or degeneration.

Objectives: To elucidate the involvement of synaptic dysfunction in delirium pathophysiology, we tested the association between CSF neurogranin concentration and delirium in hip fracture patients with different AD-biomarker profiles, while comparing them to cognitively unimpaired older adults (CUA) and AD patients.

Methods: The cohort included hip fracture patients with (n=70) and without delirium (n=58), CUA undergoing elective surgery (n=127) and AD patients (n=46). CSF was collected preoperatively and diagnostically in surgery and AD patients respectively. CSF neurogranin concentrations were analyzed in all samples with an in-house ELISA. Delirium was assessed pre-and postoperatively in hip fracture patients by trained investigators using the Confusion Assessment Method. Hip fracture patients were further stratified based on pre-fracture dementia status, delirium subtype and AD fluid biomarkers.

Results: No association was found between delirium and CSF neurogranin concentration (main analysis: delirium vs no delirium, p=0.68). Hip fracture patients had lower CSF neurogranin concentration than AD patients (p=0.001) and CUA (p=0.035) in age-adjusted sensitivity analyses.

Conclusion: The findings suggest that delirium is not associated with increased CSF neurogranin concentration in hip fracture patients, possibly due to advanced neurodegenerative disease and age and/or because synaptic degeneration is not an important pathophysiological process in delirium.

INTRODUCTION

Delirium is a severe neuropsychiatric syndrome characterized by acute disturbances in attention, awareness and cognition. It affects up to 50% of hospitalized older adults [1], and arises as a result of a medical condition or substance intoxication or withdrawal [2]. Cognitive impairment due to underlying neurodegenerative disorders (NDD) is a major risk factor [3, 4]. Serious deleterious outcomes are associated with delirium, including incident dementia and acceleration of existing cognitive symptoms and dementia [5-8]. The underlying pathophysiology of delirium is poorly understood. In recent years, biomarkers of importance in NDD have been explored in relation to delirium pathophysiology, suggesting a bilateral relationship between delirium and NDD [9-11].

Regulation of synaptic signaling is essential for the coordinated relay of information in the brain. Reduced synaptic density and efficacy has been linked to several NDD. For instance, in the early phases of Alzheimer's disease (AD), cognitive impairment has been associated with hippocampal synaptic dysfunction, prior to definite neuronal cell death [12]. Jarquin-Valdivia and Major hypothesize that synaptic disruptions, and particularly changes in Hebbian Spike-timing-dependent plasticity, are key pathological etiologies in both delirium and neurodegenerative disease [13]. An experimental mouse study showed that increasing axonal and synaptic pathology were associated with a higher risk of acute cognitive impairment, as seen in delirium [14], but no further synaptic degeneration was observed following the episode of acute cognitive impairment. This suggests that synaptic degeneration may be involved in delirium, but that the pathophysiological mechanisms causing delirium do not aggravate synaptic degeneration. As proposed by Maldonado, neurobehavioral symptoms of delirium may be explained by a temporary breakdown of functional integration between connected brain systems, resulting in pathological signaling and altered neurotransmitter

homeostasis, which may further trigger neurotoxic signaling with ensuing neuronal apoptosis, leading to long-term cognitive symptoms [15].

Neurogranin is a postsynaptic protein which has a central role in long-term potentiation through regulation of calmodulin availability [16]. It is expressed mainly in neurons in the hippocampus, associative cortex and amygdala, which are main brain areas affected by pathological changes in AD [17]. Studies have shown that neurogranin expression may be regulated through synaptic activity in hippocampal cell cultures [18] and decreases with age in mouse models [19]. At autopsy, neurogranin concentrations in the frontal cortex and hippocampus are lower in AD patients, likely reflecting reduced synaptic density [20]. In cerebrospinal fluid (CSF), neurogranin concentration increases from the early asymptomatic stages of AD [21-23] and predicts cognitive decline [24] and increased brain atrophy in early AD and mild cognitive impairment [23]. The increase may indicate ongoing synaptic loss and/or dysfunction with leakage to the CSF. The topographical distribution of neurogranin in the brain may explain that changes in neurogranin appear to be specific for AD [21] and Creutzfeldt-Jacobs disease [25]. These areas include neuroanatomical structures that are likely involved in delirium [14]. Alternatively, there may be increased neurogranin release from AD-affected neurons, possibly in response to Aβ pathology, by similar mechanisms as proposed for the AD-specific increase in cerebrospinal fluid (CSF) phosphorylated tau (p-tau) and total tau (t-tau) protein concentration [26].

To our knowledge, the relationship between delirium and neurogranin, as a biomarker of postsynaptic integrity, has never been studied. We hypothesized that increased levels of CSF neurogranin were associated with delirium in hip fracture patients (figure 1), either as a

marker of the processes causing delirium and/or contributing to the patient's vulnerability to delirium.

[Figure 1 – Graphical abstract]

Our main study population consisted of demented and non-demented hip fracture patients with and without delirium. We chose this patient group because the prevalence of delirium is high in these patients and extraction of CSF may be coupled with the onset of spinal anesthesia. We performed subgroup analysis based on dementia status and core AD biomarkers - since dementia is a main risk factor for delirium, and time of delirium onset – to better untangle the pathophysiological implications of a possible association between delirium and neurogranin. Two contrast groups were included: AD patients and CUA, to help dissociate changes in CSF neurogranin concentration due to delirium, from changes related to AD in the hip fracture population.

METHOD:

Cohorts

Hip fracture cohort

Patients with hip fractures (n=332) were enrolled in the Oslo Orthogeriatrics Trial, a randomized controlled trial evaluating the effect of orthogeriatric care on cognitive function, at Oslo University Hospital from September 2009 to January 2012, as described previously [27, 28]. Patients with terminal illness or high-energy trauma were excluded. The orthogeriatric intervention did not influence delirium incidence [27] and all participants were

assembled in the present study. 130 participants had available CSF, of which two were excluded due to missing delirium status, yielding a final sample of 128 hip fracture patients.

The presence of delirium was assessed daily by trained investigators in all participants preoperatively and until the fifth postoperative day (all) or discharge (patients with delirium), using the Confusion Assessment Method (CAM) [29]. The study physician or study nurse scored CAM based on a 10- to 30-minute interview with participants and information from relatives, nurses and hospital records. Delirium status was defined as a binary variable (delirium/no delirium). The group without delirium consisted of patients who did not develop delirium at any time point during the study. In our main analysis (delirium vs no delirium), patients with subsyndromal delirium (SSD), were included in the no delirium group. SSD was defined as fulfilling at least two, but not all required CAM criteria for the full syndrome. Within the group with delirium, participants were classified as having preoperative or incident delirium, depending on the time of delirium onset. Delirium severity was evaluated using The Memorial Delirium Assessment Scale (MDAS) [30].

One geriatrician and one old age psychiatrist independently evaluated whether participants met the ICD-10 criteria for dementia prior to the fracture, based on all prevailing data at baseline and 12-month follow-up (except delirium status during admission), including the Informant Questionnaire on Cognitive decline in the Elderly (IQCODE) and hospital records. The inter-rater consensus agreement upon the dementia diagnosis was acceptable (kappa 0.87) and disagreements were resolved through discussion.

Control group of cognitively unimpaired older adults (CUA)

The control group included 172 patients admitted for elective gynecological, orthopedic or urological surgery in spinal anesthesia, aged 65 years or older the year of inclusion, who were

recruited to the COGNORM-study from 2012-2013 at Oslo University Hospital and Diakonhjemmet Hospital, Oslo, as previously described [31]. Exclusion criteria were dementia, previous stroke with sequelae, Parkinson's disease and other acknowledged or suspected brain disease likely to influence cognition. Participants were assessed with cognitive tests prior to surgery to assure the absence of cognitive impairment, as described elsewhere [31]. Participants with a baseline Mini Mental Status Examination score [32] of <28 (n=16) or suspected undiagnosed dementia (based on test scores and clinical data) with referral to a memory clinic by a geriatrician during six years of follow-up (n=14) were excluded. Furthermore, 15 participants did not have available CSF samples. All patients were free from delirium at the time of CSF sampling, based on the cognitive tests prior to surgery. In addition, we examined case notes (all sections) to confirm that no patients had developed delirium in the time from cognitive testing to the day of surgery (mean 11 days). The final sample consisted of 127 CUA.

Contrast group of patients with Alzheimer's disease

The Norwegian Registry of Persons Assessed for Cognitive Symptoms (NorCog) is a consent-based national registry and contains clinical data for patients referred for examination of dementia in outpatient clinics [33]. The patients go through cognitive testing and tests of physical function, and blood tests and a MRI/CT of the brain are performed, as previously described [33, 34]. As a contrast group of patients with AD, 46 patients enrolled in NorCog at Oslo University Hospital from 2009 to 2012, fulfilling the core clinical NIAA-criteria for probable anamnestic AD dementia [35] were eligible for analyses of neurogranin in the CSF. Cut-offs used for CSF AD-biomarkers were as follows: amyloid β ($A\beta_{42}$)<700 pg/mL, p-tau >80 pg/mL and t-tau >300 (age <50 years), >450 (50-70 years) and > 500 (>70 years) pg/mL[34].

CSF Sampling and biochemical analyses

Hip fracture patients and CUA

Cerebrospinal fluid (CSF) was collected in propylene tubes in conjunction with and prior to administration of spinal anesthesia in both surgical cohorts. CSF samples were centrifuged, aliquoted and stored at – 80° C, as previously described [31, 36]. Samples were sent on dry ice for analyses at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital (Mölndal, Sweden). CSF AD biomarkers ($A\beta_{42}$, p-tau and t-tau) were determined using INNOTEST enzyme-linked immunosorbent assays (ELISA; Fujirebio, Ghent, Belgium) by board-certified laboratory technicians masked to clinical data.

AD patients

Lumbar punctures were performed before 11 am. CSF was collected in cryotubes and centrifuged, as previously described [34]. Samples were frozen overnight at -20°C or sent the same day to the laboratory at Akershus University Hospital (AHUS) for analysis of CSF AD biomarkers. CSF AD biomarkers were analyzed with the INNOTEST enzyme-linked immunosorbent assays (ELISA; Fujirebio, Ghent, Belgium). Due to inter-lab variation, different cut-off levels or AD biomarkers are in use at the different laboratories [34, 37] and AD biomarkers measured in the AD-cohort were not directly comparable to measurements in the surgical cohorts. Frozen samples of CSF (-80°C) were later sent on dry ice to Sahlgrenska University Hospital for analysis of neurogranin.

For all three cohorts, CSF neurogranin was measured using in-house ELISA, based on the NG2 and NG36 antibodies, as described previously in detail [38]. All analyses were performed by board-certified laboratory technicians, who were blinded to the clinical

information, at the Clinical Neurochemistry Lab, Sahlgrenska University Hospital, Mölndal, Sweden. Samples were run as duplicate measures, using the same batch of reagents, and following strict criteria for run acceptance. CVs were 5.0% for the duplicate measures.

Statistical methods

Data in either cohort were not normally distributed and fit to the normal distribution did not improve with transformation. Continuous variables were analyzed using Mann-Whitney U test and Kruskal-Wallis. Correlations were calculated with Spearman's ρ. Categorical variables were analyzed using Chi square (χ^2) statistics. Post-hoc linear regression analyses were performed adjusting for age.

In the hip fracture cohort:

First, data from the hip fracture cohort were analyzed depending on delirium status (delirium yes/no), delirium subgroups (no delirium/ SSD/ preoperative delirium/ incident delirium) and delirium severity (MDAS). Subsequently, we tested for an association between dementia and neurogranin levels in the hip fracture patients. Subgroup analysis were performed on the hip fracture patients based on pre-fracture dementia status and on CSF AD biomarkers ($A\beta_{42}$, total tau (t-tau) and phosphorylated tau (p-tau)) according to the A/T/N classification [39]. The following cutoff points were applied to assess the presence of amyloid pathology A+< $A\beta_{42}$ 530 pg/mL ≤A-, aggregation of phosphorylated tau T+> p-tau 60 pg/mL ⩾ T- and neurodegeneration N+> t-tau 350 pg/mL ⩾N-, as established for the laboratory [37].

Comparisons between cohorts and correlation with age: Finally, comparisons between the hip fracture population and the control groups were performed, with sensitivity analyses according to dementia status within the hip fracture cohort. Due to the age-difference between the cohorts and the evidence suggesting age-associated changes in neurogranin

expression, we analyzed whether CSF neurogranin correlated with age, and reported age-adjusted analyses.

All statistical analyses were performed using SPSS Statistics version 26 (IBM, Armonk, NY, USA). Graphs were designed using GraphPad Prism 8 *(https://www.graphpad.com/scientific-software/prism/)*.

Ethical standards

The study was conducted in accordance with the World Medical association Declaration of Helsinki. The data and CSF samples were collected after informed and written consent from the patient and/or proxy (if patients were unable to consent due to cognitive impairment), as approved by the Regional Committee for Medical and Health Research Ethics (South-East Norway; REK 2009/450; REK 2011/2052 and REK 2017/371).

RESULTS

Demographic characteristics

The hip fracture patients were older than the CUA and AD patients, and female participants were overrepresented in the hip fracture cohort compared to CUA (see table 1 55% (n=70) of all hip fracture patients had delirium. 74 % (n=52) of patients with delirium had dementia, whereas only 17% (n=10) of patients without delirium had dementia. . Median [IQR] IQCODE among the hip fracture patients with dementia (n=61, 1 missing) was 4.75 [4.3-5.0] and was significantly higher than in the AD patient group (8 missing, 3.7 [3.5-4.1], $p<0.001$), reflecting advanced stages of dementia among hip fracture patients. Core AD biomarkers have previously been reported for the hip fracture cohort [40].

A positive correlation (ρ=0.20, p=0.022) was found between age and CSF neurogranin, but only in CUA (hip fracture patients ρ=0.077, p=0.39, AD patients ρ=-0.14, p=0.057).

[Table 1 - Population demographics and biomarkers]

Hip fracture patients

Association between neurogranin and delirium/dementia status

Main analyses:

No difference in CSF neurogranin concentration was found between patients with and without delirium (median [IQR] 201 [150,248] vs 197 [146,235]; p=0.68, table 1, figure 2). No correlation was detected between delirium severity and CSF neurogranin concentration (ρ=-0.075, p=0.47). Adjusting for age did not alter any of the findings significantly.

[Figure 2 CSF neurogranin concentration in hip fracture patients with and without delirium.]

Sensitivity analyses:

Neurogranin in delirium subtypes: We further explored whether preoperative vs incident delirium or presence/absence of symptoms at any time (preoperative delirium vs incident delirium vs SSD vs no delirium ever) affected neurogranin concentration at time of sampling. No differences were found between the four subgroups of delirium (nodelirium (excluding SSD), SSD, preoperative delirium and incident delirium ($\chi^{2=}$ 0.185, p=0.98, df=3). Adjusting for age did not alter the findings significantly.

Neurogranin and delirium depending on dementia status:

Dementia being a major risk factor for delirium [4], we repeated the analyses stratified on dementia status. No significant difference in CSF neurogranin concentration was observed in hip fracture patients with (n=62) or without dementia (n=66), median [IQR] 198 [144,227] vs 203 [150,257], p=0.30. In subgroup analysis, no differences in CSF neurogranin concentration

were found in relation to delirium status in patients with dementia (p=0.91) or without dementia (p=0.092). Adjusting for age did not alter any of the findings significantly.

Neurogranin and delirium depending on AD-biomarkers and A/T/N classification

Four patients had missing $A\beta_{42}$ and p/t-tau values. Demographics of biomarker positivity in the hip fracture population are described in table 2.

[*Table 2. CSF neurogranin concentration and delirium in relation to core Alzheimer's Disease (AD) biomarkers in the hip fracture population*]

We found no difference in neurogranin between participants with and without delirium after stratification for biomarker positivity (table 2): A+ (p=0.24), A- (p=0.36), T+ (p=0.72), T- (p=0.58), or N+ (p=0.88) groups. In the N- group, patients with delirium tended to have slightly higher neurogranin concentration (median [IQR] 227 [192-279] vs 221 [186-269], p=0.058). Age-adjustment did not significantly alter results for any biomarker group.
No difference in neurogranin concentration in relation to delirium status was found in the AT- groups: A-T- (p=0.51), A+T- (p=0.91) and A+T+ (p=0.50). In the A-T+ group, the samples were too small for comparison.

Cognitively unimpaired older adults (CUA, control group)

No significant difference in CSF neurogranin concentration was initially found between CUA and hip fracture patients (median [IQR] 199[148-235] vs 203[167-261], p=0.17) (see demographics table 1, figure 3). After adjusting for age, CUA were found to have significantly higher concentrations of neurogranin than hip fracture patients (β= 22, p=0.035).

In post-hoc analyses adjusting for age, only hip fracture patients with dementia were found to have significantly lower neurogranin than CUA (β= 34, p=0.01).

[

AD patients (control group)

AD patients (median [IQR] 248 [183-306]) had significantly higher CSF neurogranin concentration than all hip fracture patients (p=0.001) and CUA(p=0.012) (figure 3). They also had higher neurogranin concentration than hip fracture patients with dementia (198 [144-227], p=0.001) and without dementia (203 [150-257], p=0.012). The results survived age-adjustment, except for the comparison between AD patients and hip fracture patients without dementia (age-adjusted p= 0.063).

DISCUSSION

In contrast to our main hypothesis, we did not find that delirium was associated with synaptic failure, as measured by CSF neurogranin concentration. With worsening neurodegenerative changes and cognitive impairment, the risk of delirium appears to gradually increase [14]. One might therefore expect that increasing CSF levels of neurogranin, as a measure of synaptic dysfunction and/or deterioration, might indicate an increased risk of delirium. In fact, a recent study found that blood neurogranin was elevated in critically ill patients prior to and at the time of delirium, compared to controls [41]. Importantly, neurogranin was measured in blood, and plasma neurogranin does not correlate with CSF neurogranin nor clinical outcomes in neither AD [42] nor acute stroke [43], likely due to extracerebral sources of neurogranin and proteolytic activity in blood [42]. Moreover, a study showed that the apical tree of CA1

neurons in aged mice was remodeled in response to acute stress such as during delirium [44]. The authors hypothesized that synaptic dysfunction in delirium may initially be adaptive. However, under pathological conditions with reduced synaptic plasticity, we advocate that the transient remodeling may become more permanent and lead to synaptic dysfunction and loss. Synaptic loss [45] and higher CSF neurogranin [46] have been shown to correlate with cognitive decline in early stages of AD. An association between delirium and neurogranin could thus suggest that synaptic loss caused by the mechanisms resulting in delirium might contribute to accelerated dementia and/or incident dementia after delirium.

However, our negative findings suggest that although neurogranin is expressed in neuroanatomical structures that are likely involved in delirium symptomatology [14], symptoms present in delirium are complex, and widespread cerebral dysfunction in other key areas may be more prominent. In addition, even though synaptic dysfunction with release of neurogranin may theoretically occur as a result of delirium, the rise in CSF neurogranin may be too insignificant to be registered in the hip fracture population, as discussed below.

Our second aim was to compare CSF neurogranin in the hip fracture population, which included patients with and without dementia, to neurogranin concentrations in CUA and AD patients. CUA serve as a control group for both of the other cohorts. In contrary to acutely admitted hip fracture patients, they were thoroughly tested prior to CSF sampling and represent a group that with a high degree of certainty have neither dementia nor delirium. Increased concentrations of CSF Neurogranin have been demonstrated repeatedly in AD [21-23]. Comparing CSF neurogranin concentrations in AD and hip fracture patients, may help discriminate changes in CSF neurogranin due to delirium from changes related to AD, particularly to hip fracture patients with dementia. While the AD patients from the memory clinic underwent thorough clinical and biochemical testing confirming probable AD, the type

of dementia was only registered for a minor subset of hip fracture patients. Other undetermined etiologies, such as cerebrovascular disease, might therefore have caused dementia in the hip fracture cohort. Accordingly, in agreement with previous studies, we found that AD patients had significantly higher levels of neurogranin than CUA and hip fracture patients [21-23]. Despite undetermined dementia etiologies, based on existing demographics concerning the prevalence of dementia subtypes in the oldest population [48], one might assume that AD or mixed pathology involving AD-specific changes were the leading etiologies also in the hip fracture patients. We were therefore surprised to find that after adjusting for age, hip fracture patients had lower levels of neurogranin than CUA, and that this difference seemed to be driven by lower neurogranin in hip fracture patients with dementia. We suggest that neurogranin expression in the brain likely needs to be of a certain magnitude for concentrations of neurogranin to increase detectably in CSF. The hip fracture patients with dementia were in clinically advanced disease stages based on informant questionnaires (IQCODE) and scored significantly higher on IQCODE than the AD patients enrolled at the Memory Clinic. Advanced AD in the hip fracture patients may result in reduced neurogranin expression in the brain and/or neurogranin release to CSF due to reduced neurogranin production, loss of synapses and/or low disease intensity. In line with this, decreased levels of neurogranin have been detected at autopsy in cortical regions in the AD brain [20], with greater decreases in late stages of AD [49]. Furthermore, while levels of neurogranin have been shown to rise in early phases of AD-pathology [22], several studies have reported a negative correlation between AD duration and CSF levels of neurogranin [21, 46], possibly due to depletion of neurogranin as a result of extensive neurodegenerative changes and/or reduced intensity of disease in late stages of AD. In our study, only hip fracture patients with delirium and normal levels of t-tau (N-) tended to have higher levels of neurogranin in delirium, supporting that underlying neurodegenerative changes could masque

delirium-associated changes in synaptic function and neurogranin. Lastly, neurogranin expression has been shown to decrease with age in mouse models [19], which suggests that the release of neurogranin to the CSF may not be as prominent in synaptic dysfunction in the oldest old. The hip fracture patients were significantly older than participants in the two control groups (table 1). Although we found a positive correlation between age and neurogranin in the CUA, we did not find any correlation in the hip fracture patients, possibly due to an age-related plateau effect. Taken together, CSF neurogranin may not be representative of the degree of synaptic dysfunction and/or degeneration in the hip fracture population, comprising the oldest old, including dementia patients likely to suffer in part from advanced AD.

Limitations of our study include retrospective diagnosis of dementia with missing dementia etiology in most hip fracture patients. Characterization of dementia etiology is important as neurogranin appears to be AD specific. Analysis of CSF core AD biomarkers ($A\beta_{42}$, t-tau and p-tau) at two different laboratories impeded direct comparison between cohorts. Although the hip fracture cohort was large in the setting of delirium research, lack of power may affect results, especially in subgroup analyses. The use of two contrast/control groups was a strength of our study. Also, neurogranin was analyzed at the same time at the same laboratory in all participants. Furthermore, delirium was assessed daily based on validated instruments by trained investigators.

The findings suggest that neurogranin may not be a useful biomarker in assessing pathophysiological mechanisms involved in delirium in hip fracture cohorts and/or that synaptic degeneration is not an important pathophysiological mechanism in delirium. Studies on neurogranin as a biomarker for synaptic dysfunction in delirium pathophysiology should be repeated, possibly in a younger and cognitively healthier population.

ACKNOWLEDGMENTS

We thank patients, staff, research nurses and Professors Wyller and Engedal at the Geriatric, Orthopedic, Gynecology, Urology and Anesthesiology Departments at Oslo University Hospital and Diakonhjemmet Hospital, Oslo and laboratory technicians at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital. We acknowledge NorCog for access to patient data and CSF.

Conflict of interest statement

N.B. Halaas, A-V. Idland, and L.O. Watne explicitly report no disclosures.

AB. Knapskog has been principal investigator on clinical trials for Roche (BN29553) and Boehringer-Ingelheim (1346.0023).

K. Blennow has served as a consultant at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the work presented in this paper.

H. Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the work presented in this paper.

Author contributions

Nathalie Bodd Halaas: Data collection and design of the COGNORM-study. Interpretation of the data. Preparation of manuscript.

Henrik Zetterberg: Analyses of Aβ-42, tau and neurogranin in CSF. Interpretation of the data and revision of manuscript.

Ane-Victoria Idland: Initiation and design of the COGNORM-study. Interpretation of the data and revision of manuscript.

Anne-Brita Knapskog: Conducted the clinical assessment of the AD patients. Interpretation of the data and revision of manuscript.

Leiv Otto Watne: Initiation and design of the COGNORM study and Oslo orthogeriatric trial. Data collection. Interpretation of the data and revision of manuscript.

Kai Blennow: Analyses of Aβ-42, tau and neurogranin in CSF. Interpretation of the data and revision of manuscript.

Sponsor's role

The study was funded by the Norwegian Health Association, the South-Eastern Norway Regional Health Authorities, the Medical Student Research Program of Norway and the Olav Thon Foundation. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. H.Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862) and the UK Dementia Research Institute at UCL. K. Blennow is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the

Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986) and European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236).

AVAILABILITY OF DATA AND MATERIALS

Legal restrictions, imposed by the owners of the Norwegian Registry of Persons Assessed for Cognitive Symptoms and the ethical committee, prevent us from publicly sharing the de-identified dataset regarding the AD-patients due to sensitive patient information. The clinical data may be requested at e-mail: post@aldringoghelse.no. However, data availability is dependent on approval from the REC South East, contact at e-mail: post@helseforskning.etikkom.no. The data that supports the findings in the hip fracture patients and the cognitively unimpaired control group are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

[1] Inouye SK, Westendorp RG, Saczynski JS (2014) Delirium in elderly people. *Lancet* **383**, 911-922.

[2] (2013) *Diagnostic and Statistical Manual of Mental Disorders*, Washington, DC.

[3] Khan BA, Zawahiri M, Campbell NL, Fox GC, Weinstein EJ, Nazir A, Farber MO, Buckley JD, Maclullich A, Boustani MA (2012) Delirium in hospitalized patients: implications of current evidence on clinical practice and future avenues for research--a systematic evidence review. *J Hosp Med* **7**, 580-589.

[4] Gual N, Morandi A, Pérez LM, Brítez L, Burbano P, Man F, Inzitari M (2018) Risk Factors and Outcomes of Delirium in Older Patients Admitted to Postacute Care with and without Dementia. *Dement Geriatr Cogn Disord* **45**, 121-129.

[5] Davis DHJ, Terrera GM, Keage H, Rahkonen T, Oinas M, Matthews FE, Cunningham C, Polvikoski T, Sulkava R, MacLullich AMJ, Brayne C (2012) Delirium is a strong risk factor for dementia in the oldest-old: a population-based cohort study. *Brain* **135**, 2809-2816.

[6] Krogseth M, Watne LO, Juliebo V, Skovlund E, Engedal K, Frihagen F, Wyller TB (2016) Delirium is a risk factor for further cognitive decline in cognitively impaired hip fracture patients. *Arch Gerontol Geriatr* **64**, 38-44.

[7] Krogseth M, Wyller TB, Engedal K, Juliebo V (2011) Delirium is an important predictor of incident dementia among elderly hip fracture patients. *Dement Geriatr Cogn Disord* **31**, 63-70.
[8] Fong TG, Davis D, Growdon ME, Albuquerque A, Inouye SK (2015) The interface between delirium and dementia in elderly adults. *Lancet Neurol* **14**, 823-832.
[9] Halaas NB, Blennow K, Idland AV, Wyller TB, Ræder J, Frihagen F, Staff AC, Zetterberg H, Watne LO (2018) Neurofilament Light in Serum and Cerebrospinal Fluid of Hip Fracture Patients with Delirium. *Dement Geriatr Cogn Disord* **46**, 346-357.
[10] Cunningham EL, McGuinness B, McAuley DF, Toombs J, Mawhinney T, O'Brien S, Beverland D, Schott JM, Lunn MP, Zetterberg H, Passmore AP (2019) CSF Beta-amyloid 1-42 Concentration Predicts Delirium Following Elective Arthroplasty Surgery in an Observational Cohort Study. *Ann Surg* **269**, 1200-1205.
[11] Fong TG, Vasunilashorn SM, Libermann T, Marcantonio ER, Inouye SK (2019) Delirium and Alzheimer's Disease: A Proposed Model for Shared Pathophysiology. *Int J Geriatr Psychiatry*.
[12] Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* **298**, 789-791.
[13] Jarquin-Valdivia AA, Major RJ (2019) A unifying hypothesis for delirium and hospital-acquired weakness as synaptic dysfunctions. *Med Hypotheses* **124**, 105-109.
[14] Davis DHJ, Skelly DT, Murray C, Hennessy E, Bowen J, Norton S, Brayne C, Rahkonen T, Sulkava R, Sanderson DJ, Rawlins JN, Bannerman DM, MacLullich AMJ, Cunningham C (2015) Worsening Cognitive Impairment and Neurodegenerative Pathology Progressively Increase Risk for Delirium. *American Journal of Geriatric Psychiatry* **23**, 403-415.
[15] Maldonado JR (2018) Delirium pathophysiology: An updated hypothesis of the etiology of acute brain failure. *Int J Geriatr Psychiatry* **33**, 1428-1457.
[16] Huang KP, Huang FL, Jäger T, Li J, Reymann KG, Balschun D (2004) Neurogranin/RC3 enhances long-term potentiation and learning by promoting calcium-mediated signaling. *J Neurosci* **24**, 10660-10669.
[17] Represa A, Deloulme JC, Sensenbrenner M, Ben-Ari Y, Baudier J (1990) Neurogranin: immunocytochemical localization of a brain-specific protein kinase C substrate. *J Neurosci* **10**, 3782-3792.
[18] Garrido-García A, de Andrés R, Jiménez-Pompa A, Soriano P, Sanz-Fuentes D, Martínez-Blanco E, Díez-Guerra FJ (2019) Neurogranin Expression Is Regulated by Synaptic Activity and Promotes Synaptogenesis in Cultured Hippocampal Neurons. *Mol Neurobiol* **56**, 7321-7337.
[19] Mons N, Enderlin V, Jaffard R, Higueret P (2001) Selective age-related changes in the PKC-sensitive, calmodulin-binding protein, neurogranin, in the mouse brain. *J Neurochem* **79**, 859-867.
[20] Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W, Jr., Kaye J, Manczak M (2005) Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J Alzheimers Dis* **7**, 103-117; discussion 173-180.
[21] Wellington H, Paterson RW, Portelius E, Törnqvist U, Magdalinou N, Fox NC, Blennow K, Schott JM, Zetterberg H (2016) Increased CSF neurogranin concentration is specific to Alzheimer disease. *Neurology* **86**, 829-835.
[22] Palmqvist S, Insel PS, Stomrud E, Janelidze S, Zetterberg H, Brix B, Eichenlaub U, Dage JL, Chai X, Blennow K, Mattsson N, Hansson O (2019) Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *EMBO Mol Med* **11**, e11170.

[23] Portelius E, Zetterberg H, Skillbäck T, Törnqvist U, Andreasson U, Trojanowski JQ, Weiner MW, Shaw LM, Mattsson N, Blennow K (2015) Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. *Brain* **138**, 3373-3385.
[24] Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Öhrfelt A, Andersson K, Brinkmalm G, Lannfelt L, Minthon L, Hansson O, Andreasson U, Teunissen CE, Scheltens P, Van der Flier WM, Zetterberg H, Portelius E, Blennow K (2015) Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. *Alzheimers Dement* **11**, 1180-1190.
[25] Blennow K, Diaz-Lucena D, Zetterberg H, Villar-Pique A, Karch A, Vidal E, Hermann P, Schmitz M, Ferrer Abizanda I, Zerr I, Llorens F (2019) CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD. *J Neurol Neurosurg Psychiatry* **90**, 846-853.
[26] Sato C, Barthélemy NR, Mawuenyega KG, Patterson BW, Gordon BA, Jockel-Balsarotti J, Sullivan M, Crisp MJ, Kasten T, Kirmess KM, Kanaan NM, Yarasheski KE, Baker-Nigh A, Benzinger TLS, Miller TM, Karch CM, Bateman RJ (2018) Tau Kinetics in Neurons and the Human Central Nervous System. *Neuron* **97**, 1284-1298.e1287.
[27] Watne LO, Torbergsen AC, Conroy S, Engedal K, Frihagen F, Hjorthaug GA, Juliebo V, Raeder J, Saltvedt I, Skovlund E, Wyller TB (2014) The effect of a pre- and postoperative orthogeriatric service on cognitive function in patients with hip fracture: randomized controlled trial (Oslo Orthogeriatric Trial). *BMC Med* **12**, 63.
[28] Wyller TB, Watne LO, Torbergsen A, Engedal K, Frihagen F, Juliebø V, Saltvedt I, Skovlund E, Ræder J, Conroy S (2012) The effect of a pre- and post-operative orthogeriatric service on cognitive function in patients with hip fracture. The protocol of the Oslo Orthogeriatrics Trial. *BMC Geriatr* **12**, 36.
[29] Inouye SK, van Dyck CH, Alessi CA, Balkin S, Siegal AP, Horwitz RI (1990) Clarifying confusion: the confusion assessment method. A new method for detection of delirium. *Ann Intern Med* **113**, 941-948.
[30] O'Sullivan R, Meagher D, Leonard M, Watne LO, Hall RJ, Maclullich AM, Trzepacz P, Adamis D (2015) A comparison of the revised Delirium Rating Scale (DRS-R98) and the Memorial Delirium Assessment Scale (MDAS) in a palliative care cohort with DSM-IV delirium. *Palliat Support Care* **13**, 937-944.
[31] Idland AV, Sala-Llonch R, Borza T, Watne LO, Wyller TB, Braekhus A, Zetterberg H, Blennow K, Walhovd KB, Fjell AM (2017) CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults. *Neurobiol Aging* **49**, 138-144.
[32] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.
[33] Braekhus A, Ulstein I, Wyller TB, Engedal K (2011) The Memory Clinic--outpatient assessment when dementia is suspected. *Tidsskr Nor Laegeforen* **131**, 2254-2257.
[34] Knapskog AB, Braekhus A, Engedal K (2019) The Effect of Changing the Amyloid beta42 Cut-off of Cerebrospinal Fluid Biomarkers on Alzheimer Disease Diagnosis in a Memory Clinic Population in Norway. *Alzheimer Dis Assoc Disord* **33**, 72-74.
[35] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on

Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[36] Watne LO, Hall RJ, Molden E, Raeder J, Frihagen F, MacLullich AM, Juliebo V, Nyman A, Meagher D, Wyller TB (2014) Anticholinergic activity in cerebrospinal fluid and serum in individuals with hip fracture with and without delirium. *J Am Geriatr Soc* **62**, 94-102.

[37] Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol* **5**, 228-234.

[38] Kvartsberg H, Lashley T, Murray CE, Brinkmalm G, Cullen NC, Höglund K, Zetterberg H, Blennow K, Portelius E (2019) The intact postsynaptic protein neurogranin is reduced in brain tissue from patients with familial and sporadic Alzheimer's disease. *Acta Neuropathol* **137**, 89-102.

[39] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539-547.

[40] Idland AV, Wyller TB, Stoen R, Eri LM, Frihagen F, Raeder J, Chaudhry FA, Hansson O, Zetterberg H, Blennow K, Bogdanovic N, Braekhus A, Watne LO (2016) Preclinical Amyloid-beta and Axonal Degeneration Pathology in Delirium. *J Alzheimers Dis* **55**, 371-379.

[41] Wanderlind ML, Gonçalves R, Tomasi CD, Dal-Pizzol F, Ritter C (2020) Association of neurogranin with delirium among critically ill patients. *Biomark Med* **14**, 1613-1617.

[42] Kvartsberg H, Portelius E, Andreasson U, Brinkmalm G, Hellwig K, Lelental N, Kornhuber J, Hansson O, Minthon L, Spitzer P, Maler JM, Zetterberg H, Blennow K, Lewczuk P (2015) Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimers Res Ther* **7**, 40.

[43] De Vos A, Bjerke M, Brouns R, De Roeck N, Jacobs D, Van den Abbeele L, Guldolf K, Zetterberg H, Blennow K, Engelborghs S, Vanmechelen E (2017) Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. *BMC Neurol* **17**, 170.

[44] Richwine AF, Parkin AO, Buchanan JB, Chen J, Markham JA, Juraska JM, Johnson RW (2008) Architectural changes to CA1 pyramidal neurons in adult and aged mice after peripheral immune stimulation. *Psychoneuroendocrinology* **33**, 1369-1377.

[45] Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ (2007) Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* **68**, 1501-1508.

[46] Molinuevo JL, Ayton S, Batrla R, Bednar MM, Bittner T, Cummings J, Fagan AM, Hampel H, Mielke MM, Mikulskis A, O'Bryant S, Scheltens P, Sevigny J, Shaw LM, Soares HD, Tong G, Trojanowski JQ, Zetterberg H, Blennow K (2018) Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol* **136**, 821-853.

[47] Bos I, Vos S, Verhey F, Scheltens P, Teunissen C, Engelborghs S, Sleegers K, Frisoni G, Blin O, Richardson JC, Bordet R, Tsolaki M, Popp J, Peyratout G, Martinez-Lage P, Tainta M, Lleo A, Johannsen P, Freund-Levi Y, Frolich L, Vandenberghe R, Westwood S, Dobricic V, Barkhof F, Legido-Quigley C, Bertram L, Lovestone S, Streffer J, Andreasson U, Blennow K, Zetterberg H, Visser PJ (2019) Cerebrospinal fluid biomarkers of neurodegeneration, synaptic integrity, and astroglial activation across the clinical Alzheimer's disease spectrum. *Alzheimers Dement.*

[48] Emiliano Albanese SB, Sujith Dhanasiri, Jose-Luis Fernandez, Cleusa Ferri, Martin Knapp, Paul McCrone, Martin Prince, Tom Snell, Robert Stewart (2007) Personal Social Services Research Unit (PSSRU) at the London School of Economics and the Institute of Psychiatry at King's College London, pp. 4, 18-19.
[49] Davidsson P, Blennow K (1998) Neurochemical dissection of synaptic pathology in Alzheimer's disease. *Int Psychogeriatr* **10**, 11-23.

FIGURES (3) AND TABLES (2)

Figure 1 Graphical abstract

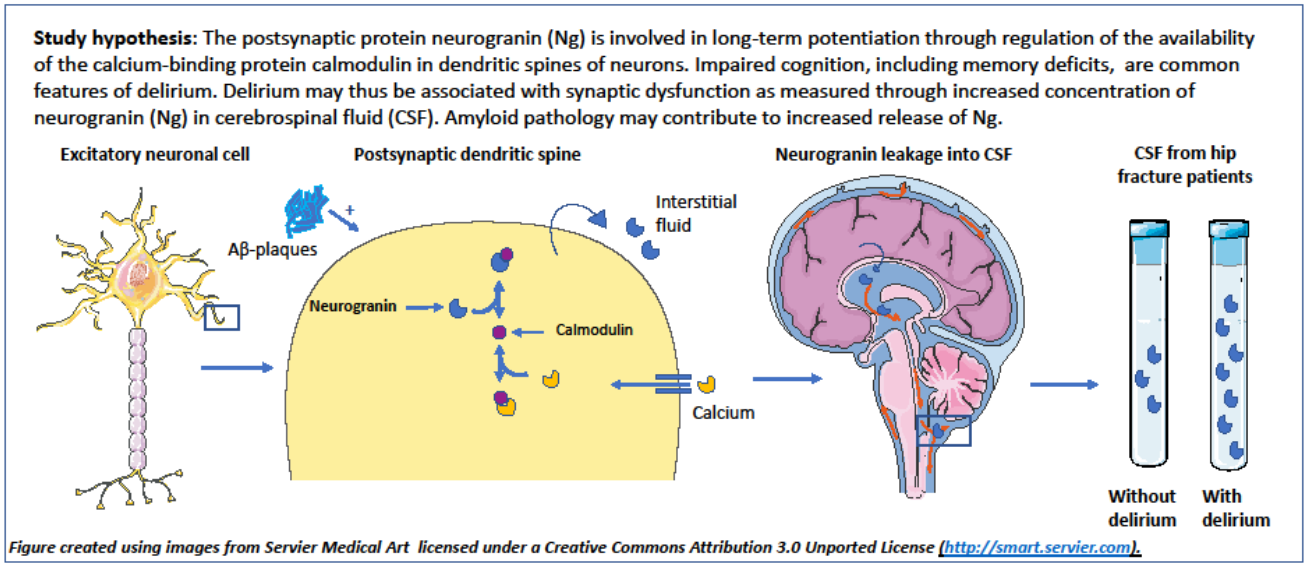

Figure 2 CSF neurogranin concentration in hip fracture patients with and without delirium. The black lines represent the median with the interquartile range. The p-value stems from Mann Whitney *U* analysis.

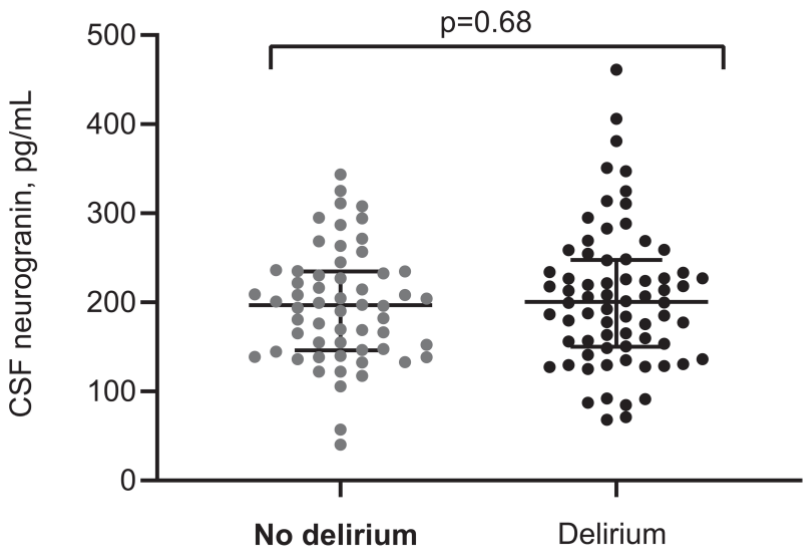

Figure 3 CSF neurogranin concentration in hip fracture patients, cognitively unimpaired older adults and patients with Alzheimer's disease. The black lines represent the median with the interquartile range. The p-values stem from Mann Whitney *U* analyses.

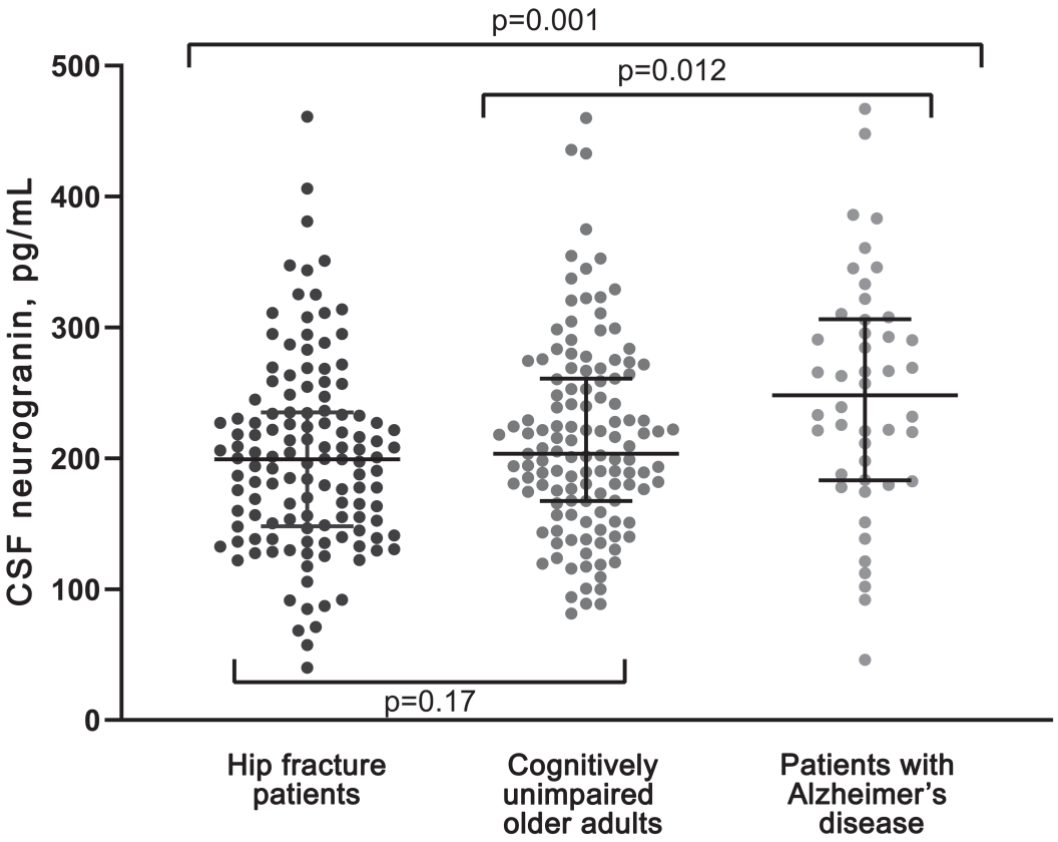

Table 1 Population demographics and biomarkers

	All hip fracture patients (n=128)‡	No delirium (n=58)‡	Delirium (n=70)‡	p-value *	Cognitively unimpaired older adults (n=127)‡‡	p-value **	Patients with Alzheimer's disease (n=46)‡‡‡	p-value ***
Age	85 (79-80)	84 (71-88)	85(81-89)	0.031	72 (68-77)	<0.001	67 (62-73)	<0.001
Female sex	93 (73)	45 (78)	43 (70)	0.26	64 (50)	<0.001	28 (61)	0.14
Dementia †	62 (48)	10 (17)	52 (74)	<0.001	-	-	-	-
IQCODE	3.7 (3.1-4.8)	3.2 (3.0-3.6)	4.5 (3.6-4.9)	<0.001	-	-	3.7 (3.5-4.1)	<0.97
MDAS	16 (7-24)	6 (3-8)	20 (14-26)	<0.001				
Neurogranin	199 (148-235)	197 (146-235)	201 (150-248)	0.68	203 (167-261)	0.17¶	248 (183-306)	0.001¶¶
Aβ42	330 (230-496)	461 (318-685)	266 (195-359)	<0.001	739 (515-857)	-	-	-
p-tau	58 (41-79)	55 (40-75)	59 (42-82)	0.29	58 (46-72)	-	-	-
t-tau	398 (288-584)	351 (266-481)	438 (309-719)	0.004	343 (267-283)	-	-	-

†Consensus diagnosis of dementia assessed retrospectively in the hip fracture patients by two independent expert physicians.

‡Hip fracture patients (Oslo Orthogeriatric Trial); ‡‡Elective surgery cohort of cognitively unimpaired older adults (CUA, COGNORM); ‡‡‡ Patients with probable anamnestic Alzheimer's disease dementia according to the clinical NIAA-criteria [33] (NORCOG).

* Delirium vs no delirium in hip fracture patients (assessed with Confusion Assessment Method)

** All hip fracture patients vs CUA

***All hip fracture patients vs Alzheimer's disease patients

¶ & ¶¶ Unadjusted value listed. The age-adjusted p-values were 0.035 (¶) and <0.001(¶¶) respectively.

Results are given as median (interquartile range) or n (%). All biomarkers are measured in the CSF and stated in pg/mL. Four hip fracture patients and two CUA had missing values for Alzheimer's

disease (AD) biomarkers (Aβ42 and t/p-tau). AD biomarkers for the AD cohort are not listed as they were analyzed in a different laboratory and were thus not directly equivalent to measurements in the two other groups. Values for IQCODE were missing for one hip fracture patient and eight AD patient.

Table 2. CSF neurogranin concentration and delirium in relation to core Alzheimer's disease (AD) biomarkers in the hip fracture population

Classification into AD biomarker groups †	n (%)	Incidence of delirium, n (%)	Neurogranin concentration [Ng] †, median (IQR)	[Ng] depending on biomarker positivity (p-values) *	[Ng] depending on delirium status within biomarker groups (p-values) **
A+	96 (75)	61 (64)	193 (145-232)	0.33	0.24
A-	28 (25)	6 (21)	204 (155-269)		0.36
T-	73 (60)	38 (52)	156 (130-203)	<0.001	0.58
T+	51 (40)	29 (57)	247 (208-294)		0.72
N-	49 (40)	21 (43)	141 (126-184)	<0.001	0.058
N+	75 (60)	46 (61)	222 (190-269)		0.88

All biomarkers are measured in the CSF and stated in pg/mL. All p-values were obtained using the Mann Whitney *U* test.

†Cut-offs for pathological concentrations of CSF AD-biomarkers were established by the laboratory as follows (in pg/mL): A- at amyloid β ($A\beta_{42}$) ≥ 530 and A+ at $A\beta_{42}$ <530; T- at phosphorylated tau (p-tau) ≤ 60 and T+ at p-tau> 60; N- at total tau (t-tau) ≤ 350 and N+ at t-tau> 350. Four hip fracture patients had missing values for Alzheimer's disease (AD) biomarkers (Aβ42 and t/p-tau).

*Difference in neurogranin concentration [Ng] in participants classified as A+ vs A-, T+ vs T- and N+ vs N-.

** Difference in [Ng] in delirium vs no delirium in the six AD-biomarker groups