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Inflammation in older subjects with early- and late-onset depression in the NESDO study: a cross-sectional and longitudinal case-only design

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\textsuperscript{e}Amsterdam UMC, Vrije Universiteit Amsterdam, GGZ inGeest / Department of Psychiatry and Amsterdam Public Health Research Institute, Amsterdam, The Netherlands
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\textbf{ARTICLE INFO}

Keywords: Depression, Late-onset, Old age, Inflammation, CRP, Cytokines

\textbf{ABSTRACT}

Objective: Different biological mechanisms may underlie depression beginning in early life (early-onset) and depression beginning later in life (late-onset). Although the relation between inflammation and depression has been studied extensively, the distinct role of inflammation in early and late-onset depression in older patients has not been addressed before. In the cross-sectional part of this study, we explored differences in levels of circulating inflammatory markers and cytokine levels in lipopolysaccharide (LPS) stimulated whole blood between older subjects with a late-life onset depression (\geq 60 years) and older subjects with an early-onset depression (< 60 years). Secondly, in a 2-year follow-up study, we examined if circulating and stimulated inflammatory markers influenced the change in Inventory of Depressive Symptomatology (IDS) scores, and if this relation was different for early- and late-onset depression.

Methods: The study was part of the Netherlands Study of Depression in Older Persons (NESDO). We included 350 patients, all aged 60 and older, with a depressive episode in the previous 6 months: 119 with a late-onset depression and 231 with an early-onset depression. Blood samples were collected and CRP, IL-6, NGAL, GDF15, and LPS plasma levels were determined and whole blood was LPS stimulated and cytokine levels IL-1\textbeta, IL-6, TNF\alpha, IFN\gamma, IL-10, and IL-1 receptor antagonist (IL-1ra) were determined.

Results: After adjustment for demographics, health indicators, and medication use, increased plasma CRP levels were more strongly associated with late-onset depression than early-onset depression (OR [95% CI]: 1.43 [1.05–1.94]). In the longitudinal analyses, higher circulating IL-6 levels were associated with a significantly slower decline in IDS scores in the crude and the adjusted models (p < 0.027). This relation was not different between late- and early-onset depression. Other circulating and stimulated inflammatory markers were not associated with late- and/or early-onset depression.

Conclusions: This study provides preliminary evidence that low-grade inflammation is more strongly associated with late-onset than early-onset depression in older adults, suggesting a distinct inflammatory etiology for late-onset depression. Cytokine production capacity did not distinguish between early- and late-onset depression.

1. Introduction

Depression in old age is common with a prevalence of approximately 15% (Power et al., 2016). Although the prevalence of depression appears to remain unchanged over age (World Health Organization, 2016; Luppa et al., 2012), it has been suggested that depression that begins early in life (early-onset) is etiologically different from depression beginning at higher ages (late-onset depression) (Brodaty et al., 2001). Exploring the etiological differences between early-onset and late-onset depression is clinically relevant, given the potential implications for depression treatment at higher ages. So far, the only well-replicated etiological difference between early- and late-onset...
The Netherlands: Amsterdam, Leiden, Groningen, Nijmegen, and
the previous six months according to the DSM-IV criteria) were re-

years and older. Details on inclusion and methods are described in more

course and consequences of depressive disorders in persons aged 60

2. Material and methods

lesser extent with stimulated cytokine production.

chomotor retardation, disturbed sleep, and social withdrawal (Miller

fl

rise in both the peripheral in

and Raison, 2016; Slavich and Irwin, 2014). With higher age there is a

rise in both the peripheral inflammatory activity, as evidenced by in-

creased level of circulating inflammatory markers (Bruunsgaard et al.,

2001), as well as increased inflammatory activity in brain, which is

reflected by elevated numbers of activated microglial cells and in-

creased levels of cerebral pro-inflammatory markers (Sparkman and

Johnson, 2008). Whether this age-related shift towards a pro-in-

flammatory state is pertinent for late-onset depression has not been

addressed before (Alexopoulos and Morimoto, 2011; Morimoto and

Alexopoulos, 2011).

The purpose of this study is to explore potential differences in peripheral inflammatory markers between early- and late-onset de-

pression. No official consensus exists as to which cut-off age distin-

guishes early- from late-onset depression. A cut-off of 60 years has however been adopted in the majority of studies performed since the

1980’s and we classified early- and late-onset depression accordingly

(Ulrich et al., 2013). We divided a well-characterized population of

patients with depression derived from The Netherlands Study of De-

pression in Older persons (NESDO) in two groups: a group with a late-

onset depression (at 60 years or later) and a group of subjects of

comparable age with an early-onset depression (before age 60) as a

comparison. The aim of our study was twofold. In the cross-sectional

part of this study, we explored if levels of circulating inflammatory

markers and cytokine levels in lipopolysaccharide (LPS) stimulated

whole blood were different between older subjects with a late-life onset depression (≥60 years) and those with an early-onset depression (<60 years). Secondly, in a 2-year follow-up study, we examined if circu-

lating and stimulated inflammatory markers influenced the change in

Inventory of Depressive Symptomatology (IDS) scores, and if this

relation was different for early- and late-onset depression. In contrast to

circulating inflammatory markers, LPS stimulated cytokine production capacity is under tight genetic control (De Craen et al., 2005).

Considering the diminished importance of genetic factors in the develop-

ment of late-onset depression, we postulated that late-onset depression was mainly associated with circulating inflammatory markers and to a lesser extent with stimulated cytokine production.

2. Material and methods

2.1. Participants

NESDO is a multi-site naturalistic cohort study, aiming to study the course and consequences of depressive disorders in persons aged 60 years and older. Details on inclusion and methods are described in more
detail elsewhere (Comijs et al., 2011). In short, from 2007 until 2010, 378 depressed patients aged 60–93 years (depression diagnoses within the previous six months according to the DSM-IV criteria) were re-
cruited from mental healthcare settings (n = 326) and general practi-
tioner settings (n = 52). Subjects were recruited from five regions in The Netherlands: Amsterdam, Leiden, Groningen, Nijmegen, and Apeldoorn/Zutphen.

Excluded were persons with a Mini Mental State Examination score (MMSE) below 19, a primary diagnosis of dementia according to the clinician, severe mental disorders other than unipolar depressive dis-
order (primary psychotic disorders and bipolar disorders), and in-
sufficient command of the Dutch language. Out of 378 participants, those with a fever during the week before blood drawing were excluded from the analyses in the current study because of the disturbing effect on the whole blood cytokine production capacity (three subjects with late-
onset depression and eleven subjects with early-onset depression), eight subjects were excluded because age of onset was unknown, six subjects were excluded because no serum samples were available (three subjects with late-onset depression and three subjects with early-onset depression). In total 231 depressed patients with an onset before the age of 60 years and 119 patients with an onset at or after the age of 60 years were included in the cross-sectional and longitudinal analyses.

2.2. Inflammatory markers (exposure)

Fasting blood samples of the participants were obtained on the morning of the day of the clinical interview to minimize circadian variation at the different recruitment sites. Blood samples were im-

mediately transferred to a local laboratory where it was aliquotted and

kept frozen at −80 °C until transfer to the Clinical Chemistry depart-

ment of the VU University Medical Center. C-reactive protein (CRP) levels were determined in a high-sensitivity immunoturbidimetric assay

(Tina-quant CRP, Roche Diagnostics, Mannheim, Germany) at the Clinical Chemistry department of the VU University Medical Center. Intra-
and inter-assay coefficients of variation of the CRP test were both

2%. Plasma IL-6 levels were assayed using a high sensitivity ELISA

(PeliKine Compact ™ ELISA, Sanquin, Amsterdam, The Netherlands) at

the Pathology department of the VU University Medical Center. Intra-
and inter-assay coefficients of variation for IL-6 were 8% and 12%,

respectively. Quantification of NGAL from plasma was performed via a

constructed sandwich ELISA using human Lipocalin-2/NGAL ELISA (R&D Systems) according to the manufacturers’ protocol. Plasma was di-

luted 1:100. The intra- and inter-assay coefficients of variation were 3%

and 5%, respectively. In order to obtain a normal distribution of the plasma NGAL concentrations, eight positive outliers were trimmed at the mean level plus three SD (60.246 + 3 × 24.836), resulting in an

acceptable skewness of 1.58 and kurtosis of 0.76. GDF-15 was analyzed

on a novel automated assay on Abbott Architect, with a reported limit

of detection of 10 pg/mL. Reagents were provided by Abbott. Assay

linearity analysis was established by dilution of two samples in five
increments up to 16-fold. To test inter-assay reproducibility, 7 samples

were measured on 2 days, yielding a coefficient of variation of 4.9%.

The intra-assay coefficient of variation was 3.8%, obtained by analysis

of three plasma samples in 2–3 replicates, in three independent mea-

surements. Recovery analysis was measured by three concentrations of

spiked calibrator protein. The mean recovery was 109% (n = 29).

Whole blood samples were used to determine the capacity of white

blood cells to produce cytokines (ng/mL) upon ex vivo stimulation with lipopolysaccharide (LPS)(Van Der Linden et al., 1998). In short, within

30–60 minutes after collection of blood in heparinized tubes, E. coli-

derived LPS (Sigma; L-2654) was added (10 pg/ml) to 3 ml of the blood

sample, where after the samples were incubated for 5 h at 37 °C and 5% CO2. After centrifugation (1500x g; 21 °C; 20 min.), the supernatants

were aliquoted at the different recruitment sites and stored at −80 °C at

the Clinical Chemistry department of the VU University Medical Center, until they were assayed for cytokine levels. Cytokine levels in the sti-
lulated heparin blood samples were determined by Mesoscale Dis-

covey (MDS; Rockville, MD, USA), using a combination of 4-plex (hu IL-6, IL-8, IL-10 and TNFα; V-plex Panel II), 2-plex (hu IFNγ + IL-1β; V-

plex Custom hu cytokine) as well as single plex (hu IL-1ra; MS96) assay

kits. Two heparin blood samples were stimulated with LPS identical to the patient samples, and multiple aliquots were stored at −80 °C and
used as an internal control in each Mesoscale run (QC1 and QC2). The inter-assay variation (%CV) for QC1 and QC2 in 14 runs, were 138% and 7.7% for IL-1β; 120% and 8.3% for IL-6; 131% and 6.6% for IL-8; 7.5% and 107% for IFNγ; 138% and 7.9% for TNFα; 26.1% and 12.0% for IFN-γ; 5.9% and 5.6% for IL-10. Lab technicians were blinded to any knowledge of clinical data.

2.3. Depression (outcome)

Diagnosis of depression, lifetime prevalence of depression, and age of onset of first depressive episode, all according to DSM-IV-R criteria, were obtained using the Composite International Diagnostic Interview conducted by trained psychologists and mental health care nurses. The CIDI is a structured clinical interview that is designed for use in research settings and has high validity for depressive disorders (Wittchen et al., 1991). Early- and late-onset depression were defined as a unipolar depressive disorder of which the first episode was respectively diagnosed before age 60, or at age 60 or older. Severity of depression was assessed using the Inventory of Depressive Symptomatology Self-Rated (IDS-SR) (Rush et al., 1996). All items were rated on a scale from 0 (symptom is not present) to 3 points (strongest impairment). In the self-rated version (IDS-SR) a cut-off point of 18 or above indicates the presence of clinically relevant depressive symptomatology. During follow-up participants were invited to fill in the IDS-SR at 6 months intervals. In the present study we used the IDS-SR scores at 6, 12, 18, and 24 months. Number of participants with early-onset and late-onset depression for whom follow-up measurements were available was respectively 208 and 101 (6 months), 185 and 83 (12 months), 186 and 85 (18 months), 180 and 87 (24 months). When analyses were restricted to depression present within the preceding month the numbers were 167 and 76 (6 months), 150 and 61 (12 months), 148 and 62 (18 months), and 145 and 68 (24 months).

2.4. Covariates

We considered the following characteristics at baseline as potential confounders of the relation between inflammatory markers, and, early- and late-onset depression: sex, age, BMI, educational level, depression severity, physical activity level, smoking, alcohol consumption, number of chronic diseases, use of Selective Serotonin Reuptake Inhibitors (SSRI) and Non-Selective Monoamine Reuptake Inhibitors use (“Tricyclic Antidepressants” (TCA), and anti-inflammatory medication. BMI was calculated as weight (in kilograms) over height (in centimeters) squared. Physical activity was estimated using the International Physical Activities Questionnaire (IPAQ) (Craig et al., 2003). Self-reported smoking behavior was measured with standard questionnaires on past and current smoking behavior. (Daily) alcohol consumption was quantified using the Alcohol Use Disorders Identification Test (AUDIT) (Babor et al., 1989). The presence of chronic diseases was assessed by means of a self-report questionnaire that has previously been used in Longitudinal Aging Study Amsterdam (LASA) (Kriegsman et al., 1996). Participants were asked whether they currently had or previously had any of the following chronic diseases or disease events: heart disease (including myocardial infarction), peripheral atherosclerosis, stroke, diabetes mellitus, COPD (asthma, chronic bronchitis or pulmonary emphysema), arthritis (rheumatoid arthritis or osteoarthritis), cancer, or any other disease. Information on SSRI use (Anatomical Therapeutic Chemical Classification System (ATC) code N06AB), TCA use (N06AA), and anti-inflammatory medication (including amino salicylic acid and similar agents (A07EC), anti-allergic agents (A07EB), systemically applied corticosteroids (H02A, H02B), as well as anti-inflammatory and anti-rheumatic products (M01)), was obtained by observation of the medication that the participants brought in.

2.5. Statistical analyses

Statistical analyses were conducted in SPSS® version 22. P values < 0.05 were regarded significant. Data were analyzed following a “complete case analysis” approach, which means that if a participant had a score missing for a particular variable, then their data were only excluded from analyses involving that particular variable. Differences between the two groups regarding the fraction of males, educational level, smoking, daily alcohol consumption, medication use, number of chronic somatic diseases, and somatic morbidity were tested using a Pearson Chi-square test. Differences between the two groups regarding depression severity, age of onset, physical exercise, body mass index, ankle/brachial-index, and levels of inflammatory markers (CRP, circulating IL-6, and stimulated IL-1β, IL-6, IL-8, TNFα, IFNγ, IL-1ra, and IL-10) were non-parametrically tested using an independent samples Mann-Whitney U test.

The relation between early- and late-onset depression, and, inflammatory markers was assessed using a binomial logistic regression. The dichotomous variable early-onset and late onset depression was included as a dependent variable and inflammatory markers were entered as independent variables. Preliminary (visual) inspection of the data provided evidence that variation in the cytokine levels existed between the recruitment sites. To take account of this variation, we included recruitment site as a dummy variable in all analyses. All inflammatory markers were first log-transformed to approach a normal distribution and subsequently transformed into Z-scores.

Covariates were entered block-wise as follows: sex, age, BMI (model 1); sex, age, BMI, educational level (categorical variable: basic, intermediate, high), depression severity, physical activity level, smoking (categorical variable: current smoking, non-smoking), alcohol consumption (continuous variable), number of chronic diseases, SSRI use (dichotomous variable: present, absent), TCA use (dichotomous variable: present, absent), anti-inflammatory medication (dichotomous variable: present, absent) (model 2).

Linear mixed models were fitted to assess the effect of inflammatory markers on the longitudinal relation between time and IDS score. To account for the correlation in the data as a consequence of the repeated measurement design, the patient identification number was included as a random factor, and random intercept and random slope were tested for improvement of the model’s goodness of fit. A random intercept only model provided the best fit. IDS scores were entered as dependent variables and follow-up time was included as an independent variable. To analyze the effect of inflammatory markers on the relation between time and IDS scores, Z-scores of inflammatory markers and the interaction term follow up time × Z-score inflammatory marker were included in the model. Subsequently, covariates were entered block-wise as described above with early-/late-onset depression as an additional covariate in model 1 and model 2. All covariates were included as fixed factors. To assess whether the relation between Z-scores of inflammatory markers and IDS scores over time were different for early-onset and late-onset depression we included the 3-way interaction term follow up time × Z-score inflammatory marker × early/late onset depression.

2.6. Additional analyses

We performed various additional analyses. We hypothesized that heightened inflammatory activity is a state aspect, as opposed to trait aspect, of depression. In our study, depression was defined as a depressive episode in the 6-month period before blood sampling. It is possible that at the time of blood sampling the depressive episode already was in full or partial remission in some of the patients, which would lead to an underestimation of the association between inflammatory activity and depression. To address this issue, we repeated the cross-sectional and longitudinal analyses described above with baseline values from a subset of cases that experienced a depression within the preceding month (n = 276, of which 184 patients had an
Table 1
Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Depression characteristics</th>
<th>Early-onset depression</th>
<th>Late-onset depression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID3 score (points)</td>
<td>22.41 ± 4.2</td>
<td>23.75 ± 4.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Mood subscale</td>
<td>6.14 ± 1.0</td>
<td>4.1 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Motivation subscale</td>
<td>3.8 ± 1.8</td>
<td>2.7 ± 2.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Somatic subscale</td>
<td>7 ± 1.4</td>
<td>6 ± 1.3</td>
<td>0.071</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>50.5 ± 10.9</td>
<td>70.5 ± 7.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2 shows the cross-sectional relation between inflammatory markers and late-onset depression compared to early-onset depression. In the adjusted models, increased levels of CRP were significantly more strongly associated with late-onset than with early-onset depression. This association remained significant when analyses were restricted to subjects with a depressive episode within the preceding month (Supplementary Table 1). Results did not materially change after exclusion of participants with CRP levels > 10 mg/ml (data not shown). Higher levels of GDF15 were more strongly associated with late-onset than early-onset depression, but this association was no longer significant after adjustment for age, sex, and BMI.

CRP levels are typically stratified into low (< 3 mg/L), intermediate (3–10 mg/L), and high risk (10 mg/L). We examined the cross-sectional association between CRP, and late- and early-onset depression using these cut-off values. Fig. 1 shows the cross-sectional relation between the increasing categories of CRP and late-onset depression compared to early-onset depression. Intermediate levels of categorized CRP were stronger related with late-onset depression than early-onset depression in all models. This association was less pronounced for the highest category of CRP levels. Restriction of the analyses to participants with a depressive episode in the preceding month did not importantly influence the results (Fig. 1B).

Table 3 shows the effect of inflammatory markers on the relation levels using according to the clinical reference values into low (< 3 mg/L), intermediate (3–10 mg/L) and high levels (> 10 mg/L) and repeated the cross-sectional and longitudinal analyses. Finally, we excluded participants with CRP levels > 10 mg/L, which could indicate infection (20 participants with early-onset depression and 12 participants with late-onset depression).

3. Results

3.1. Baseline characteristics of study population

Table 1 shows the characteristics of subjects with early-onset (first episode of depression before age 60 (mean 37.5 years)) and late-onset depression (first episode at or after age 60 (mean 70.6)). Subjects with an early-onset depression were more often male and were on average younger than subjects with a late-onset depression. IDS scores were on average higher in early-onset depression than late-onset depression. The use of TCA and SSRI was different between the group with early- and late-onset depression. Use of SSRI declined during a two year follow-up with 46.5% and 52.0% in respectively the early-onset and late-onset group. TCA use declined during a two-year follow-up with 22.3% in the early-onset group and 22.2% in the late-onset group. The two groups with early- and late-onset depression were similar regarding the number of chronic somatic diseases. GDF15 levels were higher in the group with late-onset depression. All other stimulated and circulating inflammatory markers were similar between the two groups.

3.2. Cross-sectional relation between inflammatory markers and late-onset depression compared to early-onset depression

Table 2 shows the cross-sectional relation between inflammatory markers and late-onset depression compared to early-onset depression. In the adjusted models, increased levels of CRP were significantly more strongly associated with late-onset than with early-onset depression. This association remained significant when analyses were restricted to subjects with a depressive episode within the preceding month (Supplementary Table 1). Results did not materially change after exclusion of participants with CRP levels > 10 mg/ml (data not shown). Higher levels of GDF15 were more strongly associated with late-onset than early-onset depression, but this association was no longer significant after adjustment for age, sex, and BMI.

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3.3. Longitudinal relation between inflammatory markers and IDS scores in late-life depression

We also assessed the influence of inflammatory markers on the longitudinal relation between time and IDS scores during a two-year follow-up. After a two-year follow-up, data were available for 180 participants with an early-onset depression and 87 with a late-onset depression. The fully adjusted mean decrease in Inventory Depression Rating Scale was 0.95 points per 6 months (95% CI: -1.47 − -0.44; p < 0.001) for the late-onset group. In the early-onset group there was an excess decline of 0.31 points per 6 months, yet this excess decline was not significantly different (95% CI: -0.94 − 0.32; p = 0.33). Table 3 shows the effect of inflammatory markers on the relation...

Unless stated otherwise, data are presented as median values with interquartile range. *IDS: Inventory of Depressive Symptomatology.
The production of IL-1ra was associated with a significantly slower decline in IDS scores during follow-up. A higher stimulated IL-6 was associated with a slower decline in IDS scores in the fully adjusted model. However, this relation was no longer significant in the fully adjusted model (Supplementary Table 3). All interactions between time, inflammatory markers, and, early- and late-onset depression were non-significant (data not shown).

4. Discussion

4.1. Main findings

The aim of this study was twofold. First, in a cross-sectional setting, we examined the relation between levels of circulating and stimulated inflammatory markers and late-onset depression (≥ 60 years) compared to early-onset depression (< 60 years). Secondly, we investigated the influence of inflammatory markers on the relation between IDS scores over time during a two-year follow-up and assessed whether this association was different between late- and early-onset depression. In the cross-sectional analysis, we found that after adjustment for demographics, health indicators, and medication use, higher CRP levels were more strongly associated with late-onset than early-onset depression. When CRP levels were categorized according to clinical reference values, CRP levels between 3-10 mg/L were significantly more strongly associated with late-onset than early-onset depression, whilst CRP levels > 10 mg/L were not. Other inflammatory markers showed no association with late- or early-onset depression. In the longitudinal analyses, higher circulating IL-6 levels were associated with a slower decline in IDS scores during follow-up. This relation was not different between late- and early-onset depression. Other circulating and stimulated inflammatory markers were not associated with change in IDS score over time.

4.2. Cross-sectional association between inflammatory markers, and, early- and late-onset depression

Meta-analyses have confirmed a relation between circulating inflammatory markers and depression, although this association is not consistent in all studies and for all cytokines (Howren et al., 2009; Irwin et al., 2003; Young et al., 2014). Previously, the Netherlands Study of Depression in Adults (NESDA), a study comparable to the NESDO study regarding design, recruitment methods, and determinants, examined the association between presence of depression and inflammation in a large middle-aged cohort of depressed persons and non-depressed controls. This study found higher levels of CRP among middle-aged male with a depression (Vogelzangs et al., 2012). This difference was mostly driven by cases with a higher age of depression onset. The present study adds to these earlier observations by showing that late-onset depression was associated with higher CRP levels when compared to early-onset depression. Elevated serum levels of NGAL, a newly discovered adipokine, have previously been found in age-related disorders like cardiovascular diseases, chronic kidney disease (Boligano et al., 2010; Nuade et al., 2013). Recently NGAL was found to be associated with late-life depression, but only in a subgroup of patients with pathologically increased waist circumference (Nuade et al., 2013). GDF15, an inflammatory protein from the Tumor Growth Factor-β family, was elevated in subjects with a late-onset depression, although after correction for life-style characteristics this association was no longer significant (Teunissen et al., 2016). Comparably, in our study the relation between NGAL, GDF15 and late-onset depression was confounded by age, sex and, weight.

Our findings seemingly agree with an accumulating body of evidence suggesting that elevated CRP is involved in late-onset depression. Two longitudinal studies found that elevated serum levels of CRP at higher ages were predictive of incident depression (Luikinen et al., 2010; Van Den Biggelaar et al., 2007), which suggests a causal role of CRP in the pathogenesis of late-onset depression. In our study, moderately elevated CRP levels (3–10 mg/L) were distinctive of late-onset

Table 2

Association between inflammatory markers and late-onset depression compared to early-onset depression.

<table>
<thead>
<tr>
<th></th>
<th>Crude OR (95% CI)</th>
<th>Model 1 OR (95% CI)</th>
<th>Model 2 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Circulating inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (≥ 23)</td>
<td>(0.98–1.54)</td>
<td>(1.08–1.81)</td>
<td>(1.05–1.94)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.95</td>
<td>0.97</td>
<td>0.93</td>
</tr>
<tr>
<td>(0.69–1.30)</td>
<td>(0.69–1.34)</td>
<td>(0.64–1.38)</td>
<td></td>
</tr>
<tr>
<td>NGAL</td>
<td>1.20</td>
<td>1.05</td>
<td>1.11</td>
</tr>
<tr>
<td>(0.95–1.51)</td>
<td>(0.81–1.83)</td>
<td>(0.82–1.50)</td>
<td></td>
</tr>
<tr>
<td>GDF15</td>
<td>1.34</td>
<td><strong>0.011</strong></td>
<td>1.10</td>
</tr>
<tr>
<td>(1.07–1.68)</td>
<td>(0.87–1.49)</td>
<td>(0.78–1.53)</td>
<td></td>
</tr>
<tr>
<td><strong>Stimulated inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.93</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>(0.69–1.27)</td>
<td>(0.71–1.37)</td>
<td>(0.73–1.64)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.95</td>
<td>0.97</td>
<td>0.83</td>
</tr>
<tr>
<td>(0.69–1.30)</td>
<td>(0.69–1.34)</td>
<td>(0.64–1.38)</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>1.03</td>
<td>1.10</td>
<td>0.56</td>
</tr>
<tr>
<td>(0.77–1.38)</td>
<td>(0.80–1.51)</td>
<td>(0.78–1.89)</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>0.89</td>
<td>0.90</td>
<td>0.53</td>
</tr>
<tr>
<td>(0.65–1.20)</td>
<td>(0.65–1.25)</td>
<td>(0.59–1.25)</td>
<td></td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.89</td>
<td>0.92</td>
<td>0.55</td>
</tr>
<tr>
<td>(0.70–1.14)</td>
<td>(0.70–1.21)</td>
<td>(0.64–1.22)</td>
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</tr>
<tr>
<td>IL-1α</td>
<td>1.00</td>
<td>0.98</td>
<td>0.88</td>
</tr>
<tr>
<td>(0.77–1.31)</td>
<td>(0.74–1.30)</td>
<td>(0.68–1.43)</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>1.03</td>
<td>0.92</td>
<td>0.64</td>
</tr>
<tr>
<td>(0.74–1.44)</td>
<td>(0.65–1.31)</td>
<td>(0.69–1.61)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as crude and estimated odds ratios (OR) for late- versus early-onset depression with 95% confidence intervals (95% CI) per standardized increase (z-scores) in log transformed inflammatory marker. Model 1 was adjusted for sex, age, and BMI. Model 2 is similar to model 1, additionally adjusted for education level, IDS score at baseline, physical activity level, smoking, alcohol consumption, number of chronic diseases, use of SSRI, TCA, and anti-inflammatory medication. Numbers of participants included in the analyses with late-onset and early onset respectively was: 228/117 for CRP, 227/117 for IL-6, 231/118 for NGAL, 230/118 for GDF15, 227/117 for stimulated IL-1β, IL-6, IL-8, TNFα, IFNγ, IL-1α, and IL-10.

between time and IDS scores. The interaction term for time and circulating IL-6 was positive in both the crude and the adjusted models, indicating that increased levels of IL-6 were associated with a slower decline in IDS scores during follow-up. A higher stimulated IL-6 was associated with a slower decline in IDS scores in the fully adjusted model. However, when the analyses were restricted to subjects with a depression within the preceding month, the interaction between time and IL-1α was no longer significant (Supplementary Table 2).

Results did not materially change after exclusion of participants with CRP levels > 10 mg/ml (data not shown). We also assessed whether the relation between inflammatory markers and change in IDS score was different for early- and late-onset depression. Most interactions between time, inflammatory markers, and, early- and late-onset depression were non-significant (p > 0.05), signifying that the effect of inflammatory markers on the change of IDS scores was not different for early- and late-onset depression. An exception was the interaction between time, GDF-15 and early/late-onset depression in those with a depression within the preceding month (p = 0.041).

Additionally, we assessed the longitudinal relation between categorized levels of CRP (low (< 5 mg/L), intermediate (3–10 mg/L), and high (> 10 mg/L)) and change in IDS score over time. The mean IDS score diminished during follow-up (all p values < 0.001). The interaction term between categories of CRP and time was not significant in the crude and the adjusted models, signifying that CRP levels did not influence the change in IDS score over time. When we restricted the analyses to participants with a depressive episode in the preceding month, the highest CRP category showed a significantly slower decline in IDS score when compared to the lowest CRP category. However, this relation was no longer significant in the fully adjusted model (Supplementary Table 3). All interactions between time, inflammatory markers, and, early- and late-onset depression were non-significant (data not shown).
depression, while levels above 10 mg/l were not. This finding may further point towards a role of subclinical inflammation in the development of late-onset depression, which is marked by moderately elevated inflammatory markers, while excessively high levels of CRP are typically due to infections and/or traumatic events. However, considering that other inflammatory markers were not different between the early- and late-onset depression, a cautious interpretation of the association between CRP levels and late-onset depression is warranted. The relation between CRP and late-onset depression in our study was independent of conventional determinants of heightened inflammatory activity, such as body mass index, smoking, and somatic morbidity. This raises the question what factors underlie the increased CRP levels in old-age depression. An additional explanation may be the relation between social stressors and inflammation (Berk et al., 2013). Perceived danger signals elicit the release of norepinephrine by the sympathetic nervous system. By acting on primary and secondary lymphoid organs norepinephrine promotes peripheral inflammatory activity (Irwin and Cole, 2011). This evolutionary conserved mechanism presumably facilitates recovery from physical injury, by activating the immune system in advance of an assault that could lead to a pathogen-related infection (Slavic and Irwin, 2014). Activation of this pathway is not confined to physical dangers, but also to (psychosocial) stress (Audet et al., 2014) (Rohleder, 2014). A higher number or more severe form of social stressors, for example social isolation or loneliness, among the participants with a late-onset depression could hypothetically account for the higher circulating inflammatory markers in this group.

A paucity of studies that investigated the relation between stimulated cytokine production capacity and depression yielded mixed results: two studies report a higher cytokine production capacity (Seidel et al., 1995; Vogelzangs et al., 2016), another study observed no difference in production capacity between depressed subjects and healthy controls (Irwin et al., 2003). We did not find a difference in the stimulated production capacity of inflammatory cytokines between early- and late-onset depression. To our knowledge, this is the first study examining differences in stimulated cytokine production capacity between early- and late-onset depression.

4.3. Longitudinal association between inflammatory markers and, early- and late-onset depression

Higher levels of circulating IL-6 were predictive of a lower decline in IDS scores during follow-up. This aligns with the findings from a previous study in the same cohort, which showed that metabolic syndrome, a disorder marked by a pro-inflammatory state, negatively affected the prognosis of depression in older people (Marijnissen et al., 2017). In our study, CRP levels were associated with late-onset depression in the cross-sectional analyses. However, CRP levels were not predictive of changes in depression severity. This could imply that CRP is a determinant for the development of depression, but is not associated with the course of depression. This agrees with the observation from population-based cohorts that CRP is predictive of incident depression (Luukinen et al., 2010).

**Fig. 1.** Cross-sectional association between categorized C-reactive protein (CRP) levels and late-onset depression compared to early-onset depression (A) for all participants and (B) restricted to participants with a depressive episode within the preceding month. CRP levels were stratified into low (<3 mg/L), intermediate (3–10 mg/L), and high levels (30 mg/L). Data are presented as crude and estimated odds ratios (OR) for late-over early-onset depression with 95% confidence intervals (95% CI) for the different categories of CRP (mg/L). Model 1 is adjusted for sex, age, and BMI. Model 2 is similar to model 1 and additionally adjusted for education level, IDS at baseline, physical activity level, smoking, alcohol consumption, number of chronic diseases, use of SSRI, use of TCA, and anti-inflammatory medication. Numbers of participants included in the analyses with late-onset and early onset respectively was: 158/69 (< 3 pg/ml), 50/36 (3–10 pg/ml), 20/12 (> 10 pg/ml) (figure A); and 125/52 (< 3 pg/ml), 38/26 (3–10 pg/ml), 19/12 (> 10 pg/ml) (figure B).

<table>
<thead>
<tr>
<th>CRP (mg/l)</th>
<th>Odds ratio (95% CI)</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;3</td>
<td>&lt;3</td>
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<tr>
<td></td>
<td></td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Crude</td>
<td></td>
<td>3-10</td>
<td>3-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.67 (1.00 - 2.80)</td>
<td>2.17 (1.22 - 3.87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.45 (0.67 - 3.15)</td>
<td>1.85 (0.80 - 4.28)</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reference</td>
<td>reference</td>
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<tr>
<td></td>
<td></td>
<td>3-10</td>
<td>3-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.17 (1.22 - 3.87)</td>
<td>2.67 (1.31 - 5.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.85 (0.80 - 4.28)</td>
<td>2.40 (0.88 - 6.52)</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-10</td>
<td>3-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.67 (1.31 - 5.44)</td>
<td>2.84 (1.23 - 6.57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.40 (0.88 - 6.52)</td>
<td>3.19 (1.08 - 9.41)</td>
</tr>
</tbody>
</table>
4.4. Methodological considerations

A major strength of the present study is the methodological design.

Cytokine stimulation assays require fresh blood samples. Therefore, a case-control design is preferred over a longitudinal design, considering that a longitudinal design requires a lengthy follow-up before a sufficient number of outcomes have occurred. A challenge inherent to the case-control design is the selection of appropriate controls. Choosing subjects conditioned on the presence or absence of a disease, inevitably introduces the risk of selecting other characteristics that are closely associated with the disease or its absence. To solve this challenge we selected patients with a late-onset depression and compared them to subjects of comparable age with an early-onset depression. Thus, cases and controls have the same clinical diagnosis, and are therefore more likely comparable in other aspects that could confound the relation between inflammation and depression. A number of limitations of our study should be considered. A case-control design is the selection of appropriate controls. Choosing subjects conditioned on the presence or absence of a disease, inevitably introduces the risk of selecting other characteristics that are closely associated with the disease or its absence. To solve this challenge we selected patients with a late-onset depression and compared them to subjects of comparable age with an early-onset depression. Thus, cases and controls have the same clinical diagnosis, and are therefore more likely comparable in other aspects that could confound the relation between inflammation and depression.

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A number of limitations of our study should be considered. A

Data are presented as crude and estimated change in Inventory of Depression Severity (IDS) score with 95% confidence intervals (95% CI) per standardized increase (z-scores) in log transformed inflammatory marker. Model 1 was adjusted for sex, age, BMI and early-/late-onset depression. Model 2 is similar to model 1, additionally adjusted for education level, IDS score at baseline, physical activity level, smoking, alcohol consumption, number of chronic diseases, use of SSRI, TCA, and anti-inflammatory medication. Numbers of participants included in the analyses with late-onset and early onset respectively was: 228/117 for CRP, 227/117 for IL-6, 231/118 for NGAL, 230/118 for GDF15, 227/117 for stimulated IL-1β, IL-6, IL-8, TNFα, IFNγ, IL-1ra, and IL-10.

Table 3
Longitudinal association between inflammatory markers and IDS scores in late-life depression during 2 year follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Crude Change IDS (95% CI)</th>
<th>p</th>
<th>Model 1 Change IDS (95% CI)</th>
<th>p</th>
<th>Model 2 Change IDS (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Depression within preceding 6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Circulating inflammatory markers</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CRP</td>
<td>0.18 (−1.24 to 1.59)</td>
<td>0.81</td>
<td></td>
<td>−0.31 (−1.75 to 1.31)</td>
<td>0.67</td>
<td>−0.32 (−1.47 to 0.84)</td>
</tr>
<tr>
<td>Time x CRP</td>
<td>0.12 (−0.16 to 0.40)</td>
<td>0.39</td>
<td></td>
<td>0.12 (−0.15 to 0.40)</td>
<td>0.38</td>
<td>0.08 (−0.27 to 0.43)</td>
</tr>
<tr>
<td>IL-6</td>
<td>−0.51 (−1.93 to 0.91)</td>
<td>0.48</td>
<td></td>
<td>−0.75 (−2.17 to 0.68)</td>
<td>0.30</td>
<td>−0.67 (−1.88 to 0.54)</td>
</tr>
<tr>
<td>Time x IL-6</td>
<td>0.36 (0.08−0.64)</td>
<td>0.011</td>
<td>0.36 (−0.08−0.64)</td>
<td>0.11 (−0.42 to 0.91)</td>
<td>0.42</td>
<td>0.42 (0.05−0.80)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.48 (−0.95 to 1.92)</td>
<td>0.51</td>
<td>0.57 (−0.90−2.04)</td>
<td>0.45</td>
<td>−0.26 (−1.42 to 0.91)</td>
<td>0.67</td>
</tr>
<tr>
<td>Time x NGAL</td>
<td>0.08 (−0.22 to 0.37)</td>
<td>0.61</td>
<td>0.07 (−0.22 to 0.36)</td>
<td>0.63</td>
<td>−0.07 (−0.43 to 0.29)</td>
<td>0.71</td>
</tr>
<tr>
<td>GDF15</td>
<td>1.05 (−0.39 to 2.48)</td>
<td>0.15</td>
<td>1.22 (−0.29 to 2.73)</td>
<td>0.11</td>
<td>−0.27 (−1.43 to 0.89)</td>
<td>0.65</td>
</tr>
<tr>
<td>Time x GDF15</td>
<td>0.33 (0.03−0.63)</td>
<td>0.029</td>
<td>0.33 (0.03−0.63)</td>
<td>0.030</td>
<td>0.26 (−0.09 to 0.61)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Stimulated inflammatory markers

IL-1β 0.72 (−1.11 to 2.55) 0.44 0.61 (−1.22 to 2.44) 0.47 0.51 (−1.77 to 0.82) 0.17

Time x IL-1β 0.16 (−0.13 to 0.45) 0.29 0.16 (−0.14 to 0.45) 0.29 0.26 (−0.10 to 0.64) 0.58

Time x IL-6 0.72 (−1.14 to 2.59) 0.45 0.49 (−1.37 to 2.34) 0.61 0.36 (−1.62 to 0.90) 0.89

Time x GDF15 0.14 (−0.16 to 0.44) 0.85 0.14 (−0.16 to 0.44) 0.35 0.31 (−0.05 to 0.67) 0.91

IL-8 1.00 (−0.74 to 2.74) 0.26 0.72 (−1.10 to 2.45) 0.42 0.46 (−1.88 to 0.95) 0.52

Time x TNFα 0.22 (−0.07 to 0.50) 0.14 0.21 (−0.07 to 0.50) 0.14 0.25 (−0.16 to 0.65) 0.24

TNFα 0.68 (−1.19 to 2.54) 0.48 0.33 (−1.53 to 2.19) 0.63 0.30 (−1.55 to 0.95) 0.64

Time x TNFα 0.07 (−0.22 to 0.37) 0.63 0.07 (−0.22 to 0.37) 0.63 0.23 (−0.13 to 0.59) 0.21

IL-1ra 0.50 (−0.95 to 1.95) 0.50 0.27 (−1.25 to 1.79) 0.73 0.00 (−1.15 to 1.15) 1.00

Time x IL-1ra 0.19 (−0.48 to 0.10) 0.19 0.19 (−0.47 to 0.11) 0.22 0.06 (−0.41 to 0.29) 0.74

IL-10 0.80 (−0.83 to 2.42) 0.34 0.53 (−1.11 to 2.17) 0.52 0.55 (−1.79 to 0.69) 0.38

Time x IL-10 0.28 (−0.01 to 0.60) 0.062 0.28 (−0.01 to 0.58) 0.061 0.47 (0.10−0.84) 0.012

Time x IL10 1.52 (−0.40 to 3.44) 0.12 1.67 (−0.24 to 3.57) 0.086 0.14 (−1.16 to 1.45) 0.83

Time x IL10 0.01 (−0.30 to 0.27) 0.41 0.01 (−0.30 to 0.27) 0.92 0.19 (−0.17 to 0.56) 0.30
corollary to selection of early-onset depression among persons aged 60 and older, is that many subjects in the early-onset group are likely to have had a first episode before the age of 60. Thus, recurrent depressive disorders may be overrepresented in the group with an early-onset depression. Moreover, selecting older subjects with an early-onset depression carries the risk of preferential inclusion of patients with a chronic depression. Information on the number of recurrences and duration of the depressive episode were however not available for our study.

4.5. Conclusion and clinical implications

The view that depression represents a single disease entity is increasingly hard to entertain. More plausible is that the diagnosis depression encompasses a variety of disorders each with a distinct etiology or partly overlapping etiologies, which ultimately converge into a common clinical presentation as measured in our descriptive diagnostic system. The present study provides preliminary support for low-grade inflammation, but not cytokine production capacity, as a distinctive characteristic of late-onset depression in older adults. Since inflammation is already amenable to intervention, this study could eventually contribute to the improved treatment, and possibly prevention, of late-onset depression.

Ethics

The study protocol of NESDO has been approved centrally by the Medical Center, University Medical Center Groningen and the Radboud University Medical Center in Nijmegen. Before participating in the study, all persons were provided with oral and written information. Written informed consent was obtained from all participants at the start of the baseline assessment.

Conflict of interest

The authors have no conflict of interests to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.08.029.

References


