ORIGINAL RESEARCH

Differences in potential biomarkers of delirium between acutely ill medical and elective cardiac surgery patients

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Background/aims: The pathophysiology of delirium is poorly understood. Increasing evidence suggests that different pathways might be involved in the pathophysiology depending on the population studied. The aim of the present study was to investigate potential differences in mean plasma levels of neopterin, amino acids, amino acid ratios and homovanillic acid between two groups of patients with delirium.

Methods: Data from acutely ill medical patients aged 65 years and older, and patients aged 70 years and older undergoing elective cardiac surgery, were used. Differences in biomarker levels between the groups were investigated using univariate ANOVA with adjustments for age, sex, comorbidities, C-reactive protein (CRP) and the estimated glomerular filtration rate (eGFR), where appropriate. Linear regression analysis was used to identify potential determinants of the investigated biochemical markers.

Results: Eighty patients with delirium were included (23 acutely ill medical patients and 57 elective cardiac surgery patients). After adjustment, higher mean neopterin levels (93.1 vs 47.3 nmol/L, $P=0.001$) and higher phenylalanine/tyrosine ratios (1.39 vs 1.15, $P=0.032$) were found in acutely ill medical patients when compared to elective cardiac surgery patients. CRP levels were positively correlated with neopterin levels in acutely ill medical patients, explaining 28.4% of the variance in neopterin levels. eGFR was negatively correlated with neopterin in elective cardiac surgery patients, explaining 53.7% of the variance in neopterin levels.

Conclusion: In this study, we found differences in mean neopterin levels and phenylalanine/tyrosine ratios between acutely ill medical and elective cardiac surgery patients with delirium. Moreover, our findings may suggest that in acutely ill medical patients, neopterin levels are mainly determined by inflammation/oxidative stress whereas in elective cardiac surgery patients, neopterin levels are mainly driven by renal function/fluid status. These findings suggest that the markers and pathways that might be involved in the pathophysiology of delirium may differ between specific groups of patients.

Keywords: delirium, inflammation, neopterin, oxidative stress, renal function

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Introduction

Delirium – an acute neuropsychiatric syndrome characterized by disturbances in attention, awareness and cognition – is a frequent disorder in older hospitalized patients.[1–3] It is associated with poor clinical outcomes including prolonged hospital stay, loss of independence, increased risk of cognitive decline and mortality.[4] Although it is widely accepted that the cause of delirium is multifactorial with a complex interplay between predisposing factors (eg, advanced age and dementia) and precipitating factors (eg, acute medical illness and surgery), the pathophysiology of delirium is still poorly understood.[3]

Increasing evidence suggests that delirium might have different pathophysiological mechanisms depending on the precipitating factor and the health status of the patient (acutely ill or relatively healthy).[5,6] Several mechanisms may play a role and include, among others, activation of the immune system, oxidative stress and disturbances in serotonergic and dopaminergic neurotransmission.[7] During immune system activation, monocytes and macrophages are stimulated to produce neopterin.[8] The association between this novel potential biomarker, neopterin, and delirium has been investigated in three previous studies which included acutely ill medical patients,[9] elective patients undergoing cardiac surgery[10] and acute hip fracture patients undergoing surgery.[11] In all three studies, neopterin levels were elevated in patients with delirium. However, when comparing the results of the studies, large differences are observed in mean neopterin levels between the different studies. Considerably lower mean neopterin levels were found in surgical patients with delirium[10,11] than in acutely ill medical patients with delirium.[9] Also interestingly, neopterin levels measured in patients *with* delirium after cardiac surgery[10] were in the same order as neopterin levels measured in acutely ill medical patients *without* delirium.[9]

Disturbances in serotonergic and dopaminergic neuro-transmission may also contribute to delirium.[7] The synthesis of serotonin in the central nervous system is, among others, dependent on the availability of its precursor, the large neutral amino acid (LNAA) tryptophan. Tryptophan competes with the other LNAAs (phenylalanine, tyrosine, valine, leucine and isoleucine) for transport across the blood–brain barrier. The amount of tryptophan that eventually enters the brain is determined by the ratio of tryptophan to the sum of the other LNAAs.[12] Therefore, a reduced ratio may result in a decreased synthesis of serotonin. Several studies have found decreased levels of tryptophan and tryptophan/LNAAs ratios in patients with delirium,[10,12–15] whereas other studies did not report differences in these parameters between patients with and without delirium.[16–18]

It has also been suggested that an increased dopaminergic neurotransmission plays a role in delirium. The amino acids phenylalanine and tyrosine are the precursors of dopamine and the ratios of phenylalanine and tyrosine to the other LNAAs are suggestive of the amount of phenylalanine and tyrosine that enters the brain. In patients with delirium, increased ratios,[17] as well as increased plasma levels of dopamine's metabolite homovanillic acid (HVA), have been found,[10,16] but the results are inconclusive.[19]

In addition, serotonin and dopamine are synthesized from tryptophan, phenylalanine and tyrosine in enzymatic reactions that use tetrahydrobiopterin (BH_4) as an essential cofactor.[20] A change in the availability of BH_4 may therefore affect the synthesis of these neurotransmitters. The ratio of phenylalanine to tyrosine (Phe/Tyr ratio) can be used as an indirect measure of BH_4. This ratio reflects the activity of the peripherally located enzyme phenylalanine hydroxylase, an enzyme that requires BH_4 to convert phenylalanine into tyrosine.[20,21] An increased Phe/Tyr ratio suggests a reduced availability of BH_4. In a previous study, an increased ratio was found in patients with delirium,[19] whereas another study reported no difference between patients with and without delirium.[10]

These controversial findings can be caused by differences in analytical procedures but can also be suggestive of potential differences in biochemical profiles and pathophysiological pathways between patient groups with delirium. Adequate knowledge regarding possible differences in the pathophysiology is required to improve delirium prevention and treatment.

The aim of this exploratory study was to investigate potential differences in mean plasma levels of neopterin, amino acids, amino acid ratios and HVA between two specific groups of patients with delirium, ie, acutely ill medical patients with delirium and patients with a delirium after elective cardiac surgery.

Methods
Study populations
In this study, we used data from the Delirium In The Old (DITO) study[9,19] and the DElirium pathogenesis, Cognition and Outcome (DECO) study.[10] In both studies, mean levels of neopterin, several amino acids, amino acid ratios and HVA in patients with and without delirium were compared and the results can be found elsewhere.[9,10,19] Only patients with delirium were included in the present study. The most relevant procedures of the two studies will be described here; detailed information can be found in previous publications.[9,10,19]

The DITO study was a cross-sectional study in patients aged 65 years and older who were acutely ill and admitted to the wards of Internal Medicine and Geriatrics of the Erasmus University Medical Center (Erasmus MC) and the ward of Geriatrics of the Harbour Hospital, Rotterdam, the Netherlands. Exclusion criteria were a diagnosis of Lewy body dementia, Parkinson's disease, neuroleptic malignant syndrome, tardive dyskinesia, ongoing treatment with antipsychotics or other psychiatric medications except haloperidol and benzodiazepines, aphasia, insufficient understanding of the Dutch language and a Mini-Mental State Examination (MMSE) score <10 points out of 30.

The DECO study was a prospective cohort study in patients aged 70 years and older who underwent elective cardiac surgery (coronary artery bypass graft [CABG], valve surgery or both) at the Department of Cardiothoracic Surgery of the Erasmus MC. Exclusion criteria were surgery in which deep hypothermia circulatory arrest or an emergency procedure was required, insufficient understanding of the Dutch language, preoperative delirium and insufficient adherence to the protocol.

In both studies, written informed consent was obtained from all participants or their representatives. The study protocols were approved by the Medical Ethics Committee of the Erasmus MC. All methods were performed in accordance with the relevant guidelines and regulations.

Procedures

In both studies, participants were assessed daily for the presence of delirium by the nursing and medical staff and by members of the research teams until discharge or until 7 days after surgery.

In the DITO study, the 13-items Delirium Observation Screening scale was used to screen for a change in behavior.[22] The diagnosis of delirium was made by a geriatrician, according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (*DSM-IV*),[1] and was based on psychiatric examination of the patient, the medical and nursing records, the Delirium Observation Screening scale scores and information given by the patient's closest relative. Cognitive functioning was assessed in the absence of delirium, at a later moment during the hospital stay, using the MMSE.[23] When it was impossible to score the MMSE during admission because the patient was too ill, the cognitive functioning was discussed with a clinician or assessed with information from the available medical records. When the clinical opinion was that the patient would have a MMSE score $\geq$10, the patient was not excluded from the study.

In the DECO study, the diagnosis of delirium was made by a senior psychiatrist and/or a trained researcher in daily assessments between 10:00 and 12:00 hours using the Confusion Assessment Method for the Intensive Care Unit (which follows the criteria for delirium of the *DSM-IV*)[24] and was based on psychiatric examination of the patient and information in the medical and nursing records. Delirium lasting >1 day was considered clinically relevant. The first day after surgery was not taken into account because of possible residual effects of anesthesia. On the day before surgery, cognitive functioning was assessed using the MMSE.

In the DITO study, blood samples were collected within 48 hours after admission. When a patient developed delirium during the hospital stay, new blood samples were collected within 24 hours after the onset of delirium and were used instead of the first blood samples for the statistical analyses. In the DECO study, blood samples were collected on the day before surgery and the second day after surgery.

In the present study, we compared the biochemical data collected in the DITO study with the postoperative biochemical data from the DECO study.

Biochemical measurements

In both studies, non-fasting blood was collected preferably between 8 and 10 am in an 8-mL tube containing ethylene diamine tetra-acetic acid. After blood sampling, the tubes were protected from light to prevent oxidative loss of neopterin[25] and stored at room temperature to prevent changes in the transfer of amino acids between plasma and blood cells.[26] Within 3 hours, the blood was centrifuged for 20 minutes at 2,650 g and 20°C. The obtained plasma was stored at −80°C until analysis.

In both studies, neopterin, amino acids and HVA were determined by the same procedures and by the same analytical staff. Plasma neopterin levels were determined by HPLC after acid oxidation.[21] Plasma amino acid levels were measured using HPLC with automated pre-column derivatization with *ortho*-phthalaldehyde,[26] and plasma HVA levels were determined by reversed-phase HPLC and electrochemical detection, as previously described for the measurement of serotonin.[27]

C-reactive protein (CRP) levels and the estimated glomerular filtration rate (eGFR) were taken from the medical records. In case of the DECO study, the 2-day postoperative levels were recorded. The eGFR was determined by the following Modification of Diet in Renal Disease formula: $175 \times [\text{serum creatinine (μmol/L)} \times 0.0113]^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female).

Statistical analysis

Differences in participant characteristics between acutely ill medical and elective cardiac surgery patients with delirium were evaluated using the chi-squared test or Fisher's exact test for categorical variables and the Mann–Whitney U-test or the Student's t-test for continuous variables, depending on the distribution of the data.

Univariate ANOVA was used to investigate potential differences in mean levels of biochemical parameters (dependent variable) between acutely ill medical and elective cardiac surgery patients. For this purpose, biochemical parameters that were not normally distributed were logarithmically transformed (neopterin, HVA, all amino acids and amino

acid ratios). Analyses were adjusted for age, sex and Charlson Comorbidity Index (CCI). The model including neopterin was additionally adjusted for CRP and eGFR, since neopterin is an inflammatory marker which is mainly excreted by the kidneys.[8] In additional analyses, neopterin, HVA, all amino acids and amino acid ratios were also adjusted for MMSE score. All mean levels and corresponding 95% CIs presented in this manuscript are the back-transformed $\log_{10}$-values.

Unadjusted linear regression analysis was performed to identify potential determinants of biochemical parameters, which were statistically significantly different between the two groups. Second, variables with a P-value ≤0.10 were included in a multiple linear regression analysis and stratified for study, to determine the relative contribution of each variable to the total variance in the biochemical parameter. Semi-partial (part) correlation coefficients were squared to calculate the percentage of total variance that was explained by each variable.

Due to the exploratory nature of this study, no correction for multiple comparisons was applied. All statistical analyses were performed using SPSS, version 21.0 (IBM Co., Armonk, NY, USA). Results were considered statistically significant at a two-sided $P<0.05$. Figures were constructed using GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

Results
Participant characteristics
Of the 211 patients enrolled in the two studies, 81 were diagnosed with delirium. One participant from the DECO study was excluded due to withdrawal of consent. In total, 80 patients with delirium were included, 23 acutely ill medical patients and 57 elective cardiac surgery patients. Of the 23 acutely ill medical patients with delirium, 21 had delirium on admission and 2 developed delirium during the hospital stay. Table 1 presents the demographic and clinical characteristics

Table 1 Characteristics of the study participants

	DITO (n=23)	DECO (n=57)	P-value
Male, n (%)	10 (43.5)	34 (59.6)	0.188[a]
Age, years, mean ± SD	85.9±4.0	76.8±3.8	<0.001[b]
MMSE score, median (IQR)[c,d]	20.0 (18.0–25.0)[e]	27.0 (24.0–28.0)	<0.001[f]
CCI score, median (IQR)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.550[f]
CCI score, n (%)			
0	1 (4.3)	11 (19.3)	
1	8 (34.8)	12 (21.1)	
2	7 (30.4)	15 (26.3)	
≥3	7 (30.4)	19 (33.3)	
CRP, mg/L, median (IQR)[g]	36.0 (10.0–103.0)	100.0 (60.0–148.0)	0.013[f]
eGFR, mL/min, mean ± SD[g]	49.4±25.1	48.9±23.2	0.930[b]
Acute kidney injury, n (%)[h]	2 (8.7)	8 (14.0)	0.716[i]
Reasons for admission, n (%)[j]			
Infection	17 (73.9)	–	
Electrolyte disorder	5 (21.7)	–	
Dehydration	4 (17.4)	–	
Heart failure	2 (8.7)	–	
Carcinoma	2 (8.7)	–	
Urinary retention	2 (8.7)	–	
Atrial fibrillation	1 (4.3)	–	
Adverse drug event	1 (4.3)	–	
Multiple organ dysfunction	1 (4.3)	–	
Fever of unknown origin	1 (4.3)	–	
Elective cardiac surgery	–	57 (100%)	

Notes: Values are expressed as mean ± SD for normally distributed continuous variables, median (IQR) for not normally distributed continuous variables and n (percentages) for categorical variables. [a]Chi-squared test. [b]Student's *t*-test. [c]Range 0 (severe cognitive impairment) to 30 (no cognitive impairment). [d]DITO: measured in the absence of delirium, DECO: measured on the day before surgery. [e]Four values missing. [f]Mann–Whitney *U*-test. [g]DITO: measured during delirium (on the day of neopterin sampling), DECO: measured during delirium 2 days after cardiac surgery. [h]Defined as a decrease in eGFR of >50% in comparison to baseline or the preoperative situation. [i]Fisher's exact test. [j]Percentages do not add up to 100% due to multiple admission reasons per patient.
Abbreviations: CCI, Charlson Comorbidity Index; CRP, C-reactive protein; DITO, Delirium In The Old; DECO, DElirium pathogenesis, Cognition and Outcome; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MMSE, Mini-Mental State Examination.

of the participants. Acutely ill medical patients with delirium were older (mean 85.9 vs 76.8 years, $P<0.001$), had lower MMSE scores (median 20 vs 27, $P<0.001$) and lower CRP levels (median 36.0 vs 100.0 mg/L, $P=0.013$) than elective cardiac surgery patients with delirium.

Analyses of biochemical parameters

The adjusted mean levels and corresponding 95% CIs of the investigated biochemical parameters in acutely ill medical and elective cardiac surgery patients are presented in Table 2. After adjustment for age, sex, CCI, eGFR and CRP levels, mean neopterin levels were significantly higher in acutely ill medical patients (93.1 nmol/L, 95% CI: 69.3–125.3) than in elective cardiac surgery patients (47.3 nmol/L, 95% CI: 40.1–55.8) ($P=0.001$). Furthermore, mean Phe/Tyr ratios were significantly higher in acutely ill medical patients (1.39, 95% CI: 1.22–1.58) than in elective cardiac surgery patients (1.15, 95% CI: 1.07–1.24) ($P=0.032$). No statistically significant differences were found in the mean levels of the other biochemical parameters. Estimates remained unchanged after additional adjustment for MMSE scores (Table S1).

Determinants of neopterin

Unadjusted linear regression analysis revealed that neopterin levels were positively correlated with CRP levels in acutely ill medical patients with delirium and negatively correlated with eGFR in elective cardiac surgery patients with delirium (Table 3, Figure 1). Trends toward lower neopterin levels were found for increasing age ($r=-0.399$, $P=0.060$) and increasing eGFR ($r=-0.396$, $P=0.061$) in acutely ill medical patients (Table 3, Figure 1). No correlation was found between neopterin, CCI and MMSE scores in both groups of patients ($P>0.10$) (Table 3).

Multiple linear regression analysis revealed that a model based on age, CRP and eGFR could explain 53.2% of the variance in neopterin levels in acutely ill medical patients with delirium (Table 4). In this model, CRP levels remained positively correlated with neopterin levels and explained 28.4% of the variance in neopterin levels. Age and eGFR explained 8.53% and 3.96%, respectively of the variance in neopterin levels, but were not statistically significant determinants of neopterin. In elective cardiac surgery patients with delirium, the same model explained 56.7% of the variance in neopterin levels (Table 4).

Table 2 Adjusted mean levels of biochemical parameters

	DITO (n=23)	DECO (n=57)	P-value
Neopterin, nmol/L	84.1 (57.0–123.9)	49.4 (40.0–61.0)	0.038
Neopterin, nmol/L[a]	93.1 (69.3–125.3)	47.3 (40.1–55.8)	0.001
Homovanillic acid, nmol/L	120.2 (79.6–181.1)[b]	160.3 (128.5–199.5)[b]	0.279
Glutamic acid, µmol/L	46.7 (37.3–58.3)	35.4 (31.3–39.9)	0.059
Serine, µmol/L	80.2 (68.4–94.0)	75.5 (69.2–82.2)	0.555
Glycine, µmol/L	182.0 (157.0–210.9)	198.2 (182.8–214.3)	0.375
Citrulline, µmol/L	24.0 (19.3–29.8)	23.1 (20.6–26.0)	0.803
Arginine, µmol/L	35.4 (28.1–44.6)	44.0 (38.7–49.9)	0.152
Taurine, µmol/L	35.7 (27.2–47.1)	33.6 (28.9–38.9)	0.722
Tyrosine, µmol/L	56.6 (47.2–67.9)	66.2 (60.0–73.1)	0.189
Valine, µmol/L	208.4 (182.8–237.7)	202.8 (188.8–217.8)	0.758
Methionine, µmol/L	22.4 (18.4–27.3)	28.5 (25.6–31.8)	0.063
Tryptophan, µmol/L[c]	30.4 (23.5–37.2)	33.8 (30.1–37.5)	0.440
Phenylalanine, µmol/L	78.5 (70.0–88.3)	76.0 (71.4–81.1)	0.665
Isoleucine, µmol/L	55.8 (47.6–65.5)	67.6 (61.9–73.6)	0.069
Leucine, µmol/L	121.9 (104.5–142.2)	123.9 (113.8–134.6)	0.875
Phenylalanine/tyrosine ratio	1.39 (1.22–1.58)	1.15 (1.07–1.24)	0.032
Tryptophan/LNAAs ratio × 100[c]	5.35 (4.37–6.32)	6.11 (5.59–6.64)	0.228
Tyrosine/LNAAs ratio × 100	11.4 (9.8–13.2)	13.1 (12.1–14.2)	0.152
Phenylalanine/LNAAs ratio × 100	16.5 (14.6–18.6)	15.3 (14.4–16.4)	0.363
Citrulline/arginine ratio	0.68 (0.52–0.89)	0.53 (0.45–0.61)	0.153

Notes: Values are expressed as mean (95% CI) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex and CCI. [a]Additionally adjusted for the eGFR and CRP. Analysis was performed in 76 patients with delirium (DITO n=23 and DECO n=53) due to missing CRP levels. [b]One value missing. [c]Values were not logarithmically transformed.
Abbreviations: CCI, Charlson Comorbidity Index; CRP, C-reactive protein; DITO, Delirium In The Old; DECO, DElirium pathogenesis, Cognition and Outcome; eGFR, estimated glomerular filtration rate; LNAAs, large neutral amino acids.

Table 3 Unadjusted linear regression analyses of potential determinants of neopterin and Phe/Tyr ratio

Variable	Log$_{10}$ (neopterin [nmol/L]) in DITO				Log$_{10}$ (neopterin [nmol/L]) in DECO			
	B	95% CI	P-value	R²	B	95% CI	P-value	R²
Age	−0.032	(−0.064; 0.001)	0.060	0.159	0.006	(−0.015; 0.026)	0.583	0.006
Sex	−0.085	(−0.370; 0.199)	0.539	0.018	−0.018	(−0.176; 0.139)	0.816	0.001
CCI	0.039	(−0.043; 0.122)	0.332	0.045	0.022	(−0.024; 0.069)	0.339	0.017
CRP	0.002	(0.001; 0.004)	0.002	0.364	0.001	(0.000; 0.002)	0.221	0.029
eGFR	−0.005	(−0.010; 0.000)	0.061	0.157	−0.009	(−0.011; −0.007)	<0.001	0.537
MMSE	0.001	(−0.031; 0.034)	0.940	0.000	0.004	(−0.023; 0.031)	0.756	0.002
	Log$_{10}$ (Phe/Tyr ratio) in DITO				Log$_{10}$ (Phe/Tyr ratio) in DECO			
Age	−0.006	(−0.021; 0.009)	0.436	0.029	−0.001	(−0.007; 0.005)	0.765	0.002
Sex	0.049	(−0.072; 0.170)	0.411	0.032	−0.010	(−0.056; 0.035)	0.647	0.004
CCI	0.023	(−0.012; 0.057)	0.189	0.081	0.003	(−0.011; 0.016)	0.705	0.003
MMSE	0.000	(−0.008; 0.008)	0.988	0.000	0.000	(−0.014; 0.014)	0.966	0.000

Notes: Age was measured in years, sex was coded as 1= male, 2= female, CCI was measured in points, CRP was measured in mg/L, eGFR was measured in mL/min and MMSE was measured in points. B, regression coefficient; R², squared correlation coefficient.
Abbreviations: CCI, Charlson Comorbidity Index; CRP, C-reactive protein; DITO, Delirium In The Old; DECO, DElirium pathogenesis, Cognition and Outcome; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Examination; Phe, phenylalanine; Tyr, tyrosine.

In this model, eGFR remained negatively correlated with neopterin levels and explained 53.7% of the variance in neopterin levels. Age and CRP explained 0.18% and 2.76% of the variance in neopterin levels, respectively, and were not statistically significant determinants of neopterin levels.

Determinants of Phe/Tyr ratio

Unadjusted linear regression analysis revealed that age, sex, CCI and MMSE scores were not statistically significantly correlated with Phe/Tyr ratios in both acutely ill medical and elective cardiac surgery patients with delirium (Table 3). Also no trends toward correlation were found.

Discussion

In this study, we found higher mean levels of neopterin and higher Phe/Tyr ratios in acutely ill medical patients with delirium than in patients who developed a delirium after elective cardiac surgery. These findings suggest that the markers and pathways that might be involved in the pathophysiology of delirium in acutely ill medical patients may differ from those in elective cardiac surgery patients.

Delirium frequently occurs in conditions in which the immune system is activated, and this suggests that inflammatory mediators may play a role in the pathophysiology. Several cytokines and inflammatory markers have already been found to be increased in acutely ill medical[9,28] and cardiac surgery patients[29] with delirium. Neopterin can be considered as a marker of cell-mediated immune system activation and oxidative stress as it is primarily produced by activated monocytes and macrophages upon stimulation

with the pro-inflammatory cytokine interferon-gamma (IFN-γ). Previous studies, including the DITO and DECO study, have found increased neopterin levels in plasma and cerebrospinal fluid prior to and during delirium[9–11] and this consistency in findings suggests that neopterin might indeed play a role in the pathophysiology of delirium. Nevertheless, in the present study we found a large difference in mean neopterin levels between acutely ill medical and elective cardiac surgery patients with delirium, where all neopterin measurements were performed in the same laboratory using identical procedures. This finding remained significant after adjustment for potential confounders.

Substantial differences in determinants of neopterin levels were found between the two groups of patients with delirium. CRP levels were the strongest determinant of neopterin levels in acutely ill medical patients with delirium, explaining 28.4% of the variance in neopterin levels, whereas the eGFR was the strongest determinant of neopterin levels, explaining 53.7% of the variance in neopterin levels, in elective cardiac surgery patients with delirium. These findings may suggest that, in acutely ill medical patients, neopterin levels are mainly determined by inflammation/oxidative stress, whereas in elective cardiac surgery patients, neopterin levels are mainly driven by renal function/fluid status. However, it should be noticed that the majority of the medical patients was admitted with an infection.

In both groups of patients with delirium, the model based on age, CRP and eGFR explained approximately half of the variance in neopterin levels and therefore the remaining variance might be determined by other factors, eg, the severity
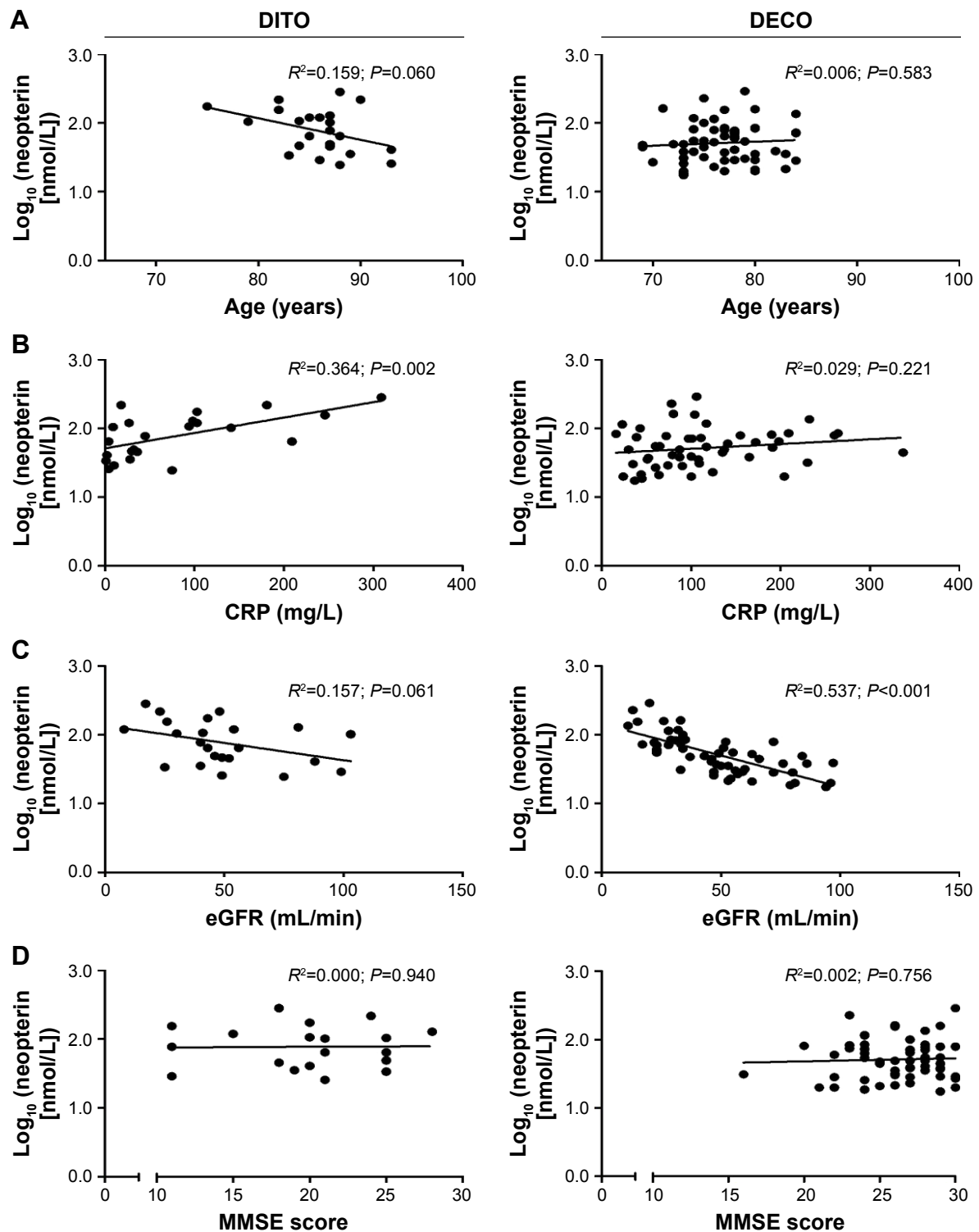
Figure 1 Scatterplots showing the relation between neopterin and age (**A**), CRP (**B**), eGFR (**C**) and MMSE score (**D**) in acutely ill medical patients with delirium (DITO) and patients with delirium after elective cardiac surgery (DECO).
Abbreviations: CRP, C-reactive protein; DITO, Delirium In The Old; DECO, DElirium pathogenesis, Cognition and Outcome; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Examination.

of delirium,[11] the presence of comorbid cognitive disorders, other medical conditions[8] or the use of specific drugs, such as aspirin[30] and statins.[31] Several studies have shown that a decrease in cognitive performance is accompanied by an

increase in neopterin levels.[32,33] In the present study, we found that acutely ill medical patients with delirium had significantly lower MMSE scores than patients who developed a delirium after elective cardiac surgery, but it should

Table 4 Multiple linear regression analyses of potential determinants of neopterin

Variable	Log$_{10}$ (neopterin [nmol/L]) in DITO					Log$_{10}$ (neopterin [nmol/L]) in DECO				
	B	95% CI	P-value	Sr^2	Variance explained, %	B	95% CI	P-value	Sr^2	Variance explained, %
Intercept	3.923	–	–	–	–	2.336	–	–	–	–
Age	−0.024	(−0.051; 0.003)	0.078	0.085	8.53	−0.003	(−0.018; 0.011)	0.654	0.002	0.18
CRP	0.002	(0.001; 0.003)	0.003	0.284	28.4	0.001	(0.000; 0.001)	0.084	0.028	2.76
eGFR	−0.003	(−0.007; 0.002)	0.221	0.040	3.96	−0.009	(−0.012; −0.007)	0.000	0.531	53.1
	R^2=0.532; $F_{(3,19)}$ = 7.188; P=0.002					R^2=0.567; $F_{(3,49)}$ = 21.412; P=0.000				

Notes: Age was measured in years, CRP was measured in mg/L and eGFR was measured in mL/min. B, regression coefficient; Sr^2, squared semi-partial correlation coefficient.
Abbreviations: CRP, C-reactive protein; DITO, Delirium In The Old; DECO, DElirium pathogenesis, Cognition and Outcome; eGFR, estimated glomerular filtration rate.

be noted that in acutely ill medical patients MMSE scores were obtained after the delirium episode and therefore, the episode can have had residual effects on the MMSE score. Nevertheless, this finding might also suggest that acutely ill medical patients were cognitively more impaired than the elective cardiac surgery patients. However, when we added MMSE score as covariate to the analyses, estimates did not change and therefore, we are not able to confirm that the difference in mean neopterin levels is caused by a difference in cognitive performance between the two groups.

In the present study, we found no correlation between CRP and neopterin levels in elective cardiac surgery patients, although CRP levels were significantly higher in elective cardiac surgery patients than in acutely ill medical patients. A previous study, performed in patients who underwent CABG, showed that both CRP and neopterin levels increased after on-pump CABG, but the increase was significantly greater for CRP than for neopterin.[34] This finding suggests a poor correlation between CRP and neopterin after on-pump CABG, which is in line with our findings. Since CRP is produced by other cells than neopterin, it might be possible that CRP levels reflect the activity of several other components of the immune system in elective cardiac surgery patients than in acutely ill medical patients.

BH$_4$ functions as an essential cofactor in several enzymatic reactions involved in the production of serotonin and dopamine,[20] two neurotransmitters that may play a role in delirium.[7] The Phe/Tyr ratio is an indirect measure of the BH$_4$ status, as it reflects the activity of the enzyme phenylalanine hydroxylase, an enzyme that uses BH$_4$ as an essential cofactor.[20,21] An elevated ratio of phenylalanine to tyrosine might be suggestive of a decreased BH$_4$ availability. To the best our knowledge, BH$_4$ status in relation to delirium has only been investigated in the DITO and DECO studies. In the DITO study, an increased Phe/Tyr ratio was found in acutely ill patients with delirium compared to those

without,[19] whereas in the DECO study, no differences were found in both the BH$_4$ level and the Phe/Tyr ratio between patients who developed a delirium after elective cardiac surgery and those who did not.[10] In the present study, we found higher Phe/Tyr ratios in acutely ill medical patients with delirium than in patients who developed a delirium after elective cardiac surgery. This finding might suggest a decreased availability of the essential cofactor BH$_4$ in the production of serotonin and dopamine in acutely ill medical patients with delirium. No determinants of the Phe/Tyr ratio could be identified.

Although we found differences in the mean levels of neopterin and Phe/Tyr ratios and in the determinants of neopterin between the two groups of patients, the potential role of these markers in the pathophysiology of delirium needs to be elucidated in future studies. It remains speculative why mean neopterin levels in surgical patients with delirium are 1) lower than mean neopterin levels in acutely ill medical patients with delirium and 2) comparable with mean neopterin levels measured in acutely ill medical patients without delirium.[9] This observation might suggest different threshold levels of neopterin in the development of delirium. Also, it cannot be excluded that factors other than the inflammatory response, eg, a low intraoperative perfusion pressure, hypoxia or changes in cerebral autoregulation,[35,36] play a more dominant role in the pathogenesis of delirium after cardiac surgery. This hypothesis is in line with the results of a previous study in which the magnitude of the inflammatory response after cardiac surgery did not show a relationship with the development of postoperative cognitive dysfunction.[37]

This study has some limitations. First, the two studies used in the present study were not developed to compare these with each other. Inclusion and exclusion criteria were different and this might have influenced the results. Although we have adjusted our analyses for several covariates, we cannot exclude residual confounding. Second, considering

the relatively small groups of patients with delirium, the findings need to be confirmed in a larger study. Third, due to the exploratory nature of this study, we did not adjust for multiple comparisons; therefore, we cannot exclude the possibility that the findings were obtained by chance. Using a Bonferroni correction for multiple comparisons, a P-value <0.002 would be required to denote statistical significance. In this case, the difference in Phe/Tyr ratio would be no longer statistically significant. Fourth, it might be possible that the degree of cognitive functioning has influenced our results. Although MMSE scores were obtained after delirium in acutely ill medical patients, the delirium episode can have had residual effects on the MMSE score. However, we have adjusted our statistical analyses for the CCI; therefore, we believe that we have provided an indirect adjustment for dementia. Fifth, the blood samples in the DITO and DECO studies were not analyzed at the same time and therefore, one might speculate that this can have introduced some bias. However, we do not think that this has influenced our results. In each HPLC run, several samples of the former run were reanalyzed to check for precision. If the values were within a range of 5% of the results of the former analysis, the run was defined as good/accurate. Additionally, depending on the compound measured, all HPLC runs included external/internal standards and recovery measurements, as previously described.[21,26,27] Sixth, we used CRP as indirect marker of inflammation in the analyses performed to identify possible determinants of neopterin. However, CRP is an acute phase reactant and might be a poor marker of cell-mediated immune system activation. Therefore, it might be possible that cell-mediated immune system activation and oxidative stress have played a larger role in both patient groups. Adjustment for inflammation markers other than CRP (eg, IFN-γ) would probably have given more insights in the results.

The present study has several strengths. First, the investigated markers were determined in the same laboratory using identical procedures; therefore, potential differences in mean levels of the investigated markers due to differences in the assay are unlikely. Second, in the two studies, patients were intensively monitored for clinical symptoms of delirium and the diagnosis of delirium was made by a geriatrician or psychiatrist using *DSM-IV* criteria. This makes it less likely that delirium was missed or that symptoms were misdiagnosed.

Conclusion

In this study, we found that acutely ill medical patients with delirium had higher levels of neopterin and higher Phe/Tyr ratios than patients who developed delirium after elective cardiac surgery. Differences in determinants of neopterin levels were found between the two groups. Our findings may suggest that, in acutely ill medical patients, neopterin levels are mainly determined by inflammation/oxidative stress whereas in elective cardiac surgery patients, neopterin levels are mainly driven by renal function/fluid status. These findings could suggest that the markers and pathways that might be involved in the pathophysiology of delirium in acutely ill medical patients may differ from those in elective cardiac surgery patients. Longitudinal studies with repeated measurements over time are needed to investigate whether the pathophysiology of delirium is different among groups. A major step forward in delirium research would be a large study, including several health care settings (eg, surgical, medical and intensive care units), in which potential biomarkers and other factors are compared across different groups of patients.

Acknowledgments

Disclosure

The authors report no conflicts of interests in this work.

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Supplementary material

Table S1 Mean levels of biochemical parameters after additional adjustment for MMSE score

	DITO (n=19)[a]	DECO (n=57)	P-value
Neopterin, nmol/L	97.9 (68.4–140.3)	47.3 (39.9–56.0)	0.002[b]
Homovanillic acid, nmol/L	111.2 (65.9–187.9)[c]	162.6 (129.1–204.6)[c]	0.252
Glutamic acid, µmol/L	42.2 (31.9–55.8)	36.7 (32.4–41.7)	0.436
Serine, µmol/L	76.6 (63.0–93.3)	76.6 (70.0–83.8)	0.994
Glycine, µmol/L	179.1 (149.6–214.3)	198.2 (182.8–214.8)	0.376
Citrulline, µmol/L	20.7 (15.9–26.9)	24.2 (21.5–27.2)	0.349
Arginine, µmol/L	35.1 (26.2–47.0)	44.5 (38.9–50.8)	0.204
Taurine, µmol/L	33.7 (23.8–47.8)	33.6 (29.2–40.1)	0.946
Tyrosine, µmol/L	54.3 (43.8–67.5)	67.0 (60.7–74.0)	0.130
Valine, µmol/L	205.6 (174.2–242.1)	204.2 (189.2–219.8)	0.950
Methionine, µmol/L	21.7 (17.1–27.6)	28.4 (25.5–31.7)	0.079
Tryptophan, µmol/L[d]	32.0 (23.4–40.7)	33.5 (29.6–37.4)	0.787
Phenylalanine, µmol/L	78.0 (67.9–89.3)	76.6 (71.9–81.5)	0.837
Isoleucine, µmol/L	56.9 (46.5–69.5)	67.5 (61.5–74.0)	0.183
Leucine, µmol/L	121.1 (99.8–147.2)	124.7 (114.3–136.1)	0.816
Phenylalanine/tyrosine ratio	1.44 (1.21–1.69)	1.14 (1.06–1.23)	0.035
Tryptophan/LNAAs ratio × 100[d]	5.63 (4.40–6.86)	6.06 (5.51–6.62)	0.576
Tyrosine/LNAAs ratio × 100	10.9 (9.2–13.1)	13.2 (12.2–14.3)	0.104
Phenylalanine/LNAAs ratio × 100	16.5 (14.3–19.1)	15.3 (14.4–16.4)	0.438
Citrulline/arginine ratio	0.59 (0.43–0.82)	0.54 (0.47–0.63)	0.684

Notes: Values are expressed as mean (95% CI) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex, CCI and MMSE score. The model including neopterin is adjusted for age, sex, CCI, the eGFR, CRP and MMSE score. [a]Analyses were performed in 19 patients instead of 23 patients due to missing MMSE scores. [b]Analysis was performed in 72 patients with delirium (DITO n=19 and DECO n=53) due to missing CRP levels. [c]One value missing. [d]Values were not logarithmically transformed.
Abbreviations: CCI, Charlson Comorbidity Index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LNAAs, large neutral amino acids; MMSE, Mini-Mental State Examination.