

Preclinical Amyloid- β and Axonal Degeneration Pathology in Delirium

Idland A-V; Wyller TB; Stoen R; Eri LM; Frihagen F; Raeder J; Chaudhry FA; Hansson O; Zetterberg H; Blennow K; Bogdanovic N, Brækhus A, Watne LO.

¹Oslo Delirium Research Group, Department of Geriatric Medicine, University of Oslo, Oslo, Norway

²Institute of Clinical Medicine, University of Oslo, Oslo, Norway

³Research Group for Lifespan Changes in Brain and Cognition, Department of Psychology, University of Oslo, Oslo, Norway

⁴Department of Anesthesiology, Oslo University Hospital, Oslo, Norway

⁵Department of Urology, Oslo University Hospital, Oslo, Norway

⁶Department of Orthopedic Surgery, Oslo University Hospital, Oslo, Norway

⁷Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

⁸Department of Clinical Sciences, Lund University, Lund, Sweden

⁹Memory Clinic, Skåne University Hospital, Lund, Sweden

¹⁰Clinical Neurochemistry Lab, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

¹¹Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK

¹²Memory Clinic, Department of Geriatric Medicine, Oslo University Hospital, Oslo, Norway

¹³Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust,
Tønsberg

¹⁴Department of Neurology, Oslo University Hospital, Oslo, Norway

Correspondence: Ane-Victoria Idland, Oslo University Hospital, Ullevål, Department of Geriatric Medicine, PB 4956 Nydalen, 0424 Oslo, Norway. Telephone: + 47 – 91881081, E-mail: av.idland@gmail.com.

Authors' contributions:

Study concept and design: Idland, Ræder, Hansson, Brækhus, Wyller, Watne.

Acquisition of clinical data: Idland, Støen, Eri, Frihagen, Brækhus, Wyller, Watne.

Analysis of CSF: Zetterberg, Blennow.

Data analysis: Idland, Bogdanovic, Wyller, Watne.

Interpretation of data: Idland, Støen, Eri, Frihagen, Ræder, Chaudhry, Hansson, Zetterberg, Blennow, Bogdanovic, Brækhus, Wyller, Watne.

Drafting of the manuscript: Idland, Wyller, Watne.

Critical revision of the manuscript for important intellectual content: Idland, Støen, Eri, Frihagen, Ræder, Chaudhry, Hansson, Zetterberg, Blennow, Bogdanovic, Brækhus, Wyller, Watne.

Statistical analysis: Idland, Watne.

Obtained funding: Wyller.

Administrative, technical or material support: Idland, Støen, Eri, Frihagen, Brækhus, Wyller, Watne.

Conflicts of interest: Dr. Watne has given a lecture on delirium for Lilly. Dr. Blennow has served on Advisory Boards for IBL International and Roche Diagnostics. Prof. Wyller has given lectures on delirium for Pfizer, Roche, AstraZeneca and Nycomed. No other disclosures reported.

Word count: 2991

ABSTRACT

Importance: A large proportion of cognitively normal elderly have biomarker evidence of brain β -amyloidosis, but it is not settled whether this represents preclinical Alzheimer's disease (AD) or have any clinical consequences. Delirium and dementia are closely

interrelated, and common pathophysiological mechanisms are believed to exist. Clinical studies on elderly developing delirium may thus give pathophysiological clues.

Objective: To determine whether the AD cerebrospinal fluid (CSF) biomarkers A β 42, total tau (T-tau) and phosphorylated tau (P-tau) can indicate an increased risk of delirium in hip fracture patients with and without dementia.

Design: Prospective cohort study carried out from September 2009 to January 2012.

Setting: University-affiliated hospitals in Oslo, Norway.

Participants: A consecutive cohort (n=143) of participants in the Oslo Orthogeriatric Trial (n=332) with CSF samples collected in conjunction to spinal anesthesia (n=143).

Main Outcome and Measures: Delirium was assessed using the Confusion Assessment Method (CAM) once daily in all hip fracture patients, preoperatively and until the fifth postoperative day (until discharge for delirious patients). The diagnosis of dementia at admission was based upon clinical consensus. CSF levels of A β 42, A β 40, T-tau, and P-tau were analyzed.

Results: Preoperative CSF samples from 70 hip fracture patients with and 59 without delirium were analyzed. Markedly CSF A β 1-42 levels (median [range], 267 [125 to 713] ng/L vs 461 [125 to 1086] ng/L, p<.001), mildly increased T-tau levels (median [range], 439 [91 to 1380] ng/L vs 360 [133 to 1034] ng/L, p=.006), but no change in P-tau were found in hip fracture patients who developed delirium compared to those who remained lucid. In analyses stratified on dementia status, differences were only statistically significant in the dementia-free stratum, and they remained so after adjusting for cognitive impairment in logistic regression analyses.

Conclusions and Relevance: The marked reduction in CSF A β 42 and mild increase in T-tau in cognitively normal elderly hip fracture patients developing delirium suggest that preclinical brain amyloidosis is clinically relevant. These biomarkers may also possibly be used clinically to identify patients at risk of developing delirium.

INTRODUCTION

The application of biomarkers for Alzheimer's disease (AD) pathogenic processes have allowed clinical studies exploring at what time-point during the course of the disease β -amyloidosis, tau pathology and neurodegeneration occur, for review see (ref 11 – Jack 2013). It is recognized that a large proportion (up to 28% at age 75) of cognitively normal elderly have brain β -amyloidosis (Jack Jr CR, Lancet Neurol 2014;13:997–1005), but it is not settled whether this represents preclinical AD or have any clinical consequences.

Delirium represents an acute and fluctuating reduction in attention, awareness and cognition, commonly precipitated by an acute somatic condition. Dementia represents decline in memory and at least one other cognitive domain which interferes with independence in everyday activities.¹ AD accounts for around 60 % of all dementia cases.² Cognitive impairment and dementia are strong risk factors for delirium,³ and delirium is independently associated with an increased incidence of chronic cognitive decline and dementia.³ Thus, delirium and dementia are closely interrelated, and common pathophysiological mechanisms are believed to exist.^{3,4} The pathophysiological links are, however, not known.³

A β 42, total tau (T-tau) and phosphorylated tau (P-tau) are the core AD cerebrospinal fluid (CSF) biomarkers AD, and are believed to reflect brain pathophysiology. Reduced CSF A β 42 concentration reflects accumulation of aggregated A β in amyloid plaques.^{5,6} Increased T-tau level reflects the intensity of axonal degeneration,^{7,8} while increased P-tau level correlates with the amount of tangle pathology in the brain.⁹ A β 42 decreases at least 5-10 years before progression to AD dementia, while P-tau and T-tau are later markers.^{10,11} This implies that cognitively normal elderly and mild cognitive impairment (MCI) patients may have neuropathological features of AD in their brains.

Because delirium and dementia are clinically and epidemiologically interrelated, these CSF biomarkers may predict the occurrence of delirium, but studies so far show conflicting results.^{13,14}

The aim of our study was to examine if the AD the CSF biomarkers can indicate an increased risk of delirium in hip fracture patients with and without dementia.

METHODS

Participants

Patients were recruited from The Oslo Orthogeriatric Trial (OOT): a randomized, controlled, trial comparing orthogeriatric care with usual orthopedic care. Delirium incidence was a secondary outcome, and there was no difference in delirium rates between intervention and control group. A predefined secondary aim was to collect CSF in conjunction with spinal anesthesia in order to elucidate pathophysiological mechanisms in delirium. The OOT assessed for eligibility all patients that were acutely admitted to the Ullevaal Clinic of Oslo University Hospital with a hip fracture from September 2009 to January 2012. Patients were excluded if regarded as moribund and if the hip fracture was part of a high energy trauma. Details have been published previously.^{15,16}

A reference group was recruited from February 2012 to June 2013. Aged 65 years or older, they were undergoing elective surgery in spinal anesthesia. Exclusion criteria were dementia,

Parkinson's disease, previous stroke, or other central nervous system (CNS) disorders likely to affect cognition. The participants underwent cognitive testing before surgery, and CSF samples were collected in conjunction to spinal anesthesia.

Assessments

Relatives or health professionals were interviewed regarding pre-fracture status. Pre-fracture cognitive status was assessed using the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)¹⁷ and pre-fracture Activities of Daily Living (ADL) performance was assessed by the Barthel Index.¹⁸ Acute Physiology and Chronic Health Evaluation (APACHE) II score¹⁹ at admittance and the American Society of Anesthesiologists (ASA) score²⁰ before hip fracture surgery were also recorded. Delirium was assessed once daily by the study geriatrician (LOW) or a study nurse using the Confusion Assessment Method (CAM),²¹ both preoperatively and postoperatively. All patients were screened until the fifth postoperative day, and delirious patients were screened until discharge. CAM scores were based on an interview with the patient, including tests of cognition, attention and alertness (digit span test, orientation and delayed recall), information from close relatives and nurses, and review of hospital records from the previous 24 hours. The diagnosis of dementia at admission was based upon consensus between an experienced old age psychiatrist (KE) and an experienced geriatrician (TBW) who independently assessed whether the hip fracture patients fulfilled the ICD-10 criteria¹ for dementia. They were allowed access to relevant information extracted from clinical records, e.g. previous dementia diagnoses and cognitive test results, as well as scores on IQCODE, Clinical Dementia Rating (CDR),²² and Nottingham Extended ADL Scale (NEADL)²³ at admission and cognitive test results, CDR

score, and NEADL score at one-year follow-up. They were blind to records of delirium status during the hospital stay. Inter-rater agreement was very good (kappa 0.87), and disagreements were resolved through discussion.

The cognitive assessment prior to surgery in the reference group included the Clock Drawing Test,²⁴ Word List Memory Task from the Consortium to Establish a Registry for Alzheimer's Disease battery,²⁵ Trail Making Test A and B,²⁶ Kendrick Object Learning Test,²⁷ Controlled Oral Word Association Test (FAS version),²⁸⁻³⁰ and Animal Naming.^{30,31} A relative or friend filled in the IQCODE. Only cognitively normal older individuals, defined as maximum one abnormal score on the tests, were included in the reference group. A score < 4/5 on the Clock Drawing Test, and scores more than 2 standard deviations below the mean for age, gender and educational level for all other tests, were considered abnormal.

Cerebrospinal Fluid Collection, Selection and Analyses

CSF samples were obtained from 143 of 332 patients included in OOT. 14 patients were later excluded (eFigure 1). Demographics, delirium incidence, IQCODE scores, and proportion with dementia were not statistically significantly different in the 129 patients left for present analyses compared with the remaining OOT patients. CSF was obtained from 155 of the 172 subjects in the reference group, and after exclusion of cognitively abnormal subjects, 121 cognitively normal older individuals remained for present analyses (eFigure 1).

CSF was collected in polypropylene tubes, centrifuged, the supernatant aliquoted in polypropylene vials, frozen as soon as possible, and stored at -80°C pending analyses. CSF from hip fracture patients was thawed, aliquoted once more in polypropylene vials and frozen again before analyses.

In December 2013, the frozen supernatant samples of CSF were sent on dry ice to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, for analyses. A β 1-42, T-tau and P-tau concentrations were determined using INNOTEST enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium). The INNOTEST A β 42 method employs antibodies that specifically detect the neo-epitopes of the first and last amino acid of the 42 amino acid-long A β sequence.³² INNOTEST A β 42 will hereafter be designated A β 1-42. A β 42 concentrations were also measured using the Meso Scale Discovery (MSD) A β Triplex Assay (Meso Scale Discovery, Rockville, Maryland). This assay uses end-specific antibodies to capture A β peptides ending at amino acid 38, 40 and 42, respectively, and 6E10 (specific to amino acids 3 to 8) to detect them. MSD A β will hereafter be designated A β X-38, A β X-40 and A β X-42. Values outside the quantification limits were imputed as the quantification limit, 125 ng/L for A β 1-42 (4 patients) and 1380 ng/L for T-tau (3 patients). All analyses were performed by board-certified laboratory technicians, who were masked to clinical data, using one batch of reagents with intra-assay coefficients of variation below 10%.

Statistical analyses

Due to a non-normal distribution of the data, Mann-Whitney U-tests were used for analyses of continuous variables. Chi-Square or Fisher's Exact Tests were used for analyses of categorical variables. We calculated ratios of A β 1-42 to T-tau and P-tau because they may increase the predictive value of the CSF biomarkers.³³ A β 40 is thought to reflect the total A β peptide production;^{34,35} we therefore calculated a ratio of A β X-42 to A β X-40 to account for inter-individual differences in A β 42 levels. ELISA concentrations for A β 42 were used, except in the A β 42 to A β 40 ratio where we used the Triplex concentration. Subjects were classified as A β 1-42+ (< 530 ng/L) or A β 1-42- (\geq 530 ng/L), T-tau+ (> 350 ng/L) or T-tau- (\leq 350 ng/L), and P-tau+ (\geq 60 ng/L) or P-tau- (< 60 ng/L) according to cut-off values suggested by Hansson et al.³⁶ Logistic regression analyses were conducted to adjust for potential confounding of the association between biomarker concentrations and delirium.

Ethical considerations

The studies were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patients. If a hip fracture patient was unable to give an informed consent, a presumed consent in combination with assent from the closest relative was obtained. Sampling of both cohorts was approved by the Regional Committee for Ethics in Medical Research in Norway (REK S-09169a and REK 2011/2052).

RESULTS

Delirium versus no delirium

Demographics of the included patients and the reference group are given in Table 1. Seventy (54%) of the patients with available CSF developed delirium in conjunction to the hip fracture, either preoperatively or postoperatively (Table 1). These patients were older, had higher morbidity, and were more dependent in ADL. They also showed a greater prevalence of dementia and nursing home residency, compared with the hip fracture patients who did not develop delirium. CSF A β 1-42 levels were markedly lower (36% of control levels) in patients who developed delirium, and 91% of patients with delirium were A β 1-42+ (Table 1). CSF T-tau levels were slightly higher in patients with delirium (126% of control levels), and the proportion of patients being T-tau+ did not significantly differ from those without delirium (Table 1). CSF P-tau levels and proportion of P-tau+ patients did not differ between patients who did and did not develop delirium. CSF A β X-42, A β X-40, and A β X-38 concentrations were significantly lower in hip fracture patients with delirium than in those without (Table 1).

Preoperative delirium versus postoperative delirium

Forty-three of the patients with delirium developed delirium preoperatively, while 22 patients developed delirium postoperatively (preoperative delirium status missing in five patients). The CSF biomarker levels were not significantly different between patients who developed delirium before or after surgery (data not shown).

Dementia versus no dementia

Fifty-four/sixty-four (84 %) demented hip fracture patients developed delirium compared with 16/65 (25 %) among non-demented patients ($p < 0.001$). Abnormal CSF biomarker levels were more prevalent in demented patients than in non-demented patients : 59/64 (92 %) vs 41/65 (63%) were A β 1-42+, and 43/64 (67 %) vs 36/65 (55%) were T-tau+.

Delirium versus no delirium stratified on dementia status

In stratified analyses a marked reduction in CSF A β 1-42 was found in patients with delirium as compared with those without, while T-tau showed a mild increase and P-tau no significant difference (Table 2 and Figure 1). No CSF biomarker showed statistically significant differences for the demented stratum (Table 2 and Figure 1). CSF A β X-42, A β X-40, and A β X-38 concentrations were not associated with delirium in any of the strata (Table 2). Because the degree of cognitive impairment might have confounded the association despite the stratification procedure, we entered CSF biomarkers and biomarker ratios, age, gender, and IQCODE scores into logistic regression models with delirium status as the dependent variable. This analysis was limited to the dementia-free stratum, where the associations had been observed in bivariate analyses. CSF levels of A β 1-42 and T-tau and the ratios of A β 42 to A β X-40, Tau and P-tau, remained significantly associated with delirium status in these analyses. CSF P-tau levels were not significantly associated with delirium in the adjusted model (Table 3).

Combinations of amyloid and tau pathology

Patients were then divided into four groups according to the presence of amyloid pathology (A β 1-42+ or A β 1-42-) and/or tau pathology (P-tau+ or P-tau-). Seventy-three % of the patients in the “A β 1-42+ and P-tau+” group developed delirium, 56 % of the “A β 1-42+ and P-tau-” did so, and among patients with negative A β 1-42, 13 % of the P-tau+ and 29 % of the P-tau- developed delirium (Figure 2a). Also for these analyses, statistically significant differences were seen only in the dementia-free stratum (Figure 2b and c). In that stratum, 56 % of the patients with evidence of both amyloid and tau pathology developed delirium, while the three other groups had a delirium occurrence below 20 % (Figure 2b). In the stratum with dementia, around 85 % of the A β 1-42+ patients developed delirium (Figure 2c).

Comparison of hip fracture patients and cognitively normal older individuals

CSF concentrations in hip fracture patients with delirium were 36 % of those in cognitively normal older individuals for A β 1-42 and 127 % of those in cognitively normal older individuals for T-tau. The hip fracture patients without delirium were older, frailer, had more dementia and other comorbid disorders than the cognitively normal older individuals (Table 1). CSF levels of A β 1-42 and the ratios of A β 42 to A β X-40, T-tau, and P-tau were significantly lower in hip fracture patients without delirium compared to cognitively normal older individuals (Table 1). There were, however, no differences in CSF T-tau and P-tau levels between these two groups (Table 1).

DISCUSSION

This is, to our knowledge, the first study to examine the relationship between preoperative CSF levels of A β 42, T-tau and P-tau and delirium in both demented and non-demented patients. It is also the first study to show a convincing association between these biomarkers and delirium. We found lower CSF A β 1-42 levels and also clearly lower ratios of A β 42 to A β X-40, T-tau and P-tau, together with an increase in CSF T-tau levels in hip fracture patients who developed delirium compared to those who remained delirium free.

In analyses stratified on dementia status, we found that CSF A β 1-42, T-tau and all ratios were significantly associated with delirium in non-demented hip fracture patients, irrespective of age, gender and IQCODE score. This indicates that preclinical AD pathology increases the risk of delirium before the patient experiences a chronic cognitive impairment. It is well established that reduced CSF A β 42 concentration reflects accumulation of aggregated A β in amyloid plaques in the brain,^{5,6} also in cognitively normal individuals,³⁷ but there is very limited data on whether amyloid pathology in non-demented elderly has any clinical consequences. An important finding is therefore that non-demented elderly with clinically silent amyloid pathology were found to develop symptoms (i.e. delirium) when exposed to physical stress. Amyloid pathology has been associated with dysfunction in brain networks in cognitively normal individuals,³⁸⁻⁴² and a breakdown of network connectivity in the brain has been suggested to be the neurobiological substrate of delirium.⁴³ It is likely that axonal degeneration and tangle pathology reflected by CSF T-tau and P-tau also have detrimental effects on brain network connectivity.⁴² We found that non-demented patients with positive A β 1-42 and P-tau were more likely to develop delirium than patients with an abnormality in only one of these two biomarkers, thus the vulnerability to delirium seems to increase with an increasing degree of neuropathology. [Hela stycket ovan är jättebra !!]

Although delirium is an independent risk factor for incident dementia,³ our findings raise the question of whether delirium simply unmasks a preclinical dementia. Also hip fracture patients without delirium had more pathological CSF biomarkers than the cognitively normal older individuals. This indicates that hip fracture patients constitute a frail population with a high prevalence of preclinical dementia even in patients who do not develop delirium.

Cavallari and colleagues did not find an association between any MRI measures of white-matter damage, global brain, and hippocampal volume and the incidence of delirium in a population of non-demented patients.⁴⁴ A possible explanation could be that most non-demented patients have insufficient structural brain pathology at a macroscopic level to be detected by MRI. CSF biomarkers may have a greater potential for detecting early brain pathology relevant for delirium.

We found no significant associations between the CSF biomarkers and delirium in hip fracture patients with dementia. Patients with dementia have more extensive brain pathology, and in our study almost all demented patients had abnormal CSF biomarkers and developed delirium. The abnormalities in biomarkers have likely reached a plateau in dementia,¹¹ thus the differences in biomarker levels between demented patients are likely to be small.

Witlox and colleagues performed a prospective cohort study in elderly hip fracture patients similar to our study.¹³ However, they excluded patients with a dementia diagnosis. They found no association between biomarkers and delirium. The biomarker levels in their study,

both in patients with and without delirium, were much closer to the normal values than we found. This might indicate that AD brain pathology was less prevalent in their study population. Xie and colleagues found CSF levels of A β 42 and T-tau more similar to our findings.¹⁴ Like Witlox and colleagues, they did not find an association between preoperative CSF biomarker levels and postoperative delirium in a population ≥ 63 years old having elective total hip or knee replacement. However, when they divided the participants into quartiles according to A β 40/T-tau and A β 42/Tau ratios, they found a higher delirium incidence in the lowest quartile.

Strengths of our study include the daily and bedside assessment of delirium with validated instruments and the inclusion of patients both with and without dementia. A larger number of patients included than in other delirium biomarker studies made subgroup analyses on dementia and time of delirium debut possible. CSF was analyzed in a laboratory with an extensive experience on A β 42, T-tau and P-tau analyzes in CSF.

Some limitations deserve a comment. The study might have been underpowered to detect biomarker differences by delirium status in the demented stratum, because demented patients had a high occurrence of delirium and a high prevalence of abnormal CSF biomarkers. Dementia diagnoses based on a cognitive assessment before the patients became hospitalized would be better than our retrospective dementia diagnoses. However, since hip fracture patients are acutely admitted to the hospital, this was not feasible. We also believe that our consensus diagnosis of dementia is more accurate than merely IQCODE cut-offs. The dementia diagnosis is, however, not etiological, thus even though a high proportion of the patients with dementia likely have AD dementia, they may also have other dementias.

Larger studies of different populations are needed to more firmly assess the potential association between A β 42, T-tau and P-tau and delirium.

CONCLUSION

We demonstrate a marked reduction in CSF A β 42, indicating β -amyloidosis, together with a mild increase in T-tau, indicating neurodegeneration, in cognitively normal elderly hip fracture patients developing delirium. These findings indicate that preclinical brain amyloidosis have clinical consequences and suggest that these patients have preclinical AD. The CSF biomarkers may be analyzed preoperatively in hip fracture patients, thereby allowing an increased focus on delirium prevention in elderly patients with abnormal biomarkers.

ACKNOWLEDGEMENTS

Ms. Idland had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Authors' contributions:

Study concept and design: Idland, Ræder, Hansson, Brækhus, Wyller, Watne.

Acquisition of clinical data: Idland, Støen, Eri, Frihagen, Brækhus, Wyller, Watne.

Analysis of CSF: Zetterberg, Blennow.

Data analysis: Idland, Bogdanovic, Wyller, Watne.

Interpretation of data: Idland, Støen, Eri, Frihagen, Ræder, Chaudhry, Hansson, Zetterberg, Blennow, Bogdanovic, Brækhus, Wyller, Watne.

Drafting of the manuscript: Idland, Wyller, Watne.

Critical revision of the manuscript for important intellectual content: Idland, Støen, Eri, Frihagen, Ræder, Chaudhry, Hansson, Zetterberg, Blennow, Bogdanovic, Brækhus, Wyller, Watne.

Statistical analysis: Idland, Watne.

Obtained funding: Wyller.

Administrative, technical or material support: Idland, Støen, Eri, Frihagen, Brækhus, Wyller, Watne.

Conflicts of interest: Dr. Watne has given a lecture on delirium for Lilly. Dr. Blennow has served on Advisory Boards for IBL International and Roche Diagnostics. Prof. Wyller has given lectures on delirium for Pfizer, Roche, AstraZeneca and Nycomed. No other disclosures reported.

Funding/support: The study was mainly funded by the Research Council of Norway through the program "Improving mental health of older people through multidisciplinary efforts" (grant no 187980/H10). Further, we have received funding from Oslo University Hospital,

The Sophies Minde Foundation, The Norwegian Association for Public Health, Civitan's Research Foundation, South-Eastern Norway Regional Health Authority, the Medical Student Research Program at the University of Oslo, The National Association for Public Health's dementia research program, the Knut and Alice Wallenberg Foundation, the Research Council, Sweden, and the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences

Role of Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review or approval of the manuscript; and decision to submit the manuscript to publication.

Additional Contributors: Knut Engedal prof. emeritus, MD, PhD, affiliated with the Institute of Clinical Medicine, Department of Geriatrics, University of Oslo and Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust, Tønsberg, contributed to the dementia consensus diagnoses. The study nurses Elisabeth Fragaat and Tone Fredriksen, affiliated with Department of Geriatrics, Oslo University Hospital, Oslo, Norway, Anette Hylen Ranhoff professor, MD, PhD affiliated with the Department of Internal Medicine, Diakonhjemmet Hospital, Oslo, Norway and the Department of Clinical Science, Kavli Research Center for Geriatrics and Dementia, University of Bergen, Bergen, Norway, Gry Torsæter Dahl, MD, affiliated with the Department of Anesthesiology, Diakonhjemmet Hospital, Oslo, Norway, Arne Myklebust, MD, and Dagfinn Tore Kollerøs, MD, affiliated with the Department of Anesthesiology, Oslo University Hospital, Oslo, Norway, contributed to data acquisition. We also acknowledge the contributions of the Department of Gynecology, the Department of Urology, the Department of Orthopedic

Surgery and the Department of Anesthesiology at Oslo University Hospital, the Department of Orthopedic Surgery and the Department of Anesthesiology at Diakonhjemmet Hospital in Oslo, Norway.

REFERENCES

1. Organization WH. *The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organization. ; 1992.
2. Kalaria RN, Maestre GE, Arizaga R, et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *The Lancet Neurology*. 2008;7(9):812-826.
3. Fong TG, Davis D, Growdon ME, Albuquerque A, Inouye SK. The interface between delirium and dementia in elderly adults. *The Lancet Neurology*. 2015;14(8):823-832.
4. Eikelenboom P, Hoogendijk WJG. Do delirium and Alzheimer's dementia share specific pathogenetic mechanisms? *Dement. Geriatr. Cogn. Disord*. 1999;10(5):319-324.
5. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*. 2003;60(4):652-656.
6. Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol. Sci*. 2015;36(5):297-309.
7. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci. Lett*. 2001;297(3):187-190.
8. Riemenschneider M, Wagenpfeil S, Vanderstichele H, et al. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. *Mol. Psychiatry*. 2003;8(3):343-347.
9. Tapiola T, Alafuzoff I, Herukka SK, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Arch. Neurol*. 2009;66(3):382-389.
10. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal Fluid Levels of beta-Amyloid 1-42, but Not of Tau, Are Fully Changed Already 5 to 10 Years Before the Onset of Alzheimer Dementia. *Arch. Gen. Psychiatry*. 2012;69(1):98-106.
11. Jack CR, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216.
12. Zetterberg H, Blennow K, Hanse E. Amyloid beta and APP as biomarkers for Alzheimer's disease. *Exp. Gerontol*. 2010;45(1):23-29.
13. Witlox J, Kalisvaart KJ, de Jonghe JF, et al. Cerebrospinal fluid beta-amyloid and tau are not associated with risk of delirium: a prospective cohort study in older adults with hip fracture. *J.Am.Geriatr.Soc*. 2011;59(7):1260-1267.
14. Xie Z, Swain CA, Ward SAP, et al. Preoperative cerebrospinal fluid β -Amyloid/Tau ratio and postoperative delirium. *Annals of Clinical and Translational Neurology*. 2014;1(5):319-328.
15. Watne LO, Torbergsen AC, Conroy S, et al. 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*. 2014;12:12.
16. Wyller TB, Watne LO, Torbergsen A, et al. 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*. 2012;12:36.

17. Jorm AF. A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation. *Psychol.Med.* 1994;24(1):145-153.
18. Mahoney FI, Barthel DW. FUNCTIONAL EVALUATION: THE BARTHEL INDEX. *Md. State Med. J.* 1965;14:61-65.
19. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit. Care Med.* 1985;13(10):818-829.
20. Dripps R. New classification of physical status. *Anesthesiology.* 1963;24(24):111.
21. Inouye SK, van Dyck CH, Alessi CA, Balkin S, Siegel AP, Horwitz RI. Clarifying confusion: the confusion assessment method. A new method for detection of delirium. *Ann.Intern.Med.* 1990;113(12):941-948.
22. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br. J. Psychiatry.* 1982;140:566-572.
23. Lincoln NB, Gladman JR. The Extended Activities of Daily Living scale: a further validation. *Disabil. Rehabil.* 1992;14(1):41-43.
24. Shulman KI. Clock-drawing: is it the ideal cognitive screening test? *Int. J. Geriatr. Psychiatry.* 2000;15(6):548-561.
25. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology.* 1989;39(9):1159-1165.
26. Reitan RM. The Relation of the Trail Making Test to Organic Brain Damage. *J. Consult. Psychol.* 1955;19(5):393-394.
27. Kendrick DC, Gibson AJ, Moyes ICA. REVISED KENDRICK BATTERY - CLINICAL STUDIES. *Br. J. Soc. Clin. Psychol.* 1979;18(SEP):329-340.
28. Strauss E, Sherman EMS, Spreen O, Spreen O. *A compendium of neuropsychological tests : administration, norms, and commentary.* Oxford; New York: Oxford University Press; 2006.
29. Benton AL, Hamsher, K.de S. *Multilingual aphasia examination manual.* University of Iowa, Iowa City, IA; 1989.
30. Tombaugh TN, Kozak J, Rees L. Normative Data Stratified by Age and Education for Two Measures of Verbal Fluency: FAS and Animal Naming. *Arch. Clin. Neuropsychol.* 1999;14(2):167-177.
31. Rosen WG. VERBAL FLUENCY IN AGING AND DEMENTIA. *J. Clin. Neuropsychol.* 1980;2(2):135-146.
32. Andreasen N, Hesse C, Davidsson P, et al. Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch. Neurol.* 1999;56(6):673-680.
33. Holtzman DM. CSF biomarkers for Alzheimer's disease: current utility and potential future use. *Neurobiol. Aging.* 2011;32:S4-S9.
34. Wiltfang J, Esselmann H, Bibl M, et al. Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. *J. Neurochem.* 2007;101(4):1053-1059.
35. Shoji M, Matsubara E, Kanai M, et al. Combination assay of CSF tau, A beta 1-40 and A beta 1-42(43) as a biochemical marker of Alzheimer's disease. *J. Neurol. Sci.* 1998;158(2):134-140.
36. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* 2006;5(3):228-234.
37. Landau SM, Lu M, Joshi AD, et al. Comparing positron emission tomography imaging and cerebrospinal fluid measurements of beta-amyloid. *Ann. Neurol.* 2013;74(6):826-836.
38. Sheline YI, Raichle ME, Snyder AZ, et al. Amyloid Plaques Disrupt Resting State Default Mode Network Connectivity in Cognitively Normal Elderly. *Biol. Psychiatry.* 2010;67(6):584-587.
39. Drzezga A, Becker JA, Van Dijk KR, et al. Neuronal dysfunction and disconnection of cortical hubs in non-demented subjects with elevated amyloid burden. *Brain.* 2011;134(Pt 6):1635-1646.

40. Hedden T, Van Dijk KR, Becker JA, et al. Disruption of functional connectivity in clinically normal older adults harboring amyloid burden. *J. Neurosci.* 2009;29(40):12686-12694.
41. Mormino EC, Smiljic A, Hayenga AO, et al. Relationships between beta-amyloid and functional connectivity in different components of the default mode network in aging. *Cereb. Cortex.* 2011;21(10):2399-2407.
42. Wang L, Brier MR, Snyder AZ, et al. Cerebrospinal fluid $\text{A}\beta_{42}$, phosphorylated tau181, and resting-state functional connectivity. *JAMA Neurol.* 2013;70(10):1242-1248.
43. Sanders RD. Hypothesis for the pathophysiology of delirium: role of baseline brain network connectivity and changes in inhibitory tone. *Med.Hypotheses.* 2011;77(1):140-143.
44. Cavallari M, Hshieh TT, Guttmann CR, et al. Brain atrophy and white-matter hyperintensities are not significantly associated with incidence and severity of postoperative delirium in older persons without dementia. *Neurobiol. Aging.* 2015;36(6):2122-2129.

FIGURE LEGENDS

Figure 1. CSF $\text{A}\beta_{1-42}$, $\text{A}\beta_{X-42}/\text{X-40}$ ratio, T-tau and $\text{A}\beta_{1-42}/\text{T-tau}$ ratio in non-demented patients with and without delirium.

Footnotes: Dementia status was based upon consensus between two experienced clinicians. There were 16 patients with delirium and 49 without. P-values were calculated using Mann-Whitney U-tests.

Figure 2. Delirium occurrence according to combinations of amyloid and tau pathology.

Explanatory legends: Percent of patients who developed delirium during the hospital stay according to grouping by positive and negative $\text{A}\beta_{1-42}$ and P-tau values. Subjects were classified as $\text{A}\beta_{1-42}+$ (< 530 ng/L) or $\text{A}\beta_{1-42}-$ (≥ 530 ng/L), and P-tau+ (≥ 60 ng/L) or P-tau- (< 60 ng/L). P-values were calculated using Chi-Square and Fisher's Exact Tests. a) all hip fracture patients, b) hip fracture patients without dementia c) hip fracture patients with dementia.

eFigure1. Selection of CSF samples

Explanatory legends: Cognitively normal older individual was defined as maximum one abnormal score on cognitive tests.

TABLES

Table 1. Baseline Characteristics.

	Cognitively normal older individuals (n=121)	Hip Fracture Patients (n=129)		P value ^a	P value ^b
		Without Delirium (n=59)	With Delirium (n=70)		
Age, median (range)	72 (64 to 93)	83 (60 to 94)	85 (68 to 101)	.02	<.001
Male, n (%)	59 (49)	13 (22)	21 (30)	.31 ^c	.001 ^c
IQCODE, median (range) ^c	3.00 (2.69 to 3.56)	3.19 (3.00 to 5.00)	4.53 (3.00 to 5.00)	<.001	<.001
Dementia, n (%) ^f	0 (0)	10 (17)	54 (77)	<.001 ^c	-
Nursing home, n (%)	-	9 (15)	34 (49)	<.001 ^c	-
APACHE II, median (range) ^g	-	8 (5 to 14)	9 (6 to 21)	.004	-
Barthel ADL, median (range)	-	19 (4 to 20)	15 (3 to 20)	<.001	-
ASA score ^h	-			-	-
I, n (%)		1 (2)	0 (0)		
II, n (%)		31 (54)	19 (28)		
III, n (%)		25 (44)	47 (69)		
IV, n (%)		0 (0)	2 (3)		
Stroke or TIA, n (%)	-	9 (15)	13 (19)	.62 ^c	-
Diabetes mellitus, n (%)	15 (12)	8 (14)	11 (16)	.73 ^c	.83 ^c
Ischemic heart disease, n (%)	16 (13)	13 (22)	18 (26)	.63 ^c	.13 ^c
A β 1-42, ng/L, median (range)	737 (275 to 1175)	461 (125 to 1086)	267 (125 to 713)	<.001	<.001
A β 1-42+ (< 530 ng/L), n (%)	32 (26)	36 (61)	64 (91)	<.001 ^c	<.001 ^c
T-Tau, ng/L, median (range)	347 (114 to 826)	360 (133 to 1034)	439 (91 to 1380)	.006	.92
T-tau+ (> 350 ng/L), n (%)	59 (49)	31 (53)	48 (69)	.06 ^c	.63 ^c
P-tau, ng/L, median (range)	59 (25 to 115)	55 (18 to 118)	60 (20 to 201)	.25	.35
P-tau+ (\geq 60 ng/L), n (%)	55 (46)	25 (42)	35 (50)	.39 ^c	.70 ^c
A β X-42, ng/L, median (range) ⁱ	417 (88 to 1106)	225 (25 to 1029)	123.5 (38 to 612)	<0.001	<0.001
A β X-40, ng/L, median (range) ⁱ	4873 (2220 to 10187)	3488 (779 to 8535)	2916 (1130 to 6490)	0.003	<0.001
A β X-38, ng/L, median (range) ⁱ	2054 (828 to 4994)	1548 (260 to 3867)	1120 (353 to 3732)	0.004	<0.001
A β X-42/X-40, median (range) ⁱ	0.09 (0.03 to 0.13)	0.07 (0.02 to 0.14)	0.04 (0.02 to 0.14)	<.001	.001
A β 1-42/T-tau, median (range)	2.07 (0.44 to 4.30)	1.38 (0.15 to 5.73)	0.56 (0.09 to 2.45)	<.001	<.001

Aβ1-42/P-tau, median (range)	13.09 (3.40 to 24.72)	8.96 (1.37 to 31.60)	4.40 (0.86 to 14.71)	<.001	<.001
------------------------------	-----------------------	----------------------	----------------------	-------	-------

Abbreviations: IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly. APACHE II = Acute Physiology and Chronic Health Evaluation II. Barthel ADL = Barthel Index of Activities of Daily Living. ASA-score = Score of The American Society of Anaesthesiologists' classification of Physical Health. TIA = Transient Ischemic Attack. P-values were calculated using Mann-Whitney U-test if not otherwise specified. Aβ 1-42 was measured using INNOTEST enzyme-linked immunosorbent assays (ELISA) if not otherwise specified.

^a Comparing hip fracture patients with and without delirium

^b Comparing cognitively normal older individuals with hip fracture patients without delirium

^c Chi-square test

^d Fischer's exact test

^e Missing in two patients with hip fracture without delirium and in two cognitively normal older individuals

^f Based upon consensus in an expert panel

^g APACHE II score without hematocrit and blood gas values

^h Missing in two patients with delirium and two patients without delirium

ⁱ AβX-42, AβX-40 and AβX-38 were measured with Aβ Triplex Assay

Table 2. CSF markers in relation to delirium and dementia status.

	No dementia		P value
	Without Delirium (n=49)	With Delirium (n=16)	
Aβ1-42, ng/L	489 (133 to 1086)	310 (125 to 633)	.006
T-Tau, ng/L	351 (132 to 808)	505 (187 to 1266)	.02
P-tau, ng/L	54 (18 to 118)	72 (25 to 154)	.06
AβX-42, ng/L	230 (57 to 1029)	142.5 (90 to 612)	0.085
AβX-40, ng/L	3488 (967 to 8535)	3281.5 (1598 to 6490)	0.69
AβX-38, ng/L	1548 (276 to 3867)	1434 (477 to 3732)	0.67
AβX-42/X-40	.08 (.02 to .14)	.04 (.03 to .14)	.004
Aβ1-42/T-tau	1.59 (.16 to 5.73)	.52 (.25 to 2.45)	<.001
Aβ1-42/P-tau	9.96 (1.37 to 31.60)	3.89 (1.82 to 14.71)	.001
	Dementia		P value
	Without Delirium	With Delirium	

	(n=10)	(n=54)	
Aβ1-42, ng/L	317.5 (125 to 675)	257.5 (125 to 713)	.43
T-Tau, ng/L	441 (114 to 1034)	410 (91 to 1380)	.67
P-tau, ng/L	61 (19 to 109)	56 (20 to 201)	.94
AβX-42, ng/L	146.5 (25 to 447)	116.5 (38 to 472)	0.24
AβX-40, ng/L	3414 (779 to 5590)	2466 (1130 to 6210)	0.20
AβX-38, ng/L	1356.0 (260 to 2241)	987 (353 to 3274)	0.24
AβX-42/X-40	.05 (0.02 to 0.08)	.04 (0.02 to 0.11)	.24
Aβ1-42/T-tau	.96 (0.15 to 1.41)	.61 (0.09 to 2.39)	.17
Aβ1-42/P-tau	7.21 (1.42 to 10.68)	4.61 (0.86 to 14.34)	.24

Footnotes: Values are median (range). P-values were calculated using Mann-Whitney U-test.

Table 3. Logistic regression analyzes controlling for age, gender and cognition.

Explanatory legend: Logistic regression analyzes controlling for potential confounders of the association between delirium and biomarkers in patients without dementia

	Unadjusted		Adjusted by age, gender and IQCODE score	
	OR	95 % CI	OR	95 % CI
Aβ1-42, ng/L	.995	.992 to .999	.993	.989 to .998
T-tau, ng/L	1.004	1.001 to 1.007	1.003	1.000 to 1.006
P-tau, ng/L	1.023	1.002 to 1.045	1.019	.987 to 1.042
AβX-42/X-40 ratio x10	.058	.005 to .615	.043	.002 to .863
Aβ1-42/T-tau ratio	.18	.06 to .56	.16	.04 to .61
Aβ1-42/P-tau ratio	.76	.63 to .92	.75	.60 to .93

Footnotes: The table displays the odds ratio of developing delirium per unit change in the biomarkers and ratios.

AbX-42/X-40 ratio is multiplied with 10 in order to get a more meaningful OR. Abbreviations: OR = Odds

Ratio. CI = Confidence Interval. IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly.

Removal of outliers, defined as standard residuals $> \pm 3$ or Cook's distance > 1 , did not change the odds ratios or significance of the biomarkers considerably, and outliers were therefore kept in the model.