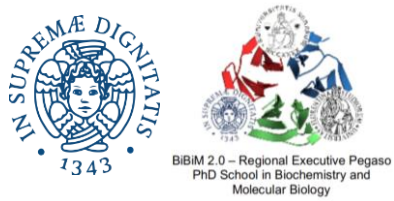


Federico Mucci
Signed by Federico Mucci

UNIVERSITÀ DEGLI STUDI DI SIENA

DIPARTIMENTO DI BIOTECNOLOGIE, CHIMICA E FARMACIA
DEPARTMENT OF BIOTECHNOLOGY, CHEMISTRY AND PHARMACY

DOCTORAL COURSE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY - BIBIM 2.0

Cycle XXXV

Coordinator: Prof. Lorenza Trabalzini

Circulating levels of vitamin D, vitamin B12, folate, homocysteine, and major haemato-chemical parameters in mood-disorder patients: a comparative cross-sectional study

Tutor:
Prof. GINO GIANNACCINI

PhD Candidate:
Dr. FEDERICO MUCCI

Academic year: 2021/2022

⁂

Table of Contents

Abstract

The potential involvement of the immune and inflammatory systems, together alterations of their biochemical markers, has been extensively studied in mood disorders such as major depression and bipolar disorders. This topic in clinical research is currently providing important results. Indeed, an ever-growing body of evidence is showing the presence, within patients, of activated inflammatory patterns resulting from altered neurotransmission and abnormally regulated neuroendocrine response to stress; bidirectionally, inflammatory patterns have been found to provoke impaired neurotransmission, stress coping and metabolism in mood disorders. Moreover, several clinical surveys have reported that plasma levels of some pro-inflammatory cytokines and acute phase proteins can vary depending on the phases of these disorders. As well, patients have been found to display disrupted redox systems and nutritional status. Despite these findings and despite the fact that the pathogenetic role of altered immunologic and metabolic profiles in mood disorders is being confirmed in many current studies, there is still a general lack of consensus about it, due to results that do not always agree. This implies the need to extend previous investigations.

In such a context, the present study aimed to appraise peripheral metabolic parameters and plasma/serum levels of essential nutrients in a group of inpatients affected by mood disorders for comparison with the same biological indexes measured in a control sample. Specifically, the study compared, in these two groups, blood glucose, lipoproteins, triglycerides, uric acid, blood urea nitrogen (BUN), transaminases and other haemato-chemical parameters, together the plasma/serum levels of some vitamins (vitamin D, B12, folate) and homocysteine, a key substrate of methionine metabolism which is involved in crucial pleiotropic enzyme reactions in the body. Results showed that most patients, despite their heterogeneity in diagnosis and clinical presentation, were highly defective in circulating vitamin D levels, not only in respect to control subjects ($P < .0001$) but even in respect to the general population normative cut-off values. This vitamin D result was paralleled by strongly increased serum homocysteine concentrations in patients vs. controls ($P<.0001$), indicating an imbalance in their methionine metabolism, a finding suggesting the impairment at the level of methylation patterns and redox regulatory fluxes in affected subjects. Surprisingly, homocysteine levels were negatively correlated with vitamin D, vitamin B12 and folate values in control subjects but not in patients, suggesting that changes of methionine metabolism and vitamin deficits were occurring independently in patients. In addition, patients displayed higher blood glucose and lower BUN than controls, indicating an impaired protein-to-carbohydrate metabolism and/or altered nutritional/dietary status, such as the preferential assumption of carbohydrates rather than proteins. Briefly, we provide herein further substantiation to the idea that mood disorder patients are a population where vitamin deficits, dysmetabolism and/or dietary defects are very common features. As a corollary, we want to emphasize herein that people affected by

major depressive disorder (MDD) or bipolar disorders (BDs) are exposed to a greater risk of a variety of somatic illnesses than the general population.

In summary, this cross-sectional investigation, albeit preliminary, contributes to improve the characterization and the monitoring of the clinical status of mood disorder patients. Our results are the starting point to the identification of new molecular targets for more tailored treatments, as well as to the implementation of more pointed health-care interventions, such as nutritional measures and the co-administration of essential substrates with psychotropic drugs and/or psychological therapies.

1. Mood disorders: Introduction

Major depressive disorder (MDD) is a commonly occurring psychiatric condition affecting approximately 220 million people worldwide. It is a highly disabling disease and can impair almost any aspect of daily living [1]. The overall impact of depression in the global population is now emerging as a social issue: the World Health Organization (WHO) estimates that it will be the second most common reason for disability in the general population, resulting in huge economic consequences, especially impacting Western society [2, 3].

A study of the adult population by the National Institute of Mental Health (NIMH) found that the lifetime risk of MDD varies from 5-12% for men and 10-25% for women; the lifetime prevalence ranges from 2-3% for men and from 5-9% for women [3, 4].

Mood disorders are currently classified in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as:

- Depressive disorders, which include MDD and other depressive-related disorders (i.e., persistent depressive disorder (dysthymia), disruptive mood dysregulation disorder, substance/medication-induced depressive disorder, premenstrual dysphoric disorder, depressive disorder as a result of another medical condition, other specified depressive disorders, and unspecified depressive disorders) [5].
- Bipolar and associated disorders, including bipolar disorder (BD) of type I and II (BDI and BDII) and other mood-related disorders (i.e., substance/medication-induced bipolar and related disorder, cyclothymic disorder, bipolar and related disorders stemming from another medical condition, other specified bipolar and related disorders, and unspecified bipolar and related disorders) [5].

A detailed and in-depth discussion of the various depressive-related disorders and mood-related disorders subtypes is not within the scope of this dissertation. Thus, as MDD and of BDI and BDII are the most frequently occurring disorders among those mentioned, the following paragraphs present a brief review of them.

1.1 Major depressive disorder

Major depression can occur as a single episode or, more often, is recurrent in 50-85% of cases; in this case, seasonal trends are possible with recurrences in spring or autumn. There is usually a gradual onset, and full-blown illness is usually preceded by prodromal symptoms that last for days or even weeks, such as unstable mood, energy decline, difficulty concentrating, insomnia, lack of appetite, and/or somatic complaints [5].

When there is full-blown symptomatology, the mood can be sad, depressed, discouraged, and hopeless. There is often a lack of interest or pleasure, and individuals may report not being able to enjoy activities that they had previously

enjoyed. Increased irritability may also be present and accompanied by getting frustrated at seemingly trivial occurrences; there can also be a tendency to respond to events in an angry manner. In terms of psychomotor functioning, slowing, lack of energy, exhaustion, or – more rarely – agitation may be present [1-5].

Cognitive symptoms include slower thought, blunting, and a reduced ability to concentrate. Thinking becomes pessimistic, with a negative perspective of oneself, of the world, and of the future; mood-congruent (guilt, worthlessness, personal inadequacy, disease, deserved punishment, nihilism) or mood-incongruent delusions (persecutory delusion, delusion of control) can also occur. Patients can experience deep sadness, pessimism, emotional indifference, and hopelessness, leading to depression and the consideration of death as the only escape from suffering. 50% of patients with depression have suicide ideation and 15% will attempt suicide [5, 6].

The risk of suicide is present continually during depressive episodes. However, factors that have been linked with increased risk of suicide include a history of suicide attempts, a family history of suicide, being of male sex, being single, or living alone. Borderline personality disorder also increases the risk

Neurovegetative symptoms are also present, including insomnia (or hypersomnia), poor appetite (or hyperphagia), diminished libido, with symptoms worsening during the day. The depressive symptomatology can also be associated with anxiety disorders or complicated by alcohol or substance abuse. Comorbidity worsens the prognosis and complicates the treatment [1-6].

If left untreated, MDD will usually last 6-12 months and normal functioning returns after recovery, although residual symptoms can impair social and professional functioning. Sometimes the depressive symptoms may last for over two years (persistent depressive disorder in DSM-5). Factors associated with prolonged cases are comorbidities, onset at an early age, unfavourable psychosocial conditions, poor global functioning and/or early interruption of treatment. After two months, patients can expect very little chance of reoccurrence; however, the chance is greater in younger people, in those who have already had several episodes, and in those where the preceding episode was severe [1-6].

Treatment for MDD includes both pharmacological and non-pharmacological interventions [6]. Double-blind controlled studies that compare the efficacy of different AD classes have not found any differences between compounds. Consequently, in clinical practice the selection of AD takes into account any potential side effects and the presence of specifiers and/or comorbid disorders. Table 1 shows a summary of the currently available compounds for treating depression:

Table 1. Antidepressants commonly used for treatment of major depressive disorder.

Drug	Mean dose (mg/day)
SSRI	

Citalopram, fluoxetine, paroxetine	20-60
Fluvoxamine	150-300
Sertraline	50-200
Escitalopram	10-20
TCA	
Amitriptyline, Clomipramine, Imipramine	100-250
Nortriptyline	150
Trimipramine	100-300
SNRI	
Duloxetine	60-120
Venlafaxine	75-300
Dopamine and norepinephrine reuptake inhibitor	
Bupropion	150-300
NaRI	
Reboxetine	4-8
Serotonin modulator	
Trazodone	75-300
NaSSA	
Mirtazapine	30-45
MAOI	
Phenelzine	15-60
Tranylcypromine	30-60
Serotonin modulator and stimulator	
Vortioxetine	5-20

Standard non-pharmacological approaches to depression include psychotherapy (cognitive-behavioural, interpersonal, or brief dynamic psychotherapy), electroconvulsive therapy (ECT) and other somatic treatments (vagus nerve stimulation, transcranial magnetic stimulation, chronotherapy, and deep brain stimulation) [6].

Although there have been many advances in the psychopharmacological treatment of major depression and newly introduced new-generation AD, 10%-15% of patients still fail to respond to pharmacological therapies and a further 30-40% only have partial remissions [6].

1.2 Bipolar disorders

BD is a disorder that has greater severity and complexities than MDD, as measured by a greater incidence of lifelong suffering and increased comorbidities

with psychiatric disorders, and in particular disorders involving substance abuse and anxiety [7-9]. Moreover, it also has associations with serious somatic disorders including cardiovascular disease, hypertension, and diabetes [10, 11]. This goes some way to explaining the correspondingly higher mortality rates amongst those with bipolar disorder; however, the suicide risk is lower in type I bipolar patients than in depression [10, 11].

The term BD(s) refers to highly debilitating, chronic disorders encompassing various conditions that affect mood, bio-rhythmicity, and psychomotricity [5, 7]. Patients usually experience the alternation of *phases* characterised by the elevation or lowering of these symptoms [9]. Currently, the DSM-5 includes four main clinical pictures within the BDs chapter, all characterised by unusual shifts in mood, activity, and energy levels that may, or may not, significantly impair daily activities. In particular, the DSM-5 recognises BD of type I (BDI), BD of type II (BDII), the *so-called* cyclothymia (also called cyclothymic disorder), and "other specified and unspecified bipolar and related disorders", all of which involve mood swings and variations in activity or energy levels. Essentially, BDI and BDII can be distinguished by the presence of one or more episode(s) of mania and hypomania, respectively [5, 9].

The definition of mania and hypomania are as a set of signs and symptoms characterised by significant elevations of mood levels, abnormally increased energy levels, consistent feelings of happiness or irritability, an increase in impulsivity and risky behaviours, and a lower need for sleep. They can be distinguished in accordance with the severity of symptoms and/or psychosis being present [5, 9, 12].

Conversely, depressive periods are characterised by apathy, melancholy, and significant reductions in activity and energy levels [5, 9, 13]. Indeed, bipolar depressive episodes occur a minimum of three time as frequently and last at least three times longer than (hypo) manic episodes [13]. Moreover, compared to (hypo)mania, depression is linked to a higher risk of suicide and with higher disability levels in social, work, and family functioning [13]. The diagnostic criteria and the specifiers reported in the DSM-5 are the same as those for unipolar depression [5]. However, authors have hypothesised that bipolar and unipolar depressions could represent distinct nosological entities, underlaid by various biological substrates, and tried to distinguish them from a phenomenological perspective (table 2). Research on this subject has so far been inconclusive, and a dimensional and probabilistic approach (mood spectrum model) is the prevailing viewpoint today. This approach states that potentially bipolar episodes include all depressive episodes that, although they do not alternate with spontaneous manic or hypomanic phases, present with at least one of the following characteristics:

a) early or post-partum onset;

b) atypical symptoms (hypersomnia, significant increase in weight or an increased appetite, leaden paralysis, interpersonal rejection sensitivity)

or mood-incongruent psychotic symptoms (persecutory delusion, delusion of control);

c) co-occurrence of sub-threshold excitatory symptoms;

d) seasonal recurrence;

e) high relapse frequency.

Table 2. Differences in symptom profiles between bipolar and unipolar depression

Symptoms more frequent in bipolar than in unipolar depression	Symptoms more frequent in unipolar than in bipolar depression
• Mood lability • Psychomotor retardation • Atypical features • Psychotic features • Irritability • Mixed features • Tension / fearfulness • Late insomnia • Hypersomnia	• Somatic complaints • Appetite loss • Initial insomnia • Pain sensitivity • Anxiety within episode

Some authors have suggested considering potential bipolar episodes as also being unipolar depressions followed by (hypo)manic episodes induced by antidepressants, for example treatment-resistant depressions and depressions occurring in those who have a family history of bipolar disorder or with cyclothymic or hyperthymic premorbid temperament [12, 13]. Treating bipolar depression is complex, highly controversial and challenging. There is currently no specific treatment that suits all patients; therefore, practice clinicians should personalise treatment after carefully evaluating the patient's clinical features and specifiers of the current episode. Pharmacological options include antidepressants, mood stabilisers, second generation antipsychotics, and dopamine agonists [13]. However, antidepressants (ADs) in the treatment of bipolar disorders remains a controversial area in psychiatry. There is little evidence of their efficacy and safety (risk of switch to (hypo)mania and/ or of suicidality), with international guidelines suggesting that AD monotherapy is inappropriate for bipolar depression, and particularly bipolar I depression. Yet, ADs are the prevalent short-term treatment for bipolar depression in clinical practice (see Table 1 for compounds and dosages).

Manic episodes are characterised by mood changes and alterations to cognition, activity and vegetative functioning. This state is usually preceded by prodromal symptoms including an unusual sensation of well-being, irritability, heightened energy and sexual activity, and a diminished need for sleep. Onset can be sudden [5, 7, 12, 13].

In the presence of full-blown symptomatology, the individual is usually elated and appears euphoric, self-confident, happy, joyful, and sometimes feels

"beatitude" and "illumination" [5, 12, 13]. Occasionally, symptoms can also include dysphoria with grumpiness, irritability and aggression. Continuous mood variations with rapid shifts over short periods of time from euphoria to dysphoria/irritability or depression ("mood lability") is typical. Cognitive functioning displays changes in both form and content. The subjective sensation of the patient is that of heightened intellectual efficiency, although poor concentration and distractibility are objectively observable. Thought is accelerated and frequently fragmented and can even result in loss of association ("flight of ideas"). Thoughts can tend towards grandiosity with an exaggerated sense of one's own physical strength, aesthetic appearance, intellectual abilities, and economic possibilities. Delusions are present in 45-75% of patients and can persist with the typical manic themes of grandiosity ("mood-congruent psychotics features") or not ("mood-incongruent psychotics features"). Hallucinations and catatonic symptoms generally occur less frequently than delusions. Motor activity is greatly increased, so individuals appear impulsive and excessively plan and participate in multiple activities. This heightened energy means that affected individuals conduct even highly difficult tasks with no sense of fatigue and, in less severe forms, it determines an increase in goal-directed activity. As the disorder progresses, there is a loss in the capacity to achieve intended aims. When motor excitation is more intense, inhibition is lost, and rage bursts with verbal and physical aggressiveness and impulsive suicide attempts are possible. There is a reduction in the need to sleep, even complete insomnia, increased appetite and sexual drive are usually observed. Denial and lack of insight are typical of mania and explain, at least in part, the patient's poor treatment's compliance [5, 7, 8, 10, 12].

The average duration of an untreated manic episode is 4-6 months, although it can vary from several days to several years. In 5-10% of patients, mania can become chronic (chronic mania) [7, 12].

Hypomanic symptoms partially overlap with those of manic episodes, but they are less severe. Individuals in hypomania appear happy, lively, self-confident, and assertive. As in mania, mood in hypomania may be unstable, with frequent sadness or irritability. Individuals with hypomania have a heightened productive capacity and a limited need for rest and sleep. The degree of activation can sometimes be so intense and subjectively unpleasant that use of alcohol or benzodiazepines for self-medication purposes is present. However, the hypomanic experience is usually pleasant, and the patients may either be unaware or in denial of it. Consequently, individuals in a hypomanic state do not spontaneously seek treatment, unless complications develop, a comorbid disorder is present, or they are urged by family members to do so [5, 12].

Pharmacological treatment includes different classes of drugs including mood stabilizers (lithium), anticonvulsants, first- and second-generation antipsychotics, benzodiazepines and other experimental agents (Table 3).

Table 3. Drugs commonly used for treatment of manic and hypo-manic episodes.

Drug	Mean dose (mg/day)

Mood stabilizers	
Lithium	900-1800 (plasma level 0.5-1.2 mEq/L)
Anticonvulsants	
Valproic acid	500-1500 (plasma level 50-125 μg/mL)
Carbamazepine	400-1200 (plasma level 4-12 mg/mL)
Oxcarbazepine	600-1800
First-generation antipsychotics	
Haloperidol	5-30
Chlorpromazine	25-300
Levomepromazine	25-300
Second-generation antipsychotics	
Amisulpride	400-1200
Aripiprazole	10-30
Asenapine	10-20
Clozapine	25-400
Cariprazine	3-12
Lurasidone	74-148
Olanzapine	5-20
Paliperidone	6-12
Quetiapine	200-800
Risperidone	3-12
Ziprasidone	80-160

Note: *Doses are usually high in mania and in mania with mixed features, low in hypomania and depression with mixed features.*

Mood disorders include other complex presentations defined as “mood disorders with mixed features” (or “mixed states”) characterized by the co-occurrence of manic and depressive and symptoms. Such clinical scenarios are challenging for clinicians at the classification, diagnostic and therapeutic levels [5, 14].

Mixed states, defined as the co-occurrence of depressive and manic symptoms, are complex presentations of mood disorders that that are challenging for clinicians at the classification, diagnostic and therapeutic levels. In the previous edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV-TR), the diagnosis of mixed state required the full criteria for a manic and a depressive episode to also be present for at least one week [14, 15]. Therefore, by definition, this diagnosis was restricted to bipolar I disorder. These narrow criteria are not very useful, as they excluded patients with “mixed depression” – i.e., with full criteria for depression and subthreshold manic symptoms – the

mixed states more commonly observed in clinical practice. In the DSM-5, where the categorical diagnoses are supplemented with a dimensional approach, the diagnosis of mixed state has been removed and the specifier with mixed features has been provided for manic, hypomanic, and unipolar or bipolar depressive episode with subthreshold non-overlapping symptoms of the opposite polarity (see section Diagnostic criteria). So, by definition, this specifier is applied to bipolar I, bipolar II and major depressive disorders (table 2) [14, 15].

However, a number of clinicians experienced in mood disorders have indicated that the DSM-5 criteria for "major depressive episode with mixed features" remain unsatisfactory because they fail to capture the core feature of "mixed depression". In fact, these criteria include, as non-overlapping manic symptoms, euphoria and grandiosity, which are rarer among patients with mixed depression and do not include mood lability, psychomotor agitation and irritability, the core features of mixed depression observed in clinical practice. Some authors have therefore proposed and validated criteria for mixed depression based on their clinical experience and broader than those of DSM-5 (table 4) [8, 14, 15].

Table 4. DSM-5 criteria of mood disorders with mixed features.

Hypo-Manic episode with mixed features	Depressive episode with mixed features
1. dysphoric or depressed mood 2. diminished interests or pleasure 3. psychomotor retardation 4. fatigue or loss of energy 5. worthlessness or inappropriate guilt 6. recurrent thoughts of death	1. elevated or expansive mood 2. inflated self-esteem 3. talkativeness 4. flight of ideas 5. increased energy or goal directed activity 6. excessive involvement in risky activities 7. decreased need for sleep

Note: *A manic or hypomanic episode with mixed features is diagnosed if the full criteria are met for a manic or hypomanic episode and three or more of the symptoms listed on the left are present. A depressive episode with mixed features is diagnosed if the criteria for a major depressive episode are met together and at least three of the symptoms listed on the right.*

Approximately 30% of mood episodes, in both major depression and in bipolar disorders, have a mixed presentation, and occur more commonly in women than in men [5, 14].

Table 5. Koukopoulos diagnostic criteria for mixed depression.

Full criteria for major depressive episode are met together with at least three of the following symptoms:

1. inner tension/agitation
2. racing or crowded thoughts
3. irritability or unprovoked feelings of rage
4. absence of signs of retardation
5. talkativeness
6. dramatic description of suffering or frequent spells of weeping
7. mood lability and marked emotional reactivity
8. early insomnia

The clinical presentation of mixed states varies between a single opposite-state symptom found within an otherwise "pure" manic or depressive episode (*e.g.*, depressive mood during mania or racing thoughts during depression) to more complex mixtures of mood, thought, and behaviors. The usual clinical picture is characterized by dysphoric mood that alternated with depressed and elevated mood (*i.e.*, mood lability), racing thoughts, suicidal ideation (30-50% of cases), psychomotor agitation, severe insomnia. Up to 50% of mixed manic episodes include psychotic symptoms such as delusions (persecutory, referential, religious, somatic), auditory hallucinations, formal thought disorder, depersonalization/derealization [5, 14].

Some authors have hypothesized that mixed presentation of a mood episode (any polarity) can be caused or made or worse through the use/abuse of alcohol, substance, psychostimulants, benzodiazepine or drugs acting on central nervous system (CNS) (antidepressants [ADs], first generation antipsychotics [FGAs], corticosteroids, antihypertensive agents), or by the presence of a neurological disease. Mixed presentation of a mood episode may be also a family trait [14].

Compared to episodes without mixed features, episodes with mixed features (any polarity) usually last longer and have a lower inter-episode remission rate, a higher risk of future mixed relapses, a poorer prognosis and a poorer response to treatments [14].

Treating mood episodes with mixed features is challenging for clinicians because: a) current international guidelines, with some exceptions, do not recommend specific treatments for these conditions; b) data on the treatment of mixed depression are scarce, c) data on the treatment of mixed mania were extrapolated from trials including patients with pure mania, with the assumption of a comparable response with this sub-population. Clinicians usually select treatment in terms of the primary diagnosis (mania, hypomania or depression) and the number and severity of the symptoms of the opposite polarity. After treatment has begun, regular and careful evaluation of the number and severity of manic and depressive symptoms is required to monitor the response and modify treatment. All drug classes for manic and depressive episodes should be used in mixed states and, as these conditions are usually resistant to monotherapy, so that combined treatment is often required.

Finally, rapid cycling (RC) is a specific bipolar course, initially described for bipolar patients with four or more distinct mood episodes over the course of the previous year [16, 17]. Subsequently, the DSM-IV and the DSM-5 have adopted

the “rapid cycling” denomination for those specific cases of bipolar disorder of type I and II, characterized by at least four mood episodes meeting the criteria for major depression, mania, hypomania, or mixed state (in DSM-IV) over a period twelve months [16]. The episodes of the same polarity must be separated by a period of partial or complete remission for at least 2 months, while switching between episodes of opposite polarity can be abrupt. The DSM definition, however, only partially covers the rapid cycling spectrum, excluding cases of ultrafast cycling (where the episodes last days) and ultradian cycling (oscillations within 24 hours) [5, 16].

Scholars agree that, more often, depression may reoccur (up to 50-85% of cases) [6, 18]. Thus, different authors and clinicians have identified MDD as a part of a wider *continuum* encompassing depressive and manic symptomatology at different severity levels on a longitudinal course. Yet, in several patients, the onset of depression can result in mania or, even more frequently, depressive symptoms appear together with manic symptoms within the same episode [18]. The concept of a *continuum* between depressive and manic states has been defined as “bipolar spectrum” and widens the number of bipolar subtypes, by up to 50% of all mood disorders [18]. Mood disorders remain challenging for clinicians, where there is a significantly high risk of periodic relapses (up to 80-90% of cases) despite the efficacy of currently available treatment strategies. Therefore, clinicians, when diagnosing bipolar disorder, should not only think about a therapy for the acute phase, but also evaluate the opportunity of a "preventive" treatment to reduce the risk of future relapses.

One of the main limitations slowing the progress of scientific research in the treatment of mood disorders is the paradigm of considering such pathologies as strictly "mental" (or at least limited to the CNS) instead of as an epiphenomenon of systemic diseases. Gradually, evidence has begun to suggest that mood disorders are multifactorial disorders as they include a combination of intertwined genetic, epigenetic, transcriptional and/or acquired factors to define a variety of biological endophenotypes and clinical pictures [19]. These factors involve not only the brain, but also the whole organism. Consequently, mood disorders should be considered as systemic diseases [19]. Moreover, changes in the monoamine system, described at the dawn of biological hypotheses of mood disorders, are now gradually being integrated with a more complex and comprehensive model of mood disorders, including immune/inflammatory processes, oxidative and nitrosative stress, and changes in peripheral tissues and organs as gut, at the level of the so-called enteric nervous system (ENS). Proinflammatory compounds and molecular patterns can in fact impact the intestinal mucosa at the level of its integrity and absorption properties, as well as at the level of the diversity of the main phyla, classes, orders, genera and species of colonizing bacteria [20]. In turn, changes of the gut microbiota, including variations of specific phyla ratios and even of single species, can relevantly alter the host global metabolic abilities and well-being. The cross-talks between the brain-body axis can be relevantly affected in mood disorders. Indeed, alterations of these signaling networks are being increasingly revealed in different psychopathological conditions, such as psychotic disorders, eating disorders,

obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD) and autism spectrum disorders (ASDs). The results may be able to shed light on the neural basis of mood disorders phenotypes, within an integrative framework between central and peripheral systems in this field. Moreover, from a clinical perspective, the evaluation of measurable biological markers, could help to improve patients' diagnostic and follow-up procedures by identifying new healthcare approaches as well as new targets for therapeutic strategies.

2. Biological hypotheses of mood disorders

2.1 Mood disorders and inflammation

Although amongst psychiatric syndromes MDD is the most common cause of disabilities worldwide [21], paradoxically, the causes of such a "*quasi pandemic*" condition remain unknown. Therefore, over the decades different models have been developed that have focused on monoaminergic systems, dysfunctions of the hypothalamus-pituitary-adrenal (HPA) axis circadian rhythms, inflammation and dysfunction of the immune system, structural or functional abnormalities of emotional circuits (i.e., the *so-called* "limbic cortical model" and the "cortico-striatal model" [22-29]. It is now evident that MD is a complex multifactorial entity that perhaps involves a combination of a genetic vulnerability interacting with different biological, psychological, genetic and social factors [26].

Understanding of the disorder's pathophysiology is still limited. For years, after the serendipitous discovery of the first two agents with antidepressant action, iproniazide and imipramine, changes to monoamine neurotransmission (monoamine deficiency, alterations of monoamine receptors) is deemed the most relevant pathophysiological mechanism of MDD. Thus, the enhancement of monoamine neurotransmission, including serotonin (5-HT), norepinephrine and dopamine, has been the only pharmacodynamic mechanism used to develop novel antidepressants (ADs) [1, 2]. However, data supporting the postulated MDD-related monoamine alterations are inconclusive and the monoamine hypotheses are insufficient for explaining the whole picture of the disorder [30, 31]. The lack in the understanding of the neurobiological mechanisms underlying MDD also limits the efficacy of the treatments. In fact, although the discovery of the selective serotonin reuptake inhibitors (SSRIs) and the dual-acting serotonin/norepinephrine reuptake inhibitors (SNRIs) improved the outcome and prognosis of MDD with specific benefits of old generation antidepressants, particularly in terms of side effects, the treatment of the disorder is unsatisfactory [1, 2, 30, 31]. Consequently, there remain relevant unmet clinical needs:

a) side effects leading to drug discontinuation, including sexual dysfunctions, gastrointestinal distress and weight gain;
b) the long latency of the antidepressant action, which increases suicide risk and due to lack of efficacy can provoke early discontinuations;
c) the response rate, as available ADs are effective in only two thirds of MDD patients and are part of the relapse process after initial improvement or even after a sustained period of remission, even if the drug is continued.

It is generally agreed amongst researchers that impairments of cerebral serotonergic activity pose an important risk for developing MDD in vulnerable people, and/or that 5-HT system dysfunctions may underlie symptom clusters or dimensions in MD and even in a number of other neuropsychiatric conditions

[1, 30, 31]. One or more abnormalities in different processes of serotonergic activities could be important to initiate or trigger depression, such as deranged 5-HT synthesis, release, reuptake, metabolism, eventually involving changes to the activation of various pre- and post-synaptic 5-HT receptors, as shown by different research tools.

According to recent hypotheses, auto receptors and some postsynaptic agents regulating neurotransmitter synthesis are involved in blocking the reuptake mechanism that occurs immediately after antidepressant administration [32, 33]. Prolonged exposure to antidepressants triggers the activation cascade of the modulation of several genes involved in expressing and synthesizing different trophic factors [34].

Consequently, antidepressant drugs (and mood stabilizers) may restore neuronal circuit functionality involved in regulating mood, emotions and cognitive processes via a partial recovery of their trophism [34]. Moreover, the loss of neuronal trophism reduces the ability to adapt to the environmental stimuli of the brain areas that regulate emotions. Experimental studies have revealed how the plastic properties of neurons are linked to changes in cell morphology that can increase or decrease the formation of synapses and dendritic spines, as well as in the extension or retraction of dendrites. [35-37] Together, these data contributed to the definition of the "neurotrophic hypothesis", proposed in the late 1990s, based on the concept of trophism loss of neurons in some brain areas after prolonged stressful periods. Experimental research carried out on rats subjected to chronic stress showed that neurons of the *nucleus accumbens*, hippocampus and prefrontal cortex undergo a reduction in the density of the dendritic spines and of the arborization of the synapses, as well as of a loss in volume, which is called "shrinking" [38, 39]. Shrinking, a phenomenon also observed in depressed patients and in subjects who underwent chronic stress, is significantly improved by antidepressants (ADs) [40]. Such compounds specifically activate different genes that may express both trophic factors and immune system mediators that ultimately result in restoring normal neuronal trophism [40].

The neurotrophins primarily involved in depression pathophysiology are the Brain-Derived Neurotrophic Factor (BDNF) and, to a lesser extent, the Glial-Derived Neurotrophic Factor (GNDF). The recent development of molecules that can overcome the blood brain barrier and exert a direct action on neurons, via epigenetic mechanisms, and therefore modulate the functioning of specific genes that can express trophic and/or molecules of the immune system appear to represent an opportunity for the development of potential new, more effective psychotropic compounds in the near future. However, the available data are now not encouraging, because of their severe side effects [41, 42].

It is of interest that the latest neurobiological research, such as that examining inflammatory pathways, continues to produce and improve serotonergic hypothesis of depression. However, it is generally agreed that mood disorder

pathophysiology involves different systems and organs, so that depression should be considered a systemic disease [30, 31, 43, 44].

Under this light, psychiatric disorders could be considered a “broad family” of multifactorial pathologies, whereby lifestyle or environmental changes relating to genetic and/or acquired vulnerabilities defining various biological endophenotypes and symptomatic clinical pictures [44-46]. For complete management of MDD, BDs and their related resistant or chronic forms, it may be necessary to control all neurochemical, neurobiological and metabolic factors that “shape” their presentations [44, 45]. This new conceptualization has been consolidatory because of the technological advances and new knowledge in neuroscience [1, 2]. Specifically, progress in this field stems from four interrelated pathways of biological research on depression:

1. molecular and cellular factors underlying and facilitating the onset of the depressive episode (etiological factors, molecular genetics, epigenetics, basic research, animal models);
2. pathogenic mechanisms defining the clinical presentation of mental disorders and its temporal evolution (central and peripheral pathogenic molecular correlates, molecular genetics, epigenetics, brain imaging techniques, animal models);
3. pharmacological and biological research in the therapeutic field (new drug development, research of new therapeutic strategies, patient lifestyle assessment);
4. response to antidepressant agents (central and peripheral molecular mechanisms that underlie the pharmacological response, therapeutic drug monitoring, pharmacogenetics).

From a neurobiological perspective, the neurotrophic theory and monoaminergic hypothesis are factors in etiopathogenesis of depression alongside other systems involved: these are mainly stress-related neuroendocrine systems [1, 2, 43-45]. HPA axis hyperactivity has long been studied in mood disorders, but only recently has the role of this system in melancholy in psychotic characteristics, and in the risk of suicide, been returned, in conjunction with molecular studies that have revealed peripheral factors including genetic variants of neurotrophic factors and pro-inflammatory cytokines [47-49]. Consequently, dysfunction of the neuroendocrine pathways of stress is a vital molecular mechanism that integrates central and peripheral monoaminergic activity, neurotrophic function and pro-inflammatory cellular and humoral factors as pathogenic effectors of depression [50, 51].

In summary, 5-HT system changes that have previously been acknowledge in depression are now being integrated with wider physiological data resulting in a more complex and comprehensive mood disorders model, which includes oxidative and nitrosative stress, immune inflammatory processes and changes in peripheral organs and tissues (i.e., the gut). These hypotheses can produce a mood disorder model that not only includes systemic systems, but also one which involves whole body processes.

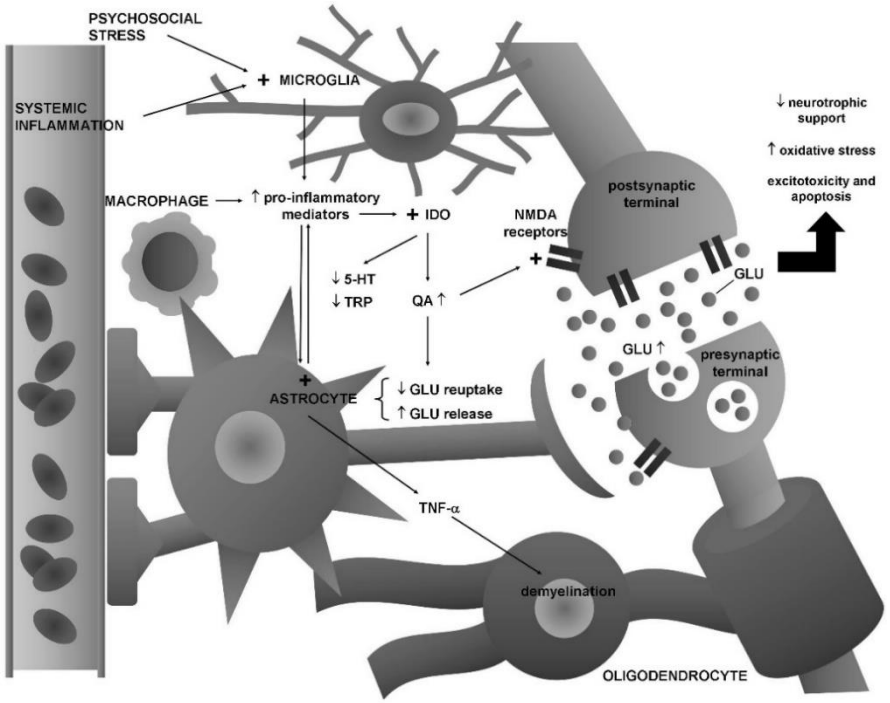
Inflammation is caused by internal and external stressors that may contribute to disease onset, and represents the body's defence response. This definition is widely accepted for the majority of somatic diseases with and also applies to neuropsychiatric disorders, especially in mood disorders [26, 51].

The cytokine hypothesis states that stressful stimuli contribute to mood disorder development by activating inflammatory pathways that then alter the physiological functioning of 5-HT system and the HPA axis [26, 44, 51-54]. According to this concept, the latter are consequences rather than etiologic factors of mood disorders [44, 55]. Over the past 20 years, increased blood and CSF concentrations of pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, interferon-γ (IFNγ) and TNFα, have been found in MDD and BDs [30, 31, 44]. In the Netherlands Study of Depression and Anxiety, which included a large cohort of controls (N = 494) and currently (N = 1132) or remitted (N = 789) MDD patients, currently depressed men showed higher levels of CRP and IL-6, as compared to euthymic subjects [56].

The activation of microglia and astrocytes (glial cells) with an increase in expression of pro-inflammatory mediators and neurotoxic factors, including superoxide, nitric oxide (NO) and tumor necrosis factor α (TNFα), are responsible for CNS inflammation (Figure 1).

Neuroinflammation can be found after acute brain damage (trauma, infections) where inflammatory pathways being activated is required for host defense. However, neuroinflammation is also characteristic of neurodegenerative diseases (*i.e.*, Parkinson's and Alzheimer's disease) [57], where microglia overactivation has detrimental effects and results in neurotoxicity [52, 57, 58]. What triggers toxic neuroinflammation remains unclear. Immunological alterations during critical periods of growth could potentially prime microglia, and subsequent stimuli can result in severe and prolonged neuroinflammation and subsequently brain damage [53]. Furthermore, systemic inflammation can induce neuroinflammation by activating glia [57]. In fact, cytokines can pass the blood-brain barrier and once there they stimulate immune and endothelial cells to produce further inflammatory mediators [26, 51] (Figure 1). This may help to explain the high rates of comorbidity between mood disorders and inflammatory diseases, for example inflammatory bowel disease, rheumatoid arthritis, coronary heart disorder, multiple sclerosis and human immunodeficiency virus (HIV) infection, [1, 30, 31, 44-46].

Figure 1. Molecular processes involved in neuroinflammation [30, 31].



Inflammatory mechanisms that are activated in the brain by external and internal stressors can trigger the neurodegenerative processes observed in MDD and BD. In fact, it is now the belief that neurodegeneration contributes to the development of these disorders, as structural alterations in several brain areas of depressed and bipolar patients have been found, such as prefrontal cortex, anterior cingulate, hippocampus, amygdala and basal ganglia [59-61].

Long-term exposure to cytokines occurs prior to onset of MDD. MDD develops as a result of a high rate of subjects under immune-therapy with INF-α, and these rates are even higher when subthreshold syndromes are considered [62-65]. Furthermore, in animals, administering cytokines or cytokine inducers, including lipopolysaccharide (LPS), induces depressive-like behaviors [66]. Specifically, LPS or IL-6 can induce a behavioral syndrome, known as "*sickness syndrome*", that resembles the clinical picture of MDD: animals develop behavioral disturbances, decreased locomotor activity and exploration, anhedonia, sedation and anorexia [67]. In humans, administration to healthy volunteers of lymphocyte activators and cytokine release promoters, such as LPS or *Salmonella typhi* vaccine, can induce anxiety and depression. It has been recently suggested that the so called "leaky-gut" condition may contribute to the development of MDD and BD symptoms, thus shaping the patients' clinical presentation. It must be indeed pointed out that the enteric nervous system (ENS) and the variability of gut microbiota can be affected in its beneficial functions by an unbalanced brain-peripheral neuroendocrine axis of stress response. Excess of glucocorticoids and cortisol release, accompanied by a glucocorticoid-receptor downregulation and loss of negative feed-back from the periphery to hypothalamic centers of CRH production, can concur to maintain the raise in alarm systems generating chronic mild-inflammation states.

The gut can be particularly sensitive and vulnerable to such a condition since it is richly innervated by the enteric plexus and the vagus nerve [68]. Moreover, the gut is the main productor of 5-HT in the body [69]. The "leaky-gut" is characterized by the irritation of the mucosa as well as by a modified permeability of the intestinal wall, generating an incorrect nutrient and xenobiotic selection [68]. This may alter microbiota composition and viability, leading, in the host, to: a) essential compound deficits, b) release of bacterial molecules entering into the bloodstream together other proinflammatory/adaptogen substrates, c) the increase of cytokine release, thus affecting mood and finally promoting the "*sickness syndrome or behavior*". "Sickness behavior" in humans is a typical condition present during infections, fever and flu, that is characterized by a state of prostration [70-72]. Precisely, this condition is featured by the reorganization of the host priorities during infection, consisting essentially in a general reduction of physical activities, sleepiness, and altered food or water intake. Specifically, an increased translocation of LPS from intestinal gram-negative bacteria may induce peripheral inflammation and results in disorder onset [70]. Thus, higher IgM and IgA serum levels against six enterobacteria have been reported in MDD patients, compared to control subjects [70, 71]. The increased blood LPS levels would induce both systemic and CNS inflammation, with the onset of depressive symptoms and sickness behavior. An intestinal barrier damage would be responsible of the translocation of LPS from the gut into the blood, similarly to what happens during recurrent inflammatory reactions with increased INF-γ and IL-1 levels [70-72].

A literature analysis reveals that, in depressed patients, IL1β, IL-6, TNF and reactive protein C (CRP) are the most reliable biological markers for inflammation. Polymorphisms of pro-inflammatory cytokine genes, including also IL-1β, TNF and CRP, are also linked to depression and the responses to its treatment. Positive and negative correlations were discovered between the high sensitivity CRP levels in the serum of women with depression, and are also associated with a high risk of cardiovascular disease [26, 51].

Administrating pro-inflammatory cytokines, for example IFNα or their inducers (such as endotoxins or anti-typhoid vaccination) results in depressive symptoms in healthy individuals. Conversely, blocking TNF-type cytokines or other aspects of inflammation (such as cyclo-oxygenase 2) results in a significant reduction in depressive symptoms in patients suffering from other pathologies, for instance psoriasis, rheumatoid arthritis, and cancer. The same results were found in patients with major depression [73]. In addition, biological markers of inflammation appear elevated in a subset of depressed patients as well as in people with other neuropsychiatric disorders such as obsessive-compulsive disorder, anxiety and schizophrenia [46, 73].

Therefore, it was suggested that the impact of inflammation on specific symptoms should be verified by diagnoses that align with the research criteria presented by the National Institute of Mental Health, rather than on depression "tout-court". These symptoms refer to anhedonia, fatigue and psychomotor impairment, increased sensitivity to threats resulting in alarm, excitement, and

anxiety [26, 51]. Inflammation appears to lead to a reduction in the response to ADs, as seen in a recent study in which almost half of resistant subjects, who failed to respond to conventional treatments, showed a CRP level > 1- 3mg/L (indicative of elevated inflammatory status) [74, 75]. The percentage of patients with high CRP levels varies according to the population being examined, and is higher in people with history of depression and treatment resistance, or who have suffered from child abuse or of other medical pathologies and metabolic syndromes [74]. It has been shown that CRP activates the intracellular multiprotein complexes named inflammasomes, as the *Nucleotide-binding domain and Leucine-rich repeat Receptor containing a Pyrin domain 3* (NLRP3) complex [76]. Inflammasomes activates in turn the protein caspase-1 that promote the maturation of pro-inflammatory cytokines [76].

The activation of inflammasomes can occur also through the release of the *so-named* danger-associated molecular patterns (DAMPs) and related receptors. DAMPs are endogenous molecules derived from self that increase after cellular stress, tissue damage and even during the coping response to psychosocial stress. These molecules are chemical sensors released in extracellular compartments, being able to promote peculiar inflammation responses induced by the exposition to different triggering agents/stimuli. Once released they are recognized by receptors named pattern recognition receptors (PRR): the binding of DAMP molecules with PRR leads to specific sterile inflammation states [77]. Sterile inflammation, DAMPs release and PRR-DAMP binding produced by psychological stress are capable to stimulate the NLRP3 complex. This phenomenon occurs under physiological responses to stress but the disruption of its fine-tuned regulatory mechanisms has been found to underlie mood disorders and other psychiatric conditions [78]. Research on DAMPs is currently spreading among researchers working on adaptive response to stressors: extracellular heat-shock protein 72 (Hsp72), uric acid, high-mobility group box 1 (HMGB1), adenosine triphosphate (ATP) and even reactive oxygen species (ROS) have been found to act as DAMPS.

In terms of the role of the immune system on depressive syndrome, research has shown how T-cells protect laboratory animals from depression and stress [54]. In fact, an antidepressant phenotype was obtained by transferring T-cells to chronically stressed animals. This data was related to pro-inflammatory cytokine activity in the serum, moving towards a neuroprotective M2 phenotype in microglia and an increase in hippocampal neurogenesis [54]. Similar results were found in mice which were subjected to acute stress where T cell migration into the choroid plexus (located in the brain ventricles) resulted in an induction of glucocorticoids to the expression of adhesion molecular pattern membrane proteins, as the ICAM1 molecule, reducing anxiety [54]. The immunization of animals prone to anxiety with a specific antigen linked to T cell activity in the brain, was able to reverse anxious behaviour provoking the increase of neurogenesis and blocking the development of stress-related depression. The mechanism by which T-cells influence resilience is related to their IL-4 production in the meningeal space which stimulates astrocytes to produce BDNF and

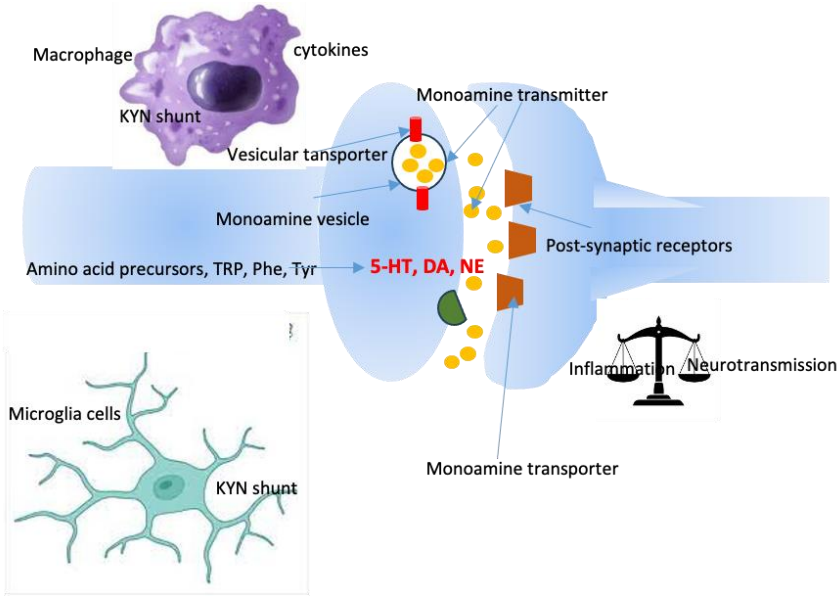
promotes the conversion of meningeal monocytes and macrophages from a pro-inflammatory M1 phenotype to a less inflammatory M2 phenotype [54].

The movement of T cells in the brain, including the meningeal space, is now of interest in describing the previously unknown brain lymphatic system. T-Reg cells can play a part in reducing inflammation and supporting neuronal integrity during stress [27, 54].

2.2 Effects of inflammation on 5-HT system

The monoamine hypothesis of MDD states that if the amount of monoamine neurotransmitter activity is reduced, depleted or dysfunctional, as with decreased plasma concentrations of the 5-HT precursor L-TRP, the disorder will occur.

Figure 2. Monoamine metabolism (modified from [26]).



Low TRP plasma levels have been observed in MDD and are related to the presence of an increased production of IL-1β, TNF-α and INFγ (Figure 2) [30, 31, 44]. In fact, pro-inflammatory cytokines stimulate inflammatory signalling pathways, including the nuclear factor (NF) κB, p38 mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT) 1a, that in turn activate the enzyme indoleamine 2,3-dioxygenase (IDO). The latter cleaves TRP into TRP catabolites along the IDO pathway (TRYCATs), including kynurenine (KYN) [1, 30, 31, 44]. In fact, in patients with hepatitis C treated with IFN-α who develop depressive symptoms, decreased TRP and increased KYN blood concentrations have been observed [1, 44]. The importance of activating IDO in the pathophysiology of depression and mood disorders is therefore supported by animal models [30, 44]. The activation of the molecular signaling inflammatory pathways by pro-inflammatory cytokines, with IDO activation and decreased TRP plasma levels, might also explain the decreased concentrations of 5-HT observed in some MDD patients. Thus, the 5-HT hypothesis of MDD can be altered from the classic concept of TRP and 5-HT depletion to that of inflammation-

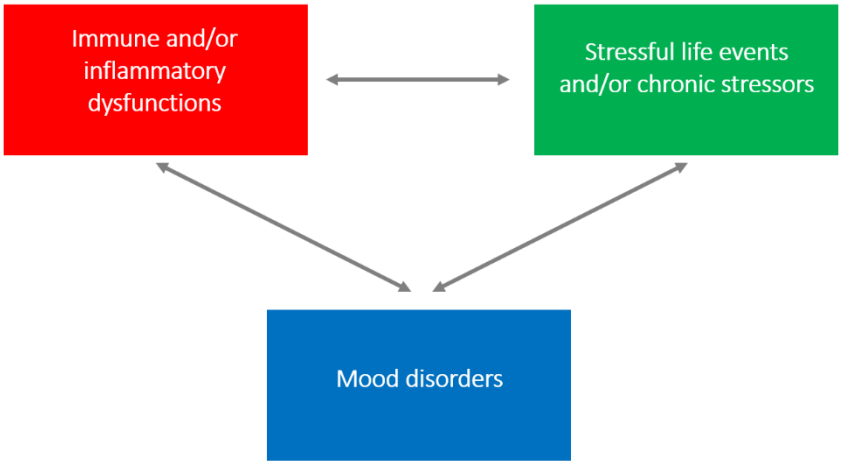
induced TRP degradation and TRYCATs production [1, 31]. In fact, after activating IDO more TRP is converted into TRYCATs, with a decrease in both 5-HT and TRP. TRYCATs cause depressive and anxious behaviors in mice [1, 30] In addition, TRYCATs have neurotoxic effects: KYN can be converted into quinolinic acid (QA), a potent N-methyl-D-aspartate (NMDA) agonist which stimulates glutamate release while resulting in lipid peroxidation [1, 30, 31, 44]. In addition to the low-serotonin concept in MDDs, an imbalance in the serotonergic/glutamatergic system has been also proposed in these disorders, involving the sensitization status of 5-HT/glutamate receptors, the composition and status of membrane-bound G proteins, and impaired intra- and extracellular signaling mechanisms, a condition that is supposed to be concomitant, at least in subgroups of patients, to peculiar activated inflammatory patterns [44].

2.3 Stress-induced activation of the inflammatory response

What are the triggering factors for neuroinflammation that contribute to the onset of mood dysregulation [26, 44]? In the depressive states that occur in the context of somatic illnesses (autoimmune/inflammatory diseases, tissue damage, infectious), the factors that form the basis of primary illness are the same that subtend the neuroinflammation development. Conversely, in the forms of depression that occur in subjects without somatic diseases, the triggering factors of neuroinflammation are less clear. In this case, stressful life events appear to have an important role in the onset of mood disorders: indeed, epidemiological studies found in clinical and community samples show a significant and strong association between the occurrence of early and recent stressful events and the presence of MDD and BDs (Figure 3)[12-14, 26, 44].

Psychosocial stressors are shown to be robust activators of central and peripheral inflammation [26, 44, 51]. Indeed, increased concentrations of IL-1β and IL-6 have been found in the blood and brain of rats under stressful conditions [30, 31].

Figure 3. Bidirectional interactions amongst mood disorders, immune/inflammatory dysfunctions and acute/chronic stress.



It was Maes et al. who first discovered that inflammatory pathways can be activated by psychological stressors; indeed, they described stress as coming from increased production of IFNγ and TNFα in stressful conditions [52-54]. Significant increases of NFκB DNA binding were found in peripheral blood mononuclear cells of healthy volunteers who had been exposed to stressors such as public speaking and mental arithmetic [53]. Chronic stress, including familiar conflicts and perceived stress, are related to increased levels of CRP and IL-6 [30, 31, 51-54]. Increased CRP levels have also been found in subjects exposed to severe early life events, such as childhood abuse [12-14, 30, 31, 26, 44]. Furthermore, increased IL-6 and NF-κB response to antigenic challenge and psychosocial stressors were detected in MDD patients [12-14, 30, 31, 26, 44]. The hypothalamus is the area that appears to have the most involvement in the genesis of the inflammatory response as a result of psychosocial stress [44]. Specifically, psychosocial stressors, including events of loss, work adversities, interpersonal conflicts, and social isolation can lead to the hypothalamic release of catecholamines and cortisol-release hormone (CRH). Within the immune system catecholamines interact with α and β adreno-receptors which increases NFκB DNA binding with the consequent release of inflammatory mediators [26, 44]. Cytokines enter the brain and, at the level of microglia, activate inflammatory signalling pathways and cause neuroinflammation [44, 51]. Conversely, the cytokine-induced activation of CRH and, then, of HPA axis, that usually inhibits inflammatory signalling pathways in pathological conditions, as in the case of MDD or chronic stress, loses its inhibitory action. Glucocorticoids lose their ability to inhibit the inflammatory pathways activation and to limit cortisol release. This condition, called glucocorticoid resistance appears related to the effects of cytokines on glucocorticoid receptors, resulting in neuroinflammation and hyperactivation of the HPA axis [44]. These phenomena appear to disrupt the brain structures and may underlie the brain damage which underlie or at least accompany, the presence of MDD and BDs: in fact, they cause excitotoxicity, decrease neurotrophic supports and alter monoamines metabolism [1, 26, 44].

Different researchers have found increased levels of cortisol in the urine, plasma and saliva of depressed patients, which results in an increased volume of pituitary and adrenal glands [79]. The alterations of the HPA axis, the presence of hypercortisolaemia and the induction of inflammatory patterns, the stimulation of specific leukocyte populations and the alteration of factors related to cardiovascular risk (such as CRP) and platelet reactivity, could be part of the same pathogenetic mechanism in depression. Mineralocorticoid receptors (MRs) could also have a role in the HPA axis dysfunction at multiple levels in depression (i.e., desensitisation and loss of inhibition in HPA feed-back) [80].

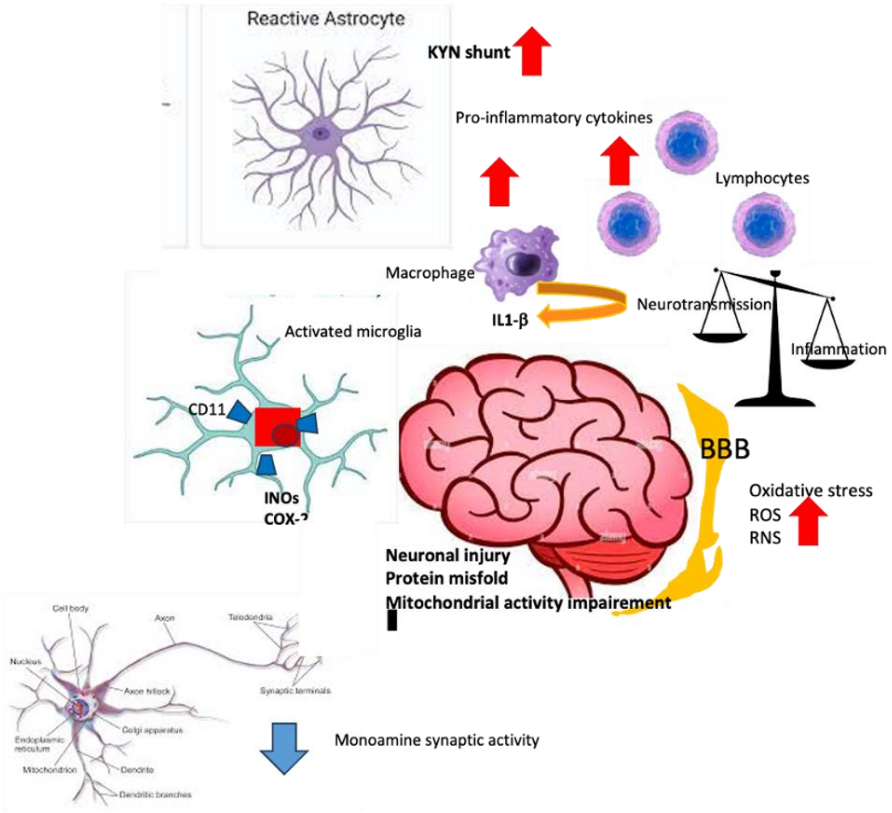
The significance of this imbalance and resistance to glucocorticoids adds to the continual secretion of pro-inflammatory cytokines. These cytokines the activate specific metabolic pathways, such as the shunt of tryptophan (also known as "kynurenine pathway"), resulting in the formation of quinolinic acid in the NAD^+ pathway and that ultimately results in a stimulation of the glutamatergic system, with a dramatic lowering of 5-HT production [74], and, consequently, of 5-HT-deriving methoxy-indole compounds, as the circadian molecules N-acethyl-5-HT (NAS) and melatonin [81].

Before concluding this paragraph, a disregarded aspect linked to the role of neuroendocrine response to stress and inflammation in mood disorders should be instead also mentioned: this concerns the influence of the functioning of the brain-body adaptive axis on feeding behavior, by means of the change of individual tastes and their choice of nutrients [82]. These variations have indeed a profound physiological relevance, namely, that to fully prepare the subject to cope with new dangerous or potentially dangerous stimuli and events: thus, that enabling to adopt the "fight-or-flight" behavior rather than the "rest-and-digest" one [83]. This implies that alterations of the physiology of the neuroendocrine stress axis, as occur in mood disorders, can lead to significant individual variations of taste, feeding and nutritional conditions.

2.4 Mood disorders and oxidative stress

Despite the numerous studies in this field, the exact processes that underlie the altered redox responses within the different depression types are still partially unknown.

Figure 4. The oxidative stress state induces neuroinflammation and neurodegeneration (modified from [76]).



It has been hypothesized that the failure of the HPA axis, of the monoaminergic and of the neurotrophic systems may affect the metabolic and redox status of the patients. Other mechanisms have been found to have relevance in redox system impairments of mood disorder patients, all linked to the presence of altered feeding behaviors and inadequate lifestyle. As afore mentioned, it is worth noting that chronic stress or a maladaptive coping of stressors can even alter taste and nutritional choices, as shown in animal models [84]. Taste perception is determined by genetic, hormonal, metabolic parameters, and even by the individual psychological state. Some studies have shown that the time of perception of bitter, sour and sweet tastes is shortened during acute stress in healthy volunteers; as well, the total amounts of these different tastes was found significantly decreased after psychological stress [82]. On the other side, healthy subjects exposed to stress have shown increased sensitivity to the bitter taste of saccharin [85]. Moreover, mood distress and anxiety have been also found to impinge taste [83, 86]. Besides, mood disorder patients commonly display altered feeding and psychomotor behaviors [87]. Essentially, dysfunctional responses to stressors may have relevant links to dietary habits in addition to a modified physical activity, to smoking habit or alcohol abuse, with consequences for the anti-oxidant response and on depressed patients' nutritional and metabolic conditions of the subjects [88, 89].

Oxidative stress (reactive oxygen species, or ROS, and nitrosative, reactive nitrogen species or RNS, Figure 4), alongside an immune-inflammatory response, could play an important part in the pathogenesis of several psychiatric disorders, including bipolar disorder and depression. Indeed, oxygen and nitrogen

could change some vital brain functions by modulating neurotransmitters (glutamate-type) involved in the neurobiology of depression [88, 78].

The antioxidant system is divided into two main components: non-enzymatic antioxidants and enzymatic antioxidants [88, 89]. Non-enzymatic antioxidants are substances with low or high molecular weight, which naturally exert antioxidant activity and neutralize the free radicals produced and residues of the oxidative metabolism, such as glutathione and other thiols (R-SH), plasma proteins, uric acid (UA), Vitamin C, Vitamin E, Zinc and coenzyme Q10 [88, 89]. Using various techniques, this component can be measured in the plasma and serum of the test subjects, such as the determination of Ferric-Reducing Antioxidant Power (FRAP) or total antioxidant power of the plasma. The FRAP measure specifically assesses the presence of uric acid, bilirubin, vitamin E, vitamin C, and other plasma components that counterbalance the accumulation of ROS or RNS. The second component of antioxidant system is represented by enzymes with specialized function to neutralize potentially toxic radical or reactive species, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and reductase (GPR), as well as thioredoxin/peroxiredoxin systems [88, 89]. Various preclinical and clinical studies have reported that depression could be related to altered levels of oxidative stress markers, with reduced concentrations of the various non-enzymatic and enzymatic antioxidants, which can be normalized with the use of antidepressants. In addition, some antioxidants display antidepressant properties (Zinc, N-acetylcysteine, Omega-3 free fatty acids) [90].

The outcome of oxidative imbalance appears to be related to an excessive production of ROS that function as signaling molecules at low physiological concentration, and which have a fundamental role in the immunological responses that regulate the activity of different cells. At high concentrations, however, ROS tend to have a damaging effect on cellular components, including proteins (enzymes and receptors), lipids and DNA, that results in apoptosis and cell death. Furthermore, oxidative stress and pro-inflammatory mechanisms seem to be significant in the aging process and in developing serious pathologies such as cancer, cardiovascular and neurodegenerative diseases (Parkinson's, Alzheimer's disease and Huntington's chorea), and psychiatric disorders including depression, schizophrenia and BDs [88-90]. The CNS, and particularly the cerebral cortex, is the tissue that most requires oxygen for oxidative metabolism, mitochondrial activity, and ATP production, in order to carry out the elevated rate of protein turn-over in neurons as well as to ensure an adequate axonal flow, so that it produce high amounts of ROS and is also highly vulnerable to oxidative damage [88-90]. On this basis, some authors have proposed the hypothesis that depression may be due to an activation of both inflammatory and oxidative/nitrosative pathways.

In addition to tissue damage, ROS and RNS may trigger an autoimmune response by changing the chemical structure of several molecules that result in the production of modified and highly autoimmune epitopes. The nitration of proteins, for example, results in nitrotyrosine, an immunogenic neoepitope. In

turn, fatty acid autoepitope oxidation, normally ignored by immune system cells, could lead to their being recognized when the components of the lipid membrane have been damaged by such oxidative processes [88-90].

A sample of depressed patients was used to examine the relationship between depression and lipid peroxidation: a decrease in PUFAs (polyunsaturated fatty acids) of the red blood cells' lipid membranes was observed, which indicates an increase in long-chain degradation by peroxidation [91, 92]. The index of oxidative potential (OPI) has been used to assess trends of fatty acids to oxidate. Depressed patients show a decreased OPI that has been interpreted as a reduced activity of phospholipids, following an increased membrane potential that would lead to a degradation. The oxidative potential index (OPI) is calculated and measures the tendency of fatty acids to oxidize, and seemed to reduce during depressive states, which indicates a decreased oxidation capacity by the phospholipids and a consequent increase in OPI, resulting in degradation of the long chain [91, 92]. Moreover, lipid peroxidation markers have also been found to decrease after successful antidepressant therapy [91, 92]. In acute forms of depression, however, an increase in oxidation was detected, as well as a consequent damage to DNA in the blood, urine and also in the brain tissue [91, 92].

Antioxidant enzymes that are expressed both peripherally and centrally are the main systems that protect the brain from any damage caused by free radicals. This activity from enzymes including copper-zinc superoxide dismutase, catalase and glutathione peroxidase, in patients with MDD differs from that found in healthy subjects. The majority of the data shows an increase in superoxide dismutase enzyme (SOD) activity in depression [93], although opposite findings have also been published [94]. However, ADs seem able to lower SOD activity, and is positively related to the severity of depression [93]. In MDD the activity of glutathione-reductase and catalase appears increased, while the glutathione-peroxidase response seems decreased; in any case, ADs normalise the activity of such enzymes [93].

Patients who had one depressive episode were also investigated and it was found that the total antioxidant capacity was lower than the oxidative stress index in healthy participants, but that normalizes after specific therapy [90]. Furthermore, a significant increase in advanced product of protein oxidation (AOPP) and a significant decrease in plasma antioxidant defenses (FRAP, catalase activity and total thiols) have been reported in 30 geriatric depressed patients in comparison with a sample of age-matched elderly controls [95]. These results reflect, overall, a change in antioxidant system reactivity in the various forms and presentations of the disease, also based on the pharmacological response.

2.5 Mood disorders and vitamins

Specific deficiencies of vitamins may eventually lead towards or accompany consistent symptoms of psychiatric disorders [96]. As a matter of facts, symptoms of thiamine (vitamin B1) deficiency - that is common in alcoholism - may

include confusion, psychotic symptoms, and neurological signs. In several cases, vitamin B-12 and folate deficiency have been linked to symptoms of disorientation, depression or psychosis: as such, measurement of these compounds is often included as part of routine dementia work-ups. Pyridoxine (vitamin B6) deficiency has been related to seizures, while the effects of high-dose supplementation with pyridoxine in disorders like depression and anxiety are currently under evaluation [97]. During the past decades, the use of vitamins has been more prominent in the treatment of schizophrenia than of other disorders; however, if the available scientific literature is scarce as regards the use of vitamins for the treatment of schizophrenia, it even appears skimpy as regards other mental pathologies such as mood disorders. This could be because, over the years, placebo-controlled trials have substantially failed to show robust evidence of efficacy of vitamin therapies for the treatment of major psychiatric conditions. Although, at the present date, vitamin therapy is no longer practiced, the role of vitamins in the development of psychiatric symptoms or as target-substrates in biological psychiatry remains pivotal [96]. The renewed interest in the study of the role of vitamins in mental disorders basically lies on the fact that a role of diet and gut microbiota in the pathogenesis of mental disorders is currently recognized; moreover, on the fact that the metabolic and psycho-neuro-endocrine alterations found in patients, also including taste and olfactory changes, can co-exist with altered feeding behaviors, imbalanced intestinal absorption and, potentially, with essential element deficiencies, also present during drug therapies which act at delayed times. Thus, this would imply a direct health-care usefulness, deriving from the co-administration of specific drugs and vitamins, and, possibly, also from the assessment of the plasma levels of both drugs, vitamins or other substrates and essential elements during the patient's follow-up period.

2.5.1 Vitamin D

Vitamin D, also known as calciferol, is a fat-soluble vitamin that can be obtained from a few foods, added to others, and taken as a dietary supplement. It is also synthesized endogenously through exposure to ultraviolet (UV) rays from sunlight. Vitamin D obtained from these sources is biologically inert and requires two hydroxylation reactions in the body to become active. The first hydroxylation occurs in the liver, which converts vitamin D to 25-hydroxyvitamin D (calcidiol). The second hydroxylation takes place primarily in the kidney and results in the formation of 1,25-dihydroxyvitamin D (calcitriol), the physiologically active form of vitamin D. Vitamin D plays a crucial role in promoting calcium absorption in the gut and maintaining adequate serum calcium and phosphate concentrations, which are necessary for normal bone mineralization and to prevent tetany due to hypocalcemia. It also facilitates bone growth and remodeling by osteoblasts and osteoclasts. Insufficient vitamin D levels can result in thin, brittle, or misshapen bones, leading to conditions such as rickets in children and osteomalacia in adults. Vitamin D and calcium together help protect older adults from osteoporosis. In addition to its role in bone health, vitamin D has various other functions in the body, including reducing inflammation and modulating processes such as cell growth, neuromuscular and immune function, and

glucose metabolism. Vitamin D receptors are present in many tissues, and some tissues can convert 25-hydroxyvitamin D to the active form, 1,25-dihydroxyvitamin D. Vitamin D is available in two main forms, D2 (ergocalciferol) and D3 (cholecalciferol), which differ only in their side-chain structures. Both forms are well-absorbed in the small intestine through passive diffusion and a mechanism that involves intestinal membrane carrier proteins. The presence of dietary fat enhances vitamin D absorption, although some vitamin D can still be absorbed without it. Vitamin D absorption from the gut is not affected by aging or obesity [98-115].

Despite vitamin D is essential for maintaining bone structure and regulating calcium and phosphorus levels in the body while also playing a central role in cell proliferation and differentiation, apoptosis, and some immune system functions, during recent years scientific community has become increasingly interested in this pleiotropic hormone because of its involvement in several functions of the CNS [98-103]. Animal studies have reported that vitamin D seems pivotal during the early stages of life by regulating brain development throughout the growth of new nerve cells (axogenesis) and the production of several neurotrophic factors, including the nerve growth factor (NGF) [99-102]. Furthermore, vitamin D may be helpful in protecting the brain from damage throughout preventing calcium from entering cells and inhibiting the production of nitric oxide [102,103]. On the other hand, although such great amount of data on its importance, vitamin D deficiency turns out to be a widespread issue among the general population: this appears particularly worrisome knowing that the fulfilment of the recommended vitamin D status is a goal that still remains far to be achieved in various Western countries [104]. As far as the neuropsychiatric field is concerned, an increasing number of reports that relate vitamin D deficiency to several mental disorders, such as psychotic disorders, eating disorders, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD) and autism spectrum disorders (ASDs) [106-122]. As for mood disorders, the literature data available to date are limited and controversial albeit far from being trivial. Two studies [117,118] highlighted a significant association between depressive symptoms and low vitamin D levels in a sample of young adults with MDD and in 196 hospitalized depressed patients, respectively. Another study involving 1892 patients with current or past depressive disorders reported vitamin D deficiency in about one-third of subjects, and such deficiency resulted more severe in patients with worse clinical symptoms. Those over 2070 elderly patients (≥ 65 years) [123,124]. Two different investigations underlined a significative vitamin D deficiency in manic episodes and bipolar psychosis in subjects with BD [125,126]. However, another study on mood disorders has questioned the results reported so far [127]. The hypothesis of the administration of vitamin-D as an enhancement treatment in mood disorders has not been examined without controversy. Notably, some scholars found out that an eight-week supplementation with vitamin D3 could improve the symptoms of mania; on the other hand, another investigation did not replicate such results neither subsequent meta-analyses [128-130]. Only a more recent meta-analysis confirmed that vitamin D enhancement could provide a significant

improvement of depressive symptoms in mood disorders in its results [131]. Other, more recent, investigations showed that implementation with 50,000 IU of vitamin D for 8 weeks turned out to be beneficial for subjects with mild-moderate depression, in the overall quality of life and illness severity, with women showing a more significant improvement than men [132-133].

2.5.2 Vitamin B12, folic acid and homocysteine

Folate and vitamin B12 are crucial essential nutrients whose insufficiency constitutes a serious public health concern worldwide, affecting all age groups and leading to various complications, including anemia, birth defects, and neurological disorders [134-141]. Furthermore, a low concentration of these vitamins is associated with high homocysteine levels, which is a recognized risk factor for cardiovascular disease, cognitive decline, and adverse pregnancy outcomes [134-141]. Inadequate dietary intake, medication use (such as methotrexate and anticonvulsants), alcoholism, and medical conditions that increase cell turnover are some of the causes of folate deficiency. In addition, genetic polymorphisms can also contribute to lowered folate levels. Conversely, vitamin B12 insufficiency is primarily due to gastrointestinal disorders that lead to impaired vitamin B12 absorption, although it can also be caused by intestinal parasitosis and genetic polymorphisms, albeit with less frequency [134-141].

Vitamin B12, also known as cobalamin, is a member of the B-group of vitamins, which plays an essential role in regulating a variety of bodily functions. Unlike other vitamins, B12 cannot be synthesized by the human body and thus requires dietary intake [134]. It is mainly found in animal-sourced foods such as meat, eggs, and certain dairy products, although its bioavailability can be influenced by other factors, including dietary habits and health status. Once ingested, B12 is released from animal proteins through the action of gastric factors such as hydrochloric acid and pepsin. It then binds to a protein called haptocorrin, which is later destroyed in the intestine, allowing B12 to bind to the intrinsic factor (IF) produced by gastric parietal cells. The B12-IF complex then binds to the cubulin receptor in the ileum and is transported through endocytosis to the plasma, where it binds to transcobalamin and, to a greater extent, to haptocorrin, before being transported to cells or stored in the liver. Vitamin B12 is a critical cofactor of two enzymes: methionine synthase and methylmalonyl CoA mutase. The former is a cytoplasmic enzyme that transfers a methyl group from methyltetrahydrofolate to convert homocysteine to methionine. The latter is located in the mitochondria and plays a crucial role in converting methylmalonyl CoA to succinyl CoA, which is involved in the oxidation of odd-chain fatty acids and catabolism of ketogenic amino acids. Therefore, B12 is essential for DNA synthesis. The recommended daily intake of cobalamin is approximately 5.94 mcg in men, 3.78 mcg in women, and between 3.76 and 4.55 mcg in younger age groups. Adequate dietary intake of vitamin B12 is crucial to prevent deficiency-related conditions and maintain optimal bodily functions [134-146].

Vitamin B12 deficiency can result from various diseases such as malabsorption syndromes (inflammatory bowel diseases, ileo-gastric surgery, pernicious anemia), dietary restrictions, alcohol abuse, pancreatic insufficiency, intake of specific drugs (e.g., metformin, proton pump inhibitors), and disorders affecting its biochemistry [134-137]. Moreover, certain populations, including extreme vegetarians [138] and pregnant women [139], appear to be at a higher risk of developing vitamin B12 deficiency.

The deficiency of vitamin B12 may cause various hematological abnormalities, including altered synthesis of red blood cells, which may lead to megaloblastic anemia [136,137]. However, recent studies have shown that such deficiency can also affect several neurological functions by causing myelin damage or inflammation, potentially resulting in dementia, depression, and weakened cognitive performance [140-143].

Folate, also known as vitamin B9 belonging to the B vitamins group, is composed of three distinct albeit linked together chemical moieties. Specifically, a pterin heterocyclic ring, which is a 2-amino-4-hydroxy-pteridine, is connected via a methylene bridge to a *p*-amino-benzoyl group. This *p*-amino-benzoyl group is bonded through an amide linkage to either glutamic acid or poly-glutamate, while the N5 nitrogen atom of the pteridine ring and/or the N10 nitrogen atom of the *p*-amino-benzoyl group may be attached with one-carbon units in a variety of oxidation states. A deficiency of folate can hinder DNA synthesis and cell division, having the greatest impact on hematopoietic cells and neoplasms because of their higher frequency of cell division [136-138].

Homocysteine is an α-amino acid that differs from cysteine for an additional methylene bridge (-CH2-). It is synthesized from methionine by removing its terminal Cε methyl group. Initially, methionine receives an adenosine group from ATP through a reaction catalyzed by S-adenosyl-methionine synthetase to form S-adenosyl methionine (SAM-e). SAM-e then transfers the methyl group to an acceptor molecule, such as norepinephrine during epinephrine synthesis or DNA methyltransferase during DNA methylation. The adenosine is then hydrolyzed to produce L-homocysteine. L-homocysteine has two primary fates: 1) conversion via tetrahydrofolate (THF) back into L-methionine through a process that utilizes N5-methyl tetrahydrofolate as the methyl donor and cobalamin (vitamin B12)-related enzymes as methionine synthase (MS), serine hydroxymethyl transferase and methylene tetrahydrofolate reductase (MTHFR) [142,143]; there is also an alternative path for methionine recovery which utilizes betaine, deriving from choline oxidation, and the enzyme betaine: homocysteine methyltransferase; 2) conversion into the protein thiol amino acid cysteine by the sequential action of two vitamin B6 (pyridoxal phosphate)-dependent enzymes, first cystathionine □-synthase (CBS), which catalyzes the condensation of homocysteine with serine giving raise to the intermediate cystathionine, then cystathionine □-lyase or cystathionase (CGL or CTH), which hydrolyzes cystathionine into cysteine, α-ketobutyrate and ammonia. These two cytoplasmic and tetrameric enzymes irreversibly produce cysteine from homocysteine enabling cells to fuel thiol reserves for protein synthesis, for disulfide bridges formation,

for glutathione, taurine and other key sulfur-containing metabolic substrates [144]. Their regulation is therefore crucial under specific physiological requirements [144; 145].

Vitamin B12, in combination with folic acid, plays a crucial role in homocysteine metabolism, which is an essential metabolic process consisting of three fundamental processes: folate metabolism, homocysteine remethylation cycle, and transsulfuration pathway. During the former, one-carbon units are transferred to tetrahydrofolate, while in the latter, methionine is synthesized from homocysteine through one-carbon units transferred from folate [142]. Thus, B12 and folic acid serve as key cofactors in the conversion of homocysteine to methionine, and their deficiencies can lead to hyperhomocysteinemia [143], which may increase the risk of metabolic syndrome and mood disorders (MDs), primarily depression [73]. This interdependence appears to impact multiple neuronal pathways that are more or less directly controlled by vitamin B12. As a result, vitamin B12 has been associated with the onset of several neuropsychiatric symptoms/disorders, such as depression, anxiety, hallucinations, and delirium [146-150].

The most consistent findings have linked vitamin B12 to MDs, particularly major depressive disorder (MDD) [146-157]. Low B12 levels have been observed in up to 30% of depressed patients [147,148], or in more than half of another large sample [149]. An inverse correlation has also been highlighted between B12 levels and illness severity [148]. A recent meta-analysis has concluded that dietary intake of B12, along with that of other B-group vitamins, was inversely associated with the risk of depression, particularly in women, although the number of studies included was small [149].

Conversely, the available literature regarding the relationship between vitamin B12 and bipolar disorders (BDs) - a group of mood disorders characterized by alternating depressive and manic/hypomanic episodes and high suicide risk - is insufficient. The limited studies available mainly consist of sporadic case reports [158-162], particularly during manic episodes. Further research is needed to better understand the role of vitamin B12 in BDs.

In summary, we can conclude this chapter by pointing out that depression and mood disorders are common complex disorders at multifactorial etiology. Both molecular factors, i.e., genetic, epigenetic, protein translational, and psychosocial and cultural factors may concur to the onset of these disorders. As concerns the molecular mechanisms involved, it should be considered that these encompass multiple branches, such as: 1) the regulation of neurotransmitter systems as the 5-HT and noradrenergic ones, and/or that of neuropeptides; 2) the degree of activation of the sympathetic nervous system, generating the release of catecholamines, in turn provoking the consequent immunomodulation of subsets of lymphocytes and leukocytes, together the enhancement of specific glycolipid metabolic patterns; 3) the degree of activation of hypothalamic and thalamic centers; 4) the degree of activation of the HPA axis, the glucocorticoid response and the ensuing regulation of inflammation; 5) the integrity and

activity of the enteric nervous system with its microbiota response; 6) the release of DAMPs from cells and tissues as well as the resulting activation state of the inflammasome signaling; ultimately, 7) nucleic acid and protein methylation states which can impact the epigenome, transcriptome, proteome and metabolome. Therefore, anomalies of these responses are supposed to reduce or unbalance the release of neurotrophins, generating immune cell activation, increased cytokine secretion, IDO/TDO-mediated induction of kynurenine-derivative metabolism at the expense of 5-HT and melatonin synthesis, glutamate system dysregulation, and, concomitantly, the abnormal production of resilience-mediating molecules in patients. Much effort is still required to piece together this puzzle and to understand the specific imbalances leading to depletion of neuropeptides, neurotrophic factors, neurotransmitters, resilience-mediating molecules and other adaptogen factors in mood disorders. As mentioned afore, it has been hypothesized that more than one pathway can affect metabolism under acute and chronic psychological distress: the activation of SNS and HPA axis, the inflammation response, hormones, the ENS and gut microbiota reactions, and even, let's not forget, changes of taste perception and nutritional conditions. So, if the acute and chronic stress underlie different networks, patients' vulnerabilities can differentially shape these brain-body axis responses. Such variability could thus represent the molecular bases of the multiple endophenotypes of depression and mania.

3. Study

3.1 Study aim

This study aimed to explore a panel of peripheral nutritional, metabolic and redox parameters in a group of patients with mood disorders. We therefore aimed to appraise the association between mood affective illnesses and vitamin D, B12 and folate deficiencies. The parameters were plasma or serum levels of:

- vitamins D, B12 and folate;

the sulfur-containing amino acid homocysteine, deriving from methionine, an essential amino acid involved in epigenetic, gene transcription regulatory systems, and in redox responses;

- glucose;
- total cholesterol (TC);
- total triglycerides (TD);
- transaminases (GOT, GPT and □□GT);
- uric acid;
- creatinine;
- azotemia or blood urea nitrogen (BUN);
- Na^+ and K^+
- The following hematological analyses were also carried out:
- white blood cell (WBC) count;
- red-blood cell (RBC) count;
- platelet (PLT) count.

Vitamin B12, folate and homocysteine had been taken into consideration since, accordingly to current scientific literature, they are involved in crucial redox/energy metabolism reactions; besides, they are among the most promising metabolic correlates of mood disorders, needing to be further investigated.

We have already examined the circulating levels of homocysteine, vitamin B12 and folate in a previous investigation [2], conducted to possibly find relationships between mood disorders and these parameters. Although the trend looked attractive, no significant correlation was found in that survey. However, that study was retrospective, with homocysteine, vitamin B12 and folate evaluated in patients only, being thus simply compared to normative values and lacking of a control group evaluated by means of the same clinical tools and the same analytical-chemistry methodologies [2].

To improve this previous study, present investigation aimed at comparing such peripheral metabolic markers to a sample of healthy subjects, thus without any psychiatric symptom, matched for age.

3.2 Subjects

A total of 100 subjects was enrolled into the present investigation during the period January 2020 - December 2022. Sixty-nine of them were inpatients hospitalized for a mood disorder and recruited by examining the hospital charts of first-time admissions at the "Casa di Cura Morana", Marsala (TP), Sicily (Italy). This health care facility is the largest public-and-private neuropsychiatric hospital of northern Sicily. All recruited subjects had approved their participation to the study accordingly to the guidelines of the Ethical Committee of the "Casa di Cura Morana". Psychiatric diagnoses were conducted in accordance with DSM-V criteria, by using the Mini International Neuropsychiatric Interview (MINI) [163]. Symptom severity, as recorded in the medical charts, were assessed using a battery of rating scale, in particular the Hamilton rating Scale for Depression (HRSD) [164], the Young Mania Rating Scale (YMRS) [165], and the Clinical Global Impression-severity (CGI-s) [166]. All recruited patients always underwent the psychiatric examination at the beginning of their hospitalization or, in some case, just few days after, but never after a pharmacological therapeutic change.

Control subjects were enrolled from healthy professionals or students who volunteered to participate in the present study. None of the control subjects had past history of major psychiatric illness neither had ever been prescribed psychiatric drugs. Two controls admitted occasional recreational use of alcohol and cannabinoids, two others admitted occasional recreational use of alcohol, one more reported the occasional recreational use of cocaine, another one of gambling, all disclosing, however, that such consumptions or habits had taken place in the past, many years before their enrolment in this study.

In particular, the subject exclusion criteria were: age <18 and > 65 years; active cancer; presence of overt heart, liver or kidney diseases; presence of hematological or neurological illnesses; current substance abuse; pregnancy. Additional exclusion criteria were the use of non-steroidal anti-inflammatory agents, hypotensive drugs or compounds that interfere with carbohydrate-lipid metabolism (insulin, oral hypoglycemic compounds, statins), as well as current or past allergy towards xenobiotics.

3.3 Psychometric rating scales

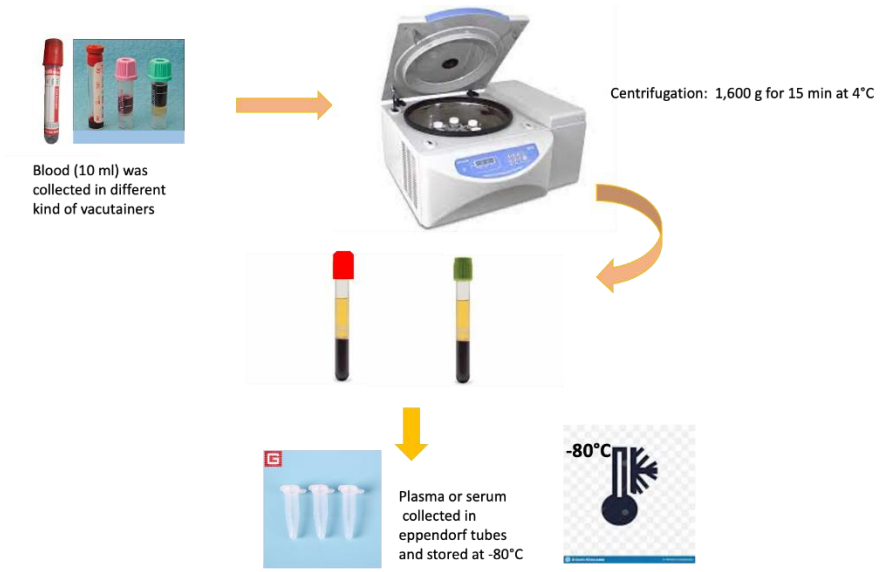
All recruited subjects were evaluated by skilled psychiatrists using suitable psychometric scales. After examination, each questionnaire provides a final numeric score, enabling clinicians to make or exclude diagnoses as well as to appraise the patient's clinical status. Precisely, all subjects were examined by the Mini International Neuropsychiatric Interview (MINI) [163], a brief structured clinical interview that allows researchers to diagnose psychiatric disorders according to the DSM-IV, DSM-V, ICD-10, or ICD-11; the Hamilton Rating Scale for Depression (HRSD) [164] which provides a quantitative assessment of the severity of depressive symptoms and their changes over time. The cut-off is defined by the following reported scores: 25 = severe depression; 18-24 moderate depression; 8-17 mild depression; < 7 no depression; the *Young Mania Rating Scale* (YMRS) [165], which is an 11-item scale that explores the key symptoms

of mania that are generally present throughout the entire clinical course (from the most modest to the most severe phases). The YMRS is similar to the HRSD in its structure and must be performed by an experienced clinician. The mean YMRS total score of the patients in the manic phase was 40+5. The assessment of severity is carried out on the basis of what the patients report on their condition within the last 48 hours and on the observation of the behavior by the doctor during the interview (with a relative priority for the latter); the CGI-S, which is a questionnaire that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis [166]. The scale is endowed with three global scales (items): two of the items, 'Severity of Illness' and 'Global Improvement' are rated on a 7-point scale, while the third, 'Efficacy Index', requires a rating of the interaction of therapeutic effectiveness and adverse reactions: 1= healthy patient; 2= borderline; 3= slightly ill; 4= moderately ill; 5= markedly ill; 6= severely ill; 7= very high severity level.

3.4 Serum and plasma preparation

All recruited subjects, the same days of psychiatric examinations, also underwent a blood withdrawn for routine monitoring, always carried out between 8.00 and 9.00 *am*, to avoid circadian variations.

Figure 5. Serum and plasma separation from collected venous blood



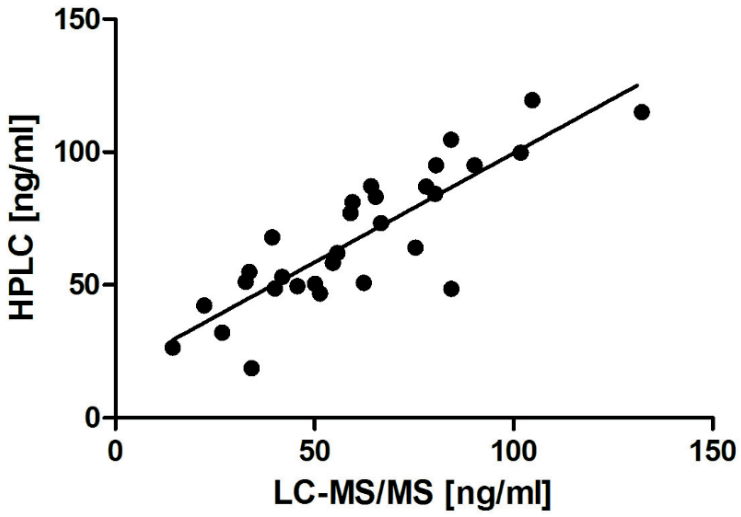
Blood samples (10 ml, antecubital vein) were obtained in both clot-activator and anticoagulant-containing vacutainers from fasting subjects, sitting and relaxing at a constant room temperature. for homocysteine and vitamin determinations, blood was then centrifuged at 1,600 x g for 15 min at 4°C and the ensuing plasma was collected and kept at -80°C until the assay. All other parameters were prepared and measured by means of standard gold methodologies certified for clinical chemistry laboratories (Figure 5).

3.4.1 Plasma Vitamin D assay

Many different methodologies are available to measure vitamin D in biological fluids. For instance, this assay can be conducted by competitive binding methods, high-performance liquid chromatography (HPLC), and radioimmunoassay (RIA). Specifically, the RIA kit developed by DiaSorin S.p.A (Saluggia, Italy) was the method used in the present investigation. The DiaSorin 25-hydroxy-vitamin-D assay involves a 2-step procedure that includes rapid extraction of 25-hydroxy-vitamin-D and other hydroxylated metabolites from serum or plasma, followed by a competitive RIA procedure that utilizes an antibody with specificity for 25-hydroxy vitamin D [167, 168]. This method has been validated showing a good agreement with both LC-MS/MS and LC-UV methodologies.

In our study, we evaluated plasma samples. The day of the analysis plasma was thawed and extracted following the procedure described by the assay kit.

Figure 6. Comparison between DiaSorin RIA vitamin D assay and a LC-MS/MS method [169].



3.4.2 Serum Vitamin B12 assay

Serum vitamin B12 assays measure the total amount of both haptocorrin-bound and transcobalamin-bound B12, including holotranscobalamin. Although the most precise way to assess vitamin B12 status would be to use sequential assay selection algorithms or to combine multiple markers into a single diagnostic indicator, Nexo et al. [170] provided a detailed description of an ELISA procedure that reliably enables the measurement of both total transcobalamin and holotranscobalamin. The procedure carried out by our laboratory strictly follows the description provided by Nexo et al. [170], using γ-globulins from two polyclonal rabbit antibodies against recombinant human transcobalamin as both capture and detection antibodies. Additionally, human recombinant transcobalamin was used as a calibrator.

3.4.3 Serum homocysteine assay

The measurement of serum homocysteine was performed by liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS), accordingly to the procedure described by Rafii et al. [171]. This procedure precisely identifies and quantifies homocysteine levels, using the deuterated internal standards methylmalonic acid. A reduction step is required to ensure the measurement of total homocysteine. This reduction step allows all forms of homocysteine, including homocysteine-cysteine mixed disulfides and other homocysteine adducts, to be converted to free homocysteine, which may be therefore accurately measured. The deuterated internal standards helped to minimize potential variations in the sample preparation and instrument analysis, enabling the accurate quantification of this analyte.

3.4.4 Plasma folate assay

Conducting epidemiological and bioavailability studies for folate or folic acid using existing analytical methods may be a challenging and time-consuming task due to laborious sample preparation and lengthy chromatographic analysis. To address this issue, Zayed and colleagues [172] developed a chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) method to determine unmetabolized folic acid in human plasma. This method utilizes folic acid-d4 as an internal standard and involves a sample preparation step of protein precipitation, with a total run time of 3.5 minutes. Separation of the analytes was achieved on a C18 column (3 µm; 50 × 3.00 mm) using an isocratic elution procedure with a mobile phase composed of ammonium acetate (1 mM)-acetic acid-acetonitrile (9.9:0.1:90, v/v/v).

3.5 Statistical analyses

All laboratory, clinical and demographic data for continuous variables were given in terms of mean ± standard deviation (SD). Categorical variables were given as frequencies (number) and percentages. The normality of variable distribution was determined using the Kolmogorov-Smirnov test. Non-parametric statistical comparisons were employed herein. Mann-Whitney U-tests were used to compare variables between the two subject groups under investigation. The Kruskal-Wallis analysis of variance was used to analyze differences among controls and sub-groups of patients. Correlations among the biological parameters and the different groups were carried out using the Spearman test. For all calculations and statistic evaluation the Graph-Pad Prism Software (Version 7.0.2, San Diego, CA, USA) was employed. The two-tailed statistical threshold was set at $p \leq .05$. To avoid type II statistical errors, we also considered trend to significant p values those fitting within $.05 < p \leq .1$.

4. Results

4.1. Demographic and clinical results

A total of 100 subjects was enrolled and divided in two branches: patients and healthy controls. The patient group (n=69) consisted of 41 women and 28 men (mean age: 44.64 ± 14.06 years). Similarly, the control group (n=31) consisted of 16 women and 15 men (mean age: 43.97 ± 14.70 years). Actually, there was no significant difference in the mean age of the two groups showing that, in both groups, the age distribution matched inside the 30s, 40s, or early 50s. No significant difference in age was found between men and women in the two groups, just a trend toward higher age in control men vs control women was reported (p= .09).

As for the patient group, 27 were diagnosed with BD type I (BDI), 15 with BD type II (BDII), 16 with schizoaffective disorders, and 11 with major depressive disorder (MDD). Additionally, as per the MINI diagnostic tool, 36 patients were experiencing a depressive episode, 20 of them an episode mixed features, seven a hypomanic episode, and four a full-blown manic episode. Psychotic symptoms were reported in 36 patients, including 19 with BDI (8 in manic phase, 8 in mixed state, and 3 in depressive phase), 13 with schizoaffective disorder (6 in hypomanic phase, 3 in manic phase, and 4 in depressive phase), and 4 with MDD. Forty patients had no psychiatric comorbidity, while 18 of them confirmed a past substance use disorder (primarily cannabis misuse, but also psychostimulants and hallucinogens); 11 of them had at least one anxiety disorder, and 10 (14.50%) presented neurodevelopmental traits (5 of which autism spectrum disorders and 5 attention-deficit/hyperactivity disorder, ADHD). Additionally, 36 patients were heavy smokers. In terms of family history, 57 patients had at least one relative with a psychiatric disorder, and 9 had a family history of suicide. Furthermore, 40 patients had at least one medical comorbidity, and 11 patients (23.19%) presented allergic reactions to xenobiotics (Table 1). The CGI-S scale reported that all recruited patients had a high severity score, ranging from 5 to 7.

Table 1. Diagnoses of a group of 69 bipolar inpatients

	Total sample (N= 69)	Women (N= 41)	Men (N= 28)
Bipolar I disorder	27 (39.1%)	15	12
Bipolar II disorder	15 (21.7%)	10	5
Schizoaffective disorder	16 (23.2%)	9	7
Major depressive disorder	11 (15.9%)	7	4
Polarity of the episode			
Manic	6 (8.7%)	2	4

Hypomanic	7 (10.1%)	4	3
Depressive	36 (52.2%)	25	11
Mixed	20 (29.0%)	10	10
Psychotic symptoms	36 (52.2%)	21	15

During the observation period, the majority of patients in the study were taking psychotropic drugs from different classes, based on their current episode and possible longitudinal psychiatric disorder. Only seven patients were not taking any psychotropic medications. Among the patients, 67 were treated with a mood stabilizer, such as valproic acid, lithium salts, gabapentin, pregabalin, lamotrigine, oxcarbazepine, or carbamazepine, of whom 54 were receiving at least two mood stabilizers. Additionally, 27 patients were treated with antidepressants, including selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclics, mirtazapine, trazodone, or bupropion. Furthermore, 63 patients were receiving antipsychotics, including both first- and second-generation antipsychotics (FGAs and SGAs), and 20 were taking benzodiazepines. Finally, 4 patients were being treated with anticholinergic drugs, such as biperiden. Table 2 provides a summary of the psychotropic medications being used by the patients in the study.

Table 2. Current treatments of the patients

Drugs	N (%)
Mood stabilizers	67 (97.1%)
One mood stabilizer	13 (21.7%)
Two or more mood stabilizers	54 (78.3%)
Lithium	52 (75.4%)
Valproic Acid	51 (73.9%)
Pregabalin	12 (17.4%)
Lamotrigine	6 (8.7%)
Oxcarbazepine	5 (7.2%)
Carbamazepine	4 (5.8%)
Gabapentin	2 (3.0%)
Antipsychotics	63 (91.3%)
One antipsychotic	45 (83.9%)
Two or more antipsychotics	18 (26.1%)
FGAs	
Haloperidol	7(10.1%)
Trifluoperazine	1 (1.4%)

Clotiapine	1 (1.4%)
SGAs	
Olanzapine	27 (39.1%)
Quetiapine	20 (29.0%)
Clozapine	11 (15.9%)
Lurasidone	6 (8.7%)
Risperidone	5 (7.2%)
Aripiprazole	5 (7.2%)
Chlorpromazine	1 (1.4%)
Paliperidone	1 (1.4%)
Antidepressants	27 (39.1%)
One antidepressant	23 (94.2%)
Two or more antidepressants	4 (5.8%)
SSRIs	14 (20.3%)
SNRIs	10 (14.5%)
TCIs	2 (2.9%)
Mirtazapine	2 (2.9%)
Trazodone	2 (2.9%)
Bupropion	1 (1.4%)
Vortioxetine	1 (1.4%)
Benzodiazepines	20 (29.0%)
Anticholinergic drugs (Biperiden)	4 (5.8%)

4.2. Biochemical results

Table 3 reports all biochemical results obtained in the patient and control groups. The mean values of clinical-chemistry parameters were always inside their bloodstream physiological limits, except for the patients' homocysteine and vitamin D levels, the former of which were found to be above and the latter below the normal ones. As concerns gender differences, these were appraised in total subjects only, due to the relatively small sample size and the prevalence of women in patients: we reported higher levels of red blood cells, uric acid and creatinine in men than women (* and *** at the significance test), reflecting the gender differences in these blood parameters; higher levels in men were reported also for GPT and γ-GT, perhaps due to gender-related somatic vulnerabilities and/or lifestyle. No significant difference between patients and controls was reported for most biochemical parameters, except for vitamin D, homocysteine, glycemia and BUN values.

Table 3. Clinical-chemical parameters in the two group under investigation

	Patients	Controls	P	Mann Whitney U
Number of patients	69	31		
Mean age (years)	44.64 ± 14.06	43.97 ± 14.07	>.05	1062
Red blood cells (*106/µL)	4.65 ± 0.56	4.59 ± 0.48	>.05	995
White blood cells (*103/µL)	7.53 ± 1.8	7.53 ± 2.24	>.05	1008
Platelets (*103/µL)	218.7 ± 48.56	239.9 ± 72.82	>.05	913
Cholesterol (mg/dL)	177.35 ± 38.60	172.0 ± 35.73	>.05	1049
Triglycerides (mg/dL)	132.20 ± 88.64	123.3 ± 40.61	>.05	969
Uric acid (mg/dL)	4.45 ± 1.24	4.50 ± 1.62	>.05	1059
Glycemia (mg/dL)	92.25 ± 22.83	85.42 ± 8.98	.05*	809
GOT (U/L)	17.14 ± 5.58	23.42 ± 19.4	>.05	866
GPT (U/L)	19.17 ± 9.27	18.65 ± 12.82	>.05	921
γ -GT (U/L)	22.59 ± 27.70	17.13 ± 9.11	>.05	935
Azotemia (BUN) (mg/dL)	28.71 ± 8.12	32.16 ± 6.44	.0194*	757
Creatinine (mg/dL)	0.83 ± 0.13	0.84 ± 0.16	>.05	982
Folic Acid (ng/mL)	7.22 ± 3.31	8.31 ± 4.67	>.05	964
Homocysteine (µmol/L)	13.43 ± 7.34 (46)	8.74 ± 3.12	<.0001****	326
Sodium (mEq/L)	141.20 ± 2.23	140.8 ± 2.69	>.05	960
Potassium (mEq/L)	4.28 ± .034	4.34 ± 0.40	>.05	932
Vitamin B12 (pg/mL)	343.72 ± 183.87	405.9 ± 234.4	>.05	909

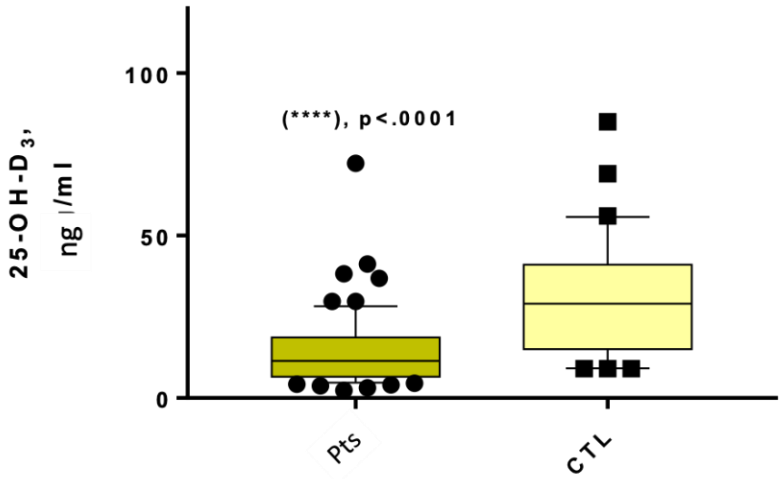
Vitamin D (ng/mL)	14.58 ± 11.27	30.97 ± 17.87	<0.0001****	401

Legend: * Significance, ** high significance, *** higher significance, **** even higher significance

4.2.1 Vitamin D

Comparison of vitamin D levels in the two groups revealed that mean values were found significantly and strongly reduced in the patients' group (Table 3; Figure 7). The standard deviation of vitamin D levels in the patients' sample was also lower, indicating that there was less variation in vitamin D levels among the patients. The range of vitamin D levels was also slightly narrower within the patients (2.4-72.3 ng/ml) compared to the values of the controls (9-85 ng/ml), further suggesting a significant difference in vitamin D levels between the two groups. Also, a statistically significant difference ($t = -11.36$ and $p < .001$) was observed between the mean vitamin D levels of the general population (30 ng/ml) and the mean vitamin D value reported in patients enrolled in this study. Of the 69 patients, 17 (24.64%) had severe critical levels (<6.5 ng/ml), 18 (26.09%) displayed critical deficiency levels (<12 ng/ml), 19 (27.54%) exhibited insufficient vitamin D levels (12-20 ng/ml), 11 (15.94%) had instead sufficient levels (20-30 ng/ml), and only 4 (5.80%) showed optimal levels (≥30 ng/ml).

Figure 7. Mann-Whitney U-test comparison between vitamin D levels in patients (Pts) vs. controls (CTL).



No significant differences were observed among the groups in terms of a family history of psychiatric disorders, specific psychopharmacologic treatment, medical comorbidities, affective episodes, diagnosis, or gender (data not shown).

4.2.2 Vitamin B12

The range of vitamin B12 levels were 80-1104 pg/ml in the patient group and 183-1078 pg/ml in the healthy control group. As reported in Figure 8, despite

the mean vitamin B12 level was lower in the patient group (343.7 ± 183.9 pg/ml) than in the control group (405.9 ± 234.4 pg/ml), the difference was not statistically significant (p = 0.2764; Mann-Whitney U = 909). Amongst the patients, notably, nine showed a critical deficiency (< 200 pg/ml, 144.78 ± 36.44), 25 insufficient (201-300 pg/ml, 258.88 ± 31.31) levels, 34 optimal values (301-950 pg/mL, 439.21 ± 159.67 pg/ml) and two presented excessive levels (> 950 pg/mL, 1552 ± 633.57 pg/ml). Interestingly, patients with a family history of suicide (7 women and 2 men, of whom 4 BDII, 3 BDI, 2 MDD; 6 in depressive episode, 2 in manic phase and 1 in mixed state; 3 with psychotic symptoms; 3 with acute onset) showed lower levels of vitamin B12 (302.1 ± 189.6 pg/ml) compared to the rest of the sample (350.1 ± 142 pg/ml). Albeit not statistically significant, these results indicate anyway a decreasing trend in mean vitamin B12 levels from the control group to the patient group without suicide familiarity and then to the patients with a suicide family history subgroup (see figure 9). Possibly, the investigation of a larger subgroup of patients with suicide family history may eventually lead towards a more precise conclusion.

Vitamin B12 levels did not significantly differed among groups, but we report here the graphical representation of results, since a trend towards lower values in patients can be observed.

Figure 8. Mann-Whitney U-test comparison of vitamin B12 values between patients (Pts) and controls (CTL).

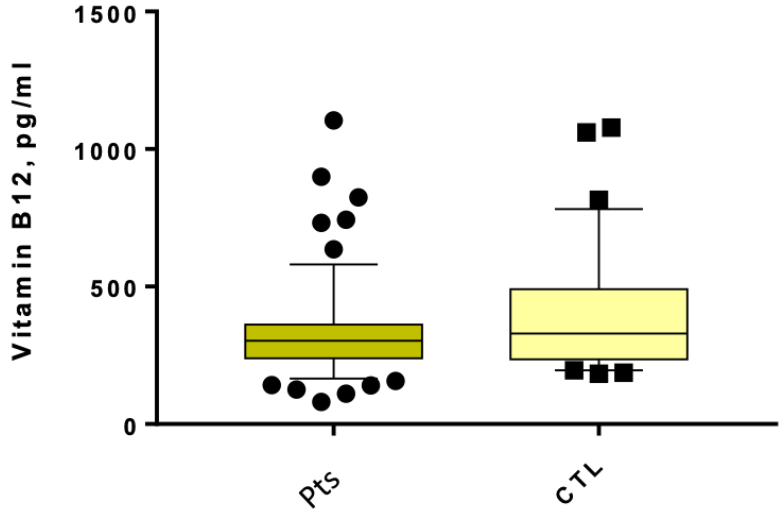
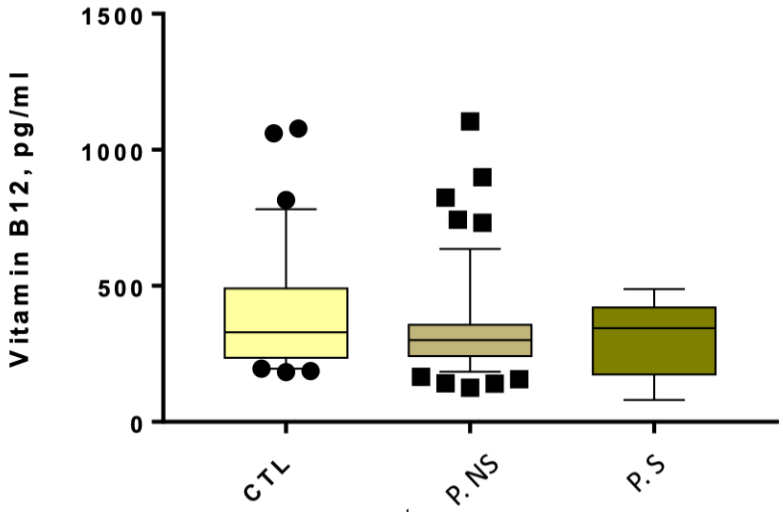


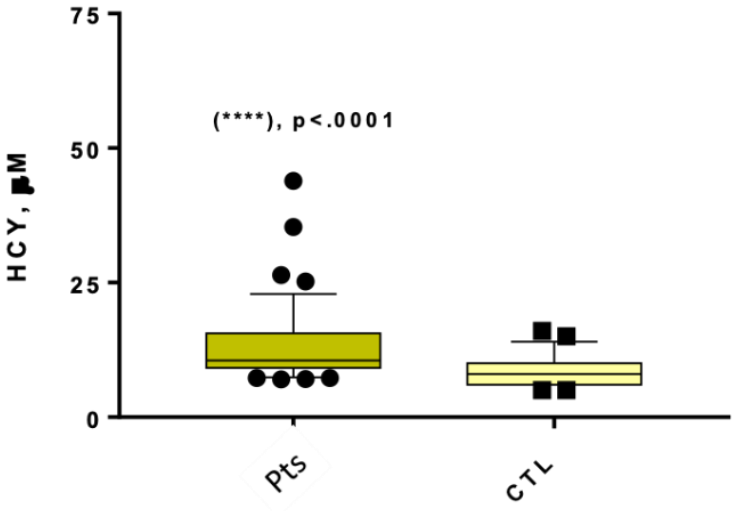
Figure 9. Kruskal-Wallis's test for vitamin B12 in controls and two patients' subgroups, based on suicide familiarity. P. NS: mood disorder patients without familiarity for suicide; P. S: mood disorder patients with familiarity for suicide.



4.2.3 Homocysteine

Homocysteine levels were measured in n=46 patients. The mean homocysteine level was strongly and significantly higher in the patients' group than in control group, as evidenced by the mean homocysteine levels of 13.43 µmol/L and 8.742 µmol/L, respectively. Furthermore, the standard deviation of homocysteine levels was found to be significantly higher in the patients with mood disorders group compared to the healthy controls group (SD = 7.34 µmol/L for patients with mood disorders and SD = 3.119 µmol/L for healthy controls). The Mann-Whitney U test showed that such difference in homocysteine levels between the two groups was significant (U = 326, p < 0.0001, two-tailed), thus suggesting strong evidence for higher homocysteine levels people with mood disorders.

Figure 10. Mann-Whitney U-test comparison of homocysteine values between patients (Pts) and controls (CTL).



In patients, higher levels of homocysteine were reported in men vs women (p=.0094), suggesting that men are more prone to altered methionine metabolism fluxes.

4.2.4 Folic Acid

The control group had a higher mean folic acid level (8.31 ± 4.67 ng/mL) than the patients' group (7.22 ± 3.307 ng/mL). The median folic acid level was 8.5 ng/mL in the control group and 6.6 ng/mL in the patients' group, further indicating that the folic acid levels in the control group were generally higher than in the patients group. The Mann-Whitney U-test showed that there was no significant difference in folic acid levels between the two groups (U = 964, p = 0.4348, two-tailed).

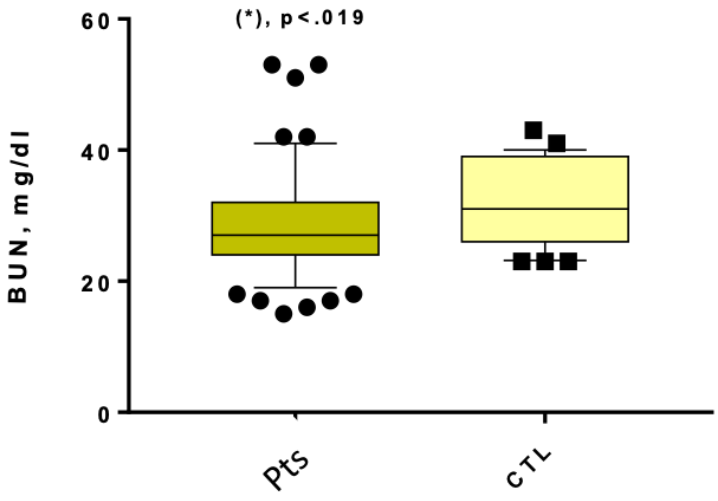
4.2.5 Azotemia (BUN) and glycemia

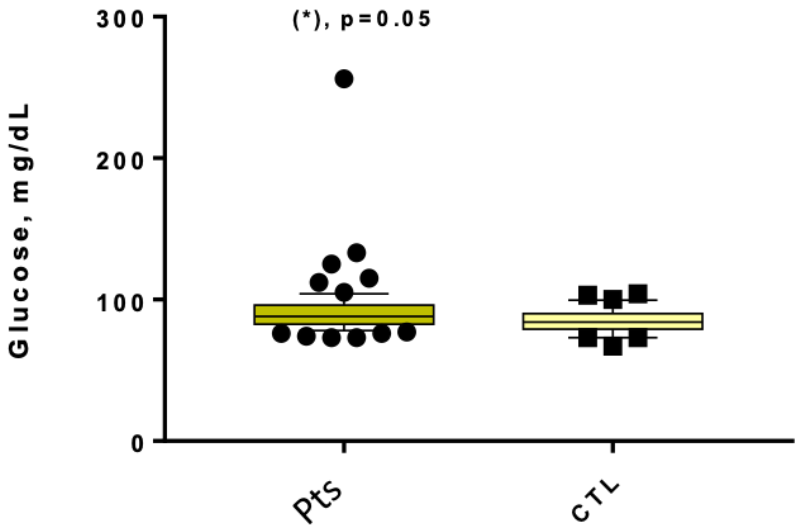
The descriptive analysis also revealed that the mean BUN level was 32.166.44 mg/dL in the control group, while it was 28.71 ± 8.121 mg/dL in the patient group. The Mann-Whitney test employed to compare the two groups showed a statistically significant difference (p=0.0194), with a two-tailed exact p-value of 0.0388 and a Mann-Whitney U value of 757.5, indicating that the control group had higher BUN levels than the patients. On the other hand, the mean glycemia was higher in the patients' group (92.25 ± 22.83 mg/dL) than the control group (85.42 ± 8.98 mg/dL). In this case, the Mann-Whitney test showed a p-value of 0.005, indicating a strong significant difference in glycemia values between the two groups.

The significantly reduced BUN, together significantly higher values of blood glucose, reported in patients vs. controls, is also shown in Figure 11.

Besides, in patients, we also reported a gender effect on glycemia values: higher levels of this parameter were obtained in men vs women (p=. 047). Also, a trend towards higher levels of BUN was obtained in men than women (p=.09).

Figure 11. Mann-Whitney U-test comparison of BUN and blood glucose values between patients (Pts) and controls (CTL).





4.2.6 Correlations among biochemical parameters

Table 4 reports all the Spearman-correlation results concerning the biochemical data measured in the whole group of subjects enrolled. Many significant correlations were found. First of all, we observed that vitamin D positively correlated with vitamin B12 and negatively with homocysteine, showing a high statistical significance, while being negatively correlated with uric acid and nearly positively correlated with folate. In addition to the correlation with vitamin D, vitamin B12 also strongly and positively correlated with folate and negatively with homocysteine; moreover, folate also correlated negatively with homocysteine, showing a trend for negative correlations with blood glycemia and sodium; homocysteine also correlated positively with uric acid and BUN, while revealing a trend for a positive correlation with glycemia; glycemia also positively correlated with circulating triglycerides, γ-GT and sodium, while displaying a trend for a positive correlation with uric acid; total cholesterol resulted positively correlated with triglycerides, uric acid and γ -GT while being nearly positively correlated with BUN and white blood cells ; triglycerides positively correlated with uric acid, γ-GT and white blood cell counts, being almost positively correlated with BUN and natremia; uric acid also positively correlated with creatinine, BUN, γ-GT, and red blood cells; creatinine positively correlated with BUN, white and red blood cell counts; BUN additionally was found positively correlated with white blood cell counts; GOT positively correlated with GPT and γ -GT, while almost negatively with platelet counts; GPT also positively correlated with γ-GT, blood potassium, and red blood cells, while reporting a trend towards negative correlation with platelet counts; γ-GT also showed a positive correlation with white blood cell counts; blood potassium showed a positive correlation with platelet cell counts; finally, white blood cell counts were found also positively correlated with red blood cells platelet counts. We also performed correlations between age and biological parameters considering the totality of recruited subjects: positive correlations were reported for total

cholesterol, triglycerides, glycemia, uric acid, BUN, creatinine and homocysteine (* and *** at the significance test).

Table 4. Spearman correlations between biochemical parameters in all subjects (n=100)

	Vit. B12 pg/ml	FA ng/ml	HCY mmol/l	GLY mg/dl	TCH mg/dl	TG mg/dl	UA mg/dl	Crea mg/dl	BUN mg/dl	GOT U/l	GPT U/l	γ-GT U/l	Na mEq/L	K mEq/L	WBC *103/µl	RBC *106/µl	PLT *103/µl
Vit. D ng/ml	r=0.26 p=.008 **	r=0.18 p=.067°	r=-0.33 P=.004**	r=-0.142 p>.05	r=-0.136 p>.05	r=-0.0365, p>.05	r=-0.2062, p=.0.4 *	r=0.078, p>.05	r= 0.0215, p>.05	r=0.049, p>.05	r=-0.053, p>.05	r= -0.122, p>.05	r=-0.16, p>.05	r=0.14, p>.05	r= -0.038, P>.05	r=-0.05, p>.05	r=0.131, p>.05
Vit.B12 pg/ml	-	r=0.35, p=.0004 ***	r=-0.27, p=0.016 *	r=-0.135, p>.05	r=0.07, p>.05	r=0.029, p>.05	r=-0.114, p>.05	r=0.064, p>.05	r=0.015, p>.05	r=0.15, p>.05	r=-0.021, p>.05	r=0.036, p>.05	r=-0.126, p>.05	r=0.06, p>.05	r=0.06, p>.05	r=0.07 p>.05	r=0.093, p>.05
FA ng/ml	-	-	r=-0.295, p=.0091 **	r=-0.18, p=.067°	r=0.011 p>.05	r=-0.12, p>.05	r=-0.081 p>.05	r=0.011, p>.05	r=-0.07, p>.05	r=0.144, p>.05	r=-0.02, p>.05	r=-0.13, p>.05	r=-0.19, p=.056°	r=-0.086, p>.05	r=-0.05, p>.05	r=0.03, p>.05	r=-0.05, p>.05
HCY mmol/l	-	-	-	r=0.21, p=.058°	r=0.16, p>.05	r=0.07, p>.05	r=0.37, p=.001 **	r=0.17, p>.05	r=0.23, p=.045 *	r=-0.06, p>.05	r=0.08, p>.05	r=0.04, p>.05	r=-0.13 p>.05	r=-0.02, p>.05	r=.047, p>.05	r=-0.04, p>.05	r=0.02, p>.05
GLY mg/dl	-	-	-	-	r=0.05, p>.05	r=0.20, p=.043 *	r=0.19, p=.058°	r=-0.022, p>.05	r=-0.143, p>.05	r=0.05, p>.05	r=0.13, p>.05	r=0.23, p=.02, *	r=0.22, p=.031 *	r=-0.15, p>.05	r=0.055, p>.05	r=-0.13, p>.05	r=-0.09, p>.05
TCH mg/dl	-	-	-	-	-	r=0.45, p<.0001 ****	r=0.35, p=.0003 ***	r=0.049, p>.05	r=0.183, p=.06°	r=0.155, p>.05	r=0.15, p>.05	r=0.36, p=.0002 ***	r=0.07, p>.05	r=-0.009, p>.05	r=0.18, p=.07°	r=0.09, p>.05	r=0.06, p>.05
TG mg/dl	-	-	-	-	-	-	r=0.35, p=.0004 ***	r=0.07, p>.05	r=0.18, p=.07°	r=0.082, p>.05	r=0.03, p>.05	r=0.23, p=.0215 *	r=0.177, p=.08°	r=-0.05, p>.05	r=0.24, p=.0148 *	r=0.08, p>.05	r=0.033, p>.05
UA mg/dl	-	-	-	-	-	-	-	r=0.34, p=.0005 ***	r=0.333, p=.0007 ***	r=0.02, p>.05	r=0.08, p>.05	r=0.26, p=.0099 **	r=0.089, p>.05	r=-0.08, p>.05	r=0.12, p>.05	r=0.23 p=.02 *	r=-01, p>.05
Crea mg/dl	-	-	-	-	-	-	-	-	r=0.48, p<.0001 ****	r=-0.007, p>.05	r=0.007, p>.05	r=0.07, p>.05	r=0.08, p>.05	r=-0.027, p>.05	r=0.19, p=.053*	r=0.23 p=.023 *	r=0.15, p>.05
BUN mg/dl	-	-	-	-	-	-	-	-	-	r=0.08, p>.05	r=0.11, p>.05	r=0.03, p>.05	r=0.004, p>.05	r=-0.04, p>.05	r=0.21, p=.03 *	r=0.04 p>.05	r=0.06, p>.05
GOT U/l	-	-	-	-	-	-	-	-	-	-	r=0.63, p<.0001 ****	r=0.197, p=.049 *	r=0.054, p>.05	r=0.02, p>.05	r=0.138, p>.05	r=0.13 p>.05	r=-0.17, p=.077 (°)
GPT U/l	-	-	-	-	-	-	-	-	-	-	-	r=0.41, p<.00001 ****	r=0.009, p>.05	r=0.21, p=.0347 *	r=0.05, p>.05	r=0.23 p=.02 *	r=-0.18, p=.068°
γ-GT U/l	-	-	-	-	-	-	-	-	-	-	-	-	r=0.049, p>.05	r=0.07, p>.05	r=0.24, p=.018 *	r=0.09 p>.05	r=0.12, p>.05
Na mEq/L	-	-	-	-	-	-	-	-	-	-	-	-	-	**r=0.10, p>.05**	**r=0.10, p>.05**	**r=0.07 p>.05**	**r=-0.017, p>.05**
K mEq/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r=0.02, p>.05	r=0.09 p>.05	r=0.29, p=.003 **
WBC *103/µl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r=0.22 p=.025*	r=0.42p <.0001 ****
RBC *106/µl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r=0.12 p>.05

Spearman correlation results were reported as r= coefficient of correlation and p= statistical significance scores.

Red values report significant correlations, with: * significance, ** high significance, *** higher significance, **** even higher significance;

Blue values report nearly significant correlations, with: ° trend towards significance, .05 < p ≤ 1.

FA: folate or folic acid; **HCY:** homocysteine; **GLY:** glycemia; **TCH:** total cholesterol; **TG:** triglycerides; **UA:** uric acid; **Crea:** Creatinine; **BUN:** blood urea nitrogen; **GOT:** glutamic oxaloacetic transaminase; **GPT:** glutamic pyruvic transaminase; □**-GT:** □-glutamyl transpeptidase; **WBC:** white blood cells; **RBC:** red blood cells; **PLT:** platelets

In addition, separate correlations in controls and patients were carried out (data not shown), obtaining different patterns of results in the two groups under investigation. The positive correlations between different vitamins and the negative correlations between vitamins and homocysteine reported in all recruited subjects were found only in control subjects. The positive correlation between vitamin D and vitamin B12 was greater in control than total subjects, r= 0.622, p= .0002. The relationships between lipid and purinergic metabolism, liver function, and kidney function were present in both patients and controls, with the exception of the negative correlation between vitamin D and uric acid, which was observed in controls only. Positive correlations between homocysteine and creatinine, r= 0.389, p= .0075, or between uric acid and white blood cells, r= 0,252, p= .037, were instead found in patients only, while correlations between glycemia and triglycerides or uric acid or γ-GT, between GOT and PLT, or between γ-GT and white blood cells, resulted more pronounced in patients than in total subjects (* and ** at the significance test). In controls we reported an exclusive negative correlation between folate and BUN, r=-0.48, p=.0057, between vitamin B12 and BUN, r=-0.402, p=.023, as well as positive correlations between homocysteine, total cholesterol and BUN and total cholesterol, BUN and triglycerides (* and ** at the significance test). Always in the controls, positive correlations with age were reported for uric acid and homocysteine (* and *** at the significance test), while reporting a negative correlation with age for vitamin D and folic acid (* at the significance test). In patients a different pattern was obtained: positive correlations with age were found with total cholesterol, glycemia and creatinine (* and ** at the significance test), without any relationship with triglycerides, homocysteine or vitamins.

5. Discussion and conclusions

The present investigation is a preliminary report conducted on a group of mood disorder patients with a heterogeneous diagnosis, all sharing an overall high global severity score due to the degree of impaired mood and mental status, with the main scope to screen their metabolic, vitamin and nutritional state, this last in particular related to the metabolic fluxes of the essential amino acid methionine, as compared to a control group of subjects without any psychiatric symptom. Due to the contrasting data present in the current literature on this topic and, at the meantime, due to the relevance of results showing vitamin deficits and other metabolic impairment for improving treatments and assistance of mood disorder patients, our study acquires direct importance. Indeed, we observed that patients presented specific metabolic alterations together vitamin deficiencies, in particular as concerns vitamin D and sulfur amino acid metabolism. Thus, we focus the results' discussion overall on those parameters found altered in patients vs. controls, and related metabolites.

5.1 Vitamin D

The objective of the present study was to evaluate the levels of vitamin D in a cohort of adult patients who were hospitalized for a mood episode of bipolar disorders (BDs), major depressive disorders (MDD) and schizoaffective disorder, as diagnosed by means of suitable psychometric tools. Specifically, we compared the vitamin D levels of these patients with those reported in a group of age-matched controls.

The current study reports further evidence to the existing literature on the relationship between vitamin D deficiency and psychiatric conditions. A main result of the present survey is that almost 80% of patients exhibited low vitamin D levels. This is a very important information. Indeed, our finding support previous research indicating that vitamin D deficiency might play a role in the pathophysiology of BDs [125;126], which represent about the 60% of our patient sample. Patients may be at a higher risk for vitamin D deficiency compared to controls, which may have implications for the management and treatment of mood disorders. Other studies have also reported low vitamin D levels in patients with acute manic episodes [121] and bipolar psychotic patients [122]. Some research suggests that the incidence of hypovitaminosis in bipolar patients is similar to those with other psychiatric disorders [123].

The impact of vitamin D on major depressive disorder (MDD) is also a topic of debate due to the relatively limited available evidence. Some studies have shown a correlation between lower vitamin D levels and depressive symptoms, such as in a sample of young adults [117] and in 196 hospitalized depressed patients [118]. A subsequent study involving 1892 patients with current or remitted depressive disorders matched with a group of healthy controls confirmed the presence of vitamin D deficiency in about one in three subjects, with

the highest prevalence among the most severe patients [119]. These findings were also replicated in a study of over 2070 elderly participants (aged ≥ 65 years) [120].

The potential impact of vitamin D supplementation as an adjunct to psychotropic drugs remains a subject of controversy [124-130]. One study found that adjunctive vitamin D supplementation may be beneficial as a single parental dose (3,00,000 IU) for depressive symptoms, as well as overall quality of life and illness severity [131]. These controversies need to be clarified, considering also that women “per se” have vulnerability towards low level of vitamin D, so that women with a mood disorder are supposed to be particularly exposed towards such a relevant nutritional deficiency. It should be pointed out that a greater improvement in depressive symptoms following three months of vitamin D supplementation at a dose of 50,000 I.U. has been observed in women [132].

The impact of vitamin D supplementation may vary according to other variables, such as the presence of comorbidities and clinical features that accompany depressive symptoms. Recent studies have supported this assertion, as some research has shown that patients with vitamin D deficiency experienced an improvement in anxiety symptoms even in the absence of depressive symptoms [174]. Additionally, given the high prevalence of vitamin D deficiency among the elderly population, medical comorbidities, which are common in these patients, may represent another variable that can affect the response to vitamin D supplementation. An improvement in depressive symptoms was recently demonstrated in a group of patients with type 2 diabetes, with concomitant improvement in metabolic parameters such as insulin and HbA1c, as vitamin D may facilitate insulin release from pancreatic beta-cells [175].

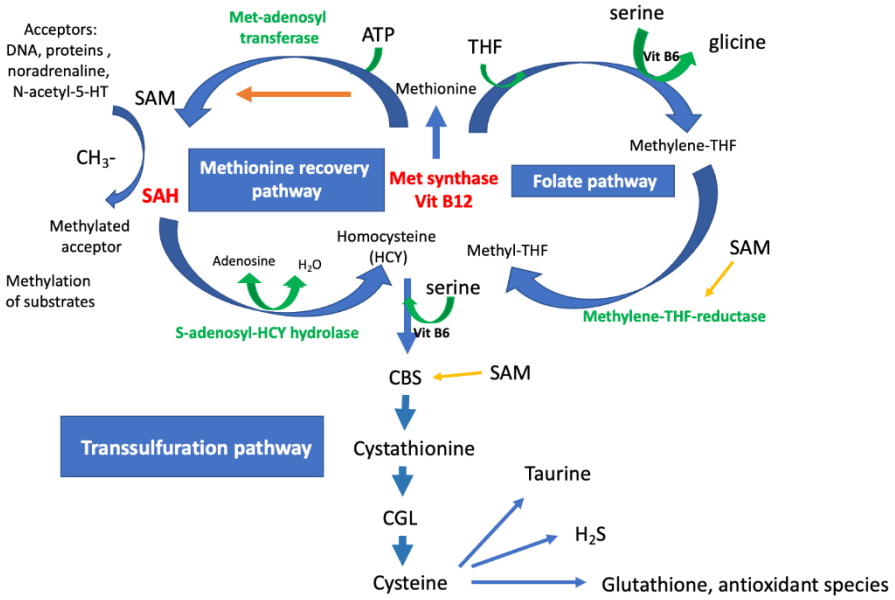
The discovery of nuclear vitamin D receptors (VDRs) and vitamin D response elements (VDRE) in the promoter regions of several genes has broadened the scope of vitamin D’s activities [176,177]. VDRs are believed to play a critical role in embryonic brain development, given their expression in different brain regions, including dopaminergic neurons in the substantia nigra [178] and various subtypes of brain cells such as microglia, astrocytes, and oligodendrocytes [179,180]. The distribution of VDRs in the brain provides an explanation for the multiple roles of vitamin D in various fundamental brain processes, such as axon development, promotion of neurotrophic factors (i.e., NGF and BDNF) [173, 181,182], and neuroprotection by inhibiting nitric oxide (NO) production. In fact, these processes can be altered in mood disorders [178-182].

5.2 Vitamin B12, folic acid and homocysteine

The present study also aimed to evaluate the levels of vitamin B12, homocysteine, and folic acid in a large sample of patients diagnosed with mood disorders disorder, who have a relevantly impaired mood tonus. These three parameters are involved in methionine metabolism, in particular: homocysteine is a main

substrate at the crossroad between its remethylation to methionine and transsulfuration reactions towards cysteine; acid folic and vitamin B12 are instead involved in the reactions of homocysteine remethylation, going back towards methionine [183, Figure 12]. The main enzymes involved in these pathways are methionine synthase (MS) which uses vitamin B12 as coenzyme and 7-methylenetetrahydrofolate reductase (MTHFR), which is part of the vitamin B12-folate cycle [184].

Figure 12. The inter-relationships between vitamin B12, folate, methionine remethylation from homocysteine, transsulfuration of homocysteine to cysteine, and the S-adenosyl-methionine (SAM) regulation [modified from 183]. CBS: cystathionine β-synthase; CGL: cystathionine γ-lyase.



Nearly half of the recruited patients (n=34) had significantly lower vitamin B12 levels than the normative values. Of these patients, 9 presented a critical deficiency (less than 200 pg/mL), while 25 had insufficient levels (between 201-300 pg/mL). Vitamin B12 is present in the bloodstream under different forms. It is therefore possible that heterogeneous compositions of blood vitamin B12, heterogeneity of diagnosis and methodological issues are susceptible to variability of results. Therefore, our observation of no significant difference between patients and controls in vitamin B12 values can be due to the small cohort of subjects recruited, even if lower mean levels were found in patients. This suggests to increase the sample size of both groups for a more accurate analysis. Previous studies have primarily focused on major depressive disorder (MDD) or isolated mood symptoms, and have reported rates of B12 deficiency in approximately 1 out of 2-3 depressed patients [150,151]. Consistent findings were observed with increased plasma homocysteine levels in a study of 30 depressed subjects conducted a decade ago [183]. The relationship between B12 deficiency and mood disorders has also been observed in both young [185] and elderly patients in other studies [184], as well as in obesity [186].

While some controversies exist, the available literature suggests that lower levels of vitamin B12 may be associated with an increased risk of developing Major Depressive Disorder (MDD) [150,151]. However, the potential benefits of supplementing or augmenting antidepressants with vitamin B12 remain unclear, and large population studies are needed to clarify this issue. The literature on BDs is more limited, with case reports from some decades ago and a few thereafter, primarily focusing on manic episodes [187-192]. Recently, a 16-year-old male adolescent with a clinical picture characterized by mixed mood disorder symptoms, psychotic features, and marked extrapyramidal symptoms possibly due to B12 deficiency was successfully treated with intramuscular B12 and risperidone [190]. It is worth noting a peculiar case report, even if this was not a case of bipolar disorder: a 27-year-old girl with a diagnosis of schizophrenic paranoia did not improve with standard therapies, being thus judged as clozapine-resistant, but remitted after vitamin B12 supplementation [193]. In another case, a 38-year-old male patient with depressive and psychotic symptoms, as well as suicidal thoughts, showed improvement after receiving a combination of intramuscular B12, sertraline, and risperidone [194]. The patient had a B12 deficiency secondary to bariatric surgery, which is a common occurrence in these patients [195]. Despite these findings, the possible therapeutic efficacy of vitamin B12 in BDs remains a mere suggestion that requires substantiation through more robust data.

In a previous investigation we found that patients with a family history of suicide had significantly lower vitamin B12 levels than the rest of the sample [2]. However, we could not confirm this result, since we could extend only the patient sub-group without familiarity for suicide. Some case reports suggest a possible link between reduced serum B12 levels and suicidal behaviors [196, 197]. Therefore, the replication of this finding is necessary, since valuable predictors of suicide, a common complication of Mood Disorders (MDs), and especially BDs, are strongly required. The estimated suicide risk in MDs is around 15%, with up to 800,000 deaths per year [198-200]. Patients with BDs have a suicide rate 20-30 times higher than the general population, with BD II patients being at greater risk [201]. While we could not find lower vitamin B12 levels in patients, the present investigation instead revealed much higher values of homocysteine levels in them, displaying values even higher than the normative ones (n=14). These findings are in agreement with previous studies investigating homocysteine levels in BD patients. For instance, a recent study found that roughly one in eight BD patients had higher homocysteine levels, particularly among men. Additionally, the study found that higher homocysteine levels were significantly associated with a higher prevalence of mixed episodes, further underscoring the importance of investigating this potential biomarker [202].

Similarly, a previous study involving 112 BD patients with a depressive episode found that almost half (45%) of the participants exhibited hyper-homocysteinemia. This study also found an inverse correlation between homocysteine levels and plasma B12 and folic acid levels, further emphasizing the need for comprehensive studies examining multiple biomarkers in BD patients [203]. This

correlation suggests that patients have increased homocysteine due to a reduced remethylation of homocysteine to methionine, because of, at least in part, the loss of both folate and vitamin B12 of the so-called folate cycle [144]. However, in the present cross-sectional survey, we could not directly relate the very high homocysteine levels with lower vitamin B12 and folate.

Regarding manic episodes, a recent meta-analysis revealed hyper-homocysteinemia during both manic episodes and euthymic phases in BD patients. However, the issue of homocysteine as a reliable biomarker for treatment response in BDs remains controversial, with previous studies pointing out the need for randomized clinical trials and consistent methodology to clarify the issue [204,205]. In light of these findings, further research is necessary to fully understand the role of homocysteine as a potential biomarker in BDs.

The majority of our patients (66 out of 69) had normal folic acid levels, which is consistent with some studies [206,207], and inconsistent with others that reported lower mean folic acid levels in bipolar depressed patients or in MDD [208, 209]. A more recent meta-analysis found that bipolar patients may have low folate levels, but this conclusion should be interpreted with caution given the potential impact of confounding variables [210]. It seems surprising that the very high plasma levels of homocysteine obtained in mood disorder patients are not linked to low circulating levels of folate and vitamin B12, due to their metabolic relationships. However, it is also possible that the severe unbalance of homocysteine levels reported herein in patients can be rather caused by an altered trassulfuration path and lowered cysteine formation from homocysteine. Cysteine formation from homocysteine is limited by the induction of the enzyme cystathionine-β-synthase (CBS) and this pathway is irreversible, unlike the remethylation branch: thus, the amount of homocysteine entering into transsulfuration cannot be regenerated again. Moreover, cysteine, in addition to being a protein component, is a semi-essential amino acid belonging to the thiol-compound pool in the body. Cysteine is a redox-regulating thiol, being the precursor of many antioxidant species as glutathione; it is a main substrate for the formation of iron-sulfur clusters, which have been found altered in some neurodegenerative disease. This amino acid also gives raise to the gasotransmitter H_2S, involved in the protection from cardiovascular diseases [144]. Then, cysteine is also involved the synthesis of coenzyme A, a key intermediate in energy Krebs cycle, and lipid metabolism, and taurine, an anti-inflammatory and detoxification compound in the body.

So, trans-sulfuration is a crucial pathway, whose down-regulation or reduction can provoke not only the accumulation of homocysteine but also the induction of multiple and severe metabolic impairments of cell and tissue redox systems. This explanation can also provide further support to those investigations reporting lowered plasma/serum thiol dynamics in geriatric depression [95] and in bipolar disorders [211]. The methionine transmethylation product S-adenosylmethionine (SAM) is a main regulator of both the vitamin B12/folate-dependent remethylation of homocysteine to methionine (methyl donor) and the

homocysteine transsulfuration (CBS activator) towards cysteine. Thus, it is important to mention here that diet can influence the metabolic fates of homocysteine at this level [212]. If diet is low in methionine, this provokes low SAM levels, overall impacting trans-sulfuration; indeed, remethylation of homocysteine to methionine can also occur through an alternative pathway, involving betaine and the enzyme betaine-homocysteine methyltransferase [212]. If diet is rich in methionine, the CBS induction can occur, leading to trans-sulfuration rather than remethylation [213]. The understanding of hyperhomocysteinemia in mood disorder patients requires a proper deepening. In fact, high homocysteine in blood has been related to an increased risk of cardiovascular diseases, diabetes, cancer, autism and neurodegeneration, among other illnesses, all often found in co-morbidity with mood distress. Besides, accumulation of homocysteine can lead to the toxic compound homocysteine thiolactone (HCTL), a highly reactive molecule capable of modifying the structural properties of proteins [212]. Also, limited levels of methionine and pyridoxal vitamin B6 could have contributed to the higher homocysteine levels reported in patients, but these two molecules were not analyzed herein.

5.3 Other parameters and correlation results

The report of low BUN and higher plasma glucose in our patients would further indicate a disturbed metabolism in mood disorders. An altered feeding behavior of patients could also explain this result: this suggests that they would rather prefer carbohydrate assumption than protein foods, which would potentially lower the assumption of methionine and reduce trans-sulfuration reactions [212, 213]. Moreover, a tendency towards insulin resistance could be present in mood disorder patients.

It is also important to discuss some negative results obtained herein as that of uric acid and WBC. Uric acid is a product of the metabolic breakdown of purine nucleotides and may also act as a redox species. As underlined in some studies, uric acid would belong to pathways and networks implicated in the pathogenesis of different neuropsychiatric disorders including mood disorders [214]. A study carried out in our department showed that uric acid levels were significantly higher in patients suffering from a manic/mixed episode, than in those euthymic or during a depressive phase. Further, these levels were related to the Clinical Global Impression-Bipolar Version (CGI-BP) scale score for the severity of manic symptoms. A positive correlation was found also with male sex and with serum lithium levels: this might explain even the lowest levels of uric acid in the female patients of the current study [214]. This is also consistent with previous data supporting a possible role of uric acid levels as a specific state-dependent marker of mania [215-218]. Regarding uric acid, no significant difference was found, unlike what had been observed in another previous investigation of ours [2]. This may be attributed to the fact that uric acid has been observed to vary in subjects with mood disorders depending on the type of episode (manic or depressive rather than past or present) and based on the clinical characteristics of patients [219]. However, the group under examination is heterogeneous with regard to mood

disorders (i.e., the various phases), and investigations with larger samples or case studies comprising less heterogeneous samples would be necessary to obtain more robust results.

As regards our negative finding concerning WBC, this is also is in conflict with some previous studies conducted in patients with mood disorders reporting higher leukocytes in subjects with specific symptoms [220; 221]. The discrepancy between these studies and ours can reside on the different design of the present study, where patients were not distinguished on the basis of their specific symptoms, also displaying, as mentioned afore for uric acid results, heterogenous diagnoses.

Regarding the significant correlations or trends to significant correlations presented in Table 4, these showed expected relationships between the levels of vitamin B12, folate, vitamin D and homocysteine, due to the existing metabolic and nutritional connections of these nutrients and metabolites; moreover, we revealed links among lipoproteins, glycemia, liver and kidney functions. Some unexpected correlations were found between metabolic parameters and blood cell counts, maybe due to peculiar inflammatory states within recruited subjects. It is instead quite surprising that in patients there were no more significant positive correlations between related parameters as folic acid and vitamin B12, or between vitamin D and other vitamins, or negative correlations between vitamins and homocysteine; meantime, those between liver and kidney functions, lipid metabolism and uric acid were reported in both patients and control subjects, revealing however slightly different patterns. The fact that vitamins and homocysteine levels were no more correlated to each other in patients could be due to the different variability of these parameters in the two groups under investigation, suggesting also that other factors (e.g., gender, age or other unknown causes) were affecting the metabolism of these nutrients and metabolites. In patients, a positive relationship between homocysteine and blood creatinine was reported. This perhaps suggests that higher levels of homocysteine would also underlie vulnerabilities to a decreased kidney function in patients, presumably in male patients. This aspect is important since the relationship between impaired methionine metabolism, reduced homocysteine metabolism/clearance and kidney function [222], could also reflect altered drug detoxification pathways, a condition that may also affect drug responses [223]. In controls, we found instead negative correlations between vitamins and BUN as well as between BUN and lipoproteins, suggesting a different influence of dietary habits on vitamin availability in the two groups of subjects under investigation. Interestingly, no effect of age or gender was found on vitamin D levels in patients, implying that the low content of this important vitamin found in this group was influenced by other factors, probably more related to their psychiatric diagnoses and severe mental conditions.

In conclusion, this study suggests that patients with mood disorders and a current severe episode, can present relevant deficiencies of essential nutrients as vitamin D and accumulation of potentially toxic substrates as homocysteine.

Therefore, these results pave the way towards a personalized intervention for patients' management, including, beside improved pharmacological treatments, the prescription of specific vitamins and nutritional care.

5.4 Limitations

This cross-sectional investigation presents some limitations. First, the small number of enrolled subjects and, overall, control subjects. Then, the lack of patients' information, such as their body mass index, dietary habit, or more details concerning their peculiar symptomatology, as mood episode specifiers. Also, the heterogeneity of diagnoses included. The extension of this investigation in specific groups of patients including the measure of other vitamins and cofactors, such as vitamin B6, antioxidant agents as total thiols, glutathione and others redox species are believed to enable the appraise of new correlations between metabolic patterns and symptoms, leading to a better characterization of clinical presentations.

6. References

[1]. Dell'Osso L, Carmassi C, Mucci F, Marazziti D. Depression, serotonin and tryptophan. Curr Pharm Des. 2016; 22(8):949-954. doi:10.2174/1381612822666151214104826

[2]. Mucci F, Marazziti D, Della Vecchia A, Baroni S, Massimetti G, Morana P, Mangiapane P, Morana F, Carpita B, Morana B, Dell'Osso L. Inflammatory and metabolic markers in patients with mood disorders. World J Biol Psychiatry. 2021 Mar;22(3):228-235. doi: 10.1080/15622975.2020.1775891. Epub 2020 Jun 18. PMID: 32468900.

[3]. Kessler RC, Berglund P, Demler O, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA. 2003; 289(23):3095-3105. doi:10.1001/jama.289.23.3095

[4]. Hasin DS, Sarvet AL, Meyers JL, et al. Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. JAMA Psychiatry. 2018; 75(4):336-346. doi:10.1001/jamapsychiatry.2017.4602

[5]. American Psychiatric Association Committee on Nomenclature and Statistics. Diagnostic and Statistical Manual of Mental Disorders, 5th ed.; American Psychiatric Press: Washington, DC, 2014.

[6]. [Musetti L, Tundo A. Major depressive disorder. In: Marazziti D ed. Clinical Psychopharmacotherapy. Rome: Giovanni Fioriti Editore; 2019: 35-49

[7]. Mucci F, Avella MT, Marazziti D. ADHD with Comorbid Bipolar Disorders: A Systematic Review of Neurobiological, Clinical and Pharmacological Aspects Across the Lifespan. Curr Med Chem. 2019; 26(38):6942-6969. doi:10.2174/0929867326666190805153610

[8]. Koukopoulos A, Sani G. DSM-5 criteria for depression with mixed features: a farewell to mixed depression. Acta Psychiatr Scand. 2014; 129(1):4-16. doi:10.1111/acps.12140

[9]. Mucci F, Toni C, Favaretto E, et al. Obsessive-compulsive Disorder with Comorbid Bipolar Disorders: Clinical Features and Treatment Implications. Curr Med Chem. 2018; 25(41):5722-5730 doi:10.2174/0929867324666171108145127

[10]. Osby U, Brandt L, Correia N, Ekbom A, Sparen P. Excess mortality in bipolar and unipolar disorder in Sweden. Arch Gen Psychiatry. 2001; 58(9):844-850. doi:10.1001/archpsyc.58.9.844

[11]. Angst J. The bipolar spectrum. Br J Psychiatry. 2007; 190:189-191. doi:10.1192/bjp.bp.106.030957.

[12]. Tundo A. Manic and hypomanic episodes. In: Marazziti D ed. Clinical Psychopharmacotherapy. Rome: Giovanni Fioriti Editore; 2019: 59-71.

[13]. Tundo A. Bipolar depression. In: Marazziti D ed. Clinical Psychopharmacotherapy. Rome: Giovanni Fioriti Editore; 2019: 49-59.

[14]. Tundo A, Dell'Osso L. Mood disorders with mixed features. In: Marazziti D ed. Clinical Psychopharmacotherapy. Rome: Giovanni Fioriti Editore; 2019: 71-81.

[15]. American Psychiatric Association. Highlights of changes from DSM-IV-TR to DSM-5. American Psychiatric Press: Washington, DC, 2013. doi:.10.1176/appi.books.9780890425596.changes

[16]. Piccinni A, Veltri A. Rapid cycling bipolar disorder. In: Marazziti D ed. Clinical Psychopharmacotherapy. Rome: Giovanni Fioriti Editore; 2019: 81-87.

[17]. Dunner DL, Fieve RR. Clinical factors in lithium carbonate prophylaxis failure. Arch Gen Psychiatry. 1974; 30(2):229-233. doi:10.1001/archpsyc.1974.01760080077013

[18]. Akiskal HS, Pinto O. The evolving bipolar spectrum. Prototypes I, II, III, and IV. Psychiatr Clin North Am. 1999; 22(3):517-534, vii. doi:10.1016/s0193-953x(05)70093-9

[19]. Mucci F, Marazziti D, Della Vecchia A, Baroni S, Morana P, Carpita B, Mangiapane P, Morana F, Morana B, Dell'Osso L. State-of-the-Art: Inflammatory and Metabolic Markers in Mood Disorders. Life (Basel). 2020 Jun 6;10(6):82. doi: 10.3390/life10060082. PMID: 32517269; PMCID: PMC7345093.

[20]. Rinninella E., Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019; 7(1): 14.

[21]. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388(10053):1545-1602. doi:10.1016/S0140-6736(16)31678-6

[22]. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003; 301(5631):386-389. doi:10.1126/science.1083968

[23]. Haeffel GJ, Gibb BE, Metalsky GI, et al. Measuring cognitive vulnerability to depression: development and validation of the cognitive style questionnaire. Clin Psychol Rev. 2008; 28(5):824-836. doi:10.1016/j.cpr.2007.12.001

[24]. Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry. 2007; 12(4):331-359. doi:10.1038/sj.mp.4001949

[25]. Jones SG, Benca RM. Circadian Disruption in Psychiatric Disorders. Sleep Med Clin. 2015; 10(4):481-493. doi:10.1016/j.jsmc.2015.07.004

[26]. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016; 16(1):22-34. doi:10.1038/nri.2015.5

[27]. Mayberg HS. Limbic-cortical dysregulation: a proposed model of depression. J Neuropsychiatry Clin Neurosci. 1997; 9(3):471-481. doi:10.1176/jnp.9.3.471

[28]. Graham J, Salimi-Khorshidi G, Hagan C, et al. Meta-analytic evidence for neuroimaging models of depression: state

or trait? J Affect Disord. 2013; 151(2):423-431. doi:10.1016/j.jad.2013.07.002

[29]. Lopez-Duran NL, Kovacs M, George CJ. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology. 2009; 34(9):1272-1283. doi:10.1016/j.psyneuen.2009.03.016

[30]. Catena-Dell'Osso M, Fagiolini A, Marazziti D, Baroni S, Bellantuono C. Non-monoaminergic targets for the development of antidepressants: focus on neuropeptides. Mini Rev Med Chem. 2013; 13(1):2-10.

[31]. Catena-Dell'Osso M, Marazziti D, Rotella F, Bellantuono C. Emerging targets for the pharmacological treatment of depression: focus on melatonergic system. Curr Med Chem. 2012; 19(3):428-437. doi:10.2174/092986712803414277

[32]. Metts AV, Rubin-Falcone H, Ogden RT, et al. Antidepressant medication exposure and 5-HT1A autoreceptor binding in major depressive disorder. Synapse. 2019; 73(6):e22089. doi:10.1002/syn.22089

[33]. Dold M, Kasper S. Evidence-based pharmacotherapy of treatment-resistant unipolar depression. Int J Psychiatry Clin Pract. 2017; 21(1):13-23. doi:10.1080/13651501.2016.1248852

[34]. Levy MJF, Boulle F, Steinbusch HW, van den Hove DLA, Kenis G, Lanfumey L. Neurotrophic factors and neuroplasticity pathways in the pathophysiology and treatment of depression. Psychopharmacology (Berl). 2018; 235(8):2195-2220. doi:10.1007/s00213-018-4950-4

[35]. Chavez-Castillo M, Nunez V, Nava M, et al. Depression as a Neuroendocrine Disorder: Emerging Neuro-psycho-pharmacological Approaches beyond Monoamines. Adv Pharmacol Sci. 2019; 2019:7943481.

[36]. Sigitova E, Fisar Z, Hroudova J, Cikankova T, Raboch J. Biological hypotheses and biomarkers of bipolar disorder. Psychiatry Clin Neurosci. 2017; 71(2):77-103. doi:10.1111/pcn.12476

[37]. Cai S, Huang S, Hao W. New hypothesis and treatment targets of depression: an integrated view of key findings. Neurosci Bull. 2015; 31(1):61-74. doi:10.1007/s12264-014-1486-4

[38]. Qiao H, Li MX, Xu C, Chen HB, An SC, Ma XM. Dendritic Spines in Depression: What We Learned from Animal Models. Neural Plast. 2016; 2016:8056370. doi:10.1155/2016/8056370

[39]. Tang M, Huang H, Li S, et al. Hippocampal proteomic changes of susceptibility and resilience to depression or anxiety in a rat model of chronic mild stress. Transl Psychiatry. 2019; 9(1):260. doi:10.1038/s41398-019-0605-4

[40]. Sapolsky RM. Depression, antidepressants, and the shrinking hippocampus. Proc Natl Acad Sci U S A. 2001; 98(22):12320-12322. doi:10.1073/pnas.231475998

[41]. Popova NK, Ilchibaeva TV, Naumenko VS. Neurotrophic Factors (BDNF and GDNF) and the Serotonergic System of the Brain. Biochemistry (Mosc). 2017; 82(3):308-317. doi:10.1134/S0006297917030099

[42]. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther. 2013; 138(2):155-175. doi:10.1016/j.pharmthera.2013.01.004

[43]. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol Lett. 2008; 29(3):287-291.

[44]. Rosenblat JD, McIntyre RS. Bipolar Disorder and Immune Dysfunction: Epidemiological Findings, Proposed Pathophysiology and Clinical Implications. Brain Sci. 2017; 7(11). doi:10.3390/brainsci7110144

[45]. Leuchter AF, Hunter AM, Krantz DE, Cook IA. Intermediate phenotypes and biomarkers of treatment outcome in major depressive disorder. Dialogues Clin Neurosci. 2014; 16(4):525-537.

[46]. Marazziti D, Mucci F, Fontenelle LF. Immune system and obsessive-compulsive disorder. Psychoneuroendocrinology. 2018; 93:39-44. doi:10.1016/j.psyneuen.2018.04.013

[47]. Husain MI, Strawbridge R, Stokes PR, Young AH. Anti-inflammatory treatments for mood disorders: Systematic review and meta-analysis. J Psychopharmacol. 2017; 31(9):1137-1148. doi:10.1177/0269881117725711

[48]. Strawbridge R, Young AH, Cleare AJ. Biomarkers for depression: recent insights, current challenges and future prospects. Neuropsychiatr Dis Treat. 2017; 13:1245-1262. doi:10.2147/NDT.S114542

[49]. Young AH. The effects of HPA axis function on cognition and its implications for the pathophysiology of bipolar disorder. Harv Rev Psychiatry. 2014; 22(6):331-333. doi:10.1097/HRP.0000000000000020

[50]. Leonard BE. The concept of depression as a dysfunction of the immune system. Curr Immunol Rev. 2010; 6(3):205-212. doi:10.2174/157339510791823835

[51]. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuro-psycho-pharmacology. 2012; 37(1):137-162. doi:10.1038/npp.2011.205

[52]. Maes M, Bosmans E, Meltzer HY, Scharpe S, Suy E. Interleukin-1 beta: a putative mediator of HPA axis hyperactivity in major depression? Am J Psychiatry. 1993; 150(8):1189-1193. doi:10.1176/ajp.150.8.1189

[53]. Maes M, Smith R, Scharpe S. The monocyte-T-lymphocyte hypothesis of major depression. Psychoneuroendocrinology. 1995; 20(2):111-116. doi:10.1016/0306-4530(94)00066-j

[54]. Maes M, Van Bockstaele DR, Gastel A, et al. The effects of psychological stress on leukocyte subset distribution in humans: evidence of immune activation. Neuropsychobiology. 1999; 39(1):1-9. doi:10.1159/000026552

[55]. Cowen PJ. Cortisol, serotonin and depression: all stressed out? Br J Psychiatry. 2002; 180:99-100.doi:10.1192/bjp.180.2.99

[56]. Vogelzangs N, Duivis HE, Beekman AT, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012; 2:e79. doi:10.1038/tp.2012.8

[57]. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet. 2007; 370(9590):851-858. doi:10.1016/S0140-6736(07)61415-9

[58]. Michaud K, Wolfe F. Comorbidities in rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2007; 21(5):885-906. doi:10.1016/j.berh.2007.06.002

[59]. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A. 1996; 93(9):3908-3913. doi:10.1073/pnas.93.9.3908

[60]. Sheline YI, Gado MH, Price JL. Amygdala core nuclei volumes are decreased in

recurrent major depression. Neuroreport. 1998; 9(9):2023-2028. doi:10.1097/00001756-199806220-00021

[61]. Campbell S, MacQueen G. An update on regional brain volume differences associated with mood disorders. Curr Opin Psychiatry. 2006; 19(1):25-33. doi:10.1097/01.yco.0000194371.47685.f2

[62]. Bonaccorso S, Puzella A, Marino V, et al. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res. 2001; 105(1-2):45-55. doi:10.1016/s0165-1781(01)00315-8

[63]. Bonaccorso S, Marino V, Biondi M, Grimaldi F, Ippoliti F, Maes M. Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus. J Affect Disord. 2002; 72(3):237-241. doi:10.1016/s0165-0327(02)00264-1

[64]. Bonaccorso S, Marino V, Puzella A, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J Clin Psychopharmacol. 2002; 22(1):86-90. doi:10.1097/00004714-200202000-00014

[65]. Capuron L, Gumnick JF, Musselman DL, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology. 2002; 26(5):643-652. doi:10.1016/S0893-133X(01)00407-9

[66]. Yirmiya R. Endotoxin produces a depressive-like episode in rats. Brain Res. 1996; 711(1-2):163-174.doi:10.1016/0006-8993(95)01415-2

[67]. Anisman H, Poulter MO, Gandhi R, Merali Z, Hayley S. Interferon-alpha effects are exaggerated when administered on a psychosocial stressor backdrop: cytokine, corticosterone and brain monoamine variations. J Neuroimmunol. 2007; 186(1-2):45-53. doi:10.1016/j.jneuroim.2007.02.008

[68]. Bonaz B, Bazin T, Pellissier S. The vagus nerve at the interface of microbiota-gut-brain axis. Front Neurosci. 2018; 12: 49.

[69]. Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Mol.

[70]. Maes M, Kubera M, Leunis JC. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuro Endocrinol Lett. 2008; 29(1):117-124.

[71]. Borowski T, Kokkinidis L, Merali Z, Anisman H. Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. Neuroreport. 1998; 9(17):3797-3802. doi:10.1097/00001756-199812010-00006

[72]. Bluthe RM, Michaud B, Poli V, Dantzer R. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. Physiol Behav. 2000; 70(3-4):367-373.doi:10.1016/s0031-9384(00)00269-9

[73]. Marazziti D, Rutigliano G, Baroni S, Landi P, Dell'Osso L. Metabolic syndrome and major depression. CNS Spectr. 2014; 19(4):293-304. doi:10.1017/S1092852913000667

[74]. Arteaga-Henriquez G, Simon MS, Burger B, et al. Low-Grade Inflammation as a Predictor of Antidepressant and Anti-Inflammatory Therapy Response in MDD Patients: A Systematic Review of the Literature in Combination With an Analysis of Experimental Data Collected

in the EU-MOODINFLAME Consortium. Front Psychiatry. 2019; 10:458.doi:10.3389/fpsyt.2019.00458

[75]. Osimo EF, Baxter LJ, Lewis G, Jones PB, Khandaker GM. Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. Psychol Med. 2019;49(12):1958-1970.

[76]. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. Int J Mol Sci. 2019;20(13):3328.

[77]. Rock KL, Latz E, Ontiveros F, Kono H. The sterile inflammatory response. Annu Rev Immunol. 2010;28:321-42.

[78]. Kwon S, Cheon SY. Influence of the inflammasome complex on psychiatric disorders: clinical and preclinical studies. Expert Opin Ther Targets. 2021;25:897-907. doi: 10.1080/14728222.2021.2005027

[79]. Pariante CM. The glucocorticoid receptor: part of the solution or part of the problem? J Psychopharmacol. 2006; 20(4 Suppl):79-84. doi:10.1177/1359786806066063

[80]. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008; 31(9):464-468. doi:10.1016/j.tins.2008.06.006

[81]. Palego L, Betti L, Rossi A, Giannaccini G. Tryptophan Biochemistry: Structural, Nutritional, Metabolic, and Medical Aspects in Humans. J Amino Acids. 2016; 2016:8952520. doi:10.1155/2016/8952520

[82]. Nakagawa M, Mizuma K, Inui T. Changes in taste perception following mental or physical stress. Chem Senses. 1996;21(2):195-200.

[83]. Al'Absi M, Nakajima M, Hooker S, Wittmers L, Cragin T. Exposure to acute stress is associated with attenuated sweet taste. Psychophysiology. 2012;49(1):96-103.

[84]. Okamoto A, Miyoshi M, Imoto T, Ryoke K, Watanabe T. Chronic restraint stress in rats suppresses sweet and umami taste responses and lingual expression of T1R3 mRNA. Neurosci Lett. 2010; 486: 211-214.

[85]. Dess NK, Edelheit D. The bitter with the sweet: the taste/stress/temperament nexus. Biol Psychol. 1998;48(2):103-119.

[86]. Kazour F, Richa S, Desmidt T, Lemaire M, Atanasova B, El Hage W, Olfactory and gustatory functions in bipolar disorders: A systematic review, Neurosci Biobehav Rev 2017; 80: 69-79.

[87]. Koning, E., Vorstman, J., McIntyre, R., & Brietzke, E. Characterizing eating behavioral phenotypes in mood disorders: A narrative review. Psychological Medicine 2022; 52:2885-2898.

[88]. de Melo LGP, Nunes SOV, Anderson G, et al. Shared metabolic and immune-inflammatory, oxidative and nitrosative stress pathways in the metabolic syndrome and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2017; 78:34-50. doi:10.1016/j.pnpbp.2017.04.027

[89]. Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4(+)T Cells in Neurodegenerative Diseases. Front Cell Neurosci. 2018; 12:114. doi:10.3389/fncel.2018.00114

[90]. Siwek M, Sowa-Kucma M, Dudek D, et al. Oxidative stress markers in affective disorders. Pharmacol Rep. 2013; 65(6):1558-1571. doi:10.1016/s1734-1140(13)71517-2

[91]. Guu TW, Mischoulon D, Sarris J, et al. International Society for Nutritional Psychiatry Research Practice Guidelines for Omega-3 Fatty Acids in the Treatment of Major Depressive Disorder.

Psychother Psychosom. 2019; 88(5):263-273.doi:10.1159/000502652

[92]. Firth J, Teasdale SB, Allott K, et al. The efficacy and safety of nutrient supplements in the treatment of mental disorders: a meta-review of meta-analyses of randomized controlled trials. World Psychiatry. 2019; 18(3):308-324.doi:10.1002/wps.20672

[93]. Liu T, Zhong S, Liao X, et al. A Meta-Analysis of oxidative stress markers in depression. PLoS One. 2015; 10(10):e0138904. doi:10.1371/journal.pone.0138904

[94]. Vavakova M, Durackova Z, Trebaticka J. Markers of oxidative stress and neuroprogression in depression disorder. Oxid Med Cell Longev. 2015; 2015:898393. doi:10.1155/2015/898393

[95]. Carpita B, Betti L, Palego L, Bartolommei N, Chico L, Pasquali L, Siciliano G, Monzani F, Franchi R, Rogani S, Mucci F, Elefante C, Lattanzi L, Marazziti D, Giannaccini G, Dell'Osso L. Plasma redox and inflammatory patterns during major depressive episodes: a cross-sectional investigation in elderly patients with mood disorders. CNS Spectr. 2021;26:416-426.

[96]. Petrie WM, Ban TA. Vitamins in psychiatry. Do they have a role? Drugs. 1985;30:58-65. doi: 10.2165/00003495-198530010-00006. PMID: 3896744.

[97]. Field DT, Cracknell RO, Eastwood JR, Scarfe P, Williams CM, Zheng Y, Tavassoli T. High-dose Vitamin B6 supplementation reduces anxiety and strengthens visual surround suppression. Hum Psychopharmacol. 2022;37(6):e2852.

[98]. Bičíková M, Dušková M, Vítků J, Kalvachová B, Řípová D, Mohr P, Stárka L. Vitamin D in anxiety and affective disorders. Physiol Res. 2015;64(Suppl 2):S101-S103. doi: 10.33549/physiolres.933082.

[99]. Parker GB, Brotchie H, Graham RK. Vitamin D and depression. J Affect Disord. 2017;208:56-61. doi: 10.1016/j.jad.2016.08.082.

[100]. Marazziti D, Parra E, Palermo S, Barberi FM, Buccianelli B, Ricciardulli S, Cappelli A, Mucci F, Dell'Osso L. Vitamin D: A pleiotropic hormone with possible psychotropic activities. Curr Med Chem. 2021;28(19):3843-3864. doi: 10.2174/0929867328666201210104701.

[101]. Umar M, Sastry KS, Chouchane AI. Role of Vitamin D beyond the skeletal function: A review of the molecular and clinical studies. Int J Mol Sci. 2018;19(6):1618. doi: 10.3390/ijms19061618.

[102]. Diaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: Possible implications for prevention and therapy. Cancer Res. 2000;60:2304-2312.

[103]. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: Lessons learned from genome-wide analysis. Front Physiol. 2014;5:151. doi: 10.3389/fphys.2014.00151.

[104]. Bivona G, Gambino CM, Iacolino G, Ciaccio M. Vitamin D and the nervous system. Neurol Res. 2019;41:827-835. doi: 10.1080/01616412.2019.1622872.

[105]. Chabas JF, Alluin O, Rao G, Garcia S, Lavaut MN, Risso JJ, Legre R, Magalon G, Khrestchatisky M, Marqueste T, Decherchi P, Feron F. Vitamin D2 potentiates axon regeneration. J Neurotrauma. 2008;25(10):1247-1256. doi: 10.1089/neu.2008.0593.

[106]. Neveu I, Naveilhan P, Jehan F, Baudet C, Wion D, De Luca HF, Brachet P. 1,25-dihydroxyvitamin D3 regulates the synthesis of nerve growth factor in

primary cultures of glial cells. Brain Res Mol Brain Res. 1994;24(1-4):70-76. doi: 10.1016/0169-328X(94)90119-8.

[107]. Brown J, Bianco JI, McGrath JJ, Eyles DW. 1,25-dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. Neurosci Lett. 2003;343(2):139-143. doi: 10.1016/S0304-3940

[108]. Ibi M, Sawada H, Nakanishi M, Kume T, Katsuki H, Kaneko S, Shimohama S, Akaike A. Protective effects of 1 alpha,25-(OH)(2)D-3 against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. Neuropharmacology. 2001;40(6):761-771. doi: 10.1016/s0028-3908(01)00009-0.

[109]. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab. 2002;13(3):100-105. doi: 10.1016/s1043-2760(01)00547-1.

[110]. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull. 2014;39:322-350. doi: 10.1111/nbu.12108.

[111]. [Nerhus M, Berg AO, Kvitland LR, Dieset I, Hope S, Dahl SR, Weibell MA, Romm KL, Faerden A, Andreassen OA, Melle I. Low vitamin D is associated with negative and depressive symptoms in psychotic disorders. Schizophr Res. 2016;178(1-3):44-49. doi: 10.1016/j.schres.2016.08.024.

[112]. Modan-Moses D, Levy-Shraga Y, Pinhas-Hamiel O, Kochavi B, Enoch-Levy A, Vered I, Stein D. High prevalence of vitamin D deficiency and insufficiency in adolescent inpatients diagnosed with eating disorders. Int J Eat Disord. 2015;48(6):607-614. doi: 10.1002/eat.22347.

[113]. Veronese N, Solmi M, Rizza W, Manzato E, Sergi G, Santonastaso P, Caregaro L, Favaro A, Correll CU. Vitamin D status in anorexia nervosa: A meta-analysis. Int J Eat Disord. 2015;48(7):803-813. doi: 10.1002/eat.22370.

[114]. Marazziti D, Barberi FM, Fontenelle L, Buccianelli B, Carbone MG, Parra E, Palermo S, Massa L, Tagliarini C, Della Vecchia A, Mucci F, Arone A, Dell'Osso L. Decreased Vitamin D levels in OCD patients. CNS Spectr. 2021;1-24. doi: 10.1017/S1092852921000821.

[115]. Terock J, Hannemann A, Van der Auwera S, Janowitz D, Spitzer C, Bonk S, Völzke H, Grabe HJ. Posttraumatic stress disorder is associated with reduced vitamin D levels and functional polymorphisms of the vitamin D binding-protein in a population-based sample. Prog Neuropsychopharmacol Biol Psychiatry. 2020;96:109760. doi: 10.1016/j.pnpbp.2019.109760.

[116]. Siracusano M, Riccioni A, Abate R, Benvenuto A, Curatolo P, Mazzone L. Vitamin D deficiency and autism spectrum disorder. Curr Pharm Des. 2020;26(21):2460-2474. doi: 10.2174/1381612826666200415174311.

[117]. Black LJ, Jacoby P, Allen KL, Trapp GS, Hart PH, Byrne SM, Mori TA, Beilin LJ, Oddy WH. Low vitamin D levels are associated with symptoms of depression in young adult males. Aust N Z J Psychiatry. 2014;48(5):464-471. doi: 10.1177/0004867413512383.

[118]. Woo YS, Kim S, Jeong JH, Jung YE, Kim MD, Bahk WM. Vitamin D Deficiency/insufficiency among inpatients with depressive symptoms. Clin Psychopharmacology Neurosci. 2019;17(1):121-124. doi: 10.9758/cpn.2019.17.1.121.

[119]. Geng C, Shaikh AS, Han W, Chen D, Guo Y, Jiang P. Vitamin D and depression: Mechanisms, determination and application. Asia Pac J Clin Nutr. 2019;28(4):689-694. doi: 10.6133/apjcn.201912_28(4).0003.

[120]. Stewart R, Hirani V. Relationship between vitamin D levels and depressive symptoms in older residents from a national survey population. Psychosom Med. 2010;72(7):608-612. doi: 10.1097/PSY.0b013e3181e9bf15.

[121]. Altunsoy N, Yüksel RN, Cingi Yirun M, Kılıçarslan A, Aydemir Ç. Exploring the relationship between vitamin D and mania: Correlations between serum vitamin D levels and disease activity. Nord J Psychiatry. 2018;72(3):221-225. doi: 10.1080/08039488.2018.1424238.

[122]. Boerman R, Cohen D, Schulte PF, Nugter A. Prevalence of vitamin D deficiency in adult outpatients with bipolar disorder or schizophrenia. J Clin Psychopharmacol. 2016;36(6):588-592. doi: 10.1097/JCP.0000000000000580.

[123]. Grønli O, Kvamme JM, Jorde R, Wynn R. Vitamin D deficiency is common in psychogeriatric patients, independent of diagnosis. BMC Psychiatry. 2014;14:134. doi: 10.1186/1471-244X-14-134.

[124]. Bersani FS, Ghezzi F, Maraone A, Vicinanza R, Cavaggioni G, Biondi M, Pasquini M. The relationship between Vitamin D and depressive disorders. Riv Psichiatr. 2019;54(6):229-234.

[125]. Sikoglu EM, Navarro AAL, Starr D, Dvir Y, Nwosu BU, Czerniak SM, Rogan RC, Castro MC, Edden RA, Frazier JA, Moore CM. Vitamin D3 supplemental treatment for mania in youth with bipolar spectrum disorders. J Child Adolesc Psychopharmacol. 2015;25(5):415-424. doi: 10.1089/cap.2014.0110.

[126]. Marsh WK, Penny JL, Rothschild AJ. Vitamin D supplementation in bipolar depression: A double blind placebo controlled trial. J Psychiatr Res. 2017;95: 48-53. doi: 10.1016/j.jpsychires.2017.07.021.

[127]. Li G, Mbuagbaw L, Samaan Z, Falavigna M, Zhang S, Adachi JD, Cheng J, Papaioannou A, Thabane L. Efficacy of vitamin D supplementation in depression in adults: A systematic review. J Clin Endocrinol Metab. 2014;99(3):757–767. doi: 10.1210/jc.2013-3450.

[128]. Gowda U, Mutowo MP, Smith BJ, Wluka AE, Renzaho AMN. Vitamin D supplementation to reduce depression in adults: Meta-analysis of randomized controlled trials. Nutrition. 2015;31:421–429. doi: 10.1016/j.nut.2014.06.017.

[129]. Hansen JP, Pareek M, Hvolby A, Schmedes A, Toft T, Dahl E, Nielsen CT. Vitamin D3 supplementation and treatment outcomes in patients with depression (D3-vit-dep). BMC Res Notes. 2019;12(1):203. doi: 10.1186/s13104-019-4218-z.

[130]. Vellekkatt F, Menon V. Efficacy of vitamin D supplementation in major depression: A meta-analysis of randomized controlled trials. J Postgrad Med. 2019;65(2):74–80. doi: 10.4103/jpgm.JPGM_571_17.

[131]. Kaviani M, Nikooyeh B, Zand H, Yaghmaei P, Neyestani TR. Effects of vitamin D supplementation on depression and some involved neurotransmitters. J Aaffect Disord. 2020;269:28–35. doi: 10.1016/j.jad.2020.03.029.

[132]. Vellekkatt F, Menon V, Rajappa M, Sahoo J. Effect of adjunctive single dose parenteral Vitamin D supplementation in major depressive disorder with concurrent vitamin D deficiency: A double-blind randomized placebo-controlled trial. J Psychiatr Res. 2020;129:250–256. doi: 10.1016/j.jpsychires.2020.07.037.

[133]. Alghamdi S, Alsulami N, Khoja S, Alsufiani H, Tayeb HO, Tarazi FI. Vitamin D supplementation ameliorates severity of major depressive disorder. J Mol Neurosci. 2020;70(2):230–235. doi: 10.1007/s12031-019-01461-2.

[134]. Watanabe F, Bito T. Vitamin B12 sources and microbial interaction. Exp

Biol Med. 2018;243(2):148-158. doi: 10.1177/1535370217734330.

[135]. Andres E, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, Noblet-Dick M, Maloisel F, Schlienger JL, Blicklé JF. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ. 2004;171(3):251-259. doi: 10.1503/cmaj.1031155.

[136]. Stabler SP. Clinical practice. Vitamin B12 deficiency. N Engl J Med. 2013;368(2):149-160. doi: 10.1056/NEJMcp1113996.

[137]. Green R, Mitra AD. Megaloblastic anemias: nutritional and other causes. Med Clin North Am. 2017;101(2):297-317. doi: 10.1016/j.mcna.2016.08.005.

[138]. Berkins S, Schiöth HB, Rukh G. Depression and vegetarians: association between dietary vitamin B6, B12 and folate intake and global and subcortical brain volumes. Nutrients. 2021;13(6):1790. doi: 10.3390/nu13061790.

[139]. Saravanan P, Sukumar N, Adaikalakoteswari A, Goljan I, Venkataraman H, Gopinath A, Bagias C, Yajnik CS, Stallard N, Ghebremichael-Weldeselassie Y, Fall CHD. Association of maternal vitamin B12 and folate levels in early pregnancy with gestational diabetes: a prospective UK cohort study (PRiDE study). Diabetologia. 2021;64(10):2170-2182. doi: 10.1007/s00125-021-05494-5.

[140]. Devalia V, Hamilton MS, Molloy AM, British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol. 2014;166(4):496-513. doi: 10.1111/bjh.12959.

[141]. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr. 2009;89(2):693S-696S. doi: 10.3945/ajcn.2008.26947D.

[142]. Coşar A, Ipçioğlu OM, Ozcan O, Gültepe M. Folate and homocysteine metabolisms and their roles in the biochemical basis of neuropsychiatry. Turk J Med Sci. 2014;44(1):1-9. doi: 10.3906/sag-1208-113.

[143]. Andersson A, Brattström L, Israelsson B, Isaksson A, Hamfelt A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. Eur J Clin Invest. 1992;22(2):79-87. doi: 10.1111/j.1365-2362.1992.tb01447.x.

[144]. Palego L. Betti L, Giannaccini G. Sulfur Metabolism and Sulfur-Containing Amino Acids: I- Molecular Effectors. Bochem & Pharmacol 2015, 4:1

[145]. Palego L. Betti L, Giannaccini G. Sulfur Metabolism and Sulfur-Containing Amino Acids: II- Autism Spectrum Disorders, Schizophrenia and Fibromyalgia. . Bochem & Pharmacol 2015, 4:1

[146]. Lindenbaum J, Healton EB, Savage DG, Brust JCM, Garrett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med. 1988;318(26):1720–1728.

[147]. Coppen A, Bolander-Gouaille C. Treatment of depression: time to consider folic acid and vitamin B12. J Psychopharmacol. 2005;19(1):59–65.

[148]. Sangle P, Sandhu O, Aftab Z, Anthony AT, Khan S. Vitamin B12 supplementation: preventing onset and improving prognosis of depression. Cureus. 2020;12(10):e11169.

[149]. Markun S, Gravestock I, Jäger L, Rosemann T, Pichierri G, Burgstaller JM. Effects of vitamin B12 supplementation on cognitive function, depressive symptoms, and fatigue: a systematic review, meta-analysis, and meta-regression. Nutrients. 2021;13(3):923.

[150]. Sahu P, Thippeswamy H, Chaturvedi SK. Neuropsychiatric manifestations in vitamin B12 deficiency. Vitam Horm. 2022;119:457–470.

[151]. Morris AL, Thomas AB, Francis JK, Jerome L, Loren RM, Richard W. Megavitamin and orthomolecular therapy in psychiatry: excerpts from a report of the American Psychiatric Association Task Force on vitamin therapy in psychiatry. Nutr Rev. 1973;32:44–47.

[152]. Airola P. Hypoglycemia: A Better Approach. Phoenix (Arizona): Health Plus Publishers; 1977.

[153]. Todorov A, Chumpalova-Tumbeva P, Stoimenova-Popova MY, Popova VS, Todorieva-Todorova DK, Tzvetkov N, Hristov IG, Georgiev G, Valtchev V, Krasteva NA, Ilieva RG, Dimitrova E, Tumbev LZ, Ruseva AL. Correlation between depression and anxiety and the level of vitamin B12 in patients with depression and anxiety and healthy controls. J Biomed Clin Res. 2017;10(2):140–145.

[154]. Skarupski KA, Tangney C, Li H, Ouyang B, Evans DA, Morris MC. Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. Am J Clin Nutrit. 2010;92(2):330–335.

[155]. Wu Y, Zhang L, Li S, Zhang D. Associations of dietary vitamin B1, vitamin B2, vitamin B6, and vitamin B12 with the risk of depression: a systematic review and meta-analysis. Nutr Rev. 2022;80(3):351–366.

[156]. Nielsen J, Steiness I. Vitamin B 12 deficiency and mental illness. Int Pharmacopsychiatry. 1968;1:129–133.

[157]. Roos D, Willanger R. Various degrees of dementia in a selected group of gastrectomized patients with low serum B12. Acta Neurol Scand. 1977;55(5):363–376.

[158]. Goggans FC. A case of mania secondary to vitamin B12 deficiency. Am J Psychiatry. 1984;141(2):300–301.

[159]. Gomez-Bernal GJ, Bernal-Perez M. Vitamin B12 deficiency manifested as mania: a case report. Prim Care Companion J Clin Psychiatry. 2007;9(3):238.

[160]. Tufan AE, Bilici R, Usta G, Erdoğan A. Mood disorder with mixed, psychotic features due to vitamin B12 deficiency in an adolescent: case report. Child Adolesc Psychiatry Ment Health. 2012;6(1):25.

[161]. Majumder S, Soriano J, Louie Cruz A, Dasanu CA. Vitamin B12 deficiency in patients undergoing bariatric surgery: preventive strategies and key recommendations. Surg Obes Relat Dis. 2013;9(6):1013-1019.

[162]. Morillo Z, Guzmán DS, Ortiz Y. Intento suicida y psicosis debida a déficit de vitamina B12, a propósito de un caso. Ciencia y Salud. 2018;2(3):55-61.

[163]. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998; 59 Suppl 20:22-33;quiz 34-57.

[164]. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960; 23:56-62. doi: 10.1136/jnnp.23.1.56

[165]. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry. 1978; 133:429-435. doi: 10.1192/bjp.133.5.429

[166]. Guy W. ECDEU assessment manual for psychopharmacology. US Department of Health, Education, and Welfare: Rockville, MD. 1976.

[167]. Arneson WL, Arneson DL. Current Methods for Routine Clinical Laboratory

Testing of Vitamin D Levels. Laboratory Medicine. 2013 Feb;44(1):e38-e42.

[168]. Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK. HPLC method for 25-hydroxyvitamin D measurement: comparison with contemporary assays. Clin Chem 52, 1120-1126, 2006Clinical Chemistry 52(6):1120-6

[169]. Galunska BT, Gerova DI, Boncheva MI, Svinarov DA (2014) HPLC method for measuring the circulating levels of 25-hydroxy vitamin D: Validation and comparison with ID LC/MS/MS and immunoassay. Integr Food Nutr Metab 1: DOI: 10.15761/IFNM.1000112

[170]. Nexo E, Christensen AL, Petersen TE, Fedosov SN. Measurement of transcobalamin by ELISA. Clin Chem. 2000 Oct;46(10):1643-9. PMID: 11017944.

[171]. Rafii M, Elango R, House JD, Courtney-Martin G, Darling P, Fisher L, Pencharz PB. Measurement of homocysteine and related metabolites in human plasma and urine by liquid chromatography electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Oct 15;877(28):3282-91. doi: 10.1016/j.jchromb.2009.05.002. Epub 2009 May 13. PMID: 19481985.

[172]. Zayed A, Bustami R, Alabsi W, El-Elimat T. Development and Validation of a Rapid High-Performance Liquid Chromatography–Tandem Mass Spectrometric Method for Determination of Folic Acid in Human Plasma. Pharmaceuticals (Basel). 2018 May 27;11(2):52. doi: 10.3390/ph11020052. PMID: 29861484; PMCID: PMC6027057.

[173]. Kennedy DO, Haskell CF. Vitamins and cognition: what is the evidence? Drugs. 2011;71(15):1957-1971. doi: 10.2165/11594130-000000000-00000.

[174]. Zhu C, Zhang Y, Wang T, Lin Y, Yu J, Xia Q, Zhu P, Zhu DM. Vitamin D supplementation improves anxiety but not depression symptoms in patients with vitamin D deficiency. Brain Behav. 2020;10(11):e01760. doi: 10.1002/brb3.176.

[175]. Omidian M, Mahmoudi M, Abshirini M, Eshraghian MR, Javanbakht MH, Zarei M, Hasani H, Djalali M. Effects of vitamin D supplementation on depressive symptoms in type 2 diabetes mellitus patients: Randomized placebo-controlled double-blind clinical trial. Diabetes Metab Syndr. 2019;13(4):2375-2380. doi: 10.1016/j.dsx.2019.06.011.

[176]. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-281. doi: 10.1056/NEJMra070553.

[177]. Haussler MR, Haussler CA, Jurutka PW, Thompson PD, Hsieh JC, Remus LS, Selznick SH, Whitfield GK. The vitamin D hormone and its nuclear receptor: Molecular actions and disease states. J Endocrinol. 1997;154 Suppl:S57-S73.

[178]. Cui X, Pelekanos M, Liu PY, Burne THJ, McGrath JJ, Eyles DW. The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. Neuroscience. 2013;236:77-87. doi: 10.1016/j.neuroscience.2013.01.035.

[179]. Melcangi RC, Panzica G. Neuroactive steroids: An update of their roles in central and peripheral nervous system. Psychoneuroendocrinology. 2009;34(Suppl 1):S1-S8. doi: 10.1016/j.psyneuen.2009.11.001.

[180]. Landel V, Stephan D, Cui X, Eyles D, Feron F. Differential expression of vitamin D-associated enzymes and receptors in brain cell subtypes. J Steroid Biochem Mol Biol. 2018;177:129-134. doi: 10.1016/j.jsbmb.2017.09.008.

[181]. Marini F, Bartoccini E, Cascianelli G, Voccoli V, Baviglia MG, Magni MV, Garcia-Gil M, Albi E. Effect of 1alpha,25-dihydroxyvitamin D3 in embryonic hippocampal cells. Hippocampus. 2010;

20(6):696-705. doi: 10.1002/hipo.20670.

[182]. Shirazi HA, Rasouli J, Ciric B, Rostami A, Zhang GX. 1,25-dihydroxyvitamin D3 enhances neural stem cell proliferation and oligodendrocyte differentiation. Exp Mol Pathol. 2015; 98(2):240-245. doi: 10.1016/j.yexmp.2015.02.004

[183]. Ebesunun MO, Eruvulobi HU, Olagunju T, Owoeye OA. Elevated plasma homocysteine in association with decreased vitamin B(12), folate, serotonin, lipids and lipoproteins in depressed patients. Afr J Psychiatry. 2012;15(1):25-29.

[184]. Hayden MR, Tyagi SC. Impaired Folate-Mediated One-Carbon Metabolism in Type 2 Diabetes, Late-Onset Alzheimer's Disease and Long COVID. Medicina 2022, 58(1), 16

[185]. Esnafoglu E, Ozturan DD. The relationship of severity of depression with homocysteine, folate, vitamin B12, and vitamin D levels in children and adolescents. Child Adolesc Ment Health. 2020;25(4):249-255.

[186]. Sozer K, Gorgulu Y, Bulent Sonmez M, Kose Cinar R. Psychotic depression after obesity surgery and recovery with vitamin B12 replacement. Düşünen Adam. 2019;32:65–68.

[187]. Goggans FC. A case of mania secondary to vitamin B12 deficiency. Am J Psychiatry. 1984;141:300-1

[188]. Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med. 1988;318:1720-8.

[189]. Gomez-Bernal GJ, Bernal-Perez M. Vitamin b_{12} deficiency manifested as mania: a case report. Prim Care Companion J Clin Psychiatry 2007;9:238.

[190]. Tufan AE, Bilici R, Usta G, Erdoğan A. Mood disorder with mixed, psychotic features due to vitamin b12 deficiency in an adolescent: case report. Child Adolesc Psychiatry Ment Health. 2012 ;6:25.

[191]. Issac TG, Soundarya S, Christopher R, Chandra SR. Vitamin B12 deficiency: an important reversible co-morbidity in neuropsychiatric manifestations. Indian J Psychol Med. 2015 ;37:26-9.

[192]. Olagunju AT, Morgan JA, Aftab A, Gatchel JR, Chen P, Dols A, Sajatovic M, Regenold WT. A Review of the Evidence Base for Nutrition and Nutritional Supplements in Older Adults with Bipolar Disorder: A report from the OABD task force. J Frailty Aging. 2021;10:241-246.

[193]. Raveendranathan D, Shiva L, Venkatasubramanian G, Rao MD, Varambally S, Gangadhar BN. Vitamin B12 deficiency masquerading as clozapine-resistant psychotic symptoms in schizophrenia. J Neuropsychiatry Clin Neurosci 2013; 25:2

[194]. Al Mansoori A, Shakoor H, Ali HI, Feehan J, Al Dhaheri AS, Cheikh Ismail L, Bosevski M, Apostolopoulos V, Stojanovska L. The Effects of Bariatric Surgery on Vitamin B Status and Mental Health. Nutrients. 2021;13:1383.

[195]. Friedman KE, Applegate K, Portenier D, McVay MA. Bariatric surgery in patients with bipolar spectrum disorders: Selection factors, postoperative visit attendance, and weight outcomes. Surg Obes Relat Dis. 2017;4:643-651

[196]. Engström G, Träskman-Bendz L. Blood folate, vitamin B12, and their relationships with cerebrospinal fluid monoamine metabolites, depression, and personality in suicide attempters, Nordic Journal of Psychiatry 1999; 53:2, 131-137

[197]. Hadinezhad P, Hosseini SH, Shekarriz R, Zarvani A. A case report of vitamin B12 deficiency with symptoms of depression, hallucination and delusion. J Babol Univ Med Sci 2016; 1: 61-65

[198]. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. [accessed 17 June 2021]. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf?sequence=1&isAllowed=y.

[199]. [199] World Health Organization. Mental Health Atlas 2017. [accessed 10 June 2019]. https://apps.who.int/iris/bitstream/handle/10665/272735/9789241514019-eng.pdf?ua=1.

[200]. Plans L, Barrot C, Nieto E, Rios J, Schulze TG, Papiol S, Mitjans M, Vieta E, Benabarre A. Association between completed suicide and bipolar disorder: a systematic review of the literature. J Affect Disord. 2019 Apr 1;242:111-122. doi: 10.1016/j.jad.2018.08.081. Epub 2018 Aug 31. PMID: 30266010.

[201]. Schaffer A, Isometsä ET, Tondo L, Moreno DH, Sinyor M, Kessing LV, Turecki G, Weizman A, Azorin JM, Ha K, Reis C, Cassidy F, Goldstein T, Rihmer Z, Beautrais A, Chou YH, Diazgranados N, Levitt AJ, Zarate CA Jr, Yatham L. Epidemiology, neurobiology and pharmacological interventions related to suicide deaths and suicide attempts in bipolar disorder: part I of a report of the International Society for Bipolar Disorders Task Force on Suicide in Bipolar Disorder. Aust N Z J Psychiatry. 2015 Sep;49(9):785-802. doi: 10.1177/0004867415595063. PMID: 26275435.

[202]. Ozdogan MG, Aydin EF, Ustundag MF, Ceyhun HA, Oral E, Bakan E. Homocysteine, chronotype and clinical course in bipolar disorder patients. Nord J Psychiatry. 2020 Aug;74(5):340-345. doi: 10.1080/08039488.2020.1726542. PMID: 32266825.

[203]. Permoda-Osip A, Dorszewska J, Skibinska M, Chlopocka-Wozniak M, Rybakowski JK. Hyperhomocysteinemia in bipolar depression: clinical and biochemical correlates. Neuropsychobiology. 2013;68(4):193–196.

[204]. Ghanizadeh A, Singh AB, Berk M, Torabi-Nami M. Homocysteine as a potential biomarker in bipolar disorders: a critical review and suggestions for improved studies. Expert Opin Ther Targets. 2015 Jul;19(7):927-39. doi: 10.1517/14728222.2015.1039593. PMID: 25959508.

[205]. Salagre E, Vizuete AF, Leite M, Brownstein DJ, McGuinness A, Jacka F, Dodd S, Stubbs B, Köhler CA, Vieta E, Carvalho AF, Berk M, Fernandes BS. Homocysteine as a peripheral biomarker in bipolar disorder: a meta-analysis. Eur Psychiatry. 2017 Aug;43:81-91. doi: 10.1016/j.eurpsy.2017.02.487. Epub 2017 May 15. PMID: 28511604.

[206]. Chiarani F, Tramontina JF, Ceresér KM, Kunz M, Paim L, Vargas CR, Sitta A, Machado SP, Wyse AT, Kapczinski FP. Homocysteine and other markers of cardiovascular risk during a manic episode in patients with bipolar disorder. Braz J Psychiatry. 2013 Mar;35(2):157-60. doi: 10.1590/S1516-44462013000200014. PMID: 23828536.

[207]. Tunbridge EM, Attenburrow MJ, Gardiner A, Rendell JM, Hinds C, Goodwin GM, Harrison PJ, Geddes JR. Biochemical and genetic predictors and correlates of response to lamotrigine and folic acid in bipolar depression: analysis of the CEQUEL clinical trial. Bipolar Disord. 2017 Sep;19(6):477-486. doi: 10.1111/bdi.12503. PMID: 28984385.

[208]. Lerner V, Kanevsky M, Dwolatzky T, Rouach T, Kamin R, Miodownik C. Vitamin B12 and folate serum levels in newly admitted psychiatric patients. Clin Nutr. 2006;25(1):60-67.

[209]. Gilbody S, Lightfoot T, Sheldon T. Is low folate a risk factor for depression? A

meta-analysis and exploration of heterogeneity. J Epidemiol Community Health. 2007;61(7):631-637.

[210]. Hsieh YC, Chou LS, Lin CH, Wu HC, Li DJ, Tseng PT. Serum folate levels in bipolar disorder: a systematic review and meta-analysis. BMC Psychiatry. 2019;19(1):305.

[211]. Erzin G, Kotan VO, Topçuoğlu C, Özkaya G, Erel Ö, Yüksel RN, Ürer E, Aydemir MÇ, Göka E. Thiol/disulphide homeostasis in bipolar disorder. Psychiatry Res. 2018 Mar;261:237-242.

[212]. Rehman T, Shabbir MA, Inam-Ur-Raheem M, Manzoor MF, Ahmad N, Liu ZW, Ahmad MH, Siddeeg A, Abid M, Aadil RM. Cysteine and homocysteine as biomarker of various diseases. Food Sci Nutr. 2020 Aug 12;8(9):4696-4707.

[213]. Tsiami A, Obersby D, Vitamins Intake and Plasma Homocysteine in Vegetarians,Ed(s): François Mariotti; Vegetarian and Plant-Based Diets in Health and Disease Prevention,Academic Press ,2017

[214]. Muti M, Del Grande C, Musetti L, Marazziti D, Turri M, Cirronis M, Pergentini I, Corsi M, Dell'Osso L, Corsini GU. Serum uric acid levels and different phases of illness in bipolar I patients treated with lithium. Psychiatry Res. 2015 Feb 28;225(3):604-8..doi:10.1016/j.psychres.2014.11.038

[215]. Black CN, Bot M, Scheffer PG, Snieder H, Penninx B. Uric acid in major depressive and anxiety disorders. J Affect Disord. 2018; 225:684-690. doi:10.1016/j.jad.2017.09.003

[216]. De Berardis D, Conti CM, Campanella D, et al. Evaluation of plasma antioxidant levels during different phases of illness in adult patients with bipolar disorder. J Biol Regul Homeost Agents. 2008; 22(3):195-200.

[217]. Machado-Vieira R. Purinergic system in the treatment of bipolar disorder: uric acid levels as a screening test in mania. J Clin Psychopharmacol. 2012; 32(5):735-736. doi:10.1097/JCP.0b013e318268391d

[218]. Kesebir S, Suner O, Yaylaci ET, Bayrak A, Turan C. Increased uric acid levels in bipolar disorder: is it trait or state? J Biol Regul Homeost Agents. 2013; 27(4):981-988.

[219]. Lu Z, Wang Y, Xun G. Individuals with bipolar disorder have a higher level of uric acid than major depressive disorder: a case-control study. Sci Rep. 2021 Sep 15;11(1):18307. doi: 10.1038/s41598-021-97955-4

[220]. Köhler O, Sylvia LG, Bowden CL, Calabrese JR, Thase M, Shelton RC, McInnis M, Tohen M, Kocsis JH, Ketter TA, Friedman ES, Deckersbach T, Ostacher MJ, Iosifescu DV, McElroy S, Nierenberg AA. White blood cell count correlates with mood symptom severity and specific mood symptoms in bipolar disorder. Aust N Z J Psychiatry. 2017;51(4):355-365.

[221]. Shafiee M, Tayefi M, Hassanian SM, Ghaneifar Z, Parizadeh MR, Avan A, Rahmani F, Khorasanchi Z, Azarpajouh MR, Safarian H, Moohebati M, Heidari-Bakavoli A, Esmaeili H, Nematy M, Safarian M, Ebrahimi M, Ferns GA, Mokhber N, Ghayour-Mobarhan M. Depression and anxiety symptoms are associated with white blood cell count and red cell distribution width: A sex-stratified analysis in a population-based study. Psychoneuroendocrinology. 2017;84:101-108.

[222]. van Guldener C, Stehouwer CD. Homocysteine and methionine metabolism in renal failure. Semin Vasc Med. 2005;5(2):201-8. doi: 10.1055/s-2005-872405.

[223]. Dreisbach AW, Lertora JJ. The effect of chronic renal failure on drug metabolism and transport. Expert Opin Drug Metab Toxicol. 2008 Aug;4(8):1065-74. doi: 10.1517/17425255.4.8.1065.