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Immune system dysregulations in major depressive disorder: The role of innate immune function and response prediction to standard and anti-inflammatory therapy – challenges towards an individualized medicine.

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II List of abbreviations

AA	arachidonic acid
ABCA1	ATP-binding cassette 1
ABCG1	ATP-binding cassette sub-family G member 1
AP1	activating protein 1
BDNF	brain-derived neurotrophic factor
BMI	body mass index
CCL	CC-chemokine ligand
CNS	cental nervous system
Cox-2	cyclooxygenase-2
CRF	corticotrophin-releasing factor
CRP	C-reactive protein
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
DUSP2	dual specificity phosphatase
EGR	early growth response protein
ELISA	enzyme-linked immunosorbent assay
GABA	gamma-aminobutyric acid
GK	gesunden Kontrollen
GR	glucocorticoid receptor
HAMD	Hamilton Rating Scale for Depression
HC	healthy controls
HIDS	hyperimmunoglobulinemia D syndrome
HPA	hypothalamic–pituitary–adrenal
IDO	indoleamine-2,3-dioxygenase
IFN-γ	interferon gamma
IFN-α	interferon-alpha
IL-1	interleukin-1
IL-1β	interleukin-1 beta
IL-6	interleukin-6
IL-18	interleukin-18

iNOS	inducible nitric oxide synthase
LPS	lipopolysaccharides
LXR	liver X receptor
MADRS	Montgomery–Åsberg Depression Rating Scale
MAFF	MAF BZIP Transcription Factor F
MAO-I	monoamine oxidase inhibitors
MCP	monocyte chemoattractant protein
MDD	major depressive disorder
MIF	macrophage migration inhibitory factor
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
MVK	mevalonate kinase
MVKD	mevalonate kinase deficiency
NDRI	norepinephrine-dopamine reuptake inhibitors
NF-kB	nuclear factor kappa B
NK	natural killer
NLRP3	NLR family pyrin domain containing 3
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NR1H3	nuclear receptor subfamily 1 group H member
NSAIDs	non-steroidal anti-inflammatory drugs
oxLDL	oxidized low density lipoprotein
PGE2	prostaglandin E2
q-PCR	quantitative-polymerase chain reaction
ROS	reactive oxygen species
SARI	serotonin antagonist and reuptake inhibitors
SMD	standard mean difference
SNRI	serotonin-norepinephrine reuptake inhibitors
SSRI	selective serotonin reuptake inhibitors
TAU	treatment as usual
TCA	tricyclic antidepressants
TDO	tryptophan 2,3-dioxygenase
TeCA	tetracyclic antidepressants
Th	T helper

Th2	T helper 2
Th17	T helper 17
TNFα	tumor necrosis factor alpha
TRD	treatment-resistant depression
3HK	3-hydroxykynurenine
7β-HC	7β-hydroxycholesterol
7-KC	7-ketocholesterol
25-HC	25-hydroxycholesterol
27-HC	27-hydroxycholesterol

III List of publications – original work

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IV Contribution to the publications

iv.i Publication I (Simon et al., 2021a)

Data for the first publication was collected within the EU-Moodinflame consortium at the three university psychiatric clinics of Katholieke Universiteit Leuven, Ludwig-Maximilians-Universität Munich, and Westfälische Wilhelms-Universität Münster. Funding from the EU 7th Framework program (grant number EU-FP7-CP-IP-2008-222963) and Horizon 2020 (grant number H2020-SC1-2016-2017/H2020-SC1-2017-Two-Stage-RTD) grants was received by the coordinator at Erasmus Medical Center Rotterdam. My role in this project mainly includes data management, data evaluations, data presentation and discussion within the consortium, shipment of samples to the responsible laboratory, and support for inquiries and reportings. My role in the present publication included organization of new determinations with lab technician at the coordinating center, preparation of the database/ data cleansing, data analysis, data interpretation and drafting/ revising the manuscript in close collaboration with the coordinator, data presentation and discussion at scientific conferences prior to the final draft, and submission to and correspondence with the journal. Please also refer to the author contribution section of the publication.

iv.ii Publication II (Simon et al., 2021b)

Data for the second publication was also collected within the EU-Moodinflame consortium at the university psychiatric clinics of Ludwig-Maximilians-Universität Munich and Westfälische Wilhelms-Universität Münster. Recruitment was terminated after the inclusion of only two participants at Münster, therefore only data from Munich went into this publication. Funding from the EU 7th Framework program (grant number EU-FP7-CP-IP-2008-222963) and Horizon 2020 (grant number H2020-SC1-2016-2017/H2020-SC1-2017-Two-Stage-RTD) grants was received by the coordinator at Erasmus Medical Center Rotterdam. Further, additional funding from the foundation ‘Immunität und Seele’ was received by the local coordinator at Munich site. The data collection from study participants was completed by the time I was recruited into the project. Nevertheless, I engaged in data collection for other projects during the time as

doctoral candidate. My role in the present publication included preparation of the database/ data cleansing, data analysis, data interpretation, drafting and revising the manuscript, and submission to and correspondence with the journal. Please also refer to the author contribution section of the publication.

0 Summary

Depression is one of the biggest health problems worldwide. A couple of treatment options like antidepressants or psychotherapy exist, which are well implemented. So far, their application is guided by disease severity; however, the complex underlying biological pathophysiology is barely taken into consideration. Especially, mounting evidence about immuno-metabolic aspects of the disease, which are present in a subgroup of patients, has not been incorporated into official guidelines yet. The present work provides an overview of the current state of research and thereby integrates different areas of research on biological underpinnings in a comprehensive model, which has not been available so far. In doing so, new developments in the field of immuno-psychiatry emanating from the results of own original works are placed into this context. Relevant components in the interplay and circuitry among each other are elevated cytokine and neurotoxic kynurenine metabolite levels, an inflammatory monocyte gene expression, a dysregulated hypothalamic-pituitary-adrenal axis, and glucocorticoid resistance. For the first original work monocyte gene expression was determined in patients with diagnosed major depressive disorder, where a low-grade inflammatory signature and upregulated apoptosis and cholesterol pathway markers were found in general, as well as an additional exacerbated inflammation together with an impairment in mevalonate degradation under the premise of childhood adversity. This underlines the relevance of innate immune function. Understanding the pathophysiology as comprehensively as possible serves as the basis to open up and implement new treatment strategies. Thus, the present work also focusses on the efficacy of drug therapy, its deficits, therapy response prediction, and the deduction of relevant implications regarding alternative anti-inflammatory therapeutic approaches. For the second original work circulating inflammatory compounds like cytokine levels were determined in patients with diagnosed major depressive disorder who underwent treatment with an antidepressant or an augmentation by an anti-inflammatory agent which blocks stimulators of neurotoxic kynurenine metabolization. Especially differential macrophage migration inhibitory factor levels emerged as an indicator of treatement success after each therapy. It is important to bear in mind that subgroups of the patient population are to be identified in order to strive for an individualized and tailored treatment. Both original works pursue this approach by, on the one hand, characterizing patient groups with different monocyte gene expression

profiles and, on the other hand, investigating biomarker levels to predict treatment success. Thus, both publications significantly contribute to this field of research.

0 Zusammenfassung

Depression ist eines der größten Gesundheitsprobleme weltweit. Es gibt eine Reihe von Behandlungsmöglichkeiten, wie Antidepressiva oder Psychotherapie, die gut implementiert sind. Deren Anwendung richtet sich bisher nach dem Schweregrad der Erkrankung; die komplexe zugrundeliegende biologische Pathophysiologie findet dabei aber kaum Berücksichtigung. Vor allem zunehmende Evidenz zu immuno-metabolischen Aspekten der Erkrankung, die sich bei einer Subgruppe von Patienten zeigen, hat bisher keinen Eingang in offizielle Leitlinien gefunden. Die hier vorliegende Arbeit bietet einen Überblick über den Stand der Forschung und integriert dabei verschiedene Forschungsbereiche zu biologischen Grundlagen in einem umfassenden Modell, welches so bisher noch nicht verfügbar war. Dabei werden neue Entwicklungen im Bereich der Immuno-psychiatrie, die aus den Ergebnissen der eigenen Originalarbeiten hervorgehen, in diesen Kontext eingeordnet. Erhöhte Zytokin- und neurotoxische Kynureninmetaboliten-Level, eine inflammatorische Monozyten Genexpression, eine dysregulierte Hypophysen-Hypothalamus-Nebenniereninden-Achse und Glucocorticoidresistenz stellen relevante Komponenten im Wechselspiel und in Regelkreisen miteinander dar. Für die erste Originialarbeit wurde Monozyten Genexpression bei Patienten mit diagnostizierter Majorer Depression bestimmt, wobei generell eine geringgradige inflammatorische Signatur und hochregulierte Apoptosemarker und Marker des Cholesterinstoffwechselwegs gefunden wurden. Weiterhin wurde zusätzlich eine exazerbierte Inflammation zusammen mit einer Beeinträchtigung des Mevalonatabbaus unter der Voraussetzung des Vorliegens kindlichen Traumas gefunden. Dies unterstreicht die Relevanz des angeborenen Immunsystems. Das Verständnis eines möglichst umfangreichen Bildes der Pathophysiologie dient als Grundlage für die Eröffnung und Implementierung neuer Behandlungswege. Daher werden in der vorliegenden Arbeit ebenfalls die Wirksamkeit medikamentöser Behandlung, deren Defizite und die Vorhersage des Behandlungsansprechens thematisiert, sowie wichtige Implikationen bezüglich alternativer anti-inflammatorischer Behandlungsansätze abgeleitet. Für die zweite Originalarbeit wurden zirkulierende inflammatorische Stoffe wie Zytokine bei Patienten mit diagnostizierter Majorer Depression bestimmt, die sich einer Behandlung mit einem Antidepressivum oder einer Augmentation mit einem anti-inflammatorischen Wirkstoff unterzogen, welcher Stimulatoren der Metabolisierung neurotoxischer Kynurenine

blockt. Unterschiedliche Level des Macrophage Migration Inhibitory Factor stellten sich insbesondere als Indikatoren für Behandlungserfolg nach der jeweiligen Therapie heraus. Um einer individuell zugeschnittenen Behandlung näher zu kommen, muss dabei stets beachtet werden, dass Subgruppen der Patientenpopulation identifiziert werden. Beide Originalarbeiten verfolgen diesen Ansatz, indem zum einen Charakteristika von Patientengruppen mit verschiedenen Monozyten Genexpressionsprofilen herausgearbeitet wurden und zum anderen Biomarkerlevel zur Vorhersage von Behandlungserfolg untersucht wurden. Beide Publikationen leisten daher einen signifikanten Beitrag zu diesem Forschungsfeld.

1 Background

Major depressive disorder (MDD) is one of the leading causes for disability-adjusted life years: results from the Global Burden of Diseases, Injuries, and Risk Factors Study show that among all age groups depressive disorders rank at 19 of leading causes in 1990 and at 13 in 2019 (GBD 2019 Diseases and Injuries Collaborators, 2020). In the age group 10-24 years depressive disorders are even placed at rank eight in 1990 and rank four in 2019, and in the age group 25-49 years at rank eight in 1990 and rank six in 2019 (GBD 2019 Diseases and Injuries Collaborators, 2020). Thus, a clear trend of a growing impact in relation to other diseases emerged throughout the last 30 years. In 2004, a meta-analysis revealed a 12-month prevalence of 6.9% (18-65 years) for major depression among the European population which was confirmed in 2010 extending the age range to 14-65 years (Wittchen et al., 2011; Wittchen & Jacobi, 2005). Further, the 12-month prevalence of 7.4% in major depressive disorder did not change in the German population in 1997-1999 compared to 2009-2012 (Bretschneider et al., 2018). These epidemiological studies strongly suggest a stable occurrence of this disease while its significance in the health system increases. Noteworthy, the diagnostic criteria for major depression according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) changed only slightly over time (American Psychiatric Association, 1987, 1994, 2000, 2013) indicating that epidemiological data are rather not ascribable to different confirmation of diagnosis. Another aspect contributing to the burden is the recurrent nature of depression. Although seemingly no recent data exist, research clearly shows that recurring episodes occur frequently ranging from 21-40% in specialized mental health care or primary care settings within 1-1.5 years and 42-75% within 5 years (Hardeveld et al., 2010). In the general population, recurrence occurred after one year with 2.5%, at 5 years with 13.2%, at 10 years with 23.2%, and at 20 years with 42.0% (cumulative values; Hardeveld et al., 2013). Apart from recurrence, there is a considerable number of patients that fall under the term treatment-resistant, here defined by at least two failed pharmacotherapeutic treatment attempts for at least 15 days (Mahlich et al., 2018; Szegedi et al., 2009). 12% of pharmacologically treated major depressed subjects developed treatment-resistant depression (TRD) within 12 months (Mahlich et al., 2018). Another study found a 12-month prevalenc of even 30.9% (Zhdanava et al., 2021). Noteworthy, diverging definitions of TRD exist (Trevino et al., 2014). Last, higher risk for mortality has been found in depression not only due to suicide but also related to cardiovascular disease

(Lépine & Briley, 2011). All of the above evidence underpins that major depression poses a global health burden hence the stability of prevalence, the high recurrence throughout life, the prevalence of treatment resistant cases, and the disease burden. Furthermore, this also leads to a substantial economic burden as to health-related costs such as workdays lost, loss of productivity, treatment costs, and costs due to mortality (Amos et al., 2018; Greenberg et al., 2015; Wang et al., 2003). Therefore, reducing prevalence of major depression still remains a major health care goal.

2 Mechanism of disease

2.1 Etiological theories

To date different psychological and biological theories about the pathogenesis of depression exist. Psychological theories focus on patterns of behavior and thought. Very prominent examples are the Lewinsohn's social reinforcement theory, the learned helplessness, and the cognitive model (Beck, 1979; Lewinsohn, 1974; Seligman, 1972). These theories share common ground as they are based on learning experiences. In particular, absence of linkage of behavioral action with positive consequences as well as generated dysfunctional cognitive patterns characterize the mechanism of pathogenesis and retention of depression (Beck, 1979; Lewinsohn, 1974; Seligman, 1972). However, the mere exposure to such experiences does not seem sufficient for developing symptoms as resilient cases demonstrate. The vulnerability-stress-model as described by Wittchen and Hoyer (2006) considers acute stressful experiences as one pathogenetic factor next to individual and environmental vulnerabilities. Thereby, crucial learning experiences can contribute to shaping vulnerabilities and modulate the burden of stressful events to the individual (Wittchen & Hoyer, 2006). Furthermore, this model incorporates biological factors and describes the interaction of psychological, social, and biological factors (Wittchen & Hoyer, 2006). By now, a substantial body of evidence exists about biological underpinnings of depression examining different pathogenic angles.

Even before the above described prominent behavioral theories were published, the monoamine hypothesis of depression was already being discussed. According to this theory, an existing lack of the neurotransmitters serotonin, noradrenaline, and dopamine in the brain is associated with depression and leads to typical symptoms (e.g., Coppen et al., 1972; McClure, 1973; Schildkraut, 1965). Although this theory is popular and widely accepted, critique has been raised as well. Liu et al. (2017) argue that the serotonin hypothesis is questionable due to conflicting research results: for example, delayed effects of antidepressants and failure to induce depression by lowering serotonin. Similar issues are being discussed about the monoamine hypothesis in general (Boku et al., 2018). Thus, at least additional explanations for the underlying pathophysiology of depression are necessary. After the monoamine hypothesis, the neuroplasticity and neurogenesis hypothesis developed implicating altered brain morphology by degeneration and lack of newly developed cells in the hippocampus, respectively (Boku et al., 2018). These

changes seem to be induced through neurotoxic effects of elevated glucocorticoid levels caused by hypothalamic–pituitary–adrenal (HPA) axis hyperactivity as a response to stress (Boku et al., 2018; Liu et al., 2017). Another approach discusses the tryptophan breakdown as an underlying mechanism of disease. Here a lack of serotonin in neurotransmission may be explained by a shift of tryptophan metabolism towards the breakdown along the neurotoxic kynurenine pathway (Müller & Schwarz, 2006; Myint, 2012). In 1970 already, higher metabolization of administered l-tryptophan into kynurenine and 3-hydroxykynurenine levels were found in depressed compared to non-depressed women (Curzon & Bridges, 1970). While this theory somewhat strengthens the serotonin monoamine hypothesis in contrast to the neuroplasticity and neurogenesis hypotheses, it also supports the idea of neurotoxic processes in the brain. This shift to neurotoxic kynurenine metabolism was also found in the hippocampus (Parrott et al., 2016a, 2016b). Although it is difficult to establish causality, these findings point into the direction that the lack of serotonin may not necessarily be the leading underlying pathology but rather one of multiple components. The neurotoxic activities of kynurenine metabolites and hypercortisolemia seem promising explanations.

Referring to the vulnerability-stress-model, the association between psychosocial factors and immune dysfunction, especially in depression, has been investigated back in 1987, already (Dorian & Garfinkel, 1987). Over the past two decades, a constantly increasing interest has been paid to the relation of depression with inflammatory processes. As early as 1990/1991, indications for immune dysregulations in depression were published (Maes et al., 1990-1991). Around that time, first indications of elevated pro-inflammatory cytokine levels in depressed patients were observed (e.g., Seidel et al., 1995). By now, a vast body of evidence has emerged in this area leading to the inflammation theory of depression of which the kynurenine pathway and the HPA axis seem to be a part of (Gałecki & Talarowska, 2018). The rising field of immuno-psychiatry considers inflammation and dysbalances of the immune system as an important underlying pathology of depression. The following section will give an overview of the current state of knowledge.

2.2 Immune profiling in depression

Firstly, attention needs to be raised to the fact that the here described abnormalities do not apply to the whole population of depressed individuals. Inflammatory activation is only present in a substantial subgroup of MDD patients (Raison & Miller, 2011; Rothermundt et al., 2001; Schiweck et al., 2020a). This strengthens the view of different etiological pathways leading to a pattern of symptoms categorized as depression. Different components have been the focus of previous investigations (see also figure 1):

Low-grade inflammation. Multiple reports demonstrate elevated circulating pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) in depression on a low to moderate level (Dowlati et al., 2010; Köhler et al., 2017). This pro-inflammatory activation has been termed low-grade inflammation and its relation to depression is now widely accepted. Measured by C-reactive protein (CRP), low-grade inflammation occurred in 27% percent of patients with depression which was significantly more frequent than in healthy controls (Osimo et al., 2019). Raison et al. (2006) measured elevated CRP levels in about one-third of depressed patients. Depression and other diseases like metabolic syndrome and cardiovascular disease share this low-grade inflammation pathology (Silverman & Sternberg, 2012). Furthermore, other immune components have also been shown to contribute to and/or express this upregulated pro-inflammatory phenotype in depression (see below).

Kynurenine pathway. As described above, the kynurenine pathway has been repeatedly discussed regarding depression and is placed in the context of inflammation here. The enzyme indoleamine-2,3-dioxygenase (IDO) metabolizes tryptophan into kynurenine which itself is stimulated by pro-inflammatory cytokines and the hormone prostaglandin E2 (PGE2; Müller, 2014; Myint, 2012; Oxenkrug, 2010a). Kynurenine metabolites may then induce apoptosis (immunosuppression) but also may have pro-inflammatory effects (quinolinic acid) depending on the enzymatic activity of the cell type and therefore the prevailing metabolite (Maes et al., 2011). Thus, low-grade inflammation promotes the availability of kynurenine and neurotoxic downstream metabolites to induce more inflammation. Next to the neurotoxic pathway (e.g., 3-hydroxy kynurenine and quinolinic acid), kynurenine may also be metabolized to neuroprotective kynurenic acid (Savitz, 2020). Hippocampal atrophy, also seen in depression with HPA axis hyperactivity, may be related to an imbalance of the neurotoxic and neuroprotective arms of the kynurenine pathway (Miura et al., 2008).

HPA axis. Psychological stress has been shown to induce inflammation (Myint & Kim, 2003). Likewise, as described above, psychological stress also leads to HPA axis hyperactivity. The HPA axis may therefore contribute to the pathophysiology of depression as it is also linked to the immune system, reciprocally (Maes, et al., 1995). Normally, proinflammatory cytokines and HPA axis form a feedback loop: activation of the HPA axis by proinflammatory cytokines leads to an increased release of glucocorticoids, which in turn elicit immunosuppressive effects (Miura et al., 2008). In more detail, cytokine production is downregulated as glucocorticoids induce apoptosis of immune cells and suppress nuclear factor kappa B (NF-kB) signaling (Slavich and Irwin, 2014). However, a defect of this regulatory process has been observed in depression, which seems to be characterized by both, high levels of proinflammatory compounds and cortisol (Medina-Rodriguez, 2018). An immune cell desensitization to glucocorticoid immunosuppression (glucocorticoid insensitivity/resistance; Silverman & Sternberg, 2012), while higher inflammation (cytokine levels) inhibits the glucocorticoid receptor function in turn are being discussed as mechanisms (Miller and Raison, 2016; Slavich and Irwin, 2014). Consequently, proinflammatory cytokines disturb the negative feedback effect of glucocorticoids leading to HPA hyperactivity and elevated glucocorticoid levels (Boku et al., 2018; Oxenkrug, 2010b). Further, healthy individuals with glucocorticoid resistance after chronic stress failed to downregulate proinflammatory cytokine production (Cohen et al., 2012). In sum, a defect of the negative feedback loop of glucocorticoids on the HPA axis itself, disturbed by high proinflammatory levels, and on cytokine output, disturbed by chronically high glucocorticoid levels, seems to be a prominent dysfunction in depression. These phenomena are not only present in depression but also in inflammatory-metabolic diseases like metabolic syndrome or cardiovascular disease (Silverman & Sternberg, 2012). With respect to the above mentioned overlap between HPA axis and kynurenine pathway, it is worthy to note that glucocorticoids induce tryptophan 2,3-dioxygenase (TDO) and IDO, both of which stimulate the kynurenine pathway (Maes et al., 2011). Thus, as glucocorticoid levels are elevated in depression, the interplay of these components contribute to pathophysiological dysbalances in which inflammation seems to play a prominent role.

Lymphocytes. Beginning in the 80ies, researchers started to pay attention to lymphocyte deregulations in patients with depression. First findings of deficient lymphocyte function were demonstrated by lower numbers of T and B cells and a

decreased mitogenic activity of lymphocytes in depressed patient groups (e.g., Kronfol et al., 1983; Schleifer et al., 1984). Until now, a more diverse but also conflicting picture emerged by studying lymphocyte subpopulations. For example, natural killer (NK) cells were shown to be increased in depressed and dysthymic subjects compared to non-depressed controls (Hernandez et al., 2010; Ravindran et al., 1995). Other studies found reduced NK, T helper 17 (Th17), T helper 2 (Th2), and T regulatory cell proportions in patients with major depressive disorder compared to healthy controls (Grosse et al., 2016a; Grosse et al., 2016b; Toben & Baune, 2015). Regarding cytotoxic T cells, contrary findings exist (Toben & Baune, 2015). Interestingly, serotonin acts as an immune modulator thereby exerting an influence on the function of pre-B cells and T cells, macrophages, and NK cells (Ahern, 2011). Regarding the generally observed lymphocytopenia in depression, T helper (Th) cells seem to show an accelerated apoptosis in depressed patients which is likely related to activated IDO and kynurenine pathway with tryptophan depletion (Miller, 2010). Furthermore, a suppression of adaptive immune function was described in more detail: lymphocyte apoptosis, downregulation of T cell function, and downregulated cytotoxic T cell function and NK cell function in particular, as well as an increased differentiation of T naïve cells into T regulatory cells (which inhibit immune responses) are stimulated by IDO and kynurenine metabolites (Savitz, 2020). Such adaptive immune suppression implies higher proneness to infection (Savitz, 2020). Another approach considers the inhibition of T cell function by glucocorticoids in depression. Glucocorticoids may also induce apoptosis in T cells and contribute to lymphocytopenia in depression (Maes et al., 1991; Miller, 2010). A later work suggests that lymphocyte glucocorticoid resistance is associated with T cell activation and inhibited glucocorticoid induced apoptosis (Maes et al., 2011). This seems somewhat contrary to an immunosuppression but is in line with the above described observations of high glucocorticoid and inflammatory cytokine levels. How the lymphocytic system is dysregulated exactly, has not yet been clarified completely and may be dependent on other interacting factors contributing to the heterogeneity of results.

Monocytes. As early as the beginning of the 90ies, the involvement of monocytic activation together with lymphocytic activation in depression was firstly proposed (Maes et al., 1995; Smith, 1991). One of the main indicators leading to that theory were higher levels of pro-inflammatory cytokines released by immune cells, thereby not clearly distinguishing the individual role of monocytes (Maes et al., 1995; Maes, 2011). After all, increased monocyte counts were found in depressed patients (Seidel et al., 1996).

Since then, more indications for pro-inflammatory activation of these innate immune cells in depression have emerged (Musil et al., 2011; Nowak et al., 2019; Suarez et al., 2003). One cytokine that has gained some attention as contributor to the activation of inflammasome and pathogenesis of depression is the macrophage migration inhibitory factor (MIF; Petralia et al., 2020). Noteworthy, MIF is not only released by monocytes/macrophages, but also by other innate and adaptive immune cells or endothelial cells (Günther et al., 2019). MIF upregulates the cytokine production, such as interleukin-1 beta (IL-1β), TNFα, IL-6, interferon gamma (IFN-γ), or the enzyme cyclooxygenase-2 (Cox-2; Günther et al., 2019) leading to the stimulation of IDO. IL-1β may also induce PGE2 (Maier 2003), an activator of IDO (Braun et al., 2005). Within circulating macrophages an increased activation of the kynurenine pathway (kynurenine, 3-hydroxykynurenine (3HK), and quinolinic acid) during inflammatory processes has been shown (Savitz, 2020). Regarding the role of monocytes/macrophages for HPA axis activity, it seems that macrophage released cytokines, i.e., interleukin-1 (IL-1) and IL-6, facilitate the release of corticotrophin-releasing factor (CRF) in the hypothalamus (Miura et al., 2008). Thus, activated macrophages may be substantially responsible for stimulated HPA and subsequent glucocorticoid activity through their cytokine output. Following activation of HPA axis, MIF is released from the anterior pituitary and adrenal gland and exerts in an inhibitory effect on the anti-inflammatory glucocorticoid action on immune cells (Petralia et al., 2020). Therefore, MIF seems to be an important inductor of steroid resistance (Petralia et al., 2020). MIF production in macrophages normally follows a regulation principle: low glucocorticoid concentrations have an inductive effect leading to suppression of anti-inflammatory glucocorticoid action while MIF levels decrease at high glucocorticoid levels (Bucala, 1996; Calandra et al., 1995). It is questionable whether this particular regulatory mechanism may also be disturbed in depression since substantially higher MIF levels were found in the presence of depressive symptoms and were associated with decreased glucocorticoid stress response (Bloom & Al-Abed, 2014). Furthermore, monocytes of drug-free depressed patients showed signs of glucocorticoid resistance (Hasselmann et al., 2018). Thus, it is tempting to speculate that monocytes/macrophages and their mediators are crucially involved in the above described dysbalances in depression. Few studies point out the relationship of monocyte activity with lymphocyte abnormalities. For example, the pro-inflammatory state of monocytes was associated with reduced percentage of T regulatory cells (Grosse et al., 2016b). In general, a pro-inflammatory monocyte gene expression and T cell defects co-occurred in

these patients with MDD (Grosse et al., 2016b). This seems in line with the above mentioned conclusion of an innate immune upregulation and an adaptive immune suppression in depression at the same time (Savitz, 2020). To further support this hypothesis, a gene expression study found that in patients with MDD, as compared to healthy controls, most upregulated genes were related to the innate immune response while downregulated genes were related to adaptive immunity and T cell and NK cell function, in particular, and that these complementary expressions were both present in single patients (Leday et al., 2018). Among other cells from the myeloid lineage, a strongly correlated upregulated gene cluster was present in monocytes (Leday et al., 2018). A study investigating the expression of target pro-inflammatory monocyte genes in MDD patients found an increased gene expression signature as compared to healthy controls (Grosse et al., 2015). In a follow-up study, this was found particularly related to a history of childhood adversity (Schiweck et al., 2020a). In sum, monocytes show signs of low-grade inflammation, dysregulated tryptophan breakdown along the neurotoxic kynurenine pathway, glucocorticoid deregulations, and are increased in amount in depression. They play an essential role in homeostasis and especially in regulating inflammatory processes (Wynn et al., 2013). Therefore, monocytes/macrophages should be a prime target of research in depression. Noteworthy, monocytes have the capability of differentiating into different types of macrophages with distinct function, such as pro-inflammatory M1 type, the anti-inflammatory M2 type, or atheroprotective M(Hb) type, among others (Chistiakov et al., 2015).

2.3 Shared factors in depression and inflammation

Strengthening the view of an inflammatory pathophysiology in depression, different depression-related characteristics are also associated with a pro-inflammatory activation. Some of special interest are described.

Sex. A sex-specific risk for having depression and a sex-specific immune response are well described (Slavich & Sacher, 2019). A recent study confirmed substantially higher self-reported and physician-diagnosed depression rates in women compared to men (Asselmann et al., 2019). Additionally, in brain regions relevant for cognition and emotions, like hippocampus and amygdala, estrogen and progesterone receptors are highly expressed, and ovarian hormones are able to act on serotonin, dopamine, gamma-

aminobutyric acid (GABA), and N-methyl-D-aspartate (NMDA) receptors (Slavich & Sacher, 2019). In another study, the interaction of female sex and elevated inflammation predicted higher depression symptoms after two years (Moieni et al., 2015). Further analyses revealed that inflammatory activation was associated with higher depression scores in females but not in males, while this difference did not exist in low inflammatory activation levels (Moieni et al., 2015). Immune cells express receptors for sex hormones and estrogens stimulate pro-inflammatory activity (Slavich & Sacher, 2019). However, the specific effect on immune function is complex and difficult to disentangle (Slavich & Sacher, 2019). In response to stress, females of different species show greater physiological and neurochemical reactivity than males (Goel et al., 2014). Though females seem to be primed for a greater release of glucocorticoids supporting sex differences in HPA stress response, human studies show some inconsistencies, and research about sex-dependent differential deregulations in depression is scarce (Goel et al., 2014). Some evidence shows that women present a greater HPA axis dysregulation than men in major depression (Young & Korszun, 2010). With regard to the neurotoxic kynurenine breakdown, women showed an exaggerated IDO induction, and a significant association of IDO activity and depressive symptoms was found in women but not in men (Elovainio et al., 2012; Maes et al., 2011). Furthermore, depressed and healthy women had reduced neuroprotective kynurenic acid serum levels as compared to men, while women with oral contraceptive use showed even lower levels than women without such female hormone medication intake (Meier et al., 2018).

Childhood adversity. Research indicates an increased risk for MDD and inflammation by the experience of (recent) stressful life events (Slavich & Irwin, 2014). It is therefore interesting to set focus on childhood adverse experiences as an intensive form of stress in early life. Inflammation and depression were particularly found after the experience of a stressful childhood environment (such as traumatic experience; Müller et al., 2019; Slavich & Irwing, 2014). In more detail, a 32-year prospective cohort study showed that childhood maltreatment and social isolation led to a higher risk of developing depression and elevated inflammatory activation in adulthood, where childhood adversity was the driver of depression and inflammation in about one third and 13% of cases, respectively (Danese et al., 2009). Furthermore, after controlling for developmental risk factors (family history of depression and heart disease, low birth weight, high childhood body mass index (BMI)) and concurrent circumstances and behaviors (low socioeconomical status, smoking, physical inactivity, poor diet), childhood adverse

experiences predicted a greater number of age-related-disease risk factors in individuals as young as 32 years (Danese et al., 2009). This points to an earlier onset of complications in this population usually seen at older age. Several studies have investigated IL-6, TNFα, and CRP as pro-inflammatory markers, where those have repeatedly been detected to be increased in individuals with childhood adversity (Deighton et al., 2018; Grosse et al., 2016c; Müller et al., 2019). However, a more recent review on MDD patients revealed that elevated IL-6 levels were consistently found in individuals with childhood adversity as compared to healthy controls and/or MDD without childhood adversity, while elevated TNFα was found in the majority of studies for this population, and results for CRP are inconsistent (Gill et al., 2020). Furthermore, changes in the stress response system particularly in depression with childhood adversity but not without were found, as well as an association of glucocorticoid resistance with childhood adversity (Heim et al., 2008). Even though depression is more prevalent in women than in men, rates of adverse childhood experience are balanced between the sexes, while girls rather experience sexual trauma and boys rather experience physical trauma (Heim et al., 2008). Given these and the above described sex differences, it can be speculated that women may have a more prone system to develop an inflammatory and depressed phenotype after stress.

Age. The onset of depression is especially prevalent in the young age group but continues throughout life while attenuating in later life (Andrade et al., 2003; Kessler et al., 2012). This trend is also seen in the 12-month prevalence per age group in a German sample (10% at 18-34 years, 7,2% at 35-49 years, 5,2% at 50-64 years, 4,4% at >65 years; Jacobi et al., 2015). Data from developed countries worldwide show a similar picture at somewhat lower rates (7.0% at 18-34 years, 6.0% at 35-49 years, 5.1% at 50-64 years, 2.6% at >65 years; Kessler et al., 2010). Contrary to the declining prevalence, chronic low-grade inflammation, alterations of the innate immune system and of immune cell composition become more prevalent with aging (Sorgdrager et al., 2019). These changes indicating the aged state of the immune system have been termed immunosenescence. A key component of this process is inflammaging, i.e., the presence of low-grade inflammation with aging by upregulation of pro-inflammatory cytokines, particularly of IL-1, IL-6, and TNFα, and compounds like the enzyme Cox-2 (Chung et al., 2009; Rea et al., 2018). Such signs of inflammaging are found in depression across the age span as described above and in major age-related diseases like atherosclerosis, diabetes, rheumatoid arthritis, cancer, or Alzheimer's disease (Chung et al., 2009; Rea et al., 2018). Another typical characteristic of immunosenescence is an altered composition of the T

cell compartment, especially reduced T naïve cells and increased T memory cells in healthy older adults (>60 years), but not in the younger population (18-44 years; Rodriguez et al., 2021). In MDD, a similar pattern emerges: reduced T naïve cells were found, though the significant difference was driven by the oldest age group (>50 years), and increased T memory cells across age groups emerged as compared to healthy controls (Schiweck et al., 2020b). Thus, immunosenescence is present in earlier ages already leading to the conclusion of an early aging of the immune system in depression.

BMI. The comorbidity between depression and overweight/obesity is a commonly known phenomenon since a considerable prevalence of obesity in depression exists and vice versa. As shown in a meta-analysis, depression was 32% more likely to occur in obese people than in eutrophic people (Pereira-Miranda et al., 2017). Another study found an increasing prevalence of depression among normal weight, overweight, and obese individuals (Carey et al., 2014). A possible critical point is that overweight/obesity may at least partly be due to psychotropic induced weight gain. However, overweight and obesity were already found in drug-naïve first episode MDD patients with considerable rates of 56.0% and 3.7%, respectively (Si et al., 2021). Thus, both conditions are bidirectionally related where both increase the risk for developing the other (Luppino et al., 2010; Milaneschi et al., 2019). Obesity is also considered an inflammatory state where pro-inflammatory cytokines are released from adipose tissue, in particular (Berk et al., 2013). In a sample of male participants, the chance of having developed abdominal obesity over 11 years was 4.28 times more likely when low-grade inflammation and depressive symptoms were present compared to men without these conditions (Valtonen et al., 2012). Similarly, depression and overweight increased the risk of elevated hsCRP levels individually and even more pronounced in combination in a mixed sample (McLaughlin et al., 2021). Therefore, these three characteristics seem to be linked closely.

Metabolic/cardiovascular disease. Like the relationship between depression and BMI, cardiovascular disease and metabolic syndrome are related bidirectionally with depression (Chan et al., 2019; Halaris, 2017). Metabolic syndrome and cardiovascular diseases thereby share the low-grade inflammation pathology with depression (Chan et al., 2019; Silverman & Sternberg, 2012). It is therefore difficult to disentangle the causal relationship in the pathogenesis of this comorbidity. As the involvement of the kynurenine pathway in depression has been demonstrated (see above), the presence of several kynurenine metabolites was also related to cardiovascular disease (Zuo et al., 2016). Further, the cytokine MIF, which has been implicated in depression and

glucocorticoid resistance (see above), has also been functionally related to metabolic and cardiovascular diseases (Grieb et al., 2010; Zernecke et al., 2008). Thus, shared underlying mechanisms of this comorbidity support the clinical relevance of such inflammatory abnormalities also in mental illness.

Smoking. Another related factor in the interplay of depression and inflammation is smoking. In depression, cigarette smoking occurs more frequently than in non-depressed individuals and this was found repeatedly (Berk et al., 2013). Further, it is associated with increased CRP and typical pro-inflammatory cytokines like they are found in depression (Berk et al., 2013), as well as cardiovascular disease (Ambrose & Barua, 2004).

In sum, several factors contribute to inflammation and depression. Most of these characteristics are mechanistically linked to inflammation and are risk factors for the development of depression. Therefore, it seems reasonable to conclude that low-grade immune dysbalances occur before the onset of depression, followed by an exacerbation towards metabolic/cardiovascular diseases through their bidirectional relationship. Thus, it is important to consider such factors when studying biological underpinnings of depression.

3 Interventions

According to the psychological theories of depression, a variety of psychotherapeutic treatments exists with similar efficacy as pharmacotherapy (Cuijpers et al., 2013). As the present work focusses on biological underpinnings in depression, this section illustrates biological therapies, i.e., psychotropic medication. Based on the observation of mood regulating capacities of substances that act on the neurotransmitter system, the monoamine hypothesis of depression was stated (see section "etiological theories") and drug development followed to focus on the neurotransmitter mechanisms (Hirschfeld, 2000; Owens, 2004). Today, selective serotonin reuptake inhibitors (SSRI) are the most prescribed antidepressants across multiple countries worldwide, followed by tricyclic antidepressants (TCA), serotonin-norepinephrine reuptake inhibitors (SNRI), tetracyclic antidepressants (TeCA), serotonin antagonist and reuptake inhibitors (SARI), norepinephrine-dopamine reuptake inhibitors (NDRI), and monoamine oxidase inhibitors (MAO-I) in alternating order (Bauer et al., 2008; Chee et al., 2015; Grover et al., 2013; Noordam et al., 2015; Treviño et al., 2017; Wilkinson & Mulder, 2018). According to the S3-guideline, efficacy of placebo and active antidepressive treatment are hardly distinguishable in mild depression and are therefore not recommended for those patients (Leitliniengruppe Unipolare Depression, 2017). In moderate to severe depression, the difference is more pronounced, where antidepressants exert a superior effect in up to 30% of the most severe cases (Leitliniengruppe Unipolare Depression, 2017). In more detail, studies revealed efficacy for different antidepressant classes as follows: response to SSRIs is 37% and to TCAs 26% more likely than to placebo in primary care (Arroll et al., 2005). Another meta-analysis found response to SSRIs being more likely by 9-29% than to placebo, depending on the dose (Furukawa et al., 2019). Ranges for SNRI (venlafaxine) and TeCA (mirtazapine) were 15-64% and -5-28% (lower efficacy of mirtazapine compared to placebo by 5% up to higher efficacy of mirtazapine compared to placebo by 28%), respectively (Furukawa et al., 2019). When established antidepressants were compared with each other, efficacy was only minimally different with 88% of studies showing no significant difference (Hansen et al., 2005). A head-to-head meta-analysis also found only few differences between antidepressants overall, with considerable variability of effects (Cipriani et al., 2018). Further, the use of placebo mostly accounts for heterogeneity and inconsistency of efficacy trial results (Cipriani et al., 2018). This demonstrates that the overall efficacy of antidepressants is insufficient,

whereat placebo effects are considerably high and variable. Some criticism on studies investigating the superiority of antidepressants over placebo has been raised. On a continuous depression rating scale, some evidence questions the clinical relevance of the score reduction between verum and placebo as this appeared rather small, especially when unpublished data was included (Moncrieff & Kirsch, 2005). No evidence-based cut-off exists defining a clinically relevant depression score reduction difference between verum and placebo (Moncrieff & Kirsch, 2005). Larger differences between verum and placebo groups, as found when response outcome was categorical, can be explained by the creation of a larger gap through dichotomization when score reduction difference between both groups is small and many individuals show a score reduction around the response criterion (Moncrieff & Kirsch, 2005). However, besides pointing to lacking efficacy of antidepressants over placebo, the authors provide no explanation for why a small difference appears at all. Potential factors driving response can be expectancy of a positive treatment effect and a caring therapeutic setting throughout the trial (Rutherford & Roose, 2013), both of which are present independently of treatment condition. Next to the large placebo effect, antidepressant efficacy is unsatisfactory and meta-analytic efficacy results are rather overestimated due to publication bias (Pigott et al., 2010), but still a considerable group of patients profit from such treatment.

Thus, it is necessary to identify factors that differentiate patients responding to antidepressants from patients not responding at all, neither to placebo nor to verum. Therefore, efficacy analyses in subgroups are essential in order to advocate individual treatment choices. Unfortunately, a clear characterization of patients benefitting from certain drugs is lacking in clinical practice, but an algorithm considering sociodemographic and disease history variables seemed promising (DeRubeis et al., 2014; Guideline Development Panel for the Treatment of Depressive Disorders, 2019). The section above about immune profiling in depression was dedicated to elaborating on pro-inflammatory activation and immune deregulations in a respectful subgroup of patients. Such abnormalities have been associated with treatment resistance which occurs in about 22-56% of cases and treatment resistant depression (TRD) is associated with higher comorbidity with cardiovascular disease (CVD) and diabetes type II (Miller, 2010; Rizvi et al., 2014; Souery et al., 2007; Strawbridge et al., 2019; Thomas et al., 2013; Zaninotto et al., 2013). Noteworthy, TRD is defined heterogeneously among such studies which is discussed in the scientific community (Berlim & Turecki, 2007; Rybak et al., 2021; Trevino et al., 2014). According to a Delphi expert consensus, TRD should be

defined by at least two different and failed (<50% symptom reduction) antidepressant treatment trials for 4-6 weeks each with adequate dosage (Rybak et al., 2021). Even though patients do not fulfill these criteria, studies already reveal non-response at earlier stages in depressed patients being associated with inflammation. Alternative treatment trials have been the subject of many studies targeting the immune system to achieve higher response rates. A meta-analysis investigating the pooled efficacy of add-on anti-inflammatory treatment strategies (in addition to standard antidepressant drug regimen) found an improvement of response and remission rates in MDD patients (RR = 1.76 and RR = 2.14, respectively; Köhler-Forsberg et al., 2019). Regarding symptom reduction by drug class, non-steroidal anti-inflammatory drugs (NSAIDs; celecoxib, standard mean difference (SMD) = -0.82), glucocorticoids (SMD = -0.90), and statins (SMD = -0.73) were superior to placebo as add-ons, while cytokine inhibitors (SMD = -0.65), minocycline (SMD = -1.06), and again NSAIDs (SMD = -0.29) were superior to placebo as monotherapy (Köhler-Forsberg et al., 2019). The more recent systematic review and meta-analysis by Bai et al. (2020) confirms these results (SMD = -0.55) and shows pooled superiority also for omega3 fatty acids (SMD = -0.35), as well as for add-on pioglitazone to citalopram in a single trial (95% vs. 40% response rate; Sepanjnia et al., 2012). Last, one study found superior response rate to N-acetylcysteine (36.6%) compared to placebo (25.0%) added to treatment as usual (TAU; Berk et al., 2014). These results seem promising with respect to efficacy as compared to placebo conditions (sole placebo or placebo augmentation to standard treatment), however head-to-head comparisons with other antidepressants are lacking. From the results above it becomes apparent, that anti-inflammatory monotherapy and add-on therapy show small to large effect sizes when compared to placebo conditions which is in about the range of antidepressant efficacy. However, add-on trials at least provide evidence for a potential additional gain of efficacy over TAU. Further, these studies are also affected by heterogeneity. Such shortcomings may be a result of non-stratified group comparisons where the anti-inflammatory mechanism of action is assumed in all subjects, whether they actually have a pro-inflammatory signature or not. Thus, it is only reasonable to apply such agents in depressed patients with inflammation. Köhler-Forsberg et al. (2019) conclude similarly but did not provide any supporting analyses. A systematic review analyzed the predictive capability of circulating pro-inflammatory compounds (CRP, cytokines) for treatment response to different treatment regimen (Arteaga-Henríquez et al., 2019). It seems that depressed patients (major depression and bipolar depression were included) with low

inflammatory state responded better to predominantly serotonergic agents compared to subjects with moderate to high inflammatory state (Arteaga-Henríquez et al., 2019). Further, for predominantly noradrenergic, dopaminergic, glutamatergic, and add-on anti-inflammatory agents the opposite was observed, while the cut-off to classify moderate to high inflammatory state tended to be considerably higher for anti-inflammatory drugs than for the antidepressants (Arteaga-Henríquez et al., 2019). Thus, CRP and pro-inflammatory cytokines may serve as biomarkers for staged treatment regimen choices to improve response rates. Another investigated immune parameter is leukocyte gene expression. Two studies showed that higher TNF, IL-1ß, and MIF gene expression were present in non-responders than responders to SSRI or TCA (Cattaneo et al., 2013; Powell et al., 2013). With regard to lymphocyte counts, non-responders showed higher cytotoxic T cell and reduced NK cell percentages compared to responders to venlafaxine or imipramine (Grosse et al., 2016a). Inconsistent results about lymphocyte counts (see section "immune profiling in depression") may arise from non-stratified analyses, i.e., likely, again only a subgroup of depressed patients is affected and may then exhibit differential response patterns. Though the anti-inflammatory treatment approach seems reasonable and has shown some efficacy, many uncertainties remain. Official treatment guidelines have refrained from incorporating recommendations for anti-inflammatory treatment options so far (Guideline Development Panel for the Treatment of Depressive Disorders, 2019; Leitliniengruppe Unipolare Depression, 2017).

4 Objectives of the original work

MDD still poses a substantial health burden nowadays, one of the reasons being non-sufficient treatment options or undifferentiated treatment allocation. Standard psychotropic therapy arising from the monoamine hypothesis is highly debated due to doubtful efficacy over placebo and considerably high rates of treatment resistant individuals. Through the neuroplasticity/ neurogenesis and kynurenine hypothesis the link of depression pathophysiology to glucocorticoids and inflammatory processes evolved. By now several compounds have been investigated that are dysregulated in depression: low-grade inflammation, characterized by elevated pro-inflammatory cytokines and CRP, and the interlinked HPA dysfunction and activation of the kynurenine pathway, where pro-inflammatory cytokines and glucocorticoids serve as stimulators for disrupted counter-regulatory processes. Such inflammatory processes seem especially prominent in females, in the presence of childhood trauma, and in overweight/obese individuals contributing to cardiovascular risk. Further, signs of immunosenescence (early aging of the immune system) are present in depression. Lymphocyte studies present heterogeneous results but may point to a suppression of adaptive immunity. Further, an upregulation of innate immunity was found, i.e., glucocorticoid resistance and pro-inflammatory upregulation in monocytes, where MIF seems to play an important role. Following these studies and the fact that monocytes are main immune drivers, they should be a prime target of research on biological underpinnings and molecular dysregulations in depression. Gene expression studies allow targeted investigations of monocyte/macrophage profiles and mechanistic pathways but are understudied so far, also with regard to relevant processes beyond the mere pro-inflammatory signature. New treatment options target such inflammatory upregulation, so far also with limited effective results. Some evidence points to an additional effect to TAU, but there is still potential to increase response rates. Thus, these treatment strategies have not found their way into clinical care and treatment guidelines, yet. Though the literature is scarce, the necessity of investigating patient subgroups regarding their immune profile and its relation to treatment responsiveness becomes apparent in order to identify and establish reliable biomarkers.

The here presented clinical studies aim at contributing to a better understanding and uncovering of biological underpinnings of MDD, to identifying distinct disease pathologies, and to predicting treatment response by such distinct profiles. This shall

facilitate developing tailored treatment regimen. In more detail, this work targets two main aspects: 1) The study of monocyte gene expression of pro-inflammatory, cholesterol metabolism, and apoptosis pathways, thereby going beyond the investigation of only the inflammatory machinery. Relevant above mentioned patient characteristics are taken into account in order to characterize subgroups. 2) The study of levels of inflammatory monocyte activation markers determined by MIF, neopterin, and TNFα, thereby stratifying for treatment response to placebo versus an anti-inflammatory agent as add-on to standard SSRI therapy. Thus, biomarker dependent response profiles shall be identified using easy to determine components. The role of other relevant patient characteristics will be explored, also.

5 Summary of the clinical studies

5.1 Publication I (Simon et al., 2021a)

In a cross-sectional observational study, monocyte gene expression was determined in MDD patients and healthy controls (HC) using quantitative-polymerase chain reaction (q-PCR). Inflammation related genes were clearly upregulated only in patients with childhood adversity with reference to HC, which was also associated with a significant downregulation of an important cholesterol pathway gene mevalonate kinase. Meanwhile, apoptosis and other cholesterol pathway related genes, as well as TNF were upregulated regardless of the presence of childhood adversity. Sex differences did not become apparent statistically when women and men were directly compared. However, on a single gene basis women showed more dysregulated gene expression compared to HC than men. The study points to an upregulated monocytic activity, mitochondrial apoptotic dysfunction, and some signs of inflammaging as a general MDD characteristic (immunosenescence), and a full-blown upregulation of inflammatory gene expression after childhood adverse experience. Please refer to the paper in annex I for full information and specifics on the background, methods, results, and discussion of this publication.

5.2 Publication II (Simon et al., 2021b)

In a phase IIa, randomized, placebo-controlled trial, cytokine levels were determined in MDD patients using enzyme-linked immunosorbent assay (ELISA). No significant difference emerged for response or remission rates between the SSRI plus placebo and the SSRI plus celecoxib groups. However, investigating cytokine levels in the four subgroups (response status per treatment arm) indicated treatment resistance to SSRI with higher MIF levels and the need for anti-inflammatory agents in such patients. Further, MIF levels trended to be lower in remitters than non-remitters in both groups after therapy. Neopterin and TNFα did not seem promising as biomarkers for treatment regimen dependent response, but the small sample size must be acknowledged. With regard to patient characteristics, lower response to SSRI was associated with weight loss

and higher age. Please refer to the paper in annex II for full information and specifics on the background, methods, results, and discussion of this publication.

5 Zusammenfassung der klinischen Studien

5.1 Publikation I (Simon et al., 2021a)

In einer Querschnittsbeobachtungsstudie wurden Monozyten Genexpressionen bei Patienten mit Majorer Depression und gesunden Kontrollen (GK) mittels quantitativer Polymerase-Kettenreaktion bestimmt. Entzündungsbezogene Gene zeigten sich nur bei Patienten mit kindlichem Trauma deutlich hochreguliert im Vergleich zu GK, was gleichzeitig mit einer signifikanten Herunterregulierung eines wichtigen Gens im Cholesterin Stoffwechselweg, Mevalonatkinase, assoziiert war. Zusätzlich waren Apoptose-bezogene und andere Gene im Cholesterin Stoffwechselweg, sowie auch TNF unabhängig des Vorhandenseins kindlichen Traumas hochreguliert. Geschlechtsunterschiede wurde statistisch beim direkten Vergleich von Männern und Frauen nicht deutlich. Allerdings zeigten Frauen im Vergleich zu GK mehr dysregulierte Genexpressionen auf Basis einzelner Gene als Männer. Die Studie weist auf eine hochregulierte Monozytenaktivität, apoptotische Dysfunktion der Mitochondrien und Anzeichen von Inflamm-Aging als eine generelle Charakteristik von Majorer Depression (Immunoseneszenz) hin, sowie einer voll ausgeprägten Hochregulierung der entzündlichen Genexpression nach dem Erleben kindlichen Traumas. Für vollständige Informationen und Details zum Hintergrund, den Methoden, den Ergebnissen und der Diskussion dieser Publikation wird auf den angehängten Artikel in Annex I verwiesen.

5.2 Publikation II (Simon et al., 2021b)

In einer Phase IIa, randomisierten Placebo-kontrollierten klinischen Prüfung wurden Zytokinlevel bei Patienten mit Majorer Depression mittels enzymgekoppelten Immunadsorptionstests bestimmt. Es ergab sich kein signifikanter Unterschied zwischen den SSRI plus Placebo und SSRI plus Celecoxib Gruppen bezüglich der Response- und Remissionsraten. Allerdings zeigte sich bei der Untersuchung der Zytokinlevel in den vier Subgruppen (Responsestatus je Behandlungsarm), dass höhere MIF Level auf eine Behandlungsresistenz mit SSRI hinweisen und damit die Notwendigkeit antientzündlicher Substanzen bei solchen Patienten andeuten. Weiterhin gab es eine Tendenz zu niedrigeren MIF Leveln bei Remittern als bei Nicht-Remittern in beiden

Gruppen nach Behandlung. Neopterin und TNFα erschienen nicht erfolgversprechend als Biomarker für behandlungsabhängige Response, aber die kleine Stichprobengröße muss eingeräumt werden. Bezüglich der Patientencharakteristika zeigte sich Assoziation von niedrigerer Response auf SSRI mit Gewichtsverlust und höherem Alter. Für vollständige Informationen und Details zum Hintergrund, den Methoden, den Ergebnissen und der Diskussion dieser Publikation wird auf den angehängten Artikel in Annex II verwiesen.

6 Discussion

The clinical studies show relevant dysregulations in monocytes of MDD patients which are not restricted to inflammation only, as well as point to MIF as potential biomarker for treatment resistance to SSRI. The study results are discussed in detail in the respective publications (see Annex I and II) and are placed into a broader context here, thereby considering different cross-links in the field and future perspectives (also see figure 1).

6.1 Inflammation

Complementing previous studies on inflammatory activation in depression, the present work shows and confirms such activation in monocytes in a subgroup of patients (Schiweck et al., 2020a; Simon et al., 2021a). In previous studies, MDD patients did not exhibit a significantly higher inflammatory monocyte gene expression than HC per se but showed an upregulated expression in interaction with age (Grosse et al., 2015). Thus, aging processes of innate immune cells are more pronounced in MDD (Grosse et al., 2015). Another recent study investigating multiple parameters characterized an inflamed subgroup of 39% of depressed patients with increased CRP and IL-6 levels, increased monocyte counts, and higher depression severity, amongst others (Lynall et al., 2020). According to the results of the own original work, this subgroup has likely experienced childhood adversity, too (Simon et al., 2021a). One study on monocyte gene expression in subjects with childhood adversity revealed increased pro-inflammatory signaling/ greater activity of the transcription factor NF-κB (Schwaiger et al., 2016). Regarding single genes, a more upregulated expression of IL-6, CC-chemokine ligand (CCL) 7, dual specificity phosphatase (DUSP2), MAF BZIP Transcription Factor F (MAFF), early growth response protein (EGR) 1, and EGR2 was found in the adversity group compared to the non-adversity group from pre to post acute stress, all of which were also upregulated in MDD patients in the own original work in the inflammatory and growth gene clusters, and some of those especially in the childhood adversity group (Schwaiger et al., 2016; Simon et al., 2021a). In fact, overexpression of EGR2 was associated with higher pro-inflammatory markers (TNF, IL-6, IL-1β, Cox-2) in macrophages in mice (Veremeyko et al., 2018). Recently, childhood adversity was suggested as an accelerator of inflammaging and immunosenescence (Merz & Turner, 2021). The own original work

also showed that patients without childhood adversity had some signs of low-grade inflammation already, especially the TNF gene was expressed in MDD patients regardless of the presence of childhood adversity (Simon et al., 2021a). In line with this, TNF production of monocytes in response to lipopolysaccharides (LPS) stimulation was related to depression and obesity, and this was mechanistically linked to a diminished suppressive activity of cortisol (Cheng et al., 2016). Noteworthy, next to the patient subgroup with upregulated inflammatory monocyte gene expression, another subgroup with an overall downregulated signature exists (Schiweck et al., 2020a). These observations seem to fit within the concept of trained innate immunity and innate tolerance, where either an increased inflammatory upregulation (long-lasting pro-inflammatory phenotype) or a downregulation after second stimulation present two distinct phenotypes of innate immune reactivity, e.g., driven by microbial stimuli, LPS, or oxidized low density lipoprotein (oxLDL; Bekkering et al., 2021). These phenotypes have been studied particularly in monocytes and seem to develop through metabolic and epigenetic reprogramming (Bekkering et al., 2021). Other adaptive programs in innate immune cells are differentiation, i.e., the long-term change of functional cell program, and priming, i.e., a residual activation level remains between first and second stimulation leading to additive or synergistic effects (Divangahi et al., 2021). Though it has been shown that trained immunity is associated with chronic low-grade inflammation, it is questionable which adaptive program is present in depression (Zhong et al., 2020). The cross-sectional data from publication I show the current state but are not suitable to demonstrate temporary fluctuations (Simon et al., 2021a). This should be the subject of future longitudinal studies.

6.2 Mitochondrial dysfunction

Next to the pro-inflammatory signature, increased apoptosis markers were found in the own original work which point to the involvement of mitochondria in such pathophysiological processes (Simon et al., 2021a). Among others, mitochondria have a regulating role for reactive oxygen species (ROS) production and apoptosis (Visentin et al., 2020). The kynurenine pathway seems to be involved in the molecular regulation of mitochondrial dysfunction: Tryptophan metabolites (quinolinic acid) along the neurotoxic kynurenine pathway stimulate high influx of calcium into mitochondria

leading to mitochondrial dysfunction and subsequently to ROS production and inflammasome activation, in turn leading to pro-inflammatory cytokine output (IL-1β, TNFα) further stimulating the kynurenine pathway (IL-1β) via IDO and TDO activation (Visentin et al., 2020). Within this process, in macrophages the inflammasome is activated by oxidized mitochondrial deoxyribonucleic acid (DNA), which is released during apoptosis (Shimada et al., 2012). Thus, aging of the immune system, and especially macrophages, is also reflected by mitochondrial dysfunction due to its contribution to cytokine output and because mitochondrial dysfunction seems to be a key component of ageing in general (Conte et al., 2020; Yarbro et al., 2020). Further, it seems likely that processes within the concepts of trained immunity and immune tolerance play a role for dysfunction of ageing macrophages (Yarbro et al., 2020). This supports the hypothesis of early ageing of the immune system in depression as indicated by the upregulation of pro-apoptotic and pro-inflammatory markers (gene expressions) obtained from MDD patients, especially with childhood adversity, in the own original work (see figure 2 and figure 4 in Simon et al., 2021a). This also demonstrates the neurotoxic potential of kynurenine metabolites given the assumption that these mechanisms are similar in brain cells. Quinolinic acid seems especially relevant in depression and its production has been suggested to mainly originate from macrophages and microglia (Steiner et al., 2012). This also makes the use of NMDA antagonist ketamine plausible as treatment option in depression to counterregulate the NMDA agonistic action of quinolinic acid (Steiner et al., 2012). Last, the role of glucocorticoids for mitochondrial apoptosis has been discussed diversely. Some findings suggest that in proinflammatory monocytes apoptosis is promoted by glucocorticoids, while others suggest anti-apoptotic effects to facilitate cell survival and reduction of inflammation (Achuthan et al., 2018; Desgeorges et al., 2019). These contradictory findings may resemble mechanisms related to different macrophage phenotypes, M1 and M2 respectively. Another possible explanation is that mitochondrial apoptosis is inhibited in case of acute glucocorticoid exposure but is promoted (amongst ROS production) by a longer and chronic exposure (Bansal & Kuhad, 2016). This idea fits with the literature described in the introduction regarding the disturbed counterregulatory processes between glucocorticoids and inflammatory levels.

6.3 Cholesterol

Cholesterol is the precursor of the oxysterols 7-ketocholesterol (7-KC) and 7β-hydroxycholesterol (7β-HC) which are essentially contained in oxLDL (non-enzymatic production) and are thought to play a role for oxidative stress and apoptosis, mediated through ox-LDL, in macrophages (Arnal-Levron et al., 2013). Further, the oxysterols 25-hydroxycholesterol (25-HC) and 27-hydroxycholesterol (27-HC) are produced intracellularly via the enzymatic route through oxLDL, of which 25-HC was also associated with apoptosis (Arnal-Levron et al., 2013; Shibata & Glass, 2010). Upon LPS stimulation, 25-HC and 27-HC are decreased, while 7-KC and 7-HC are increased (the latter formed by ROS; Mutemberezi et al., 2018). Upon oxLDL stimulation however, all those oxysterols were increased, though 25-HC and 27-HC much slightlier (Arnal-Levron et al., 2013). OxLDL is rather considered atherogenic (intracellular cholesterol accumulation), while 27-HC leads to cholesterol efflux by LXR-activation, which stimulates the cholesterol transporters ATP-binding cassette 1 (ABCA1) and ATP-binding cassette sub-family G member 1 (ABCG1; A-González & Castrillo, 2011; Arnal-Levron et al., 2013). Thus, provided a pro-inflammatory/ atherogenic stimulation, an overall less activation of cholesterol efflux related genes and more apoptosis related genes should be expressed. However, in MDD patients from the own original work both one of the liver X receptor (LXR) genes (nuclear receptor subfamily 1 group H member (NR1H3)) and the cholesterol pump genes (ABCA1 and ABCG1) were clearly upregulated indicating such a stimulated cholesterol efflux, and apoptosis-related gene expression was upregulated simultaneously (Simon et al., 2021a). After stimulation of LXRs by LXR agonists LPS, TNFα, or IL-1β some inflammatory genes such as inducible nitric oxide synthase (iNOS), Cox-2, PGE2, IL-6, IL-1β, monocyte chemoattractant protein (MCP)-1 and MCP-3, matrix metalloproteinase (MMP) 9, and TNFα are inhibited via NF-κB and activating protein 1 (AP1) mediation, whereby the downregulation of TNFα after LPS stimulation was directly linked to 25-HC, 27-HC, and 7β-HC, for example, and inflammation in general was reduced by 25-HC also independently of LXR (A-Gonzáles & Castrillo, 2011; Englund et al., 2001; Guillem-Llobat & Íñiguez, 2015; Kim et al., 2006; Schulman, 2017). Indeed, the LXR-mediated ABCA1 upregulation is a prerequisite for inflammatory inhibition by LXR-agonists (Schulman, 2017). Further, LXR activation seems to inhibit apoptosis of macrophages after infection/ inflammatory stimulation, which is also represented in an inhibition of pro-apoptotic factor EGR1 by

LXR activation (A-González & Castrillo, 2011; Guillem-Llobat & Íñiguez, 2015). The other way round, mitochondria are involved in the regulation of ABCA1-mediated cholesterol efflux (Karunakaran et al., 2015). Thus, given the above described lack of LXR stimulation upon pro-inflammatory/ atherogenic stimulation, pro-inflammatory compounds should not be inhibited and apoptotic markers should be present. Many of those pro-inflammatory and pro-apoptotic compounds were also not repressed in MDD patients from the own original work, but LXR activation was present nevertheless, thus showing the regulatory disturbance in depression (Simon et al., 2021a). A suggested potential mechanism may be that LXR activation may stimulate TNFα rather exclusively for it to stimulate apoptosis and thus reduce atherosclerotic lesion size (Millat et al., 2003). Indeed, in the own original work MDD patients without history of childhood adversity show such signature (Simon et al., 2021a). Further, though 27-HC is described as an LXR activator, it apparently also has the capability of inducing pro-inflammatory components (CCL2 and MMP9) in monocytes upon LPS stimulation (Kim et al., 2015). Interestingly, this LXR upregulation and subsequent suppression of the pro-inflammatory pathway is specific to the anti-inflammatory M2 macrophage phenotype, while pro-inflammatory M1 macrophages express Cox-2 and iNOS, and subsequently pro-inflammatory cytokines like TNFα, IL1-β, and IL-6 (Viola et al., 2019). In the own original work, it was hypothesized to have found the anti-inflammatory M(Hb) macrophage phenotype, which partly expresses similar markers like the widely known anti-inflammatory M2 macrophages (Finn et al., 2012; Simon et al., 2021a). However, the macrophages from the sample of the own original work also expressed pro-inflammatory genes (especially with presence of childhood adversity), thus suggesting a somewhat peculiar mixed inflammatory/ senescent M(Hb) macrophage type (Simon et al., 2021a). Such mixed macrophage phenotypes (M1/ M2) are known to exist in non-infectious pathologies like atherosclerosis, obesity, or in microglia in neurological diseases (A-González & Castrillo, 2011). Furthermore, a recent publication suggests that M2-like macrophages can be pro-inflammatory with signs of inflammaging, which may be driven by mitochondrial dysfunction and endoplasmatic reticulum stress (van Beek et al., 2019). This again demonstrates the possibility of mixed macrophages phenotypes.

Furthermore, it is interesting in this context that the glucocorticoid receptor (GR) also seems to be involved in the regulation of cholesterol efflux through ABCA1 in macrophages in that ABCA1 expression is GR regulated, independently from LXR-related mechanisms, i.e., ABCA1 expression was decreased by a GR agonist (Ayaori et

al., 2006). With regards to the described glucocorticoid resistance in depression, a failure of this GR-mediated ABCA1-inhibiting mechanism may additionally contribute to the high ABCA1 expression found in the MDD sample from the own original work, while also GRα and GRβ expressions were not differentially regulated as in healthy controls (Simon et al., 2021a).

6.4 Mevalonate kinase deficiency

One focus of the discussion of Simon et al. (2021a) is the observation of a downregulated mevalonate kinase (MVK) gene expression. Accumulation of mevalonate due to mevalonate kinase deficiency (MVKD) is thought to promote inflammation, trained immunity, mitochondrial dysfunction, and apoptosis/pyroptosis (Bekkering et al., 2018; Simon et al., 2021a; Tricarico et al., 2013, 2015). Interestingly, LXR activation also seems to contribute to trained immunity in macrophages (Ferreira et al., 2022). Under pro-inflammatory stimulation of already activated LXR, an increased trained immune response and induction of inflammatory activation on the long term was observed, whereby higher acetyl-CoA levels were present after LXR activation and LXR training depended on the presence of IL-1β and mevalonate pathway activity (Sohrabi et al., 2020). In line with this, caspase-1 and inflammasome (NLR family pyrin domain containing 3 (NLRP3)) expressions were induced by LXR agonists, which also points to subsequent pyroptotic (programmed cell death) processes that are caspase-1 dependent (Sohrabi et al., 2020; Tricarico et al., 2013). Molecularly, markers of epigenetic and metabolic reprogramming were found (Sohrabi et al., 2020). Thus, LXR activation has not only anti-inflammatory/anti-atherogenic capacities, but also pro-inflammatory ones and promotes trained immunity and subsequently cell death. It is unclear however, how these two contrary processes can exist simultaneously. Maybe, multiple types of LXR agonists can exert multiple reactions at the same time. Again, this phenomenon can be seen in the monocytes of MDD patients of the own original work, especially in the ones with childhood adversity (Simon et al., 2021a). A related peculiar phenomenon is the cholesterol flow itself: since mevalonate is a precursor of downstream cholesterol, this downstream pathway is blocked by MVKD (Tricarico et al., 2013). Thus, less cholesterol is synthesized. On the other hand, due to activation of LXR and the cholesterol transporters, cholesterol is pumped out of the cell at the same time (Simon et al., 2021a).

With respect to the aim of obtaining homeostasis, it seems reasonable that with higher pro-inflammatory burden due to MVKD, the cell engages in anti-inflammatory action. However, it is questionable whether the cell is acting against an intracellular cholesterol accumulation from a different source. In any way, accumulation of free cholesterol also has the capacity to induce inflammasome and IL-1β production (Ho et al., 2019). The blockage of mevalonate synthesis (and therefore also cholesterol synthesis) by statins has anti-inflammatory effects and prevents trained immunity (Bekkering et al., 2018).

6.5 MIF

MIF seems to play an important role in the inflammatory machinery: 1) MIF upregulates PGE2 and Cox-2, among others (Calandra & Roger, 2003). Interestingly, PGE2 production and Cox-2 activity are increased in aged macrophages in murine models, thus indicating immunosenescence (van Beek et al., 2019). 2) MIF is involved in the inhibition of the extrinsic apoptosis pathway, but also in cell growth and intrinsic apoptosis (Calandra & Roger, 2003). 3) MIF inhibits the inhibitory function of glucocorticoids on NF-κB-mediated inflammation and on arachidonic acid (AA; a precursor of PGE2) production, among others (Calandra & Roger, 2003). 4) MIF seems to be an important and specific regulator for inflammasome activation and inflammasome-dependent release of IL-1β and interleukin-18 (IL-18; Lang et al., 2018). 5) It has been suggested that MIF release due to oxLDL in turn stimulates further uptake of oxLDL in macrophages (Atsumi et al., 2000). Thus, MIF is an inflammatory stimulator and directly involved in the dysfunctional regulation between glucocorticoids and cytokines (see also section "immune profiling in depression"), but also may contribute to different ox-LDL mediated effects. Further, normal MIF levels contribute to macrophage apoptosis, where MIF depletion leads to aggravated apoptosis (Mitchell et al., 2002). On the other hand, during oxLDL-induced apoptosis, MIF expression was increased in plaque macrophages (Schlittenhardt et al., 2005). Indeed, MIF has been associated with mitochondrial dysfunction, where increased mitochondrial ROS led to high MIF production in macrophages after stimulation by LPS (Lee et al., 2016; Xu et al., 2020). High MIF levels may thus further aggravate apoptotic processes due to activation of apoptosis-stimulating cytokines (if even indirectly mediated through certain kynurenine metabolites; see section "immune profiling in depression"). From this, it appears as though the MIF-apoptosis-

relationship may follow an inverted u-shaped function. However, it is important to note, that MIF seems to exert different effects in other cell types, for example protects neutrophils from apoptosis (Baumann et al., 2003).

6.6 Sex differences

The role of sex differences becomes increasingly important. In the own original work, somewhat more upregulated inflammatory and cholesterol pathway related gene expression in MDD women than MDD men was observed; however, missing significances of single genes may also be an issue to power (Simon et al., 2021a). Further, higher MIF levels were found in men compared to women (Simon et al., 2021b). Thus, it seems difficult to deduce a general pattern taking different compounds into account. Previous literature describes a tendency of higher M1-polarization and reduced M2-polarization of macrophages in women with MDD compared to male counterparts (Cosma et al., 2021). Another study found that women do not express higher inflammation per se, but show a higher depressed reaction to it, thus making them more susceptible to depressed symptoms upon inflammation than men (Derry et al., 2015; Moulton et al., 2019). In contrast, men are more susceptible to inflammation-related metabolic disturbances than women (Goel et al., 2014). Thus, is seems that women and men may develop differential subsequent health problems after stress and inflammatory activation. Regarding apoptosis, women seem to be more protected from mitochondrial dysfunction than men due to estrogens (Shimamoto & Rappeneau, 2017). Since mitochondrial DNA is maternally inherited, its constitution seems rather favorable for women than for men (less ROS production, less cell death; Ventura-Clapier et al., 2017). However, there may be a deficit of estrogen in depression, thus pushing women towards the “male constitution” (Shimamoto & Rappeneau, 2017). In general, little research has been carried out establishing sex differences in the immuno-metabolic pathways of depressed patients. Presumably, they may play an even larger role than previously suspected, and their investigation must be promoted in future research.

6.7 Brain

The own original work is limited to markers measured in the periphery (Simon et al., 2021a; Simon et al., 2021b). Nevertheless, those were associated with depression as a disease thought to originate from neurotransmitter imbalances in the brain (Simon et al., 2021a; Simon et al., 2021b). Indeed, peripheral cytokines can enter the central nervous system and affect neurotransmitter metabolism of serotonin, dopamine, and glutamate (Visentin et al., 2020). Further, the pathway of dopamine synthesis can be inhibited by ROS and nitric oxide (NO) resulting from inflammation (Felger, 2017). Apparently also monocytes can infiltrate the brain and be recruited to brain sites upon microglia activation (Medina-Rodriguez et al., 2018). One suggested mechanism is an increased permeability of the blood-brain-barrier through microglia released cytokines, that allows for immune cell infiltration and infiltration of other inflammatory molecules (Medina-Rodriguez et al., 2018). Microglial cells represent the neuronal counterpart of macrophages and gain macrophage-like activity upon activation, such as secretion of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β; Tricarico et al., 2013). Further, a trained phenotype had also been found in microglia (Bekkering et al., 2021; Miura et al., 2008). Indeed, microglial activation has been found in brain regions involved in emotion regulation in MDD (Medina-Rodriguez et al., 2018). Further, activation of microglia after stress leads to an induction of the kynurenine pathway, thus resulting in a higher quinolinic acid production (Jo et al., 2015; Steiner et al., 2011). Kynurenine can cross the blood brain barrier, being then metabolized into quinolinic acid by microglia (via cytokine-activated IDO), among others (Beumer et al., 2012; Savitz, 2020). If quinolinic acid acts similar in microglia like in monocytes, mitochondrial dysfunction and apoptosis may also be induced in these brain cells (see section “mitochondrial dysfunction”). Some support for this hypothesis comes from a study in mice, where chronic stress decreased the number of microglial cells in the hippocampus after a phase of activation (Kreisel et al., 2014). Additional to the production of quinolinic acid by microglia, infiltrating macrophages can produce quinolinic acid even many times more than microglia (Espey et al., 1997). With respect to the monoamines, not only a lack of serotonin due to kynurenine pathway activation, but also a higher monoamine degradation by monoamine oxidase A and B may be a relevant process. Both monoamine oxidases are located in the mitochondrial membrane and their hyperfunction leads to oxidative stress and thus mitochondrial dysfunction (Petschner et al., 2018). In turn, mitochondrial dysfunction has been

associated with decreased neuronal transmission, as well as impaired neurogenesis and synaptic plasticity (Bansal & Kuhad, 2016).

With respect to the above mentioned considerations about the disturbed mevalonate/cholesterol pathway, imbalances in cholesterol homeostasis can also be present in the cental nervous system (CNS) and often co-occur with neurodegenerative diseases (Tricarico et al., 2013). A reduction of cholesterol levels seems to affect the synaptic transmission and an intrinsic blockade of the mevalonate pathway during brain development affects the growth of the physiological neuronal network (Tricarico et al., 2013). Patients with an inherited severe MVK deficiency (hyperimmunoglobulinemia D syndrome (HIDS) patients) show mental disabilities and phases of high autoinflammatory activation (Hoffmann et al., 1993; Ticarico et al., 2013). Most cholesterol is needed for brain functioning; thus, its abundance leads to dysfunction (Tonini et al., 2020). Cholesterol cannot pass the blood brain barrier, so all cholesterol in the brain is synthesized locally via the mevalonate pathway (Tonini et al., 2020). Assuming that the gene expression profile and mechanisms are similar in the brain, as described before in the periphery, constraining the mevalonate/cholesterol pathway through mevalonate kinase deficiency leads to neurotoxic mevalonate accumulation and a lack of cholesterol needed for functioning. Overall, literature investigating the central molecular mechanisms of depression regarding the compounds identified in the periphery and discussed in this paper is very sparse.

6.8 Psychological aspects

On the level of psychiatric symptomatology two relevant subtypes of depression have been identified, next to a third less specified group, which represent different symptom clusters within the diagnostic criteria: the atypical and the melancholic types (Lamers et al., 2010). Both subtypes are severely depressed while the melancholic patients explicitly showed decreased appetite and weight loss, as well as insomnia, worse morning mood and early morning awakening, and lack of responsiveness (Lamers et al., 2010). In contrast, the atypical patients explicitly showed increased appetite and weight gain, as well as leaden paralysis (Lamers et al., 2010). Thus, the major parameters to distinguish these subtypes are rather metabolic factors, other than just the mere psychological mood symptoms. Therefore, it is questionable whether these subtypes are of psychological

nature after all, e.g., demonstrating different emotion regulation strategies which then lead to metabolic alterations, or whether such metabolic alterations are part of the pathogenesis of depression already. Anyhow, additional characteristics of the melancholic patients were being a smoker and having a history of childhood adversity, while additional characteristics of atypical patients were being female, having a higher BMI, and a higher prevalence of metabolic syndrome (Lamers et al., 2010). Interestingly, not only metabolic dysregulations, but also inflammatory activation (as measured by pro-inflammatory cytokines) and higher kynurenine, quinolinic acid, but also tryptophan levels were present in the atypical subgroup, while HPA hyperactivity was present in the metabolic subgroup which is the larger proportion of patients than the atypical subgroup (Lamers et al., 2010, 2013; Milaneschi et al., 2021). Thus, the atypical subtype is considered the immune-metabolic depression (Lamers et al., 2020). This is somewhat conflicting with the developed model in the present work where summarized literature rather suggests that all these processes are intertwined and thus should be present in the same individuals.

Referring to the vulnerability-stress-model (Wittchen & Hoyer, 2006), that is commonly used to explain disease development and maintenance in psychological contexts, the complexity and variety of biological factors in that model now becomes very striking. However, it is still difficult to clearly place those as vulnerability or maintenance factors. Likely, they should be considered as both, assuming reciprocally reinforcing processes. Thereby, it is important to mention that other factors like sex-specific and experience-related (childhood adversity) vulnerabilities have molecular biological correlates as demonstrated in the respective paragraphs above. Thus, risk factors/ vulnerabilities should maybe not be separated into psychological, social, and biological, but are rather to be understood on different phenomenological levels.

6.9 Treatment response

As outlined in the introduction, the use of anti-inflammatory treatment options seems promising but previous research is liable to limitations such as investigating non-stratified patient subgroups regarding their inflammatory profile. Additionally, a variety of dysregulated immune components has been established raising the question which is the best target.

Even antidepressants have some anti-inflammatory capability, but this seems limited to patients that respond clinically (Visentin et al., 2020). However, higher inflammatory levels are associated with a higher probability of non-response to antidepressants (Strawbridge et al., 2015). Molecularly, increased IDO1 gene expression after LPS and interferon-alpha (IFN-α) stimulation could not be modulated by monoaminergic antidepressants (Regan et al., 2018). These findings make it difficult to draw conclusions about the level of inflammation that needs to be present to distinguish antidepressant versus anti-inflammatory drug responders. At least, pro-inflammatory cytokines are considered promising biomarkers for determining the level of inflammation in depressed patients (Visentin et al., 2020). For example, one study investigated cytokine messenger ribonucleic acid (mRNA) levels and found that among antidepressant non-responders MIF, TNFα, and IL-1β mRNA were at highest levels (Cattaneo et al., 2013). In the own original work, MIF (measured as the circulating cytokine) also stood out as a predictor of non-response to SSRI (Simon et al., 2021b). With respect to the above described mechanisms of MIF and Cox-2 in the inflammatory machinery and its involvement in mitochondrial dysfunction, celecoxib has neuroprotective properties as it restores mitochondrial function (Hunter et al., 2007). Indeed, MIF has been targeted for other human inflammatory diseases with promising treatment outcomes and thus may also be a relevant target in depression (Günther et al., 2019). However, cytokines are the most investigated compounds for response prediction and other parameters might arise increasingly. For example, SSRI non-responders were characterized by lower brain-derived neurotrophic factor (BDNF) levels, an important protein for neurodevelopment and neurogenesis, and shorter leukocyte telomere length as compared to SSRI responders (Hough et al., 2016; Wolkowitz et al., 2011). Other examples can be found in section "interventions".

Another treatment option has been the focus of efficacy studies: the NMDA antagonist ketamine. As mentioned above, ketamine can counterregulate the quinolinic acid – apoptosis/inflammation – cycle and has therefore anti-inflammatory capacities, next to its clinical antidepressant effect (see section "mitochondrial dysfunction"; Chen et al., 2018). Other potential agents that have gained some attention for their antidepressant effect are statins (Bai et al., 2020). Those may exert indirect anti-inflammatory effects by blocking the mevalonate pathway before mevalonate is synthesized and thus it cannot accumulate and induce trained immunity (Bekkering et al., 2018). Those drugs should be counted within the frame of alternative anti-inflammatory treatment options for depression.

Next to the immune compounds that can be used to predict treatment response, clinical subtypes have also been discussed as response predictors. One study found that, among others, symptoms characterizing atypical depression are predictive of poorer treatment success with an SNRI (Howland et al., 2008). On the contrary, atypical depression was not associated with outcome in response to escitalopram (SSRI) or nortriptyline (TCA), while melancholic depression was related to a slightly worse response to escitalopram (Uher et al., 2011). Also, childhood abuse (especially until the age of 7) predicted poorer response to antidepressants (sertraline, escitalopram, and venlafaxine; Williams et al., 2016). In line, a very recent study found that patients with major depressive disorder without adverse childhood experience had a significantly higher response rate than with adverse childhood experience (Yang et al., 2021). Considering the results from the own original work, a subtype of depression with childhood adversity is characterized by stronger inflammatory activation than MDD patients without childhood adversity, thus underlining the hypothesis of treatment resistance to antidepressants in those patients (Simon et al., 2021a).

An important question is whether anti-inflammatory agents should be used as single agents instead of antidepressants in MDD patients with inflammatory signature, or whether they should be added to an antidepressant regimen. So far, anti-inflammatory drugs have mostly been investigated as add-on therapy, like in the own original work (Simon et al., 2021b). To answer this question, it is necessary to establish whether two distinct subgroups exist (elevated inflammation vs normal levels) or whether those levels are rather gradual. Antidepressants were shown to have some anti-inflammatory capacity (e.g., SSRIs downregulate IL-1β, attenuation of cytokine release from microglia, sensitization of HPA glucocorticoid receptors) but seem to elicit better response rates in patients considered not having elevated inflammatory signature (Arteaga-Henríquez et al., 2019; Leonard, 2014; Więdłocha et al., 2018). Thus, a very mild anti-inflammatory effect may be advantageous in general and can be boosted by an anti-inflammatory agent in patients with elevated inflammatory signature. This also fits within the elaboration above (see section “brain”) about the intertwined mechanisms of inflammation and neurotransmission. An add-on regimen increases neurotransmission and downregulates the stimulators, i.e., inflammatory markers, of its dysfunction. Noteworthy, so far, the reason for carrying out add-on studies mainly lies in ethical aspects under the consideration that antidepressants are available and a mere placebo exposure may withhold necessary treatment from patients.

7 Limitations

This section covers some rather general limitations to this research field and research methods. Specific limitations to the results from the own original work are described in the respective publications (Simon et al., 2021a; Simon et al., 2021b).

Researching gene expression profiles, like in monocytes/ macrophages, it is difficult to determine whether these profiles originate from the same monocyte subpopulation or whether different cell types are analyzed at once. Cluster analyses at least provide a hint on the relationship of different molecular pathways but considering the existence of patient subgroups and different macrophage polarizations it is possible to obtain a biased picture. The molecular mechanisms and pathways as described in the introduction and discussion sections, and as depicted in figure 1 are assembled from the own original work and other study results, as well as literature reviews from multiple specialized fields and thus are partly investigated in other cell types than monocytes/macrophages. Mechanisms and regulatory cycles are therefore partly assumed to be similar in this cell type. Future studies should investigate patient subgroups and different biomarkers like inflammatory cytokines, monocyte gene expression, and tryptophan/kynurenine levels in an integrative approach. Another assumption in this research field is that the measurement of peripheral markers is somehow linked to pathological processes in the brain and that they may even be similar in the brain like in the periphery. At least a couple of studies provide some evidence in favor of this assumption (see section “brain”). Up until now, the causal pathophysiological pathway of dysregulations of different immuno-metabolic and stress-associated systems remains an object to speculation. Those interacting systems may change simultaneously, but initial dysregulations and their cause are not clarified. 40-60% of depressed patients show HPA dysfunction while a smaller percentage shows chronic low-grade inflammation (about one-third; Bloom & Al-Abed, 2014; Osimo et al., 2019; Raison et al., 2006). Thus, is seems possible that HPA and glucocorticoid dysregulations occur first due to chronic elevated stress experience with following increasing inflammatory levels, which then leads to a vicious circle. One major limitation to such studies and possible reason for lack of this knowledge is the bias by different time of disease until diagnoses or help seeking by patients. Further, diverse study results exist on the question whether depression or inflammation precede each other. Some studies point to low-grade inflammation as a risk factor for depression, others point to depressive symptoms as a predictor of low-grade inflammation even in healthy individuals (Franco

et al., 2017; Janssen et al., 2021). Thus, treatment choices can be guided by present symptoms or measurable inflammatory markers, but preventive measures are difficult to establish with uncertainty about causal pathways.

Regarding treatment response research, most studies used the Hamilton Rating Scale for Depression (HAMD) as an outcome measure in efficacy studies. In the own original work, the Montgomery–Åsberg Depression Rating Scale (MADRS) was used which may limit the comparability to previous study results (Simon et al., 2021b). However, MADRS was shown to be as good as HAMD to measure response to antidepressants (Hengartner et al., 2020). The large and variable placebo response makes it difficult to interpret clinical relevance of superiority in placebo-controlled studies, even if they show statistical significance (Cipriani et al., 2018; Moncrieff & Kirsch, 2005). A relapse rate of 50% for antidepressant treatment was reported, while this rate was at 33% for recovered patients after placebo (Kirsch, 2019). Similarly, the relapse rate after cognitive behavior therapy was at 36% (Kirsch, 2019). This data questions whether a substantial overlap between those treatment options exists due to mere placebo response, or whether different patient subgroups can be characterized which are responsive and prone to relapse to the one or the other treatment. In add-on studies, the superiority of anti-inflammatory augmentation over placebo plus standard therapy becomes apparent, but still the benefit over placebo remains unclear in this study design. Further, interactions between standard therapy and anti-inflammatory add-on agents may be present, so that it is not possible to determine the gain in efficacy just by the sole anti-inflammatory agent. A head-to-head trial comparing a single antidepressant with a single anti-inflammatory agent could allow for such conclusions and, if results are positive, would avoid multiple drug regimen. Another aspect is the dependency between outcome and length of treatment duration. The study designs are laid-out for antidepressive treatment duration but not necessarily for the optimal duration of anti-inflammatory treatment. Thereby, whether this should be based on an acute or chronic form of inflammation remains an open question, even though literature suggests a chronic low-grade inflammatory state. Additionally, as mentioned before, studies did not stratify patients by their inflammatory status beforehand. Such stratification would allow to randomize patients with and without inflammatory signature to (add-on) anti-inflammatory treatment versus standard therapy in groups of same size to allow for comparisons between four study arms. However, there are no established biomarkers yet to define inflammatory status. This may be due to lacking comparative studies investigating and ranking the validity of different biomarkers and lacking cut-offs

to define inflammation positive and inflammation negative cases. Anyhow, it may also be possible that the inflammatory level should be treated as a continuous variable where no clear cut-offs can be determined. This would limit the interpretation of clinical relevance and usefulness for clinical application. Another issue is the lacking assay standardization, which again limits the choice of biomarkers and their cut-off levels (Visentin et al., 2020).

The present work does not consider other factors, like exercise, diet, sleep, gut microbiome, that contribute to inflammation and can offer alternative treatment approaches from nutritional supplementation to lifestyle behaviors (Berk et al., 2013). Further, sex differences occurred in the own original work to some extent, but very obvious and distinct patterns did not emerge (Simon et al., 2021a; Simon et al., 2021b). A complication when investigating sex effects on inflammation and depression is that hormonal levels change over lifespan and depend on pregnancy and the use of hormonal contraceptives (Mattina et al., 2019). Anyhow, sex differences should also be subject of future studies to elucidate and clarify their relevance. Possibly, different treatment approaches should then be considered.

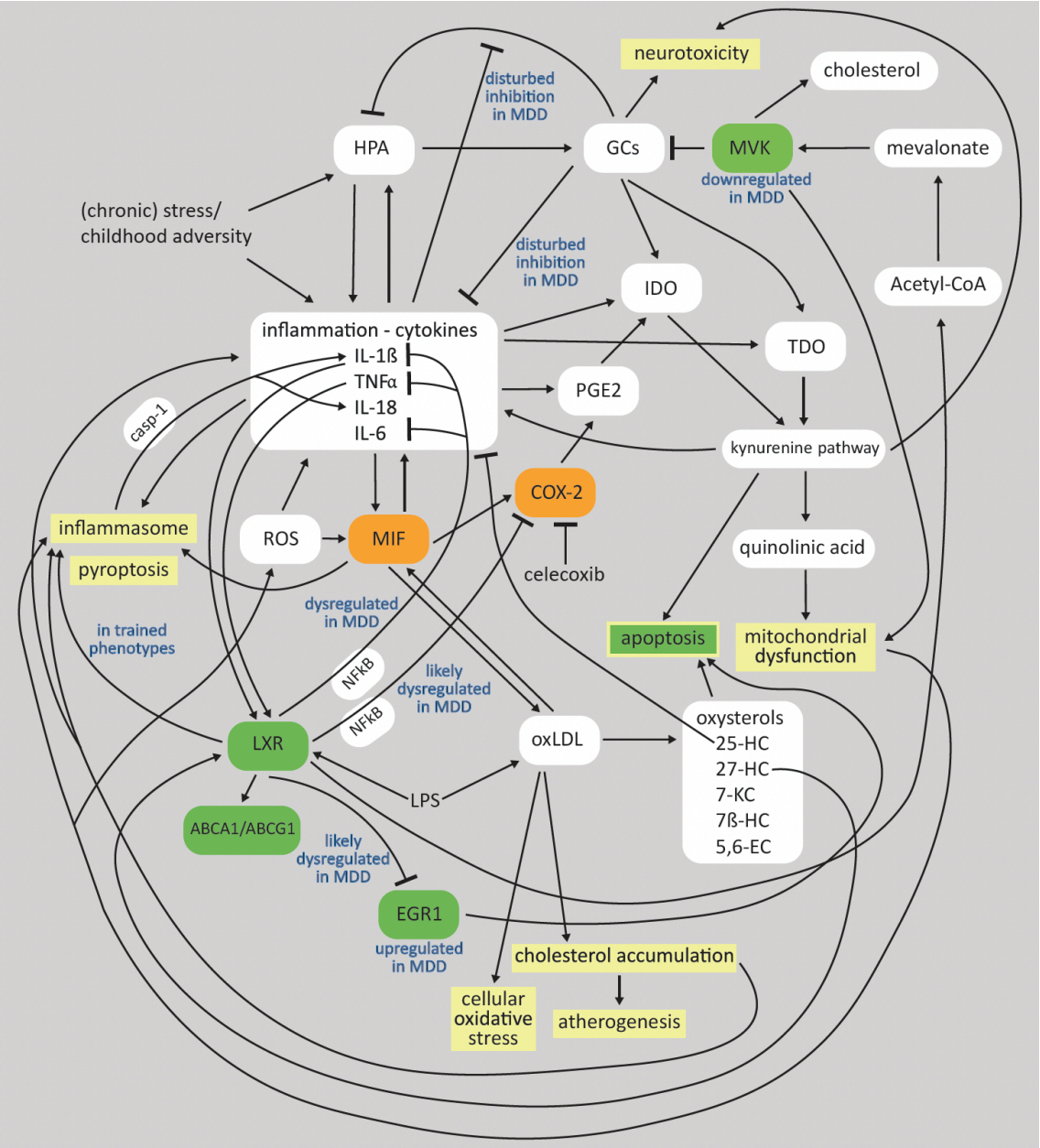
8 Conclusion

Immuno-metabolic and stress-related dysregulations in a subgroup of MDD patients are evident and require alternative treatment approaches. Subtyping is important to better understand different mechanisms of disease and deduce tailored treatment recommendations. So far, instead of stratifying for subgroups, often the whole patient group was pooled in the analyses, which is why studies can result in inconsistencies regarding biological underpinnings and efficacy of treatment methods. Subtyping still poses a great challenge due to the complexity of biological underpinnings. Cytokines are easy to determine and therefore practical to be implemented in clinical care. But which biomarkers finally are the most valid ones remains an open question until now. Anyhow, the picture increasingly emerges that there are different etiological types of depression with different underlying pathophysiology. Future aims are to develop more comprehensive models of the disease integrating different biological and psychological theories, while differentiating different etiological pathways where applicable.

The importance of this research field also becomes apparent when cardiovascular diseases are considered which are very common. For example, patients with atherosclerosis showed a trained phenotype of monocytes with elevated proinflammatory cytokines, as well as epigenetic and metabolic alterations (Bekkering et al., 2016). These changes are comparable to what can be found in depression (see section “discussion”). Maybe, stress-regulating and anti-inflammatory therapies (pharmacotherapy and psychotherapy) thus have the capability of reducing long-term CVD risk.

The results of both clinical studies (own original work) fit in well with previous research by showing that signs of immunosenescence, as well as mitochondrial and cholesterol pathway dysfunction are present in monocytes, and by pointing to the importance of stratified efficacy analyses building the grounds for future studies on stratified treatment allocation (Simon et al., 2021a; Simon et al., 2021b). Further, associated patient characteristics (childhood adversity, sex, BMI, age) and immuno-metabolic markers should be investigated comprehensively as predictors for treatment response to identify patients at risk on an individual level. Future research should also focus on factors of resilience in healthy individuals and response prediction to non-pharmacological therapies like psychotherapy.

Figure 1. Graphic summary of molecular pathways in the immuno-metabolic machinery.



Notes. Arrows represent stimulatory pathways; bars represent inhibitory pathways; yellow fields contain processes as an effect of the molecular interplay; green fields contain components from publication I (Simon et al., 2021a); orange fields contain components from publication II (Simon et al., 2021b). *ABCA1* ATP-binding cassette transporter 1; *ABCG1* ATP-binding cassette subfamily G member 1; *Acetyl-CoA* acetyl coenzyme A; *casp-1* caspase-1; *EGR1* early growth response protein 1; *GCs* glucocorticoids; *HPA* hypothalamic-pituitary-adrenal axis; *IDO* indoleamine-2,3-dioxygenase; *IL-1ß* interleukin 1beta; *IL-6* interleukin 6; *IL-18* interleukin 18; *COX-2* cyclooxygenase-2; *LPS* lipopolysaccharides; *LXR* liver x receptor; *MIF* macrophage migration inhibitory factor; *MVK* mevalonate kinase; *NFκB* nuclear factor kappa B; *oxLDL* oxidized low-density lipoprotein; *PGE2* prostaglandin E2; *ROS* reactive oxygen species; *TDO* tryptophan 2,3-dioxygenase; *TNFα* tumor necrosis factor alpha; *25-HC* 25-hydroxycholesterol; *27-HC* 27-hydroxycholesterol; *7-KC* 7-ketocholesterol; *7ß-HC* 7ß-hydroxycholesterol; *5,6-EC* 5,6-epoxycholesterol.

9 References

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10 Annex I. Publication I (Simon et al., 2021a)

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Monocyte mitochondrial dysfunction, inflammaging, and inflammatory pyroptosis in major depression

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ABSTRACT

Background: The macrophage theory of depression states that macrophages play an important role in Major Depressive Disorder (MDD).

Methods: MDD patients (N = 140) and healthy controls (N = 120) participated in a cross-sectional study investigating the expression of apoptosis/growth and lipid/cholesterol pathway genes (BAX, BCL10, EGR1, EGR2, HB-EGF, NR1H3, ABCA1, ABCG1, MVK, CD163, HMOX1) in monocytes (macrophage/microglia precursors). Gene expressions were correlated to a set of previously determined and reported inflammation-regulating genes and analyzed with respect to various clinical parameters.

Results: MDD monocytes showed an overexpression of the apoptosis/growth/cholesterol and the TNF genes forming an inter-correlating gene cluster (cluster 3) separate from the previously described inflammation-related gene clusters (containing IL1 and IL6). While upregulation of monocyte gene cluster 3 was a hallmark of monocytes of all MDD patients, upregulation of the inflammation-related clusters was confirmed to be found only in the monocytes of patients with childhood adversity. The latter group also showed a downregulation of the cholesterol metabolism gene MVK, which is known to play an important role in trained immunity and proneness to inflammation.

Conclusions: The upregulation of cluster 3 genes in monocytes of all MDD patients suggests a premature aging of the cells, i.e. mitochondrial apoptotic dysfunction and TNF "inflammaging", as a general feature of MDD. The overexpression of the IL-1/IL-6 containing inflammation clusters and the downregulation of MVK in monocytes of patients with childhood adversity indicates a shift in this condition to a more severe inflammation form (pyroptosis) of the cells, additional to the signs of premature aging and inflammaging.

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1. Introduction

A growing body of evidence points towards the involvement of an abnormal inflammatory response system in the pathogenesis of Major Depressive Disorder (MDD). In the last decades, a large number of investigations have been carried out on inflammation-regulating cytokines, since cytokines are relatively easy to determine. Meta-analyses of these investigations revealed that cytokine levels are raised in MDD patients (Köhler et al., 2017), particularly in those with a history of childhood adversity (CA; Pedrotti Moreira et al., 2018), adiposity (Gomes et al., 2019), or cardiovascular disease (Halaris, 2016). This has strengthened the inflammation theory of depression, in which early life stress leads to low-grade inflammatory activation (with inflammatory cytokines as main indicators) which in turn leads to depressive symptoms (Slavich and Irwin, 2014; Cathomas et al., 2019). Cells of the monocyte-macrophage lineage are important producers of inflammation-regulating cytokines. Thus, the inflammation theory of mood disorders has also been referred to as the monocyte/macrophage theory of depression (Smith, 1991).

In the past decade our group has extensively studied the circulating monocytes of mood disorder patients, focusing on an abnormal expression of 30–35 genes involved in the regulation of inflammation (based on the inflammatory theory of depression). In several finding pre-studies (carried out a decade ago, see Appendix A) using whole genome screening of patient versus healthy control monocytes we had selected these 30–35 inflammation-related genes because they ranked within the top over- and underexpressed genes in patient monocytes and were known to be involved in inflammation regulation as shown in Ingenuity Pathway Analyses (IPA). In later q-PCR confirmation studies we indeed showed that this set of inflammation-regulating genes was abnormally expressed in various cohorts of MDD patients, with expression being mainly higher in patient monocytes (Carvalho et al., 2014; Grosse et al., 2015; Arteaga-Henríquez et al., 2019; Schiweck et al., 2020). The most recent report shows that the expression of inflammatory genes in monocytes is particularly high in the patient group with a history of childhood adversity (Schiweck et al., 2020).

Furthermore, the IPA of the top over- and underexpressed genes carried out a decade ago (see Appendix A) also revealed that apart from inflammatory pathways other top molecular pathways were abnormally expressed, such as those related to leukocyte apoptosis, growth, and development. Top genes in these pathways were the mitochondrial apoptosis/growth regulating genes BAX, BCL10, EGR1 and EGR2 and the growth factor HB-EGF. Although apoptosis disturbances in the context of mitochondrial dysfunction and inflammation have been studied in psychiatric patients (Allen et al., 2018), studies on the expression level of mitochondrial apoptosis and growth-related genes in monocytes of well characterized groups of MDD patients are lacking. Further, their expression in relation to the previously determined inflammation-regulating genes in patient monocytes is so far unknown.

Another impetus for the study reported here is that the cholesterol metabolism in monocytes/macrophages plays an important role in the inflammation-regulation of the cells (Tall and Yvan-Charvet, 2015). An important enzyme in the lipid metabolism of monocytes is mevalonate kinase (MVK), facilitating the transition of mevalonic acid to mevalonate. Down-stream products such as the isoprenoids are anti-inflammatory (Marcuzzi et al., 2010), and a genetic deficiency of MVK leads to an inflammatory syndrome, the hyper IgD syndrome (HIDS) with strongly pro-inflammatory activated macrophages (Tricarico et al., 2013). Cholesterol pathway genes also play a role in the removal of lipids accumulated in the vessel wall of atherosclerotic plaques, and in doing so determine the inflammatory state of vessel wall infiltrating monocytes/macrophages (Westerterp et al., 2014). The gene expression state of such vessel wall infiltrating macrophages has been studied by others previously. Vessel wall infiltrating monocytes/macrophages can either be pro-inflammatory pro-atherogenic Mox macrophages (i.e. M1-like lipid-loaden foam cells) or anti-inflammatory atheroprotective M (hb)/H-mac cells (i.e. M2-like macrophages capable of pumping out cholesterol to be bound to apo-lipoproteins; Chistiakov et al., 2015). M (hb)/H-Mac cells are characterized by an active cholesterol pump machinery, such as the ABCA1 and ABCG1 pumps (Levy and Moreno, 2006), the gene expression of which is activated by the transcription factor LXR-α (coded by NR1H3) (Schulman, 2017). M(hb)/H-Mac cells are additionally characterized by the expression of the mannose receptor (MRC1), and the haptoglobin/hemoglobin receptor (CD163), while H-Mac cells additionally express HMOX (Chistiakov et al., 2015), an important anti-oxidant mechanism to counteract the pro-atherogenic effects of taken-up oxidized lipids. Despite the importance of the cholesterol pathway genes in inflammation regulation and atherosclerosis, and despite the knowledge that there is a higher risk for atherosclerotic disease in mood disorder patients (Penninx et al., 2001), studies on the expression of important cholesterol pathway genes and genes characteristic of atherosclerotic plaque macrophages are lacking in monocytes of MDD patients.

The aim of the study reported here to investigate the expression of the mitochondrial apoptosis/growth regulating genes BAX, BCL10, EGR1, and EGR2, the growth factor HB-EGF, and the cholesterol metabolism pathway genes NR1H3, ABCA1, ABCG1, MVK, CD163, HMOX1 in the monocytes of the MDD patients and healthy controls, thereby relating those to the previously studied inflammation-related genes by hierarchical clustering. Further, various sociodemographic and clinical parameters, such as sex, age, BMI, duration of illness, medication state, and childhood adversity will be taken into account analyzing gene expression data. Since childhood adversity was found to be an important determinant for a higher expression of the inflammation-regulating genes in the previous study of Schiweck et al. (2020), it will be of particular interest.

2. Methods

2.1. Participants

Using a cross-sectional study design, data were collected at three different sites using uniform procedures: the university psychiatric hospitals of Münster (Germany), München (Germany), and Leuven (Belgium). Samples of 140 MDD patients and 120 age- and sex-matched healthy controls (HC) were analyzed for gene expressions. Adult men and women patients (ages 18–65 years) were included if they were diagnosed with MDD and were free of the following diseases: clinical inflammation-related symptoms (including fever), current or recent inflammatory or infectious disease, uncontrolled systemic disease, uncontrolled metabolic disease, other uncontrolled somatic disorder affecting mood. Participants were excluded if they used somatic medication that affects mood or the immune system, e.g. statins, corticosteroids, non-steroidal anti-inflammatory drugs. Healthy controls were excluded if they were not in self-declared health (specifically lacking any form of auto-immune disease and/or atopic disease) and/or used somatic medication that affects mood or the immune system. For both the patient and control group, pregnant women or women who had delivered within the previous 6 months were excluded. The study was conducted in accordance with the declaration of Helsinki and its subsequent revisions and approved by ethical committees of the participating universities (reference numbers: Leuven: S51723; Munich: 291-09, Münster: 2009-019-f-S). Written informed consent was obtained from all participants.

2.2. Clinical assessment

Patients were diagnosed with major depression according to DSM-IV using the Mini- International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Healthy controls were screened for the presence of psychiatric symptoms using the MINI Screening version. In patients, depression severity was assessed by the Inventory of Depression

Symptoms (IDS-C30; Rush et al., 1996) in a face-to-face interview, and in healthy controls by the self-report version (IDS-SR30). Presence of adverse events during childhood was measured by the Childhood Adversity Questionnaire (CTQ; Bernstein et al., 2003). Positivity for childhood adversity (CA) was defined by presence of trauma on at least one subscale of the CTQ. Presence of CA on a single subscale was defined according to Walker et al. (1999). Body Mass Index was calculated after assessing self-reported height and body weight. Duration of illness was calculated by subtracting the date of disease onset from age at the time of study assessments. Disease onset and medication were obtained from clinical records.

2.3. Laboratory assessment

The assessment procedure of monocyte gene expression applied in this study has been described in detail before (Grosse et al., 2015). In brief, blood samples were drawn and PBMC suspensions were prepared and stored at −80 °C (Knijff et al., 2006). CD14+ monocytes were isolated by magnetic cell sorting system (Miltenyi Biotec, B.V., Bergisch Gladbach, Germany) from thawed PBMC, then RNA was isolated (RNAeasy minikit; Qiagen, Hilden, Germany). Of RNA, 1 μg was reverse transcribed (high capacity cDNA kit) to produce cDNA for quantitative-polymerase chain reaction (q-PCR) to determine monocyte gene expression by comparative threshold cycle method (CT values; Applied Biosystems, Carlsbad, CA, USA). cDNA was stored at −80 °C. Using the cDNA, the genes were determined using the probe and primer sets of Applied Biosystems (see Appendix B., Supplementary Table 1). All gene values were normalized by the value of the housekeeping gene ABL1 (ΔCT values), which is a superior housekeeping gene for leukocytes (Beillard et al., 2003). Controlling for site, for each gene the average ΔCT value of healthy controls without CA of each site was subtracted from the ΔCT values of patients of the same site resulting in corrected ΔΔCT values before data was pooled and used for analyses (Livak and Schmittgen, 2001). Negative ΔΔCT indicate upregulation, positive ΔΔCT values indicate downregulation of gene expression in reference to healthy controls without CA.

2.4. Statistical analyses

Statistical analyses were performed with R software version 3.5.2. Comparison of demographical data between MDD and HC were computed using the Chi-square test for categorical data and Wilcoxon rank sum test for continuous data. Missing gene values (5.9% of healthy control genes and 5.1% of MDD genes) were imputed using the median of patient values for missing patient values and of HC values for missing HC values for visual data presentation and initial single gene analyses. Hierarchical cluster analysis of MDD monocyte gene expression intercorrelations was conducted using Spearman rank correlation matrix and ward.d2 method. For initial analysis and graphical presentation, *p*-values of single gene expressions of 44 genes were obtained using one-sample Wilcoxon signed rank test and were adjusted for multiple testing using the Benjamini-Hochberg-method (false discovery rate). *P*-values of single gene group comparisons were calculated using Wilcoxon rank sum test (Benjamini-Hochberg-corrected). Visual data presentation follows the order resulting from hierarchical cluster analysis. For multivariate analyses, missing data were imputed by multiple imputation using chained equations (mice package). Five imputed datasets were created and analyses were run on each dataset. *P*-values were calculated based on pooled F-values from all datasets. A clusterwise multivariate analysis of co-variance (Mancova) was performed to obtain and verify pooled differences of gene expression profiles as were observed after clusterwise depiction of single gene expressions. BMI and age were included as covariates. In a second step, the effect of sex, duration of illness, and medication (binary variable anti-depressants yes/no) were also studied and added as covariates, respectively. Since we suspected differential gene expression in women and men, both groups are depicted, separately. In the Mancova analyses, we were interested in the main effects due to missing significant interaction terms and therefore used a type II sum square calculation (jmv package).

3. Results

3.1. Demographics

Table 1A shows that the distributions of age, sex, and BMI were not significantly different between patients and controls. Significant differences emerged, not surprisingly, for depression severity and CA (prevalence patients 57.0%, prevalence healthy controls 31.4%). The vast majority of patients were receiving drug treatment. Table 1B shows the varieties of medication: 11.5% were not receiving any drug treatment at the time of testing, 11.5% were receiving a benzodiazepine only, while the remaining patients often used a variety of anti-depressants.

3.2. A monocyte gene expression signature

We first conducted a hierarchical cluster analysis using the expression levels of the apoptosis/growth genes, the cholesterol pathway genes and the previously determined inflammation-regulating genes which resulted in the clusters shown in Fig. 1: The two inflammation-related gene clusters found in previously published analyses (Schiweck et al., 2020) were confirmed, and the additionally determined apoptosis/growth/cholesterol pathway genes by and large formed a separate cluster (cluster 3). Cluster 1 was composed of various cytokine production-associated genes (with important cytokine genes such as IL1 and IL6), while cluster 2 consisted of chemotaxis, adhesion, and coagulation-regulating genes. Fig. 1 futhermore shows that cluster 1 and cluster 2 inflammatory genes are in general strongly positively intercorrelated, while cluster 3 genes correlated weaker amongst themselves and to cluster 1 and 2 genes (positively and negatively). It is also of note that the newly determined M(hb) gene HMOX was not part of cluster 3 but positioned as a cluster 2 gene and correlated negatively to many of the cluster 1 inflammation-related genes in accordance with its strong anti-oxidant function (see Fig. 1).

3.3. Overexpression of cluster 3 genes is a hallmark of MDD patients, irrespective of childhood adversity. Dependency on age and BMI

Since we previously described that CA is a major determinant for the higher expression of the inflammation-related genes in this set of MDD patients (Schiweck et al., 2020), we analyzed data not only in the entire group of MDD patients, but also split into those with and without a history of CA. Fig. 2 shows the expression levels of the various genes per

Table 1A
Demographic data of patients and healthy controls.

	MDD (N)	HC (N)	*test*	*df*	*p*-value
Sex (women, %)	64.29 (140)	65.83 (120)	$\chi^2 = 0.02$	1	0.90
Age (*Md/ IQR*)	41.88/ 20.52 (140)	37.50/ 22.29 (120)	$W = 7551.00$	–	0.16
BMI (*Md/ IQR*)	23.96/ 5.09 (140)	23.59/ 4.82 (120)	$W = 7292.50$	–	0.08
IDS score (*Md/ IQR*)	31.00/ 15.00 (140)	5.50/ 6.25 (120)	$W = 287.00$	–	<0.001
CA yes (%)	57.04 (135)	31.36 (118)	$\chi^2 = 15.75$	1	<0.001
DD (*Md/ IQR*)	4.00/ 11.25 (118)	–	–	–	–
Suicide risk yes (%)	74.05 (131)	–	–	–	–

Legend. MDD Major Depressive Disorder; HC healthy control; Age in years; BMI body mass index; IDS Inventory of Depression Symptoms; CA childhood adversity; DD disease duration in years; χ2 Chi-Square statistic; Md median; IQR interquartile range; W Wilcoxon statistic; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 1B
Absolute and relative frequencies of medication taken by MDD patients.

Medication regimen	N (%)
No psychotropics	13 (11.50)
Benzodazepines only	13 (11.50)
Anti-depressants only	87 (76.99)
TCA/ TeCA	37 (32.74)
SSRI	37 (32.74)
SNRI	23 (20.35)
NARISARI	4 (3.54)
Melatonin	2 (1.77)
Neuroleptics	42 (37.17)
Lithium	13 (11.50)

Medication status was unavailable for 27 patients; cumulative frequency of anti-depressant drug regimen exceeds 100% due to multiple prescriptions; in total, 40 patients (35,40%) were taking benzodiazepines; anti-depressants only = anti-depressant drug regimen; TCA tricyclic anti-depressant; TeCA tetracyclic anti-depressant; SSRI selective serotonin reuptake inhibitor; N absolute number of patients; % percent in reference to the total patient group number.

(sub-)cluster in the monocytes of all MDD patients and those who had or had not experienced CA, respectively.

3.3.1. Cluster 1 genes

With regard to cluster 1 genes, we again showed, as in the study of Schiweck et al. (Schiweck et al., 2020), that MDD patients with a history of CA show many monocyte genes significantly upregulated (e.g. IL1B, IL1A, IL6) versus healthy controls without CA. MDD patients without a history of CA showed hardly any upregulation of cluster 1 genes. From the Mancova analysis including age and BMI, a considerably significant effect emerged for the MDD CA group, showing that the upregulation of cluster 1 is significantly linked to this mood condition, independently of the effects of age and BMI. Noteworthy, age had an important additional effect for cluster 1 genes, while BMI did not.

3.3.2. Cluster 2 genes

The Mancova analysis further showed that there existed a nearly significant ($p = 0.06$) upregulation of cluster 2 genes specific to major depression and independent from the effects of BMI and age, but only in the MDD CA group. Cluster 2 upregulation was not significantly associated with MDD without CA. It also appeared from the Mancova analysis that cluster 2 upregulation was particularly dependent on BMI and age, both in MDD patients with and without CA.

3.3.3. Cluster 3 genes

Cluster 3 was significantly upregulated in monocytes of both the MDD patient group with and without CA, independent of the effects of age and BMI. Mancova analysis showed that BMI hardly had an effect on cluster 3 expression; however, a clear age effect was present. Increasing age correlated in general to increasing expression of the cluster 3 genes.

Three subclusters of cluster 3 were observed:

3.3.4. The mitochondrial apoptosis and growth genes

Cluster 3 consisted in part of the mitochondrial apoptosis and growth regulating genes BAX, BCL10, HB-EGF, EGR1 and EGR2, of which BAX and BCL10 were significantly upregulated in MDD patients, in

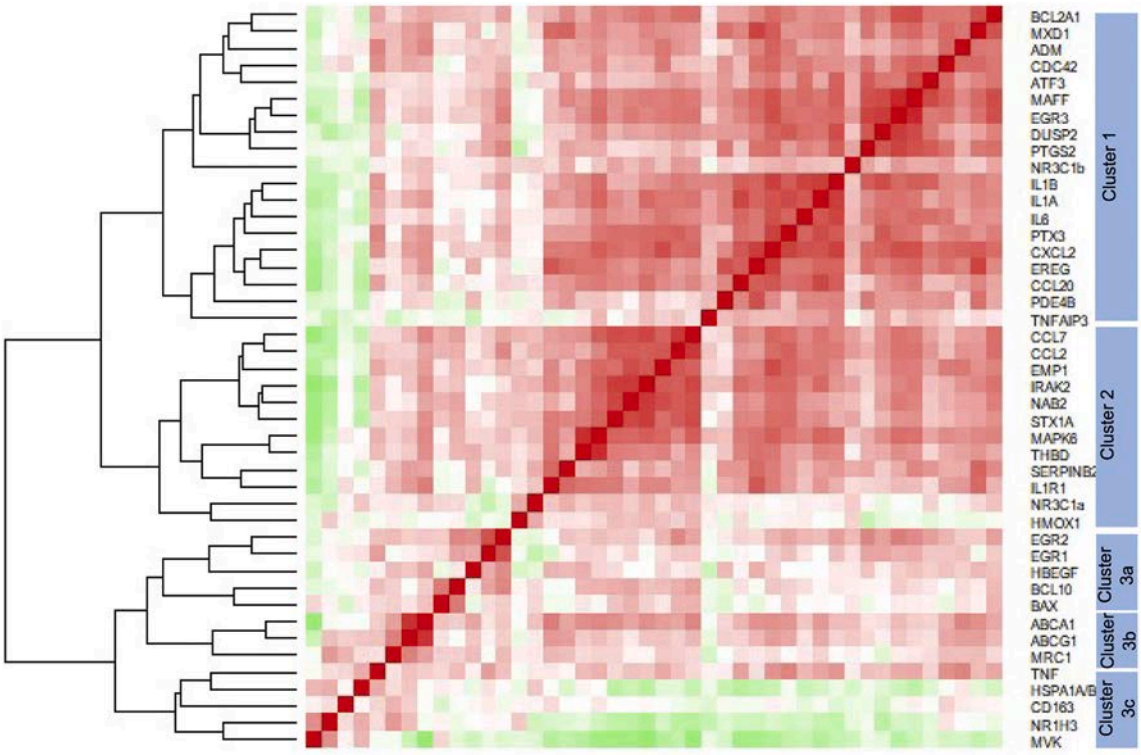
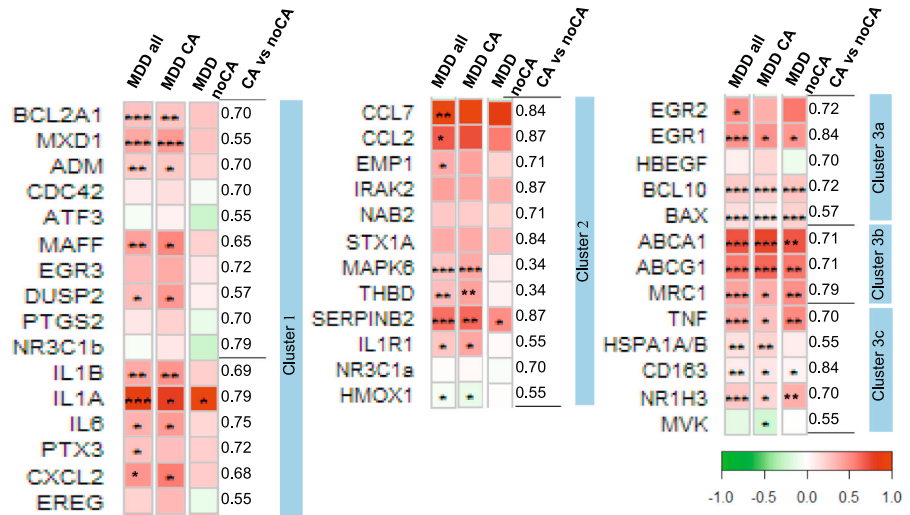


Fig. 1. Inter correlating gene clusters as expressed in monocytes of MDD patients ($N = 140$).
Legend. In this study BAX, BCL10, EGR1, EGR2, HBEGF, ABCA1, ABCG1, NR1H3, CD163, HMOX1, MRC1, and MVK were determined. In previous studies [5,6,8] ADM, ATF3, BCL2A1, CCL2, CCL20, CCL7, CDC42, CXCL2, DUSP2, EGR3, EMP1, EREG, HSPA1A_HSPA1B, IL1A, IL1B, IL1R1, IL6, IRAK2, MAFF, MAPK6, MXD1, NAB2, NR3C1a, NR3C1b, PDE4B, PTGS2, PTX3, SERPINB2, STX1A, THBD, TNF, and TNFAIP3 had been determined from the same cDNA preparations. Three main gene clusters could be identified (see dendrogram): Cluster 1 containing most of the inflammation-regulating genes (including the interleukins IL1A, IL1B and IL6). Cluster 2 comprising predominantly genes related to adhesion, coagulation, shape change and the chemotactic ability of the cells (EMP1, STX1A, THBD, CCL2, CCL7). Cluster 3 with mainly the newly determined apoptosis/growth regulating genes and cholesterol pathway genes, but also TNF and HSP1A/B. This cluster can be sub-divided in cluster 3a, 3b, and 3c.



	group				BMI				age				interpretation
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	
MDD-HCnoCA													
Cluster1	2.715	19	199	<0.001***	1.029	19	199	0.429	2.651	19	199	<0.001***	independent group effect
Cluster2	1.415	12	206	0.161	3.384	12	206	<0.001***	3.258	12	206	<0.001***	age and BMI only determinants
Cluster3	2.044	13	205	0.015*	1.755	13	205	0.035*	4.023	13	205	<0.001***	independent group effect
MDD CA-HCnoCA													
Cluster1	2.472	19	136	0.001**	1.333	19	136	0.173	3.010	19	136	<0.001***	independent group effect
Cluster2	1.832	12	143	0.048*	4.430	12	143	<0.001***	2.670	12	143	0.003**	independent group effect
Cluster3	2.091	13	142	0.018*	2.086	13	142	0.018*	4.405	13	142	<0.001***	independent group effect
MDD noCA-HCnoCA													
Cluster1	1.607	19	117	0.059^{+}	0.966	19	117	0.528	2.359	19	117	0.002**	marginal independent group effect
Cluster2	0.891	12	124	0.498	2.315	12	124	0.015*	2.516	12	124	0.010*	age and BMI only determinants
Cluster3	2.052	13	123	0.044*	1.487	13	123	0.228	1.970	13	123	0.045*	independent group effect

Fig. 2. Monocyte gene expression signature of the entire group of MDD patients ($N = 140$), and in the subgroups of patients with childhood adversity (MDD CA; $N = 77$) and without childhood adversity (MDD noCA; $N = 58$).
Legend. *MDD* Major Depressive Disorder; *CA* childhood adversity; *noCA* no childhood adversity; *HCnoCA* healthy controls without experience of childhood adversity; *BMI* body mass index. Mean values are expressed relative to the expression level of HCnoCA; the intensity of red reflects higher expression, intensity of green lower expression; stars indicate level of significance; *p*-values of group comparison are given. Pooled F-values and estimated *p*-values of the clusterwise gene expression analysis (Mancova) with group as factor and BMI and age as covariates are displayed. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p \sim 0.05$.

particular, irrespective of the presence of CA (see Fig. 2).

3.3.5. *M(hb) characteristic genes*

Cluster 3 also consisted of the genes characteristic of anti-inflammatory M(hb) cells, such as the cholesterol pump genes ABCA1/ABCG1, NR1H3 (coding LXRα, the transcription factor for the cholesterol pumps), CD163 and MRC1. Of these, ABCA1 and ABCG1 were the strongest upregulated genes in MDD patients, irrespective of the presence of CA (see Fig. 2).

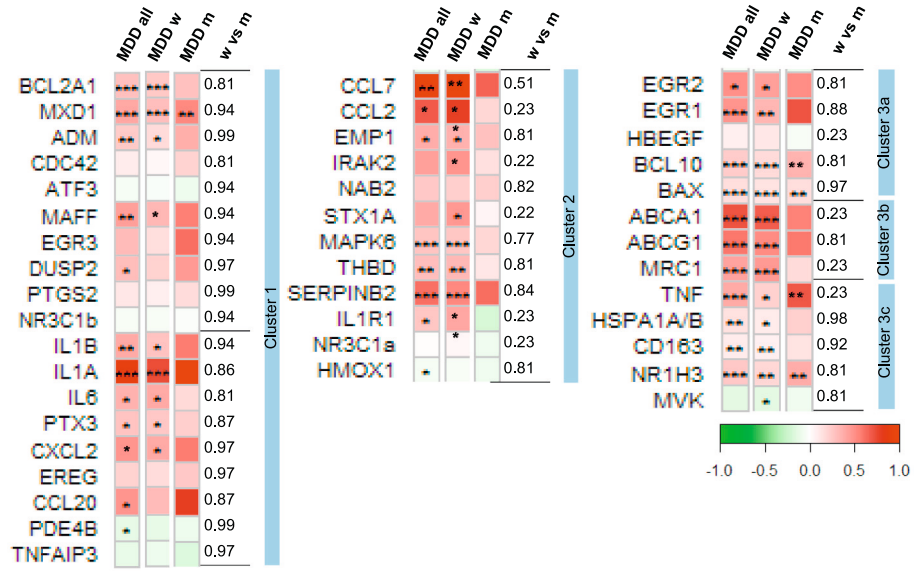
3.3.6. *Other cluster 3 genes*

Cluster 3 also contained the pro-inflammatory cytokine TNF (see Fig. 2), which was overexpressed in both the MDD group with and without CA. The overexpression of TNF in cluster 3 is peculiar regarding the M(hb) profile of cluster 3 (which is in general regarded as an anti-inflammatory profile). Interestingly, MDD patients with a history of CA showed a significant downregulation of the MVK gene (see Fig. 2), which correlated strongly but negatively to cluster 1 and 2 inflammation-regulating genes (see Fig. 1). The strongest negative correlation emerged between MVK and cluster 2 genes (Spearman-Rho ranged from −0.48 to −0.27 for 10 of the 12 cluster 2 genes; *p*-values ≤0.001).

With regard to the different subtypes of childhood adversity, Appendix C (Supplementary Fig. 1) shows that emotional abuse and emotional neglect contributed especially to the higher expression of cluster 3 genes. Schiweck et al. (2020) previously reported that emotional neglect and emotional abuse also contributed strongest to the higher expression of the inflammation-related gene clusters 1 and 2.

3.4. *Effects of sex, duration of disease, and medication on monocyte gene expression in MDD patients*

Statistically, monocytes of women and men with MDD had an equally higher expression of the three clusters of genes with reference to the monocytes of healthy controls (Fig. 3). However, men had a somewhat weaker expression level than females, as is evident from the expression levels in single gene analysis (see Fig. 3). Mancova analysis showed that age and BMI effects on monocyte gene expression were particularly evident in women. In addition, we studied the effect of disease duration and anti-depressant medication on monocyte gene



	group				BMI				age				disease duration			
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*
MDD women-HcnoCA																
Cluster1	2.074	19	134	0.009**	1.040	19	134	0.420	3.050	19	134	<0.001***	0.727	19	134	0.786
Cluster2	2.151	12	141	0.017*	3.416	12	141	<0.001***	2.590	12	141	0.004**	1.244	12	141	0.259
Cluster3	2.003	13	140	0.025*	2.440	13	140	0.005**	3.840	13	140	<0.001***	1.622	13	140	0.086
MDD men-HcnoCA																
Cluster1	2.196	19	100	0.006**	1.264	19	100	0.225	1.789	19	100	0.034	0.851	19	100	0.642
Cluster2	1.959	12	107	0.035*	1.762	12	107	0.064	1.646	12	107	0.090	1.835	12	107	0.051^{+}
Cluster3	2.104	13	106	0.020*	1.543	13	106	0.114	1.939	13	106	0.033*	1.218	13	106	0.277
	group				**BMI**				**age**				**medication**			
	F	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*	*F*	*df1*	*df2*	*p*
MDD women-HCnoCA Cluster1	2.110	19	129	0.008**	1.115	19	129	0.344	3.439	19	129	<0.001***	1.465	19	129	0.109
Cluster2	1.859	12	136	0.045*	3.680	12	136	<0.001***	3.213	12	136	<0.001***	1.916	12	136	0.037*
Cluster3	1.647	13	135	0.080	3.735	13	135	<0.001***	4.136	13	135	<0.001***	2.782	13	135	0.002*
MDD men-HCnoCA																
Cluster1	2.057	19	100	0.012*	1.125	19	100	0.339	1.792	19	100	0.034*	1.730	19	100	0.043*
Cluster2	1.974	12	107	0.034	1.389	12	107	0.182	2.044	12	107	0.027*	1.199	12	107	0.293
Cluster3	1.989	13	106	0.028*	1.305	13	106	0.221	1.921	13	106	0.035*	1.331	13	106	0.206

Fig. 3. Monocyte gene expression signature of MDD patients (N = 140), of women (MDD w; *N* = 90) and men (MDD m; *N* = 50), and after control for the additional covariates duration of disease and medication.
Legend. *MDD* Major Depressive Disorder; *w* women; *m* men; *HCnoCA* healthy controls without experience of childhood adversity; *BMI* body mass index. Mean values are expressed relative to the expression level of HCnoCA; the intensity of red reflects higher expression, intensity of green lower expression; stars indicate level of significance; p-values of group comparison are given. Pooled F-values and estimated p-values of the clusterwise gene expression analysis (Mancova) with group as factor and BMI and age as covariates are displayed. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p \sim 0.05$.

expression in this analysis. The Mancova analyses given in Fig. 3 show that an effect of disease duration on monocyte gene expression could not be detected. Medication, dichotomized as non-medicated and benzodazepine-only treated patients versus the patients with bona-fide anti-depressants, showed a significant effect for the expression of cluster 2 and cluster 3 genes in women (not in men).

4. Discussion

The results reported here are new in showing an aberrant expression of a cluster of genes in the monocytes of MDD patients composed of a subcluster of mitochondrial apoptosis/growth regulating genes (BAX, BCL10, EGR1, and EGR2), various genes previously described in M(hb) macrophages (ABCA1, ABCG1, NR1H3, MRC1, CD163), the gene for pro-apoptotic/pro-inflammatory TNF, the gene for the immune regulating/protein chaperone molecule HSP70, and the cholesterol pathway gene MVK. This gene cluster was abnormally expressed in all MDD patients irrespective of a history of CA. Here we show once more that the monocytes of the studied group of MDD patients also have a higher expression level of the inflammation-related gene clusters 1 and 2, but virtually only in the MDD cases with CA (see also Schiweck et al., 2020). In this report, we take this observation further in showing that the higher expression of cluster 1 and 2 genes correlates to the down-regulation of the cholesterol pathway gene MVK in MDD patients with CA. Fig. 4 shows an illustration of the interaction of all here tested genes and their role in monocyte/macrophage cellular functions such as

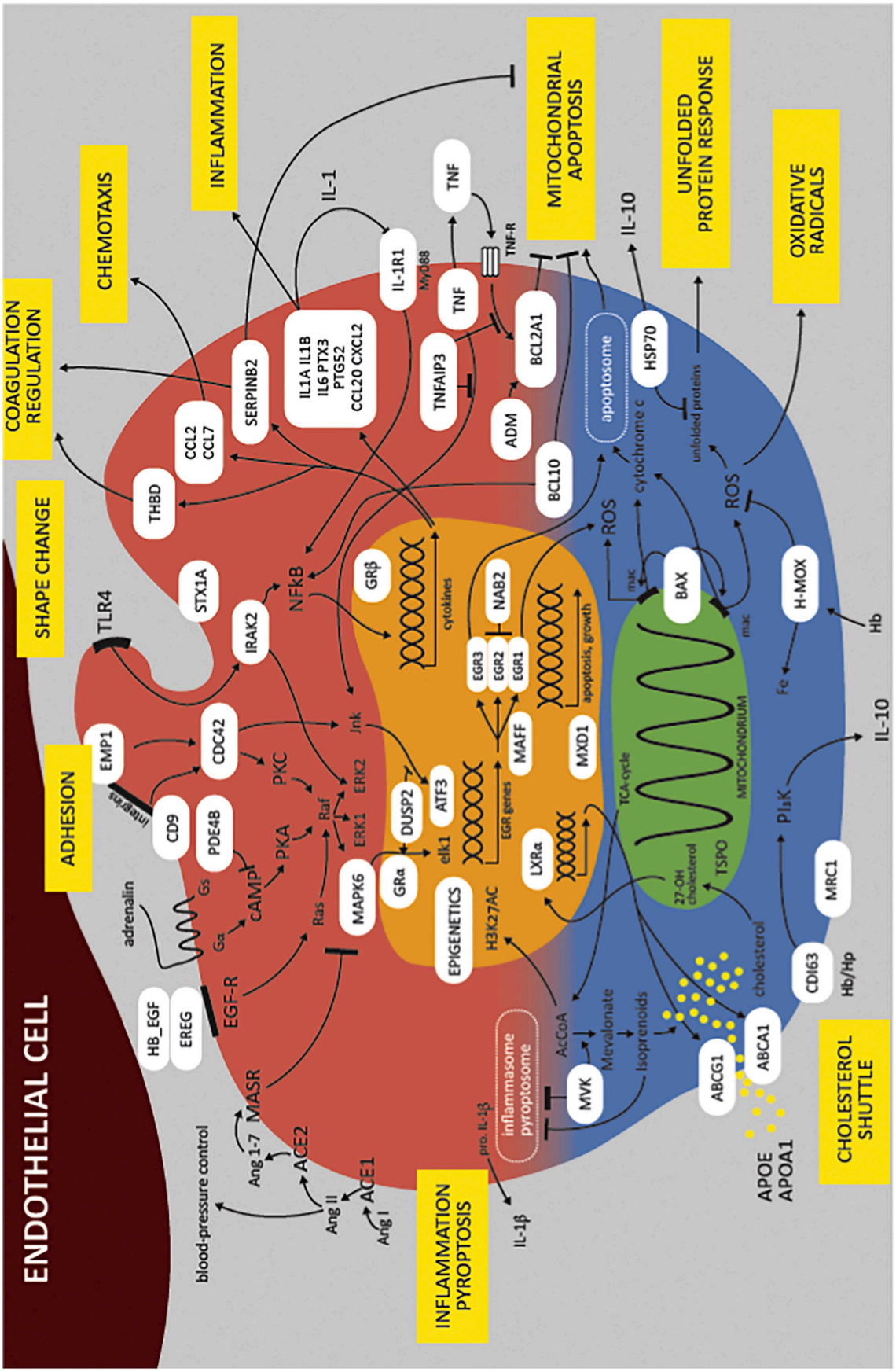


Fig. 4. Molecular gene modules activated in monocytes of MDD patients.

Legend. An illustration is shown on the interaction of the various molecules coded by the genes in the molecular modules, which are activated in monocytes of MDD patients. It is important to note that all described molecular modules contain both pro- and anti-inflammatory/apoptosis/coagulation factors and this underscores the notion that in MDD patients processes of both pro- and anti-inflammation/apoptosis/coagulation are activated. Thus, there rather exists a disequilibrium than a plain activation or inhibition of these processes in MDD monocytes. Shortly:

Mitochondrial apoptosis module, a sign of immune senescence (cluster 3a genes, lower right part of the cartoon): Includes the genes for BAX, BCL10, EGR1, EGR2 and HB-EGF (see Fig. 1).

The module of the senescence associated secretory phenotype (SASP) and unfolded protein response (cluster 3c genes, also lower right part of the cartoon): Includes the gene for TNF and HSP1A/1B. *Cholesterol metabolism module* (cluster 3b and some 3c genes, lower left part of the cartoon): includes ABCA1, ABCG1, MRC1, NR1H3 (encoding the LXR-α transcription factor) and CD163.

The module of MAP Kinases and EGRs (mainly cluster 1, upper left part of the illustration): Includes various mitogen activated protein kinases (MAPKs) such as MAPK6/ERK3, the MAPK pathway regulating genes ATF3, DUSP2, NAB2, MAFF and MXD1 and down-stream transcription factors such as EGR3. EREG feeds into this route, while PDE4B regulates the input of, e.g. adrenaline into this route.

The module of pro-inflammatory cytokines and compounds (mainly cluster 1a, upper right part of the cartoon): Activation of the MAP-kinase pathway not only leads to growth and apoptosis regulation but also to the production of pro-inflammatory cytokines and compounds. The genes IRAK-2, IL-B, IL-1A, IL-6, PTX3, PTGS2, and CXCL2 are examples.
Pyroptosis module (cluster 3c and cluster 1a, middle left part of the illustration): MVK, TNFAIP3, BCL2A1 and ADM.
Module of shape change, chemotaxis, adhesion, and coagulation (mainly top of the cartoon, all cluster 2 genes). This cluster is composed of CCL2, CCL7, EMP1, STX1A, CDC42, SERPINB2, THBD, IL-1R1, HMOX-1 and the activating GRα.
For a more detailed description of function and interaction of these separate genes (as reviewed from the literature), see Appendix D.

inflammation-regulation, chemotaxis, adhesion, shape change, coagulation regulation, mitochondrial apoptosis, growth regulation, the redox potential, the unfolded protein response, and the cholesterol shuttle (for detailed information on the separate molecules see Appendix D). The monocyte data presented here thus indicate that the immune abnormalities found in MDD are not restricted to a higher state of chronic low-grade inflammation in monocytes (which virtually only occurs in CA cases), but also involve various other disturbances of monocyte cellular functions, related to mitochondrial apoptosis, growth regulation, the redox potential, the unfolded protein response, and the cholesterol shuttle. Below we discuss the putative consequences of the abnormal expression of the cluster 3 genes in monocytes of MDD patients.

4.1. Monocyte mitochondrial dysfunction in MDD: A sign of premature monocyte aging?

One of the over-expressed cluster 3 genes in monocytes of MDD patients is BAX. BAX is a key player in inducing mitochondrial apoptosis; after oligomerization it forms the mitochondrial apoptosis-induced channel (MAC) in the mitochondrial external membrane (see Fig. 4). This channel is amongst others important for the release of Reactive Oxygen Species (ROS) and cytochrome *c* from mitochondria. Cytochrome c activates the formation of the apoptosome (Tricarico et al., 2013). The clear upregulation of the mitochondrial apoptosis regulating genes BAX and BCL10 in the monocytes of MDD patients irrespective of CA supports the view that mitochondrial dysfunction is a prime hallmark of MDD. There is pertinent literature on mitochondrial dysfunction in mood disorders showing mtDNA damage, morphological changes in the mitochondria, less ATP production, electron chain changes and higher ROS production, leading to dysregulated apoptosis, abnormal cell maturation/differentiation and proinflammatory activity (Allen et al., 2018; Bansal and Kuhad, 2016; Labra Ruiz et al., 2018). Indeed, in our study pro-apoptotic/inflammatory TNF was higher expressed as part of cluster 3.

The above described pattern of mitochondrial dysfunction, altered apoptosis, and high production of TNF is compatible with the functional and molecular profile known of senescent cells. The increased inflammatory compound production in senescent cells is known as the senescence-associated secretory phenotype (SASP) and often nicknamed "inflammaging". Senescence of monocytes/macrophages has been described, though scarcely (Ong et al., 2018). It is characterized by mitochondrial dysfunction, an increased ER stress with unfolded proteins, a high expression of MRC-1 (a characteristic of anti-inflammatory M2 like macrophages), with nevertheless a higher production of pro-inflammatory cytokines and oxidative radicals (Van Beek et al., 2019). Interestingly, the monocytes of the MDD patients in this study indeed show prime features of anti-inflammatory M2 cells such as a high expression of MRC1 and CD163 together with a high expression of the chaperone molecule HSP70 (combatting ER stress), however all in combination with a high expression of the gene for the inflammatory cytokine TNF.

To our knowledge, our study is the first indicative of a premature senescent state of monocytes of MDD patients, reinforcing the earlier expressed view that MDD belongs to the group of disorders characterized by a premature cell senescence based on shortened telomere length in circulating leukocytes (Verhoeven et al., 2014) and an over-representation of terminally differentiated pro-inflammatory senescent circulating T cells (Elwenspoek et al., 2017; Ford et al., 2020). Whether such premature immune aging is genetically programmed or the result of environmental factors such as CMV infection is under investigation (Bauer, 2008; Verhoeven et al., 2019; Reed et al., 2019; Leng et al., 2011). There are indications that CMV infection might play an important role in monocyte aging (Leng et al., 2011).

4.2. Abnormal monocyte cholesterol metabolism in MDD

Cholesterol metabolism genes known from anti-inflammatory lipid-handling M(hb) macrophages (described as athero-protective cells in atherosclerotic plaques) were also upregulated in the circulating monocytes of the MDD patients irrespective of CA. Upregulated genes were NR1H3 (LXRα) and ABCA1/ABCG1, which play prominent roles in pumping out intracellular cholesterol, a molecule that has a pro-inflammatory activity when intra-cellularly accumulated (Tall and Yvan-Charvet, 2015). An increase of the pumps can therefore be considered an anti-inflammatory event, and the upregulated expression of the M2 marker MRC1 is in accordance with such a view. However, the principally anti-inflammatory M(hb) cells show a pro-inflammatory SASP-like senescent profile with a high TNF expression (see above) in our MDD patients. It is tempting to speculate that this occurrence of the peculiar "inflammation-prone senescent M(hb) cells" is a factor in the well-known higher prevalence of atherosclerotic cardiovascular disease in MDD patients. Nevertheless, our observations support the view that the interaction between fat metabolism and immunity plays a role in the pathogenesis of MDD, as is expressed in the so-called theory of immunometabolic depression (Milaneschi et al., 2020).

4.3. Mevalonate kinase and the proneness of monocytes to induce inflammation

Our study is novel in showing that MDD patients with a history of CA and a concomitant upregulation of cluster 1 and 2 inflammation-regulating genes, show a significant downregulation of MVK. Patients with mutations in the MVK gene (HIDS patients) are known for their periodic inflammatory episodes and dysregulated pro-inflammatory cytokine levels, particularly that of IL-1β. In its most severe form, brain developmental abnormalities are at the forefront in HIDS patients (Hoffmann et al., 1993). The pathological mechanisms of auto-inflammation in mevalonate kinase deficiency are not well understood; however, reduced synthesis of isoprenoid lipids downstream of MVK are thought to play a central role (Tricarico et al., 2015). These are necessary for the prenylation (the addition of hydrophobic compounds) of small GTPases (such as CDC42, a cluster 2 gene). Reduced prenylation of GTPases results in altered autophagy, mitochondrial dysfunction, and redox balance with an over-activation of the pyroptosomes and, consequently, in a dysregulated production of IL-1β and IL-18 (Tricarico et al., 2015). Thus, it is not surprising that the reduced MVK in the monocytes of MDD patients with a history of CA is linked to the upregulation of cluster 1 and 2 genes in the patient monocytes. Furthermore, MVK also plays a prominent role in "trained immunity" (Bekkering et al., 2018). Trained immunity is the capability of monocytes/macrophages to build up a long-term non-specific memory towards danger signals via an epigenetic imprinting (Netea et al., 2016). The metabolite mevalonate is the mediator of training via activation of mTOR and subsequent histone modifications in the inflammatory pathway (Bekkering et al., 2018).

Monocytes of HIDS patients, which are deficient in MVK, accumulate mevalonate and show a trained phenotype (Bekkering et al., 2018). From our studies it can be hypothesized that CA, as an early danger signal, induces a monocyte pro-inflammatory epigenetic training program with concomitant reduced MVK activity and with long-lasting effects on the inflammatory state of monocytes/macrophages.

4.4. How do our data relate to monocyte gene expression data in previous literature?

Literature from other research groups on monocyte gene expression in MDD cases is scarce and fragmented. Zhu et al. (2019) performed gene expression studies in monocytes of monozygotic twin pairs discordant for MDD. These authors described how genes related to mitochondrial energy production, the oxidative stress response, and the cytokine secretion, and how the zinc family genes were dysregulated in MDD monocytes. Of the differentially methylated regions, those involved in apoptosis, growth, and the MAPK/ERK pathway stood out. Similarities to the profiles described here are obvious. Overexpression of inflammatory genes in monocytes of MDD patients have also been found by Chiang et al. (2019) and Hasselmann et al. (2018). Hasselmann et al. (2018) in addition described steroid resistance of the MDD monocytes, i.e. reduced expression of the GR. We did not find abnormalities in GR expression in this series of MDD patients. However, in a previous study on unmedicated older melancholic patients with a higher depression score as described here, we did find a higher expression of the inflammation-related monocyte genes together with an overexpression of the blocking GRβ and a reduction in the activating GRα correlating to the depression score (Carvalho et al., 2014). Lisi et al. (2013) stimulated monocytes of drug-free MDD patients with LPS and found a reduced PTGS2 gene expression in the presence of a trend for the IL1B and IL6 genes to be over-expressed. We found similar data, i.e. in non-stimulated MDD monocytes PTGS2 normally expressed and IL1A, IL1B, and IL6 overexpressed. Hung et al. (Hung et al., 2017; Hung, 2018) showed an inflammatory state of circulating monocytes of unmedicated MDD patients, i.e. a higher expression of inflammation-inducing toll-like receptors (TLRs) in the presence of a reduced expression of their negative regulators. The investigators specifically focused on the negative regulator TNFAIP3, one of our panel genes, which they found downregulated in unmedicated patients, but upregulated after SSRI treatment (Hung et al., 2017). We also found this gene downregulated. However, the majority of our patients was medicated. Schwaiger et al. (2016) studied monocyte gene expression after an acute stressor in healthy individuals with a history of childhood adversity. The investigators found increased gene expressions for modules of cytokine and chemokine activity/cytokine-receptor interaction and alterations in gene expression modules for steroid binding and hormone activity (Schwaiger et al., 2016). The authors also suggested that childhood adversity leads to persistent alterations in transcriptional control in monocytes, as this report also shows (Schwaiger et al., 2016).

4.5. Limitations

The present study has several limitations. The vast majority of patients was medicated by a variety of drugs and only few were not medicated at all. Although we do report data adjusted for medication, we consider outcomes as preliminary due to the low number of non-medicated patients, the high variability of multiple medication usage, and the more or less arbitrary binary coding of its use. Our outcomes show an expression of cluster 2 and 3 genes only in female MDD patients connected to the treatment with anti-depressants. This seems peculiar with respect to the general idea that anti-depressants may have anti-inflammatory effects (Dionisie et al., 2021). Probably, the use of anti-depressants in our patients indicates a genuine MDD patient group, but this is speculative. As women showed a somewhat stronger upregulation, anti-depressant use may also relate to disease severity. Future studies should evaluate the effect of anti-depressant medication on monocyte gene expression more elaborately and over time, preferably in drug-naïve patients at enrollment.

Due to a paucity of cDNA material, fewer MDD cases could be tested for the new cluster 3 genes than evaluated by Schiweck et al. (2020). Moreover, some of the genes were tested in even smaller subgroups leading to reduced statistical power and probable type II error. Further, we have not recorded the prevalence of cardiovascular abnormalities and lipid profiles in our test subjects. It is well-known that such pathologies are related to MDD (Penninx et al., 2001; Wei et al., 2020). In previous studies of our group on metabolic syndrome patients, we found the expression profile of monocyte microRNAs/mRNAs compatible with an upregulation of cluster 2 genes (Baldeón et al., 2015). Here, we also show that the BMI is a prime factor in cluster 2 gene regulation. Lastly, our study used convenience sampling. However, important subject characteristics were not different between MDD patients and HC, and the multiple sites increase generalizability of study results.

4.6. Conclusion

The gene expression profile of monocytes of MDD patients supports a view that the low-grade inflammatory state (reported by many investigators for MDD) is part of a broader immune abnormality, i.e. a premature senescence of immune cells characterized by various mitochondrial dysfunctions related to an abnormal apoptosis/growth and cholesterol metabolism, and by inflammaging (high TNF). A history of CA is related to the downregulation of MVK and an upregulation of a cluster of various pro-inflammatory genes (amongst which IL1 and IL6) in the monocytes of the MDD patients, showing a shift to more severe monocyte inflammation (pyroptosis) on top of the premature monocyte inflammaging. Thus, we here report novel observations on biological underpinnings of MDD going beyond the previous concept of chronic low-grade inflammation extending the immune abnormalities in MDD patients to monocyte mitochondrial dysfunction and early/premature aging of the myeloid system in combination with immuno-metabolic abnormalities. Uncovering these molecular mechanisms in depression pathophysiology also revealed the importance of considering the differential roles of childhood adversity for monocyte gene expression signatures. Future studies should investigate the relationship of premature aging of monocytes to the well-described premature T cell aging in MDD (Elwenspoek et al., 2017; Ford et al., 2020; Bauer, 2008) and the role of genetic susceptibility and chronic (viral) infections in monocyte aging. Further, possibilities to use the monocyte expression signature for stratifying patients for immune therapy, such as anti-inflammatory and anti-microbial interventions, should be studied as well.

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Part of the samples and genes of this report were used for prior publication as referenced in the main body text, and part of the data were presented as poster and abstract at the Innate Immune Memory (2019, Nijmegen) and EPA (2020, virtual) congresses, respectively. A previous draft of the manuscript was uploaded to the SSRN preprint server. The present work has neither been published previously nor is it currently under consideration for publication elsewhere.

Author contributions

MSS: data analysis and interpretation, drafting the manuscript. CS: provision of data analysis expertise, draft revision. GAH: support of data preparation, draft revision. SP: support of data preparation, draft revision. BCMH: support of conception, supervising material collection, draft revision. WAD: support of conception, laboratory analyses, draft revision. MS: support of conception, laboratory analyses, draft revision. EV: site coordinator, support of conception, supervising data acquisition, draft revision. OM: support of conception, supervision data collection, laboratory analyses, draft revision. SJ: supervising data acquisition, draft revision. RM: supervising data acquisition, draft revision. SC: site coordinator, support of conception, supervising data acquisition, draft revision. BTB: site coordinator, supervision data collection, draft revision. ML: deputy coordinator, support of conception, draft revision. FB: site coordinator, support of conception, collection of material, draft revision. RF: support of conception, supervising laboratory analyses, draft revision. RB: support of conception, laboratory analyses, draft revision. HdW: support of conception, laboratory analyses, draft revision. AW: laboratory analyses, draft revision. VA: site coordinator, support of conception, supervising data acquisition, draft revision. NM: site coordinator, support of conception, supervising data acquisition, draft revision. HAD: coordinator, drafting the manuscript.

All authors approved the manuscript and are accountable for the presented work.

Code availability

The R code is available from the first author upon request.

Ethical statement

The study was conducted in accordance with the standards for Good Clinical Practice (GCP) und the declaration of Helsinki and its subsequent revisions in order to protect the rights, safety, and well-being of all participants. The relevant European and national regulations were adhered to. The study was approved by the ethical committees of the participating universities (reference numbers: Leuven: S51723; Munich: 291–09, Münster: 2009–019-f-S). Before the performance of any study-related procedures, written informed consent was obtained from all participants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2021.110391.

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Appendix A.

Previously published Affymetrix whole transcriptome profiling studies using monocytes of mood disorder patients and healthy controls

In previously published studies (1-4) we have performed whole transcriptome screening search studies search for monocyte genes which are discriminative for mood disorders as compared to healthy controls. We here give a synopsis of the previously reported findings to understand the body text of the manuscript better. To perform the whole transcriptome screening studies the RNA was isolated from purified monocytes of patients and controls using minipreparation columns (RNeasy; Qiagen, Hilden, Germany) as described by the manufacturer. The RNA was first converted into complementary DNA (cDNA) and subsequently into complementary RNA (cRNA). Fragmented cRNA was hybridized to U95Av2 microarrays (Affymetrix, according to the manufacturer's protocol). For all experiments reported herein, the 5' to 3' ratios of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 2 or less (usually 0.9-1.1).

<u>The previously published whole genome screening study on bipolar patients</u>

Affymetrix analysis was performed on the MACS-purified pooled monocytes of a series of bipolar patients and healthy controls (1). Cell pools were mainly used to limit costs and to avoid interindividual differences. One pool consisted of monocytes of 7 lithium-treated bipolar patients (1 man and 6 women; mean age, 39 years; age range, 27-57 years) compared with a pool of healthy control monocytes (1 man and 6 women; mean age, 40 years; age range, 24-56 years); the other pool consisted of 7 lithium-treated bipolar patients (4 men and 3 women; mean age, 44 years; age range, 37-57 years) compared with a pool of healthy control monocytes (4 men and 3 women; mean age, 45 years; age range, 39-53 years). The raw data of the 4 pools are also available as MIAMExpress submission E-MEXP-1275 (http://www.ebi.ac.uk/miamexpress/). After Rosetta Resolver Analysis (RMA), we found 187 discriminating genes (114 upregulated and 73 downregulated) between bipolar patients and healthy controls (2-fold difference). Ingenuity Pathway Analyses (IPA) delivered that particularly these up and down regulated genes were involved in inflammation regulation. We

decided to use for the previously published studies the inflammation related genes to serve as biomarkers for the inflammatory condition of mood disorder patients. We used the top 7 genes from the list (ie, PDE4B, ATF3, MAPK6, DUSP2, TNFAIP3, CXCL2, and BCL2A1) and 6 genes with a statistically significant differential expression of more than 2-fold with a well-known involvement in inflammation, the MAPK and IL-6 pathways, and cell movement (i.e., IL1B, IL6, TNF, PTGS2, PTX3, and CCL20). In various confirmation cohorts we found these inflammatory genes indeed abnormally expressed in monocytes of bipolar disorder patients (1,2), but also those of major depressed patients (3-5).

However, Ingenuity Pathway Analyses (IPA) also delivered that apart from genes involved in inflammation also genes involved in cell growth, differentiation, survival, and apoptosis were up regulated. IPA showed for top molecular and cellular function: cell death and apoptosis of leukocytes. In the list, **BAX** took a prominent position with a more than 2-fold altered expression. Since we wanted to concentrate on this function in the present study, we selected this gene as a characteristic gene of mitochondrial apoptosis for the present study.

The previously published whole transcriptome study on melancholic MDD patients (3)

Similar Affymetrix microarray analyses as described above were performed to search for aberrantly expressed genes in monocytes on 6 monocyte pools of 3 melancholic MDD patients each and 3 monocyte pools of age/gender matched healthy controls (for patient characteristics see Carvalho et al.; 3). In the patient pools in total 10 women and 8 men were included (mean age 54 years), in the healthy control pools 4 women and 5 men, mean age 49 years. Pools were again used for minimizing inter-individual differences in mRNA expression and to reduce costs for the expensive whole genome methodology. Thus, in total 9 chips were analyzed. We analyzed the data using the modified RMA analysis as described above, and we took for IPA the genes which were statistically differentially expressed ($p<0.01$, corrected for multiple testing) between melancholic MDD patients and healthy controls. IPA showed here also for top molecular and cellular function: cell death and particular apoptosis of leukocytes. In the list

Appendix A.

BCL10 took a prominent position with a more than 2-fold altered expression. We selected this gene as a characteristic gene of mitochondrial apoptosis for the present study.

The previously whole genome screening study on MDD patients of the cohort described by Grosse et al., 2015 (4)

Affymetrix microarray analysis was furthermore performed on 2 monocyte pools of MDD patients of the cohort described by Grosse et al. (4) (in each pool 2 patients, in pool one 2 women age 19 and 23 years, in pool two a man and a woman of 24 and 25 years) against 2 monocyte pools of 2 age/gender matched healthy controls each (in pool one 2 women of 21 and 19 years, and in pool two 2 men of 22 and 24 years). MDD patients were young and were selected not to show upregulated inflammatory cluster 1 and 2 gene expression in their monocytes (4). Thus, in total 4 chips were analyzed. We analyzed the data using the modified RMA analysis described above, and we took for IPA the genes which were statistically differentially expressed ($p<0.01$, corrected for multiple testing) between MDD patients and healthy controls.

IPA showed for top molecular and cellular function: cellular development, differentiation of blood cells, and as top canonical pathway neuregulin signaling. In the IPA lists the growth and apoptosis regulating genes **EGR1 and EGR2** and the EGF-receptor ligand gene **HB-EGF** took prominent positions with a more than 2-fold altered expression. We selected these genes as characteristic genes for mitochondrial apoptosis and growth regulation.

Appendix A.

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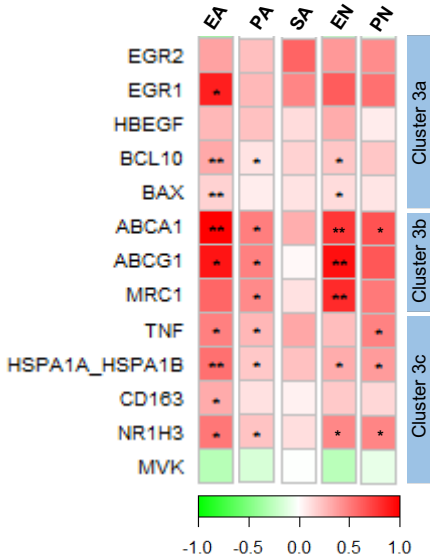
Appendix B.

Supplementary Table 1. Gene identification of Applied Biosystems.

gene symbol	assay ID
ABL1	Hs99999002_mH
BCL2A1	Hs00187845_m1
MXD1	Hs00231137_m1
ADM	Hs00181605_m1
CDC42	Hs00741586_mH
ATF3	Hs00231069_m1
MAFF	Hs00202412_m1
EGR3	Hs00231780_m1
DUSP2	Hs00358879_m1
PTGS2	Hs00153133_m1
NR3C1b	Hs00354508_m1
IL1B	Hs00174097_m1
IL1A	Hs00174092_m1
IL6	Hs00174131_m1
PTX3	Hs00173615_m1
CXCL2	Hs00236966_m1
EREG	Hs00914313_m1
CCL20	Hs00355476_m1
TNFAIP3	Hs00234712_m1
CCL7	Hs00234140_m1
CCL2	Hs00171147_m1
EMP1	Hs00608055_m1
IRAK2	Hs00176394_m1
NAB2	Hs00195573_m1
STX1A	Hs00270282_m1
MAPK6	Hs00833126_g1
THBD	Hs00264920_s1
SERPINB2	Hs00234032_m1
IL1R1	Hs00168392_m1
NR3C1a	Hs00353740_m1
HMOX1	Hs01110250_m1
EGR2	Hs00152928_m1
EGR1	Hs00166165_m1
HBEGF	Hs00174705_m1
BCL10	Hs00961847_m1
BAX	Hs00180269_m1
ABCA1	Hs01059137_m1
ABCG1	Hs00245154_m1
MRC1	Hs00267207_m1
TNF	Hs00174128_m1
HSPA1A/B	Hs00271229_s1
CD163	Hs00174705_m1
NR1H3	Hs00172885_m1
MVK	Hs00176077_m1

Appendix C.

Supplementary Figure 1. Cluster 3 gene expressions per CTQ subscale in MDD patients.



Legend. MDD Major Depressive Disorder; CTQ childhood trauma questionnaire; EA emotional abuse; PA physical abuse; SA sexual abuse; EN emotional neglect; PN physical neglect. Mean values are expressed relative to the expression level of HCnoCA (healthy control without childhood adversity); the intensity of red reflects higher expression, intensity of green lower expression; stars indicate level of significance; p-values of group comparison are given. *p<0.05 **p<0.01

Appendix D.

A detailed description of function and interaction of the separate genes in the molecular gene modules activated in monocytes of MDD patients (see Figure 4)

The Mitochondrial apoptosis module:

BAX is one of the most consistent over expressed genes in monocytes of MDD patients. It plays an activating role in mitochondrial apoptosis, via activation of the mitochondrial apoptosis complex (mac) which results in activation of the apoptosome (via cytochrome c) leading to mitochondrial apoptosis (1). It also leads to enhanced ROS production. Both apoptosis and ROS production are known of senescent cells (2).

BCL10 is equally over expressed in monocytes of MDD patients. It plays a role in suppressing mitochondrial apoptosis, thus suppressing the actions of BAX (3). It also activates NFκB, thus contributing to the stimulation of the inflammatory molecular cascade in MDD monocytes (3).

EGR1 belongs to a family of transcription factors (the EGRs) playing key roles in coordinating subsequent waves of gene expression after the immediate early gene response induced by MAPKs (4,5). EGR1 is a pro-apoptotic factor, and apoptosis can be induced amongst other mechanisms by a ROS/MAPkinase/EGR1/BAX interaction (6). EGR1 is also involved in foam cell formation, showing an M1 like pro-inflammatory function (7).

EGR2 is an anti-apoptotic growth promoting factor and involved in the M1 and M2 polarization of monocytes to macrophages (8). EGR2 expression is associated with the ability of unstimulated or M2 macrophages to respond to stimulation with inflammatory stimuli, while low levels of EGR2 expression are associated with non-responsiveness of monocytes/macrophages to activation.

HB-EGF (belonging to cluster 3, but depicted in the upper left quadrant as secreted molecule) is a member of the EGF family of ligands. HB-EGF binds to and activates EGF receptors, and thus stimulates the MAPK routes (9). Since HB-EGF was first identified as a novel growth factor secreted from a human macrophage cell line, numerous functions related to cell proliferation, migration, and inflammation have been reported. HB-EGF also plays a role in e.g. wound healing (M1 and M2 skewing), angiogenesis and adipogenesis (10).

The module of the senescence associated secretory phenotype (SASP) and unfolded protein response (cluster 3c genes, also lower right part of the figure):

TNF is expressed predominantly by immune cells and the cytokine regulates diverse cell functions, such as induction of fever, cachexia, inflammation and apoptosis, inhibition of tumorigenesis and viral replication. In this context it is relevant that TNF can downregulate mitochondrial apoptosis via BAX down regulation involving as intermediate TNFAIP3 (11).

Appendix D.

Hsp70 belongs to the heat shock proteins and protects cells from oxidative stress. Oxidative stress damages proteins, causing partial unfolding and possible aggregation (the unfolded protein response due to endoplasmic reticulum stress). Hsp70 prevents these partially denatured proteins from aggregating and inhibits them from refolding. In addition to improving overall protein integrity, Hsp70 also inhibits apoptosis by blocking the recruitment of procaspase-9 to the apoptosome complex (12). HSPs also act as immunomodulators, hsp70 is thought to have an anti-inflammatory action activating the production of IL-10 from monocytes, yet for other cells it acts as an immune-stimulant (13).

Cholesterol metabolism module (cluster 3b and some 3c genes, lower left part of the figure):

ABCA1 and ABCG1 are membrane molecules involved in the export shuttle of cholesterol from the cell to ApoE and ApoA1. Since cholesterol accumulation in the cell is a pro-inflammatory event, overexpression of ABCA1 and ABCG1 is a sign of an anti-inflammatory state (14). The molecules are also marker molecules of M(hb) cells, the athero-protective macrophages in atherosclerotic plaques clearing hemoglobin from the atherosclerotic plaque (15).

MRC1 encodes the mannose receptor (CD206), which is a C-type lectin primarily present on the surface of monocytes/macrophages/dendritic cells. It mediates the phagocytosis of certain classes of microorganisms and plays a role in antigen presentation. More importantly in this context is that it is a marker of anti-inflammatory M2 macrophages playing a role in the resolution of inflammatory responses; it is also a marker of M(hb) cells (16).

NR1H3 codes the LXR-α transcription factor, which is involved in the transcription of ABCA1 and ABCG1 (17). LXR-α transcription is induced by hydroxy-sterols (such as 27-OH cholesterol) generated from the cholesterol synthesis route, thus forming a regulatory system for intracellular cholesterol accumulation. It is also a marker of M(hb) cells (15).

CD163 is the receptor for the hemoglobin/haptoglobin complex and uptake of the complex induces and anti-inflammatory cascade in monocytes/macrophages and production of IL-10 via PI3 kinase (18). It is a marker of M(hb) cells (15).

The module of MAP Kinases and EGRs (upper left part of the figure):

The mitogen-activated protein kinases (MAPKs) take a prominent place in both the gene clusters 1 and 2 and are fundamental regulators of immune cell function (4). The 3 main classes are the extracellular signal-regulated kinases (ERKs), the p38 proteins, and the Jun N-terminal kinases (Jnks), which shuttle after an activating phosphorylation into the nucleus and initiate

the transcription of immediate Early Response Genes (EGRs). The EGRs are clearly upregulated in MDD monocytes.

PDE4B (phosphodiesterase type 4B, a cluster 1 gene) is a cyclic adenosine monophosphate (cAMP)– degrading enzyme. c-AMP coupled receptors are involved in the aberrant regulation of the MAPK pathway. c-AMP coupled receptors are a target of adrenaline. cAMP is known to be inhibitory for inflammatory cells and a high activity of PDE4B leads to a pro-inflammatory state (19,20). PDE4 inhibitors broadly inhibit functions of inflammatory cells (19,20).

Of the actual MAPKs **MAPK6/ERK3** (a cluster 2 gene) is overexpressed in MDD monocytes. EGRs are transcribed via the transcription factor Elk-1 (4), but EGR transcription can also be induced by activating transcription factor 3 (ATF3).

ATF3 (a cluster 1 transcription factor in the MAPK route) (4) is bi-directionally regulated by the Jnk (positive) and ERK (negative) pathways (21) and is induced during cellular stress.

DUSP2 (a cluster 1 gene encoding PAC1) localizes to the nucleus and is one of the most highly induced transcripts in activated immune cells. Dual-specificity phosphatases (DUSPs) regulate MAPK activity through de-phosphorylation and also anchor or shuttle MAPKs (22,23). DUSP2 has a positive function in macrophage-mediated inflammatory responses via a lowering of the level of expression of Jnks and a compensatory rise in that of ERKs and p38 (22).

NAB2 (a cluster 2 genes) is a corepressor molecule that directly binds EGR-1 and inhibits its transactivating potential (24).

MAFF and MXD1 (cluster 1 genes) are important regulators of the transcription of EGRs. MAFF regulates many processes, including proliferation (25), while the MYC/MAX/MXD1 complex antagonizes cell transformation (26).

EGR3 (a cluster 1 EGR) is induced by Elk-1; as indicated above the EGR family plays a key role in coordinating subsequent waves of gene expression after the immediate early gene response induced by MAPKs to regulate not only apoptosis, but also growth and inflammation (4,27).

EREG (epiregulin, a cluster 1 gene) (28) is a growth and chemotactic factor for monocytes and known as an M2 skewer. EREG binds to the EGF-R and is thereby able to feed back to the MAP-kinase pathway.

GRβ (the blocking glucocorticoid receptor, thus rendering a cell unresponsive to high glucocorticoids) is part of cluster 1 and correlates positively to the MAPK pathway machinery for inflammation and apoptosis. A higher expression of GR beta in MDD monocytes thus suggests that the cells are refractory to GC with respect to the activation of the MAP Kinase machinery.

Appendix D.

The module of pro-inflammatory cytokines and compounds (upper right part of the figure):
Activation of the MAP-kinase pathway not only leads to growth and apoptosis regulation but also to the production of pro-inflammatory cytokines and compounds. The cluster 1 genes **IL1B, IL1A, IL6, PTX3, PTGS2, and CXCL2** are examples of these well-known inflammatory compounds.
IRAK-2 is via the ERK and NKκB pathways involved in the signaling from TLR4 and TLR2 to a proinflammatory state of monocytes with increased pro-inflammatory cytokine production (29,30).

Pyroptosis module (middle left part of the figure):
MVK (a cluster 3 gene) is significantly reduced in MDD cases with a history of childhood adversity. MVK represents the enzyme mevalonate kinase in the cholesterol production cascade, playing a role in the transition of mevalonate to isoprenoids, the latter having a potent anti-inflammatory action. Deficiency of the enzyme leads to fever syndromes, in which the pyroptosome is activated (1).
MVK additionally plays an important role in trained immunity (31). Trained immunity is a longlasting sensitization of the macrophage to react stronger to danger signals via an epigenetic imprinting, i.e. mevalonate accumulation due to low levels of MVK leads to methylation of histon H3 (H3K4me3) with increases in the transcription of IL-6 and TNF (31,32). The trained immunity process involving MVK therefore results in a long lasting higher inflammatory responsiveness of monocytes/macrophages (31).
TNFAIP3 (a cluster 1 gene) is part of the signaling cascade downstream from the TNF-receptor (TNFR) (11). It has direct interactions with the BCL-2 family (see before) TNFAIP3 (also called A20) blocks the activation of the Jnk cascade by TNF-alpha (33) and in this way demonstrates anti-apoptotic and anti-inflammatory behavior (34).
BCL2A1 (a cluster 1 gene) is a well-known anti-apoptotic molecule (35).
ADM (Adrenomedulin, a cluster 1 gene) binds to cytoskeletal elements and is a multifunctional protein with immune and inflammation regulating properties, it promotes anti-apoptotic Bcl-2 release (36).

Appendix D.

Module of shape change, chemotaxis, adhesion and coagulation intervention (mainly top of the figure, all cluster 2 genes):

CCL2 and CCL7 are important well-known chemo-attractants for monocytes and other myeloid cells. They are produced very early in the inflammatory response, when monocytes diapedese through the endothelial layer.

EMP1 is a tetraspan transmembrane protein playing a role in cell-cell adhesion and interactions with the extracellular membrane (37). Although the function of the molecule is not entirely known, it appears to be involved in cell survival and growth (38).

STX1A is a SNARE protein involved in vesicular docking and fusion to the plasma membrane. It is thought to be instrumental in the secretion of cytokines (39). Peculiar is that it is considered neuronal specific, while we here show gene expression in monocytes.

CDC42 acts as a molecule related to the cytoskeletal organization of the cell, its motility, and chemotactic potential (40), but also as a Rho glutamyl transpeptidase–signaling molecule upstream from the MAPKs (41) and as a molecule instrumental in the secretion of cytokines.

SERPINB2 is an abundant protein in activated macrophages and upregulated by TNF (42). SERPINB2 prevents death and cessation of the innate immune response of TLR4 activated macrophages, and it is also linked to monocyte proliferation and differentiation linked to Th1 and Th2 skewing (43). SERPINB2 is also known as plasminogen activation inhibitor type 2 (PAI2), which is classically viewed as an inhibitor of fibrinolysis, it also plays a role in platelet aggregation (44).

THBD (thrombomodulin) is a potent anti-inflammatory factor produced by monocytes/macrophages stimulated by TLR4 (45). It is used as an anti-inflammatory drug counteracting septic shock with disseminated intravascular coagulation (46).

IL-1R1 (the IL-1 receptor 1) is the receptor for IL-1β and combines with the adaptor MyD88 to signal to NFκB to promote the inflammatory response (47).

HMOX-1 encodes heme oxygenase-1 (HO-1) which catalyzes the first and rate-limiting enzymatic step of heme degradation and produces carbon monoxide, free iron, and biliverdin. HO-1 has immunomodulatory and anti-inflammatory properties, it also blocks ROS production. HO-1 induction drives the phenotypic shift to M2 macrophages (48).

GRα (the activating glucocorticoid receptor, thus rendering a cell responsive to high glucocorticoid reactions during stress) is part of cluster 2 and correlates positively to cluster 2 genes involved in monocyte adhesion, shape change and chemotaxis, suggesting that these processes can be regulated by glucocorticoids in MDD monocytes.

Appendix D.

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Efficacy of Sertraline Plus Placebo or Add-On Celecoxib in Major Depressive Disorder: Macrophage Migration Inhibitory Factor as a Promising Biomarker for Remission After Sertraline—Results From a Randomized Controlled Clinical Trial

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Introduction: Previous research delivers strong indications that inflammatory activation leads to treatment resistance in a subgroup of patients with Major Depressive Disorder (MDD). Thus, tailored interventions are needed. The present study aimed to find potential biomarkers that may enable patients to be stratified according to immune activation.

Methods: A phase IIa randomized placebo-controlled trial was performed to assess levels of inflammatory compounds in responders/remitters and non-responders/non-remitters to sertraline plus celecoxib ($n = 20$) and sertraline plus placebo ($n = 23$). Levels of macrophage migration inhibitory factor, neopterin, and tumor necrosis factor alpha were determined by enzyme-linked immunosorbent assay; response and remission were measured by reduction of the Montgomery Åsberg Depression Rating Scale score.

Results: Both treatment groups showed a significant decline in depression symptoms, but no difference was found between groups. A clear pattern emerged only for macrophage migration inhibitory factor: placebo remitters showed significantly lower baseline levels than non-remitters (a similar trend was seen in responders and non-responders) while celecoxib responders showed a trend for higher baseline levels than non-responders.

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Conclusion: Small subsample sizes are a notable limitation, wherefore results are preliminary. However, the present study provides novel insights by suggesting macrophage migration inhibitory factor as a promising biomarker for treatment choice.

The trial was registered in EU Clinical Trials Register (EU-CTR): https://www.clinicaltrialsregister.eu/ctr-search/trial/2009-011990-34/DE, EudraCT-No.: 2009-011990-34.

Keywords: inflammatory, Major depressive disorder, cytokine, response, biomarker, anti-inflammatory treatment

INTRODUCTION

In clinical practice, patients' response to antidepressant treatment often remains unsatisfactory. Around 20% up to 50% of depressed patients show non-response to at least two standard antidepressant drug trials (1, 2). Furthermore, remission rates across different antidepressant treatment options are at 28% after initial treatment attempt and remission rates further decrease with each treatment failure (3). Thus, identifying patients prone to treatment resistance is important to enable early use of alternative treatment options. Previous research has consistently shown that inflammatory activation plays a role in the pathophysiology of Major Depressive Disorder (MDD) and pro-inflammatory activation has been implicated in treatment resistance to standard antidepressant medication in several studies (4). Results indicate that low-grade inflammation is present in a subgroup of MDD patients (5, 6) characterized by higher levels of circulating pro-inflammatory compounds (7–9). Overall, higher levels of these compounds were associated with depression, though results of single study show some variety (10–13). In particular, compounds such as C-reactive protein (CRP), Interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα) have been frequently described. Deficits in the T cell system and pro-inflammatory monocyte activation were also shown to be present in MDD patients (14–17).

Most studies investigate inflammatory markers from the periphery, while depressive symptoms result from dysregulations in the brain. Peripheral cytokines activate afferent nerves to the brain and can enter the brain themselves leading to further pro-inflammatory cytokine output by microglia in the brain (18–20). Besides the above-described parameters, markers of monocyte/macrophage activation (and endothelial function) have also been associated with MDD: Circulating levels of macrophage migration inhibitory factor (MIF) and neopterin were increased in depression as compared to healthy controls (21, 22). Monocytes are an important compound of the innate immune system because they are drivers of inflammation by releasing pro-inflammatory cytokines (23). Interestingly, multiple studies have confirmed a comorbidity between MDD and cardiovascular disease (24–26), and the link between MDD, low-grade inflammation, and cardiovascular events is highly suggested pointing to shared biological underpinnings (27). Thus, the use of anti-inflammatory drugs in MDD seems to be a reasonable approach to increase responsiveness. In patients with atherosclerosis, cyclooxygenase-(COX-)2, prostaglandin E receptors, and prostaglandin E synthase-1 were overexpressed in plaques and peripheral blood mononuclear cells [PBMCs; (28)]. Furthermore, stimulated macrophages exposed to high levels of oxidized low-density lipids (oxLDL) exhibit higher COX-2 (29). Thus, COX-2-inhibitors may be a promising approach because they inhibit synthesis of prostaglandin E_2 (PGE_2), which acts as a stimulator of indoleamine-2,3-dioxygenase [IDO; (30)] and mediates inflammatory response (31). IDO activation, in turn, promotes the conversion of tryptophan along the kynurenine pathway instead of serotonin and may explain the serotonin depletion and neurodegeneration hypotheses of depression (20, 32). Interestingly, depressed patients showed increased serum PGE_2 (33).

Several reviews and meta-analyses have evaluated the efficacy of anti-inflammatory treatments in MDD patients, mostly concluding an overall limited beneficial effect for clinical outcome (34–39). These overview articles included four important trials that investigated the efficacy of celecoxib (a COX-2-inhibitor) augmentation to sertraline, fluoxetine, or reboxetine and showed a greater decline of symptom severity as compared to add-on placebo [although both groups showed a significant symptom reduction; (40–43)]. Given the notion that inflammatory activation is present only in a subgroup of patients, a critical point in former analyses is the evaluation of efficacy without addressing differential inflammatory levels and the evaluation of the relation between inflammatory compounds and response across treatment arms. Consequently, potential differentiating effects of inflammatory status by treatment were lost, which is reflected by the variability of, discrepancy between, or lack of positive individual study results (4, 44). Thus, it is necessary to investigate efficacy of different treatment regimens with respect to the levels of inflammatory compounds. A systematic review revealed that higher biomarker levels (IL-6, CRP, TNFα) were associated with treatment resistance to predominantly serotonergic acting drugs and that response improved using mainly noradrenergic or dopaminergic acting drugs, as well as using anti-inflammatory drugs (45). Further, when levels of these biomarkers were low, response to several anti-inflammatory agents was even lower as compared to placebo (45). Thus, studies need to compare levels of inflammatory biomarkers depending on response status per treatment arm is needed to gain more insight into patient profiles, which may help to individualize treatment options.

Here, we evaluated data from a trial designed to investigate the relation between levels of inflammatory compounds and

response to add-on placebo vs. add-on celecoxib to standard selective serotonin reuptake inhibitor (SSRI) sertraline in MDD patients before reaching the state of treatment resistance (patients with no more than two unsuccessful treatments). In this report, we exploratively investigated serum MIF, neopterin, and TNFα levels because these compounds represent markers of macrophage and inflammatory activation. An earlier study by our group found elevated MIF levels in MDD patient, but it did not focus on patient subgroups (22). Other than that, to our knowledge MIF and neopterin have not yet been investigated in this context. Additionally, TNFα levels emerged as predictor of response to a TNF antagonist in depression (46), thus we were interested in studying this effect with celecoxib. Further, we also analyzed efficacy data independent from biomarker levels, as well as patient characteristics that are known to be related to inflammation and cardiovascular risk [i.e., smoking status, sex, body mass index (BMI), and age; (47–49)].

MATERIALS AND METHODS

This phase IIa study used a randomized, double-blind, placebo-controlled, parallel group design was used. Considering a high and variable placebo response in depression, a placebo control was chosen. Patients and investigators/study staff were blinded to treatment allocation. Psychiatric inpatients were sampled by convenience and randomly assigned in a 1:1 ratio by fixed block randomization to 6 weeks of either sertraline plus placebo or sertraline plus celecoxib treatment. The study was approved by the ethics committee of the medical faculty of Ludwig-Maximilians-University Munich (project-nr. 234-09). The trial was performed in compliance with the standards of good clinical practice and in accordance with the Declaration of Helsinki and its subsequent revisions. All participants provided written informed consent.

Participants

Patients aged between 18 and 60 years were included if they had been diagnosed with MDD by a psychiatrist (DSM-IV-TR) and had a baseline Montgomery Åsberg Depression Rating Scale [MADRS; (50)] score of 20 or above (indicating moderate to severe depression). Exclusion criteria were as follows: comorbid psychotic depression, bipolar disorder, addiction, schizoaffective disorder, schizophrenia, and other psychiatric disorders if their symptomatology was predominating. Also excluded were patients taking concomitant psychotropic drugs or anti-inflammatory pain medication such as COX-2-inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), or paracetamol; pregnant or breastfeeding women; patients with history of cardiovascular disease or heart disease, or with current cardiovascular disturbances; and patients with inflammatory or other relevant diseases were excluded from the study. For the full inclusion and exclusion criteria see **Supplementary Table A**.

Measures

Serum levels of MIF, neopterin, and TNFα were assessed at baseline and endpoint (week 6). Blood was drawn in fasting condition. All parameters were determined by enzyme-linked immunosorbent assay (ELISA) with standard curve by using the quantitative sandwich enzyme immunoassay technique (MIF, TNFα) and competitive enzyme immunoassay technique (neopterin), according to the instruction of the kit manufacturer (MIF ELH-MIF, RayBio®, Peachtree Corners, USA; TNFα HSTA00D, R&D Systems, Minneapolis, USA; neopterin EIA-2949, DRG International, Inc., Springfield, USA) and analyzed using MARS data analysis software (BMG Labtech, Ortenberg, Germany). BMI was calculated as body weight (in kg) divided by the square of body height (in m). Depression severity was assessed by trained raters at baseline and endpoint (6 weeks) with the MADRS (50, 51). Response was defined as a reduction of MADRS score of at least 50% on at endpoint, depending on the individual baseline score, and remission was defined as a score of 7 or lower at endpoint (52).

Treatment Protocol

All patients who were taking antidepressant medication prior to the study underwent a 3-day wash-out period before the start of trial treatment. In case of premedication with long half-life, a longer period since the last treatment was necessary to be included (see **Supplementary Table A**). If needed, lorazepam was administered up to 4 mg/day during wash-out and for the first 2 weeks, up to 3 mg/day for the third week, up to 2.5 mg/day for the fourth and fifths weeks, and up to 1.5 mg/day for the sixth week. Zopiclone was administered up to 7.5 mg/nightly during the wash-out period (one patient had 15 mg) and the study time, if needed. At baseline, eligible patients were randomized to one of the following treatment arms: 50–100 mg sertraline daily (one tablet/unblinded) plus celecoxib twice daily (one capsule/200 mg/blinded) or 50–100 mg sertraline daily (one tablet/unblinded) plus placebo twice daily (one capsule/blinded) The placebo capsule contained 308 mg microcrystalline cellulose coated by hard gelatin and was of the same size, weight, color, and shape as the celecoxib capsule. If a higher clinical benefit was expected, a dose of 150 mg sertraline was allowed.

Statistics

SPSS software (IBM SPSS Statistics 25) was for statistical analyses used. The trial data presented here were planned as secondary per-protocol analyses. In this report, the primary outcomes were response and remission independently of inflammatory status and cytokine serum levels according to the response and remission status. Secondary analyses, investigated the role of BMI, age, sex, and smoking status. Endpoint parameter values were subtracted from baseline parameter values to reflect the change of values over time. Therefore, positive values represent a decrease and negative values represent an increase of levels over time. Descriptive analyses are given as mean and standard deviation or median and interquartile range for continuous variables, and as frequencies (percentages) for categorical variables. Testing for significances was performed using Chi-square test for categorical data (in case of 5 or fewer observations in the cells of the contingency table, Fisher's exact test was performed), Student's *t*-test for continuous data that met the criteria for parametric testing (in case of inhomogeneous variances, Welch-test is reported; in case of non-normality,

Mann-Whitney-*U*-test was performed), or linear regression. To compare levels of inflammatory compounds between subgroups, Mann-Whitney-U-test for independent samples and Wilcoxon signed-rank test for dependent samples were used if data did not meet assumptions for parametric testing and/or small subsamples were tested. The significance level was set at 5% (two-sided) for all tests. As analyses were exploratory and subsamples were small, adjustment for multiple testing and correction for possible confounding variables were neglected in the primary analyses. Hence, the data reported here are preliminary. Due to statistical power limitations, trends are also reported ($p < 0.10$). The flow chart is given in **Supplementary Figure 1**.

RESULTS

Patient Characteristics and Levels of Immune Parameters

Table 1 shows the descriptive demographic data, depression severity, and baseline biomarker levels in each treatment group. **Table 2** shows the levels of immune parameters at baseline and endpoint in the placebo- and celecoxib-treated groups, as well as for responders/remitters and non-responders/non-remitters. Because drug treated patients were randomized but sample is of limited size, demographic data and baseline parameter values were tested for differences between the treatment arms. No statistically significant differences emerged for MADRS baseline score, MADRS endpoint score, percentage MADRS score reduction over time, age, baseline BMI, BMI at endpoint, sex distribution, smoking status, and immune parameter values at baseline between placebo and celecoxib groups (see **Supplementary Table B**).

Response and Remission Rates

Table 3 shows the proportions of responders and remitters in each treatment arm. No significant differences emerged for the distribution of response rates ($\chi^2 = 0.62$; $p = 0.43$) or remission rates ($\chi^2 = 0.49$; $p = 0.49$) between the two treatment groups. Both treatment groups showed a significant decline of MADRS scores over time (placebo: $T = 12.81$; $SE = 1.31$; $p < 0.001$; 95% $CI = [14.07; 19.51]$; celecoxib: $T = 7.86$; $SE = 2.00$; $p < 0.001$; 95% $CI = [11.53; 19.94]$). In responders, the MADRS score decreased slightly more in the celecoxib group than in the placebo group, although the difference was not significant ($T = -1.15$; $SE = 1.75$; $p = 0.26$; 95% $CI = [-5.61; 1.60]$). In non-responders, the MADRS score decreased slightly more in the placebo group than in the celecoxib group, but this difference was also not significant ($T = 1.01$; $SE = 2.45$; $p = 0.33$; 95% $CI = [-2.81; 7.77]$).

Predictive Capability of Biomarkers for Response and Remission

MIF

Figure 1 shows the results for MIF at baseline. In the placebo group (sertraline only), responders showed a trend for lower MIF levels at baseline compared with non-responders, and remitters showed significantly lower MIF levels at baseline than non-remitters. In the celecoxib group (sertraline plus celecoxib), responders showed a trend for higher MIF levels at baseline

TABLE 1 | Descriptive characteristics of patients with MDD.

	Sertraline + placebo		Sertraline + celecoxib	
MADRS baseline *Md (IQR)*	29.00 (4.00)	*N* = 23	28.00 (8.00)	*N* = 19
MADRS endpoint *M (SD)*	12.35 (7.75)	*N* = 23	13.20 (7.21)	*N* = 20
% MADRS score reduction *M (SD)*	58.91 (23.05)	*N* = 23	52.16 (26.25)	*N* = 19
Age *M (SD)*	38.78 (10.71)	*N* = 23	39.25 (12.75)	*N* = 20
BMI baseline *M (SD)*	23.28 (3.46)	*N* = 23	23.31 (3.22)	*N* = 20
BMI endpoint *M (SD)*	22.58 (3.26)	*N* = 21	22.82 (2.93)	*N* = 19
Sex women *N* (%)	12/23 (52.17)		9/20 (45.00)	
Smoking yes *N* (%)	7/23 (30.43)		11/20 (55.00)	
MIF (pg/ml) *Md (IQR)*	3484.00 (5002.25)	*N* = 22	4306.00 (4638.25)	*N* = 18
Neopterin (ng/ml) *Md (IQR)*	0.85 (0.47)	*N* = 22	0.73 (0.49)	*N* = 19
TNFα (pg/ml) *Md (IQR)*	0.78 (0.81)	*N* = 19	0.76 (0.71)	*N* = 15

BMI, body mass index; MADRS, Montgomery Åsberg Depression Rating Scale; Md, median; IQR, interquartile range; M, mean; SD, standard deviation. MADRS score at baseline was available in only 19 of the 20 patients in the celecoxib group but was available in all 20 patients at endpoint assessment.

TABLE 2 | Baseline and endpoint biomarker levels according to treatment and response status.

	Baseline *Md (IQR)*	Week 6 *Md (IQR)*	*Z (N)*	*p*
MIF (pg/ml)				
Placebo	3484.00 (5002.25)	2102.00 (2862.50)	−1.61 (22)	0.11
Celecoxib	3406.00 (4638.25)	4197.50 (3826.75)	−0.28 (18)	0.78
Responder	3484.00 (4948.25)	3078.00 (4820.00)	−0.60 (26)	0.55
Non-responder	4092.00 (5301.50)	3519.00 (2888.00)	−0.73 (13)	0.46
Remitter	2663.00 (3.697)	1207.50 (2893.00)	−1.96 (12)	0.05^{+}
Non-remitter	4321.50 (5545.75)	3736.50 (4160.50)	−0.18 (28)	0.86
Neopterin (ng/ml)				
Placebo	0.85 (0.47)	0.79 (0.44)	−0.52 (22)	0.60
Celecoxib	0.73 (0.49)	0.88 (0.21)	−2.15 (19)	0.03*
Responder	0.72 (0.50)	0.86 (0.38)	−1.21 (26)	0.23
Non-responder	0.87 (0.38)	0.91 (0.36)	−1.67 (14)	0.10
Remitter	0.80 (0.48)	0.81 (0.47)	−0.08 (12)	0.94
Non-remitter	0.76 (0.51)	0.88 (0.36)	−2.12 (29)	0.03*
TNFα (pg/ml)				
Placebo	0.78 (0.81)	0.92 (0.89)	−1.25 (19)	0.21
Celecoxib	0.76 (0.71)	0.78 (1.02)	−0.34 (15)	0.73
Responder	0.71 (0.85)	0.68 (0.94)	−0.57 (21)	0.57
Non-responder	0.96 (0.96)	1.08 (0.96)	−0.11 (13)	0.92
Remitter	0.75 (0.90)	0.63 (0.59)	−0.15 (10)	0.88
Non-remitter	0.81 (0.86)	1.07 (1.00)	−0.69 (24)	0.49

*Md, median; IQR, interquartile range; Z-test statistic Wilcoxon signed rank test. *$p < 0.05$ and $^{+}p < 0.10$.*

compared with non-responders, but no significant difference emerged between remitters and non-remitters. Statistical test results are shown in **Table 4A**. At endpoint, MIF levels were not significantly different between responders and non-responders in the placebo group but were significantly lower in remitters compared with non-remitters. In the celecoxib group, responders did not differ statistically from non-responders at endpoint, but remitters showed a trend for lower MIF levels than non-remitters. Statistical test results are shown in **Table 4B**.

Regarding the change of MIF levels over time, in the placebo group non-responders showed a trend for a decrease to endpoint ($Z = -1.86$; $p = 0.06$), but no differences of MIF change between placebo responders and non-responders were found. However, the celecoxib group showed a trend for differential change of MIF levels over time: Remitters showed a decrease while non-remitters showed an increase ($U = 14.00$; $p = 0.07$). Furthermore, the change in MIF levels was significantly different in non-responders and non-remitters to celecoxib (increase) compared with non-responders and non-remitters to placebo (decrease; $U = 6.00$; $p = 0.03$ and $U = 51.00$; $p = 0.03$, respectively).

Neopterin

No significant differences in neopterin levels emerged at baseline and endpoint between responders and non-responders as well as remitters and non-remitters in either treatment arm (see **Supplementary Tables C.1**, **C.2**). Investigating change over time, only non-responders to celecoxib showed a trend for an increase of neopterin levels ($Z = -1.95$; $p = 0.05$).

TNFα

No significances emerged for TNFα levels at baseline. At endpoint, only lower levels were found in placebo responders

TABLE 3 | Response and remission rates of MDD patients in each treatment arm.

	Responder	Non-responder
Sertraline + placebo % (*N*)	69.6 (16)	30.4 (7)
Sertraline + celecoxib % (*N*)	57.9 (11)	42.1 (8)
	Remitter	**Non-remitter**
Sertraline + placebo % (*N*)	34.8 (8)	65.2 (15)
Sertraline + celecoxib % (*N*)	25.0 (5)	75.0 (15)

TABLE 4A | Comparison of baseline MIF levels of different response statuses in the two treatment arms.

Sertraline +	Responder (*Md*)	Non-responder (*Md*)	*U*	*p*
placebo	2853.00 pg/ml	4743.00 pg/ml	29.00	$<0.10^{+}$
	Remitter (*Md*)	Non-remitter (Md)	*U*	*p*
	501.00 pg/ml	4240.00 pg/ml	15.00	$<0.01^{**}$
Sertraline +	Responder (*Md*)	Non-responder (*Md*)	*U*	*p*
celecoxib	4961.00 pg/ml	1901.00 pg/ml	15.00	0.07^{+}
	Remitter (*Md*)	Non-remitter (*Md*)	*U*	*p*
	4209.00 pg/ml	4403.00 pg/ml	24.00	0.40

Md, median; U-Mann-Whitney-U-test statistic. $^{**}p < 0.01$ *and* $^{+}p < 0.10$.

TABLE 4B | Comparison of endpoint MIF levels of different response statuses in the two treatment arms.

Sertraline +	Responder (*Md*)	Non-responder (*Md*)	*U*	*p*
placebo	1066.00 pg/ml	2924.00 pg/ml	37.00	0.28
	Remitter (*Md*)	Non-remitter (Md)	*U*	*p*
	480.00 pg/ml	2924.00 pg/ml	8.00	$<0.01^{**}$
Sertraline +	Responder (*Md*)	Non-responder (*Md*)	*U*	*p*
celecoxib	3970.00 pg/ml	4814.00 pg/ml	32.00	0.92
	Remitter (*Md*)	Non-remitter (*Md*)	*U*	*p*
	3579.00 pg/ml	4587.00 pg/ml	15.00	0.09^{+}

Md, median; U-Mann-Whitney-U-test statistic. $^{**}p < 0.01$ *and* $^{+}p < 0.10$.

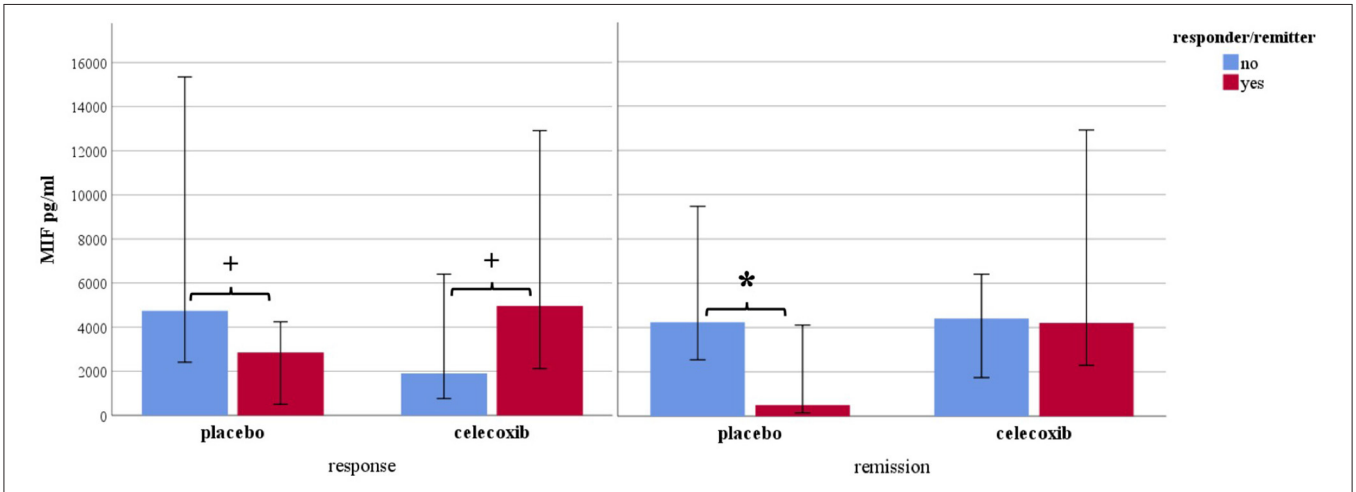

FIGURE 1 | Macrophage migration inhibitory factor levels at baseline according to response and remission status. Median levels are displayed in pg/ml; 95% confidence interval error bars are displayed; *placebo* sertraline plus placebo group; *celecoxib* sertraline plus celecoxib group. $^{*}p < 0.05$ and $^{+}p < 0.10$.

compared with placebo non-responders. For further details, see **Supplementary Tables D.1**, **D.2**. Investigating change over time, placebo non-responders showed a trend for an increase of TNFα levels over time ($Z = -1.86$; $p = 0.06$). In contrast, celecoxib non-responders showed a decrease of TNFα levels over time ($Z = -2.21$; $p = 0.03$). In the celecoxib group, responders showed differential course over time (increase) compared with non-responders (decrease; $U = 8.00$; $p = 0.03$). In non-responders, celecoxib-treated patients showed a decrease of TNF α levels over time whereas placebo-treated patients showed an increase ($U = 1.00$; $p < 0.01$).

Patient Characteristics Associated With Inflammation

The most interesting and relevant results were obtained for MIF. Therefore, possible confounders were further investigated.

BMI

No significant correlation was found between BMI and MIF baseline levels (Spearman-*Rho* = 0.23; $p = 0.16$). We found no difference in the change of BMI over time according to treatment ($T = -0.54$; $p = 0.59$) or response/ remission status across treatments ($T = -0.44$; $p = 0.66$ and $T = 0.15$; $p = 0.88$, respectively) occurred. However, in the placebo group a decrease in BMI over time significantly predicted a lower response (% MADRS score reduction) [$R^2 = 0.29$; $F_{(1, 19)} = 7.85$; ß = −22.37; $p = 0.01$].

Age

We found a significant positive correlation between age and MIF levels (Spearman-*Rho* = 0.34, $p = 0.03$) at baseline. No difference emerged in age according to response/ remission status ($T = -0.56$; $p = 0.58$ and $T = -1.04$; $p = 0.31$, respectively) across treatments. However, higher age significantly predicted a lower response (% MADRS score reduction) in the placebo group [$R^2 = 0.19$; $F_{(1, 21)} = 4.76$; ß = −0.93; $p = 0.04$].

Sex

Men had significantly higher MIF levels ($U = 115.00$; $p = 0.02$) at baseline than women. Response/remission status were independent from sex (response: $\chi^2 = 0.10$; $p = 0.75$; remission: $\chi^2 = 0.19$; $p = 0.67$). Response status was also statistically independent from sex in the placebo and celecoxib subgroups ($p > 0.99$ and $p = 0.65$, respectively). However, in the celecoxib group men showed a numerically lower reduction in MADRS score than women. Regarding changes in biomarker level over time, women as compared to men showed a significantly different course of MIF levels (women: increase, men: decrease; $U = 122.00$; $p = 0.04$). This difference emerged only in the placebo group ($U = 25.00$; $p = 0.02$) and was seen in responders as a trend ($U = 47.00$; $p = 0.06$).

Smoking Status

No significant differences in baseline MIF levels emerged between smokers and non-smokers ($U = 164.00$; $p = 0.44$). Furthermore, response/remission status were independent from smoking status (response: $\chi^2 = 0.14$; $p = 0.71$; remission: $\chi^2 = 0.14$; $p = 0.71$). Response status was also independent from smoking status in the placebo and celecoxib subgroups ($p = 0.63$ and $p = 0.66$, respectively).

Adjusted Analysis

Because MIF levels were associated with age and sex, we performed preliminary regression analyses with age and sex as covariates to further investigate the predictive capability of response status on the expression of MIF levels. In the placebo group, a trend emerged for predicting of MIF levels at baseline by response status, age, and sex [$R^2 = 0.32$; $F_{(3, 18)} = 2.82$; $p = 0.07$]. For remission, a significant model was obtained [$R^2 = 0.37$; $F_{(3, 18)} = 3.56$; $p = 0.04$]. Similarly, the model predicting MIF levels at endpoint was significant [$R^2 = 0.41$; $F_{(3, 18)} = 4.19$; $p = 0.02$]. In the celecoxib group, a trend emerged for the prediction of MIF levels at baseline by response status, age, and sex [$R^2 = 0.38$; $F_{(3, 13)} = 2.60$; $p < 0.10$]. Data on the individual predictors are given in **Supplementary Table D**. The results of the adjusted analyses are mostly in accord with the data given under 3.3. Since some models revealed a significant R^2 but partly lacking significance of single predictors, collinearity diagnostics were performed. No indication for multicollinearity was present in any of the models. Thus, this effect may result from the collective impact of more or less nearly significant predictors.

DISCUSSION

Overall, no difference in response or remission rates between the two treatment arms were found. One reason for this result might be that celecoxib add-on to sertraline is not superior to sertraline plus placebo. In contrast to our findings, a meta-analysis of previous trials found a superior effect of add-on celecoxib to standard antidepressant treatment over add-on placebo without taking inflammatory status into account (53, 54). Noteworthy, the magnitude of symptom reduction and the obtained response rates varied among the studies, even despite using the same celecoxib dose (41, 53). The studies with very high response rates to celecoxib had a higher women to men ratio in the celecoxib group than the other studies and our study (41, 42, 53). According to previous literature, inflammatory activation is associated with depression severity particularly in women (55), thus leading to a higher potential of benefitting from anti-inflammatory therapy. In line with our study, another trial investigating add-on celecoxib to SSRI compared to placebo plus SSRI in drug-naïve depressed women and found no difference in reduction of depression severity at endpoint (41). However, the authors found a superior effect of celecoxib after half the treatment phase, i.e., 4 weeks, suggesting that celecoxib might accelerate symptom reduction during early treatment phases (41). Although celecoxib add-on was not superior to standard treatment in our study, both groups had a reasonably large proportion of responders and both treatment groups showed a significant decline of depression severity over time. As compared to one of the investigated trials in the meta-analysis, we found a similar response rate in the celecoxib group while both studies used sertraline and the same celecoxib dose (43). However, the non-superiority of add-on celecoxib in our trial questions the clinical benefit that the

previous studies concluded. Because only a subgroup of patients exhibits an increased pro-inflammatory profile and patients were not stratified for inflammatory state before receiving anti-inflammatory treatment, it may be expected to find no substantial difference in response rates. This assumption is supported by studies on other (add-on) anti-inflammatory treatments, which showed that depressed patients with a low inflammatory status exhibited even lower response rates after anti-inflammatory therapy than patients with a low inflammatory status who had received (standard therapy plus) placebo (45). Therefore, patients with low levels of inflammation may have contributed to lower response rates to celecoxib. Furthermore, our study found much higher response and remission rates in the sertraline group than were found in the control groups in most of the other trials (53). In our study, almost all patients were without current premedication and about half the patients were experiencing their first episode of depression. Sertraline is a potent antidepressant and drug-naïve patients respond better to treatment than patients who have received multiple treatments (2), which together may explain the high response and remission rates to sertraline plus placebo in our study. Furthermore, many of the above-mentioned studies included outpatients, whereas our sample consisted only of inpatients (41–43). A high placebo effect may arise from receiving extensive care and attention in a hospitalized setting. Generally, given the variability of previous study results, explanations for differences of our results are rather speculative. Regarding change of biomarker levels over time, significant results for non-responders and responders or non-remitters and remitters across treatment arms, and for sertraline with add-on placebo or add-on celecoxib across response status are lacking not indicating any general effects of treatment or response status for change of biomarker levels.

Our study goes beyond the current state of knowledge by looking into response status in subgroups. A dependency of inflammatory biomarker levels and response/remission to different treatment becomes apparent, at baseline and during the course of treatment. However, a clear pattern was only observed for MIF, which is consistent with previous literature. Non-responders to sertraline plus placebo showed a trend for higher baseline levels as compared with sertraline responders. This was especially and significantly evident in non-remitters compared with remitters pointing to the well-known treatment resistance to standard serotonergic agents when pro-inflammatory levels are increased (45). Vice versa, responders to celecoxib showed a trend for higher baseline MIF levels as compared with celecoxib non-responders (who had the overall lowest levels), suggesting a beneficial effect of anti-inflammatory medication on clinical outcome when such activation is present. Interestingly, baseline MIF levels in non-responders/non-remitters to sertraline plus placebo were similar to those of responders/remitters to sertraline plus celecoxib. This oppositional relationship has also been found for CRP, IL-6, and IL-1ra (46, 56, 57). Moreover, higher MIF baseline mRNA levels were also shown to predict response to escitalopram or nortriptyline in depressed patients (58). The difference between baseline levels in placebo remitters vs. non-remitters was larger than between responders/non-responders and was still present at the end of the study. We therefore conclude that stronger or faster symptom reduction can be achieved with standard SSRIs when MIF levels are preferably low and high MIF levels after treatment indicate the persistence of clinically relevant depressive symptoms. Celecoxib non-responders and non-remitters showed the highest MIF levels of all subgroups at endpoint (see **Table 4B**). This change was significantly different from that in non-responders and non-remitters in the placebo groups and may point to a subgroup of concern that should be studied in more detail in the future. Taken together, MIF shows a predictive capability for remission (and a trend toward such capability for response) in treatment as usual (SSRI) and a trend toward predicting response to add-on celecoxib.

Regarding relevant patient characteristics, MIF levels were found to be related to age and sex but not to BMI. In the placebo group, weight loss and higher age were associated with treatment resistance. Since age was positively associated with both, MIF levels and treatment failure, the concept of immunosenscence presents a suitable explanation. Immunosenscence describes the changes of the immune system which, among others, is characterized by low-grade inflammation and all of which increase during aging (59). It is thus not surprising that these factors were related in our study. We found no effect of sex on response and no associations with smoking whatsoever. Hence, BMI, sex, and age seem to be linked to MIF levels and/or response status and should be addressed in design and statistical evaluation of future studies.

As for neopterin, baseline levels did not discriminate between responders/remitters and non-responders/non-remitters to either treatment. Previous research found that higher neopterin levels were associated with depression and the number of depressive episodes (21, 60) indicating higher disease severity. This also demonstrates that neopterin levels seem to rise with treatment resistance as the increase in non-remitters shows in our study. Further, this may be especially driven by the celecoxib non-remitters explaining the increase of neopterin levels in the whole celecoxib group (see **Table 2** and **Supplementary Tables C.1**, **C.2**). To our knowledge, no other studies have investigated baseline neopterin in relation to treatment response yet. Our study found no indication that neopterin is a potential biomarker. However, this result should be verified in a study with a larger sample size and greater power. TNFα levels were not significantly different at baseline but at endpoint in the placebo group indicating that treatment resistance to standard SSRI is accompanied by high levels of TNFα. One earlier study found significantly lower TNFα levels at baseline in responders to sertraline (at least 50% MADRS score reduction) compared with non-responders (61). Our data showed the same tendency, but the difference did not reach statistical significance. The authors of the earlier study (61) defined endpoint at 12 weeks which might better separate response status and biomarker levels. Further, Powell et al. (62) found higher levels of TNF gene expression in SSRI (escitalopram) non-responders compared to responders and this difference was even larger at endpoint (8 weeks) than at baseline, supporting our results. However, conflicting results exist (63, 64).

The involvement of the kynurenine pathway in MDD pathophysiology has been receiving growing attention. In fact, pro-inflammatory cytokines such as IFNγ, TNFα, IL-1, and the hormone PGE2, which is stimulated by MIF via COX-2-upregulation, stimulate the activity of the enzyme IDO and consequently the breakdown of tryptophan into potentially neurotoxic kynurenine metabolites (13, 65–68). This results in a lack of serotonin, a widely known characteristic of MDD. Because MIF stimulates PGE2 production, which is counteracted by a celecoxib-related decrease in IDO synthesis, MIF might act as a surrogate for COX-2 and PGE2 activity. Further, TNFα activates IDO (13), also possibly explaining the lacking serotonergic response. Furthermore, kynurenine in turn may lead to more pro-inflammatory cytokine release like TNFα (69) possibly explaining the higher levels of TNFα at endpoint. In fact, in all three markers we observed the same numeric trend of higher levels in non-responders/non-remitters at baseline and at endpoint, though not all the differences reached statistical significance. Nevertheless, this points in the direction of disturbed antidepressant action in those patients. Other previous results show that higher kynurenine/tryptophan ratio (favors kynurenine pathway) was predictive of remission after celecoxib add-on treatment in another sample of MDD patients (70). With perspective on the comorbidity of depression and cardiovascular disease, IDO activity is associated with cardiovascular risk factors (71), and celecoxib has shown beneficial effect on atherosclerotic progression (72).

This study has several limitations. Analyses in small subsamples have limited statistical power and non-significant results may therefore be a matter of type II error, which is we refrained from confident interpretation. However, we did give statistical trends some credibility. There is an ongoing debate on whether the *p*-value should be treated as a strict cut-off, and many scientists favor a non-categorical use today (73). The addition of covariates to a binary predictor in the adjusted analyses further limits power while the smaller sample size already increases variance. Future studies should aim at replicating these findings in larger samples which would also allow for multiple test correction. Due to the preliminary nature of subgroup analyses, and especially the adjusted analyses, results are rather hypothesis generating for future studies. We used convenience sampling so that even though important patient characteristics were equally distributed between the treatment arms other variables might have acted as confounders that were not accounted for. For example, childhood adverse experience was shown to be related to cytokine levels in depression (74). In addition, our sample consisted of inpatients only, so the results cannot be generalized to outpatient settings. Because antidepressant medication has some immunomodulatory effects (75), prior use of such medication might have elicited a modulating effect before the study already. Further, we did not evaluate kynurenine metabolites which will be important for future analyses to demonstrate mechanistic links as discussed above. In general, one important drawback is that there are no established cut-offs yet for categorizing levels of the investigated biomarkers as high or low. Such cut-off values are needed so that patients can be stratified by inflammatory level beforehand to investigate response to a tailored treatment allocation.

CONCLUSION

Celecoxib add-on did not lead to greater response rate at 50% symptom reduction than sertraline plus placebo regardless of inflammatory state, but patients were not stratified beforehand according to their level of pro-inflammatory activation. MIF shows potential for acting as a reliable biomarker indicating treatment responsiveness, especially remission. Response rates may be increased if such biomarkers were used to guide treatment choice or change when their monitoring during treatment indicates non-response. The present study serves as a call for future investigations, in particular on treatment remission in response to anti-inflammatory vs. standard antidepressant treatments, stratifying patients by immune activation in advance. As the trends found for response status cannot be neglected entirely, studies should also reevaluate these finding in larger samples. Therefore, cut-off values should also be established for classifying abnormal immune activation. Moreover, complex models including possible confounding variables should be performed and more biomarkers should be investigated in larger samples to predict response.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the Medical Faculty of Ludwig-Maximilians-University Munich, Germany (project-nr. 234-09). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS has prepared the data and conducted the analysis, evaluation, and interpretation of the present work, as well has drafted the present manuscript. BB and GA-H have conducted data acquisition. EW has contributed to planning the study and conducted data acquistion. PZ has conducted the laboratory analyses. RM has contributed to the interpretation of data. HD has contributed to design and planning the study and data interpretation. NM has contributed to design and planning the study, data acquisition, and evaluation. BB, EW, GA-H, PZ, RM, HD, and NM have critically reviewed and revized the content. All authors contributed to the manuscript and approved submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.615261/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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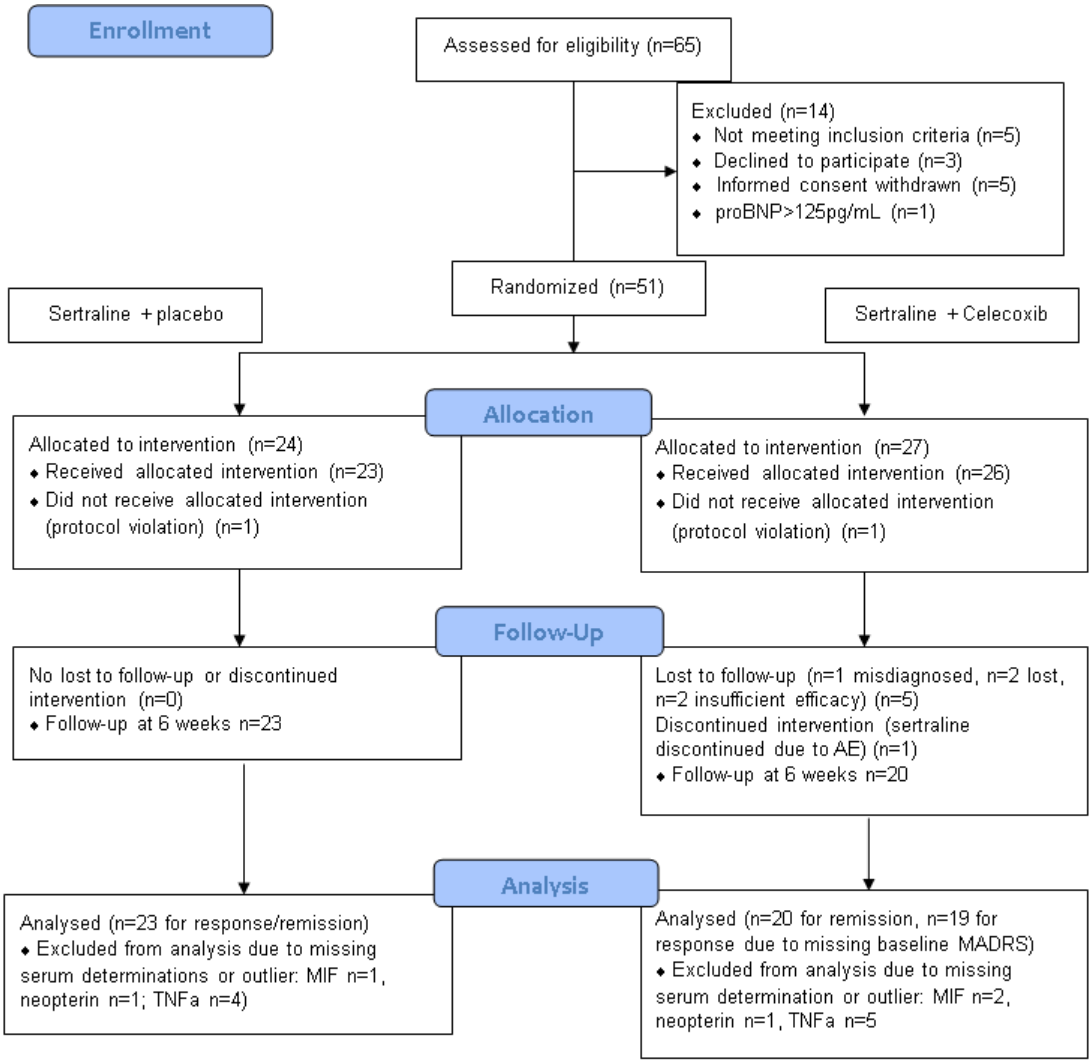
Supplementary Material

1 Supplementary Figure and Tables

1.1 Supplementary Figure

Supplementary Figure 1. Patients flow-chart according to CONSORT guideline.



1.2 Supplementary Tables

Supplementary Table A. Inclusion and exclusion criteria of the patient population.

	Inclusion criteria
(1)	Major depression diagnosed by psychiatrist.
(2)	DSM IV TR: 296.2x single depressive episode or 296.3x recurrent depressive episode.
(3)	For the present evaluation MADRS score ≥ 20 was used representing moderate to severe depression severity. Originally HamD-17 score ≥ 22 was defined; no subject was excluded due to exchange of rating scale criterion.
(4)	Informed consent.
(5)	Age between 18 and 60 years.
	Exclusion criteria
(1)	Psychotic depression or bipolar disorder, drug or alcohol addiction, schizoaffective disorders, schizophrenia. Other disorders (e.g. obsessive compulsive disorder, anxiety disorder, personality disorder) in case the symptoms predominate the clinical picture.
(2)	Unsuccessful treatment with more than 2 antidepressant medications (at therapeutically adequate doses and duration) during current episode.
(3)	Concomitant use of psychotropic drugs, including mood stabilizers, besides defined co-medication.
(4)	Immediate risk for suicidal behavior (3 on HamD-17 rating scale or 5 on MAD Rating Scale).
(5)	Women who are pregnant, breast feeding or planning to become pregnant during the course of study.
(6)	Women who are not post-menopausal (no natural menopause established in retrospect after 12 consecutive months of amenorrhea without hormone replacement therapy during the last 5 months), surgically sterilized or using a highly effective method of contraception (an implanted or injected hormonal contraceptive, some intrauterine contraceptive devices (IUDs) containing hormones, sexual abstinence, or have a vasectomized partner). Females using combined oral contraceptives should use a different or additional highly effective method of contraception as listed above.
(7)	Any history of cardiovascular disease (e.g. angina, heart attack, stroke, congestive heart failure), uncontrolled high blood pressure, documented peripheral arterial insufficiency and symptomatic, clinically significant claudication, a history of peripheral arterial embolism or cerebrovascular disease.
(8)	Patients at risk of QT/QTc interval prolongation (QTc> 450 ms, family history of long QT syndrome or use of medication prolonging QT/QTc interval).
(9)	History of coronary heart disease (CHD) or any other heart disease.
(10)	Serum NTproBNP ≥ 125 pg/mL indicating (sub-) clinical heart failure.
(11)	History of upper or lower gastrointestinal (GI) ulceration, perforation and/or obstruction.
(12)	History of upper or lower GI bleeding within the previous year.
(13)	History of inflammatory bowel disease.
(14)	Undergoing cancer chemotherapy.
(15)	Known HIV infection or clinically manifest Acquired Immune Deficiency Syndrome (AIDS), diabetes, asthma, COPD, Parkinson's or Alzheimer's disease, or any other serious condition likely to interfere with the conduct of the trial.
(16)	Clinically relevant hepatic or renal impairment (serum albumin < 25 g/L or Child-Pugh > 10 or renal GFR < 30 mL/min), or other clinically significant physical findings or clinically significant laboratory results at screening or baseline, as determined by the investigator.
(17)	History of allergy to sertraline, celecoxib, sulfonamides or closely related compounds, or excipients.
(18)	History of hypersensitivity or intolerance to pain medications.
(19)	Use of pain medication, such as a COX-2 inhibitor, NSAID (non-steroidal anti-inflammatory drug, including aspirin) or acetaminophen (syn. paracetamol) within 72 hours prior to study entry (24 hours for short-acting drugs such as aspirin or acetaminophen).
(20)	Patients currently taking warfarin.
(21)	Participation in a study of an investigational drug or device concomitantly or within 30 days prior to this study.
(22)	Patients thought to be unreliable or incapable of complying with the requirements of the protocol.
(23)	Treatment with monoamino oxidase inhibitors during the last 14 days or treatment with fluoxetine during the last 6 weeks.

Supplementary Table B. Comparison of placebo and celecoxib treatment groups.

	test (N)	SE	p	CI
MADRS baseline	U=198.50 (42)		0.61	
MADRS endpoint	T=-0.37 (43)	2.29	0.71	[-5.49;3.78]
MADRS score reduction (%)	T=0.89 (42)	7.61	0.38	[-8.62;22.13]
age	T=-0.13 (43)	3.58	0.89	[-7.69;6.76]
BMI baseline	T=-0.03 (43)	1.02	0.97	[-2.10;2.04]
BMI endpoint	T=-0.25 (40)	0.99	0.80	[-2.24;1.75]
sex	χ^2=0.22 (43)		0.63	
smoking status	χ^2=2.65 (43)		0.10	
MIF (pg/ml)	U=175.00 (40)		0.53	
Neopterin (ng/ml)	U=174.50 (41)		0.36	
TNFα (pg/ml)	U=111.00 (34)		0.27	

Notes. *MADRS* Montgomery Åsberg Depression Rating Scale *SE* standard error *CI* confidence interval.

Supplementary Table C.1. Comparison of baseline neopterin levels of different response statuses in the two treatment arms.

sertraline + placebo	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.80 ng/ml	0.90 ng/ml	38.00	0.31
	remitter (*Md*)	non-remitter (Md)	*U*	*p*
	0.55 ng/ml	0.90 ng/ml	29.00	0.10
sertraline + celecoxib	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.61 ng/ml	0.73 ng/ml	34.00	0.68
	remitter (*Md*)	non-remitter (*Md*)	*U*	*p*
	0.99 ng/ml	0.59 ng/ml	23.50	0.29

Notes. *Md* median; *U* Mann-Whitney-U-test statistic.

Supplementary Table C.2. Comparison of endpoint neopterin levels of different response statuses in the two treatment arms.

sertraline + placebo	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.66 ng/ml	0.94 ng/ml	33.50	0.18
	remitter (*Md*)	non-remitter (Md)	*U*	*p*
	0.66 ng/ml	0.90 ng/ml	33.00	0.17
sertraline + celecoxib	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.94 ng/ml	0.88 ng/ml	35.00	0.75
	remitter (*Md*)	non-remitter (*Md*)	*U*	*p*
	0.95 ng/ml	0.86 ng/ml	27.50	0.49

Notes. *Md* median; *U* Mann-Whitney-U-test statistic.

Supplementary Table D.1. Comparison of baseline TNFα levels of different response statuses in the two treatment arms.

sertraline + placebo	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.56 pg/ml	0.86 pg/ml	27.00	0.21
	remitter (*Md*)	non-remitter (Md)	*U*	*p*
	0.56 pg/ml	0.86 pg/ml	26.00	0.25
sertraline + celecoxib	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.71 pg/ml	1.08 pg/ml	16.00	0.20
	remitter (*Md*)	non-remitter (*Md*)	*U*	*p*
	0.93 pg/ml	0.76 pg/ml	20.00	0.79

Notes. *Md* median; *U* Mann-Whitney-U-test statistic.

Supplementary Table D.2. Comparison of endpoint TNFα levels of different response statuses in the two treatment arms.

sertraline + placebo	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.52 pg/ml	1.24 pg/ml	16.00	0.03*
	remitter (*Md*)	non-remitter (Md)	*U*	*p*
	0.52 pg/ml	1.05 pg/ml	27.00	0.29
sertraline + celecoxib	responder (*Md*)	non-responder (*Md*)	*U*	*p*
	0.83 pg/ml	0.60 pg/ml	17.00	0.24
	remitter (*Md*)	non-remitter (*Md*)	*U*	*p*
	0.73 pg/ml	1.10 pg/ml	18.00	0.60

Notes. *Md* median; *U* Mann-Whitney-U-test statistic; *p<0.05.

Supplementary Table E. Single predictor estimates of the preliminary adjusted analyses.

baseline MIF levels	*β*	*t*	*df*	*p*	*CI*
sertraline+placebo					
responder	-845.68	-0.35	18	0.73	[-5932.17;4240.82]
age in years	210.27	1.99	18	0.06^{+}	[-12.04;432.57]
sex	-3479.53	-1.69	18	0.11	[-7810.65;851.59]
sertraline+placebo					
remitter	-3141.23	-1.28	18	0.22	[-8283.26;2000.80]
age	156.60	1.46	18	0.16	[-68.14;381.34]
sex	-3352.00	-1.69	18	0.11	[-7511.15;807.15]
sertraline+celecoxib					
responder	5223.36	2.14	13	0.05^{+}	[-54.61;10501.33]
age	-49.13	-0.54	13	0.60	[-247.39;149.13]
sex	-4856.75	-2.19	13	0.047*	[-9644.32;-69.18]

endpoint MIF levels	*β*	*t*	*df*	*p*	*CI*
sertraline+placebo					
remitter	-3787.09	-3.51	18	0.003**	[-6057.32;-1516.86]
age	-91.05	-1.93	18	0.07^{+}	[-190.27;8.17]
sex	562.60	0.64	18	0.53	[-1273.69;2398.88]

Notes. age in years; *β* regression coefficient; t test; df degrees of freedom; CI 95% confidence interval; $^{+}$p<0.10 *p<0.05 **p<0.01

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VI Affidavit

I, Maria Susanne Simon-Strauß, hereby declare, that the submitted thesis entitled "Immune system dysregulations in major depressive disorder: The role of innate immune function and response prediction to standard and anti-inflammatory therapy – challenges towards an individualized medicine." is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given. I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Munich, March 12, 2022 Maria Susanne Simon-Strauß

VI Eidesstattliche Versicherung

Hiermit erkläre ich, Maria Susanne Simon-Strauß, an Eides statt, dass ich die vorliegende Dissertation mit dem Titel "Immune system dysregulations in major depressive disorder: The role of innate immune function and response prediction to standard and anti-inflammatory therapy – challenges towards an individualized medicine." selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe. Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

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