JCI The Journal of Clinical Investigation

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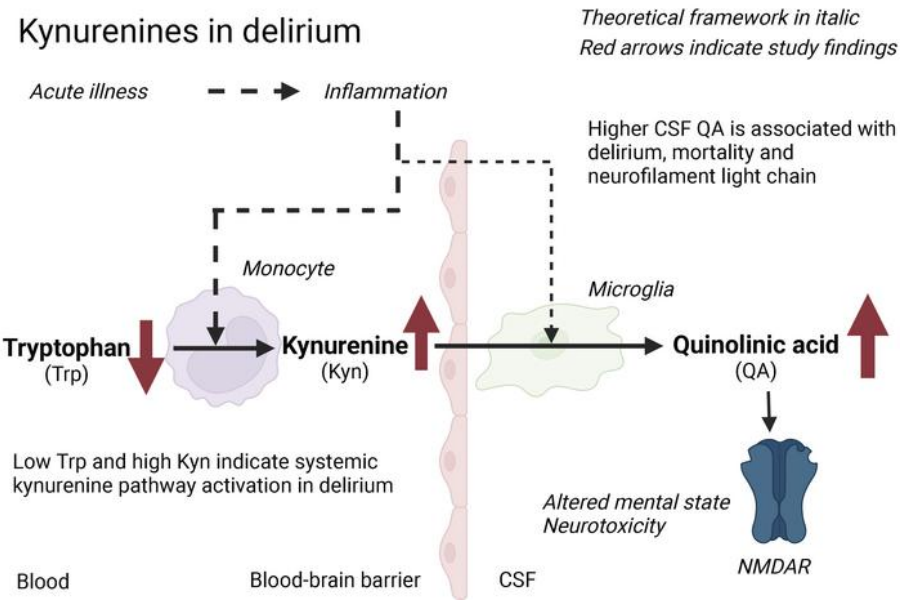
J Clin Invest. 2022. https://doi.org/10.1172/JCI163472.

Clinical Medicine | In-Press Preview | Inflammation | Metabolism

Graphical abstract



Cerebrospinal fluid quinolinic acid is strongly associated with delirium and mortality in hip fracture patients

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Declaration of Interets

Geir Selbæk: paid lecture at symposium sponsored by Biogen. Anne Brita Knapskog has been/is a principal investigator in Roche drug trial BN29553, in Boehringer-Ingelheim drug trial 1346.0023 and Novo Nordisk drug trial NN6535-4730. Henrik Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Evandro Fei Fang has CRADA arrangement with ChromaDex, and is consultant to Aladdin Healthcare Technologies, Vancouver Dementia Prevention Centre, and Intellectual Labs. All other authors have declared that no conflict of interest exists.

ABSTRACT

Background: The kynurenine pathway (KP) has been identified as a potential mediator linking acute illness to cognitive dysfunction by generating neuroactive metabolites in response to inflammation. Delirium (acute confusion) is a common complication of acute illness and is associated with increased risk of dementia and mortality. However, the molecular mechanism underlying delirium, particularly in relation to the KP, remain elusive.

Methods: We undertook a multi-center observational study with 586 hospitalized patients (248 with delirium) and investigated associations between delirium and KP metabolites measured in cerebrospinal fluid (CSF) and serum by targeted metabolomics. We also explored associations between KP metabolites and markers of neuronal damage and one-year mortality.

Results: In delirium, we found concentrations of the neurotoxic metabolite quinolinic acid in CSF (CSF-QA, OR 2.26 [1.78, 2.87], $p<0.001$) to be increased, as well as increases in several other KP metabolites in serum and CSF. In addition, CSF-QA was associated with the neuronal damage marker neurofilament light chain (NfL, β 0.43, $p<0.001$) and was a strong predictor of one-year mortality (HR 4.35 [2.93, 6.45] for CSF-QA $\geq$ 100 nmol/L, $p<0.001$). The associations between CSF-QA and delirium, neuronal damage, and mortality remained highly significant following adjustment for confounders and multiple comparisons.

Conclusion: Our data identified how systemic inflammation, neurotoxicity, and delirium are strongly linked via the KP, and should inform future delirium prevention and treatment clinical trials that target enzymes of the KP.

Funding: Norwegian Health Association and the South-Eastern Norway Regional Health Authorities

Keywords: delirium; dementia; CSF biomarkers; kynurenine pathway; quinolinic acid (QA); neurofilament light chain (NfL)

INTRODUCTION

The kynurenine pathway (KP) has been identified as a potential mediator linking acute illness to cognitive dysfunction by generating neuroactive metabolites in response to inflammation (1, 2). An imbalance of KP intermediates, exerting either neuroprotective or neurotoxic effects, has been linked to inflammation, neuronal damage, and ultimately a variety of neurodegenerative and psychiatric diseases (3, 4). Under physiological conditions, the first step in the KP is regulated by the rate-limiting enzyme tryptophan 2,3-dioxygenase (TDO), which catalyzes the conversion of tryptophan (Trp) to kynurenine (Kyn), subsequently leading to the production of several intermediates and end products, including the neuroprotective kynurenic acid (KA) and picolinic acid (Pic) as well as the neurotoxic 3-hydroxykynurenine (HK), 3-hydroxyanthranilic acid (HAA), and quinolinic acid (QA) **(Supplementary Figure 1**). However, during systemic inflammation, pro-inflammatory cytokines induce indolamine 2,3 dioxygenase (IDO) expressed in monocytes and dendritic cells, which also catalyzes the conversion of Trp to Kyn (5). Kyn is capable of crossing the blood-brain barrier (BBB) and acts as the precursor for approximately 60% of KP metabolites in the brain. Thus, systemic activation of IDO may be capable of increasing brain concentrations of metabolites such as the *N*-methyl-D-aspartate receptor (NMDAR) antagonist KA and the NMDAR agonist QA (6, 7). The NMDAR is expressed ubiquitously in the nervous system but mostly in the cortex and hippocampus(8). This could be of relevance to the pathogenesis of delirium as the NMDAR is implicated in cognitive function, psychosis, and excitotoxicity-induced neuronal cell death (2).

Delirium, a frequent and severe complication of acute illness, is characterized by sudden impairments in awareness and cognition (9). Despite its high prevalence in acutely hospitalized patients and a significant risk for mortality and future dementia (9, 10), the pathophysiology of delirium remains largely unknown. The recent COVID-19 pandemic further highlighted delirium as it is a common consequence of severe cases of COVID-19 (11). Proposed mechanisms of delirium include transient disruptions in neuroinflammatory and neurotransmitter pathways (9, 12). We propose that systemic inflammation, triggered by e.g. tissue damage or infection activates IDO, resulting in disrupted balance

of the kynurenines and, ultimately, delirium. Indeed, experimental studies have implicated the KP as a mediator of inflammation-induced cognitive dysfunction. In rats subjected to polymicrobial sepsis, IDO inhibitors prevented cognitive impairment (13). Similarly, IDO-deficient mice were protected from cognitive dysfunction following endotoxin injection (6). Clinical studies have identified an increased Kyn:Trp ratio (KTR) in blood, a potential marker of IDO activity that usually increases during inflammation, to be associated with delirium (14-16). However, to the best of our knowledge, no studies have been conducted on KP metabolites in cerebrospinal fluid (CSF), and the potentially neuroactive metabolites have not been measured, thus leaving unanswered the key question of the potential for engagement of neuroactive kynurenine metabolites with their targets in delirium.

We investigated the association between delirium and KP metabolite concentrations in CSF and serum in acutely hospitalized patients. We chose patients diagnosed with hip fracture as this group commonly develops delirium, and CSF sampling is feasible in conjunction with preoperative spinal anesthesia (17, 18). In addition, we included a group of patients with delirium triggered by another medical condition to compare kynurenine concentrations in relation to different etiologies of delirium. Cognitively unimpaired adults without delirium or dementia served as a reference group. To explore the pathophysiological relevance of the KP to delirium and outcomes for hip fracture patients, we also investigated the association between kynurenines and the neuronal damage marker, neurofilament light chain protein (NfL) (19), and one-year mortality. We hypothesized that the KP in CSF and blood was upregulated in delirium and that an accumulation of neuroactive metabolites was associated with delirium.

RESULTS

Demographics and clinical characteristics

We undertook a multi-center observational study comprising 586 participants, with CSF and serum samples collected for metabolic studies. These participants were from three study cohorts (p-values in text) with key demographics and clinical characteristics summarized in **Figure 1 and Table 1**. The hip fracture patients were older (mean age, yr (SD) 81 (11)) and included more women (68%) than medical delirium patients (42% female, 69 (12)) and the cohort of cognitively unimpaired (45% female, 73 (7)), both $p<0.001$, for both T-test and Pearson's x^2. Delirium occurred in 224 (49.8%) hip fracture patients during the hospital stay. Of these, 113 had delirium before surgery (prevalent) and 108 after surgery (incident), while this information was missing for 3 patients. An additional 44 patients had subsyndromal delirium. Hip fracture patients with delirium more often had pre-fracture cognitive impairment (IQCODE $\geq$ 3.44, 72 vs 19%), and more had severe systemic disease (American Society of Anesthesiologists (ASA) score III–IV, 68% vs 39%) compared to hip fracture patients without delirium (both $p<0.001$, Pearson's x^2).

Kynurenines and associations with delirium

Figure 2 lists the odds ratios **(**OR) for delirium in hip fracture patients according to concentrations of serum and CSF kynurenines (please also see **Supplementary Table 1** for effect sizes). In unadjusted analyses, several kynurenines in both serum and CSF were significantly associated with delirium. Importantly, only kynurenines in the CSF remained significant after adjustment for age, sex, glomerular filtration rate (GFR), Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), and ASA score. Although the kynunerines were associated with sex, GFR, IQCODE and ASA score, the effect sizes were most attenuated by age, which was the most important confounder (**Supplementary Table 2**).

Of the kynunerines, CSF-QA was most strongly associated with delirium (**Supplementary Table 3** lists the full model with CSF-QA). On the nmol/L scale, the effect declined with higher CSF-QA values

(**Figure 3**). The median CSF-QA concentrations (**Table 2**) in cognitively unimpaired adults and hip fracture patients without delirium were similar (39 and 41 nmol/L, respectively) but substantially higher in both patient groups with delirium (hip fracture, 69 nmol/L, and medical delirium, 84 nmol/L). Despite a strong CSF:serum correlation, CSF-QA was a stronger predictor of delirium compared to serum QA (**Figure 2, Supplementary Table 4**).

Quinolinic acid, other NMDAR agonists, and potential neurotoxicity

Experimental studies suggest that CSF concentrations of QA above 100 nmol/L over time are associated with neurotoxicity, and concentrations above 300 nmol/L are associated with acute neurotoxicity (20). The proportion of patients with such high QA concentrations was substantially larger in those with delirium, most notably in patients with medical delirium (**Figure 4A**). By comparison, concentrations of the endogenous NMDAR agonists glutamate and aspartate in CSF did not differ depending on delirium status in patients with hip fracture (**Figure 4B**). Of all the kynurenines in serum and CSF, CSF-QA was most strongly associated with neuronal injury, as measured with NfL (**Supplementary Table 5**), and this finding remained following adjusted analyses (**Figure 4C**; original scale **Figure 4D**). The CSF-QA NfL association was strongest in patients with dementia (IQCODE ≥ 3.44, **Figure 4E**) and comorbidity (ASA III–IV, **Figure 4F**).

Kynurenine metabolites and one-year mortality following hip fracture

Among the 450 hip fracture patients, there were 99 (22%) deaths the first year after surgery. In univariate analyses, several kynurenines in CSF and serum were strongly associated with mortality **(Supplementary Table 6**). Similar to the association with NfL, CSF-QA was again the kynurenine most strongly associated with mortality (Gini Coefficient (GC) 0.48 indicates a strong association; **Supplementary Tables 6, 7, 8**). Figure **5A** displays the Kaplan-Meier survival function of QA ≥ 100 nmol/L (hazard ratio (HR) of 4.35 [2.93, 6.45], p <0.001). The hazard for death was highest in the initial period following hip fracture (**Figure 5B**). Compared to univariate analyses (Standardized survival: **Figure 5C**, HR: 2.37 [1.87, 3.01]), CSF-QA was attenuated in adjusted analysis (Standardized survival: **Figure 5D**, HR: 1.76 [1.32, 2.33]), both p < 0.001.

Post-hoc analyses

Gaussian network models illustrating the relatedness of the kynurenines by way of partial correlations revealed that serum and CSF kynurenines were highly connected, particularly Kyn, QA, and Pic (**Supplementary Figure 4**). Following exploratory network analyses, no significant difference was found in network structure according to the presence of delirium (**Supplementary Figures 1 and 4**). However, the network structure visualizing the interrelations between the kynurenines in CSF was significantly different from serum (**Supplementary Figure 5**). We also assessed whether the kynurenine metabolites were more or less associated with the clinical presentation of delirium (i.e., subsyndromal, incident, or prevalent). CSF-QA was increasingly more strongly associated with delirium moving from subsyndromal to incident and prevalent delirium (**Supplementary Figure 2**). Further, CSF Kyn, QA, and KTR were non-significantly more strongly associated with delirium in the absence of dementia (IQCODE < 3.44, **Supplementary Figure 3**).

DISCUSSION

Our large multi-center study of CSF in hip fracture demonstrates upregulation of the kynurenine pathway (KP) in the serum that was mirrored and somewhat surpassed in the CSF of patients with delirium. While malfunction of the KP is linked to both neurodegenerative diseases (such as Alzheimer's disease (3) and Huntington's disease (7)) and mental disorders (depression, psychosis, and schizophrenia) (21), here we provide clinical evidence linking the KP, especially higher CSF quinolinic acid (CSF-QA), to delirium during acute illness. QA stimulates the *N*-methyl-D-aspartate receptor (NMDAR), implicated in the pathogenesis of psychotic disorders (22), and some delirium patients displayed what could be potentially neurotoxic concentrations of QA. Importantly, of all the investigated kynurenines, CSF-QA was most strongly associated with delirium, mortality, and the neuronal injury marker CSF-NfL. Our findings were substantiated by a similar pattern of changes in a group of patients with delirium triggered by a medical condition other than hip fracture. These findings suggest a possible explanation for how systemic inflammation in acute illness may cause a metabolic shift in the brain in association with delirium, neuronal injury, and mortality.

Our findings support previous reports wherein the Kyn:Trp ratio (KTR) in blood has been used as an indirect measure of KP activity in the brain. In a study of 84 intensive care unit (ICU) patients, elevated plasma kynurenine and KTR were associated with more days in delirium/coma (14). A more recent study of 130 ICU patients found significantly higher KTR in patients with delirium (n=65) (15). The only other study of the KP in hip fracture patients found higher KTR in plasma in patients with delirium before surgery (16). However, all these studies were performed using blood samples but missing CSF data; furthermore, no study involved performing a comprehensive detection of the kynurenines. Experimental studies investigating systemic immune activation and sepsis have demonstrated that pharmacological or genetic inhibition of IDO prevents depressive- and anxiety-like behaviors as well as cognitive deficits typically seen alongside acute inflammation (23-25). In line with this, peripheral Kyn administration induces similar deficits in a dose-dependent manner (26). Although fewer studies have

focused on cognition and QA, its injection in animals' brains has resulted in both hyperactive behavior and impairments in memory (20).

Our data suggest that concentrations of serum and CSF KP metabolites are tightly connected and that a systemic kynurenine change is likely to be a major contributor to brain KP metabolism (**Supplementary Material, Figure 4**). This raises the question of whether adaptive mechanisms, during systemic KP activation, may be impaired or overwhelmed in delirium. Ridge regression, adapted to assess highly correlated predictors, revealed that, although the odds of delirium were mostly due to the KTR in serum, it was almost exclusively related to QA in CSF (**Supplementary Material 3.6**). Further, the metabolites most strongly linked to delirium (CSF Kyn, HK, Pic, and QA) are all generated in the microglia, and microglial activation is believed to play a central role in delirium pathogenesis (9, 27). Taken together, it is plausible that excessive neuroinflammation, coupled with increased systemic kynurerine concentrations, contributes to the higher CSF kynurenine concentrations observed in delirium patients.

Although there is no known threshold for QA-induced neurotoxicity in humans, we observed that QA concentrations associated with neurotoxicity in animal studies (≥100 nmol/L and ≥300 nmol/L) (20) were much more frequent in patients with delirium. QA is as potent as glutamate and aspartate in stimulating the NMDAR (20), but these amino acids were clearly not elevated in the CSF in delirium and, if anything, tended to be lower with acute illness. Also, the concentrations of KA observed in this study, were much lower than reportedly required for neuroprotective antagonism of NMDAR(28). Unlike glutamate and aspartate, QA lacks a high-affinity uptake system at the synapses, making it prone to accumulation once quinolinate phosphoribosyltransferase (QPRT) becomes saturated (20). Consequently, QA is likely to act as an agonist of the NMDAR over longer time periods, a process that may eventually lead to receptor downregulation (29) where NMDAR hypofunction is linked to schizophrenia (30) and limbic encephalitis (31). QA is considered neurotoxic through several mechanisms (21), including some that are already believed to be involved in the pathogenesis of delirium, such as the potentiation of the inflammatory response (32), the activation of microglia (27), and excitotoxicity (9, 33). Among all the kynurenines measured in our study, CSF-QA was most strongly

associated with NfL. Further, the QA-NfL association was stronger among hip fracture patients with cognitive impairment and high comorbidity, patients who, potentially, have neuronal populations that are more vulnerable to excitotoxic damage. As CSF-QA concentrations in excess of 100 nmol/L may cause cell death in the hippocampus, striatum, and cortex (20), our study proposes neurotoxic QA concentrations as a potential mechanism linking delirium and neuronal damage to subsequent cognitive decline and increased risk of dementia (34, 35).

The kynurenines have previously been associated with all-cause mortality in population and clinical studies (36, 37). Although the mechanisms underlying these observations are not clear, they are thought to be linked to chronic inflammation, oxidative stress, and persistent immune activation. The effect sizes reported in previous studies are, however, much smaller than those we identified in our study. We believe that plausible explanations include that either QA-induced neurotoxicity contributes to mortality or that the accumulation of kynurenines in the brain reflects a more substantial failure of homeostasis than in the blood. Regardless of the mechanism, our data suggest that KP activation in the brain following a hip fracture is an independent determinant of poor outcome. Aligning with prior experimental work, our findings suggest that the KP is a potential therapeutic target in delirium. As the most important precursor of brain kynurenines is circulating Kyn, a natural consideration would be IDO inhibitors.

The present study has a number of strengths as well as limitations. This study benefits from the large CSF dataset with a substantial number of paired CSF and serum samples. The inclusion of two contrast/control groups helped to validate our findings. Delirium was assessed daily based on validated instruments administered by trained investigators. A detailed description of our diagnostic algorithm is included in the methods, as recommended for delirium studies (38). Since dementia is associated with both delirium (9) and KP metabolites (39), it was important to have information on dementia status for all patients. Due to the acute admission of patients, this was characterized using the IQCODE. Although validated and much used (40), the IQCODE is not a substitute for objective cognitive testing. Additional strengths are that all samples were analyzed simultaneously at the same laboratory by technicians

blinded to all clinical data, and metabolites were quantified with high precision using stable isotope-labeled internal standards (41). The kynurenines do have inhibitory effects on the immune system and our study is limited by a lack of data on immune function. Although repeated CSF sampling would have been desirable, such a study design would create significant ethical and practical challenges.

All in all, this study suggests that upregulation of the KP and accumulation of the potentially neurotoxic quinolinic acid in CSF are strong determinants of delirium and one-year mortality and are associated with neuronal injury. Our findings highlight a possible link between systemic inflammation, activation of the NMDAR in delirium, and neurotoxicity. Associations with mortality underscore potential pathophysiological relevance, and collectively, the results of our work should motivate studies investigating the effect of enzyme inhibitors on reducing QA formation in patients with delirium.

METHODS

Study participants

This was a multi-center observational study conducted at four hospitals in the Oslo region, Norway. Patients were included from 2009 to 2019, and CSF samples were available from 450 hip fracture patients (224 with and 226 without delirium), 24 participants from an independent cohort of medical delirium patients, and 112 cognitively unimpaired adults scheduled for elective surgery in spinal anesthesia, for a total of 586 hospitalized participants. Of these, 338 had paired serum samples available. Delirium was assessed daily according to the DSM-5 criteria and based on a standardized procedure described previously (42). The delirium diagnosis was based on an interview with the participants supplemented by information from relatives, nurses, and clinical notes. In short, level of arousal was assessed with the Richmond Agitation Sedation Scale(43) (RASS) and the Observational Scale of Level of Arousal(44) (OSLA), and attention was assessed using Months of the Year backwards (MOYB), Days of the Week backwards (DOWB), the Vigilance-A task SAVEAHAART, and counting down from 20 to 1(45). A recall test of three words (different each day) was performed at each assessment. For details regarding the delirium assessments and the diagnostic algorithm, please see **Supplementary Material,** section 2.1/appendix.

Patients with delirium were classified depending on delirium status at the time of CSF sampling as: prevalent delirium – those with delirium at the time of CSF sampling, and incident delirium – those free from delirium at the time of CSF sampling but who developed it later. Subsyndromal delirium (SSD) was defined (in regard to patients who did not fulfill all criteria for delirium) as evidence of cognitive change, in addition to any one of the following features: (a) altered arousal, (b) attentional deficits, (c) other cognitive change, or (d) delusions or hallucinations. Preoperative ASA scores were collected from hospital records. Dementia status was assessed using the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) with ≥ 3.44 as a cutoff indicating dementia (46). In the case of missing IQCODE scores (n=29 in the hip fracture cohort), dementia status was established retrospectively using

hospital records. Details regarding study participants and data collection are available as supplementary material.

CSF sampling and biochemical analyses

In the hip fracture patients and cognitively unimpaired adults, CSF was collected at the onset of spinal anesthesia, before anesthetic agents were administered. The CSF of patients with medical delirium was obtained from patients who underwent diagnostic lumbar puncture due to altered mental status, at a median of one day after CNS symptoms developed. For all participants, CSF was collected and stored in polypropylene tubes at -80°C. Prior to storage, samples were centrifuged and the supernatants aliquoted. Serum was collected by venous puncture at the time of CSF sampling, centrifuged, aliquoted, and stored at -80°C. Samples were sent on dry ice for biochemical analyses to the Bevital laboratory (Bergen, Norway) for measurement of Trp, the kynurenines, glutamate, and aspartate, and to the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital (Mölndal, Sweden) for NfL analysis. Trp, Kyn, KA, anthranilic acid (AA), 3-hydroxykynurenine (HK), picolinic acid (Pic), QA, xanthurenic acid (XA), and 3-hydroxyanthranilic acid (HAA) in CSF and serum were measured using a targeted metabolomics approach by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously (47). The lower limit of detection (LOD) for the assay ranged from 0.01–8 nmol/L, and within- and between-day CVs ranged from 3–8% and 4–10%, respectively. The KTR was calculated for both CSF and serum as 1000*µmol/µmol. XA and HAA concentrations in CSF were below the LOD in most samples and were not included in subsequent statistical analyses. CSF NfL concentrations were measured using a commercial ELISA (UmanDiagnostics, Umeå, Sweden) (48). As both QA and KA engage the NMDAR, we also measured the concentrations of the other endogenous NMDAR agonists, glutamate and aspartate in CSF and serum, by gas chromatography-tandem mass spectrometry (GC-MS/MS). The LOD for the assay was 0.5 µmol/L, and within- and between-day CVs for both amino acids were 2% and 4%, respectively (Bevital laboratory).

Statistical methods

We adjusted the main analyses (i.e., the associations of the kynurenines with delirium, NfL, and mortality in hip fracture patients) for multiple comparisons (Benjamini-Hochberg false discovery rate (FDR)) considering q-values < 0.01 significant (adjusted p-values, Stata package qqvalue) (49). Serum Trp followed a Gaussian distribution. All other metabolites were non-Gaussian, and we applied QQ plots according to the Tukey ladder of powers to identify transformations that would best approximate normality (50). Metabolites (x) in serum, CSF KA, and AA followed log-normal distributions and were log(x) transformed and the remaining CSF metabolites inverse square root transformed ($x^{-1/2}$). All metabolites and continuous covariates were standardized (i.e., z-scores).

For univariate analysis, we estimated comparable effect sizes indifferent to transformations using the area under the curve (AUC) from the Mann-Whitney U test or time-dependent ROC curves using the nearest-neighbor estimation (censored data) (51). We then calculated the Gini Coefficient (GC) as 2AUC (area under the curve) – 1 where < 0.3 is a small effect size, ≥ 0.3–0.4 is moderate, and ≥ 0.4 is large (52), aiming to normalize the AUC so that a random classifier equals 0 and a perfect classifier equals 1 (53).

For all outcomes, we performed univariate analyses and then, subsequently, adjusted for age, sex, renal function (GFR), a binary variable for the ASA score (I–II versus III–IV), cognitive function (IQCODE cutoff 3.44), and delirium status (if not the outcome). Logistic regression was used to determine the metabolite-related odds of delirium, and similarly, Cox regression was used for the hazard rate for one-year mortality. Flexible parametric survival analysis (Stata: stpm2) was used to estimate the hazard over time and standardized survival curves according to metabolite concentrations. Patients with subsyndromal delirium (n = 44), which can be classified neither as cases nor controls (54), were excluded from the analyses with delirium as the outcome but investigated in subgroup analyses presented in the supplementary material. The association between kynurenines and NfL in hip fracture patients was determined using Spearman's Rho *(R)* with adjusted analyses using linear regression (log-transformed NfL). Cohorts with medical delirium and the cognitively unimpaired adults were included as reference groups.

Methods used for several post-hoc analyses are presented in the supplementary material. All analyses were conducted using R (version 4.1.2) and Stata (version 17). BioRender was used for illustrations.

Study approval

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 was obtained 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 in Norway (REK 2009/450, REK 2011/2052, REK 2011/2578 and REK 2016/1368).

AUTHOR CONTRIBUTIONS

LOW: Initiation and design of the study. Data collection from all cohorts at all sites. Interpretation of the data and revision of manuscript. Preparation of manuscript.

CP: Data collection at Akershus University Hospital. Interpretation of the data and revision of manuscript.

BEN: Data collection, including delirium diagnostics, in hip fracture cohorts 1 and 2. Interpretation of the data and revision of manuscript.

EQP: Data collection of medical delirium cohort. Interpretation of the data and revision of manuscript.

NBH: Data collection in cohort of cognitively unimpaired adults. Interpretation of the data. Preparation of manuscript.

AVI: Data collection in cohort of cognitively unimpaired adults. Interpretation of the data. Preparation of manuscript.

BH: Planning of CSF sampling in hip fracture cohort 2. Interpretation of the data and revision of manuscript.

KH: Interpretation of the data and revision of manuscript.

ABK: Planning of the study. Interpretation of the data and revision of manuscript.

FF: Data collection at Oslo University Hospital. Interpretation of the data and revision of manuscript.

JR: Data collection at Oslo University Hospital. Interpretation of the data and revision of manuscript.

AG: Data collection at Diakonhjemmet Hospital. Interpretation of the data and revision of manuscript.

PMU: Biomarker analyses in serum and CSF at Bevital. Interpretation of the data and revision of manuscript.

AMC: Biomarker analyses in serum and CSF at Bevital. Interpretation of the data and revision of manuscript.

WF: Data collection at Bærum Hospital. Interpretation of the data and revision of manuscript.

GS: Data collection at one-year follow-up. Interpretation of the data and revision of manuscript.

HZ: Analyses of NfL. Interpretation of the data and revision of manuscript.

EFF: Guidance on the NAD^+ synthetic pathway. Interpretation of the data and revision of manuscript.

MM: Data collection at Bærum Hospital. Interpretation of the data and revision of manuscript.

LMG: Planning of the study. Statistical analysis and interpretation of the data. Preparation of manuscript.

ACKNOWLEDGMENTS

We thank the patients, staff, and research nurses at the Orthopedic, Gynecology, Urology, Neurology, Internal Medicine, and Anesthesiology Departments at Oslo University Hospital, Diakonhjemmet Hospital, Bærum Hospital, and Akershus University Hospital. We are also grateful to the laboratory technicians at Bevital and at Sahlgrenska University Hospital for their skillful technical analyses.

FUNDING

The study was funded by the Norwegian Health Association, and the South-Eastern Norway Regional Health Authorities (#2017095). Henrik Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712 and #101053962), and Swedish State Support for Clinical Research (#ALFGBG-71320). 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. Evandro Fei Fang. was supported by HELSE SØR-ØST (#2020001, #2021021), the Research Council of Norway (#262175), the National Natural Science Foundation of China (#81971327), Akershus University Hospital (#269901, #261973, #262960), the Civitan Norges Forskningsfond for Alzheimers sykdom (#281931), the Czech Republic-Norway KAPPA programme (with Martin Vyhnálek, #TO01000215), and the Rosa sløyfe/Norwegian Cancer Society & Norwegian Breast Cancer Society (#207819).

AVAILABILITY OF DATA

Due to ethical restrictions, the full data set is available to the reader upon request only. Proposals should be directed to the corresponding author at Lassegiil@gmail.com and to gain access, data requestors will need to sign a data access agreement.

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FIGURES

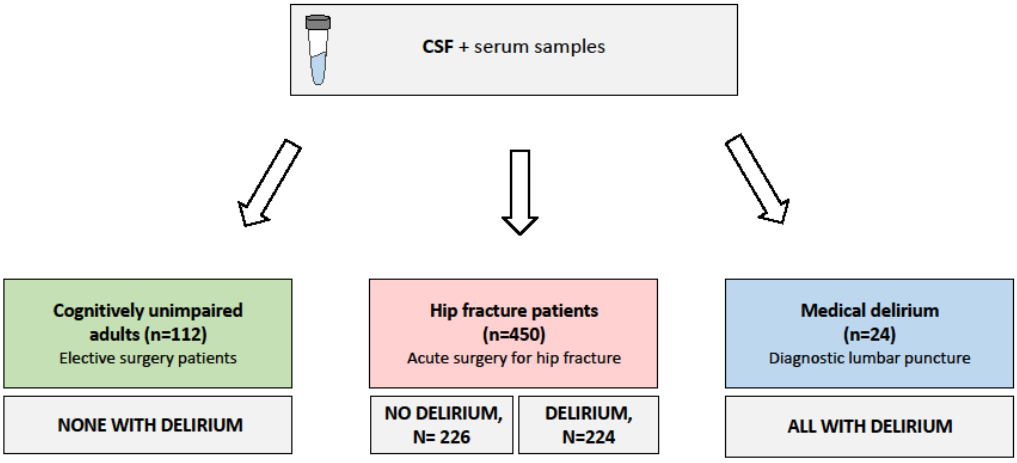

Figure 1. Overview of study design and included patients. We undertook a multi-center observational study with 586 hospitalized patients and investigated associations between delirium and kynurenine pathway (KP) metabolites measured in cerebrospinal fluid (CSF) and serum by targeted metabolomics. We also explored associations between KP metabolites and markers of neuronal damage and one-year mortality. Patients were included from 2009 to 2019, and CSF samples were available from 450 hip fracture patients (224 with and 226 without delirium), 24 participants from an independent cohort of patients with medical delirium, and 112 cognitively unimpaired adults scheduled for elective surgery in spinal anesthesia. Serum samples collected at the same time as CSF were available from 338 patients. Delirium was assessed daily according to the DSM-5 criteria and based on a standardized procedure, see supplementary section 2.1/appendix

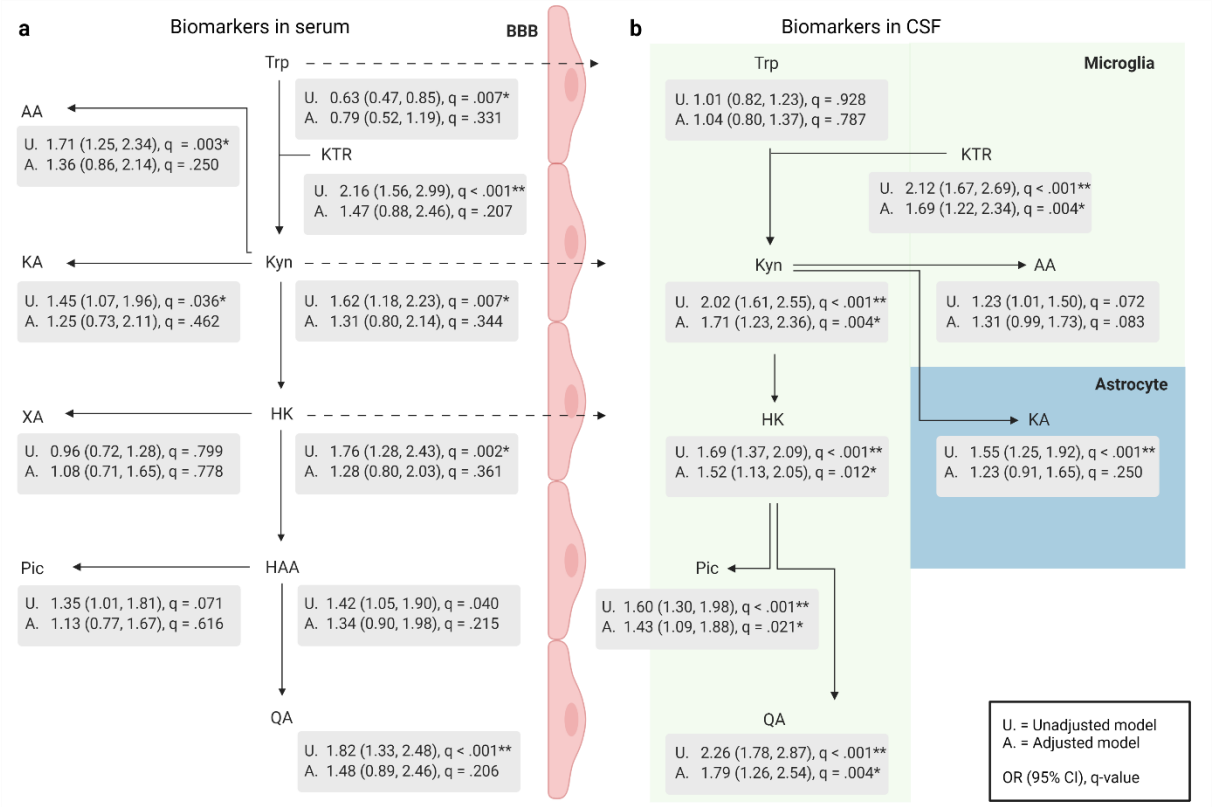

Figure 2. Tryptophan, Kynurenines, and Delirium in the Hip Fracture Cohort. Unadjusted and adjusted logistic regression with delirium as the outcome in the hip fracture cohort (224 with delirium and 182 without delirium; 44 with subsyndromal delirium excluded from analysis). Covariates were age, sex, renal function (glomerular filtration rate), ASA score (I–II vs III–IV) and IQCODE (≥ 3.44 vs. < 3.44). Serum Trp was untransformed; CSF Trp, Kyn, HK, Pic, and QA were transformed using the inverse square root transformation. All other metabolites were log-transformed. Following transformation, the metabolites were standardized to a mean of 0 and a standard deviation of 1. The inverse square root transformations generally provide somewhat lower odds ratios than log-transformations. Abbreviations: AA – anthranilic acid; ASA – American Society of Anesthesiologists Physical Status - classification; CSF – cerebrospinal fluid; HAA – 3-hydroxyanthranilic acid; HK – 3-hydroxykynurenine; IQCODE – Informant Questionnaire on Cognitive Decline in the Elderly; KA – kynurenic acid; KTR – kynurenine:tryptophan ratio; Kyn – kynurenine; Pic – picolinic acid; QA – quinolinic acid; Trp – Tryptophan. * q-value (q, p-value adjusted for multiple comparisons) < 0.05, ** q-value < 0.001.

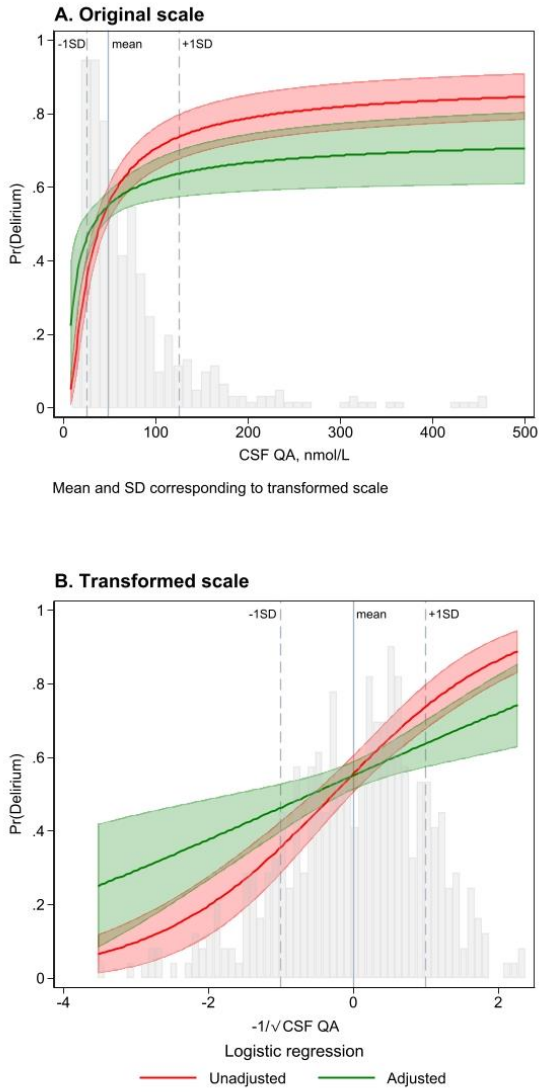

Figure 3. Quinolinic Acid Concentrations in Cerebrospinal Fluid and Delirium. A) The form of the effect size of the QA–delirium association using logistic regression is depicted on the nmol/L scale (N = 406, 224 with delirium and 182 without delirium, 44 with subsyndromal delirium excluded). The marginal predictions from logistic regression using transformed CSF-QA (**B**) have been back-transformed to the original scale of CSF-QA in nmol/L in unadjusted (red) and adjusted (green) analyses, with age, gender, glomerular filtration rate, ASA score (I–II vs. III–IV) and IQCODE (≥ 3.44 vs. < 3.44) as covariates. In **A**, CSF-QA values > 500 nmol/L (n = 4) have been left out for illustrative purposes but were included in the statistical analyses. In the background, one can see the highly skewed distribution that has been transformed to approximate normality (**B**). On the transformed scale (**B**), a 1 SD increase in CSF-QA gives an odds ratio of 2.26 (unadjusted) and 1.79 (adjusted) for delirium (**Figure 1**). However, this is highly non-linear on the nmol/L scale (**A**) where the per unit effect of CSF-QA on the probability (Pr) of delirium decreases incrementally as CSF-QA concentrations increase. This was confirmed using per 50nmol/L and per 100 nmol/L quantitative cutoffs as predictors of delirium (data not shown). Abbreviations: ASA – American Society of Anesthesiologists Physical Status - classification; GFR –

glomerular filtration rate; IQCODE – Informant Questionnaire on Cognitive Decline in the Elderly; QA – quinolinic acid.

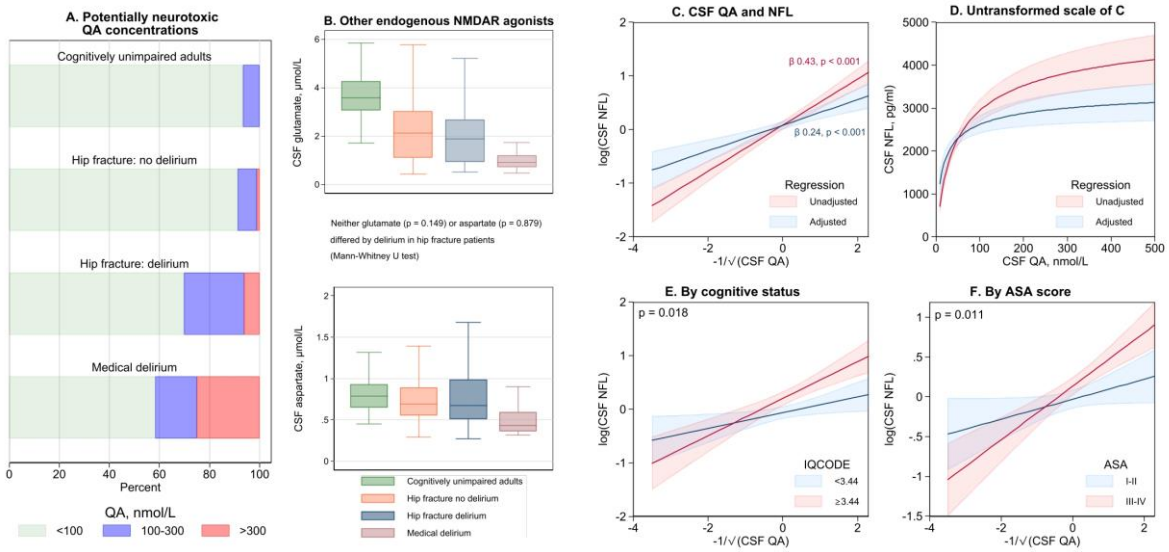

Figure 4. Quinolinic acid, NMDAR Agonists, and Potential Neurotoxicity. Potentially neurotoxic concentrations of CSF-QA, although observed in a minority of delirium patients, were much more frequent in delirium (A). Glutamate and aspartate, like QA, stimulate the NMDAR. Although there was no significant difference in CSF glutamate and aspartate concentrations in hip fracture patients according to delirium, the overall tendency was for the highest concentrations to occur in cognitively unimpaired adults and the lowest concentrations in medical delirium (B). QA was significantly associated with the neuronal injury marker neurofilament light chain protein in hip fracture patients using standardized linear regression (C and D), also adjusted for age, sex, renal function, and cognitive impairment (IQCODE ≥ 3.44), ASA score (I–II vs III–IV) and delirium status (standardized linear regression with an interaction). A and B included 406 hip fracture patients (excluding subsyndromal delirium), 112 cognitively unimpaired adults, and 24 medical delirium cases. C–F included all hip fracture patients (with subsyndromal delirium classified not delirium in adjusted analyses). However, 16 cases did not have NfL measured, and thus, there were 434 patients with hip fracture in this analysis (not excluding subsyndromal delirium cases). The association between QA and NfL was stronger in patients with cognitive impairment (E) and high ASA scores (F). Abbreviations: CSF – cerebrospinal fluid; QA – quinolinic acid.

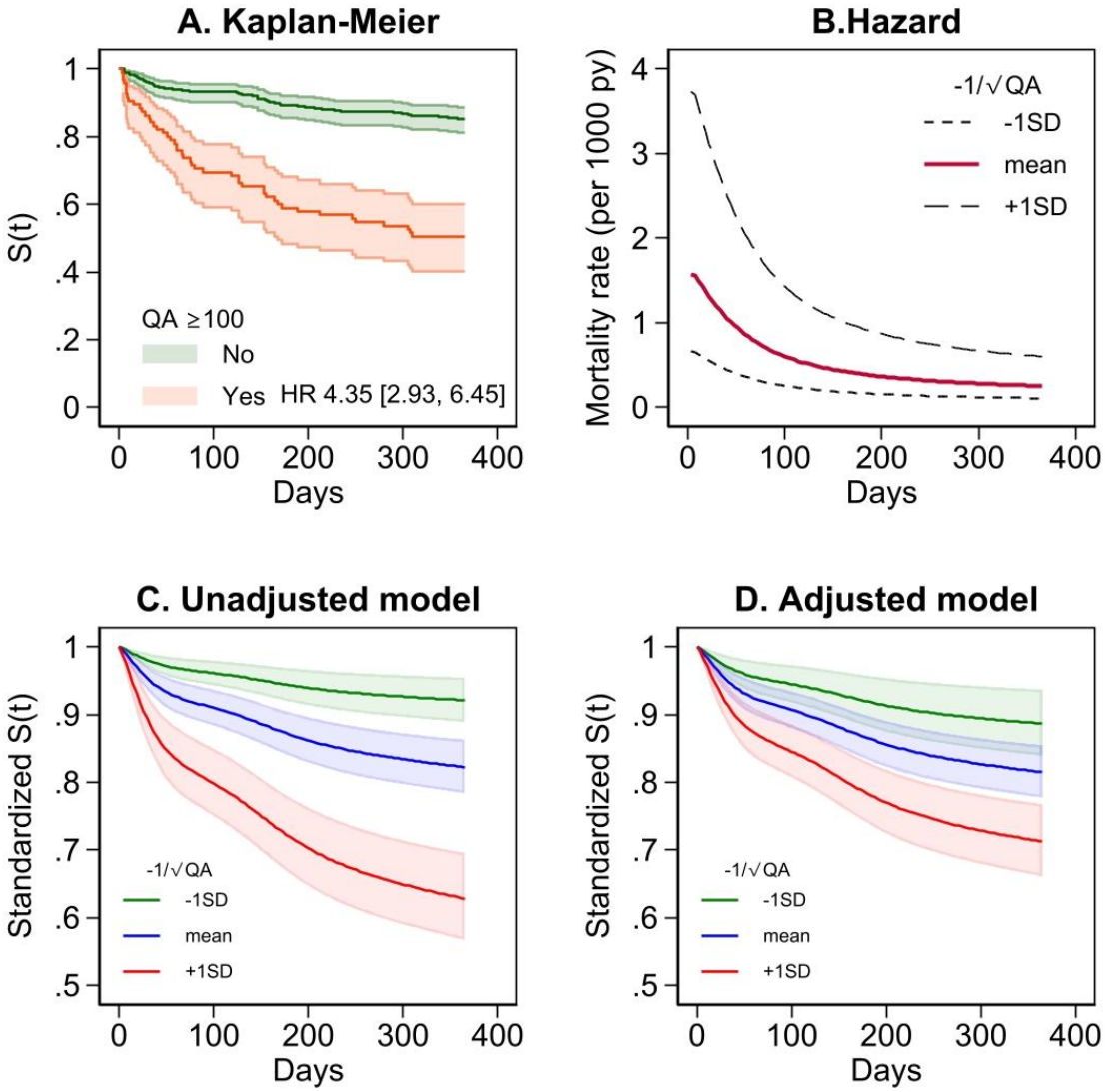

Figure 5. CSF Quinolinic Acid and One-Year Survival in Patients with Hip Fracture (n = 450, 99 events). Using a cutoff of 100 nmol/L, there was a clear survival benefit to patients with lower QA concentrations in univariate analyses (**A**) where the hazard for death was the highest in the initial period following the hip fracture (**B**). In unadjusted analyses, a 1 SD change in CSF-QA on the transformed scale resulted in a reduction in survival as illustrated in **C** (hazard ratio of 2.37); this was somewhat attenuated in adjusted analyses as illustrated in **D (**hazard ratio of 1.76). Of note, back-transforming the mean of QA to nmol/L results in 48.8 nmol/L, where -1SD is 25.7 and +1SD is 126.0. Kaplan-Meier Curve in A and Hazard ratio (HR) with [95% confidence interval] from Cox Regression. B, C and D were estimated using a standardized parametric survival analysis where the baseline hazard was estimated using restricted cubic splines.

Table 1. Study cohorts: Demographic, and clinical aspects

			Hip fracture cohort [a]			
Variables	Statistic	**Cognitively unimpaired adults** N = 112	**All** N = 450	**No delirium** N = 226	**Delirium** N = 224	**Medical delirium** N = 24
Age, years	$\bar{x} \pm SD$	73 ±7	81 ±11	76 ±12	85 ±8	69 ±12
Female	n (%)	51 (45)	307 (68)	156 (69)	151 (67)	10 (42)
GFR [b]	$\bar{x} \pm SD$	74 ±16	70 ±20	74 ±20	67 ±19	60 ±25
IQCODE [c]	$\bar{x} \pm SD$	NA	3.7 ±0.7	3.3 ±0.5	4.1 ±0.7	NA
≥ 3.44	n (%)	NA	204 (45)	43 (19)	161 (72)	NA
ASA III-IV [d]	n (%)	NA	239 (53)	87 (39)	152 (68)	NA

Abbreviations: ASA - American Society of Anesthesiologists Physical Status – classification; GFR - glomerular filtration rate; IQCODE - Informant Questionnaire on Cognitive Decline in the Elderly; n (%), number and percentage of participants in listed category; NA – not available; $\bar{x}$, mean; SD - standard deviation.

[a] The no delirium group contains 44 cases of subsyndromal delirium that were excluded from subsequent main analyses.

[b] In mL/min/m^2. 14 missing GFR in the healthy group, 6 in the hip fracture group, 1 in the medical delirium group (21 in total).

[c] Likert scale that ranges from 1 to 5 with 5 being worse cognitive function. ≥ 3.44 indicates chronic cognitive impairment.

[d] Ordinal scale indicating: I, healthy; II, mild systemic illness; III, severe but not incapacitating illness; IV, severe incapacitating illness. Here, ASA I-II is the reference group versus participants with ASA III-IV.

Table 2. Study cohorts: Metabolite concentrations

Metabolites	**Cognitively unimpaired adults**	**All**	**No delirium**	**Delirium**	**Medical delirium**
CSF	N = 112	N = 450	N = 226	N = 224	N = 24
Trp	2.8 ±0.9	2.8 ±1.0	2.8 ±0.9	2.8 ±1.2	3.3 ±1.7
Kyn	54 ±25	68 ±49	59 ±40	78 ±66	121 ±277
KA	2.9 ±2.3	3.90 ±3.9	3.58 ±3.4	4.41 ±4.8	5.7 ±8.5
AA	10 ±6.3	9.4 ±9.1	9.2 ±8.2	11 ±9.3	8.9 ±14.0
HK	4.9 ±4.1	6.6 ±7.6	5.9 ±5.7	7.7 ±9.9	10 ±27.3
Pic	21 ±11	19±13.3	16 ±13	21 ±14	58 ±78
QA	39 ±29	55 ±57	41 ±45	69 ± 74	84 ±248
KTR	20 ±7.6	24 ±15	22 ±12	29 ±20	44 ±41
Serum	N = 101	N = 214	N = 102	N = 112	N = 23
Trp	58 ±15	50 ±16	52 ±18	47 ±16	42 ±44
Kyn	1.6 ±0.6	1.6 ±0.7	1.5 ±0.7	1.7 ±0.7	1.9 ±0.9
KA	56 ±34	43 ±32	40 ±25.9	46 ±38.6	80 ±169
AA	18 ±8	17 ±9	16 ±8	19 ±10	27 ±24
HK	51 ±29	61 ±55	53 ±56	71 ±49	74 ±109
XA	13 ±8	5.4 ±6.2	5.7 ±5.3	5.4 ±6.9	14 ±16
HAA	30 ±13	24 ±16	22 ±15	25 ±18	39 ±41
Pic	34 ±15	25 ±21	23 ±19	27 ±20	92 ±82
QA	427 ±245	499 ±451	418 ±435	556 ±419	693 ±1523
KTR	26 ±11	32 ±18	30 ±16	37 ±21	45 ±55

Note: Serum Trp, Kyn and cerebrospinal fluid Trp are in µmol/L, all other kynurenines are in nmol/L with all summarized as median and IQR. Abbreviations: AA - anthranilic acid; CSF - cerebrospinal fluid; HAA - 3-hydroxyanthranilic acid; HK - 3-hydroxykynurenine; IQR - interquartile range; KA - kynurenic acid; KTR - kynurenine-to-tryptophan ratio; Kyn - kynurenine; Pic - picolinic acid; QA - quinolinic acid; $\bar{x}$, mean; SD - standard deviation. Serum Trp, Kyn and cerebrospinal fluid Trp are in µmol/L, all other kynurenines are in nmol/L.