




Deep immunophenotyping of circulating immune cells in major depressive disorder patients reveals immune correlates of clinical course and treatment response

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ABSTRACT

Major Depressive Disorder (MDD) is a widespread psychiatric condition impacting social and occupational functioning, making it a leading cause of disability. The diagnosis of MDD remains clinical, based on the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria, as biomarkers have not yet been validated for diagnostic purposes or as predictors of treatment response. Traditional treatment strategies often follow a one-size-fits-all approach obtaining suboptimal outcomes for many patients who fail to experience response or recovery. Several studies have reported an association between MDD and immune system dysregulation, but few have focused on the deep characterization of circulating cells, during the acute phase of MDD. This work aimed at immunophenotyping peripheral blood cells in the relapse phase of the disorder, to identify relevant cell populations for clinical monitoring of patients. Multiparametric analysis was performed on the peripheral blood of 60 MDD patients using flow cytometry to identify lymphocytes (naïve/effector, memory, regulatory) and myeloid cells (dendritic cells, monocytes). We studied the associations between immunophenotype and depressive symptoms, social and working functioning, and subjective quality of life during the acute phase and after three months of treatment. Multivariate analysis showed that CD4⁺ terminally differentiated effector memory (TEMRA) were associated with more depressive symptoms with a particular emphasis on anhedonic features and worse social and working functioning and quality of life. CD8⁺ TEMRA were associated with those depressive symptoms related to hopelessness. Conversely, ICOS⁺ Tregs were associated with low-intensity suicidal ideation, suggestive of a protective role. Baseline T CD4⁺ effector memory (EM) was a negative predictor of reduction of depressive symptoms after three months of treatment, whilst plasmacytoid dendritic cells (pDC) were predicting reduction of hopelessness. These results confirm the involvement of the immune system in MDD and demonstrate the existence of immunological signatures associated with the severity of major depressive episodes and treatment response that could guide clinical monitoring and future personalized therapies.

1. Introduction

Major depressive disorder (MDD) is a widespread psychiatric condition with significant implications for both individual well-being and

society. It affects approximately 340 million people worldwide, causing a considerable reduction of social and working functioning of patients and consequently ranking as a leading cause of disability with consistent direct and indirect costs (Chiriță et al., 2015). It has a profound impact

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on life expectancy with a significant reduction in lifespan, primarily due to an increased risk of suicide, as well as an elevated vulnerability to other comorbidities such as cardiovascular diseases, autoimmune diseases, diabetes, and cancer (Benros et al., 2013; Windle and Windle, 2013; Bortolato et al., 2017). Furthermore, patients with MDD tend to have poorer outcomes and responses to treatment for these medical conditions (Katon, 2011).

MDD is characterized by major depressive episodes (MDEs) that represent the acute phase of the disorder (Fava and Kendler, 2000). MDEs consist of periods of two weeks or more during which there is a persistent change in the person's usual level of functioning and are characterized by depressed mood, loss of interest, alterations in sleep and appetite, fatigue, difficulty concentrating and memory, feelings of guilt, and thoughts of death (DSM-5). Using the criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM), more than 200 combinations of symptoms can be applied to diagnose MDD, underscoring the clinical diversity of the disorder, which results in a wide range of outcomes regarding response and remission over time (Østergaard et al., 2011; Steinert et al., 2014; Belmaker and Agam, 2008). According to practical guidelines, antidepressants are the primary treatment for MDD. However, despite a wide range of effective treatments, a substantial number of MDD patients, remain nonresponsive or poorly responsive to available treatments, thus failing to reach symptomatic remission and functional and personal recovery (Nemeroff, 2020; Voineskos et al., 2020).

Precision medicine, through the assessment of individualized clinical and biological measures, could allow the identification of the most effective treatment while minimizing side effects (Stolfi et al., 2024). Understanding the complex interplay of genetic, environmental, biological, and psychological factors in MDD is crucial to improving prevention and treatment strategies for this debilitating condition.

The profiling of peripheral immune markers may contribute to defining the clinical presentation and outcomes, such as treatment response and recovery. Over the past two decades, there has been increasing evidence that MDD involves systemic immune activation regarding abnormalities in inflammatory markers, immune cell numbers, and antibody titers (Gibney and Drexhage, 2013; Müller, 2014).

Numerous studies, coming from meta-analyses of cross-sectional studies (Howren et al., 2009; Dowlati et al., 2010; Haapakoski et al., 2015; Goldsmith et al., 2016; Köhler et al., 2017) have consistently found that MDD patients often have elevated circulating levels of inflammatory biomarkers, such as C-reactive protein (CRP) and proinflammatory cytokines (i.e. interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α)) (Miller et al., 2009). Additionally, inflammation may contribute to the development of depressive symptoms, such as anhedonia (i.e. decreased motivation) and fatigue, by influencing immune system activation in both the peripheral and central nervous systems (Lee and Giuliani, 2019). Interestingly, anti-cytokine treatments have shown antidepressant effects in MDD patients with chronic inflammatory conditions (Köhler et al., 2014; Kappelmann et al., 2018).

Identifying an immune signature that effectively classifies the immune state in depressed patients during the acute phase, potentially integrating multiple markers and clinical criteria, could be a valuable step in addressing the contribution of the immune system to MDD pathogenesis. Until now, few studies have investigated simultaneously innate and adaptive immune responses in MDD patients. Though meta-analyses revealed that immune cells could be used as potential biomarkers for illness subtyping and MDD patient stratification, few works have been performed to identify in detail which are the different subtypes of immune cells that could play a role in the disorder, as well as to understand if they can be considered as predictor factors for the response to antidepressant therapy. Furthermore, most of these studies are case-control cross-sectional studies, which only focus on the acute phase and do not evaluate the response to treatment over time.

The present work aimed at performing in-depth

immunophenotyping by flow cytometry of peripheral blood cells of acute-phase MDD patients, correlating the immunological data with the severity of depressive symptoms, socio-occupational functioning, and subjective quality of life during the MDE and after three months of treatment (**Graphical abstract**). Our results revealed a specific immunological signature associated with more severe, disabling, and less responsive MDE in MDD.

2. Methods

2.1. Participants

Eighty patients with a diagnosis of MDD according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria (American Psychiatric Association. American Psychiatric Association and DSM-5 Task Force, 2013) were included in the study from February 2021 until August 2023. Patients were enrolled at the Struttura Complessa Psichiatria Universitaria, Dipartimento di Neuroscienze e Salute Mentale, Azienda Ospedaliero-Universitaria "Città della Salute e della Scienza di Torino", Turin, Italy.

Inclusion criteria were age between 18 and 70 years and a diagnosis of MDD with an ongoing MDE (DSM-5 criteria) necessitating the initiation or modification of an antidepressant treatment in an outpatient setting. Exclusion criteria were comorbidity with any other mental disorder, including intellectual disability (DSM-5), autoimmune disorders, immunodeficiencies, and infections and/or use of immunomodulatory drugs (acetylsalicylic acid, nonsteroidal anti-inflammatory drugs, and statins) in the four weeks before the baseline assessment.

MDEs were treated with serotonin selective reuptake inhibitors (sertraline 100–200 mg/die, paroxetine 20–40 mg/die, citalopram 20–40 mg/die; escitalopram 10–20 mg/die), serotonin-norepinephrine reuptake inhibitors (duloxetine 60–120 mg/die; venlafaxine extended-release 75–225 mg/die), serotonin antagonist and reuptake inhibitor (trazodone extended-release 150–300 mg/die), and serotonin multimodal antidepressant (vortioxetine 10–20 mg/die). The following augmentation strategies were tolerated: quetiapine extended-release 150–250 mg/die or aripiprazole 2.5–5 mg/die. Benzodiazepines and hypnotics were allowed only in the two months after baseline assessment.

Patients included in the study were evaluated using a semi-structured interview to assess socio-demographic and clinical features including age, gender, education, body mass index (BMI), chronic non-mental comorbidities, cigarette smoking, alcohol and/or substance abuse, duration of illness, number of previous MDEs, number of previous antidepressant treatments, and antidepressant treatments at baseline.

Eighty consecutive patients were enrolled in the study. Fourteen patients dropped out, and six blood samples of the 66 patients in follow-up were not available for analysis due to technical reasons.

Written informed consent was obtained from all subjects. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Local Research Ethics Committee (Protocol number: 0071,044).

2.2. Baseline (t_0) assessment of symptoms severity, functioning, and subjective quality of life

The severity of depressive symptoms was evaluated with the Montgomery Åsberg Rating Scale (MADRS), where higher scores indicate greater severity (Montgomery and Åsberg, 1979). Three MADRS factors were calculated according to the solution proposed by Borenstein et al. (2022): (i) affective and anhedonic symptoms (AAS) consisting of apparent sadness, reported sadness, lassitude, inability to feel; (ii) anxiety and vegetative symptoms (AVS) comprising inner tension, reduced sleep, reduced appetite, concentration difficulties; (iii) hopelessness (Hpln) composed by pessimistic thoughts and suicidal thought.

Suicidal ideation and behavior were assessed using the Columbia-

Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2011). Higher rates indicate higher suicidality.

Functioning was assessed using the Work and Social Adjustment Scale (W-SAS) (Mundt et al., 2002), where higher scores indicate a more significant functioning impairment.

The Quality-of-Life Enjoyment Satisfaction Questionnaire—Short Form (Q-LES-Q-SF, 16-item version) (Endicott et al., 1993) was employed to assess patients' quality of life and satisfaction with life. It is a self-rated questionnaire; higher scores correspond to higher subjective quality of life and satisfaction. Scores were expressed as percentages of the maximum score.

The W-SAS and Q-LES-Q-SF total scores were integrated into the recovery index (RI), which provides a standardized measure of working and social functioning and subjective quality of life. It ranges from -1.643 to 4.242 . Higher scores indicate better functioning and quality of life. The RI was calculated using an online tool (<http://tinyurl.com/RecoveryIndex>) proposed in the index validation study (IsHak et al., 2017).

2.3. Three-month follow-up (t_1) assessment of treatment response, remission, and recovery status

During the three-month follow-up visit, response to treatment, remission from the MDE, improvement of functioning and subjective quality of life were assessed. Response to treatment was evaluated with the variation (Δ) of the MADRS total score and factors (AAS, AVS, and Hpln) from baseline to the follow-up visit (Δ MADRS t_1-t_0). Clinical response was defined as a reduction of more than 50% of MADRS scores, and remission as a MADRS follow-up score <10 (Zimmerman et al., 2004).

The improvement in functioning and subjective quality of life was evaluated with the variation of the RI (Δ RI t_1-t_0).

2.4. Immunophenotyping of peripheral blood cells

Peripheral blood was collected from 80 MDD patients. All blood drawn were performed at 8:30 a.m. Two hundred microliters of blood in EDTA (BD Biosciences) were stained with a cocktail mix of 24 antibodies in Brilliant Stain Buffer (BD Horizon™) and consisted of: anti-CD183 (CXCR3) APC monoclonal antibody (mAb), anti-CD3 BUV496 mAb, anti-CD123 PECY5 mAb, anti-CD196 (CCR6) BV480 mAb, anti-CD56 BB660 mAb, anti-CD8 BUV805 mAb, anti-CD27 BV570 mAb, anti-CD25 APCR700 mAb, anti-CD197 (CCR7) BV711 mAb, anti-CD278 (ICOS) BV421 mAb, anti-CD20 BV750 mAb, anti-CD127 BV786 mAb, anti-CD11C BUV661 mAb, anti-CD24 BB515 mAb, anti-CD38 BV650 mAb, anti-IgD BB630 mAb, anti-CD16 BB780 mAb, anti-CD19 BUV615 mAb, anti-CD45 BUV395 mAb, anti-CD45RO PECY7 mAb, anti-CD194 PE-CF594 mAb, anti-CD4 BUV737 mAb, anti-CD45RA BUV563 mAb and anti-HLA-DR BV605 mAb (BD Biosciences).

Staining was performed at 37°C for 20 min. After incubation, 2 ml of a 1:10 dilution of BD Pharm Lyse™ lysing solution was added to the sample and then incubated for 10 min to lyse red blood cells. Samples were resuspended in PBS + EDTA 2 mM and centrifuged at 1500 rpm for 5 min to pellet the cells and discard cell debris. This step was repeated twice. Then, cells were resuspended in 200 μl of PBS + EDTA 2 mM strained into tube through a 70 μm filter to remove cell debris and aggregate. Finally, the sample was acquired at BD FACSymphony™ A5 after calibration with BD Rainbow beads. All reagents were purchased from Becton and Dickinson (Franklin Lakes, NJ, USA) and data were analyzed using the BD FACSDIVA™ software version 9.0. The gating strategy is shown in Supplementary materials.

2.5. Statistical analysis

Descriptive analyses were first conducted to characterize the participants' sociodemographic and clinical characteristics. Then, using the

t -test for paired samples, a comparison between the baseline symptoms, functioning, and subjective quality of life was performed. A comparison between follow-up and drop-out patients, responders and non-responders, remitters and non-remitters, was performed with one-way ANOVA for numerical variables and χ^2 test for categorical variables.

Second, we performed eleven multivariate stepwise regressions. In all regressions, the independent variables were the relative frequencies of the circulating immune cell populations identified. The dependent variables were t_0 MADRS factors and total score, CRSS intensity suicidal ideation, and RI for the baseline, and the Δt_1-t_0 of the MADRS factors and total score and of the RI. In these last five regression models, we controlled for the baseline (t_0) MADRS factors or total score or RI according to the dependent variable. In all regressions, we controlled for age, sex, education, non-psychiatric comorbidities, BMI, alcohol use, smoking status, duration of illness, number of previous MDEs, number of past antidepressants, and use of antidepressants at baseline.

All analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS Statistics), Version 29.0.1.0, with a critical p-value of .05.

3. Results

3.1. Clinical and sociodemographic characteristics of the cohort of MDD patients enrolled in the study at baseline

Table 1 reports the sample characteristics of the final 60 patients enrolled in the study with available immunophenotyping of peripheral blood cells. The mean age was 51, with a higher prevalence of women (71%). The duration of illness and the number of previous MDEs were heterogeneous, ranging from 0 to 15 episodes with a history of MDD longer than 40 years. The mean depressive symptoms were moderate (Müller et al., 2003), suicidal ideation was present in 41% of cases, the mean impairment of working and social functioning was moderate to severe (Mundt et al., 2002), and the subjective quality of life was reduced with a mean of 35% of the maximum total score (Endicott et al., 1993).

Compared to the baseline (t_0), at the three-month follow-up (t_1), patients showed significantly lower depressive symptoms and suicidal ideation and better functioning and subjective quality of life (Table 1).

The dropouts were younger than the follow-up patients (mean age 40.4 vs 52.6, $p = 0.001$; Supplementary Table 1). No significant difference in the percentages of the immune peripheral blood cell populations was found between the two groups (Supplementary Table 2).

3.2. Immune profiling of peripheral blood cells in MDD patients during MDE

Using a panel of 24 antibodies, we identified 38 distinct immune cell populations as detailed in Table 2. For the identification of these different cell populations the gating strategy depicted in Supplementary Fig. 1 was applied. Briefly, after the exclusion of doublets (FSC-H vs FSC-A), lymphocytes and monocytes were gated based on their morphological characteristic (FSC-A vs SSC-A). Mononuclear cells expressing CD45 were identified and they were divided in: classical monocytes (CD14+/CD16-), intermediate monocytes (CD14+/CD16+) and non classical monocytes (CD14-/CD16+). Lymphocytes CD45⁺ were identified and among them we gated CD3⁺ cells, CD19⁺ cells and not CD3 CD19 (CD3 vs CD19). Classical switched memory B cells (CD27+/IgD-), IgD memory (CD27+/IgDmed) and naïve B cells (CD27-/IgDhigh) were identified on CD19⁺ cells. Moreover, in B cells compartment (green box) we identified transitional B cells (CD24med/CD38+) and plasmablasts (CD24-/CD38high) and plasma cells (CD20-/CD38+).

Table 1
Sociodemographic and clinical characteristics of MDD patients enrolled (n = 60).

Sociodemographic and clinical characteristics	Baseline (t ₀)	Follow-up (t ₁)	p
Age, years	51.0 (13.2)	–	–
Gender, F	51 (70.8%)	–	–
Education, years	13.2 (3.8)	–	–
BMI 25.0–29.9 kg/m ²	17 (25.8%)	–	–
BMI ≥30.0 kg/m ²	4 (6.1%)	–	–
Chronic comorbidities	44 (66.7%)	–	–
Smoke at baseline	22 (33.3%)	–	–
Alcohol abuse	8 (12.1%)	–	–
Substance use	4 (6.1%)	–	–
Duration of illness, years	11.7 (12.3)	–	–
Number of previous MDEs	3.5 (3.0)	–	–
Number of previous antidepressant treatments	2.2 (2.0)	–	–
Antidepressant treatments at baseline	50 (75.8%)	–	–
Affective and anhedonic symptoms, MADRS factor	11.7 (4.0)	7.2 (4.0)	<.001
Anxiety and neurovegetative symptoms, MADRS factor	9.2 (3.1)	5.3 (2.6)	<.001
Hopelessness, MADRS factor	4.1 (1.9)	2.4 (1.6)	<.001
Depressive symptoms, MADRS total score	25.6 (6.7)	15.1 (7.8)	<.001
Intensity of suicidal ideation, CSSRS	5.3 (6.5)	0.6 (2.6)	<.001
Impairment in working and social functioning, W-SAS total score	19.4 (8.2)	12.3	<.001
Quality of life enjoyment and satisfaction, Q-LES-Q-SF percentage of the maximum score	35.1 (12.7)	55.3 (14.8)	<.001
Recovery Index (RI), standardized score	.58	1.50	<.001
Serotonin selective reuptake inhibitors (SSRI)	31 (51.6%)	–	–
Serotonin-norepinephrine reuptake inhibitors (SNRI)	18 (30%)	–	–
Serotonin antagonist and reuptake inhibitor (SARI)	4 (6.7%)	–	–
Serotonin modulator and stimulator (SMS)	7 (11.7%)	–	–
Quetiapine extended-release 150–250 mg/die	9 (15.0%)	–	–
Aripiprazole 2.5–5 mg/die	7 (11.7%)	–	–
Benzodiazepines and hypnotics	35 (58.3%)	–	–

3.3. Increased levels at baseline (t₀) of effector T and decreased levels of activated treg are associated with symptoms' severity, functioning, and quality of life

Multivariate regressions showed that CD4⁺ TEMRA were associated with more severe affective-anhedonic and total depressive symptoms and worse social and working functioning and subjective quality of life (RI). CD8⁺ TEMRA were associated with hopelessness. On the opposite, inducible T cell co-stimulator (ICOS) + Tregs were negatively associated with the intensity of suicidal ideation. No significant associations were found between immunophenotype cellular populations and anxiety and neurovegetative symptoms (Table 3).

3.4. Higher baseline naïve CD4⁺ T cells levels were associated with remission at follow-up

At follow-up, we found 31 responders (51.7%) and 21 remitters (35.0%). Responders presented fewer previous MDEs than non-responders; remitters exhibited higher baseline levels of naïve CD4⁺ T cells than non-remitters. These results are shown in Supplementary Table 3.

3.5. Effector memory T cells and pDC predict total depressive symptoms and hopelessness at 3 months

Multivariate regressions showed that evaluating immune cells at the relapse (t₀) may predict some outcome at 3 months. In particular, CD4⁺ EM T cells at baseline (t₀) were associated with a lower improvement of total depressive symptoms (Δ MADRS total score t₁-t₀). In contrast, pDC baseline levels were linked to a reduction of hopelessness (Δ MADRS Hpln t₁-t₀). No significant results were found for the other outcomes evaluated. These results are shown in Table 4.

4. Discussion

MDD is a common and severe mental health disorder marked by persistent sadness, hopelessness, and loss of interest. While antidepressants are the primary treatment, many patients do not achieve adequate symptom relief. Diagnosis relies on DSM-5 criteria, as no biomarkers have been validated for diagnosis or predicting treatment response (Blackburn, 2019).

Emerging research has linked MDD to immune system dysregulation, particularly involving T cells. Pro-inflammatory cytokines may contribute to MDD symptoms, and increased T cell activation markers have been observed in patients (Miller, 2010). Additionally, genetics studies have associated T cell-related genes with MDD antidepressant efficacy (Tubbs et al., 2020).

In the present work, we performed deep immunophenotyping of peripheral blood cells of MDD patients through a multiparametric flow cytometry, which is a high-throughput methodology allowing the simultaneous measurement of multiple parameters in a single specimen. We identified an immunological signature comprising both T cells and their subsets (TEMRA, EM and Tregs) and pDC that could serve as both a biomarker of severity or of treatment response for MDD.

Among T helper cells, Tregs-ICOS + subsets were associated with low intensity suicidal ideation. Hong et al. reported an imbalance between Th17 and Treg cell subsets, where an increase in Th17 cells and a decrease in Treg cells have been observed in chronic unpredictable mild stress (CUMS) mouse model, commonly used as a preclinical model of depression (Hong et al., 2012). ICOS molecule is essential for the optimal functioning of Tregs and its increased expression on Tregs enhances their suppressive capacity, contributing to better control of inflammation (Li and Xiong, 2020). We can speculate that ICOS + Tregs, through their anti-inflammatory properties, can potentially reduce the overall inflammatory burden in individuals with MDD being particularly effective in mitigating suicidal ideation due to their enhanced regulatory functions.

Moreover, among memory T cells, we found an increased percentage of CD4⁺ TEMRA cells associated with i) more severe total depressive symptoms, ii) more severe affective-anhedonic symptoms, and iii) more severe impairment of the baseline socio-occupational functioning and subjective quality of life. CD8⁺ TEMRA, instead, were associated with a greater lack of hope for the future. TEMRA are memory cells re-expressing CD45RA (i.e., a marker of naïve T cells) being able to quickly respond to previously encountered antigens. The increased presence of these TEMRA subsets in MDD patients might indicate a potential chronic activation or dysregulation of the immune system. This aligns with the growing body of research suggesting that MDD is not solely a disorder of the brain but also involves significant immune and inflammatory components (Drevets et al., 2022a). The dysregulation of immune cells, particularly TEMRA cells, may contribute to MDD by sustaining systemic inflammation, inducing neuroinflammation, and impacting mood-regulating neural circuits (Hoeks et al., 2024; Frank et al., 2004). Psychological stress in MDD can further amplify immune activation, creating a feedback loop of inflammation and depressive symptoms, while promoting premature immune aging and dysfunction (Klopach et al., 2022).

With regard to EM cells, we found that a high percentage of CD4⁺ EM

Table 2
Deep peripheral blood cells profiling.

		Immunophenotype of peripheral blood cells	M (SD)	Min-Max
T cells	Tc	CD3 ⁺ (CD45 +)	70.2 (14.1)	27.2–88.6
		CD8 ⁺ (CD3 +)	26.7 (8.6)	11.3–54.4
		CD8 ⁺ CM (CD8 + /CCR7 + /CD45RA-)	11.4 (8.2)	.8–52.8
		CD8 ⁺ naïve (CD8 + /CCR7 + CD45RA +)	32.4 (16.9)	4.7–74.4
		CD8 ⁺ EM (CD8 + /CCR7-/CD45RA-)	23.5 (11.5)	.2–46.3
	Th	CD8 ⁺ TEMRA (CD8 + /CCR7-/CD45RA +)	32.7 (15.7)	4.6–72.2
		CD4 ⁺ (CD3 +)	68.2 (9.6)	31.3–83.8
		CD4 ⁺ CM (CD4 + /CCR7 + /CD45RA-)	41.4 (13.0)	6.8–69.2
		CD4 ⁺ naïve (CD4 + /CCR7 + /CD45RA +)	40.5 (13.6)	12.3–81.1
		CD4 ⁺ EM (CD4 + /CCR7-/CD45RA-)	15.9 (8.2)	1.5–37.4
		CD4 ⁺ TEMRA (CD4 + /CCR7-/CD45RA +)	2.2 (2.5)	.0–10.6
		Th17 (CD4 + /CD196 + /CXCR3-)	22.9 (5.6)	7.7–38.1
		Th17-Th1 (CD4 + /CD196 + /CXCR3 +)	22.7 (7.4)	7.7–46.2
		Th2 (CD4 + /CD196-/CXCR3-)	24.7 (8.0)	9.3–48.6
		Th1 (CD4 + /CD196-/CXCR3 +)	29.7 (7.2)	16.5–47.4
		Tregs (CD4 + /CD127-/CD25 +)	7.1 (2.4)	3.3–16.0
		Treg memory (CD4 + /CD127-/CD25 + /CD45RO +)	70.3 (13.6)	34.3–96.9
		Treg memory ICOS (CD4 + /CD127-/CD25 + /CD45RO + /ICOS+)	34.4 (15.1)	2.2–74.8
		Treg naïve (CD4 + /CD127-/CD25 + /CD45RO-)	29.9 (13.6)	3.2–62.3
		Treg naïve ICOS (CD4 + /CD127-/CD25 + /CD45RO-/ICOS +)	5.8 (7.7)	.0–57.5
Treg ICOS (CD4 + /CD127-/CD25 + /CD45RO + /ICOS +)	26.7 (11.5)	3.5–55.7		
CD4 ⁺ memory (CD4 + /CD45RO +)	42.1 (9.1)	23.6–62.6		
B cells	CD19 ⁺ (CD45 +)	10.9 (5.1)	2.3–29.0	
	Class switched memory (CD19 + /CD27 + /IgD-)	23.4 (11.7)	3.8–66.9	
	Non class switched memory (CD19 + /CD27 + /IgDmedium)	7.2 (8.1)	.4–64.4	
	Naïve B cells (CD19 + /CD27 + /IgDhigh)	63.5 (15.9)	5.4–90.4	
	Transitional B cells (CD19 + /CD24 + /CD38 +)	4.6 (3.2)	.5–21.2	
	Plasmablasts (CD19 + /CD24-/CD38high)	1.3 (1.2)	.1–5.8	
	Plasma cells (CD19 + /CD20-/CD38 +)	2.2 (2.5)	.0–9.9	
	Innate cells	NK cells (CD3-/CD19-/CD56 + /CD16 +)	44.0 (24.6)	1.4–87.5
		NKT (CD3 + /CD56 +)	5.7 (4.4)	.1–22.9
		Activated NK cells (CD3-/CD19-/CD56 + /CD16 + /HLA-DR +)	1.6 (2.1)	.0–14.8
DC (CD3-/CD19-/CD14-/HLA-DR +)		24.7 (16.0)	.8–83.5	
mDC (CD3-/CD19-/CD14-/CD56-/HLA-DR + /CD123 + /CD11c-)		27.5 (17.0)	2.2–71.9	
pDC (CD3-/CD19-/CD14-/CD56-/HLA-DR + /CD123-/CD11c +)		14.5 (11.7)	.0–48.1	
Classical monocytes (CD45 + /CD4med/CD16-/CD14 +)		85.1 (11.1)	6.3–95.1	
Intermediate monocytes (CD45 + /CD4med/CD16 + /CD14 +)		5.1 (3.0)	.9–17.9	
Non classical monocytes (CD45 + /CD4med/CD16 + /CD14-)		4.3 (4.7)	.2–36.3	

M: mean; SD: standard deviation; Immune cell frequencies were assessed following multiparametric flow cytometry analysis and reported as percentages of the total acquired events of the parent population, using the gating strategy shown in the [Supplementary Fig. 1](#). CD8⁺ CM: CD8⁺ Central Memory; CD8⁺ EM: CD8⁺ Effector memory; CD8⁺ TEMRA: CD8⁺ Terminally differentiated Effector Memory; CD4⁺ CM: CD4⁺ Central Memory; CD4⁺ EM: CD4⁺ Effector memory; CD4⁺ TEMRA: CD4⁺ Terminally differentiated Effector Memory; Th: T helper; Th17: T helper 17; Th1: T helper 1; Th2: T helper 2; Tregs: Regulatory T cells; NK cells: Natural Killer cells; DC: Dendritic Cells; mDC: monocytes DC; pDC: plasmacytoid DC; NKT: NK T cells; ICOS: inducible T cell co-stimulator.

Table 3
Baseline regression models.

Dependent variable	Cell population at t ₀	β	95%- C.I. β	β st.	p
MADRS_AAS	CD4 ⁺ TEMRA	.490	.083; .897	.361	.020
MADRS_AVs	n.s.	n.s.	n.s.	n.s.	n.s.
MADRS_HpIn	CD8 ⁺ TEMRA	.054	.017; .092	.426	.006
MADRS_total	CD4 ⁺ TEMRA	.974	.291; 1.658	.413	.006
CSSRS-SI	Treg ICOS	-.181	-.342; -.020	-.334	.029
RI	CD4 ⁺ TEMRA	-.078	-.147; -.009	-.320	.028

MADRS: Montgomery-Asberg Depression Rating Scale; AAS: Affective and anhedonic symptoms; AVs: anxiety and vegetative symptoms; HpIn: Hopelessness; total: total score; RI: Recovery Index; CSSRS: Columbia-Suicide Severity Rating Scale SI: suicide ideation intensity; CD4⁺ TEMRA: CD4⁺ Terminally differentiated Effector Memory; DC: dendritic cell; ICOS: inducible T cell co-stimulator. n.s.: not significant.

T cells at the baseline was associated with a reduced response to treatments in terms of a lower reduction of total depressive symptoms. EM T cells are characterized by the expression of CD45RO marker and possess a high proliferative capacity compared to TEMRA, playing a crucial role in immediate defense against previously encountered pathogens (Devi et al., 2017; Niebuhr et al., 2022). The cytokines produced by EM T cells

Table 4
Regression models of the variation of the clinical variables between baseline and the 3-month follow-up.

Dependent variable	Cell population at t ₀	β	95%- C.I. β	β st.	p
Δ MADRS_AAS t ₁ -t ₀	n.s.	n.s.	n.s.	n.s.	n.s.
Δ MADRS_AVs t ₁ -t ₀	n.s.	n.s.	n.s.	n.s.	n.s.
Δ MADRS_HpIn t ₁ -t ₀	pDC	-.040	-.078; -.001	-.284	.042
Δ MADRS total t ₁ -t ₀	CD4 ⁺ EM T cells	289	006; .571	273	045
Δ RI t ₁ -t ₀	n.s.	n.s.	n.s.	n.s.	n.s.

MADRS: Montgomery-Asberg Depression Rating Scale; AAS: Affective and anhedonic symptoms; AVs: anxiety and vegetative symptoms; HpIn: Hopelessness; RI: Recovery Index; EM: Effector memory. n.s.: not significant.

can cross the blood-brain barrier and contribute to neuroinflammation (Erickson et al., 2012). This process affects brain regions involved in mood regulation, such as the hippocampus and prefrontal cortex, potentially leading to or exacerbating depressive symptoms (Rădulescu et al., 2021). Therefore, CD4⁺ EM T cells might represent a biomarker of negative response to antidepressant treatments in MDD. Conversely, baseline CD4⁺ naïve T cells were associated with remission, suggesting a

role of naïve T helpers in facilitating positive response to antidepressant treatments. This result aligns with the premature aging of the immune system hypothesis of depression and with a trend reported in a large European study on MDD (Schiweck et al., 2020).

Regarding innate immune cells, we found that at baseline pDC levels, predicted the reduction in hopelessness at three months. pDC are a small subset of DC, accounting for approximately .1%–.5% in the lymphoid organs. Initially identified as the primary producers of type I interferon (IFN) in human blood (Cella et al., 1999; Siegal et al., 1999), pDCs possess unique molecular adaptations for nucleic acid sensing, enabling their rapid type I IFN production (Ali et al., 2019). Notably, type I IFN has been linked to depressive symptoms in humans, highlighting the potential role of pDCs (Raison et al., 2006; Capuron and Miller, 2004). Conversely, our findings suggest a protective role for pDCs in depression. Noteworthy, pDCs have been linked to both disease promotion and protection. For instance, increased recruitment and activation of pDCs to arthritic joints, upon the topical application of the TLR7 agonist imiquimod, alleviated arthritis in a genetic mouse model (Nehmar et al., 2017). Furthermore, pDCs mitigated the progression of acute colitis independently of IFN-I signaling, resulting in reduced colonic production of proinflammatory cytokines (Arimura et al., 2017). pDCs also promote the differentiation of Tregs which help in suppressing inflammation and immune responses (Kuwana, 2002). In line, Chen et al. showed that pDCs protect against middle cerebral artery occlusion-induced brain injury by priming Tregs (Chen et al., 2020).

Several studies showed that NK cells are involved in depression and are associated with clinical severity (Frank et al., 2004; Park et al., 2015; Hernandez et al., 2010). In the present study, we assessed NK cell numbers without evaluating their functional activity and found no association with clinical severity or treatment response. Frank et al. and Park et al. reported that while clinical response to antidepressant treatment was not linked to changes in NK cell numbers, it was strongly associated with alterations in NK cell activity (Park et al., 2015; Frank et al., 1999). Hernandez et al. however, demonstrated a significant increase in NK cell numbers following a 52-week treatment with selective serotonin reuptake inhibitors (SSRIs). Notably, their findings were based on a cohort of only 10 patients assessed after one year of treatment, whereas our study evaluated NK cell responses over a shorter period of three months in a larger, potentially less homogeneous patient population (Hernandez et al., 2010). The absence of a similar effect in our study may be explained by differences in sample size and study duration.

This study highlights the development and validation of a multiparametric flow cytometry panel capable of analyzing over 30 immune cell populations, providing detailed insights into immune dynamics in MDD. Its prospective design enabled the evaluation of predictors of response and recovery following antidepressant therapy adjustments. In conclusion, our findings support the notion that a dysregulated immune system may significantly contribute to the elevated pro-inflammatory state associated with MDD (Drevets et al., 2022b; Lorenzo et al., 2024), and to its long-term adverse outcomes (Moylan et al., 2013), suggesting potential for targeted therapies. However, limitations include the need for larger cohorts and longer follow-ups to confirm findings. Future studies should explore immune cell populations and related cytokines as biomarkers and predictors of treatment response to improve outcomes for MDD patients.

CRedit authorship contribution statement

Fabiola Stolfi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Claudio Brasso:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Davide Raineri:** Writing – review & editing, Software, Methodology, Investigation, Data curation. **Virginia Landra:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Camilla Barbero Mazzucca:** Methodology, Data curation. **Ali Ghazanfar:**

Methodology. **Lorenza Scotti:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Riccardo Sinella:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Vincenzo Villari:** Writing – review & editing, Formal analysis, Data curation. **Giuseppe Cappellano:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition. **Paola Rocca:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Annalisa Chiochetti:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2024.100942>.

Data availability

Data will be made available on request.

References

- Ali, S., Mann-Nüttel, R., Schulze, A., Richter, L., Alferink, J., Scheu, S., 2019. Sources of type I interferons in infectious immunity: plasmacytoid dendritic cells not always in the driver's seat. *Front. Immunol.* 10.
- American Psychiatric Association. American Psychiatric Association, DSM-5 Task Force, 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5™*, fifth ed. American Psychiatric Publishing, Inc. <https://doi.org/10.1176/appi.books.9780890425596>.
- Arimura, K., Takagi, H., Uto, T., Fukaya, T., Nakamura, T., Chojookhuu, N., et al., 2017. Crucial role of plasmacytoid dendritic cells in the development of acute colitis through the regulation of intestinal inflammation. *Mucosal Immunol.* 10 (4).
- Belmaker, R.H., Agam, G., 2008. Major depressive disorder-belmaker. *N. Engl. J. Med.* 1 (358).
- Benros, M.E., Waltoft, B.L., Nordentoft, M., Ostergaard, S.D., Eaton, W.W., Krogh, J., et al., 2013. Autoimmune diseases and severe infections as risk factors for mood disorders a nationwide study. *JAMA Psychiatr.* 70 (8).
- Blackburn, T.P., 2019. Depressive disorders: treatment failures and poor prognosis over the last 50 years. *Pharmacol Res Perspect* 7 (3).
- Borentain, S., Gogate, J., Williamson, D., Carmody, T., Trivedi, M., Jamieson, C., et al., 2022. Montgomery-Åsberg Depression Rating Scale factors in treatment-resistant depression at onset of treatment: derivation, replication, and change over time during treatment with esketamine. *Int. J. Methods Psychiatr. Res.* 31 (4).

- Bortolato, B., Hyphantis, T.N., Valpione, S., Perini, G., Maes, M., Morris, G., et al., 2017. Depression in cancer: the many biobehavioral pathways driving tumor progression. *Cancer Treat Rev.* 52.
- Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon- α . *Biol. Psychiatr.* 56.
- Cella, M., Jarrossay, D., Facchetti, F., Aleardi, O., Nakajima, H., Lanzavecchia, A., et al., 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* 5 (8).
- Chen, C., Chencheng, Z., Cuiying, L., Xiaokun, G., 2020. Plasmacytoid dendritic cells protect against middle cerebral artery occlusion induced brain injury by priming regulatory T cells. *Front. Cell. Neurosci.* 14.
- Chiriță, A.L., Gheorman, V., Bondari, D., Rogoveanu, I., 2015. Current understanding of the neurobiology of major depressive disorder. *Rom. J. Morphol. Embryol.* 56 (2).
- Devi, M., Vijayalakshmi, D., Dhivya, K., Janane, M., 2017. Memory T cells (CD45RO) role and evaluation in pathogenesis of lichen planus and lichenoid mucositis. *J. Clin. Diagn. Res.* 11 (5).
- Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., et al., 2010. A meta-analysis of cytokines in major depression. *Biol. Psychiatr.* 67 (5).
- Drevets, W.C., Wittenberg, G.M., Bullmore, E.T., Manji, H.K., 2022a. Immune targets for therapeutic development in depression: towards precision medicine. *Nat. Rev. Drug Discov.* 21 (3).
- Drevets, W.C., Wittenberg, G.M., Bullmore, E.T., Manji, H.K., 2022b. Immune targets for therapeutic development in depression: towards precision medicine. *Nat. Rev. Drug Discov.* 21.
- Endicott, J., Nee, J., Harrison, W., Blumenthal, R., 1993. Quality of life enjoyment and satisfaction questionnaire: a new measure. In: *Psychopharmacology Bulletin*.
- Erickson, M.A., Dohi, K., Banks, W.A., 2012. Neuroinflammation: a common pathway in CNS diseases as mediated at the blood-brain barrier. *Neuroimmunomodulation* 19 (2).
- Fava, M., Kendler, K.S., 2000. Major depressive disorder. *Neuron* 28, 335–341.
- Frank, M.G., Hendricks, S.E., Johnson, D.R., Wieseler, J.L., Burke, W.J., 1999. Antidepressants augment natural killer cell activity: in vivo and in vitro. *Neuropsychobiology* 39 (1).
- Frank, M.G., Hendricks, S.E., Burke, W.J., Johnson, D.R., 2004. Clinical response augments NK cell activity independent of treatment modality: a randomized double-blind placebo controlled antidepressant trial. *Psychol. Med.* 34 (3).
- Gibney, S.M., Drexhage, H.A., 2013. Evidence for a dysregulated immune system in the etiology of psychiatric disorders. *J. Neuroimmune Pharmacol.* 8 (4).
- Goldsmith, D.R., Rapaport, M.H., Miller, B.J., 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol. Psychiatr.* 21 (12).
- Haapakoski, R., Mathieu, J., Ebmeier, K.P., Alenius, H., Kivimäki, M., 2015. Cumulative meta-analysis of interleukins 6 and β , tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain Behav. Immun.* 49.
- Hernandez, M.E., Martinez-Fong, D., Perez-Tapia, M., Estrada-Garcia, I., Estrada-Parra, S., Pavón, L., 2010. Evaluation of the effect of selective serotonin-reuptake inhibitors on lymphocyte subsets in patients with a major depressive disorder. *Eur. Neuropsychopharmacol.* 20 (2).
- Hoeks, C., Puijfelik, F van, Koetzier, S.C., Rip, J., Corsten, C.E.A., Wierenga-Wolf, A.F., et al., 2024. Differential Runx 3, Eomes, and T-bet expression subdivides MS-associated CD4+ T cells with brain-homing capacity. *Eur. J. Immunol.* 54 (2).
- Hong, M., Zheng, J., Ding, Z.Y., Chen, J.H., Yu, L., Niu, Y., et al., 2012. Imbalance between Th17 and treg cells may play an important role in the development of chronic unpredictable mild stress-induced depression in mice. *Neuroimmunomodulation* 20 (1).
- Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with c-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom. Med.* 71 (2).
- IsHak, W.W., Bonifay, W., Collison, K., Reid, M., Youssef, H., Parisi, T., et al., 2017. The recovery index: a novel approach to measuring recovery and predicting remission in major depressive disorder. *J. Affect. Disord.* 208.
- Kappelmann, N., Lewis, G., Dantzer, R., Jones, P.B., Khandaker, G.M., 2018. Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol. Psychiatr.* 23 (2).
- Katon, W.J., 2011. Epidemiology and treatment of depression in patients with chronic medical illness. *Dialogues Clin. Neurosci.* 13 (1).
- Klopach, E.T., Crimmins, E.M., Cole, S.W., Seeman, T.E., Carroll, J.E., 2022. Social stressors associated with age-related T lymphocyte percentages in older US adults: evidence from the US Health and Retirement Study. *Proc. Natl. Acad. Sci. U.S.A.* 119 (25).
- Köhler, O., Benros M, E., Nordentoft, M., Farkouh, M.E., Iyengar, R.L., Mors, O., et al., 2014. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatr.* 71 (12).
- Köhler, C.A., Freitas, T.H., Maes, M., de Andrade, N.Q., Liu, C.S., Fernandes, B.S., et al., 2017. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr. Scand.* 135 (5).
- Kuwana, M., 2002. Induction of anergic and regulatory T cells by plasmacytoid dendritic cells and other dendritic cell subsets. *Hum. Immunol.* 63 (12).
- Lee, C.H., Giuliani, F., 2019. The role of inflammation in depression and fatigue. *Front. Immunol.* 10.
- Li, D.Y., Xiong, X.Z., 2020. ICOS+ Tregs: a functional subset of Tregs in immune diseases. *Front. Immunol.* 11.
- Lorenzo, E.C., Figueroa, J.E., Demirci, D.A., El-Tayyeb, F., Huggins, B.J., Illindala, M., et al., 2024. Unraveling the association between major depressive disorder and senescent biomarkers in immune cells of older adults: a single-cell phenotypic analysis. *Frontiers in Aging* 5.
- Miller, A.H., 2010. Depression and immunity: a role for T cells? *Brain Behav. Immun.* 24 (1).
- Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatr.* 65 (9).
- Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. *Br. J. Psychiatry* 134 (4).
- Moylan, S., Maes, M., Wray, N.R., Berk, M., 2013. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. *Mol. Psychiatr.* 18 (5).
- Müller, N., 2014. Immunology of major depression. *Neuroimmunomodulation* 21 (2–3).
- Müller, M.J., Himmerich, H., Kienle, B., Szegedi, A., 2003. Differentiating moderate and severe depression using the Montgomery-Åsberg depression rating scale (MADRS). *J. Affect. Disord.* 77 (3).
- Mundt, J.C., Marks, I.M., Shear, M.K., Greist, J.H., 2002. The Work and Social Adjustment Scale: a simple measure of impairment in functioning. *Br. J. Psychiatry* 180 (MAY).
- Nehmar, R., Alsaleh, G., Voisin, B., Flacher, V., Mariotte, A., Saferding, V., et al., 2017. Therapeutic modulation of plasmacytoid dendritic cells in experimental arthritis. *Arthritis Rheumatol.* 69 (11).
- Nemeroff, C.B., 2020. The state of our understanding of the pathophysiology and optimal treatment of depression: glass half full or half empty? *Am. J. Psychiatr.* 177, 671–685.
- Niebuhr, M., Bahreini, F., Fähnrich, A., Bomholt, C., Bieber, K., Schmidt, E., et al., 2022. Analysis of T cell repertoires of CD45RO CD4 T cells in cohorts of patients with bullous pemphigoid: a pilot study. *Front. Immunol.* 13.
- Østergaard, S.D., Jensen, S.O.W., Bech, P., 2011. The heterogeneity of the depressive syndrome: when numbers get serious. *Acta Psychiatr. Scand.* 124 (6).
- Park, E.J., Lee, J.H., Jeong, D.C., Han, S.I., Jeon, Y.W., 2015. Natural killer cell activity in patients with major depressive disorder treated with escitalopram. *Int. Immunopharm.* 28 (1).
- Posner, K., Brown, G.K., Stanley, B., Brent, D.A., Yershova, K.V., Oquendo, M.A., et al., 2011. The Columbia-suicide severity rating scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *Am. J. Psychiatr.* 168 (12).
- Rădulescu, I., Drăgoi, A., Trifu, S., Cristea, M., 2021. Neuroplasticity and depression: rewiring the brain's networks through pharmacological therapy. *Exp. Ther. Med.* 22 (4) (Review).
- Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* 27.
- Schiweck, C., Valles-Colomer, M., Arolt, V., Müller, N., Raes, J., Wijkhuijs, A., et al., 2020. Depression and suicidality: a link to premature T helper cell aging and increased Th17 cells. *Brain Behav. Immun.* 87.
- Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., et al., 1999. The nature of the principal Type 1 interferon-producing cells in human blood. *Science* (1979) 284 (5421).
- Steinert, C., Hofmann, M., Kruse, J., Leichsenring, F., 2014. Relapse rates after psychotherapy for depression - stable long-term effects? A meta-analysis. *J. Affect. Disord.* 168.
- Stolfi, F., Abreu, H., Sinella, R., Nembrini, S., Centonze, S., Landra, V., et al., 2024. Omics approaches open new horizons in major depressive disorder: from biomarkers to precision medicine. *Front. Psychiatr.* 15 [Internet]. <https://www.frontiersin.org/articles/10.3389/fpsy.2024.1422939/full>.
- Tubbs, J.D., Ding, J., Baum, L., Sham, P.C., 2020. Immune dysregulation in depression: evidence from genome-wide association. *Brain Behav Immun Health* 7.
- Voineskos, D., Daskalakis, Z.J., Blumberger, D.M., 2020. Management of treatment-resistant depression: challenges and strategies. *Neuropsychiatric Dis. Treat.* 16.
- Windle, M., Windle, R.C., 2013. Recurrent depression, cardiovascular disease, and diabetes among middle-aged and older adult women. *J. Affect. Disord.* 150 (3).
- Zimmerman, M., Posternak, M.A., Chelminski, I., 2004. Defining remission on the montgomery-asberg depression rating scale. *J. Clin. Psychiatr.* 65 (2).