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Immune system dysfunction and its link to the monoaminergic theory of Major Depressive Disorder: Implications and therapeutic approaches

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Barcelona, 15.11.2022

Place, Date

Gara Arteaga Henríquez

Signature doctoral candidate

Para aquellos que siempre han estado a mi lado
y que siempre llevo conmigo.

Table of content

List of abbreviations

MDD: major depressive disorder

ICD-10: tenth version of the World Health Organization International Classification of Diseases

DSM-5: fifth edition of the American Psychiatric Association Diagnostic and Statistical Manual of Mental disorders

5-HT: serotonin

NA: noradrenaline

DA: dopamine

TRP: tryptophan

CSF: cerebrospinal fluid

NMDA-R: *N*-methyl-D-aspartate receptor

IRS: inflammatory response system

KYN: kynurenine

KP: kynurenine pathway

3-HK: 3-hydroxykynurenine

QUIN: quinolinic acid

KYNA: kynurenic acid

PIC: picolinic acid

TDO: tryptophan 2,3-dioxygenase

IDO: indoleamine 2,3-dioxygenase

KMO: kynurenine monooxygenase

ICCGs: pro-inflammatory cytokine-chemokine genes

ISGs: interferon-stimulated genes

HC: healthy controls

IL: interleukin

hsCRP: high-sensitivity C reactive protein

BMI: body mass index

HAMD-17: Hamilton Depression Rating Scale, 17-item version

SR: suicide risk

SSRIs: selective serotonin reuptake inhibitors

EPA: eicosapentaeonic acid

ACMSD: aminocarboxymuconate-semialdehyde-decarboxylase

1. List of publications

1.1. Publication I:

Arteaga-Henríquez G, Burger B, Weidinger E, Grosse L, Moll N, Schuetze G, Schwarz M, Wijkhuijs A, Op de Beeck G, Berghmans R, Versnel MA, Arolt V, Müller N, Drexhage HA. Activation and deactivation steps in the tryptophan breakdown pathway in major depressive disorder: A link to the monocyte inflammatory state of patients. Progress in Neuropsychopharmacology and Biological Psychiatry. 2021 Apr 20; 107:110226. doi: 10.1016/j.pnpbp.2020.110226.

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1.2. Publication II:

Arteaga-Henríquez G[*], Simon MS[*], Burger B, Weidinger E, Grosse L, Wijkhuijs A, Arolt V, Birkenhager TK, Musil R, Müller N[+], Drexhage HA[+]. Low-Grade Inflammation as a Predictor of Antidepressant and Anti-Inflammatory Therapy Response in MDD Patients: A Systematic Review of the Literature in Combination with an Analysis of Experimental Data Collected in the EU-MOODINFLAME Consortium. Frontiers in Psychiatry. 2019 Jul 09; 10:458. doi: 10.3389/fpsyt.2019.00458.

[*]These authors share first authorship

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2. Your contribution to the publications

2.1. Contribution to publication I

Data from this study originate from the EU-MOODINFLAME and CITICOX projects, initiated in 2008, and finalized in 2013. When I started to collaborate in these projects, the positive ethical decisions were already granted, the study population recruited, and laboratory parameters assessed. As I already had five years of experience working as a psychiatrist, and as I had already completed a Master of Science in Research, I already had a great experience in recruitment processes, the configuration of informed consents, and in the administration of clinical questionnaires/scales before the initiation of this doctoral thesis.

My role in these studies consisted then in first, validate all data, and configurate all databases. For this purpose, I checked all clinical, socio-demographic, and anthropometric variables, as well as all questionnaires in order to be sure that everything was well documented. In addition, I travelled regularly to Rotterdam to meet with the project coordinator, Prof.Dr. Hemmo A. Drexhage, at the Department of Immunology at the Erasmus Medical Center, with the purpose of learning key aspects about immunology and also, how all laboratory parameters were determined. Prof.Dr. Markus Schwarz also assisted me during the whole period in cases of doubts in relation to laboratory analyses, and Dr.Elif Weidinger in case of doubts in relation to the recruitment process or the existence of any inconsistencies in the data. Thereafter, I conceptualized the research questions, and conducted all data analyses by myself. For this purpose, I learned how to work with statistic programs such as SPSS and/or GraphPad. I travelled regularly to Munich to meet Prof.Dr. Norbert Müller and discuss results together. After interpretation of the data, I was able to write the manuscript and send it for consideration for publication, after all other co-authors agreed with the final version. I also was able to present my work in different congresses as oral communications and learned how to perform scientific presentations.

Prof.Dr. Norbert Müller and Prof.Dr.Hemmo A. Drexhage supervised me during the whole process.

2.2. Contribution to publication II

As in the case of Publication I, data from this study originate from the EU-MOODINFLAME and CITICOX projects, initiated in 2008, and finalized in 2013. When I started to collaborate in the projects, the positive ethical decisions were already granted, the study population recruited, and laboratory parameters assessed. As I already had five years of experience working as a psychiatrist, and as I had already completed a Master of Science in Research, I already had a great experience in recruitment processes, the configuration of informed consents, and in the administration of clinical questionnaires/scales before the initiation of this doctoral thesis.

My role in these studies consisted then in first, validate all data, and configurate all databases. For this purpose, I checked all clinical, socio-demographic, and anthropometric variables, as well as all questionnaires in order to be sure that everything was well documented. In addition, I travelled regularly to Rotterdam to meet regularly with the project coordinator, Prof.Dr. Hemmo A. Drexhage, at the Department of Immunology at the Erasmus Medical Center, with the purpose of learning key aspects about immunology and also, how all laboratory parameters were determined. Prof.Dr. Markus Schwarz also assisted me during the whole period in cases of doubts in relation to laboratory analyses, and Dr.Elif Weidinger in case of doubts in relation to the recruitment process or the existence of any inconsistencies in the data. Thereafter, I conceptualized the research questions and conducted all data analyses by myself. For this purpose, I learned how to work with statistic programs such as SPSS and/or GraphPad. I was also able to travel regularly to Munich to meet Prof.Dr. Norbert Müller and discuss results together.

This Publication also includes a Systematic Review on inflammatory parameters as response predictors of antidepressant and/or (add-on) anti-inflammatory therapy in MDD. Since this has to be done in duplicate, Maria S Simon did this together with me. She also checked all statistics that were already done by me. Since we considered that she contributed also a lot to the present work, we decided to share first authorship.

I was then able to write the manuscript and send it for consideration for publication, after all other coauthors (including Maria S Simon), agreed with it.

As in the case of Publication I, I also was able to present my work in different congresses as oral communications and learned how to perform scientific presentations.

Prof.Dr. Norbert Müller and Prof.Dr.Hemmo A. Drexhage supervised me during the whole process.

3. Introduction

With an estimated lifetime prevalence of up to 8-20%[1], and a lifetime risk of up to 15-18%, major depressive disorder (MDD) (or in other words, 'depression') is considered as the second leading contributor to the numbers of years lived with disability worldwide[2]. It has been linked to an increased medical burden and mortality rate; suffering from depression increases the risk of suffering from another comorbid medical condition, and suffering from a medical disorder increases the risk of experiencing depression at some point later in life[3,4]. In addition, MDD is considered as the major contributor to the number of suicides that occur every year[2,5].

MDD is characterized by a combination of symptoms that must be present for (at least), a period of two weeks of time, and that interfere with the person's previous functioning. It's diagnosis is therefore clinical and, as outlined in the two most commonly used classification systems (i.e., the tenth version of the World Health Organization International Classification of Diseases (ICD-10)[6], and the fifth edition of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[7]), symptoms include not only alterations in mood, pleasure and/or interests, but also in sleep, appetite, cognition, and/or psychomotor activity (**Table 1**). Depending on the way the different symptoms are combined, several specifiers can be distinguished. For example, depression 'with melancholic features' is characterized by at least one of the following symptoms: loss of interests and/or pleasure (anhedonia), lack of mood reactivity, combined with at least three of the following ones: mood that worsens in the morning, inappropriate or delusional guilt, significant appetite and/or weight loss, early-morning awakening, or psychomotor changes. On the contrary, depression 'with atypical features' is characterized by the existence of mood reactivity, together with at least two of the following ones: interpersonal rejection sensitivity, significant appetite and/or weight gain, an increase in sleep needs, and/or leaden paralysis. Other specifiers can be distinguished, such as depression 'with catatonic features', depression 'with psychotic symptoms', depression 'with anxious distress', depression 'with postpartum onset', or depression 'with a seasonal pattern'[6,7]. Therefore, MDD is considered as a complex and highly heterogeneous disorder[8].

MDD typically has its onset in young adulthood[9] and, although its clinical course may vary widely, a relapse is not uncommon, with about 75% of patients experiencing recurrent episodes usually within a period of 'sustained remission' of about two years[10-12]. Risk factors include a family history of depression (it is estimated that about 35% of the risk is hereditary), female sex[13], early life abuse and neglect, recent life stressors[14-16], early life infections[17], and/or suffering from another medical condition.

However, and despite the growing number of published research articles, and the substantial progress achieved in our understanding of the pathophysiology of the disorder, its exact etiology remains unclear, and specific objective biological hallmarks are still lacking, defaulting diagnosis and therapy stratification of patients.

Table 1. Definition of Major Depressive Disorder (MDD) according to ICD-10[6] and DSM-5[7] Diagnostic Criteria

ICD-10 criteria[6]	DSM-5 criteria[7]
-At least four of the following A criteria (**at least one** includes A1, A2 or A3): A1. Low mood A2. Loss of interest or pleasure in almost all activities A3. Tiredness, fatigue, low energy, decreased efficiency A4. Significant weight or appetite changes A5. Sleep changes A6. Psychomotor changes (agitation/retardation) A7. Feelings of worthlessness or inappropriate guilt A8. Reduced concentration and/or attention, indecisiveness A9. Suicidal thoughts, acts of self-harm, suicide attempts A10. Low self-confidence.	**-At least five** of the following B criteria (**at least one** includes B1 or B2): B1. Low mood (or irritable mood in children and/or adolescents) B2. Loss of interest or pleasure in almost all activities B3. Significant weight or appetite changes B4. Sleep changes B5. Psychomotor changes (agitation/retardation) B6. Tiredness, fatigue, low energy, decreased efficiency B7. Feelings of worthlessness or inappropriate guilt B8. Reduced concentration and/or attention, indecisiveness B9. Thoughts of death, suicidal ideation, suicide attempts.

These symptoms must be present for at least, 2 weeks (most of the day, nearly every day), and must cause clinically significant impairment in different areas of functioning. In addition, symptoms must not be attributable to the direct effects of a substance, and/or to a medical disorder, and are not better explained by schizophrenia spectrum and/or other psychotic disorders[6,7]. Importantly, criteria for a mixed episode must not be met, and a history of a manic and/or hypomanic episode must be also absent[6,7]. Both the ICD-10 and DSM-5 classification systems also distinguish between a mild, moderate and severe episode, between a single or recurrent episode, and if the episode is in partial or full remission, or not[6,7].

3.1. The monoaminergic and glutamatergic hypotheses of depression

Formulated for the first time in the mid-20th century[18,19], the monoamine theory of MDD has been the most prevailing hypothesis of depression, so far. It is based on the idea that a deficiency in the transmission within the different monoaminergic systems (i.e., serotonergic (5-HT), noradrenergic (NA) and/or dopaminergic (DA)) would be responsible of triggering depression and that, inhibition of the reuptake and/or metabolism of monoamines (especially of 5-HT) in the synaptic cleft would decrease depressive symptoms[20-24]. This hypothesis is based on two (serendipitous) discoveries that occurred nearly simultaneously, during the 1950s. One was the observation that patients treated with the antihypertensive (and monoamine depleting agent) reserpine, developed depression[25]. The second major event was the discovery that, two structurally unrelated classes of compounds (i.e., the antituberculosis agent iproniazid, and the antihistamine agent imipramine), were able to reduce depressive symptoms by, as a common mechanism, the increase in monoamine levels[26,27]. Nowadays, the best evidence for the involvement of monoamines in the pathophysiology of depression comes from studies on tryptophan (TRP) depletion. The amino acid TRP is essential for 5-HT synthesis, since it acts as its precursor. While TRP administration seems to provide beneficial effects, dietary depletion of TRP has been shown to induce a recurrence of depressive symptoms in individuals with a prior episode of depression[28,29]. In addition, decreased peripheral and/or cerebrospinal fluid (CSF) levels of TRP and/or 5-HT have been repeatedly found in individuals with depression[30,31], supporting this hypothesis. Since this theory was proposed, a large number of monoamine-based antidepressants have been developed and indeed, treatment of depression is nowadays based on the enhancement of monoaminergic neurotransmission[24]. However, response rates to monoaminergic antidepressants are still far from being optimal, with about 30% of patients not responding to treatment with one monoaminergic agent, despite adequate dose and duration[32,33].

Almost in parallel to the enunciation of the monoamine hypothesis of depression, in 1959, Crane reported the antidepressant effects of D-cycloserine[34]. While at low or moderate doses, D-cycloserine has an action as an *N*-methyl-D-aspartate receptor (NMDA-R) agonist, at high doses, it acts as an NMDA-R antagonist, decreasing the levels of the major excitatory neurotransmitter in the nervous system, glutamate[35]. Therefore, it was hypothesized that an increase in the levels of this compound would be related to the pathophysiology of depression. In support of such view, high levels of glutamate have been repeatedly found in the blood, CSF, frontal and/or occipital cortex of individuals with depression[36-39], and accumulating research has supported the antidepressant effects of NMDA-R antagonism in different animal and human studies of depression[40-42], proposing modulation of glutamatergic neurotransmission as a therapeutic promising target in, especially, individuals with depression who do not adequately respond to monoaminergic antidepressants[43,44]. However, the mechanisms by which monoaminergic and glutamatergic neurotransmission is dysregulated in MDD are still unclear.

3.2. The macrophage theory of depression

Microglial cells (also known as 'microglia'), comprise 5-10% of total brain cells[45]. However, and even thought this percentage might seem low, microglial cells are crucial for a proper brain development and function. Under steady-state 'non-inflammatory' conditions, microglia are involved in multiple processes such as phagocytosis of neurons undergoing programmed cell death, synaptic pruning, axon remodeling, and/or neurogenesis[46-50]. On the contrary, inflammatory-activated microglia are able to hamper normal brain development and to perturb important neuronal functions, (such as neurotransmitter signaling), through, among other mechanisms, an excess (or prolonged) cytokine activity[51,52].

Proposed for the first time in 1991, the 'macrophage theory of depression' postulates that chronically activated microglia are able to trigger depression through, among other mechanisms, the chronic production of pro-inflammatory compounds[53]. Increased concentrations of pro-inflammatory compounds (e.g., cytokines, chemokines and/or of acute-phase reactants) have been repeatedly demonstrated in the blood and/or CSF of individuals with depression[54-64]. In addition, several reports have demonstrated that administration of pro-inflammatory compounds is able to trigger depression even in individuals without any psychiatric diseases[65,66]. Also, imaging and histological techniques have shown an increased density of microglia in, in particular, the hippocampus of individuals with depression[67]. Pro-inflammatory activation of microglia may be part of a systemic activation of the mononuclear phagocyte system[68]. Accumulating research has demonstrated, together with the above-mentioned findings, an overall monocytosis[69], and an increased in the expression of different pro-inflammatory genes in the circulating monocytes of individuals with depression[60,62,70].

Interestingly, most of the currently available antidepressant agents show, regardless of their neurotransmission modulatory effects, immune-modulating properties[71,72]. In addition, recent research has reported that several anti-inflammatory drugs (in mono- or add-on therapy) may also exert antidepressant effects[73-75]. For example, the anti-interleukin-17A agent, ixekizumab, has been shown to induce remission of depression in up to 40% of patients with psoriasis who were experiencing an episode of depression[76]. Also, an association between several immune gene polymorphisms, and a reduced responsiveness to antidepressant therapy has been repeatedly demonstrated[77]. Taken together, this has led to the idea that response to antidepressants may (at least partially) depend on the inflammatory state of individuals with depression[78]. However, the way the inflammatory response system (IRS) interacts with the different neurotransmission systems in individuals with depression still needs to be elucidated.

3.3. The IRS and its link to neurotransmitter abnormalities in depression

TRP is catabolized into 5-HT via the 'serotonin pathway', or into kynurenine (KYN) via the 'kynurenine pathway' (KP)[79]. In fact in humans, more than 95% of TRP will be converted into KYN, (and not into 5-HT)[80]. KYN will be then catabolized into different metabolites throughout two pathways, also known as the potentially neurotoxic and neuroprotective arms of the KP[79,81]. The resulting catabolites, collectively known as 'kynurenines' are special in their capability to modulate glutamatergic neurotransmission (and also, oxidative-nitrosative pathways). Potentially neurotoxic catabolites are the NMDA-R agonists 3-hydroxykynurenine (3-HK), and/or quinolinic acid (QUIN). Potentially neuroprotective catabolites are the NMDA-R antagonists kynurenic acid (KYNA), and/or picolinic acid (PIC).

The KP is induced after activation of one of its rate-limiting enzymes, i.e., tryptophan 2,3-dioxygennase (TDO), or indoleamine 2,3-dioxygenase (IDO)[82]. TDO is mainly expressed in kidney tissues and/or in the liver, and is primarily induced by glucocorticoids or nicotidamine shortage. On the contrary, IDO is considered as an ubiquitous enzyme. It is expressed all over the human body tissues (including cells in the brain), and is induced by a number of proinflammatory stimuli, such as pro-inflammatory cytokines[83.84]. In the brain, the KP is mainly supported by microglia. Therefore, activation of IDO by pro-inflammatory-activated microglia has been suggested as the link between existence of an activated IRS and TRP (and 5-HT) deficiencies in individuals with depression[85,86]. In addition, different reports have also suggested a preferential breakdown of TRP among the potentially neurotoxic arm of the KP, since the enzyme kynurenine monooxygenase (KMO) (the rate-limiting enzyme in the conversion from KYN to 3-HK), is also activated by pro-inflammatory stimuli. This would ultimately impact on glutamatergic neurotransmission, enhancing it[87]. Supporting this idea, decreased levels of TRP, an increased KYN/TRP ratio, increased levels of KYN[88-89], together with increased levels of 3-HK and of QUIN, decreased concentrations of KYNA, and a decreased KYNA/QUIN ratio have been repeatedly demonstrated in both the periphery and blood and/or CSF of individuals with depression[90-92].

However, results have not been always concordant between studies and findings, not generalizable. For example, accumulating research has reported normal or even reduced peripheral levels of pro-inflammatory compounds[70], of KYN, 3-HK and/or QUIN[92-94], as well as reduced levels of glutamate in the anterior cingulate and frontal cortex of individuals with depression[95,96], questioning the idea of a pro-inflammatory activation of IDO and overproduction of potentially neurotoxic KP catabolites in MDD.

4. Summary

Major Depressive Disorder (MDD) is a complex and heterogeneous disease, with a so far poorly characterized underlying pathophysiology. This defaults diagnosis and therapy stratification of patients. Consequently, response rates to currently available treatments are far from being optimal, with up to 30% of patients not responding to conventional antidepressant agents. During past decades, increasing evidence has suggested that biological changes may underlie MDD. One of the established biological changes may arise from an abnormal inflammatory response system (IRS) that interacts with important neurotransmission systems (e.g., monoaminergic, glutamatergic). However, the extent and direction of these changes still remain unclear.

During the MOODINFLAME project, a European-funded cooperation project aimed to identify biomarkers of mood disorders, data about the immune cell profile, the serum inflammatory state, and the serum levels of tryptophan (TRP) and kynurenine pathway (KP) metabolites were collected in both individuals with depression and healthy controls (HC). Part one of this doctoral thesis was to analyze these data, and to study how is the inflammatory state of individuals with depression related to TRP and KP abnormalities, and to their clinical characteristics. In a second project, we explored whether the existence of a pro-inflammatory monocyte and/or serum state could be used for prediction of antidepressant response, and/or to prediction of (add-on) treatment with anti-inflammatory agents. This included conducting a systematic review on inflammatory predictors of treatment response in patients with MDD and a clinical study, where the expression levels of key pro-inflammatory genes were determined in the circulating monocytes of individuals with depression, and related to response to treatment (as measured by the % of improvement in the HAM-D 17 score).

Taken together, we found that individuals with depression that lack from other comorbid medical conditions show, in general, an increased expression of pro-inflammatory cytokine/chemokine genes in their circulating monocytes, but no serological markers of immune activation. These individuals are also characterized by decreased levels of TRP, and by a deactivation of the KP, and would benefit from treatment with primarily serotonergic antidepressants. On the contrary, individuals with depression that suffer from other comorbid medical conditions may be characterized by increased monocyte expression levels of pro-inflammatory genes, and by increased peripheral levels of pro-inflammatory compounds. In addition, they may exhibit, along with a decrease in the levels of TRP, an activation of the KP. These individuals would benefit from antidepressants and/or (add-on) therapy with anti-inflammatory/immunoregulatory agents with a strong action on other neurotransmitter systems, such as the glutamatergic system.

5. Zusammenfassung

Als Depressive Störung (oder Depression) bezeichnet man eine komplexe und heterogene Erkrankung mit einer bisher schlecht charakterisierten zugrundeliegenden Pathogenese. Letzteres erschwert Diagnose und Behandlung. Als Folge sind die Ansprechraten auf die derzeit verfügbaren Therapien bei weitem nicht optimal, da bis zu 30% der Patienten auf herkömmliche Antidepressiva nicht ansprechen.

Während der MOODINFLAME-Studie, einem europäisch finanzierten Kooperationsprojekt das darauf abzielt, Biomarker affektiver Störungen zu identifizieren, wurden nicht nur Daten zum Immunzellprofil, sondern auch zu Blutspiegeln verschiedener Entzündungsparameter und von Tryptophan- (TRP) und Kynurenin-Pfad (KP) Metaboliten bei Patienten mit Depression und gesunden Kontrollen bestimmt. Teil eins dieser Arbeit bestand daraus, diese Daten zu analysieren und zu untersuchen, inwieweit Entzündungsparameter mit TRP und KP-Auffälligkeiten, und mit unterschiedlichen klinischen Merkmalen, korreliert. In einem zweiten Projekt wurde untersucht, ob diese Parameter bei Patienten zur Vorhersage der antidepressiven therapeutischen Wirkung verwendet werden können. Zu diesem Zweck wurde vor der Behandlung mit verschiedenen serotonergen Antidepressiva, oder mit dem Entzündungshemmer Celecoxib, die Expression wichtiger Entzündungs-Genen in Monozyten von Patienten bestimmt, und mit dem Ansprechen auf die Behandlung korreliert. Darüber hinaus wurde der aktuelle Stand der Literatur zusammengefasst.

Wir konnten feststellen, dass Patienten mit einer Depressiven Störung nicht nur verringerte TRP-Spiegel in Serum zeigen, sondern auch, in etwa 30-40% der Fälle, eine erhöhte Expression von pro-inflammatorischen Zytokin/Chemokin-Genen in ihren zirkulierenden Monozyten aufweisen. In Abwesenheit komorbider Erkrankungen zeigten sich keine Auffälligkeiten bei den serologischen Markern der Immunaktivierung. Diese Patienten weisen weiterhin eine Deaktivierung des KP auf. Im Gegensatz dazu sind Patienten mit Depression, die an weiteren komorbiden Erkrankungen leiden, durch erhöhte Serum-Spiegel von Entzündungsparametern gekennzeichnet. Ausserdem zeigen sie neben der Abnahme der TRP-Spiegel, eine Aktivierung des KP. Dementsprechend fanden wir, dass sowohl die erhöhte monozytäre Expression von Entzündungs-Genen, als auch erhöhte Blutspiegel von Entzündungsparametern und verschiedener KP Metaboliten, das Nichtansprechen auf primär serotonerge Antidepressiva vorhersagen. Diese Patienten würden von Antidepressiva oder (Zusatz)-Therapie mit anti-entzündlichen/immunregulatorischen Wirkstoffen, die auch Effekte mit starker Wirkung auf andere Neurotransmittersysteme wie das glutamaterge System haben, profitieren.

6. Publication I

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Progress in Neuropsychopharmacology & Biological Psychiatry

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Activation and deactivation steps in the tryptophan breakdown pathway in major depressive disorder: A link to the monocyte inflammatory state of patients

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ABSTRACT

It is unclear how the tryptophan (TRP) breakdown pathway relates to the activated inflammatory state of patients with major depressive disorder (MDD).

We determined in two different cohorts of patients with MDD ($n = 281$) and healthy controls (HCs) ($n = 206$) collected for the EU-MOODINFLAME project:

a.) the monocyte expression levels of 5 key pro-inflammatory cytokine/chemokine genes (ICCGs), 5 type I interferon stimulated genes (ISGs), and 4 kynurenine pathway (KP) enzyme genes (i.e. *IDO-1, KMO, CCBL1/KAT II* and *CCBL2/KAT III*) by standard q-PCR,
b.) serum levels of TRP, 5-HTrp, 5-HIAA, KYN, KYNA, 3-HK, XA, PIC, and QUIN by LC-MS/MS and/or HPLC, and calculated various TRP/KP metabolism ratios.

We then correlated outcomes to each other, and to the clinical characteristics of patients.

Abbreviations: MDD, major depressive disorder; ICCGs, pro-inflammatory cytokine/chemokine-related genes; *IL-1β*, interleukin 1 beta gene; *CCL2*, chemokine C—C motif ligand 2 gene; 5-HT, 5-hydroxytryptamine; TRP, tryptophan; KP, kynurenine pathway; KYN, kynurenine; CSF, cerebrospinal fluid; IDO, indoleamine 2,3-dioxygenase enzyme; IFN, interferon; 3-HK, 3-hydroxykynurenine; QUIN, quinolinic acid; KYNA, kynurenic acid; *TNFAIP3*, tumor necrosis factor alpha-induced protein 3 gene; *CXCL2*, C-XX-chemokine ligand 2 gene; *IFI44*, interferon-induced protein 44 gene; *IFI44L*, interferon-induced protein 44 like; *IFIT3*, interferon-induced protein with tetratricopeptide repeats 3 gene; *LY6E*, E lymphocyte antigen 6 family member gene; *MX1*, interferon-regulated resistance GTP-binding protein MxA gene; HCs, healthy controls; ISGs, type I interferon-stimulated genes; *IDO-1*, indoleamine 2,3-dioxygenase gene; pSS, primary Sjögren Syndrome; *KMO*, kynurenine 3-monooxygenase gene; *CCBL/KAT*, cysteine conjugate beta lyase cytoplasmic/kynurenine aminotransferase gene; TDO, 2,3-dioxygenase enzyme; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders (4e), text revision; M.I.N·I, Mini International Neuropsychiatric Interview; SCID-I, structured clinical interview for DSM-IV Axis I disorders; IDS-C, Inventory of Depressive Symptomatology, clinician-rated version; HAM-D 17, Hamilton Rating Scale for Depression, 17-item version; PBMCs, peripheral blood mononuclear cells; cDNA, complementary deoxyribonucleic acid; qPCR, quantitative polymerase chain reaction; RT, real time; *ABL1*, Abelson murine leukemia viral oncogene homolog 1; CT, comparative threshold cycle;ELISA enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; TRB, tetramethylbenzidine; 5-HTrp, 5-hydroxytryptophan; 5-HIAA, 5-hydroxyindoleacetic acid; XA, xanthurenic acid; PIC, picolinic acid; LC-MS/MS, liquid chromatography-mass spectrometry; HPLC, high performance liquid chromatography; bmi, body mass index; ECT, electroconvulsive therapy; TPH-II, tryptophan hydroxylase enzyme; MAO, monoamine oxidase enzyme.

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Both cohorts of patients differed clinically; patients of the Munich cohort ($n = 50$) were less overweight, less medicated, were less in the current episode and showed a higher HAM-D 17 score as compared with patients of the Muenster cohort ($n = 231$).

An increased expression of ICCGs was found in the circulating monocytes of patients of both cohorts; this was in particular evident in the Munich cohort. In contrast, ISGs monocyte expression levels tended to be reduced (both cohorts).

TRP serum levels were linked to the pro-inflammatory (ICCGs) monocyte state of patients; a decrease in TRP serum levels was found in the Munich cohort; TRP levels correlated negatively to patient's HAM-D 17 score. Contrary to what expected, KYN serum levels were not increased in patients (both cohorts); and an increased KYN/TRP ratio was only found in the Munich patients (who showed the lowest TRP serum levels). *IDO-1* monocyte expression levels were decreased in patients (both cohorts) and negatively associated to their pro-inflammatory (ICCGs) monocyte state. Thus, a depletion of TRP via an ICCGs-inflammatory IDO activation is not likely in MDD.

Downstream from KYN, and regarding compounds influencing glutamate receptors (GR), reduced serum levels of KYNA (NMDA-R antagonist), 3-HK (NMDA-R agonist), and XA (mGlu2/3 agonist) were found in patients of both cohorts; PIC serum levels (NMDA-R antagonist) were increased in patients of both cohorts. Reduced QUIN serum levels (NMDA-R agonist) were found in patients of the Muenster cohort,only. 3-HK levels correlated to the monocyte inflammatory ICCG state of patients. The ultimate effect on brain glutamate receptor triggering of this altered equilibrium between peripheral agonists and antagonists remains to be elucidated.

1. Background

With a lifetime prevalence of up to 8–12% (Smith, 2014), major depressive disorder (MDD) is considered as a leading cause of disability worldwide (WHO, 2020). In addition, it is associated with numerous somatic diseases (e.g. rheumatoid arthritis, obesity) (Clarke and Currie, 2009; Preiss et al., 2013), increasing mortality rates. Importantly, MDD has been linked to an increased risk of suicidal behavior, acting as the major contributor to suicide deaths (Saxena and Fleischmann, 2014; WHO, 2020). However, understanding the exact pathogenesis of the disorder still remains challenging, making diagnosis, treatment stratification of patients and new drug discovery, difficult.

A growing body of evidence has suggested an involvement of an abnormal inflammatory response system in the pathogenesis of MDD. Altered immune cell functions and raised circulating concentrations of pro-inflammatory cytokines/chemokines have been repeatedly found in MDD (Müller et al., 1993; Connor and Leonard, 1998; Anisman and Merali, 2002; Mikova et al., 2001; Myint et al., 2005; Irwin and Miller, 2007; Dantzer et al., 2008; Wang et al., 2019). In line with this, we previously reported on the expression of a specific inflammation-related gene signature in the circulating monocytes of two cohorts of patients with MDD (Carvalho et al., 2014; Grosse et al., 2015; Grosse et al., 2016). In this signature, gene expression patterns formed two main clusters of strongly interrelated genes; one cluster was composed of a set of pro-inflammatory cytokine/chemokine-related genes (ICCGs), such as the interleukin (IL)1-beta gene (*IL-1β*), the *IL-6*, or the chemokine C—C motif ligand 2 gene (*CCL20*). In particular monocytes of the cohort of older patients with a severe and melancholic depression were characterized by an overexpression in this cluster of inflammatory genes. These patients were also characterized by elevated serum levels of pro-inflammatory cytokines/chemokines, such as IL-6, IL-8 and/or CCL2 (Weigelt et al., 2011; Carvalho et al., 2014; Grosse et al., 2015).

Whilst a functional deficit in the neurotransmitter 5-hydroxytryptamine (5-HT, also known as serotonin) has been classically mentioned as an important contributor to the pathogenesis of depression (Coopen and Doogan, 1988; Hamon and Blier, 2013), it is only in recent years that various theories have emerged to provide a mechanistic link between the altered inflammatory state of patients with MDD and neuronal functional abnormalities. One of these theories has focused on an inflammation-related enhanced breakdown of tryptophan (TRP) along the kynurenine pathway (KP) (Maes et al., 2011; Leonard and Maes, 2012: Arnone et al., 2018). TRP can be metabolized into 5-HT via the serotonin pathway, or into kynurenine (KYN) via the KP (Fig. 1) which in fact acts as the major catabolic pathway for TRP in the body (Stone et al., 2013; O'Farrell and Harkin, 2017) (Fig. 1).

An increased TRP-to-KYN breakdown ratio has repeatedly been found in the blood and cerebrospinal fluid (CSF) of individuals with depression (Maes et al., 1990; Quak et al., 2014; Baranyi et al., 2017), particularly in those at a high risk of suicide (Bradley et al., 2015; Bryleva and Brundin, 2017; Messaoud et al., 2019). Activation of the KP is mediated, −among other mechanisms-, through activation of one of its rate-limiting enzymes, indoleamine 2,3-dioxygenase (IDO) (Maes et al., 2011; Leonard and Maes, 2012; Schwarcz and Stone, 2017; Arnone et al., 2018). IDO is induced by pro-inflammatory stimuli such as interferon (IFN)-alpha and/or IL-2 (Raison et al., 2010; Leonard and Maes, 2012); thus, IDO activation in pro-inflammatory monocytes and/or microglia has been suggested as a key pathogenic mechanism that could explain the link between pro-inflammatory immune cells, TRP depletion and characteristic brain 5-HT deficiencies in MDD (Myint et al., 2007; Schwarcz and Stone, 2017). KYN is further catabolized into various downstream neuroactive metabolites throughout two degrading arms, often referred to as the potentially neurotoxic and potentially neuroprotective arms of the KP (Fig. 1). An inflammation-related catabolic imbalance between these two breakdown arms, i.e. an enhanced production of the potentially neurotoxic catabolites 3-hydroxykynurenine (3−HK) and/or quinolinic acid (QUIN) at the expense of the potentially neuroprotective catabolites kynurenic acid (KYNA) and/or picolinic acid (PIC) has also been proposed in MDD (Myint and Kim, 2003; Myint et al., 2007). This hypothesis is supported by findings of decreased KYNA and increased 3-HK and QUIN concentrations in the serum/plasma of patients with MDD (Savitz et al., 2015; Doolin et al., 2018). However, recent studies have found normal or even reduced serum/plasma levels of KYN and 3-HK in individuals with depression (Hughes et al., 2012; Wurfel et al., 2017), questioning the idea of an IDO activation and overproduction of potentially neurotoxic downstream metabolites in MDD.

The aim of the present study was to investigate how the inflammatory monocyte state of patients with MDD correlates to the breakdown of TRP to KYN, and of KYN to its potentially neurotoxic and neuroprotective downstream products.

First, we determined the monocyte expression levels of five characteristic ICCGs (i.e. *IL-1B, CCL20, IL-6*, *TNFAIP3* and *CXCL2*) (Vogels et al., 2017) and of five genes driven by type I IFNs (i.e. *IFI44, IFI44L, IFIT3, LY6E* and *MX1*) (Brkic et al., 2013) in two cohorts of patients with MDD and healthy controls (HCs) participating in the EU-MOODINFLAME study (FP7-HEALTH:222963). These type I interferon-stimulated genes (ISGs) have been shown to be positively correlated to indoleamine 2,3-dioxygenase gene (*IDO-1*) expression and to a higher serum KYN/TRP in patients with primary Sjögren Syndrome (pSS) (Maria et al., 2016), supporting the theory of an inflammation-

induced activation of the KP via IDO.

We also determined the monocyte expression levels of four genes encoding important KP degrading enzymes (i.e. *IDO-1*, kynurenine 3-monooxygenase gene (*KMO*), cysteine conjugate beta lyase cytoplasmic/kynurenine aminotransferase genes (*CCBL1/KAT I* and *CCBL2/KAT III*), as well as serum levels of various important TRP/KP metabolites (TRP, 5-HTrp, 5-HIAA, KYN, KYNA, 3-HK, XA, PIC, QUIN).

In addition, the activity of the different KP enzymes was assessed by determining various KP metabolite ratios (e.g. the KYN/TRP ratio estimating IDO and/or tryptophan 2,3-dioxygenase (TDO) enzyme activity; the KYNA/KYN ratio estimating KATs enzyme activities, the 3-HK/KYN ratio estimating KMO enzyme activity) (Fig. 1).

Finally, the serum levels of the different TRP/KP metabolites and the different KP metabolite ratios were correlated to the monocyte ICCG and ISG inflammatory states, and to the monocyte expression levels of the four KP enzyme genes.

2. Methods

2.1. Study participants

From 2010 to 2013, a total of $n = 281$ (aged 18–65 years) in- and outpatients with MDD were recruited from the Departments of Psychiatry at the University Hospital of Muenster, Germany ($n = 231$) and the University Hospital of the Ludwig Maximillian University in Munich, Germany ($n = 50$).

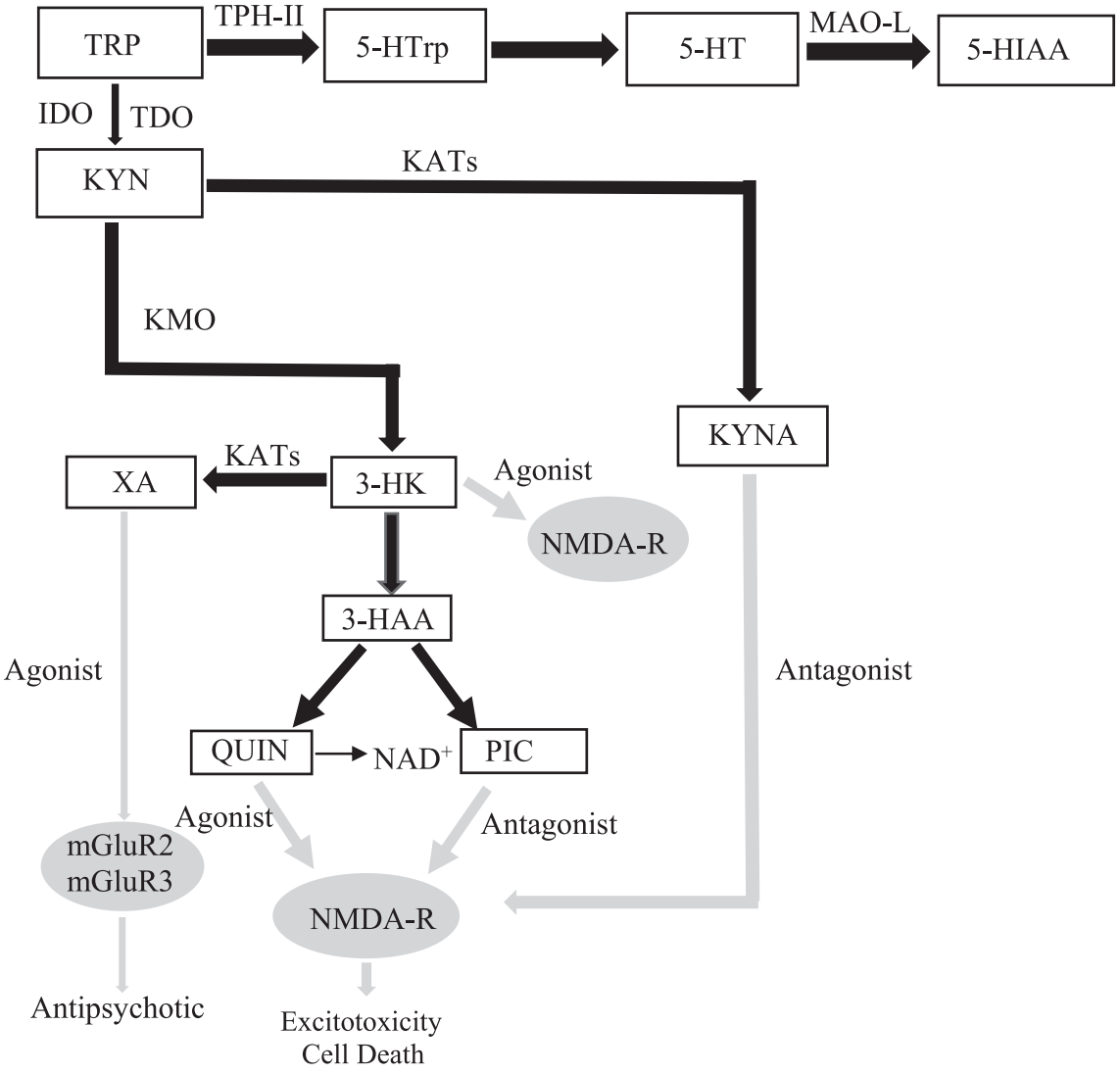

Fig. 1. The tryptophan (TRP) catabolic pathway. The main branches are towards the serotonin pathway (5-HTrp, 5-HT and 5-H-IAA) and towards the potentially neuroprotective/neurotrophic (KYNA/PIC) and potentially neurotoxic (3-HK/QUIN) arms of the kynurenine pathway. Along the arrows, the main enzymes catalyzing the reactions are given (i.e. TPH-II, MAO-L, IDO, TDO, KMO and KATs).
Abbreviations: TRP: tryptophan; 5-HTrp: 5-hydroxytryptophan; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid; KYN: kynurenine; KYNA: kynurenic acid; 3-HK: 3-hydroxykinurenine; XA: xanthurenic acid; 3-HAA: 3-hydroxyanthranilic acid; QUIN: quinolinic acid; PIC: picolinic acid; TPH-II: tryptophan hydroxylase; MAO-L: monoamine oxidase; TDO: tryptophan 2,3-dioxygenase enzyme; IDO: indoleamine 2,3-dioxygenase enzyme; KMO: kynurenine 3-monooxygenase enzyme; KATs: kynurenine aminotransferase enzymes; NMDA-R: *N*-methyl-D-aspartate receptor; mGlu-R: metabotropic glutamate receptors; NAD^+: nicotinamide adenine dinucleotide coenzyme. TRP breaks down to either 5-HT or KYN depending on IDO and/or TDO enzyme activity. KYN then breaks down towards KYNA (NMDA-R antagonist) or 3-HK (NMDA-R agonist) depending on KATs or KMO enzyme activities, respectively. 3-HK break downs to 3-HAA, which further breaks down to the potentially neuroprotective PIC, or to the potentially neurotoxic QUIN. PIC acts as an NMDA-R antagonist, while QUIN acts as an NMDA-R agonist, inducing excitotoxicity and neuronal death. QUIN (as 3-HK) is in addition a source of NAD^+, playing a role in provision of energy. Interestingly, 3-HK is also able to break down to XA, which acts as an mGlu2R/mGlu3-R agonist, blocking excitotoxicity and neuronal death.

In the EU-MOODINFLAME study, all patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (4e), Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000).

In Muenster cohort, the diagnosis was confirmed by the Mini-International Neuropsychiatric Interview (M.I.N.I) (Sheehan et al., 1998), while in the Munich cohort, the diagnosis was confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First and Pincus, 1999).

Severity of depression was assessed with the Inventory of Depressive Symptomatology, clinician-rated version (IDS-C) (Rush et al., 1986) (in the case of the Muenster cohort), and with the Hamilton Rating Scale for Depression, 17-item-version (HAM-D 17) (Hamilton, 1960) (in the case of the Munich cohort). All assessments were performed by two independent experienced clinical psychiatrists.

Excluded were patients who were unable to write or give informed consent, patients at immediate risk for suicidal behavior, and those who had another Axis I and II disorder. With the aim of homogenizing the sample, we decided not to include patients with MDD and psychotic symptoms, since it is discussed that psychotic depression has a different form of nosology than non-psychotic depression (Park et al., 2015).

Patients had to be free of any obvious major medical illness in their medical history (i.e. cardiovascular, gastrointestinal, respiratory, neurologic, hepatic, renal, neurologic, infectious, immune or inflammatory diseases as well as untreated metabolic disorders) and also of any minor medical illness, including allergic reactions or infections, in the 4 weeks before blood withdrawal. Excluded were also patients with any clinically significant physical findings (e.g. uncontrolled high blood pressure, claudication, heart murmurs, abnormal reflexes, etc.) or laboratory results (e.g. abnormal glucose, liver and kidney values, etc.), as were women who were pregnant or breastfeeding. Recent ($\leq$ 4 weeks) vaccinations were not allowed and patients taking immune-modulatory medication were excluded, too.

Only in the case of the Munich cohort, and since this cohort was used for a study on the antidepressants effects of cyclooxygenase-2 (COX-2) inhibitors in patients with MDD (Arteaga-Henríquez et al., 2019), the use of antidepressant drugs (apart from benzodiazepines/analogues) was prohibited; patients treated with monoamine-oxidase inhibitors during the last 14 days or with fluoxetine during the last 6 weeks were also excluded, as were patients currently taking warfarin or pain medications within 72 h prior to study entry. The Muenster patients used a variety of antidepressant and psychotropic drugs (Table 1).

HCs (n = 206) were recruited from the same communities as the patients. The inclusion criteria for HCs were the absence of major Axis I disorders according to DSM-IV-TR criteria; no use of antidepressants or other psychotropic drugs was allowed. Furthermore, HCs had to be in self-proclaimed good health and free of any obvious medical illness, including infections and allergic reactions, for at least 4 weeks before blood withdrawal.

The study was approved by the ethics committee of the Medical Association Westphalia-Lippe, Germany (2009-019-f-S) and the ethics committee of the medical faculty at the Ludwig Maximillian University of Munich, Germany (234–09). All participants provided written informed consent.

Table 1
Clinical and demographic data of patients with MDD of the Muenster and Munich cohorts.

	Muenster n = 231	Munich n = 50	Muenster vs Munich
	M(SD)	M(SD)	p
Number of previous depressive episodes	3.62(6.01)	1.96(1.37)	0.058
Duration of current episode (weeks)	42.98(52.38)	18.90 (15.89)	**0.001**
HAM-D 17 score	18.00(5.39)	24.46(2.48)	**<0.001**
Age (years)	40.14(12.62)	39.64 (11.61)	0.795
bmi (kg/m^2)	26.28(4.60)	23.40(3.16)	**<0.001**
	n (%)	n (%)	p
Sex			0.274
Males	96(41.60)	25(50.0)	
Females	135(58.40)	25(50.0)	
Smoking			0.638
Yes	105(45.70)	21(42.00)	
No	125(54.30)	29(58.00)	
Medication			**<0.001**
Yes	223(96.50)	38(76.00)	
No	8(3.50)	12(24.00)	
ECT			**0.020**
Yes	23(10.0)	0(0.00)	
No	208(90.0)	50(100.00)	

Values marked in bold indicate a p-value $\leq$0.05. *Abbreviations:* M: mean; SD: standard deviation; HAM-D 17: Hamilton Rating Scale for Depression, 17-item version; bmi: body mass index; ECT: electroconvulsive therapy.

2.2. Laboratory assessments

All laboratory assays were centralized in the EU-MOODINFLAME study. Monocyte gene expression levels were measured by the Department of Immunology, Erasmus Medical Center (Rotterdam, the Netherlands), TRP/KP metabolites by the Institute of Laboratory Medicine, Medical Center of the Ludwig-Maximilian-University (Munich, Germany).

2.2.1. Determination of the inflammatory activation state of circulating monocytes

Details have been given in previous publications (Carvalho et al., 2014; Grosse et al., 2015; Vogels et al., 2017), and only a synopsis of the methodology is given here. Blood was collected in sodium heparin tubes (36 ml) for immune cell preparation. From the heparinized blood, we prepared peripheral blood mononuclear cells (PBMCs) suspensions by low-density gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) within 8 h to avoid erythrophagy-related activation of monocytes, as described previously in detail (Drexhage et al.,2010). PBMCs were then frozen in 10% dimethylsulfoxide and stored in liquid nitrogen. This enabled us to test the immune cells of patients and HCs together at a later stage at the Erasmus MC. CD14$^+$ monocytes were isolated from aliquots of frozen PBMCs (from approximately 20 ml of blood) by a magnetic cell sorting system (auto MACS Pro; Milteny Biotec, B.V., Bergisch Gladbach, Germany). The man viability was 86.3 $\pm$ 10.4 (Trypan blue staining); purity of monocytes, 95.1 $\pm$ 3.0% (flow cytometry). mRNA was isolated from the purified CD14$^+$ monocytes with an RNA easy mini-kit in accordance with manufacturer's instructions (Qiagen, Hilden, Germany). The mean monocyte yield after isolation was 2.0 $\pm$ 1.6 $\times$ 10^6/participant; the mean quantity of mRNA in monocytes was 3.2 $\pm$ 1.8 µg. One µg of mRNA was reverse transcribed by a high-capacity reverse transcription kit (Applied Biosystems, Foster City, CA, USA) to produce complementary deoxyribonucleic acid (cDNA) for quantitative polymerase chain reaction (qPCR) (Taqman Arrays, format 48, Applied Biosystems, Foster City, CA, USA). qPCR was performed according to the manufacture's protocol and validated against the single real-time (RT)-qPCR method. Per fill port, 400 ng of cDNA was loaded. PCR amplification was performed with an Applied Biosystems Prism 7900HT sequence detection system with TaqMan Array block. Thermal cycler conditions were 2 min at 50 °C, 10 min at 94.5 °C, 30 s at 97 °C, and 1 min at 59.7 °C for 40 cycles. For our study, we determined five top ICCGs (i.e. *IL-1β, CCL20, IL-6*, *TNFAIP3* and *CXCL2*) as determined in the study of Vogels et al. (2017) (Supplementary Fig. 1). We verified -by using principle component analysis-, that these genes were indeed also belonging to the top ICCGs in the present study (Supplementary Fig. 2). Also in the most recent study on monocyte gene expression levels in a large cohort patients with MDD

(Schiweck et al., 2020), these genes belonged to the ICCGs signature (Supplementary Fig. 3).

In addition, we determined five key type I ISGs (i.e. *IFI44, IFI44L, IFIT3, LY6E,* and *MX1*) of the study of Brkic et al. (2013) and Maria et al. (2016), and four important KP enzyme genes (i.e. *IDO-1, KMO, CCBL1/KAT I* and *CCBL2/KAT III*). The expression of these 14 genes was normalized by the value of the housekeeping gene *ABL1* and calculated by the comparative threshold cycle (CT) method. *ABL1* has been found as being superior to other housekeeping genes for leukocyte gene level determination (Beillard et al., 2003).

2.2.2. Determination of TRP/KP metabolite serum levels

All serum samples were collected from fasting, early morning (8 a.m.-11 a.m.) venous blood samples, and immediately centrifuged, aliquoted and stored at −80 °C. TRP, 5-hydroxytryptophan (5-HTrp), 5-hydroxyindoleacetic acid (5-HIAA), KYN, KYNA, 3-HK, xanthurenic acid (XA), QUIN and picolinic acid (PIC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standards KYNA-D5, PIC-D4 and TRP-D5 were purchased from CDN Isotopes (Pointe-Claire, QC, Canada), and KYN-D4 was purchased from Buchem BV (Minden, the Netherlands). Reagents for protein precipitation, derivatization, and chromatography were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Biosolve (Valkenswaard, NL). Standards and a low- and high-quality control were established by adding defined amounts of each analyte to human serum samples obtained from a blood bank. The human serum was necessary because we had to take matrix effects into account to cover concentrations below the analyte concentrations present in healthy humans. The serum was diluted 1 + 1 with liquid chromatography-mass spectrometry (LC-MS/MS)-grade water and used as the lowest calibrator. The values of this calibrator were calculated by standard addition. Because serum is not available without these analytes, there was no blank sample. A total volume of 300 µl serum samples, calibrators, and controls was used for sample preparation.

2.2.2.1. HPLC method. In the Muenster cohort, TRP, 5-HIAA, KYN, 3-HK and KYNA ($n = 207$ MDD, 134 HC) serum levels were measured in a first set of determinations by a previously described high performance liquid chromatography (HPLC) method (Oades et al., 2010). In short, analytes were extracted from samples and calibrators/controls by using Waters Oasis MCX extraction cartridges (Waters Corporation, Milford, MA, USA). The eluent was then evaporated to dryness and reconstituted with 0.1 M phosphate buffered saline (PBS) for injection into the HPLC system. Analyses were carried out on a Waters 2695 chromatograph (Waters Corporation, Milford, MA, USA) with a 250 mm × 4 mm Supersphere 60 RP-select B, C8 column (Merck, Darmstadt, Germany), connected to a Waters 2487 dual-λ UV detector and a 2475 fluorescence detector. TRP (λex: 300 nm; λem: 350 nm) and 5-HIAA (λex: 300 nm; λem: 340 nm) were measured by fluorescence detection; KYN (365 nm), KYNA (330 nm) and 3-HK (365 nm) were measured by UV detection.

2.2.2.2. LC-MS/MS method. In a second and third round of determinations, serum levels of 5-HTrp, XA, QUIN and PIC ($n = 157$ MDD, $n = 73$ HC; Muenster cohort) and of TRP, 5-HTrp, 5-HIAA, KYN, 3-HK, KYNA, XA, QUIN and PIC ($n = 47$ MDD, 13 HC; Munich cohort) were analyzed by LC-MS/MS. The chromatographic system was composed on a Waters Acquity UPLC separation module connected to a Xevo TQ MS triple-quadrupole mass spectrometer with a *Z*-spray ESI ion source (Waters Corporation, Milford, MA, USA). Separation was performed on a Kinetex XB-C18, 2.6 µm, 2.1 × 150 mm column (Phenomenex, Torrance, CA, USA). Analytes were extracted by adding 50 µl of 2.0 M urea and 50 µl of an internal standard solution containing KYN-D4, KYNA-D5, PIC-D4, and TRP-D5. Two precipitation steps were performed by adding 200 µl methanol/ethanol (2/1 v: v) and then 800 µl acetonitrile. The supernatant was separated into two portions, which were evaporated separately. One of these portions was directly reconstituted in the mobile phase. The other portion was derivatized with 200 µl HCl/butanol at 90 °C for 60 min and then, after evaporation, also reconstituted in the mobile phase. For chromatography, 7.5 µl of the reconstituted samples, calibrators, and controls were loaded onto the LC-MS/MS system. QUIN and PIC were analyzed in the derivatized sample, while all other analytes were analyzed in the underivatized sample. For both derivatized and underivatized samples, gradient methods with a total duration of 7.5 min each were used for chromatographic separation. Mobile phase A was composed of 0.1% formic acid and 0.01% heptafluorobutyric acid in water; mobile phase B was methanol. The flow rate was set at 0.25 ml/min, and the column temperature was set at 30.0 °C. Retention times for the analytes were between 3.1 and 6.0 min. The Xevo TQ MS was operating at atmospheric pressure, and electrospray ionization was in positive mode (ESI+). Ion source settings were as follows: capillary voltage, 1.00 kV; desolvation temperature, 650 °C; source temperature, 150 °C. Nitrogen was used as the desolvation gas, at an API gas flow rate of 1200 l/h, and argon was used as the collision gas, at a flow rate of 0.15 ml/min. The analytes and internal standards were detected by multiple reaction monitoring (MRM) technique. System operation, data acquisition, and data processing were controlled with MassLynx V4.1 software (Waters Corporation, Milford, MA, USA). The lower limit of quantification (LLOQ) and lower limit of detection (LLOD) of the method described above were calculated according to DIN 32645 guidelines. The method was further validated based on the European Medicines Agency (EMEA) guidelines at the Institute of Laboratory Medicine, Medical Center of the Ludwig Maximilian University, Munich.

2.3. Statistics

Statistical analyses were performed with IBM SPSS v.21 and Microsoft Excel v.15.30 (170107) for Mac. Continuous sample characteristics are reported as mean (M) ± standard deviation (SD). Data were tested for normal distribution by the Shapiro-Wilk test ($n < 30$) and by the Kolmogorov-Smirnov test ($n \geq 30$). For group comparisons of sample characteristics (e.g. MDD vs HCs), continuous data were analyzed with Mann-Whitney *U* tests; categorical data were analyzed with Pearson's chi-square (χ^2) tests.

Since considerable differences were found between the Muenster and Munich patients regarding their clinical characteristics (disease acuteness, severity of current episode), as well as regarding their medication/ECT state, we decided to do to two different analyses: First, we compared patients of the Muenster and Munich cohorts with their respective HCs, and then, we compared patients of both cohorts between themselves. Second, comparisons between the entire group of patients with MDD and HCs, and between the small subgroup of medication/ECT-naïve patients, and their respective HCs were also made.

Group differences were tested by univariate analyses of covariance (ANCOVA), correcting for age, sex, bmi and smoking (patients vs. HCs), and for bmi, HAM-D 17 score, number of previous depressive episodes, durations of current episode, medication (yes/no), ECT (yes/no) (Muenster patients vs. Munich patients). In the case of medication/ECT-naïve subgroup of patients, we corrected only for smoking (yes/no). Bonferroni's adjustments for multiple comparisons were additionally applied in all analyses. Correlations between were determined by Spearman's-rank correlation coefficient (*rho*). All hypotheses were tested with $\alpha \leq 0.05$ (two-sided).

Due to methodological differences, and in order to correct for site-dependent differences regarding monocyte gene expression levels and serum TRP/KP catabolite levels, we derived a fold change by dividing individual values of immune parameter or metabolite by the HC average value of each item per site (e.g. MDD individual value of site Muenster divided by HC average of site Muenster). This enabled us to pool the data of the two sites for the supplementary data (all patients together and all non-medicated patients of the two sites). The calculations of the values normalized to the HC values did not alter the fold differences (and statistical significances) between patients and HCs using the original data

per site (data not shown).

3. Results

3.1. Sample characteristics

The clinical and demographic data of the study participants are shown in Table 1. In this table, all data are given per recruiting clinic (Muenster or Munich).

3.1.1. Muenster cohort

The MDD group included 96 men and 135 women with a mean age of 40 years and a mean bmi of 26 kg/m^2, indicating overweight. The IDS-C score was recorded in the 231 Muenster patients with MDD (see Section 2.1); we calculated a HAM-D 17 correlate score from this IDS-C score as described in the literature (Rush et al., 1986; Trivedi et al., 2004). Table 1 shows that this resulted in a mean HAM-D 17 score of 18, indicating –in general-, cases with a moderate depression. Patients reported a history of 4 previous depressive episodes on average; and being in the current episode since about 43 weeks. The vast majority of patients, i.e. 216 (93.5%) were on different regimens of antidepressants, 139 (60.2%) were treated with benzodiazepines/analogues, 135 (58.4%) were taking antipsychotic agents and/or mood stabilizers (15 (6.5%)); 23(10%) patients were undergoing electroconvulsive therapy (ECT).

3.1.2. Munich cohort

The MDD group included 25 men and 25 women with a mean age of 40 years and a mean bmi of 23 kg/m^2, indicating a normal weight. The mean HAM-D 17 score was 25, indicating -in general-, cases with a severe episode. Patients reported a history of 2 previous depressive episodes, and being in the current episode since 19 weeks; 38 (76%) were treated with benzodiazepines/analogues, the use of other psychotropic drugs/ECT was not allowed in the Munich patients (see Section 2.1).

Taken together, patients of the Munich cohort were less overweight and less medicated as compared with patients of the Muenster cohort. They were also characterized by a more acute and severe depressive episode (Table 1). The Muenster cohort was characterized by a longer history of MDD, were also longer in the present episode; they were using extensively more medication, and had at the time of testing less severe depression (though still being depressed), they also had a significantly higher bmi.

In Supplementary Table 3a, data are given for the entire group of patients with MDD (Muenster and Munich together, $n = 281$); in Supplementary Table 3b, data are given separately for the patients without any form of medication ($n = 20$) (also not benzodiazepines/analogues, as was allowed in the less medicated Munich group). From the total group of HCs, we selected 20 age, sex and bmi-matched HCs to be compared to the subgroup of 20 medication/ECT-free patients (Supplementary Table 3b). Where appropriate, we give immune and KP data for this subgroup underneath and in the supplementary material.

3.2. Monocyte inflammatory state of patients with MDD and HCs

Relative mRNA expression levels of ICCGs and ISGs of study participants are shown in Fig. 2a,b. In these figures, the essential data are given per recruiting clinic. Supplementary Fig. 2a,c show data of the entire group of study participants, data of the subgroup of medication/ECT-naïve patients and their respective HCs are given in Supplementary Fig. 2b,d.

3.2.1. Relative mRNA expression levels of ICCGs in circulating monocytes

The expression levels of five key ICCGs (i.e. *IL-1β, IL-6, TNFAIP3, CCL20* and *CXCL2*) were determined in the circulating monocytes of 121 patients with MDD (Muenster cohort ($n = 81$), Munich cohort ($n = 40$)), and in 124 HCs (Muenster cohort ($n = 82$), Munich cohort ($n = 42$)) (Fig. 2a). Fig. 2a shows a higher expression of ICCGs in patients with MDD of both the Muenster and the Munich cohorts as compared to their respective HCs. The same trend was found in the subgroup of medication/ECT-naïve patients (yet here, a statistical significance was however

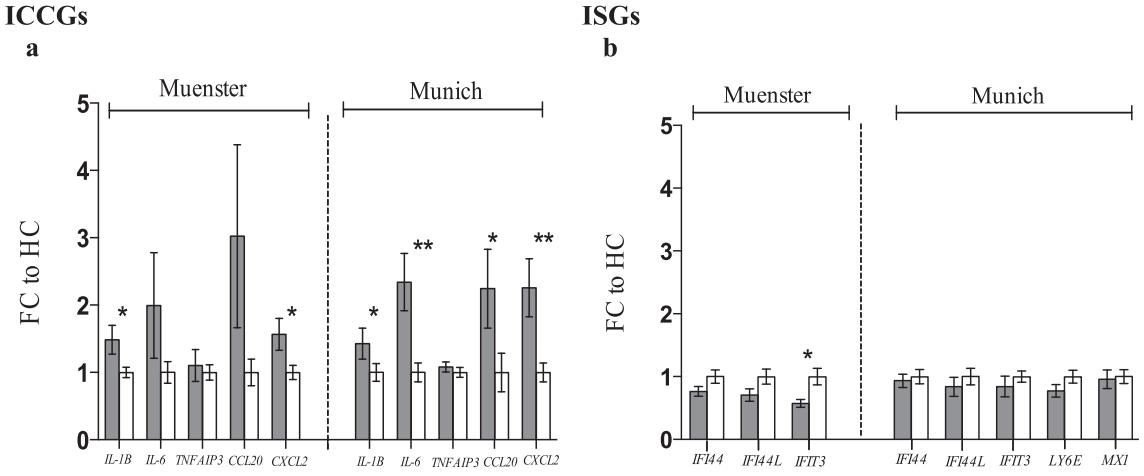

Fig. 2. a,b The relative mRNA expression levels of the indicated inflammatory genes in the circulating monocytes of patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$. *Abbreviations*: ICCGs: pro-inflammatory cytokine/chemokine genes; ISGs: type I interferon-stimulated genes; *IL-1β:* interleukin 1β gene; *IL-6:* interleukin 6 gene; *TNFAIP3:* tumor necrosis factor, alpha-induced protein 3 gene; *CCL20:* C-C-motif chemokine ligand 20 gene; *CXCL2:* C-X-X-chemokine ligand 2 gene; *IFI44:* interferon-induced protein 44 gene; *IFI44L:* interferon-induced protein 44 Like gene; *IFIT3:* interferon-induced protein with tetratricopeptide Repeats 3 gene; *LY6E:* lymphocyte antigen 6 family member E gene; *MX1:* interferon-regulated resistance GTP-binding protein MxA gene.

Compared to HCs, an increased expression of all ICCGs was found in patients of the Muenster cohort, values being statistically significant for *IL-1β* ($F(1,157) = 4.022$, $p = 0.047$,$\eta p^2 = 0.025$) and for *CXCL2* ($F(1,157) = 4.104$,$p = 0.044$,$\eta p^2 = 0.025$). The same increase was found in patients of the Munich cohort, values being in this case significant for *IL-1β* (F(1,76)=4.411,$p = 0.039$,$\eta p^2 = 0.055$), IL-6 (F(1,76)=9.571,$p = 0.003$,$\eta p^2 = 0.112$), *CCL2* (F(1,76)=5.299,$p = 0.024$,$\eta p^2 = 0.065$), and for *CXCL2* (F(1,76)=8.375,$p = 0.005$,$\eta p^2 = 0.099$). On the contrary, patients were characterized by decreased in ISGs monocyte expression levels, a statistical significance was only reached for *IFIT3* in the case of the Muenster cohort ($F(1,103) = 6.598$,$p = 0.012$,$\eta p^2 = 0.060$). No significant differences were found between patients of the Muenster and Munich cohorts in relation to their ICCGs and ISGs monocyte expression levels.

only reached for *IL-6* (Supplementary Fig. 2b). The small number of subjects in this group probably plays a role here.

Both the Muenster and Munich patients showed almost equally increased monocyte expression levels of the ICCGs (Fig. 2a). However, the increase was a little more outspoken in patients of the Munich cohort, showing statistical significant values in 4 of the 5 genes against 2 of the 5 of the Muenster cohort when compared with HCs. Interestingly, a positive correlation was found between the monocyte expression levels of all top five ICCGs and patient's HAM-D 17 score (being values statistically significant only for *IL-6* ($rho = 0.246$, $p = 0.006$)). Significant correlations were not found between ICCGs monocyte expression levels and neither medication/ECT state, nor disease acuteness (data not shown).

3.2.2. Relative mRNA expression levels of type I ISGs in circulating monocytes

We also determined the expression levels of five key type I ISGs (i.e. *IFI44, IFI44L, IFIT3, LY6E* and *MX1*) in the circulating monocytes of 92 patients with MDD (Muenster cohort ($n = 52$), Munich cohort ($n = 40$)), and 99 HCs (Muenster cohort ($n = 57$), Munich cohort ($n = 42$)) (Fig. 2b). Compared with HCs, patients with MDD of the two cohorts showed a reduced expression of all ISGs, yet a statistical significance was however only reached for *IFIT3* in the subgroup of patients of the Muenster cohort (Fig. 2b). Also in the entire group of patients, ISGs were underexpressed compared to HCs (Supplementary Fig. 2c), the same trend was found in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 2b).

Supplementary Fig. 2b also shows that treatment state had no obvious effect on this slightly reduced expression of the ISGs in monocytes. Significant correlations were not found between the monocyte expression levels of all ISGs and any of the clinical characteristics of patients (data not shown).

3.3. Serum levels of TRP/KP metabolites, KP metabolism ratios and monocyte expression levels of KP enzymes genes in patients with MDD and HCs

The TRP/KP catabolic data of study participants are shown in Figs. 3–7. In these figures, the essential data are given per recruiting clinic. Data of the entire group of patients and HCs, and the subgroup of medication/ECT-naïve patients and HCs are given in Supplementary Figs. 3–7.

3.3.1. TRP to 5-HTrp and 5-HIAA breakdown pathway

Compared with HCs, significantly decreased TRP and 5-HTrp serum levels were only found in the Munich patients (Fig. 3a,b); the same was found when compared with patients of the Muenster cohort. Considering the difference in treatment status between the Muenster and Munich patients, it is worthy to note that an effect of medication/ECT state was found for TRP and 5-HTrp in patients (i.e. not taking medication was significantly associated with lower TRP and 5-HTrp serum levels in patients ($rho = 0.140$, $p = 0.026$ and $rho = 0.289$, $p < 0.001$, respectively)). In support of this, significantly reduced TRP and 5-HTrp serum levels were found in the subgroup of medication/ECT-naïve patients as compared to HCs (Supplementary Fig. 3b,d). In addition, a significant negative correlation was also found between TRP and 5-HTrp serum levels, and patient's HAM-D 17 score ($rho = -0.279$, $p < 0.001$ and $rho = -0.363$, $p < 0.001$, respectively).

Muenster patients with MDD showed lower 5-HIAA serum levels compared to HCs and to patients of the Munich cohort (Fig. 3c). In this case, significant correlations were however not found between 5-HIAA serum levels in patients, medication/ECT state and diseases severity/acuteness (data not shown).

3.3.2. TRP to KYN breakdown pathway

Patients of both the Muenster and Munich cohorts showed reduced KYN serum levels towards HCs, though a statistical significance was not reached (Fig. 4a); a statistical significance was reached for the combined entire group of patients with MDD and HCs (Supplementary Fig. 4a). Both the Muenster and Munich patients equally contributed to the reduced KYN serum levels in patients (Fig. 4a). Accordingly, statistical significant correlations were not found between KYN serum levels and neither medication/ECT state, nor disease severity/acuteness (data not shown).

Interestingly, a significantly higher KYN/TRP ratio was found only in patients of the Munich cohort when compared with their respective HCs, and with patients of the Muenster cohort (Fig. 4b), although this did not result in higher KYN serum levels (since the precursor TRP was very low, see Section 3.3.1). A significant positive correlation was found between the KYN/TRP ratio and HAM-D17 score in MDD patients ($rho = 0.141$, $p = 0.029$).

Since IDO is an important inflammation-induced enzyme for the

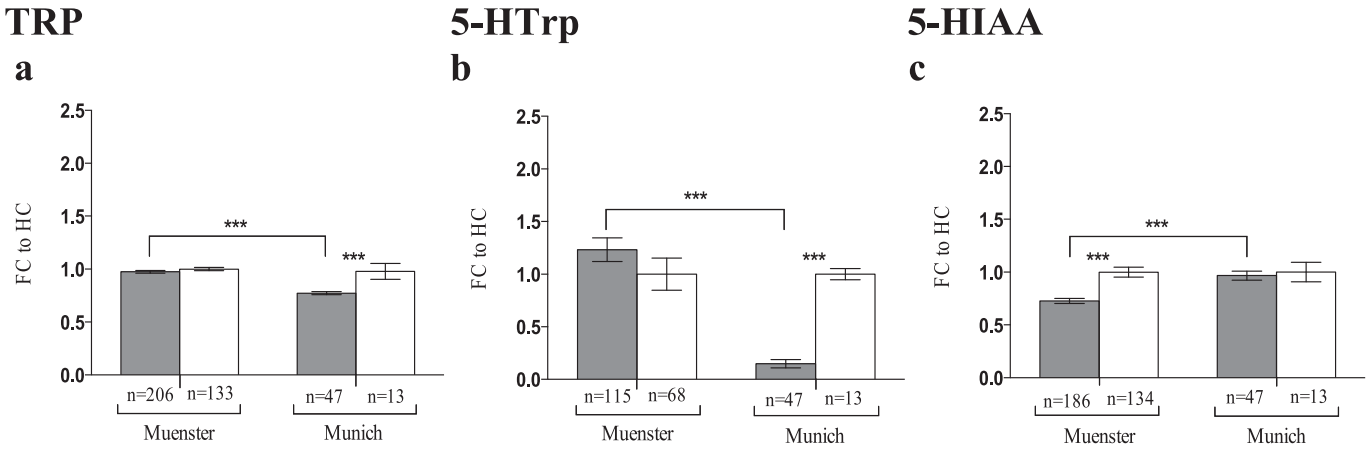

Fig. 3. a–c. Serum levels of TRP,5-HTrp and 5-HIAA in patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$.
Abbreviations: TRP: tryptophan; 5-HTrp: 5-hydroxytryptophan; 5-HIAA: 5-hydroxyindoleacetic acid.
Patients of the Munich cohort were characterized by decreased TRP and 5-HTrp serum levels as compared with HCs ($F(1,54)=20.105$,$p < 0.001$,$\eta p^2 = 0.271$ and $F(1,54)=122.455$,$p < 0.001$,$\eta p^2 = 0.694$, respectively), and with patients of the Muenster cohort ($F(1,246) = 32.480$,$p < 0.001$,$\eta p^2 = 0.117$ and $F(1,155) = 34.202$,$p < 0.001$,$\eta p^2 = 0.181$,respectively). Patients of the Muenster cohort were characterized by decreased 5-HIAA serum levels as compared with HCs ($F(1,314) = 28.589$, $p < 0.001$,$\eta p^2 = 0.083$) and with patients of the Munich cohort ($F(1,226) = 12.166$,$p = 0.001$,$\eta p^2 = 0.051$).

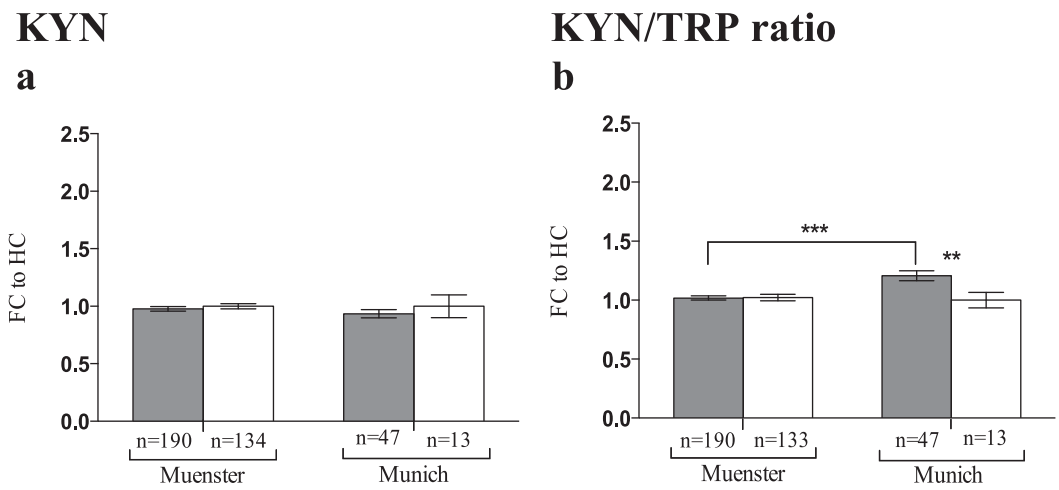

Fig. 4. a,b. Serum levels of KYN and the KYN/TRP ratio in patients with MDD and HCs. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$. *Abbreviations*: KYN: kynurenine; TRP: tryptophan.

Significant differences were not found between patients and HCs of both cohorts regarding KYN serum levels. Interestingly, patients of the Munich cohort were characterized by an increased KYN/TRP ratio as compared with their respective HCs ($F(1,54)=7.940, p = 0.007, \eta p^2 = 0.128$) and with patients of the Muenster cohort ($F(1,228) = 19.059, p < 0.001, \eta p^2 = 0.077$).

conversion of TRP into KYN, we also determined the expression levels of *IDO-1* in the circulating monocytes of 68 patients with MDD (Muenster cohort ($n = 29$), Munich cohort ($n = 39$) and 67 HCs (Muenster cohort ($n = 25$), Munich cohort ($n = 43$)) (Fig. 5).

IDO-1 monocyte expression levels did not differ between patients and HCs of both cohorts (Fig. 5); a statistical significance was in this case also not reached for the combined entire group of patients with MDD and HCs (Supplementary Fig. 5a). Significant differences were also not found between the Muenster and Munich patients in relation to *IDO-1* monocyte expression levels (Fig. 5).

Accordingly, significant correlations were not found between medication/ECT state, illness severity/acuteness and monocyte expression levels of *IDO-1* in patients (data not shown).

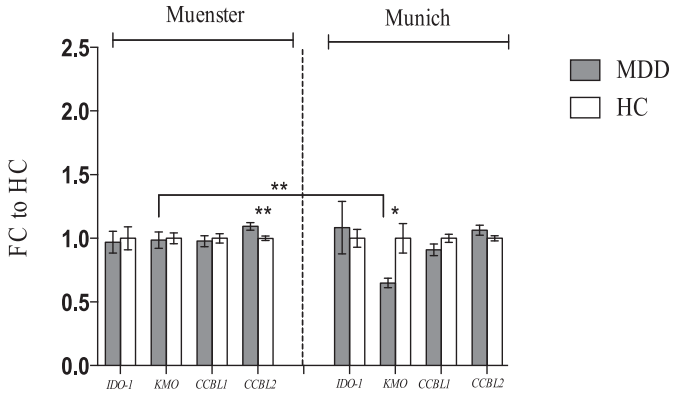

Fig. 5. The relative mRNA expression levels of the indicated inflammatory KP enzyme genes in the circulating monocytes of patients with MDD and HCs. Gray bars represent the group of patients with MDD, white bars represent the group of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$. *Abbreviations*: *IDO-1*: indoleamine 2,3-dioxygenase gene; *KMO*: kynurenine 3-monooxygenase gene; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase III gene.

Significantly decreased *KMO* monocyte expression levels were found in patients of the Munich cohort as compared with HCs ($F(1,75)=6.865, p = 0.011, \eta p^2 = 0.084$), and with patients of the Muenster cohort ($F(1,60)=9.420, p = 0.003, \eta p^2 = 0.136$). Significantly increased expression levels of *CCBL2/KAT III* were found in the circulating monocytes of patients of the Muenster cohort as compared with HCs ($F(1,109) = 7.414, p = 0.008, \eta p^2 = 0.064$).

3.3.3. KYN to KYNA breakdown arm

Significantly reduced serum levels of the NMDA-R antagonist KYNA, and KYNA/KYN ratios were found in both cohorts of patients with MDD (Fig. 6a,b). This was in particular evident in patients of the Muenster cohort (Fig. 6a,b). However, significant correlations were not found between these metabolite serum levels and neither medication/ECT state, nor disease severity/acuteness in patients (data not shown).

Since KAT enzymes are involved in the KYN to KYNA breakdown, we also determined the expression levels of *CCBL1/KAT I* and *CCBL2/KAT III* in the circulating monocytes of 94 patients with MDD (Muenster cohort ($n = 55$), Munich cohort ($n = 39$)), and 103 HCs (Muenster cohort ($n = 61$), Munich cohort ($n = 42$)) (Fig. 5).

Fig. 5 shows that – in accord with the reduced KYNA serum levels-, a reduced *CCBL1/KAT I* monocyte expression was found in patients with MDD of both cohorts, however, values did not reach statistical significance. A statistical significance was only reached in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 5b).

In contrast, an increased expression of *CCBL2/KAT III* was found in both cohorts of patients with MDD as compared to HCs; a statistical significance was however only found in the subgroup of patients of the Muenster cohort (Fig. 5) and in the entire group of patients with MDD (Supplementary Fig. 5a), in the latter irrespective of medication/ECT state (Supplementary Fig. 5b). Significant correlations were not found between *CCBL1/KAT I* and *CCBL2/KAT III* monocyte expression levels and neither medication/ECT state, nor disease severity in patients.

3.3.4. KYN to 3-HK breakdown arm

Compared to HCs, reduced serum levels of the NMDA-R agonist 3-HK (Fig. 6c), and a reduced 3-HK/KYN ratio were found in patients of both the Muenster and Munich cohorts (Fig. 6d). This was in particular evident in patients of the Munich cohort (Fig. 6c,d). Medication/ECT-naïve patients were also characterized by a decrease in serum levels of 3-HK (Supplementary Fig. 6d) and by a reduced 3-HK/KYN ratio (Supplementary Fig. 6f) as compared to their respective HCs. Accordingly, a significant negative correlation was found between 3-HK serum levels, the 3-HK/KYN ratio, and patient's HAM-D 17 score ($rho = -0.144$, $p = 0.028$ and $rho = -0.142$, $p = 0.030$, respectively). Not taking medication was also associated with lower 3-HK serum levels ($rho = 0.179$, $p =$

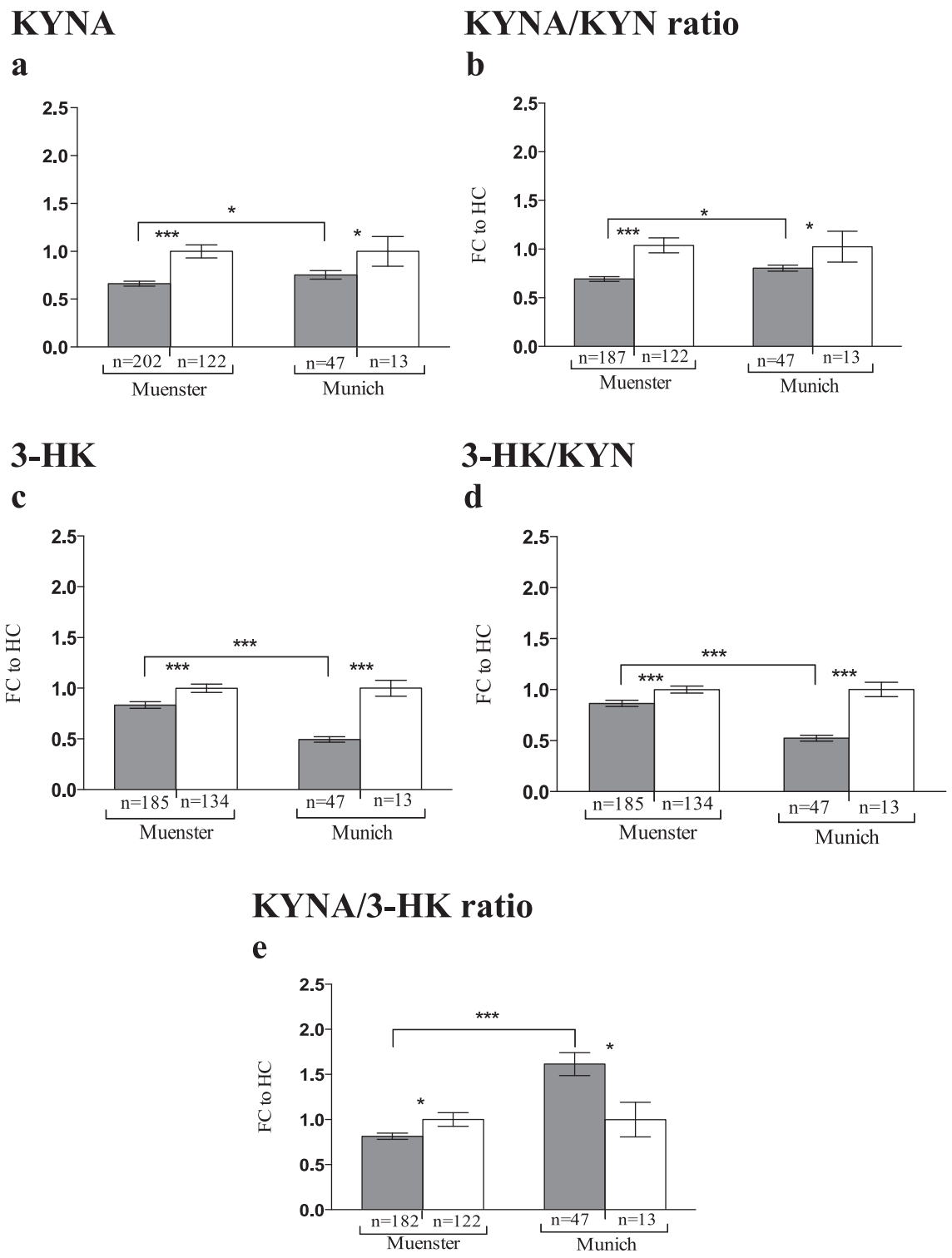

Fig. 6. a–e. Serum levels of the indicated KP metabolites and KP metabolism ratios in patients with MDD and HCs of both the Muenster and Munich cohorts. Gray bars represent the subgroup of patients with MDD, white bars represent the subgroup of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$. *Abbreviations*: KYNA: kynurenic acid; KYN: kynurenine.

Decreased KYNA serum levels and a decreased KYNA/KYN ratio were found in both cohorts of patients with MDD as compared with HCs. This was in particular evident in patients of the Muenster cohort, showing decreased KYNA serum levels ($F(1,240) = 5.333, p = 0.022, \eta p^2 = 0.022$), and a decreased KYNA/KYN ratio as compared with patients of the Muenster cohort ($F(1,225) = 5.900, p = 0.016, \eta p^2 = 0.026$). Decreased 3-HK serum levels, and a reduced 3-HK/KYN ratio were also found in both cohorts of patients with MDD. In this case, this was in particular evident in patients of the Munich cohort, showing significantly decreased 3-HK serum levels ($F(1,223) = 14.235, p < 0.001, \eta p^2 = 0.060$) and a decreased 3-HK/KYN ratio ($F(1,223) = 22.845, p < 0.001, \eta p^2 = 0.093$) as compared with patients of the Muenster cohort.

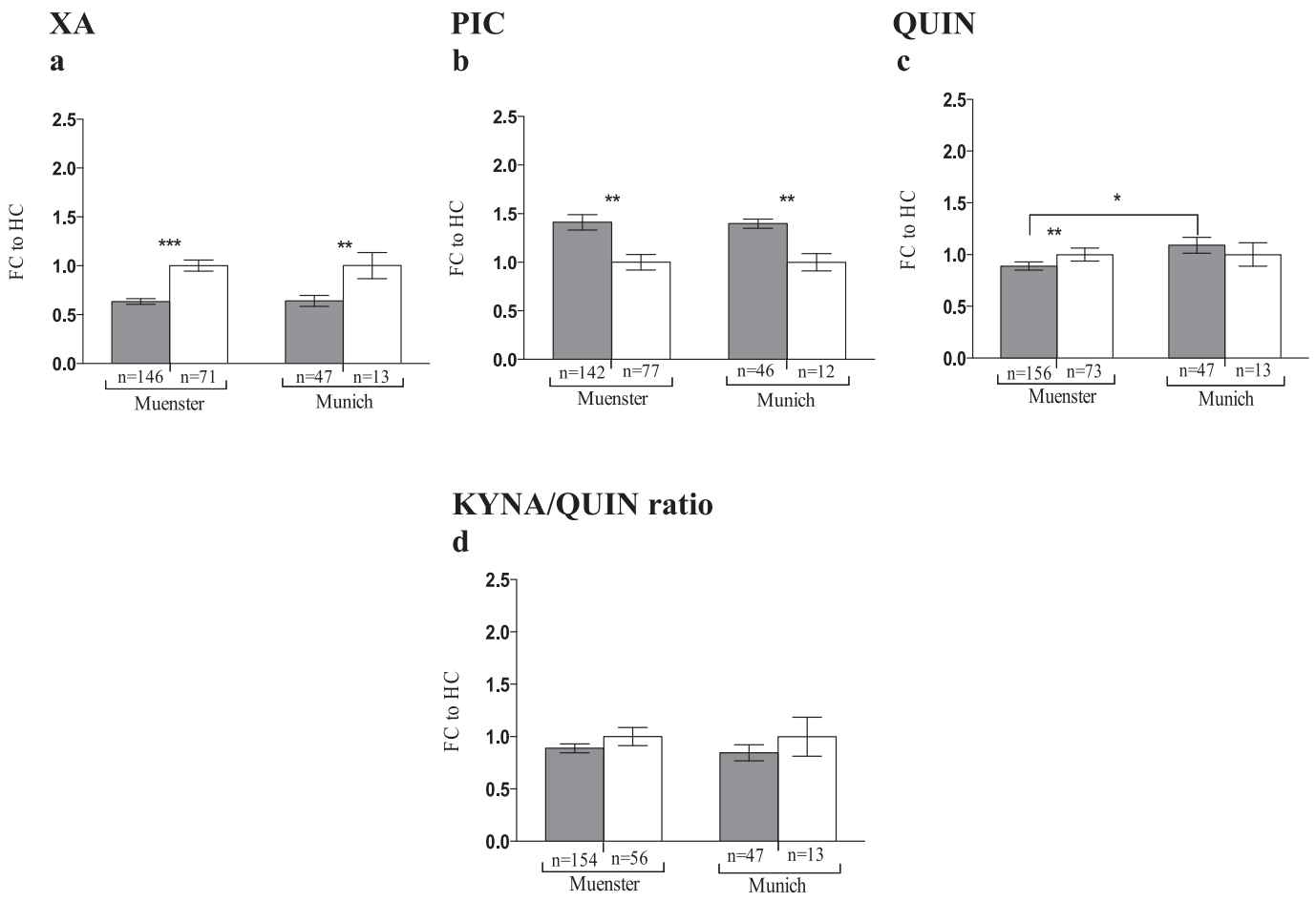

Fig. 7. a–d. Serum levels of the indicated KP downstream catabolites and the KYNA/QUIN ratio in patients with MDD and HCs. Gray bars represent the group of patients with MDD, white bars represent the group of HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). $^{*}p \leq 0.050$, $^{**}p \leq 0.005$, $^{***}p \leq 0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; XA: xanthurenic acid; QUIN: quinolinic acid; PIC: picolinic acid; KYNA: kynurenic acid. Compared with HCs, patients with MDD of both cohorts showed reduced serum levels of the mGlu2/3 agonist XA ($(F(1,211) = 41.437, p < 0.001, \eta p^2 = 0.164$ (Muenster cohort) and $F(1,54)=8.408, p = 0.005, \eta p^2 = 0.135$ (Munich cohort)). Significant differences were not found between patients of both the Muenster and Munich cohorts in relation to their XA serum levels. On the contrary, patients with MDD of both cohorts were characterized by increased serum levels of the potentially NMDA-R antagonist PIC ($(F(1,201) = 8.593, p = 0.004, \eta p^2 = 0.041$ (Muenster cohort) and $F(1,52)=10.926, p = 0.002, \eta p^2 = 0.174$ (Munich cohort)). Both the Muenster and Munich patients equally contributed to the reduced XA, and increased PIC serum levels in patients. Interestingly, patients of the Muenster cohort were characterized by significantly decreased serum levels of the NMDA-R agonist QUIN as compared with HCs ($F(1,223) = 7.647, p = 0.006, \eta p^2 = 0.033$) and with patients of the Munich cohort ($F(1,194) = 6.860, p = 0.010, \eta p^2 = 0.034$).

0.006) and with a reduced 3-HK/KYN ratio in patients ($rho = 0.165$, $p = 0.012$).

Since KMO is the enzyme involved in the KYN to 3-HK transition (Fig. 1), *KMO* expression levels were also determined in the circulating monocytes of 68 patients with MDD (Muenster cohort ($n = 29$), Munich cohort ($n = 25$)) and 67 HCs (Muenster cohort ($n = 39$), Munich cohort ($n = 42$)) (Fig. 5). Compared to HCs, *KMO* was significantly underexpressed in the circulating monocytes of the Munich patients, but not of the Muenster patients (Fig. 5). The same trend was found in the subgroup of medication/ECT-naïve patients (Supplementary Fig. 5b). We also found a significant negative correlation between *KMO* monocyte expression levels and patient's HAM-D 17 score ($rho = -0.357$, $p = 0.003$).

Since both 3-HK and KYNA serum levels were reduced in both cohorts of patients with MDD, we also determined the KYNA/3-HK ratio, in order to get an idea of the relative overweight of the potential neuroprotective over the potential neurotoxic arms of the KP. Fig. 6e interestingly shows a difference between Muenster and Munich patients regarding the KYNA/3-HK ratio; while in Muenster patients the potential neurotoxic arm was favored in comparison to HCs, the Munich patients showed an overweight of the potential neuroprotective arm. It must again be stressed here that in both cohorts, levels of 3-HK and KYNA were actually reduced.

3.3.5. 3-HK to XA breakdown arm

Compared to HCs, significantly decreased XA serum levels were found in patients of both the Muenster and Munich cohorts (Fig. 7a). Also in the entire group, XA serum levels were significantly decreased in patients as compared to HCs (Supplementary Fig. 7a), this irrespective of the medication/ECT state (Supplementary Fig. 7b). Accordingly, significant correlations were neither found between XA serum levels and medication/ECT status, nor with disease severity/acuteness in patients (data not shown).

3.3.6. 3-HK to PIC breakdown arm

Significantly increased PIC serum levels were found in patients of both the Muenster and Munich cohorts as compared to their respective HCs (Fig. 7b). Also in the entire group, PIC serum levels were significantly increased as compared to HCs (Supplementary Fig. 7c), the same trend was found in the subgroup of medicated/ECT patients (Supplementary Fig. 7d, $p = 0.053$). Accordingly, significant correlations were not found between PIC serum levels and neither medication/ECT status,

nor disease severity/acuteness (data not shown).

3.3.7. 3-HK to QUIN breakdown arm

Interestingly, patients of the Muenster cohort were characterized by significantly reduced QUIN serum levels as compared to their respective HCs, and to patients of the Munich cohort (Fig. 7c). However, significant correlations were not found between QUIN serum levels and neither medication/ECT status, nor with disease severity/acuteness (data not shown).

To investigate in more detail the apportioning of the so-called potentially neuroprotective versus potentially neurotoxic KP catabolites, we also analyzed the KYNA/QUIN ratio (Fig. 7d). Compared to HCs, this neuroprotective over neurotoxic ratio was found decreased in patients of both the Muenster and Munich cohorts, however, a statistical significance was not reached (Fig. 7d). A statistical significance was only reached if all patients were taken together (Supplementary Fig. 7g).

3.4. Correlations between the monocyte expression levels of pro-inflammatory genes and the monocyte expression levels of KP enzyme genes in patients with MDD

First, it is worthy to note that significant and negative correlations were found between the increased expression of ICCGs in patient's monocytes, and the monocyte expression levels of the 4 KP enzyme genes in patients (Table 2). This was in particular evident for *KMO* (catalyzing the transition of KYN to 3-HK), *CCBL1/KAT I* and *CCBL2/KAT III* (catalyzing the transition of KYN to KYNA). In contrast, a significant positive correlation was found between the monocyte expression levels of *IDO-1* and the monocyte expression levels of 4/5 ISGs in patients (Table 2), confirming the correlation data of Maria et al. (2016) in Sjögren's disease.

3.5. Correlations between the monocyte expression levels of pro-inflammatory genes and serum levels of TRP/KP metabolites and KP metabolite ratios in patients with MDD

Table 3 shows that the higher the expression of ICCGs in patient's monocytes, the lower the TRP serum levels in patients. In addition, a significant positive correlation was found between the monocyte expression levels of ICCGs and the KYN/TRP ratio.

Table 3 additionally shows that the expression of ICCGs in patient's monocytes significantly and negatively correlated to 3-HK serum levels and to the 3-HK/KYN ratio in patients.

Noteworthy significant correlations were not found between the expression levels of ICCGs in patient's monocytes and serum levels of KYN, KYNA, the KYNA/KYN ratio, XA, QUIN, PIC, and the KYNA/QUIN ratio in patients (data not shown). A consistent and noteworthy picture of correlations could also not be found for the monocyte expression levels of ISGs and any of the serum TRP/KP catabolites in patients.

Table 2
Correlations between the monocyte expression levels of ICCGs/ISGs and the monocyte expression levels of KP enzyme genes in patients with MDD.

	IDO-1	*KMO*	*CCBL1/KATI*	*CCBL2/KATIII*
IL-1β	−0.137	0.016	**−0.303****	**−0.369*****
IL-6	−0.175	**−0.410****	**−0.319****	**−0.236***
TNFAIP3	0.061	−0.221	**−0.207***	−0.010
CCL20	**−0.309***	**−0.372****	**−0.396*****	**−0.233***
CXCL2	**−0.337****	**−0.317****	**−0.290****	**−0.250***
IFI44	**0.404***	0.158	−0.014	−0.165
IFI44L	**0.430****	**0.317***	0.071	0.124
IFIT3	**0.458****	0.306	0.161	−0.028
LY6E	0.229	0.088	−0.304	0.148
MX1	**0.362***	0.187	0.174	0.010

Significant correlations are marked with an asterisk (*$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$) and highlighted in bold. *Abbreviations: IDO-1*:indoleamine 2,3-dioxygenase gene; *KMO*: kynurenine 3-monoxygenase gene; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase III gene; IL: interleukin; *TNFAIP3*: tumor necrosis factor, alpha-induced protein 3 gene; *CCL20*: C—C motif chemokine ligand 20 gene; *CXCL2*: C-X-X-chemokine ligand 2 gene; IFI: interferon-induced protein gene; *LY6E*: lymphocyte antigen 6 family member E gene; *MX1*: interferon-regulated resistance GTP-binding protein MxA gene.

Table 3
Correlations between the monocyte expression levels of ICCGs/ISGs, serum levels of TRP/KP metabolites and KP metabolite ratios in patients with MDD.

	TRP	KYN/TRP	3-HK/KYN	3-HK
IL-1β	−0.018	0.070	−0.084	−0.111
IL-6	**−0.220***	**0.305****	**−0.420*****	**−0.319****
TNFAIP3	**−0.264****	**0.314*****	**−0.442*****	**−0.282****
CCL20	−0.164	**0.282****	**−0.334****	**−0.245***
CXCL2	−0.179	**0.197***	−0.140	−0.170
IFI44	−0.124	−0.068	0.008	−0.173
IFI44L	−0.019	−0.218	0.070	−0.098
IFIT3	−0.055	−0.167	0.179	−0.006
LY6E	0.082	0.031	−0.286	−0.222
MX1	−0.043	−0.287	0.219	0.015

Significant correlations are marked with an asterisk (*$p \leq 0.050$, **$p \leq 0.005$, ***$p \leq 0.001$) and highlighted in bold. *Abbreviations:* TRP: tryptophan; KYN: kynurenine; 3-HK: 3-hydroxykynurenine; IL: interleukin; *TNFAIP3*: tumor necrosis factor, alpha-induced protein 3 gene; *CCL20*: C—C motif chemokine ligand 20 gene; *CXCL2*: C-X-X-chemokine ligand 2 gene; IFI: interferon-induced protein gene; *LY6E*: lymphocyte antigen 6 family member E gene; *MX1*: interferon-regulated resistance GTP-binding protein MxA gene.

4. Discussion

The TRP catabolic pathway in patients with MDD shows various activation and deactivation steps which are linked to their pro-inflammatory state. Table 4 gives an overview of the main data, we refer to Fig. 1 for the different TRP/KP catabolic pathways, and for the effects of TRP/KP metabolites on neuronal cell functions.

4.1. Tryptophan to 5-HTrp and 5-HIAA breakdown pathway

Consistent with previous findings (Maes et al., 1990; Charney, 1998; Hughes et al., 2012; Ogawa et al., 2014; Cowen and Browning, 2015; Kuwano et al., 2018), this study found reduced TRP and 5-HTrp serum levels in patients with MDD as compared to HCs, particularly in the more acute, severely affected and less medicated Munich patients with a relatively high monocyte ICCG expression. Indeed, the reduced TRP and 5-HTrp serum levels were associated with higher HAM-D 17 scores, and a higher monocyte ICCG expression (only TRP serum levels). They were also evident in the medication/ECT-naïve patients of the combined cohorts (Supplementary Fig. 3b,d).

Reduced TRP serum levels are a well-known feature of MDD and are thought to play a role in at least, part of the depressive syndrome, since TRP is – via 5-HTrp -, the ground substance for 5-hydroxytryptamine (5-HT, also known as serotonin), an essential neurotransmitter in mood regulation (Lapin and Oxenkrug, 1969; Toker et al., 2010).

Our data thus show a shortage of this ground substance particularly in non-medicated, severe and recent cases of MDD with a relatively high monocyte ICCG inflammatory state (the Munich cases), while this is was not the case in the medicated, longstanding and less severe cases of the Muenster cohort. Our observations thus support the important role of TRP and 5-HT deficiency in MDD, and a correcting effect of medication for this pathway.

4.2. TRP to KYN breakdown pathway

TRP is not only metabolized into 5-HT via the serotonin pathway, but also into KYN via the KP, which in fact acts as the major catabolic

Table 4
Synopsis of the findings.

Pro-inflammatory genes/agents	MDD Munich Early episode	MDD Muenster Late episode	Correlation to demographic characteristics	Correlation to disease severity (HAM-D 17 score)
ICCGs (monocytes)	↑	↑	Positive to age	Positive
ISGs (monocytes)	=	=	No correlation	No correlation
TRP/KP catabolites, KP catabolite's ratios and KP enzyme genes	**MDD Munich Early episode**	**MDD Muenster Late episode**	**Correlation to monocyte inflammatory state**	**Correlation to disease severity (HAMD-17 score)**
TRP	↓	=	Negative to ICCGs	Negative
5-HTrp	↓	=	No correlation	Negative
KYN	=	=	No correlation	No correlation
KYN/TRP ratio	↑	=	Positive to ICCGs	Positive
IDO-1 (monocytes)	=	=	Negative to ICCGs Positive to ISGs	No correlation
KYNA	↓	↓	No correlation	No correlation
KYNA/KYN ratio	↓	↓	No correlation	No correlation
CCBL1/KAT I (monocytes)	=	=	Negative to ICCGs	No correlation
CCBL2/KAT III (monocyten)	=	↑	Negative to ICCGs	No correlation
3-HK	↓	↓	Negative to ICCGs	Negative
3-HK/KYN ratio	↓	↓	Negative to ICCGs	Negative
KMO (monocytes)	↓	=	Negative to ICCGs	Negative
KYNA/3-HK ratio	↑	↓	Negative to ICCGs	Positive
XA	↓	↓	No correlation	No correlation
PIC	↑	↑	No correlation	No correlation
QUIN	=	↓	No correlation	No correlation
KYNA/QUIN ratio	=	=	No correlation	No correlation

↑: statistically significantly elevated compared to HCs, =: no statistically significant differences between patients and HCs; ↓: statistically significantly decreased compared to HCs. *Abbreviations:* MDD: major depressive disorder; HAM-D 17: Hamilton Rating scale for depression, 17-item version; ICCGs: inflammatory cytokine/chemokine genes; ISGs: interferon stimulated genes; TRP: tryptophan; 5-HTrp: 5-Hydroxytryptophan; KYN: kynurenine; *IDO-1*: indoleamine 2,3-dioxygenase gene; KYNA: kynurenic acid; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase II gene; 3-HK: 3-hydroxykynurenine; *KMO*: kynurenine 3-monooxygenase gene; XA: xanthurenic acid; PIC: picolinic acid; QUIN: quinolinic acid.

pathway for TRP in the body (Stone et al., 2013; O'Farrell and Harkin, 2017) (Fig. 1).

In recent years, various theories have emerged to provide a mechanistic link between the existence of an altered inflammatory state in patients with MDD and the decreased TRP availability for 5-HT synthesis in MDD. One of these theories has focused on an increased breakdown of TRP down the KP by activation of KP breakdown enzymes under the influence of inflammatory stimuli (Maes et al., 2011; Leonard and Maes, 2012; Arnone et al., 2018).

In support of such view, our study found an increased KYN/TRP ratio, but only in the subgroup of patients of the Munich cohort (characterized by a more severe episode as compared with patients of the Muenster cohort). The increased KYN/TRP ratio was found positively associated with the pro-inflammatory (ICCGs) state of patient's monocytes. This is in accord with a higher entrance of TRP into the KP in the Munich patients with a high monocyte pro-inflammatory state, supporting a view of TRP depletion down the KP at the expense of the serotonin pathway.

However, KYN serum levels (and many of the KYN downstream products) were not raised in the Munich patients. We therefore assume that it is not likely that there is a considerable drain of TRP down the KP in the Munich patients and that, initially reduced TRP levels (perhaps reduced by low uptake via the gut) may play a more important role in acting as factor for the raised KYN/TRP ratio.

4.2.1. A prominent role for IDO in the TRP to KYN breakdown pathway in patients with MDD?

IDO is one of the most well-known enzymes for the transition of TRP into KYN. It is specifically expressed in pro-inflammatory immune cells; therefore, a prominent role has been suggested for IDO in the phenomenon of inflammation-induced increased TRP to KYN degradation.

However, and against such a view of a prime role of IDO in depleting TRP levels in MDD, *IDO-1* was not overexpressed in the otherwise pro-inflammatory activated monocytes of patients. In fact, a negative correlation was found between the monocyte expression of ICCGs and *IDO-1* in patient's monocytes. Supporting our findings, Hughes et al. (2012) also found a strong TRP depletion, a high KYN/TRP ratio and normal KYN levels in the presence of a normal *IDO-1* expression in subjects with MDD, also raising doubts about an activation of IDO in MDD. Other investigators have pointed to liver TDO, activated by stress, as playing a more prominent role in the TRP to KYN transition in patients with MDD (Dantzer et al., 2011; Maes et al., 2011; Badawy, 2013; Godoy et al., 2018; Qin et al., 2018). However, as an increase in KYN serum levels was not observed in our study, it is unlikely that the depletion of TRP resulted from a stress-related increase in TDO activity.

Interestingly, a positive correlation was found between the monocyte expression levels of *IDO-1* and type I ISGs in patients, although the ISG expression was not raised (it was earlier suppressed). This positive correlation reinforces the idea that IDO is under the control of inflammation, but of another type than the ICCG expression. Since ISG inflammatory overexpression is a characteristic of patients with systemic autoimmune conditions such as pSS (Maria et al., 2016), while ICCG inflammatory over expression is a characteristic of patients with mood disorders, our present and previous findings suggest that an increased IDO mediated TRP to KYN breakdown is particularly relevant for the immune dysregulation in systemic autoimmune conditions and not for that in mood disorders. This may be of relevance since it may distinguish the MDD state from the state of depression that occurs during the course of other somatic diseases such as pSS or from depression induced by administration of exogenous cytokines such as IFN-α (Capuron et al., 2012; Raison et al., 2010).

4.3. KYN to 3-HK and KYNA breakdown arms

In the present study, and contrary to what expected (see Background section), significantly reduced 3-HK serum levels, and a significantly reduced 3-HK/KYN ratio (reflecting KMO enzyme activity) were found in both cohorts of patients with MDD as compared to HCs, although these phenomena were in particular pronounced in patients of the Munich cohort. The more reduced KMO activity in the Munich patients was reflected in a significantly reduced monocyte expression level of *KMO*. Since the expression of the ICCGs in patient's monocytes were negatively associated with the monocyte expression level of *KMO*, the 3-HK/KYN ratio, and with the 3-HK serum levels, our findings suggest that the higher the pro-inflammatory ICCGs state of patient's monocytes, the lower the potential of monocytes to catabolize KYN into 3-HK. Importantly, this decrease in the KYN to 3-HK transition was also negatively associated with a higher HAM-D 17 score in patients.

In support of our findings, Wurfel et al., 2017 also found lower 3-HK serum levels in a cohort of patients with affective disorders (i.e. MDD, bipolar disorder and schizoaffective disorder), with 3-HK serum levels

being particularly reduced in affective psychosis. In addition, the study of Clark et al., 2016 also found a reduced KP metabolism, be it not in the blood, but in the ventrolateral prefrontal cortex of individuals with depression. Hughes et al. (2012) on the other hand did neither find a deactivation nor an activation of the KP in depressed patients.

With regard to the KYN to KYNA breakdown arm, reduced serum levels of KYNA, and a reduced KYNA/KYN ratio (reflecting a reduced activity of the KAT enzyme system) were found in patients of both cohorts. With regard to the KAT enzymes involved in the breakdown of KYN to KYNA, a contrasting picture was found. In accord with a reduced activity of the KAT enzyme system, we found a tendency for a reduced expression of the *CCBL1/KAT I* in the circulating monocytes of patients of both cohorts (but only in the unmediated patients). However, there also was an overexpression of *CCBL2/KAT III* in the monocytes of the Muenster patients. We explain this inconsistency by assuming that monocyte *CCBL2/KAT III* must play a minor role in the systemic transition of KYN to KYNA in patients with MDD. Clearly more investigations are needed on the role of the three KAT enzymes in KYN-to-KYNA transition in different tissues.

Collectively, our data suggest a deactivation of both the potentially neuroprotective KYN to KYNA, and the potentially neurotoxic KYN to 3-HK breakdown arms in MDD. However, consistent signs of an involvement of the monocyte pro-inflammatory (ICCGs) state of patients was only found for the deactivation of the KYN to 3-HK breakdown arm.

4.4. 3-HK to XA breakdown arm

XA has been implicated in various central nervous system disorders such as chronic pain and/or epilepsy (Melnikova, 2003; Curto et al., 2015). In addition, it has also been shown to exert antipsychotic effects and, interestingly, it has been reported as substantially reduced in patients with schizophrenia (Curto et al., 2019). However, only few studies have measured XA serum levels in MDD (Colle et al., 2020; Ryan et al., 2020). Consistent with previous reports, reduced XA serum levels were found in both cohorts of patients with MDD as compared with HCs. This was irrespective of disease severity/acuteness, and of medication/ECT status.

XA production is also under the influence of KAT enzymes. As discussed in 4.3, a reduced monocyte expression of *CCBL1/KAT I*, accompanied by an increased monocyte expression of *CCBL2/KAT III* were found in the circulating monocytes of –especially-, patients of the Muenster cohort (and the subgroup of medication/ECT naïve patients, see Supplementary Fig. 5b). We reiterate the need of further studies to understand the relationship between the chronic low grade inflammatory state of patients with MDD, KATs expression, and XA production from 3-HK in MDD.

Our results could be however of relevance due to XA's capacity to activate metabotropic glutamate 2 and 3 (mGlu2/3) receptors and thereby, inhibit excitatory synaptic transmission (Fazio et al., 2015). In the last years, glutamatergic pathway disturbances have emerged as an important key factor in, at least, a subgroup of patients with MDD, and increasingly literature doubts about (a generalized) validity of the "serotonergic theory of MDD", pointing in the direction that low serotonin levels may be an important factor for preserving recovery from depression rather than having a primary effect on mood lowering in vulnerable people (Cowen, 2008; Cowen and Browning, 2015). Therefore, modulation of glutamate receptors has been suggested as an important new target for the treatment of MDD (Matrisciano et al., 2007; Park et al., 2015). In line with this, recent evidence has showed antidepressant-like activity of mGlu2/3 receptor agonists in animal models of depression (Feinberg et al., 2002; Matrisciano et al., 2007).

4.5. 3-HK to QUIN and PIC levels

Increased serum levels of the NMDA-R antagonist PIC were found in patients of both cohorts as compared to their respective HCs; this increase was irrespective of disease severity/acuteness and of medication/ECT status.

On the opposite, and contrary to what expected, significantly decreased serum levels of the NMDA-R agonist QUIN were found in patients of the Muenster cohort as compared with both HCs, and with patients of the Munich cohort. However, significant correlations were neither found between QUIN serum levels nor with disease severity/acuteness or medication/ECT status in patients.

4.6. Limitations of the study

The results of the present study should be interpreted in light of several important limitations.

First, the used parameters were determined in blood, and outcomes might differ from measurements in the brain or in the CSF. Of interest in this context is that McGuiness et al. (2016) showed that in the non-obese diabetic mouse model of anxiety and depression, inflammatory stimulation with lipopolysaccharides (LPS) did induce the expression of ISGs and *IDO-1* in the *brain*, but that it did not induce ISG expression in *circulating monocytes* (while it did induce ICCG expression in these cells, thus giving a monocyte profile similar to the pattern found here in patients). This shows that a different inflammatory reaction of the brain versus the periphery is possible and this thus also makes a local brain depletion of TRP due to a local *IDO-1* overexpression possible in the absence of a similar reaction in the periphery.

Furthermore, the inflammatory parameter used was the expression of inflammatory genes in circulating monocytes. We were only able to measure the circulating inflammatory compounds hsCRP and IL-6 in the Muenster patients and controls (Supplementary Table 4). Of note, we did not find significant elevations of these inflammatory compounds in patients, there did also not exist a positive correlation of these compounds with the monocyte inflammatory gene state. The latter suggests other sources of these serum inflammatory compounds than the monocytes (e.g. liver and adipose tissue). A similar discrepancy between monocyte inflammatory state and serum inflammatory serum compounds has been described for the metabolic syndrome too, where adiposity and blood lipid levels play an important role in the discrepancy (Baldeon-Rojas et al., 2016). Interestingly, KYN and QUIN serum levels did correlate to the serum levels of hsCRP and IL-6 (see legend Supplementary Table 4). All this is of relevance since various reports showed increased blood/CSF IL-6, KYN and QUIN levels in patients with MDD and suicidal behavior (Lindqvist et al., 2009; Sublette et al., 2011; Erhardt et al., 2013); high QUIN levels have also been detected in the microglia of suicide victims (Busse et al., 2015). Thus, the positive correlation between blood/CSF inflammatory compounds, QUIN, and KYN could highlight a detection possibility for an important subgroup of patients with MDD. Unfortunately, our information on patient's risk for suicidal behavior was not uniformly recorded in the patients under study here, while psychotic patients were excluded from recruitment in the MOODINFLAME study. Therefore, we could not perform studies on the effects of these variables in this sample of patients with MDD and HCs.

Also, and although we investigated a larger panel of TRP and KP metabolites than most of the previous studies, our panel is still limited. Future studies should consider other KP metabolites, such as 3-HAA and/or AA, as well as the genes for tryptophan hydroxylase (TPH)-II, monoamine oxidase (MAO), kynureninase and/or TDO.

Another limitation was the relatively small sample size of the Munich cohort compared to the Muenster cohort, yet we have tried to take this into account in our supplementary material and pooled the data of the two cohorts to be able to draw more generalized conclusions for MDD, irrespective of the phase of the episode. We additionally analyzed in this total cohort medicated and medication-free patients,separately.

Furthermore, methodologies for TRP metabolite determinations differed between both cohorts and unfortunately, various runs of assays were carried out on limited numbers of patients over time, resulting in the condition that not all variables were tested in all patients. To be able

to pool the data we harmonized data of a metabolite or immune parameter to the means of the HC value of that parameter, this harmonization had no effects on actual fold differences (and the significance level) between patients and control values of that parameter. Nevertheless, future studies on larger cohorts should try to standardize as much as possible their detection assays and include larger groups of antidepressant-treated and antidepressant-naïve patients with MDD in better defined phases of the disease to obtain more precise data on TRP metabolism.

5. Conclusions

The systemic TRP/KYN catabolic pathway shows various activation and deactivation steps in MDD which are linked to the inflammatory state of patients.

TRP serum levels were reduced in patients and linked to an increased pro-inflammatory (ICCGs) monocyte state. This was in particular evident in the subgroup of patients with the highest HAM-D 17 score (Munich patients). Contrary to what expected, KYN serum levels were not increased in patients; an increased KYN/TRP ratio was only found in the subgroup of patients with the lowest TRP serum levels (Munich patients). *IDO-1* monocyte expression levels were decreased in patients and negatively related to their pro-inflammatory (ICCGs) monocyte state. Thus, a decrease in TRP serum levels via an ICCGs-inflammatory activation of the KP is unlikely in MDD.

Downstream from KYN, various other activation and deactivation steps were detected, and linked to the inflammatory state of patients. This resulted, regarding compound capable of influencing glutamate receptors, in reduced serum levels of 3-HK (NMDA-R agonist), KYNA (NMDA-R antagonist), and XA (mGlu2/3 agonist). PIC (NMDA-R antagonist) was increased in patients, QUIN (NMDA-R agonist) was decreased only in the Muenster patients. Only 3-HK serum levels were related to the pro-inflammatory (ICCGs) monocyte state of patients; the deactivation of the KYN to 3-HK breakdown pathway was in particular evident in the cohort of patients with the highest HAM-D 17 score (Munich patients). The ultimate effect on brain glutamate receptor triggering of this altered equilibrium between peripheral agonists and antagonists needs further exploration.

Ethical statement

This study has been conducted in compliance with standards for Good Clinical Practice (GCP), assuring that the rights, safety and well-being of patients were protected in accordance with the principles that have their origin in the Declaration of Helsinki (June 1964, last amendment Tokyo 2004). Additionally, for the conduct of the study, the relevant national and European regulations were adhered to. After study procedures had been fully explained, all subjects provided written informed consent prior to performance of any screening phase evaluations. Only patients who had the cognitive abilities for the informed consent of the study participation were included. The study was approved by the ethics committee of the Medical Association Westphalia-Lippe, Germany (2009-019-f-S) and the ethics committee of the medical faculty at the Ludwig-Maximilian-University of Munich, Germany (234-09).

Declaration of competing interest

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Authors contributions

BB, EW and LG collected the data, GAH analyzed the data. GAH and HAD drafted the manuscript. NM, GS, MS, NM, LG, HAD critically reviewed the manuscript. All other authors read and approved the final manuscript.

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Appendix A. Supplementary data

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Further Reading

Ogyu, K., Kubo, K., Noda, Y., et al., 2018. Kynurenine pathway in depression: a systematic review and meta-analysis. Neurosci. Biobehav. Rev. 90, 16–25. https://doi.org/10.1016/j.neurobiorev.2018.03.023.
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	Component		
	1	2	3
IL1β	,978	-,105	-,125
CCL20	,937	-,048	-,049
IL6	,907	-,043	-,125
TNFAIP3	,870	-,282	,136
CXCL2	,739	,332	-,025
PDE4B	,734	-,053	,127
EREG	,731	,252	-,023
ATF3	,719	,054	-,069
IL1A	,678	,166	,093
PTX3	,652	,403	,077
PTGS2	,609	,177	,195
DUSP2	,578	,422	,008
TNF	,566	,041	-,094
ADM	,505	,344	-,128
BCL2A1	,435	,382	-,203
CCL7	-,046	,961	,128
NAB2	-,104	,952	-,046
PTPN7	-,056	,921	-,019
CCL2	-,109	,895	,408
STX1A	,057	,878	-,175
THBD	-,033	,801	-,192
MAFF	,156	,755	,183
IRAK2	,250	,733	,015
MAPK6	,175	,664	-,034
DHRS3	,034	,637	-,036
BTG3	,344	,605	,105
SERPINB2	,314	,526	,150
MXD1	,341	,519	,181
IL1R1	,082	,485	-,058
CDC42	,371	,447	-,332
HSPA1A/B	,210	-,301	,159
CD9	,121	,257	-,212
IFIT3	-,085	-,027	,934
IFI44	,053	,168	,861

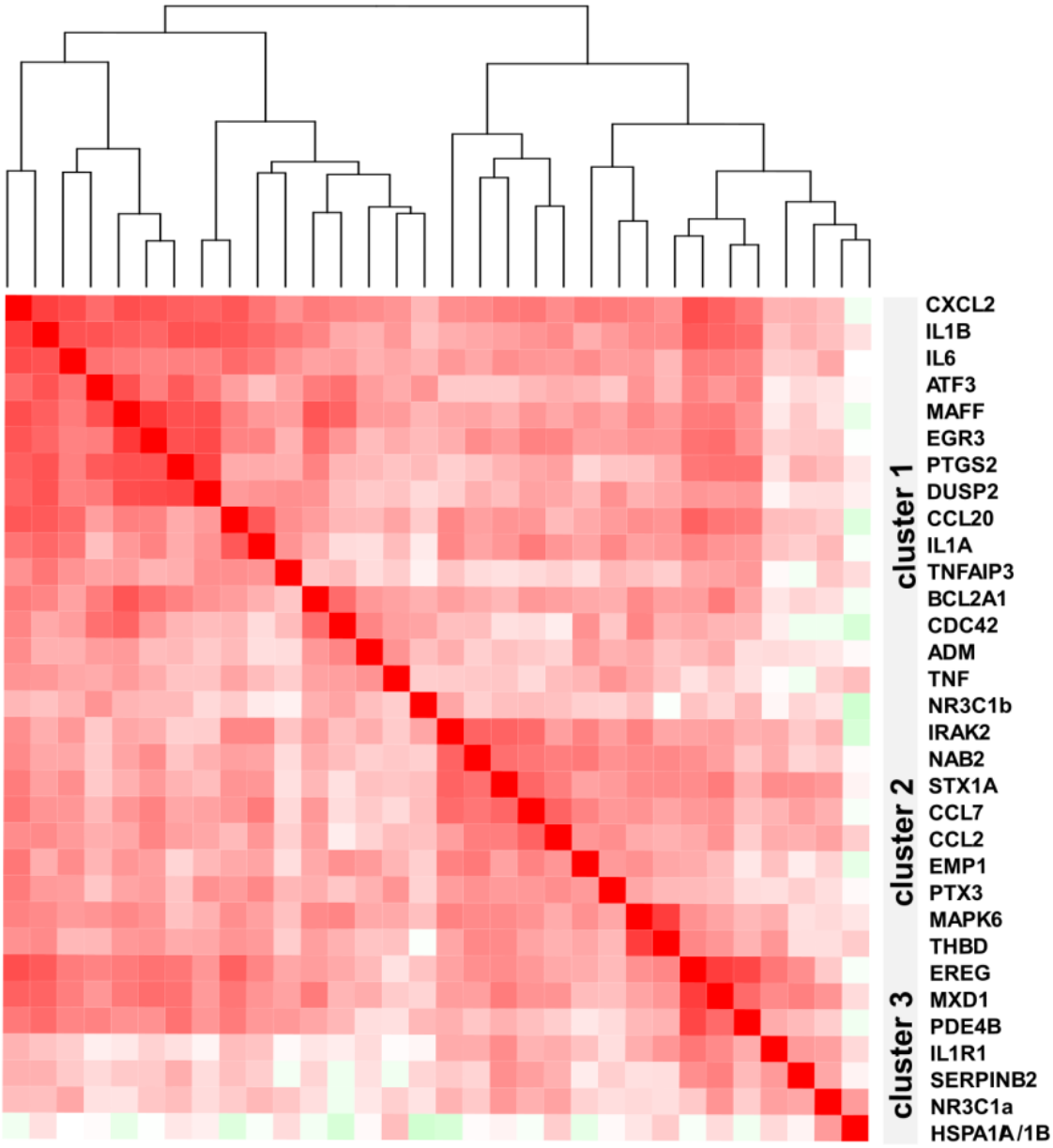
Supplementary Table 1. Principle component analysis for grouping of monocytes based on their gene expression levels (Vogels et al.,2017). Shown are the pattern matrices, values represent the correlation coefficients between genes and components. Three fixed factors were extracted for both the HCs and patient's analyses, using oblimin

rotation with a maximum of 25 iterations and listwise deletion of participants without complete data for all included genes. As can be seen, there is a cluster of mainly inflammation-related cytokines/chemokines and factors in their production (i.e. component 1, here also called as the ICCGs signature). The 5 top genes were used for the present study (cadre).

	Muenster patients:			Munich patients:		
	1	2	3	1	2	3
EGR3	,885	-,081	-,080	,986	,071	-,120
NAB2	,785	,193	,163	,978	,048	-,151
CCL2	,784	-,222	,028	,950	,115	-,143
CCL7	,766	,506	,004	,913	-,012	-,004
MAFF	,740	-,268	-,229	,902	,040	,172
PTGS2	,616	-,030	-,430	,898	,123	,098
ATF3	,554	-,342	-,230	,769	-,025	,352
EREG	,550	-,102	,024	,591	,031	,263
PDE4B	,399	-,267	-,052	,588	-,025	,517
FABP5	,012	-,810	,125	,498	,165	,417
MAPK6	,156	-,789	-,006	,424	,337	,184
HSPA1A_HSPA1B	,043	-,726	,059	,391	,219	,360
THBD	,412	-,709	,115	-,348	-,157	-,253
STX1A	,260	-,657	-,039	,012	,917	,064
IL1R1	,259	,654	,134	-,118	,902	-,189
CDC42	,283	-,649	-,226	,038	,819	,121
MXD1	,202	-,589	-,201	,401	,770	,100
DHRS3	,132	-,575	,164	,217	,697	,330
IRAK2	,185	,553	,013	,185	,621	,119
BCL2A1	,331	-,469	-,298	,263	,580	,268
SERPINB2	,196	,444	,056	-,055	,543	,298
IL1B	,085	-,050	-,860	,257	-,499	,436
CXCL2	,166	-,029	-,854	-,158	-,495	,233
TNF	-,269	,137	-,847	,103	-,113	-,085
PTX3	,115	-,278	-,778	,094	,027	,748
ADM	,044	,023	-,644	-,037	,223	,686
DUSP2	,553	-,065	-,624	,303	-,316	,661
TNFAIP3	,119	,469	-,590	-,175	,110	,643
IL1A	,396	,076	-,564	,140	,318	,617
CD9	-,331	-,048	-,504	-,510	,341	,559
IL6	,266	-,045	-,419	,356	,091	,558
PTPN7	,188	-,339	-,363	,200	-,213	,507
CCL20	-,044	-,013	-,175	,195	,309	,420

Supplementary Table 2. Principle component analysis for grouping of the monocytes based on their gene expression in the present study. Shown are the pattern matrices, values represent the correlation coefficients between genes and components. Three fixed factors were extracted for both the HCs and patient's analyses, using

oblimin rotation with a maximum of 25 iterations and list wise deletion of participants without complete data for all included genes. As can be seen, there is a cluster of mainly inflammation-related interleukins/cytokines/chemokines and factors in their production (i.e. component 3, particularly in the Muenster patients, here also called the ICCGs signature). The 5 genes used for the present study belonged to this component (cadre).



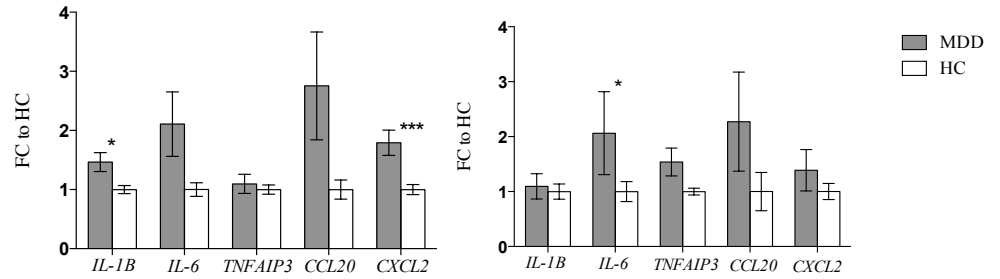
Supplementary Fig.1. Interrelated gene clusters from the study of Schiweck et al.,2020 (unpublished observations). In this study, a total of 198 patients with MDD and 208 HCs from the MOODINFLAME studies were incorporated (up till now, our largest study on monocyte gene expression levels in patients with MDD). Gene clusters are shown based on distances derived from Spearman's rank correlation matrixes. Three gene clusters were identified, of which cluster 1 comprised most of the inflammatory genes (in the present article, called as the ICCGs signature). The five genes tested in our study belonged to this cluster and are indicated.

	a.)				b.)			
	HCs n=206	MDD n=281	MDD vs. HC		HCs n=20	MDD n=20	MDD vs. HC	
	M(SD)	M(SD)	*F*	*p*	(M±SD)	(M±SD)	*F*	*p*
Number of previous depressive episodes	0(0.00)	3.33(5.53)	74.817	**<0.001**	0(0.00)	2.00(1.79)	24.863	**<0.001**
Duration of current episode (weeks)	0(0.00)	38.66(48.80)	129.281	**<0.001**	0(0.00)	33.75(32.06)	22.161	**<0.001**
HAM-D 17 score	-	19.18(5.49)	-	-	ND	20.40(4.50)	ND	ND
Age (years)	36.17(12.19)	40.05(12.43)	11.810	**0.001**	36.93(10.82)	32.08(11.52)	1.889	0.177
BMI (kg/m^2)	23.66(3.09)	25.77(4.51)	33.616	**<0.001**	23.18(3.63)	24.72(3.99)	1.622	0.211
	n (%)	n (%)	χ^2	*p*	n (%)	n (%)	χ^2	*p*
Sex								
Males	76(36.90)	121(43.10)	1.877	0.171	6(30.00)	8(40.00)	0.440	0.507
Females	130(63.10)	160(56.90)			14(70.00)	12(60.00)		
Smoking								
Yes	52(25.20)	126(45.00)	19.959	**<0.001**	4(20.00)	10(52.60)	4.509	**0.034**
No	154(74.80)	154(55.00)			16(80.00)	9(47.40)		
Medication								
Yes	0(0.00)	261(92.90)	412.308	**<0.001**	-	-	-	**-**
No	206(100.00)	20(7.10)						
ECT								
Yes	0(0.00)	23(8.20)	17.697	**<0.001**	-	-	-	**-**
No	206(100.00)	258(55.60)						

Supplementary Table 3 a,b. Clinical and demographic data of patients with MDD and HCs. a.) represents the whole group of patients with MDD and HCs, b.) represents the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. Values marked in bold indicate a *p*-value ≤ 0.05. *Abbreviations:* HCs: healthy controls; MDD: major depressive disorder; M: mean; SD: standard deviation; HAM-D 17: Hamilton Rating Scale for Depression,17-item version; bmi: body mass index; ECT: electroconvulsive therapy.

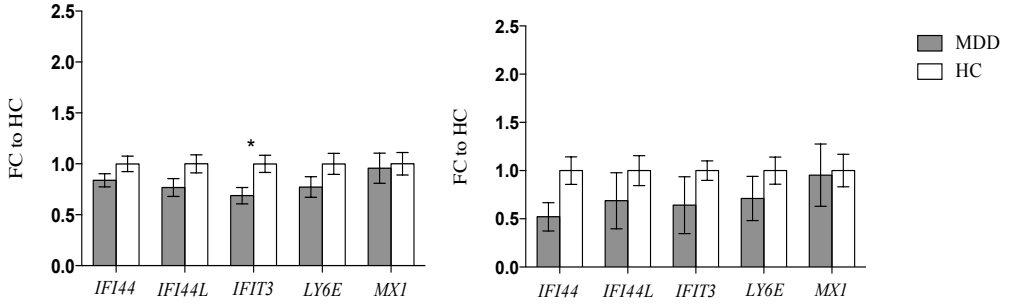
ICCGs

a **b**



ISGs

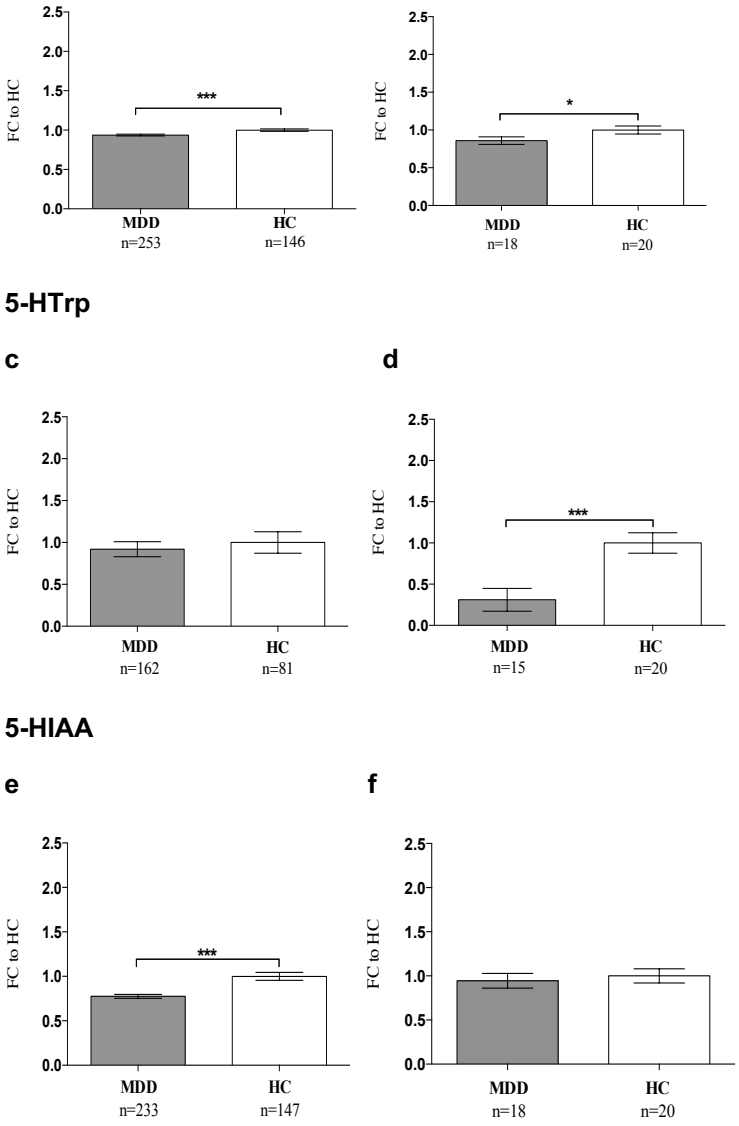
c **d**



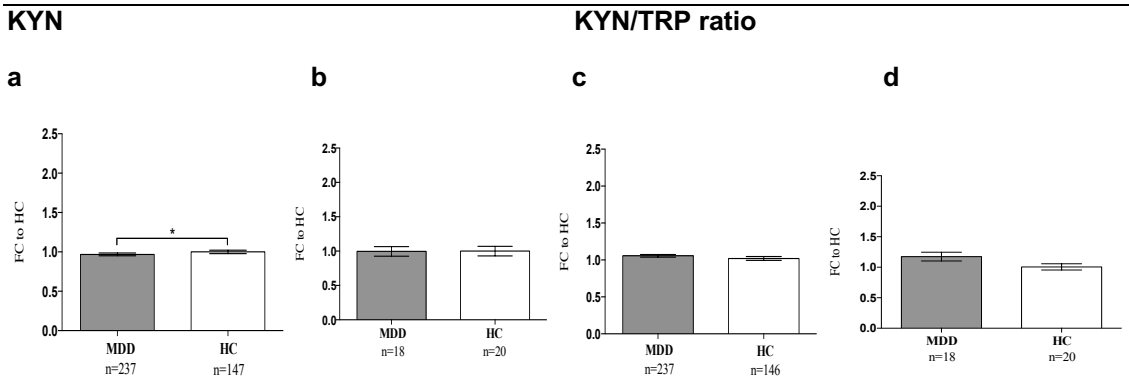
Supplementary Figs.2 a-d. The relative mRNA expression levels of the indicated pro-inflammatory genes in the circulating monocytes of patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). $^{*}p\leq0.050$, $^{**}p\leq0.005$, $^{***}p\leq0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; ICCGs : pro-inflammatory cytokine/chemokine genes; ISGs: type I interferon stimulated genes; *IL-1β:* interleukin 1β gene; *IL-6:* interleukin 6 gene; *TNFAIP3:* tumor necrosis factor, alpha-induced protein 3 gene; *CCL20:* C-C-motif chemokine ligand 20 gene; *CXCL2:* C-X-X-chemokine ligand 2 gene; *IFI44:* interferon-induced protein 44 gene; *IFI44L:* interferon-induced protein 44 Like gene; *IFIT3:* interferon-induced protein with tetratricopeptide Repeats 3 gene; *LY6E:* lymphocyte antigen 6 family member E gene; *MX1:* interferon-regulated resistance GTP-binding protein MxA gene. Compared to HCs, an increased expression of all ICCGs was found in patients, values being statistically significant for *IL-1β* ($F(1,239)=6.551, p=0.011, \eta p^2=0.027$) and for *CXCL2* ($F(1,239)=11.541, p=0.001, \eta p^2=0.046$). The same trend was found in the subgroup of medication/ECT-naïve patients (yet here, a statistical significance was however only reached for *IL-6* ($F(1,30)=4,761, p=0.037, \eta p^2=0.137$). On the contrary, decreased ISGs monocyte expression levels were found in subjects with MDD, values reaching significance for *IFIT3* ($F(1,185)=5.001, p=0.027, \eta p^2=0.026$). The same trend was again found in the subgroup of medication/ECT-patients, with values reaching a trend of statistical significance in the case of *IFI44* ($F(1,25)=3.568, p=0.071, \eta p^2=0.125$).

TRP

a **b**

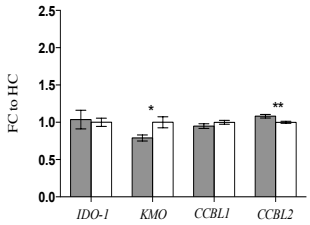


Supplementary Figs.3 a-f. Serum levels of TRP,5-HTrp and 5-HIAA in patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). $^{*}p \leq 0.050$, $^{**}p \leq 0.005$, $^{***}p \leq 0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; TRP: tryptophan; 5-HTrp: 5-hydroxytryptophan; 5-HIAA: 5-hydroxyindoleacetic acid.

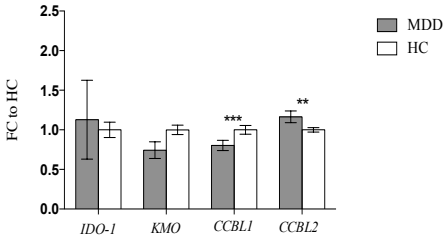


Supplementary Figs.5 a-d. Serum levels of KYN and the KYN/TRP ratio in patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. Values are expressed as the fold change expression values relative to the mean value of the HC group (=1). *p*-values are based on univariate analyses of covariance (ANCOVA) controlling for age, sex, bmi and smoking (whole group) and for smoking (medication/ECT-naïve subgroup). Bonferroni's adjustments for multiple comparisons were additionally applied. $^{*}p \leq 0.050$, $^{**}p \leq 0.005$, $^{***}p \leq 0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; KYN: kynurenine; TRP: tryptophan.

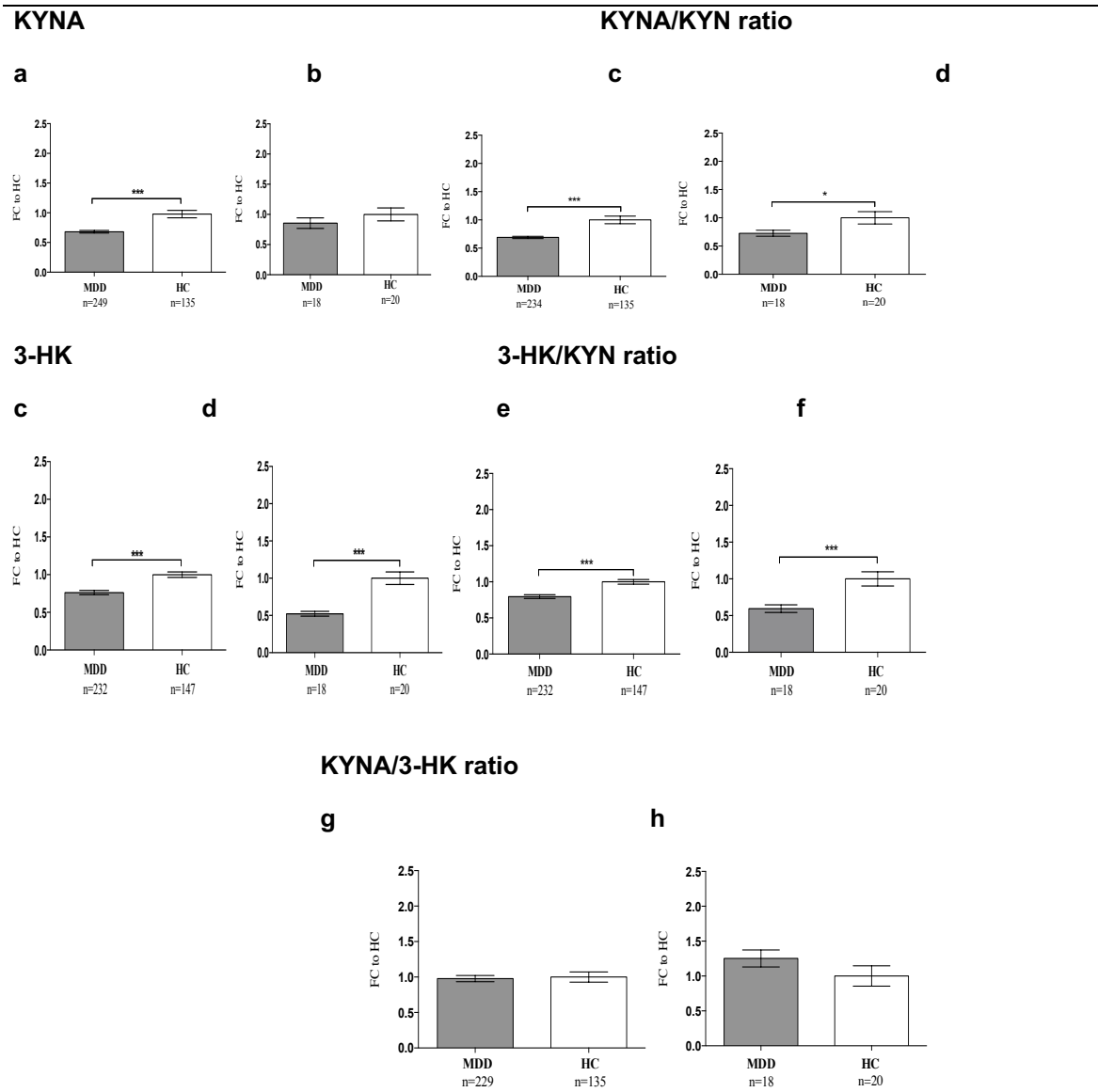
a



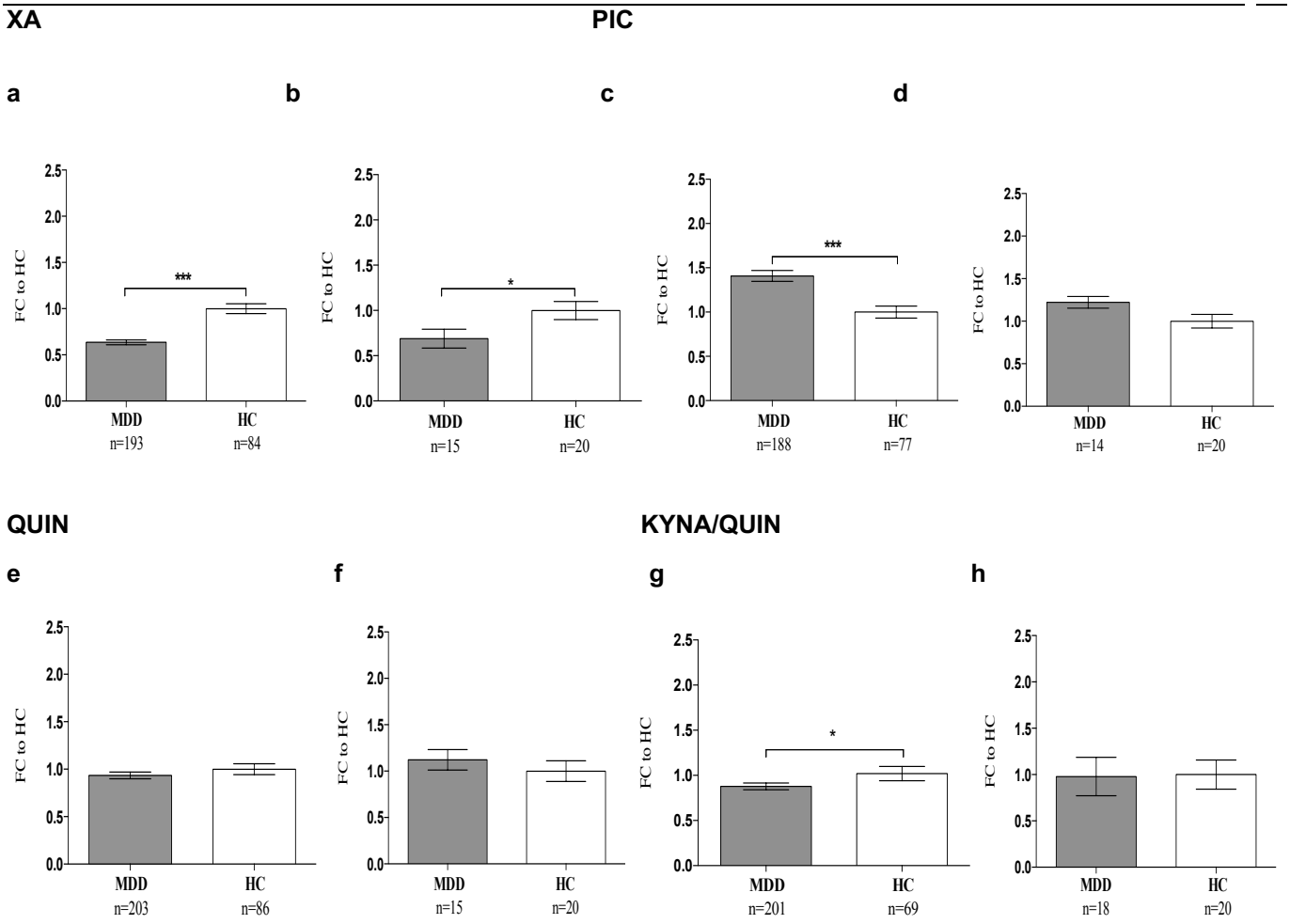
b



Supplementary Fig.5 a,b. The relative mRNA expression levels of the indicated KP enzyme genes in the circulating monocytes of patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the medication/ECT-naïve subgroup of patients and their respective age, sex and bmi-matched HCs. *Abbreviations*: *IDO-1*: indoleamine 2,3-dioxygenase gene; *KMO*: kynurenine 3-monooxygenase gene; *CCBL1/KAT I*: cysteine conjugate beta lyase cytoplasmic1/kynurenine aminotransferase I gene; *CCBL2/KAT III*: cysteine conjugate beta lyase cytoplasmic 2/kynurenine aminotransferase III gene. Compared to HCs, patients with MDD were characterized by decreased *KMO* expression levels as compared with HCs ($F(1,129)=6.319, p=0.013, \eta p^2=0.047$). The same trend was also found in the subgroup of medication-ECT patients ($F(1,22)=0.087, p=0.027, \eta p^2=0.127$).



Supplementary Fig.6 a-h Serum levels of the indicated KP metabolites and KP metabolite ratios in patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. $^{*}p \leq 0.050$, $^{**}p \leq 0.005$, $^{***}p \leq 0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; KYNA: kynurenic acid; KYN: kynurenine. Patients with MDD were characterized by decreased KYNA serum levels and by a decreased KYNA/KYN ratio as compared with HCs (($F(1,378)=40.268, p<0.001, \eta p^2=0.096$ and $F(1,363)=31.032, p<0.001, \eta p^2=0.179$, respectively). The same trend was found in the subgroup of medication/ECT-naïve patients. Significantly reduced 3-HK serum levels ($F(1,373)=40.242, p<0.001, \eta p^2=0.097$), and a reduced 3-HK/KYN ratio ($F(1,373)=36.357, p<0.001, \eta p^2=0.089$), were found in patients with MDD as compared with HCs. The same was found in the subgroup of medication/ECT-naïve patients ($F(1,35)=24.348, p<0.001, \eta p^2=0.410$ and ($F(1,35)=12.577, p<0.001, \eta p^2=0.264$, respectively).



Supplementary Figs.7a-h. Serum levels of XA, PIC, QUIN and the KYNA/QUIN ratio in patients with MDD and HCs. The left-hand columns represent the whole group of patients with MDD and HCs, the right-hand columns represent the subgroup of medication/ECT-naïve patients and their respective age, sex and bmi-matched HCs. $^{*}p \leq 0.050$, $^{**}p \leq 0.005$, $^{***}p \leq 0.001$. *Abbreviations*: MDD: major depressive disorder; HC: healthy controls; XA: xanthurenic acid; PIC: picolinic acid; QUIN: quinolinic acid; KYNA: kynurenic acid.

Significantly reduced XA serum levels ($F(1,271)=52.110, p<0.001, \eta p^2=0.164$) were found in patients as compared with HCs; this was also found in the subgroup of medication/ECT-naïve patients ($F(1,32)=6.971, p=0.013, \eta p^2=0.179$). On the contrary, patients with MDD were characterized by increased PIC serum levels as compared with HCs($F(1,259)=13.002, p<0.001, \eta p^2=0.048$). The same trend was also found in the subgroup of medication/ECT-patients ($F(1,33)=4.024, p=0.053, \eta p^2=0.109$). Significant differences were not found between patients and HCs regarding QUIN serum levels; reduced KYNA/QUIN serum levels were found in the entire group of patients with MDD ($F(1,264)=3.996, p=0.047, \eta p^2=0.015$). The same trend was found in the subgroup of medication/ECT-naïve patients ($F(1,35)=3.046, p=0.090, \eta p^2=0.080$).

	Muenster MDD n=81	Muenster HCs n=122	MDD vs. HCs	
	M(SD)	M(SD)	*F*	*p*-value
hsCRP(μg/mL)	2.08(2.64)	1.37(2.00)	0.316	0.574
IL-6 (μg/mL)	1.11(1.61)	0.83(1.19)	0.001	0.980

Supplementary Table 4. Comparison between serum levels of hsCRP and IL-6 in patients of the Muenster cohort and HCs. *p*-values are based on univariate analyses of covariance (ANCOVA) controlling for age, sex, bmi and smoking. Bonferroni's adjustments for multiple comparisons were additionally applied.

As can be seen in this sample differences between patients and HCs could not be seen. Of note is that in relation to KP metabolites, there was a positive correlation between KYN as well as with QUIN with the inflammatory compounds (IL-6-KYN (*rho*=0.357, *p*=0.004), hsCRP-KYN (*rho*=0.110,*p*=0.396), IL-6-QUIN (*rho*=0.449,*p*=0.001), hsCRP-QUIN (*rho*=0.363,*p*=0.007)

Abbreviations: MDD: major depressive disorder; HCs: healthy controls; hsCRP: high-sensitivity C Reactive Protein; IL: interleukin; M: mean; SD: standard deviation

Methods: Both hsCRP and IL-6 serum levels were quantified by enzyme-linked immunosorbent assay (ELISA) by the kit manufacturer apDia, Turnhout, Belgium. For the determination of hsCRP (reference number:740011), serum samples were diluted (1:1000) and incubated with prediluted standards (10x1:100). Microtiter strips were incubated with the diluted standards and sera for 30 min at room temperature (RT). Unbound conjugates were removed by washing, and a specific horseradish peroxidase (HRP)-conjugated antibody added to detect the antibody-antigen complex. Strips were then incubated for 30 min at RT. After removal of any unbound conjugate, a chromogen solution containing tetramethylbenzidine (TMB) was added and incubated during 10 min at RT. A blue color developed, and the reaction was stopped (0.5 M stop solution). Signals were then read at 450 nm. For IL-6 determination, pre-coated microtiter strips were incubated with standards and diluted samples (overnight, at 4ºC). Unbound serum proteins were removed by washing. Next, a monoclonal biotin conjugate was added to bind directly to the antibody-antigen complex and incubated for 60 min at 37ºC. Any unbound biotin antibodies were then removed by washing. Then, a streptavidin poly HRP conjugate was added, and strips incubated for 20 min at 37°C. After removal of unbound conjugate, a TMB containing solution was pipetted into the wells and incubated for 20 min at 37°C. Again, reaction was stopped after the development of a blue color (0,5M stop solution), and signals were read at 450 nm.

7. Publication II

frontiers in Psychiatry

ORIGINAL RESEARCH
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Low-Grade Inflammation as a Predictor of Antidepressant and Anti-Inflammatory Therapy Response in MDD Patients: A Systematic Review of the Literature in Combination With an Analysis of Experimental Data Collected in the EU-MOODINFLAME Consortium

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Low-grade inflammation plays a role not only in the pathogenesis of major depressive disorder (MDD) but probably also in the poor responsiveness to regular antidepressants. There are also indications that anti-inflammatory agents improve the outcomes of antidepressants.

Aim: To study whether the presence of low-grade inflammation predicts the outcome of antidepressants, anti-inflammatory agents, or combinations thereof.

Methods: We carried out a systematic review of the literature on the prediction capability of the serum levels of inflammatory compounds and/or the inflammatory state of circulating leukocytes for the outcome of antidepressant/anti-inflammatory treatment in MDD. We compared outcomes of the review with original data (collected in two limited trials carried out in the EU project MOODINFLAME) on the prediction capability of the inflammatory state of monocytes (as measured by inflammatory gene expression) for the outcome of venlafaxine, imipramine, or sertraline treatment, the latter with and without celecoxib added.

Results: Collectively, the literature and original data showed that: 1) raised serum levels of pro-inflammatory compounds (in particular of CRP/IL-6) characterize an inflammatory form of MDD with poor responsiveness to predominately serotonergic agents, but a better responsiveness to antidepressant regimens with a) (add-on)

noradrenergic, dopaminergic, or glutamatergic action or b) (add-on) anti-inflammatory agents such as infliximab, minocycline, or eicosapentaenoic acid, showing—next to anti-inflammatory—dopaminergic or lipid corrective action; 2) these successful anti-inflammatory (add-on) agents, when used in patients with low serum levels of CRP/IL-6, decreased response rates in comparison to placebo. Add-on aspirin, in contrast, improved responsiveness in such "non-inflammatory" patients; 3) patients with increased inflammatory gene expression in circulating leukocytes had a poor responsiveness to serotonergic/noradrenergic agents.

Conclusions: The presence of inflammation in patients with MDD heralds a poor outcome of first-line antidepressant therapies. Immediate step-ups to dopaminergic or glutamatergic regimens or to (add-on) anti-inflammatory agents are most likely indicated. However, at present, insufficient data exist to design protocols with reliable inflammation parameter cutoff points to guide such therapies, the more since detrimental outcomes are possible of anti-inflammatory agents in "non-inflamed" patients.

Keywords: major depression, inflammation, antidepressant therapy, anti-inflammatory therapy, therapy prediction

INTRODUCTION

It is well accepted that immune dysregulation plays an important role in the pathogenesis of at least a proportion of patients with major depressive disorder (MDD) (1–16). Genetic defects and/or polymorphisms, childhood trauma, and chronic stress are all capable of eliciting such immune dysregulations (17–19). In the last decades, special interest has been raised for the role of low-grade inflammation in the immune system dysregulation of MDD. Low-grade inflammation is characterized by an increase in the level of circulating pro-inflammatory compounds, such as acute phase proteins [e.g., C-reactive protein (CRP)] and cytokines [e.g., interleukin (IL)-6], and/or by a pro-inflammatory activity of circulating or tissue resident immune cells (20–23).

A wide range of medications is currently available for the treatment of MDD. First-line agents are the well-known serotonin reuptake inhibitors (SSRIs; e.g., sertraline, escitalopram, or citalopram), which show a predominantly serotonergic action (24). First-line agents are also the serotonin-noradrenaline reuptake inhibitors (SNRIs), which show a predominantly serotonergic action at low doses and a combined serotonergic–noradrenergic action at moderate to high doses (25). Tricyclic antidepressants (TCAs) show a similar mechanism of action as SNRIs regarding the dual serotonergic-noradrenergic action, but because of more side effects, they are actually used as second-line agents. Third-line agents are drugs with a predominantly noradrenergic/dopaminergic action, such as mirtazapine or bupropion, or agents with other mechanisms of action, such as ketamine [i.e., an *N*-methyl-D-aspartate (NMDA) receptor antagonist, elevating glutamate levels]. Despite this wide range of medications, response rates to treatment are still insufficient, with about half of the patients not responding adequately to an installed treatment (26, 27).

Since most of the antidepressant drugs have—next to their neurotransmission modulatory effects—also immune modulating capacities (28, 29), it is thought that the inflammatory state of patients might play a role in non-responsiveness. To enforce the mood-regulating effects of antidepressants, and being aware of the notion that low-grade inflammation plays a role, various studies have been undertaken to use anti-inflammatory agents as add-ons to regular antidepressant therapies. In this way, acetylsalicylic acid (i.e., aspirin, a COX1 and COX2 inhibitor), selective COX-2 inhibitors (e.g., celecoxib), minocycline (a tetracyclin with anti-inflammatory effects), and anti-TNF monoclonal antibodies (e.g., infliximab) have been used experimentally (30–33). Besides these anti-inflammatory agents, agents such as cholesterol-lowering fish oil (eicosapentaenoic acid) and anti-oxidative *n*-acetylcysteine have also been used (33, 34). These agents also have anti-inflammatory actions, since both the cholesterol metabolism and the anti-oxidative machinery are linked to inflammation (35, 36). Though it seems that anti-inflammatory agents did show limited beneficial effects in most of the reported studies (30–34), there is still doubt on the real validity of such interventions, particularly due to the paucity and preliminary character of the studies, while there is also the feeling that such anti-inflammatory agents might only work in a proportion of patients.

Collectively, the abovementioned notions lead to the view that there is a need for a personalized medicine approach to select patients who, in particular, will respond to first-line agents and those needing immediate step-up therapies to drugs other than the first-line drugs and/or an add-on of a first-line agent with an anti-inflammatory agent. In such an approach, it is the question whether a pre-existent state of enhanced low-grade inflammation (present in around one-third of patients) (37) indeed plays a role in non-responsiveness to antidepressants and whether such a state

is capable of predicting the outcome of the abovementioned antidepressant therapy regimens.

For this report, we have carried out a systematic review searching for the relevant literature on the prediction capability of soluble inflammatory compounds/cytokines in serum/plasma/CSF and/or the inflammatory state of circulating leukocytes for the outcome of antidepressant/anti-inflammatory treatments in MDD. We combined the outcomes of the systematic review with experimental data collected in the EU-MOODINFLAME consortium on the prediction capability of the inflammatory state of circulating monocytes (as measured by inflammatory gene expression). Two EU-MOODINFLAME trials could be evaluated, a trial carried out on patients with MDD collected at the Rotterdam site and treated in first line with venlafaxine or imipramine (38), and a small trial carried out on patients with MDD collected at the Munich site and treated with sertraline *plus* add-on celecoxib or placebo.

MATERIALS AND METHODS

Search Strategy for Systematic Review

We conducted a systematic literature search in the PubMed/MEDLINE and Web of Science databases to identify immune-inflammatory predictors for treatment response to antidepressants, anti-inflammatory agents, and/or their combination with anti-inflammatory agents (or anti-inflammatory agents alone) in MDD from inception (for anti-inflammatory) and from 2008 (for antidepressant) until August 16, 2018. To find additional relevant studies, citation lists of included articles were tracked in Google Scholar (39) or citation lists of topic-related reviews and meta-analyses were checked. The last author of a significant paper concerning celecoxib and an expert in the field (NM) was also contacted and asked of awareness of any additional studies.

The following search terms were used: (mdd OR major depressive disorder OR depression) AND (inflammation) AND (therapy OR treatment OR antidepressant drugs OR sertraline OR venlafaxine OR escitalopram OR citalopram OR tricyclic OR ssri OR snri) AND (biomarker OR cytokines OR il-6 OR t cells OR nk cells OR th17 OR leukocytes OR macrophages OR crp OR genes) AND (response OR prediction), (mood disorder OR depression OR bipolar) AND (anti-inflammatory OR inflammation) AND (therapy OR treatment OR medication OR drugs OR add-on OR adjunct OR anti TNF OR infliximab OR CRP OR aspirin OR ASA OR acetyl salicylic acid OR minocycline OR omega 3 fatty acids OR NAC OR acetylcysteine OR cox 2 inhibitor OR celecoxib) AND (biomarker OR cytokines OR macrophages OR t cells OR NK cells OR leukocytes OR CRP OR genes).

The initial search of 7,047 studies resulted in 174 relevant studies selected by title. Inclusion criteria for further selection were:

(1) publications written in the English language;
(2) human clinical trials;
(3) the diagnosis of MDD. Because of the paucity of studies in unipolar depression, both unipolar and bipolar depression were included for the studies on (add-on) anti-inflammatory agents. To make comparisons possible, we indicate in the result section (**Table 1C**, marked with **B** and **C**) which studies included bipolar depressed patients, and we discuss in the Discussion section putative differences stemming from this inclusion.
(4) the absence of severe somatic diseases (especially inflammation-related);
(5) the assessment of immune biomarkers;
(6) the use of first-line or other antidepressant agents or the use of an anti-inflammatory agent added to antidepressant treatment or alone;
(7) the assessment of symptom reduction with standardized measure [e.g., Hamilton Rating Scale for Depression (HAMD), Montgomery–Asberg Depression Rating Scale (MADRS), Beck's depression inventory (BDI)] and
(8) the analysis of responder and non-responder subgroups.

By reading the abstracts, methods, and results sections and applying the inclusion criteria and by removing duplicate records, 36 studies were selected. Further exclusion criteria were:

(1) no predictive information provided;
(2) use of parameters that are not inflammatory biomarkers in a narrower sense [e.g., serotonin and kynurenine metabolites, brain-derived neurotrophic factor (BDNF), calcium-binding protein B (S100B), macrophage-derived chemokine (MDC), platelet-derived growth factor (PDGF), and Eotaxin-1/CCL11];
(3) genetic studies were excluded except for leukocyte gene expression level studies;
(4) the use of agents whose anti-inflammatory mechanisms are not direct and even questionable (e.g., l-methylfolate, pioglitazone, modafinil).

By applying these exclusion criteria, we finally included 24 reports in the systematic review.

With the purpose of providing a comprehensive presentation, we decided to split the remaining studies into studies concerning circulating inflammatory compounds/cytokines (n = 19, see **Tables 1A–C**) and gene expression in circulating leukocytes (n = 5; see **Table 2**). For detailed information about the study selection, see **Figure 1**.

Experimental Clinical Studies

Details on the inclusion and exclusion criteria, as well as on the clinical instruments and characteristics of patients, have been published before (38, 64). In short, in- and outpatients were recruited from the Departments of Psychiatry at the Erasmus Medical Centre (ErasmusMC) in Rotterdam (The Netherlands) and at the University Hospital of the Ludwig Maximilian University (LMU) in Munich (Germany). All patients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders, Text Revision* (*DSM-IV-TR*) (65) and confirmed by using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (66). Included were patients with a minimum score of 17 (Rotterdam) or 22 (Munich) on

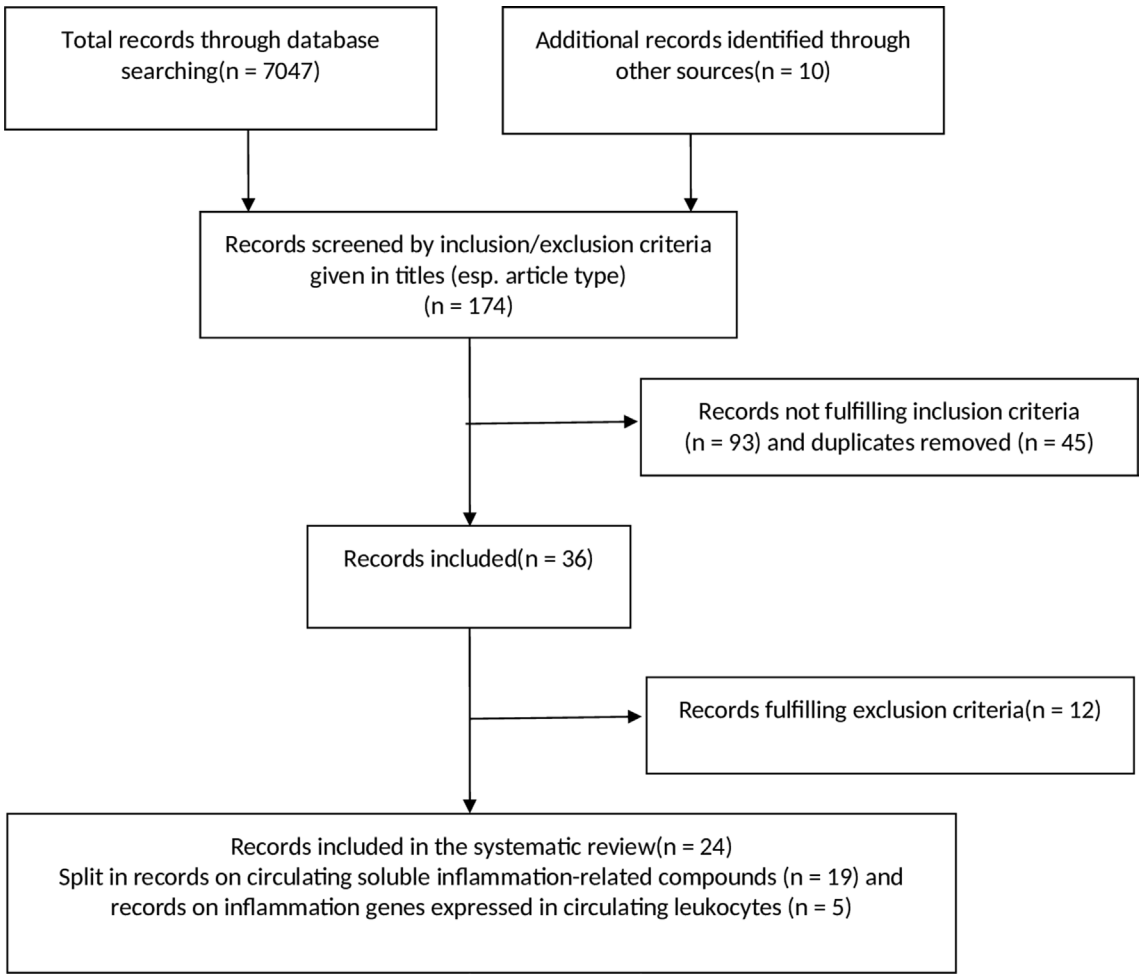

FIGURE 1 | Flow diagram of the systematic research. See materials and methods section for further explanation.

the Hamilton Rating Scale for Depression (HAMD, 17-item-version) (67).

Studies had been approved by the ethics committee of the medical faculty at the LMU, Munich (Germany), and the medical ethics committee of the ErasmusMC, Rotterdam (the Netherlands). The study was conducted in compliance with standards of Good Clinical Practice (CGP), assuring that the rights, safety, and well-being of patients were protected in accordance with the principles that have their origin in the Declaration of Helsinki (June 1964, last amendment Fortaleza 2013). Additionally, the relevant national and European regulations were adhered, too. After study procedures had been fully explained, all subjects provided written informed consent.

Healthy Controls

Healthy controls (HCs) were recruited from the same communities (Rotterdam and Munich). Details on the HC can be found in Refs. (64) and (68). In short, the inclusion criteria for HC were the absence of major *DSM-IV-TR* Axis I disorders including schizophrenia, psychotic disorders, mood disorders, anxiety disorders, or substance-related disorders according to *DSM-IV* criteria; the absence of usage of psychiatric drugs; and the absence of severe medical illness. HC had to be in self-proclaimed good health and free of any obvious medical illness for at least 2 weeks prior to the blood withdrawal, including acute infections and allergic reactions.

Treatment Protocols

Being both double-blind studies, subjects, investigators, and study staff had been blinded to the treatment assignment for the duration of the study.

Venlafaxine/Imipramine Study (Rotterdam)

Prior to the start of antidepressants, patients with MDD underwent a wash-out period for at least 1 week. The use of benzodiazepines was allowed up to a maximum daily dose of 3 mg lorazepam or the corresponding equivalent. Subsequently, patients were randomly assigned to a 7-week monotherapy with either the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine

(mean daily dose 371 mg, range dose of 300–375 mg/day) or with the TCA imipramine (mean dose 206 mg, range dose of 50–450 mg/day). The duration of the treatment trial was 7 weeks to ensure that patients treated with imipramine had adequate plasma levels for at least 4 weeks. Response to treatment was defined as ≥50% reduction of the initial HAM-D score.

Sertraline Plus Placebo/Celecoxib Study (Munich)

Prior to the start of treatment, patients with MDD underwent a washout period for 3 days. The use of lorazepam or zopiclon was allowed in this period and also during the study, up to a maximum daily dose of 3 or 15 mg, respectively. Subsequently, patients were randomly assigned in a 1:1 ratio to a 6-week therapy with either the selective serotonin reuptake inhibitor (SSRI) sertraline *plus* placebo, or with sertraline *plus* the selective COX-2 inhibitor celecoxib. The dose of sertraline was flexible and ranged between 50 and 100 mg/day. A daily dose higher than 100 mg was not recommended, but in the expectation of more clinical benefit, a daily dose of 150 mg sertraline was allowed. The daily dose of celecoxib was 400 mg (200 mg in the morning and 200 mg in the evening). Patients from the placebo group received two identical capsules (morning and evening). As in the Rotterdam cohort, response to treatment was defined as ≥50% reduction of the initial HAM-D score.

Numbers of Patients With MDD and HC

Only patients and HC with full data regarding the expression levels of all key genes for monocyte inflammatory activation could be used for the present study. The Rotterdam sample therefore consisted of 34 MDD patients and 45 HC. Of the patient group, 14 patients were treated with venlafaxine and 20 patients were treated with imipramine. The Munich sample consisted therefore of 35 MDD patients and 42 HC. Of the patient group, 19 patients were treated with sertraline *plus* placebo, and 16 patients were treated with sertraline *plus* celecoxib.

Blood Collection

Blood was collected in sodium-heparin tubes (36 ml) for immune cell preparation just prior to treatment. From the heparinized blood, peripheral blood mononuclear cell (PBMC) suspensions were prepared by low-density gradient centrifugation *via* Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) within 8 h to avoid erythrophagy-related activation of the monocytes. PBMCs were frozen in 10% dimethylsulfoxide and stored in liquid nitrogen. This enabled us to test immune cells of patients and controls together at a later stage. Tests were done at ErasmusMC.

Monocyte Inflammatory Gene Expression

$CD14^+$ monocytes were isolated from aliquots of the frozen and thawed PBMCs by a magnetic cell sorting system (auto MACS Pro, Miltenyi Biotec, B.V., Bergisch Gladbach, Germany). The average viability was 86.3 ± 10.4 (Trypan blue staining) and the purity of monocytes was 95.1 ± 3.0% (flow cytometry). RNA was isolated from the purified monocytes using RNA easy mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). On average, monocytes cell yield after isolation was $2.0 \pm 1.6 \times 10^6$/subject and the quantity of RNA in monocytes was 3.2 ± 1.8 μg. One microgram of RNA was reverse-transcribed using the cDNA high capacity reverse transcription kit (Applied Biosystems, Foster City, CA, USA). qPCR was performed using Taqman Arrays, format 48 (Applied Biosystems), according to the manufacturer's protocol and validated against the single RT-qPCR method. Per fill port, 400 ng of cDNA (converted from total RNA) was loaded. PCR amplification was performed using an Applied Biosystems Prism 7900HT sequence detection system with TaqMan Array block. Thermal cycler conditions were 2 min at 50°C, 10 min at 94.5°C, 30 s at 97°C, and 1 min at 59.7°C for 40 cycles.

Based on several previous studies on mood disorders (21, 64, 69), we decided to include in our panel the most consistently abnormally expressed inflammatory genes in the studies. Therefore, relative to the housekeeping gene ABL1, the expression of a total of up to 49 genes was determined (also because of the maximum of fill ports in the Taqman assay) and expression values were calculated using the comparative threshold cycle (CT) method [see, for technical details, Refs. (21, 64, 69)]. The mentioned earlier studies also carried out a hierarchical clustering of these genes and found two main distinct clusters of gene expression. The first cluster is found consistently in virtually all of our monocyte inflammatory gene expression studies (also besides disease conditions such as mood disorders), and this cluster is composed of well-known pro-inflammatory cytokines and chemokines and important enzymes or transcription factors to produce these compounds. For the calculation of the "positivity of this inflammatory compound cluster", we took the expression level of the top 10 genes [the most consistently overexpressed genes in all our studies thus far; see Ref. (64)] of this cluster into consideration, i.e., *IL1β*, *CCL20*, *EREG*, *IL6*, *TNFAIP3*, *CXCL2*, *PDE4B*, *ATF3*, *PTX3*, and *IL1A*. These genes accounted for 70–99% of the inflammatory cluster response. For each of the 10 genes, we determined a range of the HC gene expression (using the $2^{-\Delta Ct}$ values). The range was defined by the mean of the values for that gene in HC monocytes ± 1 standard deviation (SD). Then, we used this range as a standard of comparison for the MDD patients' gene expression. We decided to refer to a patient's top gene as upregulated, if the patient's gene expression was higher than HC's mean plus 1×SD, or downregulated when it was lower than HC's mean minus 1×SD. This was done for all 10 above given genes. Then, we declared the monocyte population of a given patient as "pro-inflammatory positive" if 6 of these 10 top inflammatory genes (or more) were upregulated. These data are given in **Table 3** in the Results section. Similar calculations/algorithms for monocyte inflammatory positivity have been used by us before (21, 69–71). Further methodological details of the calculation can be found in these publications. Original Q-PCR data have been uploaded and can be retrieved *via* the GEO repository ref number GSE132315: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132315

Statistics

Statistical analyses were performed using IBM SPSS v.21 for Mac. Continuous sample characteristics are reported as mean (± standard deviation). Group comparisons (e.g., MDD vs. HC, responders vs. non-responders) were analyzed using analysis of

variance (ANOVA) tests for continuous data (e.g., age) and using Pearson's chi-square (χ^2) tests for categorical data (e.g., gender). For group comparisons of positivity of monocyte gene expression (e.g., MDD vs. HC, responders/non-responders vs. HC, responders vs. non-responders), Pearson's chi-square (χ^2) tests were applied, too. All hypotheses were tested with $\alpha \leq 0.05$ (two-sided).

RESULTS

Systematic Review Data on the Usefulness of Circulating Serum Inflammatory Compounds

Tables 1A, **1B**, and **1C** show the data of the systematic review of the 19 selected articles (see the section Search Strategy for Systematic Review) regarding the predictive capability of inflammatory state [assessed by serum/plasma immune compounds (mainly CRP and cytokines) (only one study used CSF)] in patients with MDD for the response rates to various classes of antidepressant drugs and to anti-inflammatory agents added to an antidepressant regimen (except for one study, in which the anti-inflammatory agent was used as monotherapy) (52). For comprehensibility, we have grouped the outcomes in **Table 1** according to the regimen used.

Table 1A shows that, in three out of three studies (40–42), antidepressants with a predominant serotonergic action [i.e., escitalopram (SSRI)] induced a better response in patients with low inflammatory markers as compared to patients with high inflammatory markers in the same study. On the contrary, when inflammatory markers were high, five out of seven of the studies (40, 45–48) showed that drugs with a predominant serotonergic action (i.e., SSRIs, SNRIs, and TCAs) induced reduced response rates as compared to patients with low inflammatory markers in the same study. Cutoff points for low and high levels were defined for CRP in the reviewed studies at 1 mg/L; for IL-6 and TNFα, values depended on the actual sensitivity of the assay used in the report. Two studies formed an exception. Manoharan et al. (44) did not find any effect of pre-selection of the inflammatory state. However, this study was special in that treatment duration was of only 6 weeks, and patients had a relatively low to moderate depression severity (HAMD score ≥ 13). The other study (43) showed the opposite message (i.e., an improved response to SSRIs in patients with a high inflammatory state as compared to a low inflammatory state). This study was special, in that many patients were treated with paroxetine (SSRIs), which—apart from its serotonergic action—also exerts a considerable dopaminergic action (72).

Taken together, predominantly serotonergic agents showed, in general, insufficient response rates in those patients with signs of moderate to high inflammation as measured by circulating inflammatory compounds.

The review also delivered that, in such conditions of moderate to high signs of inflammation, drugs with another mechanism of action than primarily serotonergic do show an effect. Using nortriptyline, mirtazapine, or ketamine alone, or combinations of an SSRI with nortriptyline or bupropion resulted, in 5 out of 5 studies, in improved responses rates (40, 41, 49–51) as compared to the patients with low inflammatory markers (**Table 1B**).

Similar beneficial effects existed for combinations of antidepressant drugs with anti-inflammatory agents. **Table 1C**

TABLE 1A | Predominantly serotonergic action: higher response rates in low inflammatory state vs. moderate–high inflammatory state (prior to treatment).

INFLAMMATORY STATE	STUDY	DRUG	INFLAMMATORY TEST	RESPONSE
LOW	Jha et al., 2017 (40)	Escitalopram (SSRI) + Placebo	CRP < 1 mg/L	**Higher** response rates compared to m–h IS *
	Uher et al., 2014[a] (41)	Escitalopram (SSRI)	CRP < 1 mg/L	**Higher** response rates compared to m–h IS ***
	Eller et al., 2008 (42)	Escitalopram (SSRI)	TNFα	**Higher** response rates compared to m–h IS *
MODERATE–HIGH	Yoshimura et al., 2013 (43)	Paroxetine, Sertraline (SSRI)	IL-6	**Higher** response rates compared to low IS *
	Manoharan et al., 2016 (44)	Fluoxetine (SSRI)	IL-6	**No associations** between biomarker values and response rates
	Jha et al., 2017 (40)	Escitalopram (SSRI) + Placebo	CRP ≥ 1 mg/L	**Lower** response rate compared to low IS *
	Chang et al., 2012 (45)	Fluoxetine (SSRI), Venlafaxine (SNRI)	CRP ≥ 1 mg/L	**Lower** response rate compared to low IS *
	Haroon et al., 2018 (46)	SSRIs, SNRIs, TCA	CRP, IL-6, TNFα, sTNF-R2	**Lower** response rate compared to low IS *
	Yoshimura et al., 2009 (47)	Paroxetine, Sertraline, Fluvoxamine, Milnacipran (SSRI, SSNRI)	IL-6	**Lower** response rate compared to low IS *
	Martinez et al., 2012 (48)	Venlafaxine (SNRI)	TNFα (CSF)	**Lower** response rate compared to low IS *

SSRI, selective serotonin reuptake inhibitors; SNRI, serotonin-norepinephrine reuptake inhibitor; TCA, tricyclic antidepressant; TNFα, tumor necrosis factor alpha; IL, interleukin; CSF, cerebrospinal fluid; CRP, C-reactive protein; m–h, moderate–high; IS, inflammatory state.

**p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.*

[a]*Improvement on Montgomery–Asberg Depression Rating Scale (MADRS) score 3 points higher with nortriptyline when CRP ≥ 1 mg/L and 3 points higher with escitalopram when CRP < 1 mg/L.*

TABLE 1B | Predominantly noradrenergic, predominantly dopaminergic, and glutamatergic action: higher response rates in moderate–high inflammatory state vs. low inflammatory state (prior to treatment).

INFLAMMATORY STATE	STUDY	DRUG	INFLAMMATORY TEST	RESPONSE
LOW	Jha et al., 2017 (40)	Escitalopram (SSRI) + Bupropion (NDRI)	CRP < 1 mg/L	**Lower** response rate compared to m–h IS *
MODERATE–HIGH	Jha et al.,2 017 (40)	Escitalopram (SSRI) + Bupropion (NDRI)	CRP ≥ 1 mg/L	**Higher** response rates compared to low IS *
	Uher et al., 2014[a] (41)	Nortriptyline (TCA)	CRP ≥ 1 mg/L	**Higher** response rates compared to low IS ***
	Harley et al., 2010 (49)	Fluoxetine (SSRI) + Nortriptyline (TCA)	CRP ≥ 1 mg/L	**Higher** response rates compared to low IS ***
	Yang et al., 2015 (50)	Ketamine (NMDA Receptor Antagonist)	IL-6	**Higher** response rates compared to low IS ***
	Gupta et al., 2016 (51)	Mirtazapine (NaSSA)	TNFα	**Higher** response rates compared to low IS *

SSRI, selective serotonin reuptake inhibitors; NDRI, norepinephrine dopamine reuptake inhibitor; TCA, tricyclic antidepressant; NaSSA, noradrenergic and specific serotonergic antidepressant; TNFα, tumor necrosis factor alpha; IL, interleukin; CRP, C-reactive protein; m–h, moderate–high; IS, inflammatory state.
**p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.*
[a]Improvement on Montgomery–Asberg Depression Rating Scale (MADRS) score 3 points higher with nortriptyline when CRP ≥ 1 mg/L and 3 points higher with escitalopram when CRP < 1 mg/L.

TABLE 1C | Anti-inflammatory agents (added to an antidepressant regimen, except for one study): lower response rates in low inflammatory state (prior to treatment) versus placebo and higher response rates in moderate–high inflammatory state versus low inflammatory state (prior to treatment).

INFLAMMATORY STATE	STUDY	DRUG	INFLAMMATORY TEST	RESPONSE
LOW	Rapaport et al., 2016 (52)	Monotherapy eicosapentaenoic acid (EPA)	e.g., IL-1ra, hs-CRP	**Lower** response rate **compared to placebo** of low inflammatory state *
	Raison et al., 2013[b] (53)	Infliximab (anti-TNFα)	CRP ≤ 5mg/L	**Lower** response rate **compared to** placebo of low inflammatory state **
	Savitz et al., 2018[c] (54)	Minocycline	IL-6	**Lower** response rate **compared to placebo** of low inflammatory state [d]
	Savitz et al., 2018[c] (54)	Aspirin (NSAID)	IL-6	**Higher** response rates compared to m–h IS [d]
MODERATE–HIGH	Rapaport et al., 2016 (52)	Monotherapy eicosapentaenoic acid (EPA)	e.g., IL-1ra, hs-CRP	**Higher** response rates compared to low IS *
	Raison et al., 2013[b] (53)	Infliximab (anti-TNFα)	CRP > 5mg/L, TNFα, sTNFR I and II	**Higher** response rates compared to low IS **
	Savitz et al., 2018[c] (54)	Minocycline	IL-6	**Higher** response rates compared to low IS **[d]
	Husain et al., 2017 (55)	Minocycline	CRP > 5 mg/L	**Higher** response rates compared to low IS
	Porcu et al., 2018[c] (56)	*N*-acetylcysteine	CRP > 5 mg/L	**Higher** response rates compared to low IS *
	Hasebe et al., 2017 (57)	*N*-acetylcysteine	IL-6	**No associations** between biomarker values and response rates
	Panizzutti et al., 2018[c] (58)	*N*-acetylcysteine	CRP, IL-6, TNFα, BDNF, IL-8, IL-10	**No associations** between biomarker values and response rates
	Savitz et al., 2018[c] (54)	Aspirin (NSAID)	CRP	**No associations** between biomarker values and response rates
	Savitz et al., 2018[c] (54)	Aspirin (NSAID)	IL-6	**Lower** response rate compared to low IS [d]

TNFα, tumor necrosis factor alpha; IL, interleukin; CRP, C-reactive protein; m–h, moderate–high; IS, inflammatory state.
**p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, reported effects without significance were not tested for significance but showed a clear descriptive trend and were therefore considered noteworthy.*
[b]Mixed sample with MDD and bipolar depressed patients, [c]Bipolar depressed sample (type I/II and unspecified), [d]Personal communication.

shows that five out of seven studies (52–56) found a significant improvement of an (add-on) anti-inflammatory therapy, when patients with high signs were compared to patients with low signs of inflammation. The anti-inflammatory agents used in these studies were infliximab, minocycline, *n*-acetylcysteine, and fish oil (the latter as monotherapy, and compared to placebo).

It must be mentioned that the study of Savitz et al. (54) only noted such improving effect with minocycline; aspirin had no such effect in their study. Aspirin did work in their "non-inflamed" patients, yet had no effect or even a reduced effect in patients with high signs of inflammation, depending on the inflammatory serum marker used to determine the state of low-grade inflammation (CRP or IL-6, see **Table 1C**). The study of Savitz was also special in that both unipolar and bipolar depressed patients were included.

Table 1C additionally shows that there are also two out of three studies (57, 58) that showed that in the case of add-on *n*-acetyl cysteine, it was of no use to stratify the patients in low- or high-grade inflammation prior to therapy. Two of the studies of add-on *n*-acetyl cysteine (one showing and one not showing an effect of prior determination of the inflammatory state) involved both unipolar and bipolar depressed patients.

It was remarkable that when an add-on anti-inflammatory agent was given to patients with low signs of inflammation, reduced responses were obtained as compared to patients with high signs of inflammation and even to placebo (two out of three of such studies) (53, 54). Also, when fish oil (an agent with both lipid-correcting and anti-inflammatory properties) was given as a monotherapy to patients with a low inflammatory state, reduced responses were seen as compared to placebo (52) (**Table 1C**). As mentioned above, add-on aspirin did induce an increased response in patients with low signs of inflammation in the study of Savitz et al. (54).

Taking these literature data together, it is difficult to draw a simple conclusion on the usefulness of a prior measurement of serum inflammatory markers for the determination of the effect of (add-on) anti-inflammatory agents. There is a clear trend that (add-on) anti-inflammatory agents, such as infliximab, minocycline, and fish oil are effective if inflammatory markers are clearly present, but this does not apply to aspirin and *n*-acetylcysteine. However, it is also safe to say that special caution must be given when there is an absence of circulating inflammatory markers in patients with MDD: the chances are high that the use of the effective anti-inflammatory agents (such as infliximab, minocycline, and fish oil) in states of moderate–high inflammation actually has an opposite effect than expected in such patients, namely, a reduced responsiveness.

Systematic Review Data on Gene Expression in Circulating Leukocytes

Table 2 shows the studies we selected that dealt with the gene message for pro-inflammatory cytokine production in the circulating leukocyte pool prior to treatment and predictive for treatment outcome. We found five relevant articles.

In 2013, Powell et al. (59) described a significantly increased baseline expression of *TNF* in escitalopram non-responders (n = 21) compared to responders (n = 25) taken from the GENDEP study. In the same year, Cattaneo et al. (61) reported on data of the GENDEP study and found higher baseline mRNA levels for *IL1β*, macrophage inhibiting factor (*MIF*), and *TNF* in antidepressant (escitalopram or nortriptyline) non-responders compared to responders, the three cytokine expressions together explaining 46% of the variance of treatment response.

Belzeaux et al. (63) identified an algorithm of four mRNAs, including two cytokine genes (TNF and *IL1β*, together with *PPT1* and *HIST1H1E*) to be predictive of the treatment response in MDD. However, the weakness of their study was that a whole scale of antidepressants was used, while numbers of patients and HC were limited (16 vs. 13). Guilloux et al. (60) predicted non-remission following escitalopram treatment in MDD with an accuracy of 79.4% using a 13-gene model including four genes associated with immune and inflammatory activation (however, *TNF* was not part of the 13 genes). Mediation of cell proliferation was another important function of the remaining genes, but not exclusively. In 2016, Cattaneo et al. (62) took the data of the GENDEP study further and reported that absolute values of the message for *IL1β* and *MIF* together could predict non-responsiveness to escitalopram or nortriptyline in over 99%. These outcomes were confirmed in an independent, naturalistic replication sample.

Taken together, it is clear that non-responsiveness to an SSRI or to a TCA (nortriptyline) can likely be predicted by determining the expression level of combinations of important immune genes (*IL1β*, *MIF*, *TNF*, and *CD3*) in preparations of circulating leukocytes of patients with MDD.

TABLE 2 | The predictive capability of inflammatory state prior to therapy measured by circulating leukocyte gene expression for the response to various antidepressant regimens in MDD.

ANTIDEPRESSANT AGENT	GENE TRANSCRIPT	EFFECT	STUDY
Escitalopram (SSRI)	TNF	Higher levels in non-responders	Powell et al., 2013 (59)
Escitalopram (SSRI)	13-gene model, including immune/inflammatory genes (CD3D, CD97, IFITM3, and GZMA)	Predicting non-remission with 79.4% accuracy	Guilloux et al., 2015 (60)
Escitalopram(SSRI) or Nortriptyline (TCA)	IL1β, TNF, and MIF (relative mRNA values)	Higher levels in non-responders	Cattaneo et al., 2013 (61)
Escitalopram (SSRI) or Nortriptyline (TCA)	IL1β and MIF (absolute mRNA values)	Algorithm predictive of non-response with probability of over 99%	Cattaneo et al., 2016 (62)
Antidepressant treatment, not specified	IL1β, TNF, PPT1, and HIST1H1E	Algorithm predictive of treatment response	Belzeaux et al., 2012 (63)

SSRI, selective serotonin reuptake inhibitors; TCA, tricyclic antidepressant; TNF, tumor necrosis factor; IL, interleukin; CD, cluster of differentiation; mRNA, messenger ribonucleic acid; PPT1, palmitoyl-protein thioesterase 1; HIST1H1E, histone cluster 1 H1 family member E; MIF, macrophage inhibition factor; IFITM3, interferon-inducible transmembrane protein 3; GZMA, granzyme A.

Experimental Data on Inflammation-Related Gene Expression in Circulating Monocytes as a Predictor of Treatment Response

Prior to treatment, we could test 34 patients with MDD [mean age: 52.2 (±9.9) years, 59% females, collected at the ErasmusMC, Rotterdam] for inflammatory gene expression in their circulating monocytes. As a control group, we tested 45 HC of comparable age [mean age: 49.1 (±9.4) years] and gender (44% females). Of the 34 patients, 14 were treated with venlafaxine and 20 were treated with imipramine. An overall response rate of 11% was found in this trial, with 11/34 patients responding to treatment. The difference between the response rates for both treatment arms were not statistically significant, i.e., a response rate of 36% (5/14) for patients treated with venlafaxine and of 30% (6/20) for patients treated with imipramine. Vermeiden et al. (38) have reported extensively on this study and described that in the entire group of patients (n = 85), 45% of the patients responded to this first line of drug treatment (measured as 50% HAM-D reduction).

The other series of patients involved 35 patients with MDD [mean age: 41.4 (±10.8) years, 46.7% females, collected at the LMU, Munich] and 42 HC of comparable age [mean age: 37.9 (±11.9) years] and gender (61.9% females). Of the 35 patients, 19 were treated with sertraline *plus* placebo and 16 were treated with sertraline *plus* celecoxib. A high overall response rate was found in this trial, i.e., 26/35 (74.3%) of patients responded to treatment. The difference between the response rates for both treatment arms was not statistically significant, i.e., a response rate of 68.4% (13/19) for patients treated with sertraline *plus* placebo and 81.3% (13/16) for patients treated with sertraline *plus* celecoxib.

We determined with an already published algorithm [see the Section Monocyte Inflammatory Gene Expression and Ref. (45)] the inflammatory state of the monocytes using the top 10 cluster 1 inflammatory genes (*IL1β*, *CCL20*, *EREG*, *IL6*, *TNFAIP3*, *CXCL2*, *PDE4B*, *ATF3*, *PTX3*, and *IL1A*). We controlled the patient monocyte tests with the outcomes of the same tests carried out in HC. **Table 3** shows that, in each study group, a significant larger proportion of patients had—prior to therapy—circulating monocytes with a positive inflammatory gene signature as compared to the respective HC. Taking all patients from the four study groups together, 25 of the 69 (36%) patients with MDD had circulating monocytes with a pro-inflammatory gene signature, while only 9 of 87 (10%) HC had such monocyte signature ($p < 0.05$). This observation is in accord with earlier observations that monocytes of part of the patients with MDD show signs of a high inflammatory state (21).

For the purpose of this study, we divided the total patient group in those with a negative monocyte inflammatory gene score and those with a positive score. The data in **Table 3** show that in the response rates in three out of four patient groups, patients with a positive inflammatory gene score had a lower response rate than those without a positive score. This, however, did not apply to the sertraline *plus* placebo group, and also significant differences were not reached in any of the groups. The phenomenon of better responsiveness in "non-inflamed" MDD patients could also be seen in the total MDD patient group; patients with a positive inflammatory gene score had a lower response rate than patients without a positive inflammatory gene score (i.e., 44% vs. 59%); however, a statistical significance was not reached in the total group of patients.

DISCUSSION

The Predictive Capability of the Inflammatory State for Anti-Depressive and Anti-Inflammatory Treatment and Potential Mechanisms

The data of the systematic review and experimental monocyte data, as presented in this study, collectively point in the direction that the state of so-called low-grade inflammation does play a role in the outcome of antidepressant therapy of patients with MDD.

Low-grade inflammation is characterized by an increase in the serum level of pro-inflammatory compounds (e.g., CRP, IL-1 β, IL-6, and TNF-α) and/or an activation state of circulating or tissue resident immune cells, including the brain microglia. Both an increase in pro-inflammatory compounds in the blood of patients with MDD and a pro-inflammatory activation

TABLE 3 | Proportions of patients with a positive inflammatory gene signature of circulating monocytes prior to therapy measured in total and by response.

Predominantly serotonergic agents	HC	MDD	Inflammatory negatives		Inflammatory positives	
	Positive	Positive	Responders	Non-responders	Responders	Non-responders
Sertraline *plus* Placebo (SSRI)	5/42 (12%)	7/19 (37%)	8/12 (67%)	4/12 (33%)	5/7 (71%)	2/7 (29%)
Sertraline *plus* Celecoxib (COX-2 inhibitor)	idem	3/16 (19%)	11/13 (85%)	2/13 (15%)	2/3 (67%)	1/3 (33%)
Venlafaxine (SNRI)	2/22 (9%)	6/14 (43%)	3/8 (38%)	5/8 (62%)	2/6 (33%)	4/6 (66%)
Imipramine (TCA)	2/23 (9%)	9/20 (45%)	4/11 (36%)	7/11 (64%)	2/9 (22%)	7/9 (78%)
SUM	9/87 (10%)	25/69 (36%)*	26/44 (59%)*	18/44 (41%)*	11/25 (44%)*	14/25 (56%)*

SSRI, selective serotonin reuptake inhibitors; SNRI, sertraline–noradrenaline reuptake inhibitors; TCA, tricyclic antidepressant; COX-2, cyclooxygenase 2; HC, healthy controls; MDD, major depressive disorder.

**$p \leq 0.05$ compared to HC.*

Positivity was defined by an upregulation of 6 (or more) of the 10 cluster 1 genes.

Original Q-PCR data have been uploaded and can be retrieved via the GEO repository ref number GSE132315: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132315

of microglia and/or of myeloid cells in the periphery (i.e., monocytes) have been documented in a considerable (≈30–40%) proportion of patients with MDD (20, 73, 74). Moreover, imaging and histological techniques have shown microglial activation in the hippocampus of depressed patients (75).

By producing an array of neurotrophic factors, pro- and anti-inflammatory cytokines (e.g., IL-6), as well as axon guidance molecules, non-inflammatory activated microglia has been implicated both in white matter integrity and in the adequate development and function of important stress-regulating systems in the healthy brain (76, 77). On the contrary, inflammatory activated microglia (and/or a transfer of peripheral pro-inflammatory cytokines to the brain) is thought to hamper the normal development, growth, and synaptic function of stress-regulating systems and brain connections important for mood regulation, such as the white matter tracts between the forebrain and the limbic system. To illustrate this, raised serum pro-inflammatory cytokine levels have been associated in mood disorder patients with increased activation of threat- and anxiety-related neuro-circuits (78), reduced neural responses to negative stimuli in frontal brain regions involved in cognitive and emotional functions (79), and compromised integrity of myelin sheaths in cortico-limbic networks involved in mood regulation (80).

Importantly, low-grade inflammation has been shown to influence not only brain development and function but also neurotransmission, with excellent reviews on the inhibitory effects of pro-inflammatory cytokines, such as IL-1β and TNF-α, on the synaptic availability of monoamines and BDNF, while the same cytokines have been shown to increase extracellular glutamate, all important molecular determinants in MDD pathogenesis and response to treatment (15).

The data from the here presented systematic review on circulating inflammatory compounds indicate that patients with MDD with an activated inflammatory state (as measured by, e.g., moderate to high levels of circulating CRP, IL-6, and/or TNF-α) show reduced response rates to antidepressant regimens with a primarily serotonergic action (e.g., escitalopram), while showing improved response rates to antidepressant regimens with a primarily noradrenergic (e.g., nortriptyline), dopaminergic (e.g., bupropion, mirtazapine), or glutamatergic action (i.e., ketamine).

The systematic review data on the inflammatory gene expression in circulating leukocytes confirmed this phenomenon, showing that patients with a high gene expression level of *IL1β*, *TNF*, and/or *MIF* did not respond well to interventions with an SSRI in comparison to MDD patients with a low expression of these genes. However, gene data in circulating leukocytes disagreed with the data reported for circulating inflammatory compounds regarding TCA. While the primarily noradrenergic TCA nortriptyline did not give a satisfactory response in patients with MDD with a high gene expression level in circulating leukocytes (see **Table 2**), nortriptyline did in patients with high levels of circulating inflammatory compounds (see **Table 1B**).

Apparently, inflammatory gene expression in leukocytes does not measure the same level of inflammation than the measurement of circulating inflammatory compounds in serum/plasma; a high inflammatory gene expression might typify a state of "stronger/other" inflammation in MDD needing a treatment with drugs beyond the serotonergic and noradrenergic drugs. In other studies, we have also noted that inflammatory gene expression in circulating cells does not correlate one to one with the circulating protein gene product in serum/plasma (81). We explained this phenomenon by assuming that resident cells, such as the endothelial cells and resident macrophages in the tissues, also contribute to the level of circulating inflammatory compounds.

Although the data of our experiments on inflammatory gene expression levels in circulating monocytes (a subset of the circulating leukocytes) did not deliver statistically significant results, they were, by and large, in agreement with the above-described findings for the gene expression in all circulating leukocytes and showed that patients with "inflammatory" monocytes showed reduced response rates to predominantly serotonergic drug interventions and patients with "non-inflammatory" monocytes showed higher response rates to these type of agents.

Collectively, we deduce from these data that MDD patients with an activated inflammatory state (as measured by moderate to high circulating levels of, e.g., CRP, TNF-α, and IL-6, or a high gene expression of, e.g., *IL1β*, *TNF*, and *MIF* in circulating leukocytes) need more than a monotherapy with a predominantly serotonergic agent to improve clinically in a satisfactory way. An option then seems to be an immediate step up to agents with also a strong dopaminergic or glutamatergic action.

The reason for a better response to dopaminergic or glutamatergic drugs in the case of signs of enhanced inflammation can only be speculated on. It is possible that these drugs are needed because they also have clear anti-inflammatory actions, counteracting the detrimental effects of the high inflammatory state on the signs and symptoms of depression. There is ample evidence that dopamine and ketamine can reduce the production of pro-inflammatory cytokines and enhance that of anti-inflammatory cytokines (82, 83). On the other hand, the pro-inflammatory state itself may lead to an altered neurotransmitter metabolism, necessitating more than a primarily serotonin reuptake inhibition, but also an intervention in the dopamine or glutamate metabolism. Pro-inflammatory cytokines have been reported to activate neuronal mitogen-activated protein kinase (MAPK) pathways, increasing monoamine transporter expression and activity in general, which leads to an increased pre-synaptic reuptake of not only serotonin but also other neuroactive amines (84, 85). Furthermore, the state of enhanced inflammation is thought to lead to an enhanced tryptophan breakdown *via* the kynurenine pathway, resulting in various neuroactive compounds, among which NMDA agonists and antagonists, aggravating glutamatergic neurotransmitter imbalances (86, 87). This might also necessitate more than only a serotonin reuptake inhibition to be effective.

A step up to dopaminergic and glutamatergic antidepressants was more effective in "inflammatory" MDD patients than in "non-inflammatory" patients, and a combination of an antidepressant with an anti-inflammatory agent increased the response rates in

these "inflammatory" patients with MDD as compared to "non-inflammatory" MDD patients. Though the reviewed literature data are scarce, the best prediction results seem to be obtained for infliximab (anti-TNF-α agent), minocycline (tetracycline), and eicosapentaenoic acid (fish oil). For *n*-acetylcysteine, the inflammatory state did show conjectural prediction effects, while for aspirin (acetylsalicylic acid), a reduced response was actually seen in "inflammatory" patients as compared to "non-inflammatory" patients.

The strength or character of the anti-inflammatory agents may have played a role in this variation of predictability of the state of inflammation for the (add-on) anti-inflammatory agents. Both anti-TNF agents and minocycline are in clinical practice and are considered stronger anti-inflammatory drugs than *n*-acetylcysteine and aspirin. However, for fish oil, a high inflammatory state was also predictive for a better effect in MDD patients, while fish oil is considered a relatively weak anti-inflammatory agent. Interestingly, fish oil exerts its anti-inflammatory effects *via* changing the "bad" pro-inflammatory lipid state of individuals (88), and perhaps the high state of inflammation in MDD patients is primarily driven by a bad lipid profile, which is then best corrected by fish oil.

Also, direct or indirect neurotransmitter effects of the anti-inflammatory agents may have played a role in the success or failure to predict their improved responsiveness in "inflammatory" MDD patients. Interestingly, two of the three add-on anti-inflammatory agents (minocycline and fish oil) that worked better in "inflammatory" than in "non-inflammatory" patients possess dopaminergic activities (89, 90). *N*-acetylcysteine, of which it is conjectural whether it works better as add-on in "inflammatory" than in "non-inflammatory" MDD patients, influences both dopamine and glutamate levels in the brain (91). Add-on aspirin, in contrast, had fewer effects in "inflammatory" MDD patients as compared to "non-inflammatory" patients; interestingly, aspirin has anti-glutamatergic actions (92, 93). These varying neuro-modulating actions of anti-inflammatory drugs make complex interactions in the neuro-immune network possible, inducing varying outcomes of combinations of antidepressants and anti-inflammatory agents. Of note also is that three of the reviewed studies of add-on anti-inflammatory agents had included bipolar depressed patients (54, 56, 58). This applies in particular to the study on aspirin (54), in which a reducing effect was found in "inflammatory" versus "non-inflammatory" patients. Intrinsic differences between bipolar and unipolar depression, such as differences in the immune and the glutamate state (94–98), may have played a role here.

Despite the above-listed uncertainties, it is nevertheless tempting to postulate—based on the outcomes of the literature review—that when MDD patients are "inflammatory", (add-on) anti-inflammatory drugs are also an option to improve responsiveness and then the best results are probably obtained when anti-inflammatory agents are potent, influence lipid metabolism, and/or influence primarily dopaminergic synaptic transmission.

Regarding the use of (add-on) anti-inflammatory agents, another important message emerges from our systematic review of the literature. Interestingly, three out of four reports (52–54) indicated that "non-inflammatory" MDD patients showed a reduced response rate as compared to even placebo to the effective (add-on) intervention with an anti-inflammatory agent. In other words, the addition of the anti-inflammatory drugs effective in "inflammatory" patients was detrimental, and the anti-inflammatory drugs inhibited the effect of the antidepressants or delayed natural recovery. Such an outcome of an anti-inflammatory regimen is counterintuitive, if one assumes that inflammation contributes to depressive symptomatology (see before). The authors of one of the papers describing this phenomenon (53) explain their finding, that perhaps a small activation of the inflammatory system is needed for mental well-being and that both an extreme low and an extreme high activity of the inflammatory response system is disadvantageous for mental health. In other words, there would be an optimal set point for the inflammatory state of an individual for mental health. Downregulating this optimal state with an effective anti-inflammatory agent would, in such a view, be counterproductive and would open the way for the development of depressive symptoms.

Another explanation is that there exists a form of MDD that is non-immune and characterized by absent serological markers of immune activation. As indicated, (add-on) aspirin has a beneficial effect in patients and it can be hypothesized that it is in particular the neuro-modulating effect (anti-glutamatergic) of aspirin that induces this effect.

Based on this literature review, what appears to be the best and easiest assay system to measure the inflammatory state of MDD patients? The systematic review data on the gene expression level of cytokines in circulating leukocytes showed that two of the leukocyte gene expression studies resulted in very good accuracy rates of prediction of non-responsiveness, and algorithms could be developed, which showed high accuracies from 75% to even a 100% to predict non-responsiveness to an SSRI/TCA drug intervention (60, 62). Apparently, high levels of inflammatory cytokine gene message in circulating leukocytes are a precise sign of poor (treatment) outcome, and perhaps even better than high levels of inflammatory compounds/cytokines in serum/plasma. Nevertheless, it is technically less demanding to measure inflammatory compounds/cytokines in serum/plasma than to perform a gene expression assay in circulating leukocytes. Regarding the inflammatory markers best to be measured in serum/plasma to determine a raised inflammatory state in patients with MDD, it is worthy to note that the most consistent effects were found in our literature analysis with circulating CRP and/or IL-6 levels. These inflammatory compounds were tested in a large proportion of the here reviewed studies on serum inflammatory compounds (15/19), and outcomes and conclusions were congruent between these studies regarding these two inflammation markers.

Circulating TNF-α was measured in only six studies; hence, sufficient information on the validity of this parameter is lacking. Importantly, one of the studies showed that circulating TNF-α levels were not in agreement with the general rule, finding that a high TNF-α level was not predictive of a decreased responsiveness to an SSRI/SNRI (while a high IL-6 level in the same study was)

(43). Other circulating inflammatory compounds (e.g., IL-8, IL-10, and IL-1) have also been tested in the here reported studies, but in only very few studies, and therefore data cannot be reliably evaluated. They nevertheless showed the general trend for serum/plasma factors that, in a state of inflammation, more than a monotherapy with a predominantly serotonergic agent might be needed.

Collectively, it seems that for predicting responsiveness to regular antidepressants, the avenue exploring the usefulness of serum/plasma CRP and IL-6 determination is the easiest and clinically the most feasible and promising approach. High levels of CRP/IL-6 would indicate that treatment with a serotonergic drug is not effective enough. However, the data reviewed here also indicate that the gene expression in circulating leukocytes cannot be neglected as a predicting parameter due to the reported high levels of accuracy to predict non-responsiveness to SSRI/SNRI and TCA therapy.

Limitations

In this article, we only focused on inflammation parameters as determinants for the outcome of treatment. The various other determinants important for treatment outcome have recently been reviewed by Perlman et al. (99). The authors described not only that inflammation-related determinants are important but also that a whole array of genetic, endocrine, neuroimaging, sociodemographic, and symptom-based predictors turn out to influence outcome. However, due to heterogeneous sample sizes, effect sizes, publication biases, and methodological disparities across reviews, Perlman et al. (99) concluded that they could not accurately assess the strength and directionality of the predictors, and the authors therefore highlighted the importance of large-scale research initiatives and the use of clinically easily accessible biomarkers, as well as the need for replication studies of current findings. Clearly, we support such view and underscore the notion that our review data are also affected by the heterogeneous sample sizes, effect sizes, publication biases, and methodological disparities and that the data do not yet give a clear-cut picture. Also, our own experimental data on monocyte gene expression were underpowered and too limited to obtain clear-cut results and significances. Thus, clearly more studies are needed using standardized add-on anti-inflammatory treatments to standardized single antidepressant medications to develop a clearer picture of the actual response rates in immune and otherwise stratified patients with MDD.

Conclusions

There are excellent recent reviews on the discovered signs of low-grade inflammation in psychiatric patients that have transformed our understanding of neuropsychiatric diseases and urge for new diagnostic and therapeutic criteria in the emerging field of immuno-psychiatry (100). There are, however, at present, insufficient data and reliable concepts on the inflammation pathogenesis of MDD to design clinically applicable treatment drug protocols with reliable cutoff points for inflammatory parameters to guide therapy regimens.

Despite this limitation, a few generalizations can nevertheless be made from our study regarding inflammation as a predictor. Of the inflammation parameters, the serum CRP and IL-6 seem to be the most promising parameters for further clinical development. They are relatively easy to determine and, thus, useful in clinical studies. Using these parameters, a state of raised inflammation (as evidenced by raised serum CRP and IL-6 levels) characterizes a form of MDD with a relatively poor outcome and a non-responsiveness to agents with a predominant serotonergic action. Such cases might need a faster step-up to drug regimens with agents with dopaminergic (e.g., mirtazapine and bupropion) or glutamatergic (e.g., ketamine) effects, or a combination of a first-line antidepressant with an anti-inflammatory agent such as infliximab, minocycline, or fish oil (but not aspirin), most of them showing dopaminergic action. Varying anti-inflammatory properties of antidepressants as well as varying neuro-modulatory effects of anti-inflammatory agents (and/or complex interactions thereof) may play a role in the therapeutic success or failure of the step-ups.

A word of caution is needed regarding the regimens using as add-on the successful anti-inflammatory agents infliximab, minocycline, and fish oil: There must indeed be laboratory signs of inflammation (i.e., raised serum levels of CRP or IL-6) for this addition to be effective. If not, even response rates lower than the non-add-on situation might be obtained.

ETHICS STATEMENT

These studies were carried in compliance with standards of CGP, assuring that the rights, safety, and well-being of patients were protected in accordance with the principles that have their origin in the Declaration of Helsinki (June 1964, last amendment Fortaleza 2013). Additionally, the relevant national and European regulations were adhered, too. After study procedures had been fully explained, all subjects provided written informed consent.

AUTHOR CONTRIBUTIONS

GA and MS designed the strategy of the present review. BB and EW collected part of the study cohort, GA, AW and HD evaluated the data. GAH and MS wrote the first draft of the paper, HD and NM contributed with supervision and expert advice and critically revised the draft. All other authors contributed to the manuscript revision and approved the submitted version.

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8. Discussion

8.1. IRS abnormalities in individuals with depression

Accumulating research has reported an increase in the expression levels of different pro-inflammatory genes in the circulating monocytes of individuals with depression, suggesting a pro-inflammatory monocyte state[60,61,70,97,98]. In our study, patients with depression were characterized by increased monocyte expression levels of pro-inflammatory cytokine-chemokine genes (ICCGs), and by decreased monocyte expression levels of type I interferon-stimulated genes (ISGs) compared to healthy controls (HC)[99]. We must however highlight that patient's monocytes were not uniformly pro-inflammatory activated (i.e., monocytes of only 36% of patients showed such a pro-inflammatory gene signature[100]), something which is in line with previous literature[61,70].

Little is known about the origin of the pro-inflammatory activated monocytes in individuals with depression. In accordance with previous research[60,62], age was found as an important factor influencing monocyte expression levels of ICCGs in patients[99]. Several reports have also suggested a significant positive association with childhood trauma in individuals with depression[70]. In relation to type I ISGs, recent reports have demonstrated an increased expression of these genes in different somatic conditions, such as autoimmune diseases[101]. Interestingly, age, childhood trauma, and/or the existence of autoimmune diseases have been repeatedly related to microbiome abnormalities[102-105]. On the other hand, microbiome abnormalities have been also repeatedly associated with the expression levels of ICCGs and of type I ISGs in individuals with depression[-108]. The exact mechanisms by which all these factors are interrelated still needs to be elucidated.

Most of the available literature about inflammation and depression has not focused on the expression levels of pro-inflammatory genes, but on the concentrations of pro-inflammatory compounds in the periphery[64,109-113]. In our study, and contrary to what expected, significant differences were not found between individuals with depression and HC in relation to the serum levels of interleukin (IL)-6 and of high-sensitivity C reactive protein (hsCRP)[99]. Importantly, a significant association was also not found between the monocyte expression levels of pro-inflammatory genes and the serum levels of neither IL-6, nor hsCRP in patients. This is in accordance with previous literature, where an association between the peripheral levels of pro-inflammatory compounds, and the expression levels of inflammation-related genes has also not been found, both in healthy individuals and patients with depression[60,70,114]. Our findings may be of relevance, suggesting that: in line with previous research[115], not all patients with depression show signs of immune activation at the peripheral level and second, circulating monocytes may not be the unique source of cytokines in humans, reflecting the involvement of other cell types (e.g., adipocytes, activated endothelial cells) in the peripheral levels of these compounds. In

support of this idea, a significant positive association was found in our study between the serum levels of IL-6 and of hsCRP, and body mass index (BMI), in patients. Previous literature has also reported an association between the peripheral levels of pro-inflammatory compounds and different factors such as BMI[70,116], comorbid metabolic syndrome[70], coronary artery disease[117], smoking[118], and/or exercise[119] in individuals with depression and healthy individuals.

Taken together, our findings may be of relevance. First, they suggest a differential pro-inflammatory monocyte profile in individuals with depression. Second, our findings also suggest that not all individuals with depression show increased levels of pro-inflammatory compounds in the periphery. All this may depend on different factors, such as the existence of comorbid somatic conditions, such as overweight and/or autoimmune disorders.

8.2. The association between the existence of an abnormal IRS and neurotransmitter abnormalities in depression

Different theories have attempted to link the existence of an abnormal IRS with TRP (and 5-HT) deficiencies in individuals with depression. One of the most widely accepted hypotheses postulates the existence of an enhanced catabolism of TRP down the KP by induction of IDO, by pro-inflammatory stimuli. In our study, individuals with depression showed reduced serum levels of TRP compared to HC, and this was especially pronounced in the subgroup of individuals with depression with the highest monocyte expression levels of ICCGs[99]. However, and in line with previous research[93], significant differences were not found between patients and HC in relation to the serum levels of KYN, to the KYN/TRP ratio, and to the monocyte expression levels of *IDO-1*[99]. Indeed, a significant negative correlation was found between the monocyte expression levels of *IDO-1*, and of ICCGs in patients[99]. Interestingly, *IDO-1* monocyte expression levels were significantly positively associated to the monocyte expression levels of type I ISGs. In addition, serum levels of KYN, as well as the KYN/TRP ratio were significantly positively associated to the serum levels of IL-6 and of hsCRP in patients[99]. Taken together, this suggests an activation of the KP only in a subgroup of individuals with depression (i.e., in those patients where increased peripheral levels of pro-inflammatory markers, or where increased monocyte expression levels of type I ISGs are expected), as for example, patients with comorbid somatic conditions, such as overweight and/or autoimmune disorders. Therefore, the reason for the TRP (and 5-HT) deficiencies in MDD (in the absence of systemic inflammation, or in the absence of an activation of type I ISGs), can be only speculated on. TRP is an essential amino acid mainly derived from the diet, since mammals are unable to synthetize it[124]. Therefore, gut microbiota participates actively in TRP's metabolism, and may influence its levels in humans. In line with this, an association between microbiome abnormalities and peripheral levels of TRP have been repeatedly demonstrated[121,122].

Other theories have also attempted to link the existence of an abnormal IRS with glutamatergic imbalances in depression. One of the most accepted hypotheses has suggested an inflammation-related catabolic imbalance between the potentially neurotoxic and potentially neuroprotective

arms of the KP. In other words, pro-inflammatory stimuli would favor the production of potentially neurotoxic compounds, such as 3-HK and/or QUIN, impacting on glutamatergic neurotransmission (more specific, enhancing it)[123]. In line with this idea, individuals with depression of the here reported cohort showed reduced serum levels of the potentially neuroprotective metabolite KYNA, when compared to HC. However, and contrary to what expected, patients also showed reduced serum levels of 3-HK, a reduced 3-HK/KYN ratio, and importantly, reduced expression levels of *KMO* in their circulating monocytes, suggesting a deactivation of this pathway, rather than an activation. We would like to highlight here that the fact that *KMO* expression was reduced, makes unlikely that the existence of reduced 3-HK serum levels in patients was only due to the existence of reduced TRP serum levels. In relation to the serum levels QUIN, significant differences were not found between patients and HC. Previous research has also found reduced peripheral levels of both 3-HK and of QUIN in individuals with depression when compared to HC[92,94]. Interestingly, serum levels of 3-HK, the 3-HK/KYN ratio, and the monocyte expression levels of *KMO* were significantly negatively associated to the expression levels of ICCGs in patient's monocytes, but not to the serum levels of pro-inflammatory compounds. On the contrary, and in line with previous research[124,125], a significant positive association was found in our study between the serum levels of IL-6 and of hsCRP, and the serum levels of QUIN in patients. We thus hypothesize that an increase in the levels of potentially neurotoxic compounds (e.g., 3-HK, QUIN) is only expected in patients showing signs of peripheral inflammation. In the case of patients not showing increased peripheral levels of pro-inflammatory compounds, a deactivation of both the potentially neuroprotective and neurotoxic arms of the KP is expected.

We would like to highlight that MDD is a highly heterogeneous disorder (**Table 1**). If different inflammatory profiles are related to different neurotransmitter abnormalities, we hypothesize that this will be also reflected in the clinic. In our study, an association between the monocyte expression levels of ICCGs, and the severity of depression (as measured by the total score in the Hamilton Depression Rating Scale, 17-item version (HAMD-17)) was found in patients[99].Other researchers have also found an association between the monocyte expression levels of ICCGs and a poorer course of the disorder[60]. Different reports have also suggested an association between the levels of pro-inflammatory compounds and of TRP and/or KP metabolites, and different symptom domains in depression. For example, accumulating research has reported an association between the existence of atypical features of depression and high levels of pro-inflammatory compounds, an activation of the KP, and also high levels of potentially neurotoxic metabolites such as QUIN in individuals with depression[126]. Similar findings have been found in individuals with depression and a high suicide risk (SR), when compared to individuals with depression and no/low SR[127,128]. Interestingly, depression with atypical features, and/or depression with a high SR have been repeatedly associated to a higher BMI[129], and/or a higher incidence of different somatic conditions such as metabolic syndrome[130,131] and/or coronary disease[132], all these factors related to both an increased in the peripheral levels of pro-inflammatory compounds and to an activation of the KP. On the contrary, an association between

melancholic forms of depression, a lower BMI, and a lower prevalence of metabolic syndrome has also been demonstrated[133].

8.3. The IRS, TRP and KP imbalances, and treatment response in individuals with depression

Treatment of depression is based on the enhancement of monoaminergic neurotransmission and nowadays, there are hundreds of monoaminergic antidepressants on the market. However, response rates are far from being optimal, with about 1/3 of patients not responding to treatment. Recent research has suggested that these patients may benefit from agents targeting the glutamatergic system, such as ketamine. The reality is that none of the currently available antidepressants have been shown as objectively 'better' than all the others and that, in the absence of clear biological markers of treatment response, antidepressant prescription remains a matter of clinical judgment[134].

Collectively, the data presented in our study, together with the data of the systematic review suggest that individuals with depression showing increased expression levels of pro-inflammatory genes (e.g., ICCGs, *MIF*), and increased peripheral levels of pro-inflammatory compounds (e.g., CRP, IL-6, TNF-α) exhibit, in general, lower response rates to predominantly monoaminergic agents (e.g., selective serotonin reuptake inhibitors (SSRIs)) compared to patients without such a pro-inflammatory state. These patients may benefit from antidepressants with a strong effect on other neurotransmission systems, such as the glutamatergic system, and/or from (add-on) treatment with anti-inflammatory drugs. Considering that systemic inflammation may be associated with both an activation of the KP and an increase in the levels of potentially neurotoxic compounds (e.g., QUIN), these findings seem coherent, with patients with depression and systemic inflammation needing treatment with agents that decrease potentially neurotoxic KP metabolites. In line with this idea ketamine, (an NMDA-R antagonist that has been repeatedly tested over the last decade as a promising antidepressant in, especially, treatment resistant depression)[135,136], has been shown to be able to decrease glutamate levels by the inhibition of the expression of different pro-inflammatory genes[137], as well as the inhibition of the production of pro-inflammatory compounds[138]. Importantly, ketamine has also been shown to induce changes in microglial cells, decreasing QUIN production and levels. Indeed, QUIN plasma levels have been recently proposed as a response predictor to ketamine in individuals with depression[139]. Several anti-inflammatory agents have demonstrated a similar action on the KP. In line with this idea, the omega-3 fatty acid eicosapentaenoic acid (EPA) has been shown to influence glutamatergic neurotransmission by different mechanisms such as decreasing hepatic activity of aminocarboxymuconate-semialdehyde-decarboxylase (ACMSD) enzyme. Interestingly, ACMSD enzyme acts as a key enzyme in the KP, since it acts on the production of QUIN[140].

8.4. Limitations

A major limitation of our studies is the fact that pro-inflammatory compounds, as well as TRP and KP metabolites were determined in the blood, and may not reflect what happens in the brain. Peripheral levels of pro-inflammatory compounds may be influenced by different biological factors, such as the presence of overweight. In addition, and while TRP, KYN, and/or 3-HK easily penetrate the blood brain barrier, other metabolites such as KYNA and/or QUIN do not[141]. Therefore, future studies investigating to what extent are concentrations of pro-inflammatory compounds and/or of TRP and KP metabolites in the periphery related to CSF and/or to brain levels and also, to symptoms, are urgently needed.

9. Conclusions

Taken together, our study suggests the existence of an abnormal IRS in MDD. However, it is crucial to move away from the idea that all individuals with depression are the same, and thus, that they are uniformly “inflamed”. In other words, patients with MDD may be characterized by different inflammatory profiles that may be related to different neurotransmitter abnormalities and symptom profiles, and therefore, to different treatment strategies.

To the best of our knowledge, this is the most comprehensive study to date linking IRS abnormalities in patients with depression, to TRP and KP imbalances, and to response to treatment. Taken together, we found that individuals with depression lacking from comorbid medical conditions (e.g., obesity, autoimmune disorders) show, in general, increased monocyte expression levels of ICCGs, but no serological markers of immune activation compared to HC. These individuals are also characterized by decreased levels of TRP, and by a deactivation of the KP, and may benefit from treatment with primarily serotonergic antidepressants (e.g., SSRIs). On the contrary, individuals with depression suffering from comorbid medical conditions (and/or with a high SR) may be characterized by increased monocyte expression levels of ICCGs, together with an increased in the serum levels of pro-inflammatory compounds compared to HC. In addition, they may exhibit, along with a decrease in the levels of TRP, an activation of the KP. These individuals may benefit from antidepressants with a strong action on other neurotransmitter systems than the monoaminergic system, such as the glutamatergic system, and/or from (add-on) therapy with anti-inflammatory/immunoregulatory agents.

Despite limitations, and although these preliminary data have to be interpreted cautiously, we believe our findings are encouraging and may have a potential clinical impact. There is currently insufficient data on the inflammatory state of individuals with depression to construct clinically reliable treatment protocols with valid biological parameter cut-off points to guide therapy alternatives. Therefore, dissection of the heterogeneity of depression and matching with clear inflammatory phenotypes may increase the possibility of finding specific disturbances in the pathophysiology of the disorder that may benefit from targeted treatments. In this sense, our findings represent an important first step toward precision psychiatry and may prove to be a useful tool to be applied in future research.

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