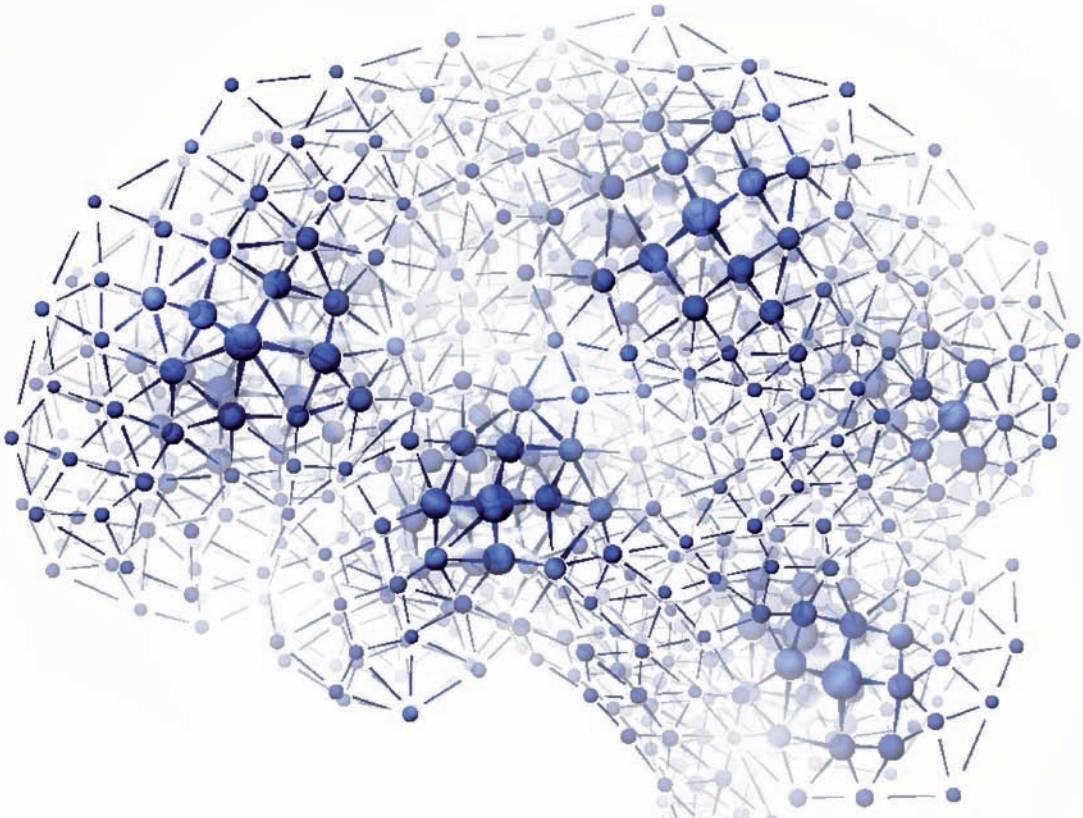

Delirium in Old Age

Pathophysiological and Pharmacological Aspects

ANGELIQUE EGBERTS

Delirium in Old Age

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Angelique Egberts

COLOPHON

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Delirium in Old Age

Pathophysiological and Pharmacological Aspects

Delirium bij Ouderen

Pathofysiologische en Farmacologische Aspecten

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‘Cui calor et tremor est, saluti delirium est.’

De Medicina, Liber II, Aulus Cornelius Celsus
- 1st century A.D. -

CONTENTS

1. Introduction

Chapter 1

General introduction and outline of this thesis

GENERAL INTRODUCTION

Delirium – an acute neuropsychiatric syndrome characterized by fluctuating disturbances in attention, awareness and cognition – is a common and severe disorder in older hospitalized patients [1]. This syndrome affects approximately 20 to 30% of all older patients admitted to acute hospital wards [2] and can affect even a higher proportion of patients in the post-operative and intensive care settings [3]. Delirium is associated with poor clinical outcomes, including prolonged hospital stay, loss of independence, cognitive decline and mortality [4, 5]. Approximately 20 to 25% of the patients who experience delirium dies within 6 to 12 months [4], but higher mortality rates can be expected based on care setting, patient characteristics [6] and duration of delirium [7-10]. In older patients admitted to a general medicine ward, the 3-month mortality risk can increase by 11% for every additional 48 hours that delirium persists [7]. Despite the high frequency and the clinical impact, many important aspects of this syndrome still need to be clarified. The pathophysiological pathways leading to delirium are poorly understood; early recognition and prediction of delirium are difficult and not supported by biomarkers, and an evidence–based effective drug to treat or prevent delirium is still not available.

Delirium is not a novel phenomenon; it has been recognized since the ancient time (4th century BC). Nevertheless, diagnostic criteria for delirium have only been available since the publication of the third edition of the Diagnostic and Statistical Manual of Mental Disorders in 1980 [11]. Until then, different terms were used to describe delirium, which complicated the detection of delirium and interpretation of research findings. The publication of the first diagnostic criteria and soon afterwards also the introduction of screening tools, strongly facilitated research in delirium [11].

Up-to-date, the high need to improve the prediction, detection and treatment of delirium, in order to reduce morbidity and mortality, is increasingly being recognized and the number of publications mentioning delirium is rising (**figure 1**). Conversely, the underlying pathophysiology remains largely understudied (**figure 1**). Adequate knowledge of the pathophysiology is required to find markers for early recognition and to improve delirium prediction, prevention and treatment.

Several mechanisms have been proposed to be involved in the pathophysiology of delirium and include, among others, inflammation, disturbances in neurotransmission, oxidative stress, loss of brain reserve, dysregulation of the hypothalamic-pituitary-adrenal axis, reduced cerebral blood flow and dysregulation of the sleep-wake cycle [12]. Considering that different combinations of predisposing and precipitating factors can result into delirium, it seems most reasonable that the pathophysiology of delirium is multifactorial due to a

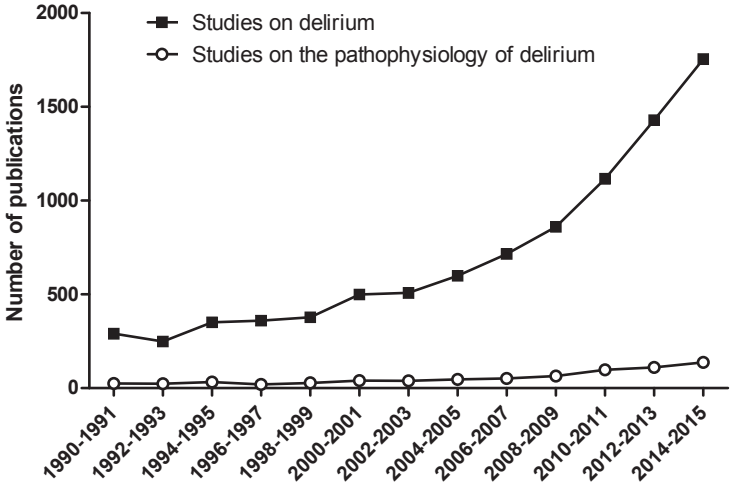

Figure 1. Number of PubMed publications per time-interval of 2 years for 'delirium' and 'delirium pathophysiology' during the period 1990-2015. Search strategies can be found in supplementary file 1.

complex interplay among several biochemical pathways. However, the extent in which the proposed mechanisms contribute to delirium is unclear and additionally, increasing evidence suggests that delirium might have different pathophysiological mechanisms depending on the precipitating factor and the health status of the patient.

Also several drugs and drug classes may play a role in the pathogenesis of delirium. Many drugs commonly used by older persons interfere with one or more of the hypothesized mechanisms underlying delirium and therefore, it is likely that some drugs may increase the risk of delirium, while others may reduce or prevent the development of delirium. Drug use is modifiable and therefore, it is important to know which drugs should be considered as a risk factor for delirium and which ones as potential protective drugs. Nevertheless, there is considerable uncertainty about the risk of delirium associated with several drugs commonly used by older persons [13, 14].

OUTLINE OF THIS THESIS

The aims of this thesis are to investigate novel biomarkers of delirium and the potential role of pharmacological agents in determining delirium. The findings can contribute to a better understanding of delirium and may help to find markers for early recognition and to improve delirium prediction, prevention and treatment.

The findings described in this thesis are based on two studies. The first study is the Delirium In The Old (DITO) study, a cross-sectional study designed to investigate several advanced biochemical blood markers in patients with and without delirium. This study included 86 patients aged 65 years and older who were acutely admitted to the wards of Internal Medicine and Geriatrics of the Erasmus Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands.

The second study is a chart-review study including 905 patients aged 65 years and older who were acutely admitted to the ward of Geriatrics of the Erasmus Medical Center. This study was performed to investigate the possible association between the use of anticholinergic drugs and delirium. Additionally, within the framework of this study, we have investigated a relatively novel and easily measurable inflammatory marker in patients with and without delirium.

The first part of this thesis, **Chapter 2**, focuses on several mechanisms that might play a role in the pathophysiology of delirium. **Chapter 2.1** reports on the association of neopterin, interleukin-6 and insulin-like growth factor-1, which are markers of the immune system, oxidative stress and brain reserve, with delirium. **Chapter 2.2** focuses on disturbances in serotonergic and dopaminergic neurotransmission as well as oxidative stress in delirium. For this purpose, levels of amino acids, amino acid ratios and dopamine's metabolite homovanillic acid (HVA) were investigated. In **Chapter 2.3**, the association of the neutrophil-lymphocyte ratio and other, more conventional, inflammatory markers (i.e. white blood cells, neutrophils, lymphocytes and C-reactive protein) with delirium is presented. **Chapter 2.4** describes differences in biochemical markers (neopterin, amino acids, amino acid ratios and HVA) between acutely ill medical and elective cardiosurgical patients with delirium.

The second part of this thesis, **Chapter 3**, focuses on the role of pharmacological agents in delirium. In **Chapter 3.1**, the potential influence of acetylsalicylic acid on neopterin and tryptophan levels in patients with delirium is described. **Chapter 3.2** describes differences in the association between anticholinergic drug use, measured with three anticholinergic drug scales, and delirium.

In **Chapter 4**, the findings described in this thesis are discussed and directions for future research are presented. Finally, an English and Dutch summary of this thesis are provided in **Chapter 5**.

SUPPLEMENTARY MATERIAL

File 1. Search strategies in PubMed

Search strategy for 'Delirium':

Query:

"Delirium"[mh] OR Delirium*[tiab]

Search strategy for 'Delirium and pathophysiology':

Query:

("Delirium"[mh] OR Delirium*[tiab])

AND

("Pathology"[mh] OR Patholog*[tiab] OR Pathophysiol*[tiab] OR Neuropathol*[tiab] OR Pathogenes*[tiab] OR Causal*[tiab] OR Etiology[sh] OR Etiolog*[tiab] OR "Biomarkers"[mh] OR biomarker*[tiab])

AND

("Blood"[mh] OR Blood[tiab] OR "Plasma"[mh] OR Plasma[tiab] OR "Serum"[mh] OR Serum[tiab] OR "Cerebrospinal fluid"[mh] OR Cerebrospinal fluid*[tiab] OR Cerebro spinal fluid*[tiab] OR "Biomarkers"[mh] OR Biomarker*[tiab] OR Biological marker*[tiab] OR Biologic marker*[tiab] OR Laboratory marker*[tiab] OR Biochemical marker*[tiab] OR Immunologic marker*[tiab] OR Immune marker*[tiab] OR "Urine"[mh] OR Urine*[tiab] OR Urine[sh])

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2. Pathophysiological markers and delirium

Chapter 2.1

Neopterin: a potential biomarker for delirium in elderly patients

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Dement Geriatr Cogn Disord. 2015;39(1-2):116-24

ABSTRACT

Background/Aim

The diagnosis of delirium is not supported by specific biomarkers. In a previous study, high neopterin levels were found in patients with a postoperative delirium. In the present study, we investigated levels of neopterin, interleukin-6 (IL-6) and insulin-like growth factor-1 (IGF-1) in acutely ill admitted elderly patients with and without a delirium.

Methods

Plasma/serum levels of neopterin, IL-6 and IGF-1 were determined in patients aged ≥ 65 years admitted to the wards of Internal Medicine and Geriatrics. Differences in biomarker levels between patients with and without a delirium were investigated by the analysis of variance in models adjusted for age, sex, comorbidities and eGFR (when appropriate).

Results

Eighty-six patients were included; 23 of them with a delirium. In adjusted models, higher mean levels of neopterin (70.5 vs. 45.9 nmol/l, p = 0.009) and IL-6 (43.1 vs. 18.5 pg/ml, p = 0.034) and lower mean levels of IGF-1 (6.3 vs. 9.3 nmol/l, p = 0.007) were found in patients with a delirium compared to those without.

Conclusion

The findings of this study suggest that neopterin might be a potential biomarker for delirium which, through oxidative stress and activation of the immune system, may play a role in the pathophysiology of delirium.

INTRODUCTION

Delirium is an acute neuropsychiatric syndrome [1] common in the elderly and associated with increased morbidity and mortality, prolonged hospital stay, loss of independence and increased risk of dementia [2, 3]. Early recognition of a delirium might be difficult and is not supported by biomarkers [4].

The pathophysiology of delirium is poorly understood, but it is widely accepted that it is multifactorial due to a complex interaction between several underlying mechanisms [5]. Activation of the immune system, oxidative stress, loss of neuroprotection and disturbances in cerebral neurotransmitter systems may all contribute to a delirium [6-9]. During immune activation, monocytes and macrophages are stimulated to produce neopterin in response to the pro-inflammatory cytokine interferon-gamma [10, 11]. Increased neopterin production has been associated with neurodegenerative disorders like Alzheimer's disease [12] and Huntington's disease [13], but also delirium after cardiac surgery [6]. To the best of our knowledge, the potential role of neopterin in delirium has never been investigated in elderly patients admitted due to acute pathology.

Immune activation is also reflected by the levels of the pro-inflammatory cytokine interleukin-6 (IL-6). Increased levels have been associated with postoperative confusion [14], postoperative delirium [15, 16] and delirium in acutely ill elderly patients [17]. For the latter category, data are limited and inconsistent [8, 17].

Moreover, it has been suggested that an underlying vulnerability of the brain may predispose patients to the development of a delirium [2, 18]. In several studies, the potential role of the neuroprotective cytokine insulin-like growth factor-1 (IGF-1) has been investigated. Low IGF-1 levels have been associated with delirium in acutely ill elderly patients [8, 19, 20].

In the present study, we investigated levels of the potential biomarkers neopterin, IL-6 and IGF-1 in elderly patients with and without a delirium.

PATIENTS AND METHODS

Participants

In this study, we included patients admitted to the wards of Internal Medicine and Geriatrics of the Erasmus Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands.

All acutely admitted patients aged ≥ 65 years were eligible to participate in the study. 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.

Written informed consent was obtained from all participants. In case of a delirium or cognitive impairment at the time of admission, informed consent was obtained from a representative of the patient. The Medical Ethics Committee of the Erasmus Medical Center approved the study protocol.

Procedures

All participants were observed daily by the nursing and medical staff and by members of the research team until discharge. To screen for a change in behavior, the 13-items Delirium Observation Screening scale was used during the first 5 days of admission [21]. The diagnosis of delirium was made by a geriatrician, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [1], and was based on the psychiatric examination of the patient, the medical and nursing records, including the Delirium Observation Screening scale scores, and information given by the patient's closest relative. When the diagnosis of delirium was doubtful, the case was discussed with the geriatric consultation team to gain consensus.

Demographic and clinical data were collected at admission. Age, sex and living situation before admission were documented. Cognitive functioning was assessed in absence of a delirium using the MMSE [22]. 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 there was enough evidence that the patient would have a MMSE score ≥ 10, the patient was not excluded from the study. Severity of comorbidities was scored using the Charlson Comorbidity Index [23]. The physical functionality was assessed using the 6-items Katz Activities of Daily Living (ADL) scale and the Barthel Index [24, 25]. The instrumental functionality was assessed using the 7-items Older Americans Resource Scale for Instrumental ADL [24]. The frailty of the patients was measured with the Identification Seniors at Risk questionnaire [26]. Blood samples of all patients were collected within 48 h after admission. When a patient developed a delirium during the hospital stay, new blood samples were collected within 24 h after the onset of the delirium and were used for the statistical analyses.

Biochemical measurements

Nonfasting blood was collected preferably between 8 and 10 a.m. in a 8-ml tube containing ethylene diamine tetra-acetic acid as well as in a 10-ml serum-separating tube. After blood sampling, the tubes were stored at room temperature and protected from light to

prevent oxidative loss of neopterin [27]. Within 3 h, the blood was centrifuged for 20 min at 2,650 *g* and 20 °C. The obtained plasma and serum were stored at –80 °C until analysis.

Plasma neopterin levels were determined by high-performance liquid chromatography after acid oxidation, as previously described [28]. The Human IL-6 Chemi Enzyme-Linked Immunosorbent Assay Kit (Invitrogen™, Life Technologies, Carlsbad, Calif., USA) was used for the quantification of serum IL-6 levels. Serum levels of IGF-1 were determined by the IMMULITE® 2000 Analyzer (Siemens Healthcare, Erlangen, Germany).

C-reactive protein (CRP) levels and the estimated glomerular filtration rate (eGFR) were taken from the medical records. These markers were used to adjust neopterin for inflammatory state and renal function. The eGFR was determined by the following Modification of Diet in Renal Disease formula: $175 \times [\text{serum creatinine } (\mu\text{mol/l}) \times 0.0113]^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female).

Statistical analysis

Medians and interquartile ranges were determined for continuous participant characteristics and proportions for categorical characteristics. Biochemical parameters with a skewed distribution were logarithmically transformed (neopterin, IL-6, IGF-1 and CRP). Univariate one-way analysis of variance was used to investigate the association between mean levels of neopterin, IL-6, CRP, eGFR and IGF-1 (dependent variable) and the presence of a delirium. Models were adjusted for age, sex and the Charlson Comorbidity Index, and those including neopterin were adjusted for age, sex, Charlson Comorbidity Index, tertiles of eGFR and additionally for CRP levels. Mean eGFR values in the tertiles were 34.5, 57.4 and 96.5 ml/min. Additional analyses were performed for neopterin, IL-6 and IGF-1 after adding also MMSE score to the models. A two-tailed $p < 0.05$ was defined as statistically significant.

Univariate one-way analysis of variance was used to compare mean neopterin levels across tertiles of eGFR. Nonlinear regression was used to fit a two-phase exponential decay model to the eGFR values and corresponding neopterin levels.

GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, Calif., USA) was used for curve fitting and to draw all graphs. Statistical Package for the Social Sciences, version 21.0 (SPSS Inc., Chicago, Ill., USA) was used to perform the other statistical analyses.

RESULTS

Participant characteristics

Table 1 represents the baseline characteristics of the 86 participants who were included in the study. Twenty-three patients were diagnosed with a delirium, of which 21 were admitted to the hospital with a delirium and 2 developed a delirium during admission.

Table 1. Characteristics of the study participants

	No delirium (n = 63)	Delirium (n = 23)
Male	47.6	43.5
Age, years	81.0 (75.0–85.0)	87.0 (84.0–88.0)
MMSE score	25.5 (22.0–28.0) [a]	20.0 (18.0–25.0) [b]
Living situation		
Home	47.6	26.1
Home care	31.7	30.4
Residential home	7.9	17.4
Nursing home	3.2	13.0
Missing data	9.5	13.0
Katz ADL score	0.0 (0.0–3.0)	2.0 (1.0–11.0)
OARS-IADL score	5.0 (0.0–10.0)	9.5 (3.5–14.0)
Barthel Index	18.0 (13.0–20.0)	16.0 (9.5–19.0)
ISAR score	4.0 (2.0–6.0)	6.0 (4.8–7.0)
Charlson Comorbidity Index	1.0 (1.0–2.0)	2.0 (1.0–3.0)

Notes: Values are expressed as median (interquartile range) or percentages. [a] Three values missing. [b] Four values missing.
Abbreviations: ADL, Activities of Daily Living; ISAR, Identification of Seniors at Risk; MMSE, Mini-Mental State Examination; OARS-IADL, Older Americans Resource Scale for Instrumental Activities of Daily Living.

Analyses of biochemical parameters

Mean levels and corresponding 95% confidence intervals (CI) of the investigated biochemical parameters in patients with and without a delirium are presented in **table 2**. In adjusted models, mean neopterin levels were significantly higher in patients with a delirium (70.5 nmol/l, 95% CI: 54.1–91.8) than in those without (45.9 nmol/l, 95% CI: 39.4–53.6) (p = 0.009; **figure 1A**). This association remained statistically significant after additional adjustment for CRP levels. For 6 patients, IL-6 data were missing (delirium, n = 1; no delirium, n = 5). Mean IL-6 levels were significantly higher in patients with a delirium (43.1 pg/ml, 95% CI: 22.5–82.2) compared to those without (18.5 pg/ml, 95% CI: 12.6–27.2) (p = 0.034;

Table 2. Mean levels of biochemical parameters

	No delirium (n = 63)	Delirium (n = 23)	p-value
Neopterin, nmol/l [a]	45.9 (39.4–53.6)	70.5 (54.1–91.8)	0.009
Neopterin, nmol/l [a,b]	47.6 (41.8–54.3)	64.7 (51.5–81.1)	0.028
IL-6, pg/ml [a]	18.5 (12.6–27.2)	43.1 (22.5–82.2)	0.034
CRP, mg/l [a]	18.5 (12.0–28.6)	33.0 (15.8–69.2)	0.196
eGFR, ml/min	65.4 (59.1–71.8)	53.9 (43.0–64.8)	0.081
IGF-1, nmol/l [a]	9.3 (8.1–10.7)	6.3 (5.0–8.0)	0.007

Notes: Values are expressed as mean (95% confidence interval). Models are adjusted for age, sex and Charlson Comorbidity Index. Models including neopterin are adjusted for age, sex, Charlson Comorbidity Index and eGFR. [a] Means and 95% confidence intervals are the back-transformed $\log_{10}$-values. [b] Additionally adjusted for log CRP.
Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6.

figure 1B). Mean IGF-1 levels were significantly lower in patients with a delirium (6.3 nmol/l, 95% CI: 5.0–8.0) than in those without a delirium (9.3 nmol/l, 95% CI: 8.1–10.7) (p = 0.007; **figure 1C**). In all additional models adjusted for MMSE score, estimates remained statistically significant (data not shown).

Tertiles of eGFR were associated with decreasing neopterin levels (**figure 2**). In the first tertile, the mean neopterin level was 78.3 nmol/l (95% CI: 61.5–99.8), in the second, 44.8 nmol/l (95% CI: 35.4–56.5) and in the third, 39.4 nmol/l (95% CI: 30.8–50.1). Mean neopterin levels were significantly lower in the second (p = 0.001) and third tertile (p = 0.000) than in the first tertile. **Figure 3** shows the two-phase exponential decay curve for neopterin levels as function of eGFR.

DISCUSSION

In the present study, we found elevated mean levels of neopterin and IL-6 as well as reduced mean levels of IGF-1 in elderly patients with a delirium. We found that patients with a delirium have increased neopterin levels, even after adjustment for inflammatory state. Since neopterin levels may reflect the amount of cell-mediated immune activation and oxidative stress [10, 11], this finding might suggest that oxidative stress plays the most important role in the induction of delirium and that cellular immune activation is not a prerequisite for delirium. In the present study, we also found that patients with a severe impaired renal function have significantly increased neopterin levels compared to patients with a less impaired renal function. This finding is in agreement with the study by Godai et al. [29], in which a negative exponential correlation was found between serum neopterin levels and creatinine clearance in younger individuals without an infection. The effect of

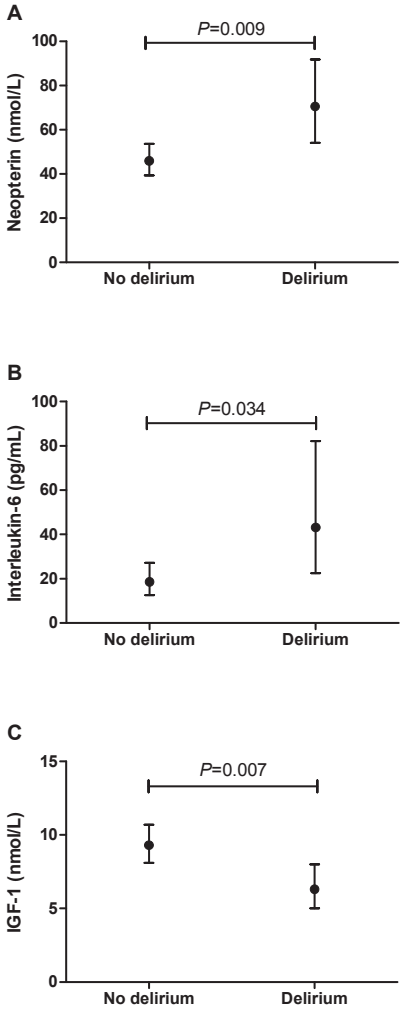

Figure 1. Mean levels and corresponding 95% confidence intervals of neopterin (**A**), interleukin-6 (**B**) and IGF-1 (**C**) in patients with and without a delirium.
Abbreviation: IGF-1, insulin-like growth factor-1.

renal function on neopterin levels has probably not influenced our results, since neopterin levels were adjusted for eGFR. Our finding of increased neopterin levels in elderly patients with a delirium is in agreement with the elevated neopterin levels that have previously been found by Osse et al. [6] in patients with a postoperative delirium.

Furthermore, we found that patients with a delirium have elevated serum IL-6 levels, suggesting an activated immune system. This finding is in line with previous results found in medical and surgical patients with a delirium [15-17]. Our results also showed that patients with a delirium have reduced serum IGF-1 levels. Since IGF-1 is a neuroprotective

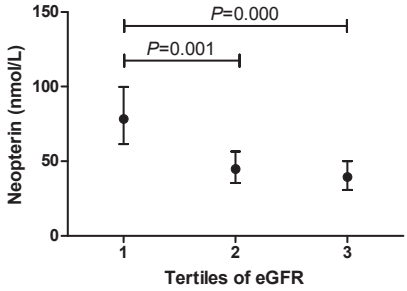

Figure 2. Mean levels and corresponding 95% confidence intervals of neopterin by tertiles of the estimated glomerular filtration rate (eGFR).

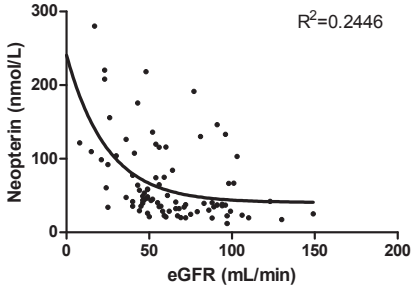

Figure 3. Two-phase exponential decay curve of neopterin levels as function of the estimated glomerular filtration rate (eGFR).

cytokine that is able to pass the blood-brain barrier [30], this finding might suggest a loss of brain reserve. Our finding is in agreement with earlier results found in elderly patients who were acutely admitted to the hospital [8, 20].

Our results of elevated levels of neopterin and IL-6 and reduced levels of IGF-1 in patients with a delirium might suggest that a disturbed activation of the innate and cellular immune system, oxidative stress and an increased vulnerability of the brain may underlie the development of a delirium. It is, however, unclear whether neopterin plays a direct role in the pathogenesis of delirium or that it reflects the involvement of other factors. In various diseases with neurological complications, such as multiple sclerosis and HIV infection with the AIDS dementia complex, elevated neopterin levels have been found in cerebrospinal fluid [31, 32]. The source of neopterin in cerebrospinal fluid in these diseases remains unclear, but an in vitro study has suggested that neopterin in the brain might derive from infiltrating macrophages and monocytes, since astrocytes, microglia and neurons were not able to produce neopterin [33]. The same study also showed that neopterin itself had no effect on the viability of brain cells, but its derivative 7,8-dihydroneopterin was able to induce apoptosis in astrocytes and neurons in a dose-dependent manner [33]. It might

be possible that neopterin is associated with delirium through the indirect induction of apoptosis in the brain.

A second mechanism exists that might explain why high neopterin levels are related to delirium. Interferon-gamma stimulates not only the enzyme guanosine triphosphate cyclohydrolase-I in macrophages [10, 11], which causes the production of neopterin, but also the enzyme indoleamine 2,3-dioxygenase [34]. This enzyme is part of the kynurenine pathway, and under normal circumstances, nearly 90% of the amino acid tryptophan is metabolized via this pathway. Induction of indoleamine 2,3-dioxygenase is responsible for an increased metabolism of tryptophan to kynurenine both in peripheral and central tissue. Kynurenine is able to pass the blood-brain barrier and can be further metabolized to kynurenic acid and quinolinic acid. The latter is a neurotoxic metabolite produced by microglia, which may cause a delirium [34, 35].

It might be possible that in some patients elevated IL-6 levels played a role in the development of a delirium. This cytokine may activate astrocytes and microglial cells, which in turn produce neurotoxic factors and pro-inflammatory cytokines [36, 37]. These factors may decrease synaptic plasticity and cholinergic neurotransmission in the brain [36, 37]. A central cholinergic deficiency is one of the most hypothesized causes of a delirium [38].

Our results also show that patients with a delirium have reduced IGF-1 levels. This neuroprotective cytokine inhibits oxidative stress and promotes neuronal survival [30]. Therefore, low IGF-1 levels might suggest that the brain is more vulnerable to the cytotoxic effects of cytokines and other neurotoxic factors [20], for example, IL-6 and quinolinic acid.

Our hypothesized mechanisms by which neopterin, IL-6 and IGF-1 are involved in the pathogenesis of a delirium might all suggest neuronal injury. This hypothesis is in line with results of previous studies, which showed that S100B, a marker of cerebral damage, is elevated in the serum of patients with a delirium [39, 40]. Neuronal injury might also be an explanation for the severe complications seen after a delirium and for the association of a delirium with dementia [3, 18].

Limitations and strengths

This study has some limitations. First, our findings were obtained in a relatively small group of patients; therefore, extrapolation to a larger population needs to be further investigated. Second, the timing of blood sampling might be a factor of significance. It is possible that the levels of the biochemical markers are dependent on the delirium duration and severity. However, we were not able to adjust for these two factors (delirium severity was not scored). Besides, it is possible that biomarker levels will fluctuate during the day in patients with a delirium, just like delirium symptoms. In the present study, blood sampling and delirium occurred on the same day. However, there is a possibility that the patients had no delirium symptoms at the moment of blood sampling. These factors might have influenced

our results. Third, it might be speculated that comorbidities might have influenced the mean levels of biomarkers; however, also after adjustment for Charlson Comorbidity Index, estimates remained statistically significant. Finally, some patients were not included in the study and this might have resulted in some selection bias. However, since this was random (both in patients and controls) we think that our results are only minimally influenced by this.

The strengths of the present study are as follows. First, the intensive monitoring of clinical symptoms of patients with a delirium until discharge and the DSM-IV diagnosis by a geriatrician makes it less likely that we missed a delirium or misdiagnosed symptoms. Second, we did not focus on one but on several possible pathways that might lead to a delirium as it has been suggested that the pathophysiology is multifactorial. Third, to the best of our knowledge, the potential role of neopterin in delirium has never been investigated in elderly patients admitted due to acute pathology.

CONCLUSION

In this study, we found that patients with a delirium had higher levels of neopterin and IL-6 and lower levels of IGF-1 than patients without a delirium. These results might suggest a potential role of neopterin, IL-6 and IGF-1 in the pathophysiology of a delirium in elderly patients. However, the pathways by which these biomarkers are associated with a delirium remain speculative and require further investigation. Moreover, larger studies are needed to investigate the clinical significance of plasma neopterin as a biomarker for delirium diagnosis next to, or instead of, peripheral levels of IL-6 and IGF-1.

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Chapter 2.2

Disturbed serotonergic neurotransmission and oxidative stress in elderly patients with delirium

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ABSTRACT

Background/Aim

Oxidative stress and disturbances in serotonergic and dopaminergic neurotransmission may play a role in the pathophysiology of delirium. In this study, we investigated levels of amino acids, amino acid ratios and levels of homovanillic acid (HVA) as indicators for oxidative stress and disturbances in neurotransmission.

Methods

Plasma levels of amino acids, amino acid ratios and HVA were determined in acutely ill patients aged ≥ 65 years admitted to the wards of Internal Medicine and Geriatrics of the Erasmus University Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands. Differences in the biochemical parameters between patients with and without delirium were investigated by analysis of variance in models adjusted for age, sex and comorbidities.

Results

Of the 86 patients included, 23 had delirium. In adjusted models, higher mean phenylalanine/tyrosine ratios (1.34 vs. 1.14, p = 0.028), lower mean tryptophan/large neutral amino acids ratios (4.90 vs. 6.12, p = 0.021) and lower mean arginine levels (34.8 vs. 45.2 µmol/l, p = 0.022) were found in patients with delirium when compared to those without. No differences were found in HVA levels between patients with and without delirium.

Conclusion

The findings of this study suggest disturbed serotonergic neurotransmission and an increased status of oxidative stress in patients with delirium.

INTRODUCTION

Delirium is a common and severe complication among elderly patients and is associated with increased morbidity and mortality, prolonged hospital stay, increased risk of post-discharge institutionalization and dementia [1, 2]. The pathophysiology of delirium is still largely hypothetical. Identifying accurate biomarkers for delirium may shed light on the pathophysiology and may help to improve delirium recognition and care.

Oxidative stress and disturbances in serotonergic and dopaminergic neurotransmission might all be involved in the pathophysiology of delirium and probably act together [3]. Within the central nervous system, tetrahydrobiopterin (BH_4) functions as an essential cofactor in enzymatic reactions responsible for the production of serotonin and dopamine. In addition, BH_4 is a cofactor for nitric oxide synthase (NOS) that catalyzes the production of nitric oxide (NO) and citrulline from arginine [4]. If BH_4 becomes limited, this could impair serotonin and dopamine synthesis. Besides, when BH_4 is partially deficient, some cellular sources of NOS may generate superoxide ($O_2^{\bullet -}$) instead of NO and citrulline [4, 5]. In patients with delirium, BH_4 status has only been investigated after elective cardiac surgery [6].

In order to assess BH_4 status, we measured amino acid levels and subsequently calculated the phenylalanine/tyrosine (Phe/Tyr) ratio. This ratio is an indicator for the BH_4 status as it reflects the activity of the enzyme Phe hydroxylase, an enzyme that uses BH_4 as a cofactor [4, 7]. An elevated ratio is suggestive for decreased BH_4 availability. Furthermore, we determined the ratios of tryptophan (Trp), Phe and Tyr to the other large neutral amino acids (LNAAs). Trp is the precursor of serotonin, while Phe and Tyr are the precursors of dopamine. The LNAAs (Trp, Phe, Tyr, valine, isoleucine and leucine) compete with each other for transport across the blood-brain barrier. Therefore, a decreased Trp/LNAAs ratio is suggestive for a decline in the amount of Trp that enters the brain and consequently for reduced synthesis of serotonin [8]. Moreover, we measured plasma levels of the dopamine metabolite homovanillic acid (HVA), approximately 30% of which is estimated to originate from dopamine neurons in the central nervous system and which is therefore thought to be a reliable indicator for central dopamine activity [9]. Finally, we measured plasma levels of arginine and citrulline to investigate the production of NO by NOS.

The aim of the study was to investigate BH_4 status, potential disturbances in serotonergic and dopaminergic neurotransmission and the production of NO in patients with and without delirium.

METHODS

Study design and participants

The present study was performed within the Delirium In The Old (DITO) study in which mean levels of neopterin, interleukin-6 and insulin-like growth factor-1 were compared between patients with and without delirium [10]. In the DITO study, a cross-sectional study, we included patients who were admitted to the wards of Internal Medicine and Geriatrics of the Erasmus University Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands. All acutely admitted patients aged ≥ 65 years were eligible to participate. 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. We excluded patients with a MMSE < 10 because it can be quite difficult to distinguish between features of severe dementia and delirium at admission as well as to measure improvement of cognitive function in this group.

Written informed consent was obtained from all participants. In case of delirium or cognitive impairment at the time of admission, informed consent was obtained from a representative of the patient. The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocol.

Procedures

All participants were observed daily by the nursing and medical staff and by members of the research team until discharge. To screen for a change in behavior, the 13-item Delirium Observation Screening scale was used during the first 5 days of admission [11]. The diagnosis of delirium was made by a geriatrician, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [12], and was based on the psychiatric examination of the patient, the medical and nursing records, including the Delirium Observation Screening scale scores, and information given by the patient's closest relative. When the diagnosis of delirium was doubtful, the case was discussed with the geriatric consultation team to gain consensus.

Demographic and clinical data were collected at admission. Age, sex and living situation before admission were documented. Cognitive functioning was assessed in absence of delirium using the MMSE [13]. When it was impossible to score the MMSE during admission because the patient was too ill, cognitive function 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 ≥ 10, the patient was not excluded from

the study. Severity of comorbidities was scored using the Charlson Comorbidity Index. This index encompasses 19 medical conditions, including dementia, and each condition is weighted with a score of 1–6 by severity [14]. Physical functionality was assessed using the 6-item Katz Activities of Daily Living scale and the Barthel index [15, 16]. Instrumental functionality was assessed using the 7-item Older Americans Resource Scale for Instrumental Activities of Daily Living [15]. Frailty was measured with the Identification of Seniors at Risk questionnaire [17]. Blood samples of all patients were collected within 48 h after admission. When a patient developed delirium during the hospital stay, new blood samples were collected within 24 h after the onset of the delirium and were used instead of the first blood samples for the statistical analyses.

Biochemical measurements

Nonfasting blood was collected preferably between 8 and 10 a.m. in an 8-ml tube containing ethylene diamine tetra-acetic acid. After blood sampling, the tubes were stored at room temperature to prevent changes in the transfer of amino acids between plasma and blood cells [18]. Within 3 h, the blood was centrifuged for 20 min at 2,650 *g* and 20 °C. The obtained plasma was stored at –80 °C until analysis.

Plasma amino acid levels were determined by high-performance liquid chromatography with automated pre-column derivatization with *ortho*-phthalaldehyde as previously described [18]. Plasma HVA levels were determined by reversed-phase high-performance liquid chromatography and electrochemical detection, as previously described for the determination of serotonin [19].

Statistical analysis

Medians and interquartile ranges were determined for continuous participant characteristics and proportions for categorical characteristics. Biochemical parameters with a skewed distribution were logarithmically transformed (all amino acids, amino acid ratios and HVA). Univariate one-way analysis of variance was used to investigate the association between mean levels of amino acids, amino acid ratios and HVA (dependent variables) and the presence of delirium. Models were adjusted for age, sex and the Charlson Comorbidity Index. Additional analyses were performed for all amino acids, amino acid ratios and HVA after also adding MMSE score to the models. A two-tailed $p < 0.05$ was defined as statistically significant.

Statistical Package for the Social Sciences, version 21.0 (SPSS Inc., Chicago, Ill., USA) was used to perform the statistical analyses. GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, Calif., USA) was used to draw all graphs.

RESULTS

Participant characteristics

Table 1 presents the baseline characteristics of the 86 participants who were included in the study. Of the 23 patients diagnosed with delirium, 21 were admitted to the hospital with delirium and 2 developed delirium during admission.

Table 1. Characteristics of the study participants

	No delirium (n = 63)	Delirium (n = 23)
Male	47.6%	43.5%
Age, years	81.0 (75.0–85.0)	87.0 (84.0–88.0)
MMSE score [a]	25.5 (22.0–28.0) [b]	20.0 (18.0–25.0) [c]
Living situation:		
Home	47.6%	26.1%
Home care	31.7%	30.4%
Residential home	7.9%	17.4%
Nursing home	3.2%	13.0%
Missing data	9.5%	13.0%
Katz Activities of Daily Living score [d]	0.0 (0.0–3.0)	2.0 (1.0–11.0)
OARS-IADL score [e]	5.0 (0.0–10.0)	9.5 (3.5–14.0)
Barthel Index [f]	18.0 (13.0–20.0)	16.0 (9.5–19.0)
Identification of Seniors at Risk score [g]	4.0 (2.0–6.0)	6.0 (4.8–7.0)
Charlson Comorbidity Index [h]	1.0 (1.0–2.0)	2.0 (1.0–3.0)

Notes: Values are expressed as medians (interquartile ranges) or percentages. [a] Range 0 (severe cognitive impairment) to 30 (no cognitive impairment). [b] Three values missing. [c] Four values missing. [d] Range 0 (no disability) to 12 (severe disability). [e] Range 0 (no disability) to 14 (severe disability). [f] Range 0 (severe disability) to 20 (no disability). [g] Scores ≥ 2 indicate a high risk of functional decline. [h] Range 0–37 (severe burden of comorbidities).
Abbreviations: MMSE, Mini-Mental State Examination; OARS-IADL, Older Americans Resource Scale for Instrumental Activities of Daily Living.

Analyses of biochemical parameters

The mean levels and corresponding 95% confidence intervals (CIs) of the investigated biochemical parameters in patients with and without delirium are presented in **tables 2 and 3**. In adjusted models, mean levels of arginine were significantly lower in patients with delirium (34.8 µmol/l, 95% CI: 28.8–42.0) than in those without (45.2 µmol/l, 95% CI: 40.6–50.5) (p = 0.022; **figure 1**). Concerning the amino acid ratios, mean Phe/Tyr ratios were significantly higher in patients with delirium (1.34, 95% CI: 1.19–1.51) than in patients without delirium (1.14, 95% CI: 1.06–1.22) (p = 0.028; **figure 1**). In addition,

mean Trp/LNAAs ratios were significantly lower in patients with delirium (4.90, 95% CI: 4.19–5.74) than in those without (6.12, 95% CI: 5.58–6.71) (p = 0.021; **figure 1**). No associations between the other amino acids and ratios and delirium were found, although citrulline (**figure 1**) and Trp levels were at the border of significance lower in patients with delirium than in those without (p = 0.052 and p = 0.067, respectively).

Table 2. Mean levels of amino acids

	No delirium (n = 63)	Delirium (n = 23)	p-value
Glutamic acid, µmol/l	46.2 (40.9–52.2)	41.3 (33.5–50.9)	0.368
Serine, µmol/l	84.5 (78.7–90.8)	81.3 (71.9–91.8)	0.596
Glycine, µmol/l	194.5 (179.5–210.9)	187.1 (162.6–214.8)	0.633
Citrulline, µmol/l	29.4 (26.1–33.3)	23.0 (18.6–28.4)	0.052
Arginine, µmol/l	45.2 (40.6–50.5)	34.8 (28.8–42.0)	0.022
Alanine, µmol/l	329.6 (297.1–364.8)	337.3 (283.1–402.7)	0.814
Taurine, µmol/l	41.3 (37.4–45.5)	38.5 (32.6–45.6)	0.496
Tyrosine, µmol/l	58.6 (54.0–63.7)	55.6 (48.2–64.1)	0.529
Valine, µmol/l	212.8 (198.2–228.0)	214.8 (190.5–241.5)	0.898
Methionine, µmol/l	22.4 (20.8–24.3)	23.3 (20.4–26.5)	0.651
Tryptophan, µmol/l	32.1 (28.8–35.7)	26.2 (21.8–31.5)	0.067
Phenylalanine, µmol/l	66.7 (62.2–71.4)	74.5 (66.2–83.8)	0.122
Isoleucine, µmol/l	59.6 (55.0–64.6)	57.7 (50.2–66.2)	0.690
Leucine, µmol/l	120.5 (111.4–130.6)	123.3 (107.6–141.3)	0.783
Ornithine, µmol/l	78.0 (71.0–85.5)	67.6 (57.5–79.4)	0.146

Notes: Values are expressed as means (95% confidence intervals) and are the back-transformed $\log_{10}$ values. Models are adjusted for age, sex and Charlson Comorbidity Index.

Table 3. Mean levels of amino acid ratios and homovanillic acid

	No delirium (n = 63)	Delirium (n = 23)	p-value
Phe/Tyr ratio	1.14 (1.06–1.22)	1.34 (1.19–1.51)	0.028
Trp/LNAAs ratio (x 100)	6.12 (5.58–6.71)	4.90 (4.19–5.74)	0.021
Tyr/LNAAs ratio (x 100)	11.8 (11.0–12.7)	11.0 (9.7–12.4)	0.342
Phe/LNAAs ratio (x 100)	13.6 (12.7–14.7)	15.3 (13.6–17.3)	0.122
HVA, nmol/l	93.3 (79.4–109.4) [a]	123.0 (93.3–162.6) [a]	0.098

Notes: Values are expressed as means (95% confidence intervals) and are the back-transformed $\log_{10}$ values. Models are adjusted for age, sex and Charlson Comorbidity Index. [a] One value missing.
Abbreviations: HVA, homovanillic acid; LNAAs, large neutral amino acids; Phe, phenylalanine; Trp, tryptophan; Tyr, tyrosine.

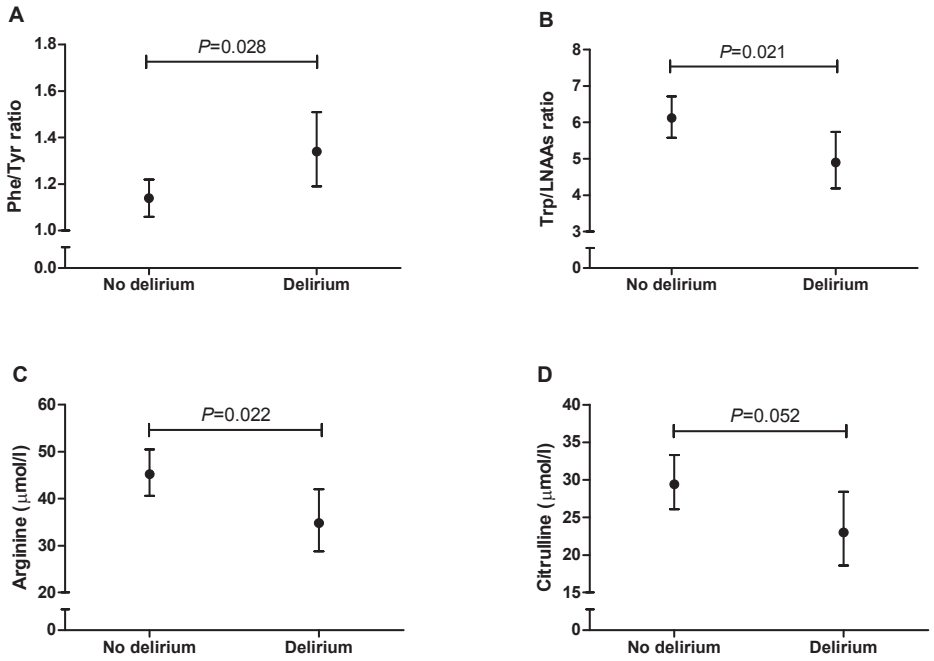

Figure 1. Mean levels and corresponding 95% confidence intervals of the Phe/Tyr ratio (**A**), the Trp/LNAAs ratio (**B**), arginine (**C**) and citrulline (**D**) in patients with and without delirium.
Abbreviations: LNAAs, large neutral amino acids; Phe, phenylalanine; Trp, tryptophan; Tyr, tyrosine.

HVA data were missing for 2 patients (delirium, n = 1 [not enough plasma]; no delirium, n = 1 [measurement failed]). Mean HVA levels were not statistically significantly different between patients with delirium (123.0 nmol/l, 95% CI: 93.3–162.6) and patients without delirium (93.3 nmol/l, 95% CI: 79.4–109.4) (p = 0.098).

In the models additionally adjusted for MMSE score, the association between arginine and delirium did not reach statistical significance (delirium: mean 36.0 μmol/l, 95% CI: 28.7–45.1 vs. no delirium: mean 44.8 μmol/l, 95% CI: 39.8–50.5, p = 0.107). Mean Phe/Tyr ratios remained borderline significantly higher in patients with delirium (1.34, 95% CI: 1.16–1.55) than in those without (1.15, 95% CI: 1.07–1.24) (p = 0.089) and mean Trp/LNAAs ratios remained borderline significantly lower in patients with delirium (5.00, 95% CI: 4.15–6.01) compared to those without (6.15, 95% CI: 5.58–6.78) (p = 0.062). Estimates for the other biochemical parameters remained statistically insignificant (data not shown).

DISCUSSION

In the present study, we found disturbed serotonergic neurotransmission and an increased status of oxidative stress in patients with delirium when compared to patients without delirium.

As far as we are aware, this is the first delirium study investigating BH_4 status and levels of arginine and citrulline in acutely ill elderly hospitalized patients. In order to assess the BH_4 status, we measured the Phe/Tyr ratio. In patients with delirium, we found an increased ratio, suggesting a deficiency in the essential cofactor BH_4 for the production of serotonin, dopamine and NO. Decreased BH_4 availability has already been found in other neuropsychiatric disorders such as Alzheimer's disease [20], Parkinson's disease [20] and schizophrenia [21]. Our finding is not in agreement with the results of a previous delirium study which showed that levels of BH_4 and Phe/Tyr ratios did not differ between patients with and without delirium [6]. However, that study included a relatively younger group of patients undergoing elective cardiac surgery.

In the present study, serotonergic neurotransmission was investigated with the Trp/LNAAs ratio and the Phe/Tyr ratio. We found that patients with delirium had a decreased Trp/LNAAs ratio, which might suggest reduced serotonin production in the central nervous system. This hypothesis is strengthened by the finding that patients with delirium had an elevated Phe/Tyr ratio, which might suggest a deficiency in the essential cofactor BH_4 in the production of serotonin. In previous studies, controversial results have been reported. Several studies found a reduced Trp/LNAAs ratio during delirium [6, 8, 22, 23], whereas two studies reported no difference in this ratio between patients with and without delirium [24, 25]. The study performed by Flacker and Lipsitz [24] included only patients with mild illnesses not requiring hospitalization. Therefore, the findings may not be generalizable to acutely ill patients who needed medical care in hospital. The study performed by van der Cammen et al. [25] included delirium patients with Alzheimer's disease. It might be possible that in those patients a disturbance in cholinergic neurotransmission played a more important role in the development of delirium than disturbances in other pathophysiological pathways [26].

Furthermore, we found no differences in Phe/LNAAs ratios, Tyr/LNAAs ratios and HVA levels between patients with and without delirium, suggesting that dopaminergic neurotransmission is not impaired during delirium. The finding that plasma HVA levels are not significantly increased in patients with delirium compared to patients without delirium is not in agreement with earlier results [6, 25]. However, those studies were performed in patients with Alzheimer's disease [25] and patients undergoing cardiac surgery [6], and therefore the results may not be generalizable. Ramirez-Bermudez et al. [27] found that cerebrospinal fluid HVA levels correlated with psychotic symptoms of delirium (hallucinations and delusions) in neurological patients. It might also be possible that we did not find

an association between the dopaminergic markers and the presence of delirium because we included patients both with and without psychotic features.

In our study, we also found reduced plasma arginine levels and borderline statistically significantly reduced citrulline levels in patients with delirium. Considering the cross-sectional study design, these findings could mean several things. First, it is possible that patients with delirium had a pre-existing arginine deficiency which might have resulted in a reduced production of citrulline by NOS (**figure 2A**). Second, when BH_4 is partially deficient, as our results do suggest, some cellular sources of NOS may generate $O_2\bullet^-$ instead of citrulline and NO from arginine (**figure 2B**) [4, 5], leading to decreased levels of both arginine and citrulline. If this latter scenario is true for delirium, this would also suggest an increased status of oxidative stress, since it favors peroxynitrite formation (**figure 2B**). If peroxynitrite is not scavenged by antioxidants, it may cause oxidative damage to cellular macromolecules [20, 28], which has already been hypothesized to occur in Alzheimer's disease [29]. However, both amino acids have been investigated previously by Osse et al. [6] in patients with delirium after cardiac surgery, but they found no differences in arginine and citrulline levels between patients with and without delirium. Since they also reported no difference in BH_4 status between patients with and without delirium, the results are probable not generalizable to our study.

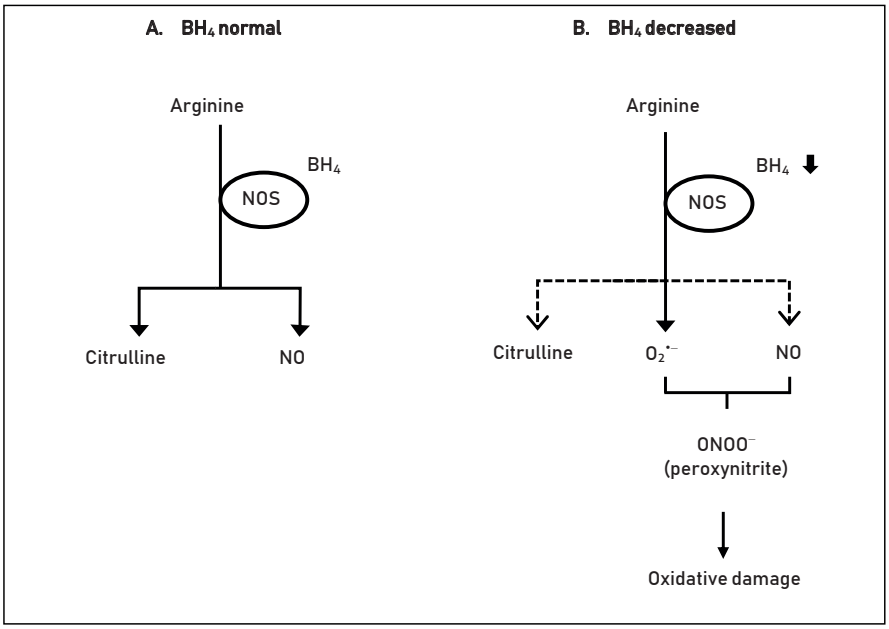

Figure 2. Schematic presentation of the role of tetrahydrobiopterin (BH_4) in the formation of citrulline, nitric oxide (NO) and superoxide ($O_2\bullet^-$) by nitric oxide synthase (NOS). **A.** Situation in which there is sufficient supply of BH_4 for NOS. **B.** Situation in which there is insufficient supply of BH_4. Some cellular sources of NOS will generate $O_2\bullet^-$ instead of NO and citrulline. Generation of $O_2\bullet^-$ and NO together will lead to the formation of peroxynitrite, which may cause oxidative damage.

Limitations and strengths

This study has some limitations. First, our findings were obtained in a relatively small group of patients; therefore, confirmation in a larger population is recommended. Second, it might be speculated that the degree of the patients' cognitive functioning influenced the mean levels of the biochemical parameters [20]. In this study, we adjusted for the Charlson Comorbidity Index, which includes dementia, and our estimates remained statistically significant. However, for our additional analysis, MMSE scores were not available for all patients; therefore, we can neither confirm nor deny that the presence of a comorbid cognitive disturbance, not diagnosed as dementia (yet), was a confounding factor. Third, the timing of blood sampling might be a factor of significance. It is possible that the levels of biochemical markers are dependent on delirium duration and severity or even fluctuate during the day in patients with delirium, just like delirium symptoms. In the present study, blood sampling and delirium occurred on the same day, but there is a possibility that the patients had no delirium symptoms at the moment of blood sampling. This might have influenced our results. Finally, some potential participants were not included in the study and this may have resulted in some selection bias; however, since this was random and occurred in both patients with and without delirium, we think that our results are only minimally influenced by this.

The present study has several strengths. First, the intensive monitoring of clinical symptoms of patients with delirium until discharge and the DSM-IV diagnosis by a geriatrician makes it less likely that we missed delirium or misdiagnosed symptoms. Second, we did not focus on one but on several possible pathways that might lead to delirium as it has been suggested that the pathophysiology is multifactorial.

CONCLUSION

In this study in older, acutely ill hospitalized patients, we found that patients with delirium had higher Phe/Tyr ratios, lower Trp/LNAAs ratios and lower levels of arginine and citrulline than patients without delirium. These findings might suggest that decreased BH_4 availability, disturbed serotonergic neurotransmission and an increased status of oxidative stress may have played a role in the pathogenesis of delirium in our patient group. Since as far as we know this is the first delirium study investigating BH_4 status and levels of arginine and citrulline in acutely ill elderly hospitalized patients, confirmation of our results in a larger, comparable population is recommended. Moreover, more research is needed to explore the potential differences in the pathophysiology of delirium in patients with and without cognitive disorders.

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Chapter 2.3

Increased neutrophil-lymphocyte ratio in delirium: a pilot study

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ABSTRACT

Background/Aim

Delirium is a common and severe complication among older hospitalized patients. The pathophysiology is poorly understood, but it has been suggested that inflammation and oxidative stress may play a role. The aim of this pilot study was to investigate levels of the neutrophil-lymphocyte ratio (NLR) – a marker of systemic inflammation and oxidative stress – in patients with and without delirium.

Methods

This pilot study was performed within a retrospective chart review study that included acutely ill patients, 65 years and older, who were admitted to the ward of geriatrics of the Erasmus University Medical Center. All patients in whom the differential white blood cell (WBC) counts as well as the C-reactive protein (CRP) level were determined within 24 h after admission were included in the present study. Differences in NLR between patients with and without delirium were investigated using univariate analysis of variance, with adjustments for age, sex, comorbidities, CRP level and total WBC count.

Results

Eighty-six patients were included. Thirteen patients were diagnosed with delirium. In adjusted models, higher mean NLR values were found in patients with delirium than in those without delirium (9.10 versus 5.18, $p = 0.003$).

Conclusion

In this pilot study, we found increased NLR levels in patients with delirium. This finding might suggest that an inadequate response of the immune system and oxidative stress may play a role in the pathogenesis of delirium. Further studies are needed to confirm the association between NLR and delirium.

INTRODUCTION

Delirium, an acute neuropsychiatric syndrome, is a common complication among older hospitalized persons and is associated with prolonged hospital stay, loss of independence, and increased risk of cognitive decline and mortality [1, 2]. The underlying pathophysiology is poorly understood and the diagnosis is still primarily based on clinical observation [3]. Identifying accurate biomarkers for delirium may shed light on the pathophysiology and potentially improve delirium recognition and prediction.

Both inflammation and oxidative stress may be involved in the pathophysiology of delirium [4, 5]. Several inflammatory markers have been investigated and were found to be associated with delirium, but time-consuming and expensive measurements make their use for research purposes and clinical practice less attractive.

The neutrophil-lymphocyte ratio (NLR), derived directly from the differential white blood cell (WBC) count, is an easily applicable marker of inflammation and oxidative stress [6]. Several studies have reported an association between increased NLR and cerebrovascular disease [7], schizophrenia [6] and Alzheimer's disease [8] as well as an association with increased severity and poor prognosis of various cardiovascular diseases [9-11] and malignancies [12]. Furthermore, NLR has been found to be a more powerful predictor of cardiovascular risk and mortality in various medical conditions in comparison with traditional infection markers, such as the total WBC count, the individual WBC subtypes and C-reactive protein (CRP) [13-16]. No previous study has investigated the possible association between the NLR and delirium.

We hypothesized that mean NLR levels would be elevated in patients with delirium; therefore, in this pilot study, we compared mean NLR levels of patients with and without delirium who were acutely admitted to a geriatric ward.

METHODS

The present study was performed within a retrospective chart review study in which the possible association between anticholinergic drug exposure and delirium, length of hospital stay, postdischarge institutionalization and in-hospital mortality was investigated [17]. In the previous study, all acutely ill elderly aged 65 and older who were admitted to the ward of geriatrics of the Erasmus University Medical Center, Rotterdam, the Netherlands, between January 1, 2012 and December 31, 2015 were eligible for inclusion. Acutely ill patients were defined as patients with an acute disease whereby a hospital admission was required for medical treatment. Patients hospitalized for less than 3 days, admitted for elective (diagnostic) procedures, or with missing data on drug use or outcome measures were not included [17]. In the present pilot study, we included all patients enrolled in the

previous study in whom the differential WBC counts as well as the CRP level were determined within 24 h after admission. The rationale to choose this time period is that drugs, such as antibiotics, started on admission can have a significant effect on CRP and WBC counts within 48 h. To our knowledge, it is unknown whether NLR levels are stable over time in acutely ill patients. To minimize the possible influence of drugs on the investigated markers, the first 24 h were chosen as the cut-off.

The study was conducted in accordance with the principles expressed in the Declaration of Helsinki. In the Netherlands, ethical approval is only required for studies in which persons are subjected to additional diagnostic procedures or treatments or are required to follow a certain behavioral strategy. No ethical approval and patient consent are required for retrospective chart review studies in which data collected during routine clinical care are extracted and analyzed anonymously.

Data collection

All data were extracted from medical records. Age, sex, place of residence before admission, and the presence of delirium during the hospital stay were documented. Severity of comorbidities on admission was calculated using the Charlson Comorbidity Index (CCI) [18]. The total WBC count, neutrophil count, lymphocyte count, and CRP level within 24 h after admission were recorded. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. WBC, neutrophil and lymphocyte counts were determined on a Sysmex XN-9000 hematology analyzer with flow cytometry (Sysmex Corporation, Kobe, Hyogo Prefecture, Japan). CRP was determined with the CRPL3 assay (immunoturbidimetric method) on a Cobas 8000 c701/702 analyzer (Roche Diagnostics, Rotkreuz, Switzerland).

Definition of delirium

Reported diagnoses of delirium were extracted from medical records. On the ward of geriatrics, the diagnosis of delirium is made by geriatricians as part of daily clinical practice, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th and 5th editions [19, 20], and is based on daily psychiatric examination, medical and nursing notes, the Delirium Observation Screening scale scores, and information given by the patient's closest relative. In the previous chart review study, delirium was defined as "present on admission" if the diagnosis was made within the first 2 days of the hospital stay. All other cases of delirium were defined as "incident delirium". In this pilot study, these two groups were combined.

Statistical analysis

Differences in baseline characteristics between patients with and without delirium were compared using the Fisher's exact test for categorical variables, the Mann-Whitney U-test for non-normally distributed continuous variables, and the Student's t-test for normally distributed continuous variables.

Univariate one-way analysis of variance was used to compare mean levels of NLR, CRP, WBC, neutrophils and lymphocytes in patients with and without delirium. Biochemical parameters with a skewed distribution were logarithmically transformed using the natural log (NLR, WBC count, neutrophil count and CRP levels). Models were adjusted for age, sex, CCI, CRP levels and total WBC count, except when one of these variables was the dependent variable. Correlations between the aforementioned inflammatory markers were analyzed using the Spearman's correlation coefficient.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 21.0 (IBM Corp., Armonk, NY, USA). Results were considered statistically significant at a two-sided p-value less than 0.05. Figures were constructed using GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Of the 905 patients enrolled in the chart review study, 862 had available data on the total WBC counts whereas 856 had available data on the CRP level upon admission. In 90 patients also the differential WBC counts were determined. Eighty-six of them had available data on the neutrophil and lymphocyte counts as well as the CRP level within 24 h after admission and these patients were included in the present pilot study.

Table 1 presents the baseline characteristics of the 86 included patients. The mean age was 80.1 ± 6.5 years; 37.2% were male. Thirteen patients were diagnosed with delirium, of whom eight had delirium on admission and five had incident delirium. The median number of days between admission and diagnosis of incident delirium was 6 (range: 3–13). No statistically significant differences were found in age, CCI score, and the other demographic characteristics between patients with and without delirium.

Analyses of inflammatory markers

Mean levels and corresponding 95% confidence intervals (CIs) of the investigated inflammatory markers in patients with and without delirium are presented in **table 2**. In adjusted models, mean levels of NLR remained statistically significantly higher in patients with delirium (9.10, 95% CI: 6.54–12.65) than in those without (5.18, 95% CI: 4.53–5.93; p

Table 1. Baseline characteristics of the study participants

Variables	No delirium (n = 73)	Delirium (n = 13)	p-value
Male	28 (38.4)	4 (30.8)	0.759 [a]
Age (years)	79.9 ± 6.5	81.2 ± 6.6	0.517 [b]
Place of residence before admission:			
Home (with or without home care)	56 (76.7)	9 (69.2)	0.726 [a]
Institutional care facility	17 (23.3)	4 (30.8)	
First time on the ward of geriatrics	45 (61.6)	9 (69.2)	0.759 [a]
Charlson Comorbidity Index	2.0 (1.0–4.0)	4.0 (1.5–5.5)	0.110 [c]

Notes: Values are expressed as mean ± SD for normally distributed continuous variables, median (interquartile range) for not normally distributed continuous variables and n (percentages) for categorical variables. [a] Fisher's exact test. [b] Student's t-test. [c] Mann-Whitney U-test.

Table 2. Mean levels of inflammatory markers

Variables	No delirium (n = 73)	Delirium (n = 13)	p-value
Total WBC count ($\times 10^9$/l)	9.33 (8.46–10.30)	7.83 (6.17–9.95)	0.186 [a,c]
Neutrophil count ($\times 10^9$/l)	6.53 (6.25–6.83)	7.04 (6.31–7.85)	0.220 [c]
Neutrophil fraction (% of WBCs)	72.7 (70.8–74.6)	78.1 (73.5–82.8)	0.037
Lymphocyte count ($\times 10^9$/l)	1.37 (1.24–1.50)	1.06 (0.75–1.38)	0.080
Lymphocyte fraction (% of WBCs)	16.3 (14.8–17.7)	11.0 (7.5–14.6)	0.008
NLR	5.18 (4.53–5.93)	9.10 (6.54–12.65)	0.003 [c]
CRP (mg/l)	9.7 (6.2–15.3)	26.3 (8.8–78.3)	0.099 [b,c]

Notes: Values are expressed as mean (95% confidence intervals) and are adjusted for age, sex, Charlson Comorbidity Index score, CRP level and total WBC count unless otherwise specified. [a] Not adjusted for total WBC count. [b] Not adjusted for CRP level. [c] Values are presented as the back-transformed natural log values.
Abbreviations: CRP, C-reactive protein; NLR, neutrophil-lymphocyte ratio; WBC, white blood cell.

= 0.003; **figure 1**). With regard to the other inflammatory markers, mean neutrophil fractions were significantly higher in patients with delirium (78.1%, 95% CI: 73.5–82.8) than in those without (72.7%, 95% CI: 70.8–74.6; p = 0.037) and mean lymphocyte fractions were significantly lower in patients with delirium (11.0%, 95% CI: 7.5–14.6) than in those without (16.3%, 95% CI: 14.8–17.7; p = 0.008). No differences were found in total WBC count, neutrophil and lymphocyte counts, and CRP level between the groups, although the lymphocyte count was at the border of significance lower in patients with delirium than in those without (p = 0.080).

In the total group of patients, NLR showed a weak positive correlation with CRP (r = 0.389, p < 0.001; **figure 2A**), a moderate positive correlation with the total WBC count (r = 0.588, p < 0.001; **figure 2B**), a strong positive correlation with the neutrophil count (r =

0.738, p < 0.001; **figure 2C**) and a strong negative correlation with the lymphocyte count (*r* = –0.638, p < 0.001; **figure 2D**).

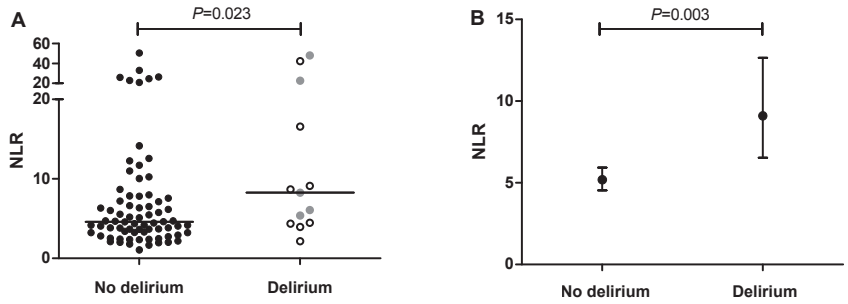

Figure 1. NLR in patients with and without delirium. (**A**) Unadjusted levels of NLR in patients with and without delirium. Lines represent medians, open dots represent patients with delirium on admission, gray dots represent patients with incident delirium. (**B**) Mean levels and corresponding 95% confidence intervals of NLR in patients with and without delirium. Values are the back-transformed natural log values. Model is adjusted for age, sex, the Charlson Comorbidity Index score, C-reactive protein level, and white blood cell count.
Abbreviation: NLR, neutrophil-lymphocyte ratio.

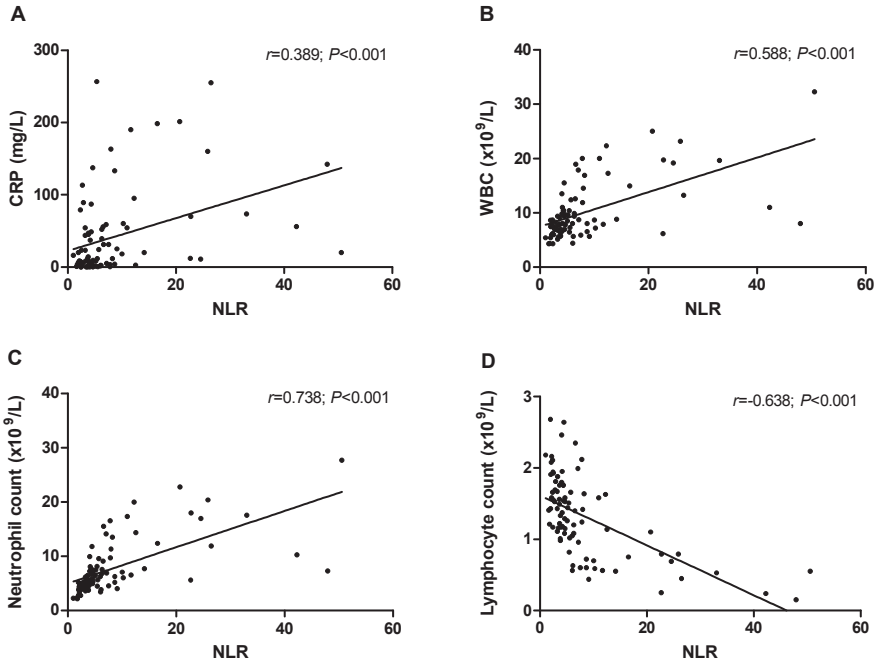

Figure 2. Correlations between the neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP) (**A**), white blood cell (WBC) count (**B**), neutrophil count (**C**) and lymphocyte count (**D**). *r* = Spearman's correlation coefficient.

DISCUSSION

In the present pilot study, we found elevated mean levels of NLR in elderly patients with delirium.

We are aware that no conclusions on causality can be drawn from this observational study; however, our results might suggest that an inadequate response of the immune system and oxidative stress might be both involved in the pathophysiology of delirium.

Activation of the immune system is a prominent feature of many conditions associated with delirium, such as infections and traumas. Several cytokines and inflammatory markers have already been detected in serum and cerebrospinal fluid during delirium [21-24]. In various stressful situations, the physiological response of the immune system is characterized by an increase in neutrophils and a decrease in lymphocytes [25, 26]. Neutrophils play an important role in the first-line defense during inflammation; once activated, they release reactive oxygen species, myeloperoxidase and proteolytic enzymes in an attempt to destroy pathogens or damaged cells [27, 28].

Disruption of the blood-brain barrier (BBB) and brain damage have previously been suggested as possible underlying mechanisms for delirium [29]. It has been found that, at an early stage of systemic inflammation, neutrophils adhere to activated endothelial cells of the BBB, migrate across the BBB, and release reactive oxygen species and proteases, which in turn causes destruction of the endothelial cell alignment [4, 28, 30, 31]. At the same time, several other processes also increase the permeability of the BBB [30]. Disruption of the BBB will lead to enhanced cytokine transport into the brain. These cytokines may activate microglia, which in turn will produce a wide range of inflammatory markers and reactive oxygen species [32]. Production of reactive oxygen species by neutrophils and microglia may lead to oxidative stress and may ultimately result in neuronal damage and apoptosis.

Lymphocytes play an important role in the regulation of an appropriate inflammatory response. Although a decrease in lymphocytes is a normal response during periods of acute stress, a chronic decrease in lymphocytes due to increased levels of catecholamine and cortisol, redistribution of lymphocytes to lymphatic tissue, and accelerated apoptosis might lead to a detrimental inflammatory state and ultimately result in poor clinical outcomes [25, 33, 34].

NLR is the balance between neutrophils and lymphocytes and integrates two components of the immune system in one marker. Since in our study the individual neutrophil and lymphocyte counts were not (or not strongly) associated with delirium in comparison to NLR, it might be suggested that it is the balance between the two that is out of range and not particularly the two individual WBC subtypes. Therefore, an increased NLR might be suggestive for a decreased physiological reserve to respond adequately to an inflammatory insult.

In various pathological conditions, elevated NLR levels have been found to be an independent predictor of disease severity and poor prognosis. Moreover, NLR has been found to be a more powerful predictor of adverse outcomes in comparison with conventional inflammatory markers, such as the total WBC count, the individual WBC subtypes and CRP [13-16]. In the present study, we found no differences in mean levels of CRP, WBC, neutrophils and lymphocytes between patients with and without delirium. Mean levels of NLR showed statistically significant increases in patients with delirium, even after adjustment for inflammatory markers. These findings might suggest an independent role of NLR in delirium above conventional inflammatory markers.

To the best of our knowledge, this is the first study investigating a possible association between NLR and delirium. A few studies have demonstrated that elevated NLR levels are associated with neuropsychiatric disorders such as Alzheimer's disease [8], Parkinson's disease [35] and schizophrenia [6] as well as with cognitive dysfunction after carotid endarterectomy [36]. Limited research has focused on the possible association of neutrophils and lymphocytes with delirium. In line with our study, Watts et al. [37] found no difference in neutrophil counts between patients with and without delirium who were admitted to an intensive care unit. Tanaka [38] found that a decreased lymphocyte count was associated with a combined outcome of perioperative delirium and acute exacerbation of behavioral and psychological symptoms of dementia. Zuliani et al. [39] found that a decreased lymphocyte count was associated with subsyndromal delirium. On the contrary, Inoue et al. [40] did not find an association between the lymphocyte count and the number of days that intensive care unit patients were free of both delirium and coma. In the present study, we found no association between the lymphocyte count and delirium, but the mean lymphocyte count seemed to be lower in patients with delirium than in those without. Of note, three of the four previous studies [38-40] did not have delirium as a clear outcome, which makes it difficult to compare the results.

Limitations and strengths

This study has some limitations. First, as this was a pilot study in a relatively small group of patients, the findings need to be confirmed in a larger study. Moreover, due to the small group of patients with delirium, we were not able to perform stratified analyses for "delirium on admission" and "incident delirium". It might be interesting to evaluate the diagnostic and predictive power of NLR in future studies. Second, the observational design with a single measurement of NLR limits the ability to identify causal associations. Repeated NLR measurements over time are required to provide evidence for a possible role of NLR in the pathogenesis of delirium. Third, it might be speculated that other comorbidities, such as cardiovascular diseases [9-11], diabetes [41], malignancies [12], infection [15], and inflammation [14], might have influenced the mean levels of NLR; however, even after

adjustment for the CCI and other inflammatory markers, estimates remained statistically significant. Fourth, only in a relatively small number of patients neutrophil and lymphocyte counts were measured on admission and this might have introduced some selection bias. Unfortunately, it is unclear why differential WBC counts were measured in these patients. The reason was probably not an increased inflammatory state, since the total WBC counts and CRP levels were lower in these patients than in the patients not included in this pilot study (data not shown).

An important strength is the use of an easily applicable marker, which combines information of several components of the immune system, as it has been suggested that the pathophysiology of delirium is multifactorial.

CONCLUSION

In this pilot study in older, acutely ill hospitalized patients, we found that patients with delirium had higher levels of NLR than patients without delirium. This finding might suggest that an inadequate response of the immune system and oxidative stress may play a role in the pathogenesis of delirium. Larger studies with repeated measurements of NLR over time are needed to confirm the possible role of NLR in the pathogenesis of delirium and to investigate whether NLR can be used as a diagnostic and predictive marker for delirium.

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Chapter 2.4

Unraveling delirium: differences in potential biomarkers between acutely ill medical and elective cardiosurgical patients with delirium

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Submitted

ABSTRACT

Background/Aim

Delirium is a major problem in older hospitalized patients whereas the pathophysiology is poorly understood. Increasing evidence suggests that different pathways may play a role in the pathophysiology depending on the etiological factors of the delirium and 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, i.e. acutely ill medical patients and patients with a delirium after elective cardiac surgery.

Methods

Data from two studies, including acutely ill medical patients aged 65 years and older (the DITO study), and patients aged 70 years and older undergoing elective cardiac surgery (the DECO study), were used. Differences in biomarker levels between these two groups were investigated using univariate analysis of variance 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 biochemical markers in the two groups.

Results

Eighty patients with delirium were included (23 acutely ill medical patients and 57 elective cardiosurgical 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 compared to elective cardiosurgical patients. Multiple linear regression analysis showed that CRP was positively correlated with neopterin in acutely ill medical patients, explaining 28.4% of the variance in neopterin, whereas eGFR was negatively correlated with neopterin in elective cardiosurgical patients, explaining 53.7% of the variance in neopterin.

Conclusion

In this study, we found differences in mean neopterin levels and phenylalanine/tyrosine ratios between acutely ill medical and elective cardiosurgical patients with delirium. Moreover, differences in determinants of neopterin were found between the two groups. Our findings suggest that, in acutely ill medical patients, neopterin levels are mainly determined

by inflammation/oxidative stress whereas in elective cardiosurgical 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 with delirium.

INTRODUCTION

Delirium – an acute neuropsychiatric syndrome characterized by disturbances in attention, awareness and cognition – is a common and severe disorder in elderly 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 (e.g. advanced age and dementia) and precipitating factors (e.g. 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], patients undergoing elective cardiac surgery [10] and acute hip fracture patients undergoing surgery [11]. In all three studies, neopterin levels were increased in patients with delirium compared to patients without delirium. However, when comparing the results of the studies, large differences are observed in mean neopterin levels between the different patient groups. 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* a delirium after cardiac surgery [10] were in the same order as neopterin levels measured in acutely ill medical patients *without* delirium [9]. These observations might suggest 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 study was to investigate potential differences in mean plasma levels of several biochemical parameters, i.e. neopterin, amino acids, amino acid ratios and dopamine's metabolite homovanillic acid (HVA), between acutely ill medical patients with delirium and patients who developed a delirium after elective cardiac surgery.

METHODS

Study populations

In this study, we used data from the Delirium In The Old (DITO) study [9, 12] and the DElirium pathogenesis, Cognition and Outcome (DECO) study [10]. 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, 12].

The DITO study was a cross-sectional study in patients aged 65 years and older who were acutely admitted to the wards of Internal Medicine and Geriatrics of the Erasmus University Medical Center (Erasmus MC) and the ward of Geriatrics of the Harbor 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 cooling, 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.

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 [13]. The diagnosis of delirium was made by a geriatrician, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th 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 absence of a delirium using the MMSE [14].

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 h using the Confusion Assessment Method for the Intensive Care Unit (which follows the criteria for delirium of the DSM-IV) [15] and was based on psychiatric examination of the patient and information in the medical and nursing records. Delirium lasting more than 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 h after admission. When a patient developed delirium during the hospital stay, new blood samples were collected within 24 h 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, nonfasting blood was collected preferably between 8 and 10 a.m. 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 [16], and stored at room temperature to prevent changes in the transfer of amino acids between plasma and blood cells [17]. Within 3 h, the blood was centrifuged for 20 min 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 high-performance liquid chromatography (HPLC) after acid oxidation [18]. Plasma amino acid levels were measured using HPLC with automated pre-column derivatization with *ortho*-phthalaldehyde [17] and plasma HVA levels were determined by reversed-phase HPLC and electrochemical detection, as previously described for the measurement of serotonin [19].

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-days postoperative levels were recorded. The eGFR was determined by the following Modification of Diet in Renal Disease formula: $175 \times [\text{serum creatinine } (\mu\text{mol/l}) \times 0.0113]^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female).

Statistical analysis

Differences in participant characteristics between acutely ill medical and elective cardiosurgical patients were evaluated using the chi-square 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 analysis of variance was used to investigate potential differences in mean levels of biochemical parameters (dependent variable) between acutely ill medical and elective cardiosurgical patients. For this purpose, biochemical parameters with a non-normal distribution 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% confidence intervals (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 groups. Secondly, variables with a p-value ≤ 0.10 were included in a multiple linear regression analysis, 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 in order to calculate the percentage of total variance that was explained by each variable.

Statistical Package for the Social Sciences, version 21.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analyses. A two-tailed $p < 0.05$ was defined as statistically significant. 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, 23 acutely ill medical patients and 57 elective cardiosurgical patients with delirium were included. **Table 1** presents the demographic and clinical characteristics of these patients. Acutely ill medical patients 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 cardiosurgical patients.

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 [e]
Age, years, mean ± SD	85.9 ± 4.0	76.8 ± 3.8	< 0.001 [f]
MMSE score, median (IQR) [a,b]	20.0 (18.0–25.0) [c]	27.0 (24.0–28.0)	< 0.001 [g]
CCI score, median (IQR)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.550 [g]
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) [d]	36.0 (10.0–103.0)	100.0 (60.0–148.0) [b]	0.013 [g]
eGFR, ml/min, mean ± SD [d]	49.4 ± 25.1	48.9 ± 23.2	0.930 [f]

Notes: Values are expressed as mean ± SD for normally distributed continuous variables, median (interquartile range) for not normally distributed continuous variables and n (percentages) for categorical variables. [a] range 0 (severe cognitive impairment) to 30 (no cognitive impairment). [b] DITO: measured in the absence of delirium, DECO: measured on the day before surgery. [c] Four values missing. [d] DITO: measured during delirium (on the day of neopterin sampling), DECO: measured during delirium 2 days after cardiac surgery. [e] Chi-square test. [f] Student's t-test. [g] Mann-Whitney U-test.
Abbreviations: CCI, Charlson Comorbidity Index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MMSE, Mini-Mental State Examination; SD, standard deviation.

Analyses of biochemical parameters

The mean levels and corresponding 95% CIs of the investigated biochemical parameters in acutely ill medical and elective cardiosurgical patients are presented in **tables 2 and 3**. 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 cardiosurgical patients (47.3 nmol/l, 95% CI: 40.1–55.8) (p = 0.001). Furthermore, mean phenylalanine/tyrosine (Phe/Tyr) ratios were significantly higher in acutely ill medical patients (1.39, 95% CI: 1.22–1.58) than in elective cardiosurgical 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 (**tables S1 and S2**, supplementary material).

Table 2. Mean levels of biochemical parameters

	DITO (n = 23)	DECO (n = 57)	p-value
Neopterin, nmol/l	93.1 (69.3–125.3)	47.3 (40.1–55.8)	0.001 [a]
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

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex and Charlson Comorbidity Index. The model including neopterin is adjusted for age, sex, Charlson Comorbidity Index, the estimated glomerular filtration rate and C-reactive protein (CRP). [a] Analysis was performed for 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.

Table 3. Mean levels of amino acid ratios

	DITO (n = 23)	DECO (n = 57)	p-value
Phenylalanine / tyrosine ratio	1.39 (1.22–1.58)	1.15 (1.07–1.24)	0.032
Tryptophan / LNAAs ratio x 100 [a]	5.35 (4.37–6.32)	6.11 (5.59–6.64)	0.228
Tyrosine / LNAAs ratio x 100	11.4 (9.8–13.2)	13.1 (12.1–14.2)	0.152
Phenylalanine / LNAAs ratio x 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
Taurine / (serine x methionine) ratio x 100	1.99 (1.42–2.79)	1.56 (1.30–1.87)	0.273

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex and Charlson Comorbidity Index. [a] Values were not logarithmically transformed.
Abbreviations: LNAAs, large neutral amino acids (tryptophan, phenylalanine, tyrosine, valine, isoleucine and leucine).

Determinants of neopterin

Unadjusted linear regression analysis revealed that neopterin levels were positively correlated with CRP levels in acutely ill medical patients, and negatively correlated with eGFR in elective cardiosurgical patients (**table 4, figure 1**). Trends towards lower neopterin levels

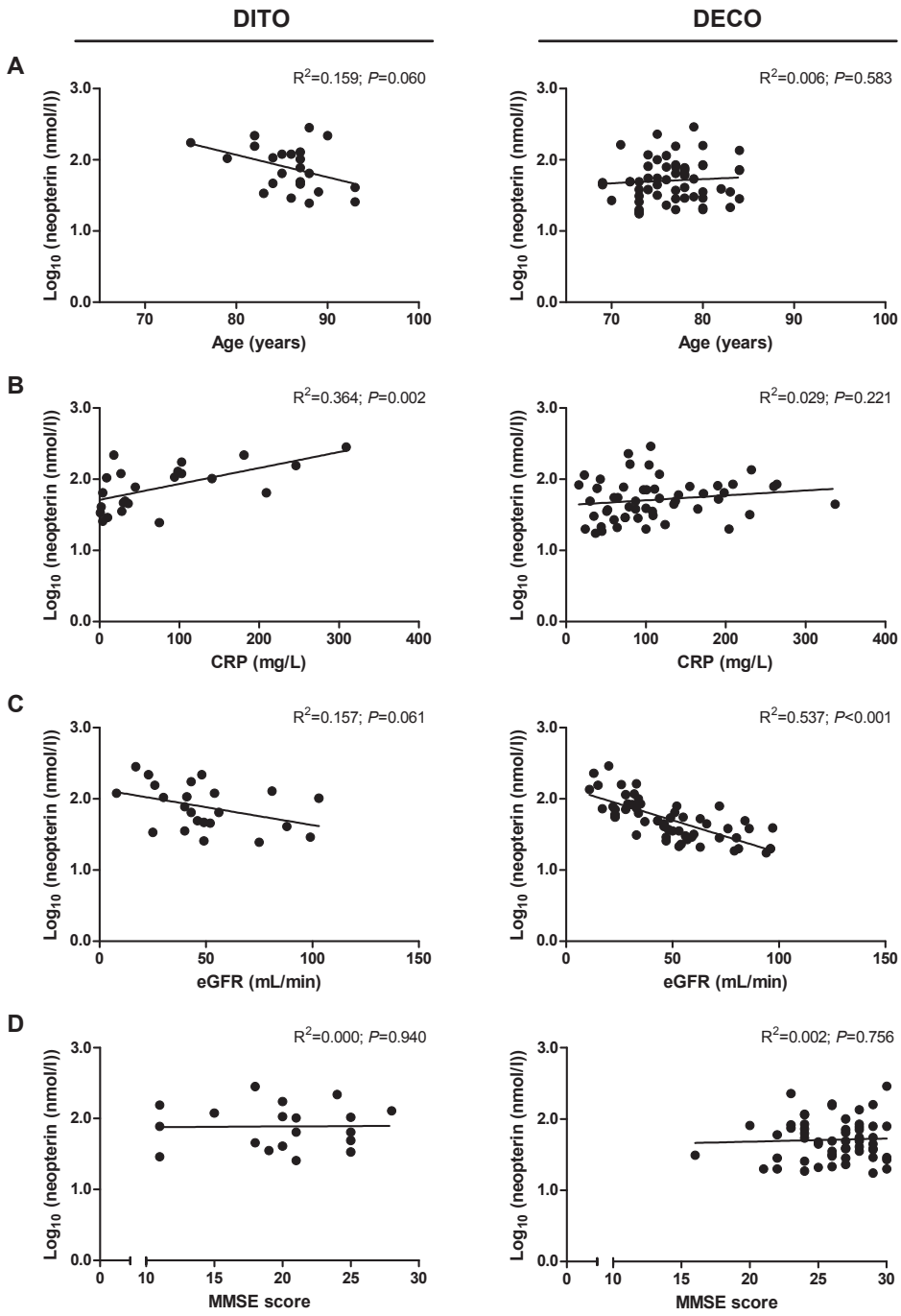

Figure 1. Unadjusted linear regression analyses between neopterin and age (**A**), CRP (**B**) eGFR (**C**) and MMSE score (**D**) in acutely ill medical patients with delirium (DITO) and patients with delirium after elective cardiac surgery (DECO).

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Examination.

were found for increasing age (p = 0.060) and increasing eGFR (p = 0.061) in acutely ill medical patients. No correlation was found between neopterin, CCI and MMSE scores in both groups of patients (p > 0.10).

Table 4. Unadjusted linear regression analyses of potential determinants of neopterin and Phe/Tyr ratio

	Log_{10} (neopterin (nmol/l)) – DITO				Log_{10} (neopterin (nmol/l)) – DECO			
Variable	B	95% CI	p-value	R^2	B	95% CI	p-value	R^2
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) – DITO				Log_{10} (Phe/Tyr ratio) – DECO			
Variable	B	95% CI	p-value	R^2	B	95% CI	p-value	R^2
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; R^2 = squared correlation coefficient.

Abbreviations: CI, confidence interval; CCI, Charlson Comorbidity Index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Examination; Phe, phenylalanine; Tyr, tyrosine.

Multiple linear regression analysis revealed that a model based on age, CRP and eGFR could explain 53.2% of the variance in neopterin in acutely ill medical patients (**table 5**). In this model, CRP levels remained positively correlated with neopterin levels and explained 28.4% of the variance in neopterin. Age and eGFR explained 8.53% and 3.96% of the variance in neopterin respectively, but remained not statistically significant determinants of neopterin. In elective cardiosurgical patients, the same model explained 56.7% of the variance in neopterin (**table 5**). In this model, eGFR remained negatively correlated with neopterin levels and explained 53.7% of the variance in neopterin. Age and CRP explained 0.18% and 2.76% of the variance in neopterin respectively and remained not statistically significant determinants of neopterin.

Table 5. Multiple linear regression analyses of potential determinants of neopterin

	Log_{10} (neopterin (nmol/l)) – DITO					Log_{10} (neopterin (nmol/l)) – DECO				
Variable	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: CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

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 cardiosurgical patients (**table 4**). Also no trends towards 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 cardiosurgical 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, 20] and cardiosurgical patients [21] 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 have found increased neopterin levels in both medical and surgical patients with delirium [9-11], and this suggests a potential role for neopterin 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 cardiosurgical patients with delirium, whereas 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 populations. CRP levels were the strongest determinant of neopterin levels in acutely ill medical patients, explaining 28.4% of the variance in neopterin, whereas the eGFR was the strongest determinant of neopterin, explaining 53.7% of the variance in neopterin, in elective cardiosurgical patients. These findings suggest that, in acutely ill medical patients, neopterin levels are potentially mainly determined by inflammation/oxidative stress, whereas in elective cardiosurgical patients, neopterin levels are mainly driven by renal function/fluid status. In both populations, the model based on age, CRP and eGFR explained approximately half of the variance in neopterin and therefore the remaining variance might be determined by other factors, e.g. the presence of comorbid cognitive disorders, other medical conditions [8] or the use of specific drugs, such as acetylsalicylic acid [22] and statins [23]. Several studies have shown that a decrease in cognitive perfor-

mance is accompanied by an increase in neopterin levels [24, 25]. In the present study, we found that acutely ill medical patients had significantly lower MMSE scores than patients who developed a delirium after elective cardiac surgery. This shows that acutely ill medical patients were cognitively more impaired than the elective cardiosurgical patients. However, the estimates did not change after additional adjustment for MMSE scores and therefore, we can not 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 cardiosurgical patients, although CRP levels were significantly higher in elective cardiosurgical 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 [26]. 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 cardiosurgical patients than in acutely ill medical patients.

Tetrahydrobiopterin (BH_4) functions as an essential cofactor in several enzymatic reactions involved in the production of serotonin and dopamine [27], 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 [18, 27]. An elevated ratio of phenylalanine to tyrosine might suggest a decreased BH_4 availability. In previous delirium studies, controversial results have been reported. In acutely ill medical patients, an increased Phe/Tyr ratio was found in patients with delirium compared to patients without delirium [12], whereas in patients who underwent cardiac surgery, no differences in BH_4 levels and Phe/Tyr ratios were found between patients with and without delirium [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.

Limitations and strengths

This study has some limitations. First, the two studies used in the present study were not developed to compare these with each other. In- 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, we used CRP as indirect marker of inflammation in 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 (e.g. IFN-γ) would probably have given more insights into the results. Third, our findings were obtained in a relatively small group of patients; therefore, the findings need to be confirmed in a larger study.

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 a delirium after elective cardiac surgery. Differences in determinants of neopterin were found between the two groups. Our findings suggest that, in acutely ill medical patients, neopterin levels are mainly determined by inflammation/oxidative stress whereas in elective cardiosurgical 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 cardiosurgical patients.

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

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex, Charlson Comorbidity Index and MMSE score. The model including neopterin is adjusted for age, sex, Charlson Comorbidity Index, the estimated glomerular filtration rate, C-reactive protein (CRP) and MMSE score. [a] Analyses were performed for 19 patients instead of 23 patients due to missing MMSE scores. [b] Analysis was performed for 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.
Abbreviation: MMSE, Mini-Mental State Examination.

Table S2. Mean levels of amino acid ratios after additional adjustment for MMSE score

	DITO (n = 19)	DECO (n = 57)	p-value
Phenylalanine / tyrosine ratio	1.44 (1.21–1.69)	1.14 (1.06–1.23)	0.035
Tryptophan / LNAAs ratio x 100 [a]	5.63 (4.40–6.86)	6.06 (5.51–6.62)	0.576
Tyrosine / LNAAs ratio x 100	10.9 (9.2–13.1)	13.2 (12.2–14.3)	0.104
Phenylalanine / LNAAs ratio x 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
Taurine / (serine x methionine) ratio x 100	2.02 (1.32–3.10)	1.57 (1.30–1.91)	0.352

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values unless otherwise specified. Models are adjusted for age, sex, Charlson Comorbidity Index and MMSE score. [a] Values were not logarithmically transformed.
Abbreviations: LNAAs, large neutral amino acids (tryptophan, phenylalanine, tyrosine, valine, isoleucine and leucine); MMSE, Mini-Mental State Examination.

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3. Pharmacological agents and delirium

Chapter 3.1

Potential influence of aspirin on neopterin and tryptophan levels in patients with a delirium

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ABSTRACT

In an in vitro study, it was found that aspirin might decrease neopterin production and tryptophan degradation. The aim of the present study was to evaluate the possible association between aspirin use and mean neopterin and tryptophan levels in patients with and without a delirium and whether the use of aspirin is associated with a decreased prevalence of delirium. Neopterin and tryptophan levels were determined previously in acutely ill admitted patients aged ≥ 65 years. The possible influence of aspirin on mean levels of neopterin and tryptophan was investigated with univariate analysis of variance in adjusted models. Eighty patients were included; 22 had a delirium. In patients without a delirium (no aspirin (n = 31) versus aspirin (n = 27)), mean neopterin levels were 47.0 nmol/l versus 43.6 nmol/l (p = 0.645) and tryptophan levels were 33.1 µmol/l versus 33.9 µmol/l (p = 0.816). In patients with a delirium (no aspirin (n = 13) versus aspirin (n = 9)), mean neopterin levels were 77.8 nmol/l versus 71.1 nmol/l (p = 0.779) and tryptophan levels were 22.4 µmol/l versus 27.3 µmol/l (p = 0.439). No difference was found in the distribution of aspirin users between patients with and without a delirium. In this study, we found that the use of aspirin had no significant effect on mean levels of neopterin and tryptophan. However, the raw data suggest that there might be a potential influence in patients with a delirium. Aspirin use was not associated with a decreased prevalence of delirium.

INTRODUCTION

Delirium, an acute neuropsychiatric syndrome, is a common, severe complication in the elderly and is associated with poor clinical outcomes including increased morbidity and mortality, prolonged hospital stay, loss of independence, and increased rates of cognitive decline [1, 2]. The pathophysiological mechanisms underlying delirium are still poorly understood, but it is widely accepted that delirium occurs due to a complex interplay among several biochemical pathways. Therefore, it might be required to interrupt in multiple biochemical pathways at the same time to prevent, treat, or to lower the severity of a delirium.

Activation of the immune system, oxidative stress, and disturbances in the serotonergic neurotransmission may all contribute to the development of a delirium. Recently, we found that acutely ill hospitalized elderly patients with a delirium have increased levels of neopterin [3]. Neopterin is produced primarily by activated monocytes and macrophages in response to the pro-inflammatory cytokine interferon-gamma (IFN-γ) and its levels reflect the amount of cell-mediated immune activation and oxidative stress [4, 5]. Furthermore, we have found that patients with a delirium have a decreased availability of tryptophan to the central nervous system. This decreased availability might result in a decreased serotonin production in the brain, since tryptophan is the precursor of serotonin [6].

A study of Schroecksnadel et al. showed that treatment of stimulated peripheral blood mononuclear cells with aspirin significantly decreased neopterin production and tryptophan degradation in vitro [7]. Therefore, the aim of the present study was to evaluate the possible association between aspirin use and mean levels of neopterin and tryptophan in patients with and without a delirium and additionally, whether the use of aspirin is associated with a decreased prevalence of delirium.

METHODS

Participants

The present study was performed within the Delirium In The Old (DITO) study in which mean plasma/serum levels of several biochemical parameters, including neopterin and tryptophan, were compared between patients with and without a delirium [3, 6]. In the DITO study, a cross-sectional study, we included patients who were admitted to the wards of Internal Medicine and Geriatrics of the Erasmus University Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands. All acutely admitted patients aged ≥ 65 years were eligible to participate. 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. Patients with a MMSE < 10 were not included because it can be quite difficult to distinguish between features of severe dementia and delirium at admission, as well as to measure improvement of cognitive function in this group. Additional exclusion criteria for the present study were unclear data regarding the use of non-steroidal anti-inflammatory drugs (NSAIDs) in the days preceding hospital admission as well as the use of aspirin concomitantly with other NSAIDs (as it might be possible that other NSAIDs interfere with aspirin's potential effect on neopterin and tryptophan levels).

Written informed consent was obtained from all participants. In case of a delirium or cognitive impairment at the time of admission, informed consent was obtained from a representative of the patient. The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocol.

Procedures

All participants were observed daily by the nursing and medical staff and by members of the research team until discharge. To screen for a change in behavior, the 13-item Delirium Observation Screening scale was used during the first five days of admission [8]. The diagnosis of delirium was made by a geriatrician, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [9], and was based on the psychiatric examination of the patient, the medical and nursing records, including the Delirium Observation Screening scale scores, and information given by the patient's closest relative. When the diagnosis of delirium was doubtful, the case was discussed with the geriatric consultation team to gain consensus.

Demographic and clinical data were collected at admission. Age and sex were documented. Cognitive functioning was assessed in absence of a delirium using the MMSE [10]. 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 ≥ 10, the patient was not excluded from the study. Severity of comorbidities was scored using the Charlson Comorbidity Index. This index encompasses 19 medical conditions and each condition is weighted with a score of 1 to 6 by severity [11]. The physical functionality was assessed using the six-item Katz Activities of Daily Living (ADL) scale and the Barthel Index [12, 13]. The instrumental functionality was assessed using the 7-items Older Americans Resource Scale for Instrumental ADL (OARS-IADL) [12]. Frailty was measured with the Identification of Seniors at Risk (ISAR) questionnaire [14]. For all participants the medication at hospital admission was reviewed for the use of NSAIDs (including low dose acetylsalicylic acid and the equivalent drug carbasalate calcium), beta-

blockers, diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, calcium channel blockers, nitrates, statins, and dipyridamole.

Blood samples of all patients were collected within 48 h after admission. When a patient developed a delirium during the hospital stay, new blood samples were collected within 24 h after the onset of the delirium and were used, instead of the first blood samples, for the statistical analyses.

Biochemical measurements

Nonfasting blood was collected preferably between 8 and 10 a.m. 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 [15], and stored at room temperature to prevent changes in the transfer of amino acids between plasma and blood cells [16]. Within 3 h, the blood was centrifuged for 20 min at 2,650 *g* and 20 °C. The obtained plasma was stored at –80 °C until analysis.

Plasma neopterin levels were determined by high-performance liquid chromatography after acid oxidation, as previously described [17]. Tryptophan levels were determined by high-performance liquid chromatography with automated pre-column derivatization with *ortho*-phthalaldehyde [16].

Statistical analyses

Depending on the distribution of the data, differences in demographic and clinical baseline characteristics between patients with and without a delirium were evaluated using the chi-square test or the Fisher's exact test for categorical variables and the Mann–Whitney U-test or the Student's t-test for continuous variables.

Levels of neopterin and tryptophan were not normally distributed and were, therefore, logarithmically transformed. Univariate one-way analysis of variance was used to investigate the association between mean levels of neopterin and tryptophan (dependent variable) and the use of aspirin in both patients with and without a delirium. For this purpose, analyses were stratified for aspirin use. Age, sex, Charlson Comorbidity Index and statin use were used as covariates. The Charlson Comorbidity Index was added since neopterin levels are found to be increased and tryptophan levels decreased in several medical conditions. Statin use was added since statins might also inhibit neopterin production and tryptophan degradation [18]. The model including neopterin was additionally adjusted for eGFR, since neopterin is excreted mainly by the kidneys [5]. All mean levels and 95% confidence intervals (CI) of neopterin and tryptophan presented in this manuscript are the back-transformed log-values. A two-tailed $p < 0.05$ was defined as statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences

(SPSS), version 21.0 (IBM Corp., Armonk, NY, USA). GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA) was used to draw the graphs.

RESULTS

Participant characteristics

Of the 86 patients enrolled in the DITO study, 80 were included in the stratified analyses to examine the effect of aspirin use on neopterin and tryptophan levels. Three patients were excluded due to unclear data regarding the use of NSAIDs in the days preceding hospital admission, 1 patient used diclofenac and 2 patients used carbasalate calcium concomitantly with another NSAID (diclofenac and etoricoxib respectively). **Table 1** represents the baseline characteristics of the included patients. Twenty-two patients were diagnosed with a delirium, of which 21 were admitted to the hospital with a delirium and 1 developed a delirium during admission. No difference was found in the number of aspirin users between patients with and without a delirium (46.6% versus 40.9%, p = 0.651, respectively).

Analysis of biochemical parameters

Mean levels and corresponding 95% CI of neopterin and tryptophan in patients with and without a delirium, stratified for the use of aspirin, are presented in **tables 2 and 3**.

In the group without a delirium, no significant difference was found in the adjusted mean levels of neopterin between patients who used aspirin (43.6 nmol/l, 95% CI: 34.5–55.0) and patients who did not use aspirin (47.0 nmol/l, 95% CI: 37.8–58.3) (p = 0.645). Also no difference was found in the adjusted mean levels of tryptophan between patients who used aspirin (33.9 µmol/l, 95% CI: 29.6–38.8) and patients who did not use aspirin (33.1 µmol/l, 95% CI: 29.2–37.6) (p = 0.816).

In the group with a delirium, unadjusted levels of neopterin seemed to be lower in patients who used aspirin than in those who did not, as shown in **figure 1**. However, in this small group the adjusted mean levels of neopterin were not statistically significantly lower in patients who used aspirin (71.1 nmol/l, 95% CI: 43.8–115.6) than in patients who did not use aspirin (77.8 nmol/l, 95% CI: 52.4–115.6) (p = 0.779). In addition, unadjusted levels of tryptophan seemed to be higher in patients who used aspirin than in those who did not (**figure 1**). However, the adjusted mean levels of tryptophan were not statistically significantly higher in patients who used aspirin (27.3 µmol/l, 95% CI: 18.4–40.5) than in those who did not (22.4 µmol/l, 95% CI: 16.2–30.9) (p = 0.439).

Table 1. Demographic and clinical baseline characteristics of the study participants

Variable	No delirium (n = 58)	Delirium (n = 22)	p-value
Male	28 (48.3)	9 (40.9)	0.555 [a]
Age in years	80.4 ± 7.5	85.8 ± 4.1	0.002 [c]
MMSE score [e]	25.0 (22.0–28.0)	20.0 (17.3–24.3)	0.000 [b]
Katz ADL score [f]	0.0 (0.0–3.0)	3.5 (1.0–11.3)	0.013 [b]
OARS-IADL score [g]	5.0 (0.0–10.0)	10.0 (3.0–14.0)	0.037 [b]
Barthel Index [h]	18.0 (13.0–20.0)	16.0 (9.0–19.0)	0.050 [b]
ISAR score [i]	4.0 (2.5–6.0)	6.0 (5.0–7.0)	0.000 [b]
Charlson Comorbidity Index [j]	2.00 (1.00–3.00)	2.00 (1.00–3.25)	0.202 [b]
eGFR (ml/min)	64.3 ± 25.3	48.0 ± 24.7	0.011 [c]
Aspirin at admission	27 (46.6)	9 (40.9)	0.651 [a]
Type of aspirin:			
Acetylsalicylic acid	12 (44.4)	5 (55.6)	
Carbasalate calcium	15 (55.6)	4 (44.4)	
Beta-blockers	17 (29.3)	6 (27.3)	0.857 [a]
Diuretics	22 (37.9)	7 (31.8)	0.612 [a]
ACE inhibitors	14 (24.1)	6 (27.3)	0.772 [a]
Angiotensin II receptor antagonists	8 (13.8)	2 (9.1)	0.719 [d]
Calcium channel blockers	13 (22.4)	3 (13.6)	0.536 [d]
Nitrates	5 (8.6)	1 (4.5)	1.000 [d]
Statins	27 (46.6)	3 (13.6)	0.007 [a]
Dipyridamole	5 (8.6)	0 (0.0)	0.315 [d]

Notes: Values are expressed as mean ± SD for normally distributed continuous variables, median (interquartile range) for not normally distributed continuous variables and n (percentages) for categorical variables. [a] Chi-square test. [b] Mann-Whitney U-test. [c] Student's t-test. [d] Fisher's exact test. [e] range 0 (severe cognitive impairment) to 30 (no cognitive impairment). [f] range 0 (no disability) to 12 (severe disability). [g] range 0 (no disability) to 14 (severe disability). [h] range 0 (severe disability) to 20 (no disability). [i] scores ≥ 2 indicate a high risk for functional decline. [j] range 0 to 37 (severe burden of comorbidities).
Abbreviations: ACE, angiotensin converting enzyme; ADL, Activities of Daily Living; eGFR, estimated glomerular filtration rate; ISAR, Identification of Seniors at Risk; MMSE, Mini-Mental State Examination; OARS-IADL, Older Americans Resource Scale for Instrumental Activities of Daily Living.

Table 2. Neopterin levels (nmol/l) in patients with and without a delirium, stratified for the use of aspirin

No delirium	No aspirin (n = 31)	Aspirin (n = 27)	p-value
Model 1	45.8 (36.2–57.9)	44.9 (34.9–57.8)	0.908
Model 2	47.0 (37.8–58.3)	43.6 (34.5–55.0)	0.645
Delirium	No aspirin (n = 13)	Aspirin (n = 9)	p-value
Model 1	88.3 (57.7–135.2)	59.3 (35.6–98.9)	0.228
Model 2	77.8 (52.4–115.6)	71.1 (43.8–115.6)	0.779

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values. Model 1: not adjusted. Model 2: adjusted for age, sex, Charlson Comorbidity Index, statin use and the estimated glomerular filtration rate.

Table 3. Tryptophan levels (µmol/l) in patients with and without a delirium, stratified for the use of aspirin

No delirium	No aspirin (n = 31)	Aspirin (n = 27)	p-value
Model 1	33.8 (29.6–38.6)	33.1 (28.7–38.2)	0.835
Model 2	33.1 (29.2–37.6)	33.9 (29.6–38.8)	0.816
Delirium	No aspirin (n = 13)	Aspirin (n = 9)	p-value
Model 1	21.6 (15.4–30.3)	28.8 (19.2–43.2)	0.269
Model 2	22.4 (16.2–30.9)	27.3 (18.4–40.5)	0.439

Notes: Values are expressed as mean (95% confidence interval) and are the back-transformed $\log_{10}$ values. Model 1: not adjusted. Model 2: adjusted for age, sex, Charlson Comorbidity Index and statin use.

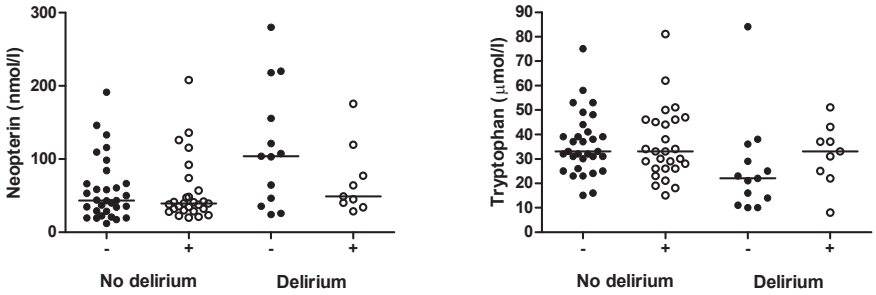

Figure 1. Unadjusted levels of neopterin and tryptophan in patients with and without delirium who used (+) or did not use (-) aspirin. Lines are medians.

DISCUSSION

In the present study, we found that the use of aspirin (exclusively low dose aspirin) was not associated with a decreased prevalence of delirium. Furthermore, we found that mean neopterin and tryptophan levels were not statistically significant affected by the use of aspirin in patients with and without a delirium.

As far as we are aware, this is the first study investigating the possible association between aspirin use and mean neopterin and tryptophan levels in patients with and without a delirium. We found that neopterin and tryptophan levels were not statistically significant affected by the use of aspirin. Therefore, we could not confirm the relationship found in vitro by Schroecksnadel et al. that aspirin decreases neopterin production as well as tryptophan degradation. These controversial findings may be caused by several factors. First, Schroecksnadel et al. found that the inhibition of neopterin production and tryptophan degradation by aspirin was dose-dependent [7]. In our study, all aspirin users used acetylsalicylic acid or carbasalate calcium in a low dose within cardiovascular risk management (80 and 100 mg a day, respectively). It might be possible that these dosages are too low for having a significant effect on neopterin and tryptophan levels. Another possibility is that

we did not find an association due to the small sample size. However, in the group with a delirium, neopterin levels seemed to be lower and tryptophan levels seemed to be higher in patients who used aspirin than in those who did not. Therefore, it might be possible that in a larger group these differences would become statistically significant. On the other hand, this trend was not seen in the group without a delirium. Schroecksnadel et al. found that aspirin did not influence tryptophan degradation and only minimally affected neopterin production in resting cells [7]. It might be possible that in patients without a delirium neopterin production and tryptophan degradation was not stimulated enough and that we, therefore, did not see a trend in this group.

The potential influence of aspirin on neopterin production and tryptophan degradation in patients with a delirium might be the result of a modulating effect of aspirin on the cytokine IFN-γ. Both the production of neopterin as well as the degradation of tryptophan is IFN-γ dependent. During immune activation, IFN-γ induces in macrophages the enzyme guanosine triphosphate cyclohydrolase-I, which is among others responsible for the production of neopterin [4, 5]. IFN-γ also induces the enzyme indoleamine-2,3-dioxygenase which converts tryptophan to kynurenine [19]. In a previous study performed in a chimeric mouse model of giant cell arteritis, aspirin has been demonstrated to be highly effective in suppressing IFN-γ production at doses of 20–100 mg/kg [20]. However, the doses used in that study were much higher than the dose used by our participants and therefore it might be expected that the findings are only generalizable to a lesser extent to the dose used in our study. Interestingly, they also found that another NSAID, indomethacin, was not able to reduce IFN-γ transcription [20]. This might suggest that NSAIDs which are structurally unrelated to aspirin are not able to affect neopterin and tryptophan levels. In line with this hypothesis, Forrest et al. found in patients with osteoporosis after two years of drug treatment that additional pain treatment with a NSAID did not decrease neopterin levels and did not increase tryptophan levels in comparison with patients who did not use NSAIDs [21]. The authors note that patients taking NSAIDs might be among the more severely affected patients in whom disease control could be difficult and this could have influenced their results [21].

Furthermore, we found that the use of aspirin was neither associated with a decreased nor with an increased prevalence of delirium, despite it has been speculated that NSAIDs increase the risk of a delirium. In a systematic review, it was found that research on the association of NSAIDs with delirium is limited and that the association remains uncertain [22]. It is important to note that in the present study the association between the use of low dose aspirin and delirium was evaluated. Therefore, it might be still possible that other NSAIDs are associated with an increased risk of a delirium.

Limitations and strengths

This study has some limitations. First, the cross-sectional design limits the ability to identify a causal relationship between aspirin use, neopterin and tryptophan levels, and the prevalence of delirium. Therefore, the results of this study should be considered as hypothesis generating. Second, the relatively small sample size decreased the ability to detect a possible association between aspirin use and mean neopterin and tryptophan levels in patients with a delirium. Third, delirium severity was not scored in our study. It might be possible that the use of aspirin does not prevent delirium, but that it will decrease delirium severity (as aspirin really inhibits neopterin production and tryptophan degradation as it seems). Fourth, tryptophan levels could be influenced by dietary intake. In this study, we were not able to adjust for dietary status and this might have influenced our results. However, since possible food intake was random and blood was collected between 8 and 10 a.m., we think that our results are only minimally influenced by this. Finally, we were not able to evaluate whether the use of NSAIDs other than aspirin will have a potential influence on neopterin and tryptophan levels. Since other NSAIDs, used to treat inflammation and pain, are only limited prescribed to elderly patients due to their negative effects on renal function, it would probably only be interesting to investigate this for diseases in a younger population in which neopterin and tryptophan are also involved and not for delirium in elderly patients.

The present study has several strengths. First, the intensive monitoring of clinical symptoms of patients with a delirium until discharge and the DSM-IV diagnosis by a geriatrician makes it less likely that we missed a delirium or misdiagnosed symptoms. Second, we have performed statistical analyses in a relatively homogeneous group of patients, since all of them used low dose aspirin.

CONCLUSION

In this study in older, acutely ill hospitalized patients, we did not find a statistically significant effect of aspirin use on neopterin and tryptophan levels in patients with and without a delirium. However, in patients with a delirium, neopterin levels seemed to be lower and tryptophan levels seemed to be higher in patients who used aspirin compared with those who did not. Larger studies might be needed to investigate this potential influence of aspirin use on neopterin production and tryptophan degradation in patients with a delirium.

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Chapter 3.2

Anticholinergic drug exposure is associated with delirium and postdischarge institutionalization in acutely ill hospitalized older patients

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ABSTRACT

Several studies investigated the possible association between anticholinergic drugs and diverse clinical outcomes in older persons, but the results are inconsistent. The aim of this study was to investigate whether anticholinergic drug exposure is associated with delirium on admission, length of hospital stay, postdischarge institutionalization and in-hospital mortality in acutely ill hospitalized older patients. In this observational chart review study, we included acutely ill patients aged 65 and older who were admitted to the geriatric ward of the Erasmus University Medical Center, Rotterdam, the Netherlands, between 2012 and 2015 (n = 905). Anticholinergic drug exposure on admission was defined as the use of anticholinergic drugs, total number of anticholinergic drugs and anticholinergic drug burden score (ADB), quantified with the Anticholinergic Risk Scale (ARS), the Anticholinergic Cognitive Burden scale (ACB) and the list of Chew et al. (Chew). Logistic regression analyses were performed to investigate possible associations between anticholinergic drug exposure and the aforementioned outcomes. Analyses were adjusted for age, sex, comorbidities, non-anticholinergic drugs and delirium, where appropriate. Moderate and high ADB measured with the ARS were associated with delirium on admission with odds ratios (OR) of 1.70 (95% confidence interval (CI): 1.16–2.49) and 1.83 (95% CI: 1.06–3.15), respectively. High ADB measured with the ARS was also associated with postdischarge institutionalization (OR = 2.43, 95% CI: 1.24–4.75). No associations were found using the ACB and Chew. Future studies are warranted to investigate the clinical usefulness of the ARS in reducing complications in older persons.

INTRODUCTION

Drugs with anticholinergic properties are commonly prescribed in older persons [1]. These drugs are associated with a wide spectrum of adverse effects including dizziness, blurred vision, urinary retention, constipation, confusion and possibly also delirium [1]. Older persons are more susceptible to those adverse effects due to an age-related increase in blood-brain barrier permeability, a reduction in hepatic and renal clearance and a decrease in cholinergic neurons and receptors [1-3].

It has been hypothesized that adverse effects of anticholinergic drugs can restrict older persons in performing daily activities and lead to hospitalizations, longer length of hospital stay (LOS) and even death (e.g., due to falls). Additionally, a decline in the ability to perform daily activities may increase the need for institutionalization in older persons [4]. Several studies have investigated the possible association between anticholinergic drugs and delirium, LOS, physical function and mortality, but the results are inconsistent [5-9]. This discrepancy might be caused by the methods used to assess anticholinergic drug use, which differ substantially between studies [6]. In some studies, anticholinergic drug use is assessed with crude measures such as 'exposed or not exposed' and the total number of anticholinergic drugs taken by a person, whereas in other studies the specific anticholinergic load of the different drugs is taken into account. However, little is known about potential differences in results between these methods and whether the results can be compared. To the best of our knowledge, no previous study has investigated the association between anticholinergic drugs and postdischarge institutionalization in acutely ill older patients.

The aim of the study was to investigate whether anticholinergic drug exposure on admission quantified according to three anticholinergic drug scales is associated with delirium on admission, LOS, postdischarge institutionalization and in-hospital mortality in acutely ill older patients admitted to a geriatric ward.

MATERIALS AND METHODS

In this observational chart review study, we included acutely ill patients aged 65 and older who were admitted to the ward of geriatrics of the Erasmus University Medical Center, Rotterdam, the Netherlands, between 1 January 2012 and 31 December 2015. Patients were excluded if they were hospitalized for less than 3 days or if data regarding drug use or outcome measures were not available. Individual persons could be included more than once as patient in the study. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Demographic and clinical variables

All data were collected from the medical records and included age, sex, the estimated glomerular filtration rate (eGFR) on admission, drug use at the time of admission and the severity of comorbidities calculated with the Charlson Comorbidity Index (CCI) [10]. The CCI encompasses 19 medical conditions weighted with a score of 1–6, with total scores ranging from 0 to 37, with higher scores indicating a more severe burden of comorbidities. Data collected to determine outcome measures were: delirium status during the hospital stay, dates of admission and discharge, place of residence before and after discharge and in-hospital mortality.

Anticholinergic drug exposure

Dispensing records from the community pharmacy were preferentially used for recording all drugs in use by a patient at the time of admission. If this information was not available, we used correspondence letters of general practitioners or other referrers, or the medication history taken in the hospital. This information was additionally combined with patients' self-reports on over-the-counter drugs (reported in the medical record). When a drug was stopped 1 or more days prior to admission, we assessed whether there was a possibility that the drug was still present in a patient's body at the time of admission by calculating a time window of 5x the elimination half-life of the drug.

Several anticholinergic drug scales have been developed previously that classify drugs according to their anticholinergic activity into four or five categories, ranging from no anticholinergic activity (score 0) to strong anticholinergic activity (score 3 or 4) [11]. Three of them, the Anticholinergic Risk Scale (ARS) [12], the updated version of the Anticholinergic Cognitive Burden (ACB) scale [13] and the list of Chew et al. [14] (hereafter called Chew), were used in the present study. Shortly, on the ARS, drugs are ranked based on their potential to cause central and peripheral anticholinergic adverse effects (score range: 0–3). Drugs assigned a score of 1 have a moderate anticholinergic potential and drugs with scores 2 and 3 have a strong and very strong potential, respectively. On the ACB, drugs are ranked based on their potential to have a negative effect on cognition (score range: 0–3). Drugs with a score of 1 are those with serum anticholinergic activity or in vitro affinity to muscarinic receptors, but without known clinically relevant cognitive effects. Drugs with established and clinically relevant cognitive anticholinergic effects were assigned a score of 2 or 3 [13, 15]. On the Chew, drugs are ranked based on in vitro serum anticholinergic activity measurements (score range: 0–3). Drugs with a score of 0.5 have an estimated anticholinergic activity of 0 at therapeutic doses, but may demonstrate some anticholinergic activity at higher doses. Drugs with a score of 1–3 demonstrate low to high anticholinergic activity across the therapeutic range [14].

In the present study, anticholinergic drug exposure on admission was defined as the use of drugs with anticholinergic properties, total number of anticholinergic drugs and total anticholinergic drug burden score (ADB), all quantified with the three anticholinergic drug scales. The ADB is the sum of scores assigned to each drug a patient is taking.

Outcome measures

The outcomes of interest were delirium on admission, LOS, postdischarge institutionalization and in-hospital mortality. On the ward of geriatrics, the diagnosis of delirium is made by geriatricians as part of daily clinical practice, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th and 5th edition [16, 17] and is based on daily psychiatric examination, medical and nursing notes, the Delirium Observation Screening scale scores, and information given by the patient's closest relative. In this study, reported diagnoses of delirium were extracted from the medical records. Delirium was defined as "present on admission" if the diagnosis was made within the first 2 days of the hospital stay. All other patients were considered as not having delirium on admission.

LOS was defined as the number of days a patient was hospitalized, with the first day of admission as day one. Patients who died during the hospital stay were not included in analyses of LOS.

Postdischarge institutionalization was defined as discharge to an institutional care facility rather than discharge to home. Patients who resided in an institutional care facility before admission and patients who died during the hospital stay were not included in analyses regarding postdischarge institutionalization.

In-hospital mortality was recorded; all patients were included in the analyses.

Statistical analyses

Differences in characteristics between patients with and without delirium on admission were compared using the Chi-square test for categorical variables, the Mann–Whitney U-test for non-normally distributed continuous variables and the Student's t-test for normally distributed continuous variables.

Logistic regression analysis was performed to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI) for delirium on admission, LOS, postdischarge institutionalization and in-hospital mortality (dependent variables) according to different measures of anticholinergic drug exposure (exposure no/yes, total number of anticholinergic drugs and categories of ADB quantified with the ARS, the ACB and the Chew). LOS was divided into two groups based on the median value found in the overall sample (8.0 days). Number of anticholinergic drugs was treated as a continuous variable. ADB was divided into three categories: no ADB (for all scales score 0), moderate ADB (ARS and ACB

score 1–2; Chew score 0.5–1.0) and high ADB (ARS and ACB score ≥ 3; Chew score ≥ 1.5); the first category was used as reference. All analyses were adjusted for age, sex, CCI and number of non-anticholinergic drugs. Analyses of LOS, postdischarge institutionalization and in-hospital mortality were additionally adjusted for delirium at any time during the hospital stay. Subsequently, analyses of LOS, postdischarge institutionalization and in-hospital mortality were repeated in the group of patients with delirium on admission. Considering the suggested cholinergic deficiency in delirium [18] and the high prevalence of prolonged LOS, postdischarge institutionalization and in-hospital mortality in patients with delirium [19, 20], we hypothesized that the effect of anticholinergic drug exposure on aforementioned outcomes would be different in acutely ill older patients with delirium. LOS was divided into two groups based on the median value found in this group (10.0 days). Analyses were adjusted for age, sex, CCI and number of non-anticholinergic drugs.

Repeated measures logistic regression models were fitted for all outcome measures using the Generalized Estimating Equations (GEE) method, to examine the effect of multiple inclusions per individual on the calculated estimates. Models were adjusted for the same covariates as the main analyses.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 21.0 (IBM Corp., Armonk, NY). Results were considered statistically significant at a $p < 0.05$.

RESULTS

A total of 1193 patients were admitted during the study period, of which 165 did not meet the inclusion criteria. Of the remaining 1028 patients, 123 were excluded: 119 were hospitalized for less than 3 days and four had unclear data regarding drug use or outcome measures. In total, 905 patients were included in the study; 215 of them (23.8%) had delirium on admission (**figure 1**). No statistically significant differences were found in sex distribution (men: 48.3% versus 41.5%, $p = 0.155$) and mean age (81.0 ± 7.0 versus 81.0 ± 7.5, $p = 0.966$) between patients who were included and those who were not. Baseline and discharge characteristics of the included patients are outlined in **table 1**. The frequency distributions of the total number of anticholinergic drugs and the ADB scores among patients are presented in **figures S1 and S2** of the Supplementary material.

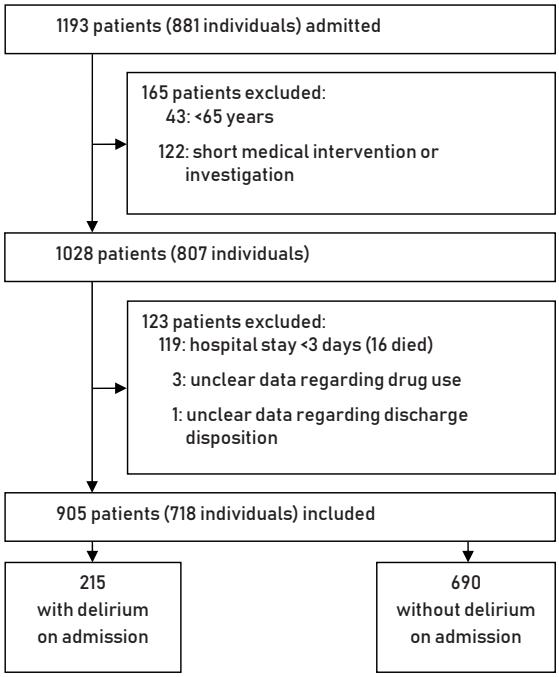

Figure 1. Flow chart of study sample selection.

Delirium and length of stay

Table 2 presents the ORs and corresponding 95% CIs for delirium on admission and LOS ≥ 9 days according to different measures of anticholinergic drug exposure. After adjustment for age, sex, CCI and number of non-anticholinergic drugs, we found that exposure to anticholinergic drugs according to the ARS was associated with an increased odds of having delirium on admission (OR = 1.73, 95% CI: 1.23–2.45). Each additional anticholinergic drug used by a patient was associated with a 38% increase in odds of having delirium on admission (OR = 1.38, 95% CI: 1.10–1.73). Both moderate and high ADB measured with the ARS were associated with an increased odds of having delirium on admission when compared to no ADB (OR = 1.70, 95% CI: 1.16–2.49 and OR = 1.83, 95% CI: 1.06–3.15, respectively). No associations were found between anticholinergic drug exposure quantified with the ACB and the Chew, and delirium.

After adjustment for age, sex, CCI, number of non-anticholinergic drugs and delirium at any time during the hospital stay, no associations were found between anticholinergic drug exposure and LOS.

Table 1. Baseline and discharge characteristics of the overall study sample and stratified for delirium on admission.

Characteristic	Overall sample (n = 905)	No delirium (n = 690)	Delirium (n = 215)	p-value [a]
Male, n (%)	437 (48.3)	316 (45.8)	121 (56.3)	0.007 [b]
Age, years, mean ± SD	81.0 ± 7.03	80.7 ± 7.1	81.9 ± 6.7	0.022 [c]
Place of residence before admission, n (%)				0.035 [b]
Home (with or without home care)	696 (76.9)	542 (78.6)	154 (71.6)	
Institutional care facility	209 (23.1)	148 (21.4)	61 (28.4)	
First time on the ward of geriatrics, n (%)	580 (64.1)	426 (61.7)	154 (71.6)	0.008 [b]
CCI, median (IQR)	2.0 (1.0–4.0)	2.0 (1.0–4.0)	3.0 (1.0–4.0)	0.678 [d]
eGFR, ml/min, median (IQR)	54.0 (35.0–75.0)	54.0 (35.0–75.0)	53.0 (36.0–74.0)	0.918 [d]
Number of drugs, median (IQR)	8.0 (6.0–12.0)	8.0 (6.0–12.0)	8.0 (6.0–12.0)	0.963 [d]
Use of at least one DAP, n (%)				
ARS	256 (28.3)	180 (26.1)	76 (35.3)	0.009 [b]
ACB	644 (71.2)	488 (70.7)	156 (72.6)	0.605 [b]
Chew	523 (57.8)	399 (57.8)	124 (57.7)	0.969 [b]
Number of DAPs, median (IQR)				
ARS	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.011 [d]
ACB	1.0 (0.0–2.0)	1.0 (0.0–2.0)	1.0 (0.0–2.0)	0.383 [d]
Chew	1.0 (0.0–1.0)	1.0 (0.0–1.0)	1.0 (0.0–1.0)	0.585 [d]
ADB score, median (IQR)				
ARS	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.015 [d]
ACB	1.0 (0.0–3.0)	1.0 (0.0–3.0)	1.0 (0.0–3.0)	0.118 [d]
Chew	0.5 (0.0–1.0)	0.5 (0.0–1.0)	0.5 (0.0–1.0)	0.474 [d]
Delirium developed during the hospital stay, n (%)	45 (5.0)	45 (6.5)	n/a	n/a
Place of residence after discharge, n (%)				< 0.001 [b]
Home (with or without home care)	448 (49.5)	389 (56.4)	59 (27.4)	
Institutional care facility	392 (43.3)	260 (37.7)	132 (61.4)	
In-hospital mortality, n (%)	65 (7.2)	41 (5.9)	24 (11.2)	0.010 [b]
Length of stay, days, median (IQR)	8.0 (5.0–11.0)	7.0 (5.0–11.0)	10.0 (7.0–14.0)	< 0.001 [d]

Notes: [a] No Delirium versus Delirium. [b] Chi-square test. [c] Student's t-test. [d] Mann-Whitney U-test.
Abbreviations: ACB, Anticholinergic Cognitive Burden scale; ADB, Anticholinergic Drug Burden; ARS, Anticholinergic Risk Scale; CCI, Charlson Comorbidity Index (range 0-37); DAP, drug with anticholinergic properties; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SD, standard deviation.

Table 2. Odds ratios for delirium on admission and prolonged length of hospital stay according to different measures of anticholinergic drug exposure.

	Delirium on admission		LOS ≥ 9 days	
Variable	Delirium/no delirium	OR (95% CI) [a]	LOS ≥ 9/LOS < 9	OR (95% CI) [b,c]
ARS				
Exposure to DAPs				
No	139/510	1.00 (ref)	274/330	1.00 (ref)
Yes	76/180	**1.73 (1.23–2.45)**	114/122	1.06 (0.77–1.48)
Number of DAPs				
Per drug	215/690	**1.38 (1.10–1.73)**	388/452	0.99 (0.79–1.24)
ADB score				
0	139/510	1.00 (ref)	274/330	1.00 (ref)
1-2	52/121	**1.70 (1.16–2.49)**	75/84	1.00 (0.69–1.45)
≥3	24/59	**1.83 (1.06–3.15)**	39/38	1.23 (0.73–2.07)
ACB				
Exposure to DAPs				
No	59/202	1.00 (ref)	113/133	1.00 (ref)
Yes	156/488	1.10 (0.77–1.59)	275/319	0.99 (0.71–1.38)
Number of DAPs				
Per drug	215/690	1.07 (0.94–1.23)	388/452	0.93 (0.82–1.06)
ADB score				
0	59/202	1.00 (ref)	113/133	1.00 (ref)
1-2	91/311	0.99 (0.67–1.46)	170/199	0.98 (0.70–1.39)
≥3	65/177	1.39 (0.89–2.18)	105/120	0.99 (0.66–1.51)
Chew				
Exposure to DAPs				
No	91/291	1.00 (ref)	161/193	1.00 (ref)
Yes	124/399	1.09 (0.78–1.51)	227/259	1.10 (0.81–1.49)
Number of DAPs				
Per drug	215/690	1.11 (0.94–1.31)	388/452	0.95 (0.82–1.12)
ADB score				
0	91/291	1.00 (ref)	161/193	1.00 (ref)
0.5-1	82/285	1.00 (0.71–1.43)	161/182	1.11 (0.81–1.54)
≥1.5	42/114	1.34 (0.85–2.11)	66/77	1.05 (0.69–1.62)

Notes: Values in bold are statistically significant ($p < 0.05$). [a] Model adjusted for age, sex, Charlson Comorbidity Index and non-anticholinergic drugs. [b] Model adjusted for age, sex, Charlson Comorbidity Index, non-anticholinergic drugs and delirium at any time during the hospital stay. [c] Patients who died during the hospital stay were excluded.

Abbreviations: ACB, Anticholinergic Cognitive Burden scale; ADB, Anticholinergic Drug Burden; ARS, Anticholinergic Risk Scale; CI, confidence interval; DAPs, drugs with anticholinergic properties; LOS, length of hospital stay; OR, odds ratio.

GEE logistic regression models for delirium on admission and LOS showed that the inclusion of individuals multiple times did not affect the estimates (data not shown).

Postdischarge institutionalization and in-hospital mortality

Table 3 presents the ORs and corresponding 95% CIs for postdischarge institutionalization and in-hospital mortality according to different measures of anticholinergic drug exposure. After adjustment for age, sex, CCI, number of non-anticholinergic drugs and delirium at any time during the hospital stay, we found that each additional anticholinergic drug used by a patient according to the ARS was associated with a 38% increase in odds of being institutionalized after discharge (OR = 1.38, 95% CI: 1.02–1.86). Additionally, a high ADB quantified with the ARS was associated with a 2.43 times higher odds of being institutionalized after discharge in comparison to no ADB (OR = 2.43, 95% CI: 1.24–4.75). No associations were found between anticholinergic drug exposure quantified with the ACB and the Chew, and postdischarge institutionalization.

After adjustment for age, sex, CCI, number of non-anticholinergic drugs and delirium at any time during the hospital stay, no associations were found between anticholinergic drug exposure and in-hospital mortality.

GEE logistic regression models for postdischarge institutionalization and in-hospital mortality showed that the inclusion of individuals multiple times did not affect the estimates (data not shown).

Analyses in patients with delirium on admission

Table 4 presents the ORs and corresponding 95% CIs for LOS ≥ 11 days, postdischarge institutionalization and in-hospital mortality according to different measures of anticholinergic drug exposure in patients with delirium on admission. The association between anticholinergic drug exposure and postdischarge institutionalization found in the total group of acutely ill patients was not maintained in this subgroup. No associations were found between anticholinergic drug exposure and LOS and in-hospital mortality.

GEE logistic regression models for the three outcome measures showed that the inclusion of individuals multiple times did not affect the estimates (data not shown).

Table 3. Odds ratios for postdischarge institutionalization and in-hospital mortality according to different measures of anticholinergic drug exposure.

	Postdischarge institutionalization		In-hospital mortality	
Variable	Institutionalized/home	OR (95% CI) [a,b]	Dead/alive	OR (95% CI) [a]
ARS				
Exposure to DAPs				
No	151/333	1.00 (ref)	45/604	1.00 (ref)
Yes	63/110	1.43 (0.95–2.14)	20/236	1.20 (0.67–2.15)
Number of DAPs				
Per drug	214/443	**1.38 (1.02–1.86)**	65/840	1.07 (0.73–1.58)
ADB score				
0	151/333	1.00 (ref)	45/604	1.00 (ref)
1-2	40/81	1.17 (0.74–1.85)	14/159	1.20 (0.63–2.29)
≥3	23/29	**2.43 (1.24–4.75)**	6/77	1.22 (0.47–3.13)
ACB				
Exposure to DAPs				
No	72/129	1.00 (ref)	15/246	1.00 (ref)
Yes	142/314	0.84 (0.56–1.25)	50/594	1.51 (0.80–2.84)
Number of DAPs				
Per drug	214/443	0.94 (0.80–1.11)	65/840	1.13 (0.90–1.41)
ADB score				
0	72/129	1.00 (ref)	15/246	1.00 (ref)
1-2	85/207	0.73 (0.48–1.12)	33/369	1.52 (0.79–2.93)
≥3	57/107	1.12 (0.68–1.86)	17/225	1.47 (0.66–3.25)
Chew				
Exposure to DAPs				
No	99/202	1.00 (ref)	28/354	1.00 (ref)
Yes	115/241	1.15 (0.80–1.67)	37/486	1.11 (0.64–1.91)
Number of DAPs				
Per drug	214/443	1.05 (0.85–1.29)	65/840	1.11 (0.84–1.46)
ADB score				
0	99/202	1.00 (ref)	28/354	1.00 (ref)
0.5-1	81/178	1.09 (0.74–1.62)	24/343	1.01 (0.56–1.83)
≥1.5	34/63	1.37 (0.80–2.36)	13/143	1.39 (0.66–2.92)

Notes: Values in bold are statistically significant ($p < 0.05$). [a] Model adjusted for age, sex, Charlson Comorbidity Index, non-anticholinergic drugs and delirium at any time during the hospital stay. [b] Patients who resided in an institutional care facility before admission and patients who died during the hospital stay were excluded.

Abbreviations: ACB, Anticholinergic Cognitive Burden scale; ADB, Anticholinergic Drug Burden; ARS, Anticholinergic Risk Scale; CI, confidence interval; DAPs, drugs with anticholinergic properties; OR, odds ratio.

Table 4. Odds ratios for prolonged length of hospital stay, postdischarge institutionalization and in-hospital mortality according to different measures of anticholinergic drug exposure in patients with delirium on admission.

	LOS ≥ 11 days		Postdischarge institutionalization		In-hospital mortality	
Variable	LOS ≥ 11/LOS < 11	OR (95% CI) [a,b]	Institutionalized/home	OR (95% CI) [a,c]	Dead/alive	OR (95% CI) [a]
ARS						
Exposure to DAPs						
No	58/67	1.00 (ref)	55/43	1.00 (ref)	14/125	1.00 (ref)
Yes	30/36	1.07 (0.57–2.00)	28/15	1.57 (0.72–3.45)	10/66	2.08 (0.79–5.50)
Number of DAPs						
Per drug	88/103	1.00 (0.68–1.49)	83/58	1.36 (0.74–2.50)	24/191	1.17 (0.64–2.14)
ADB score:						
0	58/67	1.00 (ref)	55/43	1.00 (ref)	14/125	1.00 (ref)
1-2	20/24	1.02 (0.51–2.07)	18/11	1.40 (0.58–3.39)	8/44	2.52 (0.90–7.08)
≥3	10/12	1.18 (0.44–3.12)	10/4	2.08 (0.56–7.63)	2/22	1.15 (0.21–6.33)
ACB						
Exposure to DAPs						
No	26/29	1.00 (ref)	28/18	1.00 (ref)	4/55	1.00 (ref)
Yes	62/74	0.99 (0.51–1.91)	55/40	0.85 (0.39–1.84)	20/136	2.19 (0.66–7.25)
Number of DAPs						
Per drug	88/103	0.79 (0.61–1.03)	83/58	0.75 (0.54–1.04)	24/191	1.22 (0.82–1.84)
ADB score:						
0	26/29	1.00 (ref)	28/18	1.00 (ref)	4/55	1.00 (ref)
1-2	38/41	1.05 (0.52–2.12)	32/26	0.75 (0.33–1.70)	12/79	1.94 (0.55–6.77)
≥3	24/33	0.88 (0.39–1.99)	23/14	1.12 (0.42–3.03)	8/57	2.99 (0.72–12.51)

Table 4. Odds ratios for prolonged length of hospital stay, postdischarge institutionalization and in-hospital mortality according to different measures of anticholinergic drug exposure in patients with delirium on admission. (*continued*)

	LOS ≥ 11 days		Postdischarge institutionalization		In-hospital mortality	
Variable	LOS ≥ 11/LOS < 11	OR (95% CI) [a,b]	Institutionalized/home	OR (95% CI) [a,c]	Dead/alive	OR (95% CI) [a]
Chew						
Exposure to DAPs						
No	39/43	1.00 (ref)	41/28	1.00 (ref)	9/82	1.00 (ref)
Yes	49/60	0.98 (0.53–1.79)	42/30	1.07 (0.52–2.19)	15/109	1.64 (0.62–4.33)
Number of DAPs						
Per drug	88/103	0.92 (0.67–1.26)	83/58	1.05 (0.72–1.55)	24/191	1.47 (0.92–2.35)
ADB score:						
0	39/43	1.00 (ref)	41/28	1.00 (ref)	9/82	1.00 (ref)
0.5-1	34/39	1.01 (0.52–1.95)	27/23	0.91 (0.42–1.98)	9/73	1.30 (0.45–3.77)
≥1.5	15/21	0.90 (0.39–2.08)	15/7	1.61 (0.55–4.72)	6/36	2.82 (0.80–9.95)

Notes: [a] Model adjusted for age, sex, Charlson Comorbidity Index and non-anticholinergic drugs. [b] Patients who died during the hospital stay were excluded. [c] Patients who resided in an institutional care facility before admission and patients who died during the hospital stay were excluded.

Abbreviations: ACB, Anticholinergic Cognitive Burden scale; ADB, Anticholinergic Drug Burden; ARS, Anticholinergic Risk Scale; CI, confidence interval; DAPs, drugs with anticholinergic properties; LOS, length of hospital stay; OR, odds ratio.

DISCUSSION

In this study, we found that anticholinergic drug exposure, measured with the ARS, is associated with an increased prevalence of delirium and increased postdischarge institutionalization in acutely ill hospitalized older patients.

Our finding that anticholinergic drug exposure measured with the ARS is associated with delirium, is in agreement with the results of previous studies performed in critically ill patients [21], palliative care patients [22], patients with Parkinson's disease [23] and older nursing home residents [24]. Also, previous studies found no association between anticholinergic drug exposure, measured with the ACB or the Anticholinergic Drug Scale, and delirium in older hospitalized patients [21, 25, 26]. These findings strengthen the observation that results may differ depending on which scale is used when assessing anticholinergic drug exposure.

Several studies have investigated the possible relationship between anticholinergic drug exposure and LOS in older hospitalized persons [7-9, 27, 28]. Three of them used the ARS and/or ACB and found, in line with our study, no association between anticholinergic drug exposure and LOS [7, 27, 28]. Mangoni et al. [9] also used the ARS and found that anticholinergic drug exposure was only associated with prolonged LOS in older patients who were admitted during a non-heat wave period. In contrast to our study, the previous study [9] included only older patients who were discharged home and did not exclude patients who were hospitalized for < 3 days. Therefore, it might be speculated that the patients included in the study of Mangoni et al. [9] were healthier and probably less frail than our study population. Lowry et al. [8] also found that the use of anticholinergic drugs was associated with prolonged LOS in older hospitalized persons. However, they used an alternate anticholinergic drug scale and had previously found that the ARS was not associated with LOS in the same study sample [28].

As far as we are aware this is the first study showing an association between anticholinergic drug exposure, measured with the ARS, and postdischarge institutionalization in acutely ill older patients. In a previous study, no association was found between anticholinergic drug use and nursing home admission within one year after hospital discharge [29]. However, Narbey et al. [29] did not use an anticholinergic drug scale and treatment with anticholinergic drugs can have changed over time. Therefore, caution is warranted in extrapolating their results. A possible explanation for our finding that anticholinergic drug exposure, measured with the ARS, is associated with postdischarge institutionalization, might be an underlying decrease in functional performance. Several studies have found that anticholinergic drug exposure quantified with the ARS is associated with a reduced physical function in older persons [7, 24, 28, 30, 31].

Several studies have investigated the possible association between the use of anticholinergic drugs and in-hospital mortality in older patients [8, 9, 27, 28]. Mangoni et al.

[9], Kidd et al. [27] and Lowry et al. [8] used the ARS, the ACB and the anticholinergic component of the Drug Burden Index respectively, and in line with our study, found no association. In a subgroup of older patients with hyponatremia, Lowry et al. [28] reported that high ARS scores were associated with increased in-hospital mortality.

To the best of our knowledge, no previous studies investigated the possible association between anticholinergic drug use and clinical outcomes in acutely ill hospitalized older patients with delirium. Recently, Kolanowski et al. [32] found that the use of anticholinergic drugs according to the ACB was associated with prolonged LOS and reduced physical function, but not with discharge disposition in older persons with delirium who resided in a postacute care facility. In contrast to our study, their sample size was relatively small, all participants had dementia, were not acutely ill, and in the vast majority delirium was resolving.

Although no conclusions on causality can be drawn from this observational study, our results suggest that older persons who are exposed to anticholinergic drugs are at increased risk for delirium when they become acutely ill. Additionally, they might be at increased risk for postdischarge institutionalization independently of delirium. The question remains whether anticholinergic drugs 'in general' are associated with delirium and postdischarge institutionalization, since only the ARS was associated with them. Discrepancies in results between the ARS, ACB and Chew might be related to the large variation in number and ranking of drugs within each scale, which is caused by the different methods used to develop them. In all anticholinergic drug scales, the calculation of the ADB is based on the assumption that anticholinergic effects of different drugs are additive in a linear fashion. This might not be the case and therefore, inclusion of drugs without known clinically relevant anticholinergic effects might dilute possible associations. Therefore, it might be warranted to identify only drugs with established peripheral and cognitive anticholinergic effects in future studies. Furthermore, it might be possible that in delirium not only central anticholinergic effects may play a role, if any, but also peripheral anticholinergic effects. Blurred vision, urinary retention, constipation and confusion are risk factors for delirium and might explain why the ARS was associated with delirium. However, since we did not collect data on adverse effects, this remains speculative. In patients with delirium, we found that the ARS was not associated with postdischarge institutionalization. It might be possible that the sample size was too small; other explanations could be that anticholinergic drugs play a minor role, if any, in the clinical course of delirium, or that anticholinergic drugs were stopped more frequently after admission since a cholinergic deficiency is still one of the most hypothesized causes of delirium [18].

Limitations and strengths

This study has some limitations. First, the study design limits the ability to identify causal associations between the use of anticholinergic drugs and the outcome measures. Information on any changes in drug exposure during hospitalization was not collected and we cannot exclude that the treatment approach for the acute illness has influenced our results. Moreover, other health-related factors, such as the reason for admission, the severity of illness, functional status and the degree of cognitive functioning can have influenced our results. In this study, we were not able to score and adjust for the severity of illness and physical function, since information on those items was not always available. However, we have adjusted for the CCI in statistical models; therefore, we believe that we have provided an indirect adjustment for dementia. A comorbid cognitive disturbance, not diagnosed as dementia (yet), can still be a confounding factor. Second, the three anticholinergic drug scales were developed several years ago (the ARS and the Chew in 2008; the ACB was last updated in 2012) and do not include newer anticholinergic drugs. This might have led to an underestimation of the anticholinergic drug exposure, but we believe that our results are only minimally influenced by this. Third, the three anticholinergic drug scales do not take into account daily drug dose and treatment duration. Since it is likely that anticholinergic effects will be amplified with higher drug doses and longer treatment duration, this could have influenced our results. Fourth, our results are mainly based on information on prescribed drugs; minimal information was available on treatment adherence prior to hospitalization.

The study has several strengths. First, the findings were obtained in a relatively large sample size. Second, we used three anticholinergic drug scales within the same population which makes it possible to make clear comparisons between results. Third, the ARS, ACB and Chew provide a quick and simple measure of anticholinergic drug burden and are suitable for clinical practice.

CONCLUSION

In this study, we found that anticholinergic drug exposure measured with the ARS, is associated with an increased prevalence of delirium on admission and increased postdischarge institutionalization in acutely ill hospitalized older patients.

Considering the fact that delirium and postdischarge institutionalization are associated with a very poor prognosis, future studies are needed to investigate whether regular medication reviews using the ARS are a useful tool in order to reduce complications and to preserve independent functioning in older persons.

SUPPLEMENTARY MATERIAL

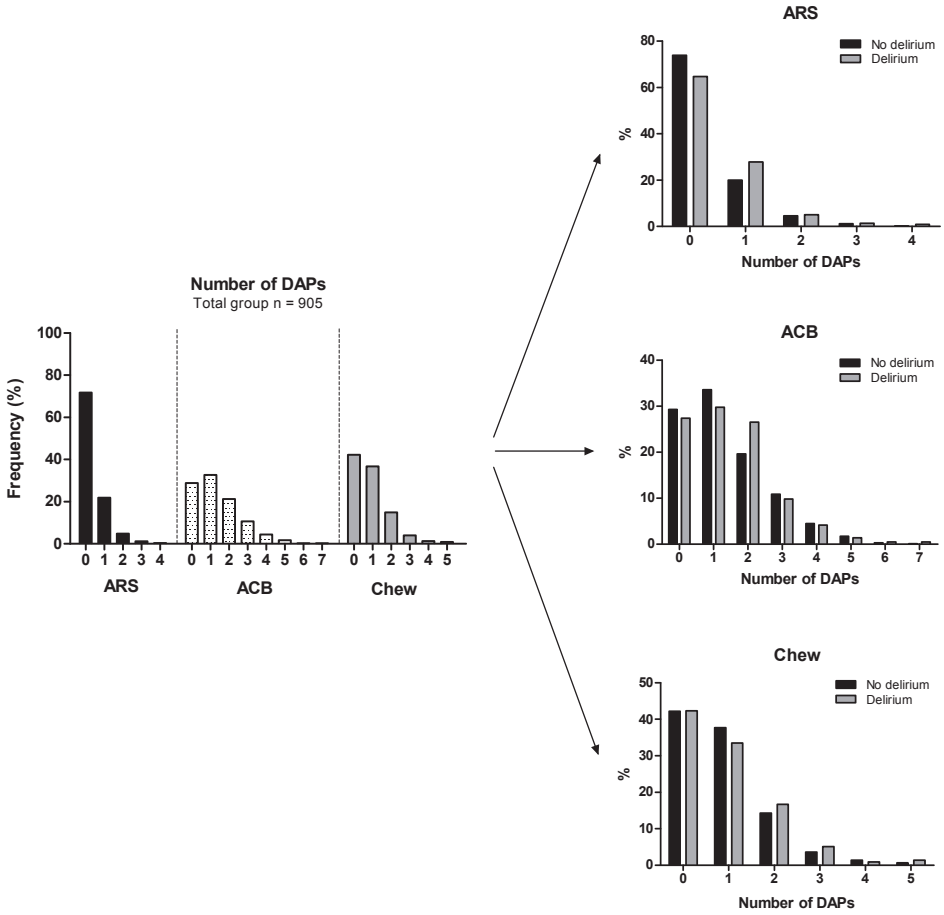

Figure S1. Frequency distributions of the number of DAPs on admission for the overall study sample and stratified for delirium on admission.

Abbreviations: ACB, Anticholinergic Cognitive Burden Scale; ARS, Anticholinergic Risk Scale; DAPs, drugs with anticholinergic properties.

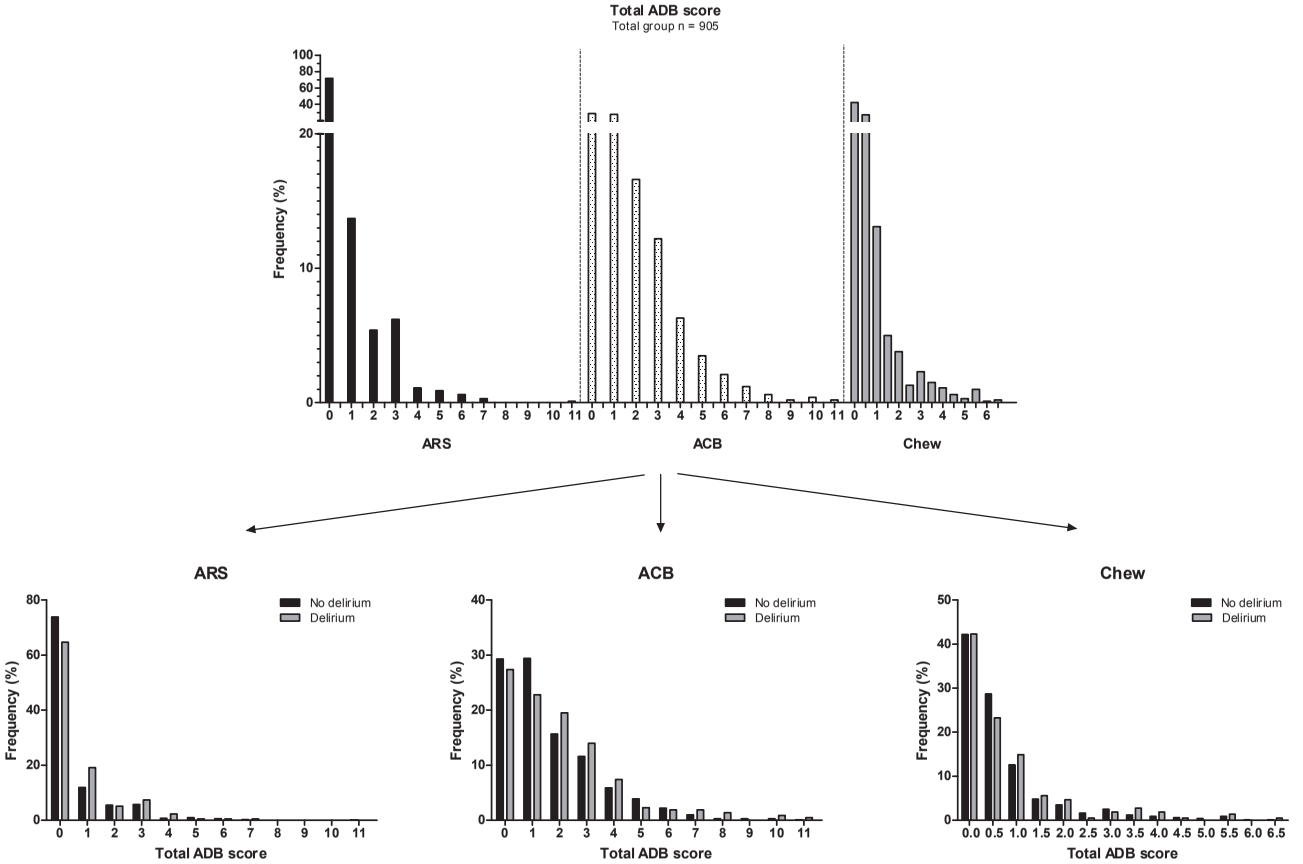

Figure S2. Frequency distributions of the total ADB scores on admission for the overall study sample and stratified for delirium.
Abbreviations: ACB, Anticholinergic Cognitive Burden Scale; ADB, Anticholinergic Drug Burden; ARS, Anticholinergic Risk Scale.

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4. Discussion

Chapter 4

General discussion, clinical implications and future directions

GENERAL DISCUSSION

Delirium, an acute neuropsychiatric syndrome, is a common disorder among older hospitalized patients and is associated with poor clinical outcomes including prolonged hospital stay, loss of independence, increased rates of cognitive decline and mortality [1-3]. However, despite the high prevalence and clinical impact, very little is known about the underlying pathogenesis. Several mechanisms have been proposed to be involved in the pathophysiology of delirium and include, among others, increased activation of the immune system, oxidative stress, loss of neuroprotection and disturbances in several cerebral neurotransmitter systems [4]. Also several drugs and drug classes may play a role in the pathogenesis of delirium. Many drugs commonly used by older persons interfere with one or more of the hypothesized mechanisms and therefore, the use of specific drugs may increase or decrease the risk of developing delirium.

The aims of this thesis were to investigate the association of several biochemical markers and pharmacological agents with delirium. The findings can contribute to a better understanding of the pathophysiology of delirium and may help to find markers for early recognition and to improve delirium prediction, prevention and treatment.

The findings described in this thesis are based on two studies. The first study is the Delirium In The Old (DITO) study, a cross-sectional study designed to investigate several advanced biochemical blood markers in patients with and without delirium. This study included 86 patients aged 65 years and older who were acutely admitted to the wards of Internal Medicine and Geriatrics of the Erasmus Medical Center and the ward of Geriatrics of the Harbor Hospital, Rotterdam, the Netherlands. The second study is a chart-review study including 905 patients aged 65 years and older who were acutely admitted to the ward of Geriatrics of the Erasmus Medical Center. This study was performed to investigate the possible association between the use of anticholinergic drugs and delirium. Additionally, within the framework of this study, we have investigated a relatively novel and easily measureable inflammatory marker in patients with and without delirium.

In this chapter, the main findings are discussed and directions for further research are presented. Part one of this chapter will focus on the potential role of inflammation, oxidative stress, loss of neuroprotection and disturbances in neurotransmitters as well as the interplay among these mechanisms in the pathophysiology of delirium. Part two will focus on the potential role of pharmacological agents in delirium.

Main findings

Biochemical markers of delirium – potential implications for the pathophysiology

Activation of the immune system is a prominent feature of many conditions associated with delirium, suggesting that inflammatory mediators may play a role in delirium. Cur-

rently, the neuroinflammatory hypothesis is one of the leading theories with regard to the pathophysiology of delirium. This hypothesis suggests that peripheral inflammation induces activation of microglia and astrocytes in the brain which in turn will release pro-inflammatory cytokines and neurotoxic factors. These factors may cause neuronal and synaptic dysfunction and these neurological changes may subsequently manifest as delirium [4].

In **chapter 2.1**, levels of neopterin, interleukin-6 (IL-6) and insulin-like growth factor-1 (IGF-1) were compared between acutely ill older patients with and without delirium, as markers for immune system activation, oxidative stress and brain reserve. Increased levels of neopterin and IL-6 and decreased levels of IGF-1 were found in patients with delirium. These findings might suggest that a disturbed activation of the innate and cellular immune system, oxidative stress and an increased vulnerability of the brain may underlie the development of delirium.

So far, limited research has focused on the potential role of neopterin in delirium, but all studies currently performed have found increased neopterin levels in plasma or cerebrospinal fluid (CSF) prior to and during delirium [5-7]. This consistency in findings suggests that neopterin might indeed play a role in the pathophysiology of delirium. The physiological functions of neopterin in the periphery and central nervous system are, however, virtually unknown. Therefore, it remains hypothetical by which mechanisms neopterin might be associated with delirium. Considering that neopterin is primarily produced by activated macrophages and monocytes (cells that produce reactive oxygen species (ROS)), it seems plausible that neopterin induces or enhances cytotoxicity, either directly or indirectly. In vitro studies have suggested that neopterin might activate ROS-sensitive transcription factors [8] and neopterin's derivative 7,8-dihydroneopterin might be able to induce apoptosis in astrocytes and neurons in a dose-dependent manner [9].

Another mechanism exists that might explain why increased neopterin levels are associated with delirium. During inflammation, neopterin production will be accompanied by an enhanced conversion of tryptophan to kynurenine in both peripheral and central tissue (**figure 1**). Kynurenine is able to pass the blood-brain barrier and can be further metabolized to kynurenic acid and quinolinic acid. During times of stress and inflammation, the kynurenine pathway preferentially produces quinolinic acid, which is a neurotoxic metabolite produced by microglia [10, 11]. Increased production of quinolinic acid can lead to neuronal injury, excitotoxicity and apoptosis [12], which may manifest as delirium. Increased activation of the kynurenine pathway has been implicated in the pathophysiology of several neurodegenerative and psychiatric disorders [13], including acute brain dysfunction during critical illness [14]. Though kynurenine levels were not measured in the studies described in this thesis, it might be possible that the observed lower tryptophan

levels and tryptophan ratios in patients with delirium (chapter 2.2) might be caused by an increased turnover of tryptophan to kynurenine.

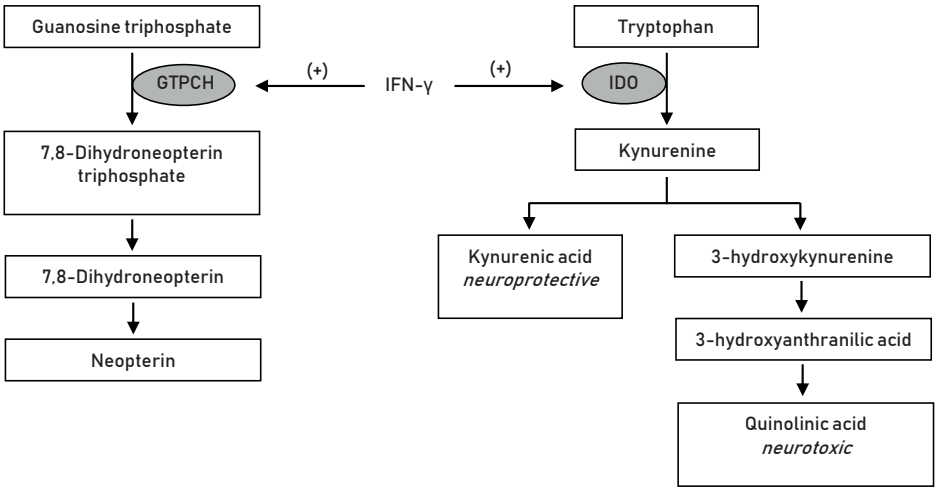

Figure 1. Schematic presentation of the formation of neopterin and kynurenine in response to the pro-inflammatory cytokine IFN-γ. During inflammation, the kynurenine pathway preferentially produces quinolinic acid under the influence of a variety of pro-inflammatory mediators which affect several enzymes not depicted in this figure.
Abbreviations: GTPCH, guanosine triphosphate cyclohydrolase I; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ.

Inflammation is a complex process in which different cytokines and other inflammatory mediators interact and the balance between pro- and anti-inflammatory responses will determine in which amount the inflammatory response will be protective or harmful. During peripheral inflammation, pro-inflammatory cytokines – in particular IL-1β, tumor necrosis factor-α (TNF-α) and IL-6 – play an important role in the upregulation of the inflammatory response. It is now well established that pro-inflammatory cytokines as well as other inflammatory mediators communicate with the brain via multiple pathways, leading to the activation of microglia and astrocytes. Once activated, these cells will secrete several cytotoxic factors, such as reactive oxygen species and proteases, and pro-inflammatory cytokines which will enhance the inflammatory response, but which may also cause neuronal and synaptic dysfunction [15].

In patients with delirium, increased levels of pro-inflammatory cytokines (e.g. IL-1β [16, 17] and IL-6 [5, 18-21]) and decreased levels of anti-inflammatory cytokines (e.g. IL-1 receptor antagonist [22, 23]) have been found in serum and CSF, but the results are inconsistent. Interestingly, a previous study reported that patients who developed delirium after elective arthroplasty had an increased ratio of pro-inflammatory to anti-inflammatory

cytokines, suggesting that an imbalance in the inflammatory response, in favor of the pro-inflammatory response, might be involved in delirium [24].

It has also been suggested that an underlying vulnerability of the brain may predispose patients to the development of delirium. IGF-1 – a growth factor with neuroprotective properties – plays an important role within the central nervous system. IGF-1 promotes neuronal proliferation, differentiation and survival; controls synaptogenesis and synaptic plasticity and protects neurons against free radical damage [25]. Therefore, decreased IGF-1 levels may suggest that the brain is more vulnerable to the cytotoxic effects of cytokines and other neurotoxic factors.

Several mechanisms might be involved in the pathophysiology of delirium and besides inflammation and an increased vulnerability of the brain, disturbances in one or more cerebral neurotransmitter systems might play a role. The neurotransmitter hypothesis suggests that a relative acetylcholine deficiency, an excess of dopamine and disturbances in serotonin and melatonin (both increased and decreased activity) may underlie the characteristic symptoms seen during delirium [4].

The measurement of cerebral neurotransmitter levels requires the performance of invasive procedures such as lumbar punctures. Alternatively, one can also measure plasma precursors and/or metabolites as indirect measures for central neurotransmitter functioning.

Serotonin synthesis in the central nervous system is, among others, dependent on the availability of its precursor, the large neutral amino acid (LNAA) tryptophan (**figure 2**). 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 (tryptophan/LNAAs ratio). Furthermore, the amino acids phenylalanine and tyrosine are the precursors of dopamine (**figure 2**). Therefore, the ratios of tyrosine and phenylalanine to the other LNAAs are suggestive for the amount of tyrosine and phenylalanine that enters the brain.

Tetrahydrobiopterin (BH_4) functions as an essential cofactor in several enzymatic reactions involved in the production of serotonin and dopamine (**figure 2**) [26]. A change in the availability of BH_4 may therefore affect the synthesis of these neurotransmitters. The phenylalanine/tyrosine 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 a cofactor [26, 27]. An elevated ratio of phenylalanine to tyrosine suggests a decreased BH_4 availability.

In **chapter 2.2**, potential disturbances in serotonergic and dopaminergic neurotransmission during delirium were investigated. For this purpose, peripheral levels of amino acids, amino acid ratios and dopamine's metabolite homovanillic acid (HVA) were investigated. Decreased tryptophan/LNAAs ratios and increased phenylalanine/tyrosine ratios were found in patients with delirium. These findings are suggestive for a decreased availability

of tryptophan and BH_4 for the synthesis of serotonin and consequently might suggest a reduced production of serotonin during delirium. No differences in phenylalanine/LNAAs ratio, tyrosine/LNAAs ratio and HVA levels were found between patients with and without delirium, suggesting that the dopaminergic neurotransmission is not impaired during delirium.

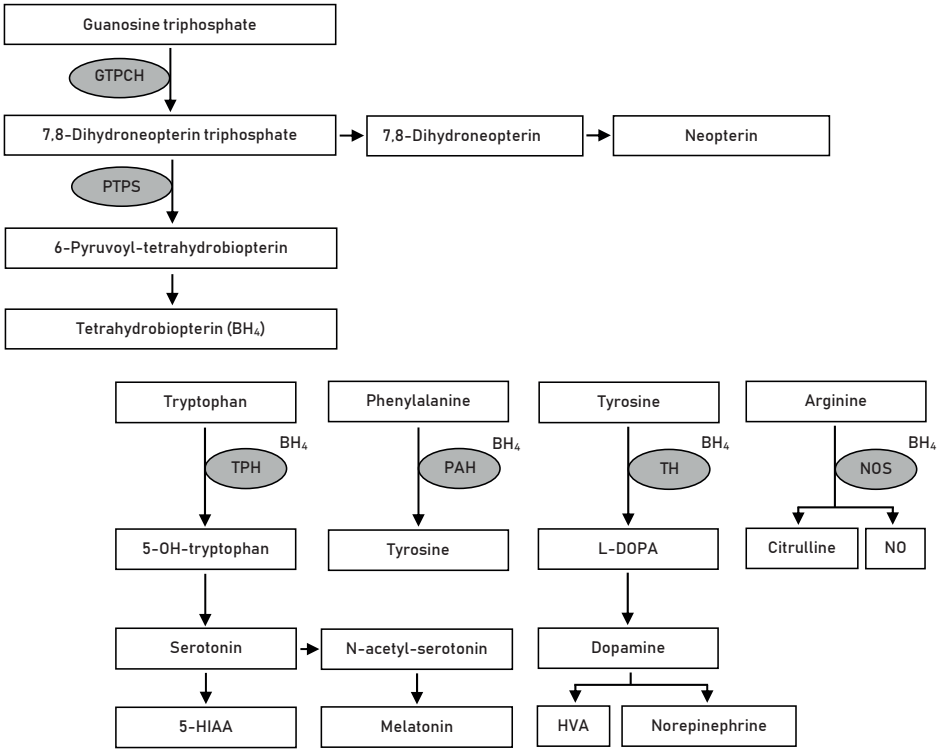

Figure 2. Schematic and simplified presentation of the biosynthesis of tetrahydrobiopterin (BH_4), monoamine neurotransmitters and nitric oxide. Due to a relative deficiency of PTPS in macrophages and monocytes, upregulation of GTPCH by IFN-γ leads to accumulation of 7,8-dihydroneopterin triphosphate in the cell, favoring the formation of neopterin. BH_4 is an essential cofactor for the aromatic amino acid hydroxylases (TPH, PAH and TH) as well as for NOS.
Abbreviations: GTPCH, guanosine triphosphate cyclohydrolase I; PTPS, 6-pyruvoyltetrahydrobiopterin synthase; TPH, tryptophan hydroxylase; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; NOS, nitric oxide synthase; 5-HIAA, 5-hydroxyindolacetic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; HVA, homovanillic acid.

Instead of determining ratios of cytokines that reflect different components of the immune system (as suggested before), it is also possible to determine ratios of different immune cells. The neutrophil-lymphocyte ratio (NLR) represents the balance between neutrophils and lymphocytes and integrates two components of the immune system in one marker.

Neutrophils play an important role in the immediate response against tissue damage and pathogens [28], whereas lymphocytes play an important role in the regulation of an appropriate inflammatory response. In various pathological conditions, elevated NLR levels have been found to be an independent predictor of disease severity and poor prognosis. Moreover, NLR seems to be a more powerful predictor of adverse outcomes than conventional inflammatory markers, such as the total white blood cell (WBC) count, the individual WBC subtypes and C-reactive protein (CRP) [29-32].

In **chapter 2.3**, a pilot study is presented in which levels of NLR were investigated in patients with and without delirium. Patients with delirium had increased NLR levels, even after adjustment for inflammatory markers and additionally, no differences were found in the levels of CRP, WBC, neutrophils and lymphocytes. These findings might suggest an independent role of NLR in delirium above conventional inflammatory markers and support the hypothesis that an inadequate response of the immune system and oxidative stress may play a role in the pathogenesis of delirium.

Considering that different combinations of predisposing and precipitating factors can lead to delirium, it seems likely that the underlying pathophysiological mechanisms may differ between groups of patients with delirium. In **chapter 2.4**, mean levels of neopterin, amino acids, amino acid ratios and HVA in acutely ill medical patients with delirium and patients with delirium after elective cardiac surgery were compared. Higher neopterin levels and higher phenylalanine/tyrosine ratios were found in acutely ill medical patients with delirium than in patients with delirium after cardiac surgery. Differences in determinants of neopterin were found between the two groups. The findings suggest that, in acutely ill medical patients, neopterin levels are mainly determined by a cell-mediated immune response and oxidative stress, whereas in elective cardiosurgical patients neopterin levels are mainly driven by renal function/fluid status. This study highlights that levels of certain potential biomarkers for delirium can vary in different groups of patients and suggests 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 cardiosurgical patients.

Pharmacological agents

Several drugs commonly used by older persons can interfere with one or more of the hypothesized mechanisms underlying delirium and therefore, the use of specific drugs may increase or decrease the risk of developing delirium.

In patients with delirium, increased plasma neopterin levels and a decreased availability of tryptophan to the brain were found (chapters 2.1 and 2.2). It has been reported that treatment of stimulated peripheral blood mononuclear cells with aspirin (acetylsalicylic acid) can significantly inhibit neopterin production and tryptophan degradation in vitro [33]. The potential influence of acetylsalicylic acid on neopterin production and trypto-

phan degradation might be the result of a modulating effect of acetylsalicylic acid on the cytokine interferon-gamma (IFN-γ), since both the production of neopterin as well as the degradation of tryptophan are IFN-γ dependent (**figure 1**). It is unknown whether the use of acetylsalicylic acid can influence neopterin and tryptophan levels in vivo.

In **chapter 3.1**, the possible association between the use of acetylsalicylic acid and mean neopterin and tryptophan levels in patients with and without delirium was investigated. The use of acetylsalicylic acid had no statistically significant effect on the mean levels of neopterin and tryptophan. However, in patients with delirium, neopterin levels seemed to be lower and tryptophan levels seemed to be higher in patients who used acetylsalicylic acid compared to those who did not.

The neurotransmitter acetylcholine is implicated in several processes that are impaired during delirium, such as attention, sleep and memory, and this has led to the hypothesis that a cholinergic deficiency might be involved in the pathogenesis of delirium [34, 35]. Drugs with anticholinergic properties are commonly prescribed in older persons and the use of these drugs will result in some degree of cholinergic deficiency, therefore anticholinergic drug use might be a risk factor for delirium.

Currently, there is no consensus on the definition of anticholinergic drugs and this has led to the development of several ranked anticholinergic drug scales. Previous studies generally used one anticholinergic drug scale to assess anticholinergic drug exposure and additionally, used them in different ways. The available scales differ, however, substantially from each other in number and ranking of drugs. Unfortunately, little is known about how these differences in measurement affect their predictive validity with respect to clinically relevant outcomes, such as delirium [36].

In the study presented in **chapter 3.2**, three anticholinergic drug scales were used within the same population to investigate whether anticholinergic drug exposure on admission is associated with delirium on admission, length of hospital stay, postdischarge institutionalization and in-hospital mortality. We demonstrated that the scale used to identify anticholinergic drugs significantly affected the results. Only anticholinergic drug exposure measured with the Anticholinergic Risk Scale (ARS), was associated with an increased prevalence of delirium on admission and postdischarge institutionalization in acutely ill hospitalized patients. The ARS is a quick measure of anticholinergic drug burden, easy to apply in clinical practice, and might be a useful tool to identify patients at increased risk for delirium. Future research is needed to investigate whether medication reviews using the ARS will lead to better clinical outcomes in older patients.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Delirium in older hospitalized patients is a major problem associated with poor clinical outcomes. Up-to-date, the high need to improve the prediction, detection and treatment of delirium is increasingly being recognized, while the underlying pathophysiology remains largely understudied. Adequate knowledge of the pathophysiology is required to find markers for early recognition and to improve delirium prediction, prevention and treatment.

Identification of accurate biomarkers for delirium will help unraveling the complex pathophysiology. In this thesis, several markers of different pathways were investigated and were found to be increased or decreased in older, acutely ill patients with delirium. The findings suggest that an inadequate response of the immune system, oxidative stress, loss of brain reserve and a disturbance in the serotonergic neurotransmission may be all involved in the pathophysiology of delirium in acutely ill older patients. These speculations need to be confirmed and larger studies are needed to investigate the clinical significance of the investigated markers as potential biomarkers for delirium diagnosis.

Several markers have been found to be increased or decreased in patients with delirium and have the potential to be a biomarker, but many of the investigated markers require time-consuming and expensive measurements and trained personnel which make their use for research purposes and clinical practice less attractive. A potential diagnostic (or predictive) biomarker for delirium requires a fast, simple and widely available method of determination. A time-consuming measurement is not preferred, given the fact that a person's prognosis becomes poorer with each day that delirium persists.

The neutrophil-lymphocyte ratio, which is directly derived from the differential white blood cell count, is an easily applicable marker of inflammation and oxidative stress. Higher NLR levels were found in patients with delirium when compared to patients without delirium. If these findings are confirmed in larger studies, NLR offers new opportunities for delirium research. It would be interesting to evaluate the discriminative power of NLR in diagnosing and predicting delirium in different populations and to investigate if NLR levels can predict poor outcomes in patients with delirium.

In this thesis, we have described differences in biochemical profile between medical and surgical patients with delirium. These findings suggest that the markers and pathways that might be involved in the pathophysiology of delirium may differ between groups of patients with delirium and highlight the importance to perform studies in different populations. It might be possible that delirium care needs different approaches in different settings. A

major step forward in delirium research would be a large study, including several health care settings (e.g. surgical, medical and intensive care units), in which potential biomarkers and other factors are compared across different groups of patients with delirium.

Based on our findings it can be concluded that the ARS might be a suitable instrument to identify patients at increased risk of delirium and postdischarge institutionalization. Previous studies have shown that medication reviews can be effective in reducing anticholinergic drug exposure in persons aged 65 years and older [37, 38]. Moreover, it has been shown that in frail, acutely ill, older patients admitted to a geriatric hospital ward, anticholinergic drug exposure can be reduced further simply by alerting the clinician of a person's ARS score and by asking the clinician to reconsider anticholinergic drug use in patients with an ARS score greater than 0 [38]. Therefore, it would be interesting to investigate whether regular medication reviews with the ARS as additional tool in every person aged 65 years and older performed in both the community and hospital setting will reduce delirium on admission and may preserve independent functioning in older persons.

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5. Summaries

Chapter 5.1

English summary

SUMMARY

Delirium – an acute neuropsychiatric syndrome – affects approximately 1 out of 3 older patients admitted to the hospital and is associated with poor outcomes, including prolonged hospital stay and increased rates of postdischarge institutionalization, cognitive decline and mortality. Despite the high frequency and clinical impact, this syndrome is still poorly understood. The aims of this thesis were to investigate the association of several biochemical markers and pharmacological agents with delirium in two groups of acutely ill hospitalized patients aged 65 years and older. The findings may contribute to a better understanding of the pathophysiology and may help to find markers for early recognition and to improve delirium prevention, prediction and treatment.

Chapter 1 gives a general introduction to the research topics of this thesis. **Chapter 2** focuses on several mechanisms that might play a role in the pathophysiology of delirium. In **chapter 2.1** the association of plasma levels of neopterin, interleukin-6 (IL-6) and insulin-like growth factor-1 (IGF-1) with delirium was investigated. Patients with delirium had statistically significantly higher levels of neopterin and IL-6 as well as statistically significantly lower levels of IGF-1 than patients without delirium. These findings suggest that activation of the immune system, oxidative stress and an increased vulnerability of the brain (due to decreased neuroprotection) might be involved in the pathogenesis of delirium.

Chapter 2.2 focuses on disturbances in serotonergic and dopaminergic neurotransmission as well as oxidative stress in delirium. For this purpose, levels of amino acids, amino acid ratios and dopamine's metabolite homovanillic acid (HVA) were investigated. Patients with delirium had higher phenylalanine/tyrosine ratios, lower ratios of tryptophan to the other large neutral amino acids (LNAAs) and lower levels of arginine and citrulline than patients without delirium. These findings suggest that a decreased availability of tetrahydrobiopterin, disturbed serotonergic neurotransmission and an increased status of oxidative stress may play a role in the pathogenesis of delirium. No differences in phenylalanine/LNAAs ratios, tyrosine/LNAAs ratios and HVA levels were found between patients with and without delirium, suggesting that the dopaminergic neurotransmission is not impaired during delirium.

In **chapter 2.3**, the association of the neutrophil-lymphocyte ratio (NLR) and other, more conventional, inflammatory markers (i.e. white blood cell (WBC) count, neutrophils, lymphocytes and C-reactive protein (CRP)) with delirium was investigated. Patients with delirium had increased NLR levels, even after adjustment for inflammatory markers and additionally, no differences were found in the levels of CRP, WBC, neutrophils and lymphocytes. These findings might suggest an independent role of NLR in delirium above conven-

tional inflammatory markers and support the hypothesis that an inadequate response of the immune system and oxidative stress may play a role in the pathogenesis of delirium.

Chapter 2.4 focuses on potential differences in blood-based markers among different categories of patients with delirium. In this chapter, mean plasma levels of neopterin, amino acids, amino acid ratios and HVA in acutely ill medical patients with delirium and patients with delirium after elective cardiac surgery were compared. Higher neopterin levels and higher phenylalanine/tyrosine ratios were found in acutely ill medical patients with delirium than in patients with delirium after elective cardiac surgery. Furthermore, substantial differences in determinants of neopterin levels were found between the two populations. C-reactive protein levels were the strongest determinant of neopterin levels in acutely ill medical patients with delirium, whereas the estimated glomerular filtration rate was the strongest determinant of neopterin in elective cardiosurgical patients with delirium. Thus, in acutely ill medical patients, neopterin levels are probably mainly determined by inflammation/oxidative stress, whereas in elective cardiosurgical patients, neopterin levels are mainly driven by renal function/fluid status. These findings suggest that delirium might have different pathophysiological mechanisms depending on the population studied.

Chapter 3 focuses on the role of pharmacological agents in delirium. In **chapter 3.1**, the possible influence of the use of acetylsalicylic acid on neopterin and tryptophan levels in patients with and without delirium was investigated. No differences were found in mean levels of neopterin and tryptophan between users and non-users. However, in patients with delirium, neopterin levels seemed to be lower and tryptophan levels seemed to be higher in patients who used acetylsalicylic acid compared to those who did not.

In **chapter 3.2**, the possible association between anticholinergic drug use and delirium on admission, length of hospital stay, postdischarge institutionalization and in-hospital mortality was investigated. Anticholinergic drug exposure on admission was quantified with three anticholinergic drug scales, i.e. the Anticholinergic Risk Scale (ARS), the Anticholinergic Cognitive Burden scale (ACB) and the list of Chew et al. (Chew). Substantial differences were found in the estimation of anticholinergic drug exposure among the ARS, ACB and Chew as well as in the presence of associations with clinically relevant outcomes. Only anticholinergic drug exposure measured with the ARS was associated with delirium on admission and postdischarge institutionalization. Considering the fact that delirium and postdischarge institutionalization are both associated with a very poor prognosis, future studies are needed to investigate the clinical utility of the ARS in reducing complications in older persons.

In the general discussion, **chapter 4**, the main findings of this thesis are discussed and suggestions for further research are presented. The results indicate that an inadequate response of the immune system, oxidative stress, loss of brain reserve and a disturbance in the serotonergic neurotransmission may be all involved in the pathophysiology of delirium in acutely ill older patients. Moreover, the 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 cardiosurgical patients, which highlights the importance to perform delirium studies in different populations. Furthermore, we demonstrated that anticholinergic drug exposure measured with the ARS was associated with delirium on admission. The ARS is a quick and simple measure of anticholinergic drug exposure, easy to apply, and might be clinically useful in identifying patients at increased risk of developing delirium. Future studies are needed to investigate whether regular medication reviews using the ARS are a useful tool in order to reduce delirium in older persons.

Chapter 5.2

Nederlandse samenvatting

SAMENVATTING

Een delirium is een acute vorm van verwardheid die voorkomt bij ongeveer 1 op de 3 ouderen die zijn opgenomen in het ziekenhuis. Een delirium is een ernstig neuropsychiatrisch syndroom dat vaak gepaard gaat met een langer verblijf in het ziekenhuis, een vermindering van zelfredzaamheid, een grotere kans op het ontwikkelen van dementie en een verhoogd 1-jaars mortaliteitsrisico. Hoewel delirium zeer frequent voorkomt bij ouderen en een grote impact heeft op zowel patiënten als de gezondheidszorg, is er nog veel onbekend over de pathofysiologie en de mogelijke rol van geneesmiddelen bij het uitlokken van delirium.

Het doel van dit proefschrift was om verschillende factoren die een rol kunnen spelen in de pathofysiologie van delirium te onderzoeken en om de relatie tussen bepaalde groepen geneesmiddelen en delirium nader te evalueren. De onderzoeken die in dit proefschrift beschreven worden, berusten op twee studies die zijn uitgevoerd bij patiënten van 65 jaar en ouder die acuut ziek waren opgenomen in het ziekenhuis. De bevindingen van dit proefschrift kunnen bijdragen aan een beter begrip van de pathofysiologie; helpen bij de zoektocht naar potentiële (bio)markers voor een vroege herkenning van het delirium en helpen bij het verbeteren van de preventie, predictie en behandeling van het delirium.

In **hoofdstuk 1** wordt een algemene inleiding over delirium gegeven en worden de onderwerpen die in dit proefschrift aan bod komen, geïntroduceerd. In **hoofdstuk 2** staan verschillende mechanismen centraal die een rol zouden kunnen spelen in de pathofysiologie van delirium. In **hoofdstuk 2.1** werden plasma spiegels van neopterine, interleukine-6 (IL-6) en insulin-like growth factor-1 (IGF-1) van patiënten met en zonder delirium vergeleken. In patiënten met een delirium werden statistisch significant hogere neopterine en IL-6 spiegels gevonden en statistisch significant lagere IGF-1 spiegels dan in patiënten zonder delirium. Deze resultaten suggereren dat een geactiveerd immuunsysteem, oxidatieve stress en een verhoogde kwetsbaarheid van het brein (door een verminderde neuroprotectie) een rol kunnen spelen in de pathofysiologie van delirium.

In **hoofdstuk 2.2** staan oxidatieve stress en verstoringen in de serotonerge en dopaminerge neurotransmissie als mogelijk onderliggende mechanismen voor delirium centraal. Om de eventuele betrokkenheid van deze mechanismen bij delirium te bestuderen, werden in het bloed van patiënten met en zonder delirium een aantal markers bepaald, namelijk diverse aminozuren (enkele zijn voorlopers van neurotransmitters) en homovanillinezuur (een metaboliet van dopamine), en werden verschillende ratio's van aminozuren berekend. Patiënten met een delirium hadden statistisch significant hogere fenylalanine/tyrosine ratio's, lagere ratio's van tryptofaan ten opzichte van de andere grote neutrale aminozuren en lagere arginine en citrulline spiegels dan patiënten zonder delirium. Deze resultaten

suggereren dat een verminderde beschikbaarheid aan tetrahydrobiopterine (een essentiële cofactor bij de vorming van serotonine en dopamine), een verstoorde serotonerge neurotransmissie en een verhoogde staat van oxidatieve stress een rol kunnen spelen in de pathofysiologie van delirium. Er werden geen verschillen gevonden in de dopaminerge markers in patiënten met en zonder delirium.

In de pilotstudie die beschreven wordt in **hoofdstuk 2.3** werden in patiënten met en zonder delirium diverse inflammatoire markers onderzocht die routinematig bepaald kunnen worden, namelijk de neutrofielen-lymfocyten ratio (NLR) en de meer traditionele inflammatoire markers, zoals C-reactive protein (CRP), het totale aantal leukocyten, neutrofielen en lymfocyten. Patiënten met een delirium hadden statistisch significant hogere NLR waarden dan patiënten zonder delirium, zelfs na correctie voor de andere inflammatoire markers. Er werden geen verschillen gevonden in CRP spiegels en het totale aantal leukocyten, neutrofielen en lymfocyten. Deze resultaten suggereren dat de NLR mogelijk een onafhankelijke en tevens sterkere rol speelt in delirium dan de traditionele inflammatoire markers. Daarnaast ondersteunen de resultaten de hypothese dat een verstoorde reactie van het immuunsysteem en oxidatieve stress een rol kunnen spelen in de pathofysiologie van delirium.

In toenemende mate wordt er gesuggereerd dat er mogelijk verschillen bestaan in biologische markers tussen verschillende groepen patiënten met een delirium. Om te onderzoeken of er verschillen bestaan, werden in **hoofdstuk 2.4** plasma spiegels van neopterine, homovanillinezuur, aminozuren en aminozuurratio's van acuut zieke patiënten met een delirium, die opgenomen waren bij de afdeling geriatrie, vergeleken met die van patiënten met een delirium na een electieve hartoperatie. In acuut zieke patiënten met een delirium werden statistisch significant hogere neopterine spiegels en hogere fenylalanine/tyrosine ratio's gevonden dan in patiënten met een delirium na een electieve hartoperatie. Daarnaast werden er grote verschillen gevonden in factoren die de neopterine spiegel bepaalden. In acuut zieke patiënten met een delirium was CRP de sterkste determinant van de neopterine spiegel, terwijl de geschatte glomerulaire filtratiesnelheid de sterkste determinant van de neopterine spiegel was in patiënten met een delirium na een electieve hartoperatie. Deze bevindingen suggereren dat neopterine in acuut zieke patiënten met een delirium voornamelijk bepaald wordt door een onderliggende ontstekingsreactie of oxidatieve stress, terwijl in patiënten met een delirium na een electieve hartoperatie, neopterine waarschijnlijk voornamelijk bepaald wordt door de nierfunctie of de vullingsstatus van de patiënt. Deze resultaten suggereren dat de pathofysiologie van delirium mogelijk verschilt tussen groepen patiënten met een delirium.

In **hoofdstuk 3** staat de mogelijke relatie tussen geneesmiddelen en delirium centraal. In **hoofdstuk 3.1** werd de mogelijke invloed van het gebruik van acetylsalicylzuur op

neopterine en tryptofaan onderzocht bij patiënten met en zonder delirium. Er werden geen verschillen gevonden in neopterine en tryptofaan spiegels tussen patiënten die wel en geen acetylsalicylzuur gebruikten. Wel leken bij patiënten met een delirium de neopterine spiegels lager en de tryptofaan spiegels hoger bij mensen die acetylsalicylzuur gebruikten dan bij patiënten die dit middel niet gebruikten, maar dit verschil was niet statistisch significant.

In **hoofdstuk 3.2** werd het mogelijke verband tussen het gebruik van anticholinerge medicatie en delirium bij opname, opnameduur, institutionalisering na ontslag en overlijden in het ziekenhuis, onderzocht. Het gebruik van anticholinerge medicatie bij opname werd vastgesteld met drie anticholinerge scoringslijsten, namelijk de Anticholinergic Risk Scale (ARS), de Anticholinergic Cognitive Burden Scale (ACB) en de lijst van Chew et al. (Chew). Er werden grote verschillen gevonden in de mate van blootstelling aan anticholinerge medicatie wanneer gebruik werd gemaakt van de drie verschillende lijsten. Daarnaast werden met de ARS, de ACB en de Chew niet dezelfde associaties met de onderzochte uitkomstmaten gevonden. Patiënten die volgens de ARS anticholinerge medicatie gebruikten, hadden een verhoogde kans op een delirium bij opname en een verhoogde kans om ontslagen te worden naar een instelling. Aangezien zowel een delirium als institutionalisering geassocieerd zijn met een slechte prognose, zijn vervolgstudies nodig om te onderzoeken of de ARS een bruikbaar hulpmiddel is om bij ouderen de kans op negatieve uitkomsten te verminderen.

In **hoofdstuk 4** worden de belangrijkste bevindingen van dit proefschrift bediscussieerd en worden suggesties voor vervolgonderzoek gepresenteerd. De resultaten van dit proefschrift laten zien dat een inadequate reactie van het immuunsysteem, oxidatieve stress, een verhoogde kwetsbaarheid van het brein en een verstoring in de serotonerge neurotransmissie waarschijnlijk allemaal een rol spelen in de pathofysiologie van delirium bij acuut zieke oudere patiënten. Daarnaast suggereren de resultaten dat bij acuut zieke ouderen mogelijk andere markers en mechanismen een rol spelen in de pathofysiologie van delirium dan bij ouderen die een electieve hartoperatie hebben ondergaan. Deze bevinding onderstreept het belang om bij verschillende populaties onderzoek te doen naar delirium. Verder hebben we laten zien dat ouderen die volgens de ARS anticholinerge medicatie gebruiken een verhoogde kans hebben op een delirium bij opname in het ziekenhuis. Met de ARS kan makkelijk en snel worden vastgesteld of iemand anticholinerge medicatie gebruikt en dus is de ARS mogelijk een bruikbaar hulpmiddel om patiënten te identificeren die een verhoogd risico hebben op het ontwikkelen van een delirium. Vervolgstudies zijn nodig om te onderzoeken of medicatiereviews waarbij aanvullend wordt gecontroleerd op medicatie van de ARS lijst, kunnen helpen bij het terugdringen van delirium bij ouderen.

Appendices

DANKWOORD

Dit dankwoord is dan wel één van de laatste hoofdstukken van mijn proefschrift, maar is daarom zeker niet minder belangrijk. Ik weet niet of het ooit onderzocht is, maar ik gok dat het dankwoord het meest gelezen hoofdstuk van een proefschrift is. Eigenlijk is dat ook wel te begrijpen, want promoveren doe je niet alleen. Via dit dankwoord wil ik dan ook iedereen bedanken die heeft bijgedragen aan de totstandkoming van dit proefschrift. Een aantal mensen wil ik graag in het bijzonder bedanken, want zonder hun hulp, kennis, suggesties, waardevolle feedback en luisterende oren was dit promotietraject toch wel een zware opgave geworden.

Allereerst wil ik mijn copromotor en tevens supervisor van mijn masteronderzoek bedanken, Francesco Mattace-Raso. Beste Francesco, op 5 juli 2012 rond kwart over drie hebben wij elkaar voor het eerst ontmoet. Op dat moment wisten wij geen van beiden dat deze dag een zeer uitdagende wending aan onze levens zou gaan geven. Ik zal deze dag in ieder geval nooit vergeten! Ik had vlak voor onze ontmoeting te horen gekregen dat het onderzoeksproject van mijn eerste keus al vergeven was en toen vertelde jij mij héél enthousiast dat je nog wel een delirium-project had. Ik deelde jouw enthousiasme totaal niet, want ik had van te voren besloten dat ik geen onderzoek wilde doen dat ook maar IETS met hersenen te maken had. Gelukkig is nee bij mij niet altijd nee en besloot ik om mij gedurende 6 maanden met dit project bezig te houden. Een keus met verstrekkende gevolgen, want op 6 mei 2013, toen ik mijn verslag kwam bespreken, liet je al vrij snel de vraag vallen of ik geen promotieonderzoek wilde doen. Ik had geen idee waar ik aan begon en jij volgens mij ook niet (Weet je het zeker, Angelique? Weet je het *echt* zeker? Moet je niet eerst een promotie bijwonen?), maar ik bleef dit keer bij mijn besluit, waar jij en ik nog steeds heel blij mee zijn! Natuurlijk was het tijdens het promotietraject niet altijd rozengeur en maneschijn. Jouw Italiaanse temperament en mijn Rotterdamse directheid konden soms flink botsen en er zijn genoeg momenten geweest waarop ik je graag achter het behang had geplakt (wederzijds ☺), maar zonder jou was het mij waarschijnlijk nooit gelukt om dit traject te doorlopen. Je stond én staat altijd voor me klaar, verkeerde soms in meer spanning dan ik wanneer we op een reactie van een tijdschrift zaten te wachten en gelukkig schreeuwde je het nog net niet van de daken hoe blij en trots je was als we weer iets moois hadden bereikt. En terwijl ik dit allemaal schrijf, hoor ik je zeggen 'less is more', dus ik ga afronden.

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Angelique

ABOUT THE AUTHOR

Angelique Egberts was born on the 4th of April 1990 in Rotterdam, the Netherlands. After her graduation from secondary school (Gymnasium, Citycollege St. Franciscus, Rotterdam) in 2008, she studied Pharmacy at the Utrecht University, the Netherlands. For her Master's thesis she conducted a research project aimed at biomarkers of delirium within the section of Geriatric Medicine at the Erasmus Medical Center in Rotterdam. Her thesis was nominated for the Vliegenthart Thesis Award 2014 (Utrecht University). The results of her research project are described in the current thesis. In 2014 she received her master's degree and started (or continued) her PhD research at the department of Internal Medicine, section of Geriatric Medicine at the Erasmus Medical Center, Rotterdam.

LIST OF PUBLICATIONS

International publications

Egberts A, Wijnbeld EHA, Fekkes D, van der Ploeg MA, Ziere G, Hooijkaas H, van der Cammen TJM, Mattace-Raso FUS. Neopterin: a potential biomarker for delirium in elderly patients. Dement Geriatr Cogn Disord. 2015;39(1-2):116-24.

Egberts A, Fekkes D, Wijnbeld EHA, van der Ploeg MA, van Saase JLCM, Ziere G, van der Cammen TJM, Mattace-Raso FUS. Disturbed serotonergic neurotransmission and oxidative stress in elderly patients with delirium. Dement Geriatr Cogn Dis Extra. 2015;5(3):450-8.

Egberts A, Fekkes D, Ziere G, van der Cammen TJM, Mattace-Raso FUS. Potential influence of aspirin on neopterin and tryptophan levels in patients with a delirium. Geriatrics. 2016;1(2), 10; doi:10.3390/geriatrics1020010

Egberts A, van der Craats ST, van Wijk MD, Alkilabe S, van den Bemt PMLA, Mattace-Raso FUS. Anticholinergic drug exposure is associated with delirium and postdischarge institutionalization in acutely ill hospitalized older patients. Pharmacol Res Perspect. 2017;5(3):e00310

Egberts A, Mattace-Raso FUS. Increased neutrophil-lymphocyte ratio in delirum: a pilot study. Clin Interv Aging. 2017;12:1115-1121.

National publications

Egberts A, Mattace-Raso FUS. Anticholinerge medicatie en delirium. Psyfar. Invited paper. Accepted for publication.

PhD PORTFOLIO

Summary of PhD training and teaching	
Name PhD student:	Angelique Egberts MSc
Erasmus MC Department:	Internal Medicine – Geriatric Medicine
PhD period:	2012 – 2017
Promotor:	Prof. J.L.C.M. van Saase
Supervisor:	Dr F.U.S. Mattace-Raso

1. PhD training	Year
General courses	
- Biostatistical methods I: basic principles (NIHES)	2016
- CPO course – Patient Oriented Research: design, conduct and analysis	2016
- Time management	2016
- Systematic literature retrieval in PubMed and other databases	2016
- BROK – Basic Course on Regulations and Organization for Clinical Investigators	2015
- Research Integrity	2015
- Good Clinical Practice (ICH-GCP)	2015
- Endnote	2015
- Analytical Laboratory Techniques	2012
Clinical courses	
- Pharmacotherapy in older persons (Ephor), Utrecht (NL)	2015-2016
Oral presentations	
- **Science Meeting Geriatric Medicine, Rotterdam (NL)** De neutrofielen-lymfocyten ratio: een toekomstige marker voor delirium?	2017
- **National congress of the Dutch Geriatrics Society, 's-Hertogenbosch (NL)** Anticholinerge medicatie en klinische uitkomsten in acuut zieke patiënten	2017
- **Symposium Frail Elderly, Aafje Schiehoven, Rotterdam (NL)** Anticholinerge belasting en delirium in de klinische praktijk	2016
- **Science Meeting Geriatric Medicine, Alkmaar (NL)** Anticholinerge belasting en delirium in de klinische praktijk	2015
- **Scientific Meetings Internal Medicine, Erasmus MC, Rotterdam (NL)** Potential biomarkers for delirium	2014
- **Scientific Meetings of the Alzheimercenter Rotterdam (NL)** Delirium In the Old – Potential biomarkers for delirium	2014
Poster presentations	
- **Science Days Internal Medicine, Erasmus MC, Antwerp (B)** Increased neutrophil-lymphocyte ratio in delirium: a pilot study	2018
- **Science Days Internal Medicine, Erasmus MC, Antwerp (B)** Anticholinergic drugs and clinical outcomes in older persons	2017

- **National congress of the Dutch Geriatrics Society, 's-Hertogenbosch (NL)** Disturbed serotonergic neurotransmission and oxidative stress in patients with delirium	2016
- **Science Days Internal Medicine, Erasmus MC, Antwerp (B)** Potential biomarkers for delirium	2014

2. Teaching	**Year**
Supervising Master's theses	
- Saskia T. van der Craats, pharmacy student, Utrecht University (NL)	2015-2016
- Melissa D. van Wijk, pharmacy student, Utrecht University (NL)	2015-2016
- Shams Alkilabe, pharmacy student, Utrecht University (NL)	2015

3. Others	**Year**
Awards and nominations	
- **Runner-up Science Prize – Dutch Geriatrics Society** National congress of the Dutch Geriatrics Society, 's-Hertogenbosch (NL)	2017
- **Runner-up Vliegenthart Thesis Award** Utrecht University (NL)	2014
Organization of research meetings Geriatric Medicine, Erasmus MC, Rotterdam (NL)	2015-