Chapter 2

Interleukin, tumor necrosis factor-α and c-reactive protein profiles in melancholic and non-melancholic depression: A systematic review

Chenghao Yang, Kim M. Tiemessen, Fokko J. Bosker, Klaas J. Wardenaar, Jie Lie
and Robert A. Schoevers

J Psychosom Res. 2018 Aug;111:58-68
Abstract

Objective: The current diagnostic criteria for major depressive disorder (MDD) do not allow prediction of prognosis and therapeutic response. A possible strategy to improve this situation is the identification of depression subtypes on the bases of biomarkers reflecting underlying pathological processes such as neuro-inflammation.

Methods: The PubMed/Medline database was searched until Apr 25th, 2017. In the initial search 1018 articles were retrieved, which were subsequently screened and only selected when the inclusion and exclusion criteria were fulfilled.

Results: Eight eligible studies were found. Overall, serum interleukin-6 and 1β values were increased in the melancholic MDD subtype compared to controls and the non-melancholic MDD subtype. C-reactive protein was increased in non-melancholic MDD in 2 out of 4 studies, while there was no difference for tumor necrosis factor-α and interleukin-2 and 10.

Conclusion: Given the paucity of eligible studies the tentative conclusion must be drawn that peripheral inflammation markers have limited added value thus far to distinguish between melancholic and non-melancholic depression. To allow for a more definitive conclusion, further research is warranted using a broader panel of inflammatory markers in MDD subtypes, preferably based on a general consensus regarding diagnostic criteria and subtype definitions.
1. Introduction

Depression is one of the most prevailing illnesses in the world, with more than 300 million people falling under this category [1]. Major depressive disorder (MDD) has been estimated to account for a total of 63.2 million disability adjusted life years (DALYs) worldwide [2], making it a high cost burden for the society. MDD is a syndrome with a broad spectrum of varying symptoms. On the basis of symptom profiles the diagnostic statistical manual (DSM) has classified MDD into several clinical subtypes. However, field trials for DSM-5 mood disorders diagnoses have shown that 6-month test-retest reliability was poor to fair (kappa 0.20–0.39) for MDD [3] and even poor using the DSM-IV criteria [4]. So, attempts to improve the reliability of these diagnoses are called for. Moreover, such classification appeared to have little predictive power with respect to prognosis and treatment outcome [3–5]. Still, this is what the field has been working with for many years despite many trials to improve it. To the best of our knowledge, no such data is available regarding the subtypes of MDD, but as subtypes are mostly based on symptoms that are also assessed in MDD diagnosis they will probably be in the same range. A more fruitful approach could be a classification of MDD and its subtypes on the basis of underlying pathological processes. Arguably, this will provide a more rational and suitable basis for improving antidepressant treatment.

Several major hypotheses of pathophysiological processes involved in MDD have been raised in the past, including dysfunctions of the monoamine system, the immune-inflammatory system, the hypothalamic-pituitary-adrenal (HPA) axis and neurogenesis/neuroplasticity related processes. Previously we have proposed a theoretical model linking clinical presentations of depression to these pathophysiological processes [6]. The present review is focused on the immune-inflammation hypothesis. It postulates that monocytes, T-lymphocytes and cytokines are involved in the pathogenesis of MDD [7, 8]. According to this theory pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-1 (IL-1) play a key role in the control of neuro-endocrine and behavioral characteristics of MDD. Growing evidence suggests indeed that the pathophysiology of depression is associated with dysregulated inflammatory processes and cytokine imbalance [9–15]. Following this line of thought, research into a possible relation of peripheral inflammatory markers with subtypes of MDD might help to pave the way for a more physiologically oriented approach to diagnosis, prognosis and treatment outcome [16]. In addition, it could contribute to developing preventative measures and adjuvant pharmacological treatment strategies [17].

A challenging problem with biomarker research is the heterogeneous character of MDD [5]. Currently, most biomarker research involves patients with divergent symptom profiles. As a consequence, the results may be mixed and possibly delude one another. The biological dysregulations found in patients with MDD have indeed varied across studies [18, 19]. This variability could be due to differences in sample size and composition (such as age and ethnicity) or to methodological differences, but it might also be attributable to the heterogeneity of MDD [20]. It is thus important to identify biological correlates of MDD subtypes, which may also enable the identification of patients “at-risk” for MDD, for instance those with silent chronic inflammation, to enable preventative measures to be taken. Yet
attempts to predict antidepressant treatment response in the STAR*D and iSPOT-D trials [5, 21] on the basis of subtypes such as melancholic depression, atypical depression and anxious depression appeared far from successful. Moreover, both trials reported a considerable overlap between these subtypes while 25-33% of the patients could not be categorized through any of them. Given the generally poorer prognosis with the anxious form of depression [22], it can also be argued that this is not a subtype but a comorbid disorder with two distinct biological correlates. In terms of clinical subtypes, the only distinction that has remained over time is between melancholic and atypical depression. These subtypes have a different clinical presentation and may also differ in course and treatment outcome [23-25]. It is important to note here that atypical depression falls under non-melancholic depression The DSM classifications categorize atypical depression by means of specific symptoms, and it often has a chronic course [26, 27], which contrasts with what is often concerned as the typical melancholic form of depression. Both subtypes of depression are relatively common amongst patients diagnosed with MDD, with 15 to 30% of patients displaying atypical features [28, 29] and 25 to 30% displaying melancholic features [29]. Several studies have suggested that melancholic and atypical depression also differ in biological characteristics, which is promising as these two subtypes have remained relatively stable and distinct from one another over time [20, 30, 31]. The biomarkers investigated in this review include interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 beta (IL-1β), TNF-α, and C-reactive protein (CRP/hsCRP), and are all important players in the human immune system (see table 1). The aim of this systematic review is to investigate whether these peripheral markers provide relevant information regarding inflammatory processes in the melancholic and non-melancholic forms of depression.
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cytokine Receptor</th>
<th>Cytokine Source</th>
<th>Cytokine Targets</th>
<th>Cytokine Main Function</th>
<th>Cytokine Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>Has two sites of binding to IL1RI and IL1R-AcP</td>
<td>Macrophages, epithelial cells, many others</td>
<td>Macrophages, thymocytes, CNS, others</td>
<td>Inflammatory; promotes activation, costimulation, and secretion of cytokines and other acute-phase proteins; pyrogenic; kills a limited number of tumor cell types</td>
<td>↑ = inflammatory bone resorption; gout; promotes Th17 response; interaction with TNF-α involving in insulin resistance</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL1RI and IL1R-AcP</td>
<td>Macrophages, epithelial cells, many others</td>
<td>Macrophages, thymocytes, CNS, others</td>
<td>Inflammatory; contributes pain sensitivity; promotes activation, costimulation, and secretion of cytokines and other acute-phase proteins; pyrogenic</td>
<td>↑ = inflammatory bone resorption; gout; promotes Th17 response</td>
</tr>
<tr>
<td>IL-2</td>
<td>IL2Rα, IL2Rb, and IL2Rγ</td>
<td>T cells</td>
<td>T, B, NK cells, and macrophages</td>
<td>Proliferation; enhancement of cytotoxicity, IFNγ secretion, and antibody production</td>
<td>↓ = lymphoproliferative disease and susceptibility to autoimmune disease; reduced Treg development. ↑ = reduced Th17 development.</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL6Rα and gp130</td>
<td>Macrophages, T cells, fibroblasts, and others</td>
<td>Wide variety of cells: B cells, T cells, thymocytes, myeloid cells, osteoclasts</td>
<td>Inflammatory and costimulatory action; induces proliferation and differentiation; synergizes with TGFβ to drive Th17</td>
<td>↓ = deficient innate immunity and acute-phase responses, lymphopenia</td>
</tr>
<tr>
<td>IL-10</td>
<td>IL10R1 and IL10R2</td>
<td>Differentiated T helper cells, Tregs, B cells, dendritic cells, others</td>
<td>Macrophages, T cells, dendritic cells, B cells</td>
<td>Immune suppression; decreases antigen presentation and MHC class II expression of dendritic cells; down-regulates pathogenic Th1, Th2, and Th17 responses</td>
<td>↓ = immune pathology due to uncontrolled inflammation. ↑ = inhibits sterile immunity to some pathogens.</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TNFR1 (CD120a) and TNFR2 (CD120b)</td>
<td>Macrophages, CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons</td>
<td>Most tissues in the body (TNFR1) and cells of the immune system (TNFR2).</td>
<td>Regulation of immune cells; inducing fever, cell apoptosis, cachexia, and inflammation; inhibition of oncogenesis and viral replication; responding to sepsis via IL1 &amp; IL6 producing cells.</td>
<td>↑ = promotes the inflammatory response. This causes many of the clinical problems associated with autoimmune disorders. Also induces fever, cell death, and shock-like symptoms. ↓ = induces cachexia.</td>
</tr>
<tr>
<td>CRP/hsCRP</td>
<td>Phosphocholine on the surface of dead or dying cells and some bacteria; FcγRI, FcγRIIa, and FcγRIIb</td>
<td>Synthesized by the liver in response to biomarkers released by macrophages and adipoocytes</td>
<td>Damaged cells, dead cells, complement system, bacteria</td>
<td>Activates the complement system, promoting phagocytosis of dead or dying cells (or bacteria) by macrophages.</td>
<td>↑ = Increasing phagocytosis and release of cytokines; binding to damaged membranes; increasing clearance of apoptotic cells; masking autoantigens from the immune system or enhancing their clearance.[32]</td>
</tr>
</tbody>
</table>

Taken and adapted from http://www.sinobiological.com/What-are-Interleukins-a-6072.html, with added information. IL-1α was only included in this overview to show the many similarities with IL-1β with respect to source, receptors, targets and main functions, but this cytokine was not specifically assessed in any of the papers.
2. Materials and Methods

The database used was Pubmed (Medline). The search string included the following terms: ((((((((("Biological Markers"[Mesh]) OR "C-Reactive Protein"[Mesh]) ) OR "Interferon-gamma"[Mesh]) OR "Interleukins"[Mesh])) OR "Tumor Necrosis Factor-alpha"[Mesh]) ) OR (biomarker*[tw] OR "inflammatory marker*"[tw] OR c-reactive protein[tw] OR CRP[tw] OR high-sensitive CRP[tw] OR hsCRP[tw] OR interferon gamma[tw] OR interleukin[tw] OR tumor necrosis factor[tw])) AND ("Depressive Disorder, Major"[Mesh] OR atypical depress*[tw] OR melanchol*[tw]).

The PubMed search was performed on Apr 25th 2017, and yielded 1018 articles (8 studies in non-human species were excluded; see figure 1 below). The titles and abstracts of the articles were scanned to see if they met the inclusion criteria. If there were any doubts whether an article should be included or not, the whole text was read. Previous review studies, including meta-analyses, were not used for this review, but their reference list was scanned for articles that might have been missed by the PubMed search. Articles that primarily focused on somatic diseases (such as cardiovascular disease, cancer or autoimmune disease) with co-morbid depression were also excluded from the study. An exception to this exclusion criterion was made for depression with co-morbid anxiety disorder as these very often co-occur [33]. It is important to note that only studies reporting baseline serum values of biomarkers were taken into consideration, thus excluding challenge studies to assess the cytokine production capacity. Some antidepressants can alter the immune response [34, 35]. Yet we have also included studies wherein part of the patients was treated with antidepressants, as long as co-variates analyses indicated that antidepressant treatment did not appreciably influence the outcome. Finally, it was required that studies included both melancholic and non-melancholic subtypes in relation to biomarker levels. References in all included studies were screened for cross references of eligible studies possibly missed by the PubMed search. The articles were then evaluated whether useful information was provided regarding inflammatory processes in the two subtypes. It is also important to note that a study not making a distinction between IL-1α and IL-1β has been excluded in this respect [36]. Finally, on the basis of the reported sample size, mean value and standard deviation Forest plots were constructed showing the Hedges’g effect sizes for the markers. Given the small sample size of some of the studies (n<20) we have used the Hedges’g formula instead of the simpler one from Cohen.
3. Results

In the 8 studies eligible for analysis, 6307 persons were included. In total, 5455 controls were compared to 852 MDD patients. Most studies used the symptom-based DSM-IV criteria to diagnose MDD and to define the subtypes, although a few used alternative methods such as the sign-based CORE measure, which assesses psychomotor and neuroendocrine disturbances instead of symptoms. Other assessments included the Diagnostic Interview for Genetic Studies (DIGS) combined with the General Health Questionnaire-12 items (GHQ-12), the Composite International Diagnostic Interview-version 2.1 (CIDI-2.1) or Latent Class Analysis (LCA). A summary of the results can be found in Table 2, while the statistically significant findings are summarized in the text below. Only 6 out of 8 studies were suitable to construct Forest plots, depicting the Hedges’g effect sizes. These are shown in figure 2 together with the number of patients, mean values and standard deviations.

![Figure 2: Forest plots depicting the differences between controls, melancholic and atypical (non-melancholic) patients for 6 out 8 studies, including number of patients, mean values and standard deviations. Notably, the Hedges’g effect sizes of IL-1 from the study by Maes et al. (38) are also included, but since it was not specified whether it concerned IL-1α or IL-1β these data were not used in the final evaluation.](image)
**IL-2 in melancholic and non-melancholic depression**

Spanemberg et al. found no statistically significant difference between melancholic and non-melancholic groups for IL-2 (p>0.05) [37]. Overall, there is no tendency for IL-2 to be increased in patients suffering from non-melancholic MDD (including atypical depression).

**IL-6 in melancholic and non-melancholic depression**

Dunjic-Kostic et al. found a tendency for increased IL-6 levels in melancholic patients [38]. The serum concentration of IL-6 was found to be higher in the melancholic subtype compared to the atypical subtype, although Fisher’s least significant difference (LSD) showed no difference in IL-6 between the two groups [38]. The only statistically significant difference was between melancholic depression and controls (p<0.05) [38].

Karlovic et al. did not find statistically significant differences in IL-6 concentrations between the melancholic group and the atypical group when multinominal logistic regression was used (p>0.003) [39]. However, levels were significantly higher in the melancholic group when compared to controls (p<0.003) [39]. Atypical IL-6 levels versus controls were not significant (p>0.003) [39].

Lamers et al. found that IL-6 levels were elevated in atypical depression when compared to melancholic depression and to healthy controls [30]. However, when multivariate models were run with adjustment for BMI, the differences between the groups were no longer significant [30]. The atypical and melancholic subtypes were identified based on a LCA rather than on DSM criteria and only those classified as having severe MDD were studied.

Spanemberg et al. found similar data, and also reported no differences in IL-6 concentrations between melancholic and non-melancholic subtypes identified using the CORE measure (p>0.05) [37]. MDD IL-6 levels were significantly higher than controls (p<0.05), levels in melancholic depression were significantly higher than controls (p<0.05), and non-melancholic versus controls levels were not significantly different (p>0.05) [37].

Glaus et al. found that IL-6 levels did not differ in healthy controls, atypical subtype and melancholic subtype (p>0.05) [40], also after adjustment for comorbid disorders, diabetes, smoking, BMI, selective serotonin reuptake inhibitors (SSRI), mood stabilizers, antipsychotics (p>0.05) [40].

**IL-10 in melancholic and non-melancholic depression**

Huang et al. did not find differences in serum levels between melancholic patients and those with non-melancholic features for IL-10 when ANCOVA with age and BMI adjustment was used (F = 2.014; d.f. = 1,40; P = 0.165) [41]. The same was found for IL-10 levels in MDD versus controls (p > 0.05) [41], but there was no direct comparison between the subtypes of MDD and controls.

Spanemberg et al., similarly, found no statistically significant difference in IL-10 levels between melancholic and non-melancholic patients (p>0.05) [37]. Using DSM-IV criteria 33 patients were diagnosed as being depressed. Patients were classified as melancholic or non-melancholic using the CORE measure. This evaluates 18 observable features of melancholia on a 4-point scale, and measures its absence or presence. A CORE score of ≥8
was taken as determining melancholia. MDD IL-10 levels versus controls were also not found to be significantly different (p>0.05) [37].

**IL-1β in melancholic and non-melancholic depression**

Huang et al. found significantly higher serum levels of IL-1β in patients with melancholic features than in those with non-melancholic features after adjusting for age and BMI using analysis of covariance (ANCOVA) (F = 5.703; d.f. = 1,40; P = 0.023) [41]. Not mentioned in this study is how many females and males were included in the separate subtypes of MDD. IL-1β levels in MDD were not significantly different from controls (p>0.05) [41].

Glaus et al. found no statistically significant differences in IL-1β levels among healthy control, atypical subtype and melancholic subtype (p>0.05) [40], both before and after adjustment for comorbid disorders, diabetes, smoking, BMI, SSRI, mood stabilizers, antipsychotics (p>0.05) [40].

**TNF-α in melancholic and non-melancholic depression**

Dunjic-Kostic et al. used Fisher’s least significant difference (LSD) and found no significant difference in TNF-α levels between patients with melancholic MDD and patients with atypical MDD, although levels were slightly higher in the melancholic group [38]. There was a significant difference in TNF-α only between the atypical group and controls (p<0.05) [38].

Karlovic et al. found no statistically significant difference for TNF-α across melancholic and atypical MDD groups [39]. No significant difference was found with control groups either [39].

Huang et al. found no difference in TNF-α levels across melancholic and non-melancholic groups (p> 0.05) [41]. Analysis of covariance (ANCOVA) was used to perform data analysis, with adjustments for age and BMI for mean group differences. TNF-α levels in MDD versus controls were not significantly different (p> 0.05) [41]. MDD had significantly higher TNF-α levels than controls after correcting for BMI (p< 0.05). Melancholic versus non-melancholic groups showed no significant difference in TNF-α levels even after age and BMI were corrected for (p>0.05) [41].

Lamers et al., on the other hand, found TNF-α levels in the atypical depression group to be significantly higher than in the melancholic group when adjusted for age, sex, educational level and smoking (p<0.05) [30]. When multivariate models were used to correct for BMI, it still remained significant (P=0.01). The atypical and melancholic subtypes were identified based on a LCA rather than on DSM criteria, and only those classified as having severe MDD were studied [30]. Differences in TNF-α levels between the melancholic groups and controls were not significant (p> 0.05). Atypical TNF-α levels were significantly higher than controls (p<0.05), also after correction for BMI [30].

Maes et al. found serum TNF-α levels to be higher in melancholic patients than in non-melancholic patients [36]. TNF-α levels were significantly higher in MDD when compared to controls (p<0.05) [36]. There was some disparity in the subtyping, where normal MDD was used (which could be non-melancholic or typical). TNF-α levels in melancholic patients were significantly higher than normal MDD levels (P<0.05) [36].
Spanemberg et al. found no significant difference in TNF-α levels between the melancholic and non-melancholic groups (p>0.05) [37]. No significant difference was found for TNF-α levels in MDD versus controls (p>0.05) [37].

Glaus et al. found that TNF-α levels did not differ in healthy control, atypical subtype and melancholic subtype (p>0.05) [40], also after adjustment for comorbid disorders, diabetes, smoking, BMI, SSRI, mood stabilizers, antipsychotics (p>0.05) [40].

**CRP in melancholic and non-melancholic depression**

Karlovic et al. found no statistically significant difference in serum CRP concentrations between melancholic and atypical MDD groups [39]. No difference was found between the atypical versus control groups (p>0.003) or in the melancholic versus atypical group (p>0.03) [39].

In contrast, Lamers et al. found CRP levels to be higher in atypical depression than in melancholic depression (p<0.05) [30]. After adjustment for age, sex, educational level and smoking, atypical depression still retained higher CRP levels, although between-group differences were no longer significant when multivariate models with adjustment for BMI were used [30]. The atypical and melancholic subtypes were identified based on a LCA rather than on DSM criteria and only those classified as having severe MDD were studied. ANOVA was used to compare the subtypes, and effect sizes (Cohen’s d) were also calculated. Melancholic versus controls were not significant for CRP levels (p>0.05) [30]. CRP levels in the atypical group were significantly higher than in controls (p<0.05) [30].

Hickman et al. found that CRP levels in atypical depression were significantly elevated when compared to non-atypical depression and to healthy controls (p<0.05) [42]. Multiple linear regressions were used to examine the association between serum CRP levels and the subtypes of MDD (demographics-adjusted, confounder-adjusted and fully-adjusted). Logistic regression was also performed. After adjusting for potential confounders, BMI, and smoking, CRP levels remained significantly higher in atypical MDD than in either non-atypical MDD or healthy controls (p<0.05) [42].

Glaus et al. found that hsCRP levels were decreased in the melancholic subtype compared to healthy controls (p<0.05), but not after adjusting for dyslipidemia, diabetes, BMI, hypertension (p>0.05) [40]. There was no difference between the atypical subtype and healthy controls. No data was provided for the comparison between melancholic and atypical subtype [40]. A summary of the most important findings can be found in table 2 below.
### Table 2. Summary of results

<table>
<thead>
<tr>
<th>Author (ref. nr)</th>
<th>N (Male/Female)*</th>
<th>Age in years (mean ± s.d)</th>
<th>Classification of MDD (subtype composition)</th>
<th>Subtype definition</th>
<th>Cytokines measured</th>
<th>Results of study shown as concentration of cytokines (mean ± s.d)</th>
<th>Assay</th>
<th>Adjustment for confounders</th>
<th>Difference in results after adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Spanemberg et al, [37]</td>
<td>Total 87 (74.1% female)</td>
<td>C: 47.4 ± 9.97</td>
<td>DSM-IV to diagnose MDD;</td>
<td>M: the CORE score of patient with MDD ≥ 8;</td>
<td>IL-2, IL-6, IL-10, TNF-α</td>
<td>IL-2: NM=M=C; C: 0.25 ± 0.08 pg/ml; M: 0.24 ± 0.07 pg/ml; NM: 0.27 ± 0.12 pg/ml</td>
<td>Cytometry</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>C: 54 (74.1% female)</td>
<td>M: 52.8 ± 10.7</td>
<td>CORE to identify subtypes</td>
<td>NM: apart from those with melancholic depression</td>
<td>MDD &gt; C; M &gt; C; NM=C</td>
<td>IL-6: MDD &gt; C; M &gt; C; NM = C</td>
<td>C: 0.88 ± 0.69 pg/ml; M: 1.45 ± 2.55 pg/ml; NM: 1.28 ± 1.10 pg/ml</td>
<td>ELISA</td>
<td>No</td>
<td>25</td>
</tr>
<tr>
<td>M: 13 (76.9% female)</td>
<td>NM: 48.4 ± 7.7</td>
<td>(M vs NM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM: 20 (90.0% female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunjic-Kostic et al, [38]</td>
<td>Total 86</td>
<td>C: 49.90 ± 4.99</td>
<td>DSM-IV</td>
<td>M: melancholic features specifier of DSM-IV</td>
<td>IL-6, TNF-α</td>
<td>IL-6: M &gt; C; AD=C; AD=M; TNF-α: M=AD; AD &lt; C; MF-C</td>
<td>ELISA</td>
<td>Yes: Age, gender, BMI, smoking</td>
<td>No</td>
</tr>
<tr>
<td>C: 39 (17/22)</td>
<td>M: 50.28 ± 7.41</td>
<td>(M vs AD)</td>
<td>AD: atypical features specifier of DSM-IV</td>
<td>AD: melancholic features specifier of DSM-IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M: 29 (13/16)</td>
<td>AD: 52.26 ± 7.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD: 18 (8/10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Total</td>
<td>C</td>
<td>M</td>
<td>AD</td>
<td>MDD</td>
<td>NM</td>
<td>IL-6, TNF-α, CRP</td>
<td>ELISA &amp; Immunoturbidimetric assay</td>
<td>Age, gender, employment status, place of living, marriage status, smoking</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td>-----</td>
<td>----</td>
<td>------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Karlovic et al.</td>
<td>73</td>
<td>C: 18 (8/10)</td>
<td>M: 32 (22/10 or 20/12**)</td>
<td>AD: 23 (15/8)</td>
<td>M: 48.6 ± 8.1</td>
<td>AD: 50.9 ± 8.3</td>
<td>M &gt; C; AD = C; AD = M</td>
<td>Yes; Age, gender, employment status, place of living, marriage status, smoking</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C: 45.0 ± 9.5</td>
<td>M: 48.6 ± 8.1</td>
<td>AD: 50.0 ± 8.3</td>
<td>MDD: 15/8</td>
<td>NM: 34.7 ± 7.6</td>
<td>TNF-α: AD = M = C</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM-IV (M vs AD)</td>
<td>AD: melancholic features specifier of DSM-IV</td>
<td>AD: atypical features specifier of DSM-IV</td>
<td>IL-6: M &gt; C; AD = C; AD = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>ELISA &amp; Immunoturbidimetric assay</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-6: M &gt; C; AD = C; AD = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>ELISA &amp; Immunoturbidimetric assay</td>
<td>IL-6: M &gt; C; AD = C; AD = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-6: M &gt; C; AD = C; AD = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>ELISA &amp; Immunoturbidimetric assay</td>
</tr>
<tr>
<td>Maes et al.</td>
<td>57</td>
<td>C: 20</td>
<td>M: 12</td>
<td>NM: 25</td>
<td>MDD: 42.0 ± 11.0</td>
<td>NM: 34.7 ± 7.6</td>
<td>TNF-α: MDD &gt; C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM-IV (M vs NM)</td>
<td>MDD: 11/15</td>
<td>NM: 10.59 ± 3.91</td>
<td>NM: 24.9 ± 21.2 pg/ml</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>TNF-α: MDD &gt; C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 40.2 ± 8.0</td>
<td>NM: 34.7 ± 7.6</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>TNF-α: MDD &gt; C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NM: 25</td>
<td>NM: 17</td>
<td>NM: 24.9 ± 21.2 pg/ml</td>
<td>NM: 10.59 ± 3.91</td>
<td>TNF-α: MDD &gt; C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>82</td>
<td>C: 40 (15/25)</td>
<td>M: 25</td>
<td>NM: 17</td>
<td>MDD: 42 (12/30)</td>
<td>NM: 17</td>
<td>IL-10, IL-1β, TNF-α</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C: 31.4 ± 3.9</td>
<td>M: 40.2 ± 8.0</td>
<td>NM: 34.7 ± 7.6</td>
<td>NM: 24.9 ± 21.2 pg/ml</td>
<td>TNF-α: MDD &gt; C; NM = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM-IV (M vs NM)</td>
<td>MDD: 11/15</td>
<td>NM: 10.59 ± 3.91</td>
<td>NM: 24.9 ± 21.2 pg/ml</td>
<td>TNF-α: MDD &gt; C; NM = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 12.2 ± 5.9</td>
<td>NM: 24.5 ± 30.0 pg/ml</td>
<td>NM: 24.9 ± 21.2 pg/ml</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-10: MDD = C; NM = M</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>IL-1β: MDD = C; M &gt; NM</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
<td>CRP: M &gt; C; AD = C; M = AD</td>
</tr>
<tr>
<td>Study</td>
<td>Total</td>
<td>C: 41.3 ± 14.6</td>
<td>M: 40.2 ± 12.1</td>
<td>AD: 39.6 ± 12.1</td>
<td>M: characterized by decreased appetite, weight loss, suicidal thought and psychomotor changes</td>
<td>IL-6, TNF-α, CRP</td>
<td>IL-6: M=C; AD &gt; C; AD &gt; M</td>
<td>CRP: AD &gt; non-AD; AD &gt; C</td>
<td>M: melancholic features specifier of DSM-IV</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------------------------------------------------------------------------------------</td>
<td>-----------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>#Lamers et al, [30]</td>
<td>Total 776</td>
<td>C: 543 (60.6% female)</td>
<td>M: 40.2 ± 12.1</td>
<td>AD: 39.6 ± 12.1</td>
<td>M: characterized by decreased appetite, weight loss, suicidal thought and psychomotor changes</td>
<td>IL-6, TNF-α, CRP</td>
<td>IL-6: M=C; AD &gt; C; AD &gt; M</td>
<td>CRP: AD &gt; non-AD; AD &gt; C</td>
<td>M: melancholic features specifier of DSM-IV</td>
</tr>
<tr>
<td>#Hickman et al, [42]</td>
<td>Total 1,791</td>
<td>C: 29.0 ± 5.8</td>
<td>Non-AD: 30.1 ± 6.0</td>
<td>AD: 29.0 ± 5.1</td>
<td>AD: characterized by overeating and weight gain</td>
<td>CRP: AD &gt; non-AD; AD &gt; C</td>
<td>CRP: AD &gt; non-AD; AD &gt; C</td>
<td>CRP: AD &gt; non-AD; AD &gt; C</td>
<td>AD: characterized by overeating and weight gain</td>
</tr>
<tr>
<td>#Glaus et al, [40]</td>
<td>Total 3355</td>
<td>Not available</td>
<td>Age only provided in total controls and total MDD patients</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
<td>Digestive enzymes combined with GHQ-12 to diagnose MDD</td>
</tr>
</tbody>
</table>

NM: 0.7 ± 2.1 pg/ml

TNF-α.
in absolute numbers unless otherwise stated. If the study only included percentages for sex, a percentage is stated instead. Where sex was not known, left blank.

* No standard deviation given in study; interquartile range provided instead. Also no measurement units given in paper; assumed to be pg/ml.

** Error in original study: conflicting number of males and females.

Abbreviations: AD = atypical depression; C = controls; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; SSRI: selective serotonin reuptake inhibitors; ELISA = Enzyme-Linked Immunosorbent Assay; CIDI-2.1: Composite International Diagnostic Interview-version 2.1; LCA = latent class analysis; M = melancholic depression; NM = non-melancholic depression; DIGS = Diagnostic Interview for Genetic Studies; GHQ-12 = combined with General Health Questionnaire-12 items; CVRFs: cardiovascular risk factors, such as smoking, diabetes, overweight.

# Participants in study were not free from antidepressant medications at the time of sampling (baseline).

Legend:

=: no significant differences in biomarker levels between these groups (p>0.05)

>: biomarker levels were significantly higher in this group (p < 0.05)

<: biomarker levels were significantly lower in this group (p < 0.05)
4. Discussion

The aim of this review was to investigate whether melancholic and non-melancholic depression display different immune patterns, thus hinting at divergent underlying pathophysiological processes. The latter would argue for future investigations into biochemically oriented profiling of MDD subtypes.

Most of the recruited articles were published in the past 10 years, indicating that immunological research into MDD subtypes is relatively new. The lack of consensus regarding the definition of MDD subtypes and the limited number of inflammatory biomarkers investigated also affirms that this field of research is still far from mature. When evaluating the studies, no differences were found for IL-2 and IL-10 in any of them, indicating that these cytokines provide little information with respect to alleged differential inflammatory processes in MDD subtypes [37, 41]. Regarding IL-6, the first two studies found similar results; this cytokine was elevated only in the melancholic group compared to healthy controls [38, 39]. In both studies, clinical variables were also examined in relation to the biomarkers, with a negative correlation between IL-6 levels and the severity of depression being found in atypical MDD patients [38, 39]. However, there was a difference with respect to the melancholic patients. Whereas Karlovic et al. found increased IL-6 levels to be associated with a more severe form of disease in this MDD subtype; this was not the case in the other study [39]. Both studies measured severity of depression with the Hamilton Depression Rating Scale (HDRS), but while one study only included severely depressed patients (HDRS scores ≥ 17) [38], the other also included moderately depressed patients (HDRS scores ≥ 8) [39]. Arguably the discrepant findings for the melancholic patients in these studies can be partly attributed to the different severity spectrum being used. CRP levels in the melancholic group were elevated compared to the healthy controls, but not to the atypical group, indicating that this marker does not provide useful information regarding putative differential inflammatory processes in MDD subtypes [39].

Inflammation is an innate immune response, and both pro-inflammatory and anti-inflammatory cytokines have been investigated in studies of depression. Examples of pro-inflammatory cytokines are IL-6, IL-1β and TNF-α. When an immunologic challenge presents itself, these cytokines are produced by innate immune cells in response [43]. Pro-inflammatory cytokines are primarily mediated by the T-helper 1 (Th1) cells [8, 44-46]. Anti-inflammatory cytokines, on the other hand, counter the pro-inflammatory response by weakening the production of the latter’s cytokines or by acting as an antagonist at their respective receptors [7]. Pro-inflammatory cytokines stimulate peripheral cells (for example, hepatocytes) to produce acute phase proteins such as CRP [47]. These acute phase proteins are responsible for the systemic inflammatory reaction [47]. Anti-inflammatory cytokines are mainly mediated by Th2 cells, and include the cytokines IL-4, IL-5 and IL-10 [44-46]. Because pro- and anti-inflammatory cytokines influence each other it can be argued that chronic inflammation is associated with a general shift in balance between these cytokine families, but it may also have consequences for the relations within in each cytokine family, thus leading to immune dysregulation. It is important to note here that Dunjic-Kostic et al. reported an interesting relationship between the pro-inflammatory cytokines IL-6 and TNF-α [38]. When compared to healthy controls, IL-6 levels were increased in melancholic depression whereas TNF-α levels were decreased in atypical depression [38]. However, there
was a positive correlation between these cytokines only in the atypical MDD group [38]. It has been proposed that IL-6 and TNF-α levels might be inversely proportionate [48], with increased IL-6 levels suppressing TNF-α secretion. Accordingly, this mechanism might apply to the melancholic patients but not to the non-melancholic (atypical) patients [38]. This might point at an important biological difference between the two patient groups. However, it could also be an artifact and more research is warranted to confirm or rule out whether or not atypical depression has a different secretory mechanism for TNF-α, or whether or not the inversely proportionate relationship between IL-6 and TNF-α is consistently disturbed in this subtype. Two other studies have found TNF-α to be elevated in MDD only [36, 41], suggesting that this cytokine may play a role in the general psychopathology of MDD rather than of specific subtypes [41].

Spanemberg et al., who used the sign-based measure CORE instead of DSM-IV criteria, reported similar findings with a significant increase of IL-6 levels in melancholic depression compared to healthy controls and atypical patients [37]. This cytokine, however, was also elevated in all MDD patients, and in contrast with the study by Dunjic-Kostic and colleagues [38] no significant differences in TNF-α, IL-2 and IL-10 levels were found for all groups [37]. Interestingly, another study contradicted the aforementioned findings regarding IL-6 and TNF-α levels [30]. This study by Lamers et al. found both IL-6, TNF-α and CRP to be elevated in patients with atypical depression compared to patients with melancholic depression as well as healthy controls [30]. A possible confounding factor is the considerably more frequent intake of anti-inflammatory medication in the atypical and especially the melancholic group compared to the healthy control group in this study [30]. Another factor could be the use of LCA instead of DSM criteria to classify the subtypes [30]. The DSM-IV definition of atypical depression includes symptoms of mood reactivity and interpersonal sensitivity [20, 49], but the validity of these symptoms in the definition of this subtype is still somewhat controversial [27, 50-52]. Past LCA studies, on the other hand, have found appetite and weight to be the most discriminating symptoms [53, 54], and LCA is believed by some to be more accurate in discriminating the subtypes. Another explanation for the discrepant findings in the studies could connect to the considerable differences in sample size (776 persons in the Lamers study compared to less than 100 persons in all the other studies, see also table with the Forest plots in figure 2). Another factor could be differences in the composition of the clinical samples, especially with respect to depression severity [30, 55]. Because IL-6, TNF-α and CRP levels were increased in atypical depression only, and there were no significant differences between the melancholic and healthy control groups, this might suggest that a crucial role of inflammatory processes may be limited to the atypical depression subtype. It must be noted, however, that the Hedges’g effect sizes in the Lamers study are only modest. Hickman et al. further collaborate this idea by reporting similar findings for CRP [42]. These findings did not change after adjustment for potential confounders such as BMI, smoking, and anxiety disorders, indicating that these are not decisive factors [42]. Yet, another study reported that hsCRP levels were significantly decreased in the melancholic subtype but not in the atypical subtype compared with healthy control, but the effect was no longer significant after adjustment for cardiovascular risk factors (CVRFs), suggesting that smoking, diabetes, and overweight contributed to the hsCRP
abnormality in melancholic MDD [40]. Nonetheless, a correlation does not necessarily imply causation, and it could also be argued that increased systemic inflammation contributes to the development of atypical depression instead of inflammation being a result of developing this depressive subtype [42]. Clearly, sample composition may also be an important factor in this discussion, because patients in the Hickman study were considerably younger than in all of the other studies (around 30 years of age versus around 40-50 years). Since atypical depression has been shown to have an earlier age of onset than other forms of depression [56, 57], it is plausible that elevations in CRP levels had not yet occurred in this patient group [42]. As it stands, however, the findings of Hickman et al. suggest that increased inflammation may indeed be a characteristic that differentiates atypical from melancholic MDD, and that this inflammation is not related to comorbid anxiety disorders [42]. In conclusion, except for TNF-α in the Maes study, effect sizes for the peripheral inflammatory markers are only modest (see the Forest plots in figure 2). Given the many discrepant findings (see also the Forest plots) and the complexity of the subject, it is clear that many methodological problems have to be resolved before biochemical profiling of MDD subtypes will ever become a serious option. Other problems that have to be addressed are presented by depression with comorbid anxiety and bipolar disorder. The first has considerable overlap with both melancholic and atypical depression [5, 58]. The latter is sometimes difficult to distinguish from MDD especially during the first depressive episode. Both are likely to have biological correlates different from MDD, which may also have consequences for their immune patterns [59].

Limitations
The most important limitation of this study is related to the lack of consensus in MDD subtype definitions. Some studies made a distinction between melancholic and non-melancholic depression while others used the terms typical and atypical depression. Because melancholic depression and atypical depression display different symptom profiles we have categorized atypical depression under the umbrella term non-melancholic depression. This is a compromise to allow comparisons between the studies and it is clear that not everybody would agree given past discussions with respect to DSM-IV classifications for the subtypes of depression [50-52, 60]. Another complicating factor is that some studies compared subtypes directly, while others made comparisons with healthy controls. Moreover, some studies used sign-based methods such as LCA instead of symptom-based DSM criteria. LCA may have some advantages but it also has its own problems [55]. Furthermore, most studies only included inpatients regularly displaying a more severe form of MDD (with increased severity, comorbidity and disability) except for the study by Lamers et al. [30]. Finally, the limited number of articles for some of the investigated markers is a serious problem. For instance, only one article for IL-2 and two articles for IL-1β that matched the criteria were found [37, 40, 41], and one cannot seriously draw conclusions on whether or not these cytokines play a role in the pathophysiology of the subtypes of MDD based on one or two studies. Although it was not our aim to discover a biomarker panel capable of distinguishing between the subtypes, it should be mentioned here that ROC analyses are mandatory to assess sensitivity and specificity of a biomarker (panel). However, none of the recruited studies has performed ROC analyses making it impossible to include this kind of validation in the current review.
5. Conclusions and future work
Given the limited number of eligible studies, the inconsistent results and the mostly modest effect sizes the tentative conclusion must be drawn that thus far peripheral inflammation markers have limited value to distinguish between inflammatory processes in melancholic and non-melancholic depression. Future research should include longitudinal studies using more than one definition for MDD subtypes in their sample (i.e. DSM-IV, CORE, LCA). Although difficult to achieve, patients should preferably be medication-free, and biomarker profiles should be assessed under unstimulated conditions. If not feasible, the effects of medication could be analysed as covariate in substantially larger cohorts. Although unavoidable in the present review the use of the umbrella term non-melancholic depression should be avoided in future studies and replaced by more clearly defined subtypes such as atypical and psychotic depression. It is well possible that a relation between depression and inflammation is confined to the atypical subtype. However, for a more definitive conclusion to be drawn further research is warranted using a broader panel of inflammatory markers in MDD subtypes, preferably based on a general consensus regarding diagnostic criteria and subtype definitions. Given the problems encountered with current symptom-driven approaches, a viable alternative could be an entirely data-driven approach based on a comprehensive assessment of a broad range of symptoms, clinical variables, biomarkers and course or treatment response [55].
Acknowledgments:
The systematic study was supported by funds from the Tianjin Finance Bureau and Tianjin Key Programs for Science and Technology Development in Health Industry (grant number: No.13KG118).
References


35. Lanquillon, S., et al., Cytokine production and treatment response in major depressive disorder.


