DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO
DOTTORATO DI RICERCA IN MEDICINA MOLECOLARE
CICLO XXXIII
Coordinatore: **Chiar.mo Prof. Vincenzo Sorrentino**

NEUROINFLAMMATORY BIOMARKERS: CLINICAL AND PHARMACOLOGICAL IMPLICATIONS IN BIPOLAR DISORDER

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Firmato il 23/06/2021

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Anno Accademico: 2020-2021

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1. Psychiatry and biomakers

Statistical and Diagnostic Manual of Mental Disorders (DSM 5), the International Classification of Diseases (ICD 11) and the Research Domain Criteria (RDoC) system, currently provide the cornerstone to elaborate a diagnosis in psychiatry. Despite the continuous updating and improvement of diagnostic criteria, often the psychopathology underlies multiple biological, genetic and environmental factors that interact with each other at multiple levels (1).

The identification of psychiatric biomarkers could represent an important instrument to defining etiology, diagnosis and severity of mental diseases and to identify multiple risk factors with the aim to detect the most suitable and personalized pharmacological treatment (2) .

Biomarkers can be defined as objective measurements that perform normal biological and pathological processes; in several hospital settings, biomarkers are used to detect pharmacotherapeutic targets, resulting important parameters of disease and necessary to monitoring the state of health (3).

Biomarkers must have exact characteristics: easy measurement, accuracy, repetitiveness, standardization and good cost-benefit ratio (4).

Adding biological markers to neuroimaging, identification of genetic, proteomic, metabolic factors, shows an advantage to formulate a personalized therapy in psychiatric settings (5,6,7).

Developments in the knowledge of cell biology and inter-neuronal communication, the potential biomarkers have grown exponentially, based on the measurement of proteins, lipids, carbohydrates and electrolytes, which regulate brain and systemic functions. Several studies have focused on the development of markers in psychiatry, with particular emphasis on genetics, epigenetics, transcriptomics, proteomics, immunological measurements and their potential relevance for neuropsychiatric diseases (8) .

Biomarkers in psychiatry can be divided into:

- **Molecular markers.** Most of the molecular markers have been measured in peripheral blood and this approach allows easy accessibility by venous sampling, low cost and reproducibility (9). The areas most studied in recent years include:
    - trophic factors: neurotrophins and other trophic factors are responsible for regulating cell dynamics and are expressed in the brain and peripheral tissues in a region-specific

way. Neurotrophins, such as brain neurotrophic factor (BDNF), insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF), have been specifically selected to explore specific alterations in psychiatric disorders. For example, to better understand the role whose these factors play in the different phases of bipolar disorder and their potential used as biomarkers (10);

- neuroinflammation developed by pro- and anti-inflammatory cytokines (explored in the next chapter);
- oxidative stress and mitochondrial function: when the production of reactive oxygen species exceeds the capacity of the endogenous/exogenous antioxidant system, a state known as ”oxidative stress” occurs, involving macromolecule targets, including proteins, lipids and DNA, affecting membrane fluidity, protein functionality and DNA structure. Oxidative stress mechanism have been recognized in post-mortem brain studies and peripheral blood cells trial in patient affected by psychiatric diseases, specifically in bipolar disorder (11);
- urinary metabolites: recent studies aim to examine potential biomarkers in the urine of patients that are specific for psychiatric disease. For example, choline, α-hydroxybutyrate, N-methylnicotinamide and isobutyrate, found in the urine of patients with bipolar disorder, were evaluated as possible markers, showing differences compared to healthy controls (12).

• **Genetic markers** are a fundamental building block of modern medicine. Several studies on families and twins have made possible to demonstrate the inheritance of psychiatric diseases (13). Genomic association studies (GWAS) have made significant methodological advances compared to previous genetic approaches, allowing to compare the allelic frequencies of thousands of nucleotide polymorphisms between cases and controls; nevertheless, to make these studies meaningful, larger samples are needed. Combining GWAS and genome-wide sequencing of patients with bipolar disorder, was possible to detect evidence regarding polymorphisms of the voltage-gated calcium channel alpha-1C subunit (CACNA1C) and anchorine 3 (ANK3) (14). Furthermore, the combination of neuroimaging with genetic markers could bring out the neural pathways that mediate genetic risk: for example, the risk allele CACNA1C rs1006737 (from G to A) appears to be related to distinct structural and functional neuroimaging results (9) .

- **Neuroimaging markers** can be distinguished in structural and functional neuroimaging and can be derived from the whole brain or from specific regions (13). Structural neuroimaging investigates regional brain volumes and white matter characteristics: for example, prominent white matter hyperintensity and increased ventricular space volume have emerged in recent bipolar disorder studies (15,16). Functional neuroimaging data range from resting state measures to task-related activation studies, among which functional magnetic resonance imaging (fMRI) studies related to emotional processing have identified a location of the cortical cognitive brain network greater activation in the ventral area of the limbic system among patients with bipolar disorder (16,17).

1.1 Biomarkers and mood disorders

Recent scientific advances in the study of markers implicated in mood disorders have allowed their application into the possibility to distinguish between normal and pathological states, and in the observation of the response to drug treatment. For example, genetic studies confirm that the risk of inheriting a major depressive disorder (DDM) is between 30% and 40%, with higher rates in women. A large genomic association study (GWAS) found 44 significant loci associated with DDM. Specific analyzes have identified neuronal genes, genes that regulate expression (such as RBFOX1), genes involved in splicing, as well as genes that are targets of antidepressant treatment (18,19).

Other important studies have investigated drug interactions and gene iso-forms in the cytochrome P450 (CYP450) pathway, which explain the fluctuations in the concentration of drugs that reaching the brain could cause beneficial or side effects in vulnerable subjects. Several commercially available kits classify patients based on their phenotype (e.g. CYP2D6, 2C19, CYP3A4), leading to the introduction of phenotypic categories: poor, intermediate, normal and ultra-rapid metabolizers, based on CYP450 isoenzyme status and their relationship with the plasma levels of drugs at fixed doses (20). In genetic studies of patients with bipolar disorder (DB), despite a consistent association with the BDNF, catechol-O-methyltransferase and serotonin transporter (5-HTT) genes, it was not possible to distinguish them as specific markers, as they have also been found in other psychiatric disorders.

Patients with bipolar disorder have shown relevant evidence regarding the polymorphisms of the alpha-1C subunit of the voltage-gated calcium channel (CACNA1C) and of anchoririne 3 (ANK3) (14). Furthermore, CACNA1C appears to be involved in disturbances in hippocampal functioning, anterior cingulate cortex and dorsolateral prefrontal cortex.

Rong H et al. (21) demonstrated that untreated manic patients were found to have down-regulated levels of micro RNA (miRNA) expression, which plays a key role in inflammation and gene expression.

Neuroimaging markers show the structural and functional abnormalities, typifying different diagnostic models used to improve the clinical approach to mood disorders. The main structural changes in mood disorders occur in the associative cortex (mainly in the dorsolateral prefrontal cortex, orbitofrontal cortex and anterior cingulate cortex), hippocampus and amygdala. Furthermore, the morphological changes in the neuronal circuits, which regulate brain function, are influenced by the pathological process and affect the density and size of neurons in the areas that form the neuronal circuits responsible for higher cognitive functions and mental processes associated with the limbic system. There was a decrease in neuronal density and nerve cell size in the associative cortex, but this type of injury was not reported in the sensory and motor cortex, which is consistent with recent neuro-functional and neuroimaging findings (22).

Nevertheless, some of the morphological abnormalities are similar for bipolar and schizophrenic patients, such as a decrease in the density of oligodendroglia in layer VI in Brodmann area 9 (23).

Major Depressive Disorder (MDD) is characterized by structural and functional brain abnormalities involving the limbic and prefrontal regions. While functional neuroanatomy of verbal working memory also exhibits distinct neural correlates in MDD and generated statistically significant diagnostic accuracy for depression, clinical significance is limited due to its low accuracy. Anyway, increased sensitivity can be generated by combining emotional processing and reward functional imaging tasks (24).

Two studies examined multiple genetic effects on the structure of the brain in patients with MDD. One study examined the impact of the BDNF, COMT and SERT genes on both gray and white matter; another study focused on COMT/MTHFR polymorphisms and putamen.

Kostic et al. found that DDM patients with all three risk polymorphisms exhibited alterations in the fronto-occipital regions (25, 26).

Finally, the molecular markers of mood disorders have been studied extensively in recent years, assumed a key role for the promising results obtained.

Among these, plasma β-amyloid (Aβ) levels were investigated as diagnostic markers of bipolar depression: patients with this diagnosis had lower plasma Aβ42 levels and a higher Aβ40/Aβ42 ratio than the control group; there was a significant negative correlation between plasma Aβ42 levels and disease duration, and a positive correlation between the Aβ40/Aβ42 ratio and depressive relapses (27).

In order to identify and differentiate mood disorders, Frye et al. (28) observed that retinol binding protein 4 (RBP-4), transthyretin (TTR complex, RBP-4/TTR), thyroxine and Vitamin A are present in the cerebrospinal fluid (CSF) and are involved in brain maturation, cognitive ability, the acquisition of concepts and behavior, demonstrating a significant difference between psychiatric patients and healthy individuals.

More controversial results emerged for corticotropin-releasing hormone (CRH): some studies observed a direct correlation between clinical severity of depression and plasma levels of CRH, although a more recent study showed low hippocampal levels of CRH mRNA in patients with major depressive disorder (29).

Piccinni et al. (30) have shown that BDNF plays an important role in neurogenesis and neuronal plasticity: healthy individuals showed BDNF levels significantly higher than patients with major depressive episodes and mixed episodes, suggesting that BDNF is a specific marker of disturbances and that can be used in future clinical and therapeutic approaches for major depression, bipolar depression, and possibly the full spectrum of mood disorders.

The role of inflammation in mood disorders is supported by the increased expression of the mRNA of pro-inflammatory cytokines (such as IL-1α, IL-1β, IL-6, IL-8, IL-10 , IFNγ, MIF and TNFα) compared to healthy controls. Inflammation is also associated with increased oxidative stress, the mRNA expression of genes that code for proteins associated with oxidative stress (e.g., cyclooxygenase-2 (COX -2), myeloperoxidase (MPO), phospholipase A2 (PLA2G2A)) was greater in the peripheral blood of patients with recurrent depressive disorder (31).

2. Neuroinflammation and bipolar disorder

Central nervous system (CNS) inflammation is common to all neurodegenerative conditions, and is often considered to be detrimental to the neurological and psychological functions.

Masgrau et al. (80) explained how the classical inflammatory processes, occurring in the CNS, could cause the initiation of a local response by the immune cells resident in the CNS: increase in the production of proinflammatory chemokines and cytokines, the further recruitment of immune cells in the primary site of the trauma, loss of the blood brain barrier and leukocyte infiltration into the brain parenchyma and, finally, the resolution of inflammation and tissue remodeling.

Several studies in recent years aimed to identifying the neuroinflammatory mechanisms of bipolar disorder (BD), also with the aim of understanding the degree to which these mechanisms could impact on the progression of the disease and on the efficacy of drug treatment. Subsequently, translational studies and more advanced technologies allowed to further understand the immune mechanisms of BD (32).

The presence of a chronic, low-grade immune-inflammatory activation is observed through multiple pathological associations in bipolare patients.

A correlation between autoimmune processes and increased expression of psychiatric disorders is supported by the increased risk of patients with systemic autoimmune diseases of developing BD (33).

Despite such chronic immune dysfunction appears to contribute significantly to developing co-morbidities in bipolar disorder, the mechanisms are unclear; for example it is not yet clear whether BD increases the risk for these conditions or whether a pre-existing inflammatory condition increases the risk of BD. The most recent hypothesis suggests a bidirectional interaction between BD and conditions related to inflammation and that these reinforce each other, moreover specific genetic and environmental factors contribute to increasing the risk (34).

Among the most important co-morbidities, in correlation with BD, cardiovascular diseases have emerged and represent the main cause of death in these patients, followed by metabolic syndrome, type II diabetes mellitus, chronic obstructive pulmonary disease (COPD) and obesity (32).

The mechanisms involved in explaining the immune-inflammatory changes in bipolar disorder are multiple:

1. Activation mediated by DAMPs (Molecular Patterns associated with Damage). Patients with bipolar disorder have higher levels of cell death than controls due to likely poor resilience or ineffectiveness mechanisms against cellular stressors (35). Peripheral blood mononuclear cells (lymphocytes and monocytes) undergo early apoptosis in patients with bD and these cells have lower levels of anti-apoptotic proteins and increased levels of caspase-3 (which deals with "breaking down" the proteins of the cell during apoptosis) (32). During apoptosis, some endogenous molecules, known as DAMPs, are released by dying cells and recognized by the innate immune system through toll-like receptors (TLR) (36). Bipolar disorder has been associated with significantly increased levels of DAMPs at peripheral level, and patients have higher levels of circulating free nuclear DNA, which is known to have great pro-inflammatory potential through binding to TLR9 or other cytosolic sensors (37).
2. Microglia activation. Microglia, produced by central nervous system macrophages, plays a vital role in brain development, homeostasis, neuroplasticity, and inflammation (38). Although their physiological functioning is crucial for the elimination of defective or unused synapses. Excessive activation of these cells (as seen in chronic inflammation), especially in its pro-inflammatory phenotype, may have deleterious consequences for CNS with consequences in cognition and behavior. Specifically, subjects affected by BD shows an imbalance between pro-inflammatory and anti-inflammatory microglia. Researchers speculated that neuronal hyperactivation is initially induced by pro-inflammatory microglia in the amygdala, which is followed by subsequent neuroinflammation extending to the prefrontal cortex (PFC) and related tissues, associated with insufficient anti-inflammatory microglial activation (39), allowed by peripheral cytokines crossing directly the blood brain barrier. Furthermore, pro-inflammatory cytokines and inflammation could interrupt the blood brain barrier and increase its permeability, potentially allowing more peripheral inflammatory mediators to reach brain tissue (40). Once in the brain, these inflammatory mediators, may affect the levels of neurotransmitters, such as serotonin, dopamine, and norepinephrine, and indirectly affect cognition, emotion, and behavior (41).

3. Dysfunction of the hypothalamic-pituitary-adrenal axis. The mechanisms associated with the regulation and function of the hypothalamus-pituitary-adrenal (HPA) axis could have important effects on the immune system and inflammatory response, indeed it has been implicated as one of the culprits of the immune dysfunction observed in patients with bipolar disorder. In particular, BD patients have a peripherally hyporesponsive glucocorticoid receptor (GR), which may be at least partially responsible for a negative feedback loop of the HPA axis (42). In addition, pro-inflammatory cytokines have also been shown to increase HPA activity and thereby increase systemic cortisol levels, contributing to chronic axis activation in the DB. The resulting chronic hypercortisolemia could have deleterious effects on the body that can trigger inflammatory responses, for example by damaging cells and releasing DAMP (43).
4. The intestinal microbiota plays a fundamental role in the proper functioning of the immune system and the intestine contains about 80% of the body's immune cells (44,45). The correlation of the gut microbiome in inflammatory changes in bipolar disorder appears to be supported by several studies suggesting that the composition of the gut microbiota may have direct consequences on the levels of cytokines produced by the gastrointestinal system (46), thus potentially contributing to the peripheral cytokine profile observed in the DB. Additionally, some studies have suggested changes in microbial translocation in DB patients (i.e., gut outflow), as evidenced by increased serum levels of antibodies to fungal pathogens and increased levels of bacterial translocation markers (47). These results indicate greater permeability of the intestinal lumen in the BD (48) and a potential exposure of intestinal microbes to the circulation. Consequently, the inflammatory response resulting from this loss can have effects in the cognitive and behavioral domains (47). However, the direct effects of microbiota alterations in behavior have yet to be explored in both the preclinical and clinical settings.
5. Genetic mechanisms appear to be significantly involved in the risk of developing bipolar disorder and in pathophysiological mechanisms, including immune-inflammatory dysfunction. According to this hypothesis, specific genetic and epigenetic markers, when associated with environmental stimuli, could give rise to the inflammatory phenotype observed in bipolar disorder. Numerous studies have associated the disease with single nucleotide polymorphisms (SNPs) located within genes that code for cytokines, including associations

with specific loci within genes that code for monocyte chemotactic protein 1 (MCP- 1), tumor necrosis factor (TNF-α), interferon gamma (IFN-γ), and interleukins such as IL-6, IL-1 and IL-10 (49, 50). There was also a different mRNA expression of cytokine genes in DB patients, such as the expression of IL-6 and CC Motif Chemokine Ligand 3 (CCL3), both of which were found to be higher in the blood of DB patients. as well as CCL1, CCL22, and IL-10, which were found to be lower in patients' blood than controls (51).

The measurement of peripheral and central inflammation markers is fundamental, both for determining the association between bipolar disorder and immune-inflammatory dysfunction, and for the possibility of introducing new early diagnostic tools as well as new therapeutic targets. Rosenblat et al. (34) hypothesized a simultaneous trend of mood levels, cognitive functions and inflammatory markers in patients with bipolar disorder. According to this scheme, cytokine levels increase chronically and can increase both during depressive episodes and during manic episodes, while the resolution of the episode is also associated with the restoration of some cognitive functions; although, evidence suggests that cognitive functions are still far from a complete remission even in periods of euthymia. Specifically Fernandes et al. in 2015 and 2016 showed an increase in C reactive protein (CRP) more markedly in the acute phase of mania (52, 53), while Klaus Munkholm et al. (54) showed an altered leukocyte component a in patients with bipolar disorder compared to the healthy control group on a sample of 300 blood draws. In the following paragraphs we will focus on the role of these two biomarkers in bipolar disorder.

2.1 CRP: role in bipolar disorder

C-reactive protein (CRP) is an acute phase protein, recognized as a useful biomarker of inflammation (55). Initially recognized as a marker of infection and cardiovascular events, more recent was elucidated an important function of CRP in inflammatory processes and host responses to infection, including the complement pathway, apoptosis, phagocytosis, release of reactive oxygen and the production of cytokines. CRP is mainly synthesized in hepatocytes and by muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes. The main inducer of CRP gene expression is interleukin 6 (IL-6) and interleukin 1 (IL-1) which enhances its effect (56). High levels of CRP have been found in various psychiatric disorders but

particularly in schizophrenia, depressive disorders, bipolar disorder (57). Several studies have been conducted in an attempt to clarify the relationship between CRP concentrations and the various emotional states of bipolar disorder, for example Cunha et al. found a correlation between high levels of CRP and manic states, while more uncertain data are available regarding euthymic and depressive states (58). The changes in CRP levels also remain doubtful following the improvement of manic or depressive symptoms, with studies showing the increase, decrease or absence of changes in CRP during the various phases of the disease (59). The role of CRP in the neuro-progression of the disease is quite debated: Dickerson et al. (60), with a cross-sectional trial on 107 subjects, demonstrated that CRP levels above the 75th percentile positively correlate with a reduction in cognitive capacity in euthymic patients with bipolar disorder; while Fernandes et al. (53), suggested that high CRP levels may increase the risk of developing bipolar disorder, but not represent a relevant cause of neuro-progression. This conclusion appears to be consistent with Balukova SM et al. (61) study, whereby high CRP levels in 84 patients with bipolar disorder were not related to a worsening of prognosis in the 15-month follow-up after recruitment. New studies will be needed to clarify whether changes in CRP concentration have a causal relationship with the onset, development and recurrence of acute phases of bipolar disorder or whether it is a simple epiphenomenon caused by a low-grade inflammatory state related to the risk factors and multiple co-morbidities commonly associated with this disease. CRP peripheral measurement may be useful in revealing the response to treatment. CRP detected in the various phases of bipolar disorder is higher in patients treated with mood stabilizers than in untreated patients, possibly due to a protective role of these drugs on the inflammatory state of patients (52, 53). In the study by van den Ameele et al. (2016) (62) emerges how bipolar patients without psychotropic therapy have disease-related inflammatory cytokine alterations, while patients in euthymia and treated with mood stabilizers, and in particular with lithium, have values similar to healthy controls, suggesting a role in normalization of immune system by lithium. Preclinical studies have also described that valproic acid has shown anti-inflammatory properties with an action both on the systemic and central nervous system level (63). Furthermore, CRP could help in the selection of the correct antidepressant, if used as an inflammatory marker to define the response to the serotonin and norepinephrine re-uptake inhibitors. Two studies conducted respectively on escitalopram and nortriptyline antidepressants and anti-inflammatory action showed that patients with

lower CRP levels have demonstrated a better response to escitalopram, while patients with higher CRP levels showed greater improvement with nortriptyline (64). In conclusion, a study written by Manish K Jha et al. (65) demonstrates that a lower baseline CRP level correlates with a better response to antidepressant treatment.

2.2 Leucocytes: role in bipolar disorder

Measuring white blood cell counts (WBCs) is widely used as indicator of inflammation (66) and can offer several advantages over other markers thanks to very reliable, inexpensive and widely available measurement techniques. Unfortunately, few studies have analyzed blood components as a marker of low-grade inflammation in bipolar disorder (66). WBC is a measure of the overall activity of the immune system, in which particularly high levels (leukocytosis) indicate a robust inflammatory response, and particularly low levels (leukopenia) indicate insufficient immune activity.

In a study conducted by Köhler et al. (67), which investigation was focused on confounding factors that could affect leukocytes and symptom severity (age, sex, BMI, smoking, and diagnosis of diabetes, hypertension, or hyperlipidemia), was found that leukocyte levels were higher or lower in association with greater symptom severity and specific symptom clusters, and differed by gender (more prominent in men than women). Similarly Klaus Munkholm et al. (66) taking into account age, gender, BMI, smoking and alcohol intake as confounding factors, in a study comparing 300 blood samples from healthy patients and patients with bipolar disorder, founding 23% higher white blood cell counts and 30% higher neutrophil counts in patients diagnosed with bipolar disorder (68). Several studies have focused on the Neutrophils/Lymphocytes ratio (NLR), which could represent an inexpensive and easily obtainable clinical biomarker, reflecting the low-grade chronic inflammation of various psychiatric pathologies (69, 71). Specifically, NLR was altered in patients with bipolar disorder than healthy controls (70). Mazza MG et al. (72) in a meta-analysis show that the NLR could be useful to detect the inflammatory activation occuring in mood disorders. Furthermore, a clinical and prognostic role of NLR emerged: higher values were associated with more frequent relapses

and hospitalizations in psychiatric setting (73), and in patients with a positive family history of suicide confirming a positive correlation between NLR and suicidal behavior (74).

3. Aim of the study

The study aims, first of all, to investigate the relationship between the inflammatory process and bipolar disorder by identifying groups of patients with altered plasma concentrations of CRP, total leukocytes and relative leukocyte formula in correspondence of the acute phase, in order to identify disease biomarkers. Secondly, to understand how drug therapy with mood stabilizers, first-line therapy in bipolar disorder, has an impact on the initial values of the CRP biomarker. In order to evaluate the differences between the acute and post-acute phase of the disease, the study evaluated the trend of the biomarkers in question over the time (T0, T1, T2). Furthermore, the study aims to observe the correlations between the trend of the identified biomarkers, both with the psychometric scales administered (CGI-D, MADRS, YMRS, HAM-A) to investigate the correlation with the clinical trend, and with the pharmacological therapy.

4. Materials and method

The observational, retrospective and monocentric study includes 104 patients diagnosed with bipolar disorder belonging to the University Psychiatry of University of Siena in ordinary hospitalization and day hospital setting.

The data relating to the study was collected by analyzing the medical records in the following times:

- the first survey corresponds to the moment of admission (T0) and the sample consists of 104 patients (n = 104);
- the second survey corresponds to day 7 ± 4 from admission (T1) and the sample consists of 104 patients (n = 104);
- the third and last survey coincides with the first day of the possible continuation of the therapeutic project in day hospital (T2) and the sample is made up of 60 patients (n = 60).

Inclusion criteria:

- Minimum age of 18;
- Diagnosis of Bipolar Disorder;
- Voluntary participation, ability to understand and sign informed consent.

Exclusion criteria:

- Primary diagnosis of schizophrenia, schizophreniform disorder, schizoaffective disorder, and delusional disorder;
- Neurodegenerative diseases, intellectual disability, neurological diseases, history of head injury
- Any clinical condition that could interfere with the reliability of the assessment: current infection, recent surgery, trauma, burns, neoplastic processes, joint rheumatism such as rheumatoid arthritis and rheumatic polymyalgia, autoimmune diseases such as SLE, inflammatory bowel disease, disease pelvic inflammatory disease, myocardial infarction, appendicitis, pancreatitis, cholangitis, pyelonephritis, gout and tuberculosis.
- Pharmacological treatment in place with anti-inflammatories or corticosteroids.

The variables considered in our study were extracted from the hospitalization and day hospital records of the participating patients:

- Socio-demographic variables of the subjects: age, sex, body mass index (BMI), smoking habit;
- Psychiatric diagnosis: carried out by psychiatric specialists with high training experience in the field of mood disorders.
- Any psychiatric and medical diagnoses in comorbidity;
- Blood tests including blood count with total leukocytes and leukocyte formula (neutrophils and lymphocytes in percentage) and C reactive protein (PCR);
- Pharmacological therapy before the admission in the hospital and changes implemented within the timeframe provided for by the study;
- Psychometric Scores:
    - **Clinical Global Impression - Severity of illness (CGI-S).** The CGI-S is a self-administration scale that requires the physician to assess the severity of the patient's disease at the time of assessment, and is composed of 7 values from 1 (no disease) to 7 (severe degree of illness).

- **Montgomery-Åsberg Depression Rating Scale (MADRS).** Self-administration scale in which the clinicians assesses the presence and severity of depressive symptoms. Made up of 10 items (each of which can be assigned a score from 0 to 6): manifest sadness and reported sadness, internal tension, reduced sleep, reduced appetite, difficulty concentrating, tiredness, inability to experience feelings, thoughts pessimistic and suicidal ideas. The score from 0 - 6 shows the absence of depressive symptoms, from 7 to 19 the presence of minimal symptoms, from 20 to 34 a moderate symptomatology and a score greater than 34 reveals the presence of severe depressive symptoms (75).
- **Young Mania Rating Scale (YMRS).** Scale for the assessment of (hypo)-maniacal symptoms in self-administration. Composed of 11 items, 4 of which with a score ranging from 0 - 8 that investigate the areas of irritability, language, thought content and disruptive/aggressive behavior; the remaining 7 questions evaluate the other areas of the manic field and have a score from 0 to 4 (76).
- **Hamilton Anxiety Rating Scale (HAM-A).** Scale for the assessment of the severity of anxious symptoms in self-administration. Made up of 14 items, each of which can be assigned a score ranging from 0 (symptom not present) to 4 (severe symptom): anxiety, tension, fears, insomnia, difficulty concentrating, depressed mood, muscle somatization (pain, stiffness, myoclonia, etc.), sensory somatization (tinnitus, blurred vision, hot or cold sensation, etc.), cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, genitourinary symptoms, autonomic symptoms, behavior during the interview (77).

The general descriptive characteristics are expressed in terms of absolute number and percentage for the qualitative variables, mean and standard deviation for the quantitative variables.

For the inferential analysis of the data were used the Pearson correlation coefficient, the univariate ANOVA analysis of variance and the repeated measures ANOVA analysis of variance. SPSS software (ver. 20.0) was used for all analyzes.

5. Results

The sample analyzed was made up of 104 patients of which 68 were women with a mean age of 48.52 years (DS ± 18.43). The average body mass index (BMI), assessed on physical examination during the patient's hospital admission, was equal to 24,78 (DS ± 4.93). 43% of patients are smokers while 4% are ex smokers. Half of the sample have bipolar I disorder, 46% have bipolar II disorder and 4% have other types of bipolar disorder. By evaluating psychiatric co-morbidities, we found that 15% of patients have obsessive compulsive disorder (OCD); 1% a panic disorder; 15% have an eating disorder; 14% have borderline personality disorder while; 7% a narcissistic personality disorder. In addition, 8% of patients abuse alcohol, 2% of drugs, 2% of both. Medical co-morbidities are present in 22% of patients: 6% suffer from diabetes mellitus; 4% hypothyroidism; 3% of osteoporosis; 2% of atopic asthma while 1% of patients report polycystic ovary syndrome, psoriasis, Mediterranean anemia, migraine, Parkinson's and prostatic hypertrophy. At T2 of the 104 patients, 60 subjects in day hospital were evaluated and among these, 56 patients had carried out the control of total leukocytes with white blood cell formula and 39 had carried out the control of CRP.

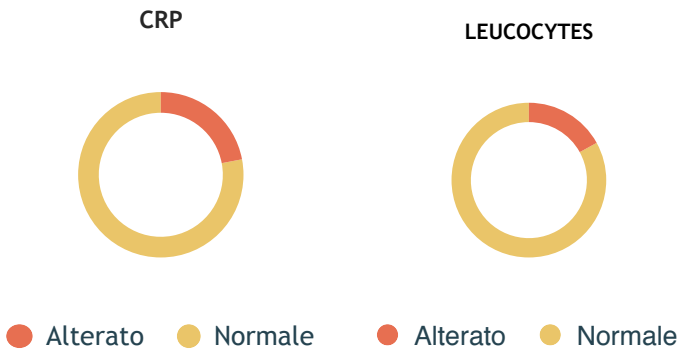
5.1 Descriptive analysis of labs data

From the medical records of ordinary hospitalization were extracted the blood tests of patients at the moment of admission (T0), including blood count and in particular total leukocytes, leukocyte formula (neutrophils and lymphocytes in percentage) and CRP. The descriptive analysis obtained can be seen in the table below (Table 1).

	CRP MG/DL	LEUCOCYTES ∗10³/MMC	NEUTROPHILS %	LIMPHOCYTES %
AVARAGE VALUE	0,31	7,38	57,6	31,17
S.D.	±0,42	±2,5	±12,24	±11,3
MIN	0,03	3,22	20,7	7,7
MAX	2,38	17,32	90	70,3

Table 1. Descriptive analysis of data at beginning of hospitalization (T0).

We found significantly higher levels of inflammatory biomarkers at T0, compared to T1. T2 was performed on a smaller sample both for CRP (*n=40*) and leukocytes (*n=57*). Specifically, at T0 we found that 21,5% of subjects had CRP values greater than 0,5 mg/dl, and 11,5 % of subjects had leukocyte alteration (Graph 1). At T1: we found that 14,4 % of subjects had CRP values greater than 0,5 mg/dl, and 13,46% of subjects had leukocyte alteration. Whereas T2 sample were different than T0 and T1 samples, we only investigated the follow-up of patients with previous alteration of CRP or leukocytes and we found a rate of regression of 72,7% for CRP and 75% for leukocytes comparing with T0.



Graph 1. Patient groups with altered plasma concentrations of CRP, total leukocytes and related leukocyte formula at T0

5.2 Descriptive analysis of pharmacotherapy

Psychiatric pharmacotherapy was evaluated in the sample (n=104), taking into account the drugs at the moment of recruitment (T0) and the therapeutic changes made on day 7 ± 4 after admission (T1) and on the first day of possible continuation of the therapeutic project (n = 60) in day hospital setting (T2). The following table describes the therapy divided by single drug associated with the respective frequency within the sample. (Table 2)

		T0 (N=104)	T1 (N=104)	T2 (N=60)
MOOD STABILZIERS (ANTIEPILETICS)	Valproic acid	40	60	39
	Lithium	31	52	32
	Carbamazepine	8	6	3
ANTIDEPRESSANTS	Paroxetine	16	14	7
	Amitriptyline	3	2	2
	Mirtazapine	6	9	5
	Trazodone	11	24	14
	Citalopram	11	17	12
	Venlafaxine	7	8	4
	Sertraline	5	6	4
	Buproprion	1	-	-
	Fluoxetine	6	2	1
	Duloxetine	5	7	3
	Fluvoxamine	1	3	2
	Reboxetine	1	-	-
	Mianserin	1	-	-
	Ketamine	-	5	4
	Clomipramine	4	4	3
	Escitalopram	5	4	1
	Vortioxetine	6	4	1
ANTIPSYCHOTICS (NEUROLEPTICS)	Olanzapine	10	12	6
	Clozapine	-	3	2
	Quetiapine	24	31	12
	Aripiprazol	14	28	17

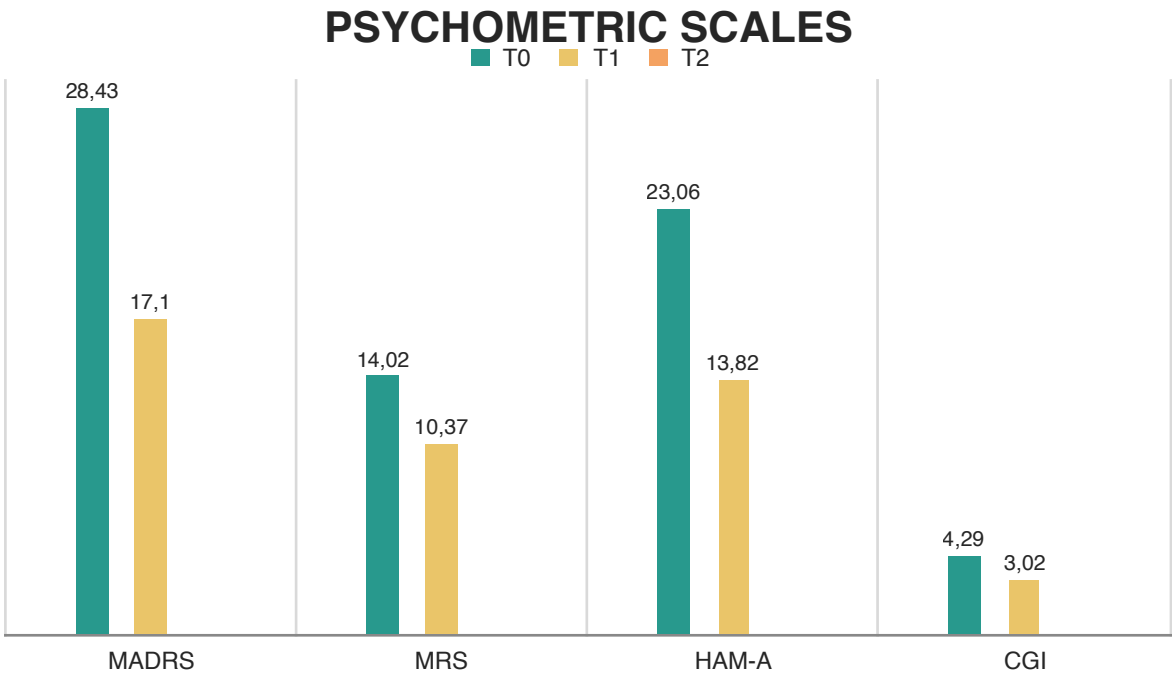
	Asenapine	2	3	3
	Clotiapine	4	6	5
	Paliperidon	2	2	1
	Lurasidon	4	2	1
	Risperidon	2	5	3
	Amisulpiride	2	1	1
	Haloperidol	2	2	1
	Perfenazine	1	2	2
	Promazine	2	4	1
	Tiapride	1	-	-
	Cariprazine	1	-	-
BENZODIAZEPINES	Lorazepam	25	47	30
	Clonazepam	11	6	2
	Alprazolam	7	2	1
	Triazolam	1	-	-
	Delorazepam	2	-	1
OTHER PSYCHOTROPIC DRUGS	Gabapentin	20	27	13
	Pregabalin	12	18	15
	Lamotrigine	9	6	4
	Sodium Oxybate	3	-	-
	Disulfiram	2	1	-
	Topiramate	4	1	1
	Idroxizine	-	1	-
	Levomepromazine	1	1	3

Table 2. Pharmacological therapy taken by the patients in the study (N) in the three times foreseen by the study (T0, T1, T2).

5.3 Descriptive analysis of psychometric scales

The patients in our sample were evaluated by psychometric scales within the time required by the study, in particular at T0 and T1 the Montgomery Asberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS), Hamilton Anxiety Rating Scale (HAM-) were

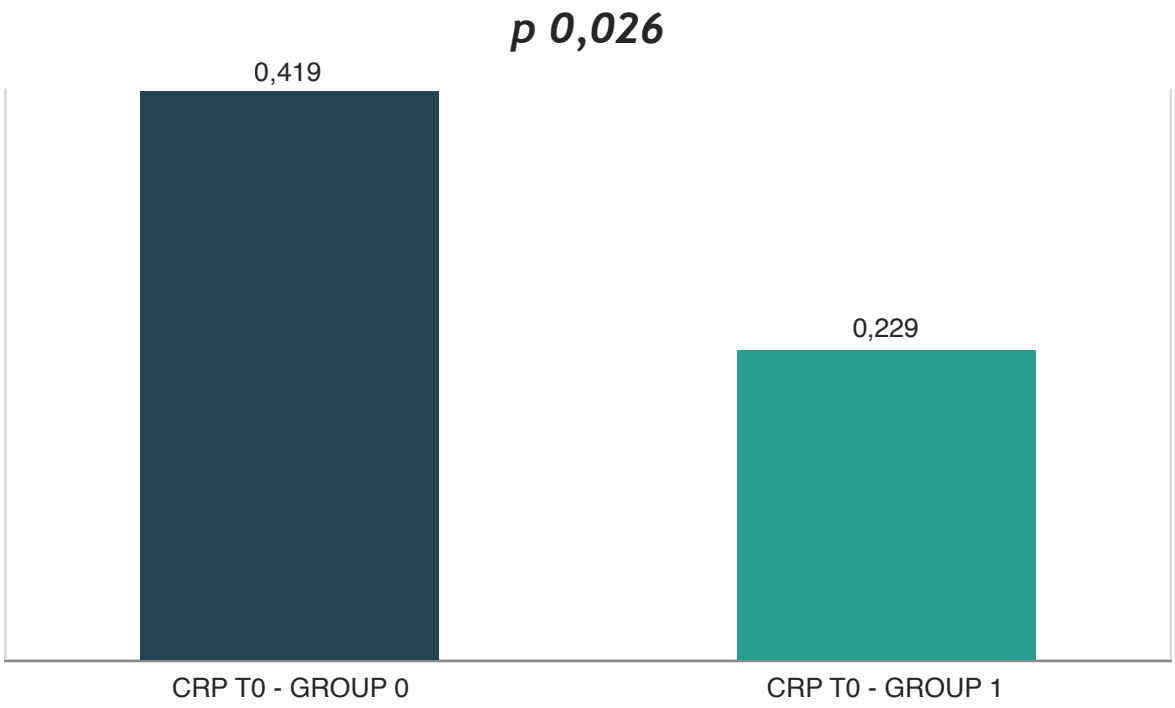
used. A) and Clinical Global Impression - Severity of Illness (CGI-S). The 60 patients studied at T2 were given the Clinical Global Impression - Severity of Illness (CGI-S)(Graph2).



Graph 2. Average value of the total obtained on the psychometric scales in the three times foreseen by the study (T0, T1, T2)

5.4 Comparison between groups: CRP and drug therapy

Having detected, as the initial hypothesis, a group of patients who presented an alteration of blood chemistry tests, we investigated a possible correlation between blood chemistry values and the variable "drug therapy", in particular focusing on the use of mood stabilizers (valproic acid, lithium and carbamazepine). The whole sample have been divided into two groups: Group 0 (n=43) patients not previously treated with mood stabilizers and Group 1 (n=61) patients already treated with mood stabilizers at the admission (T0). Through the univariate ANOVA analysis, a statistically significant difference (*p=0.026*) emerged between the means of the CRP values of the two groups. Graph shows that he patients in Group 1 had mean CRP values of 0.23 mg/dl (DS± 0.32) reduced compared to Group 0, which had mean CRP values of 0.42 mg/dl (DS ± 0.52).



Graph 3. Correlation between the mean CRP values (mg/dl) and the variable "drug therapy" in the two Groups at T0 (*p=0.026*)

5.5 CRP Biomarker

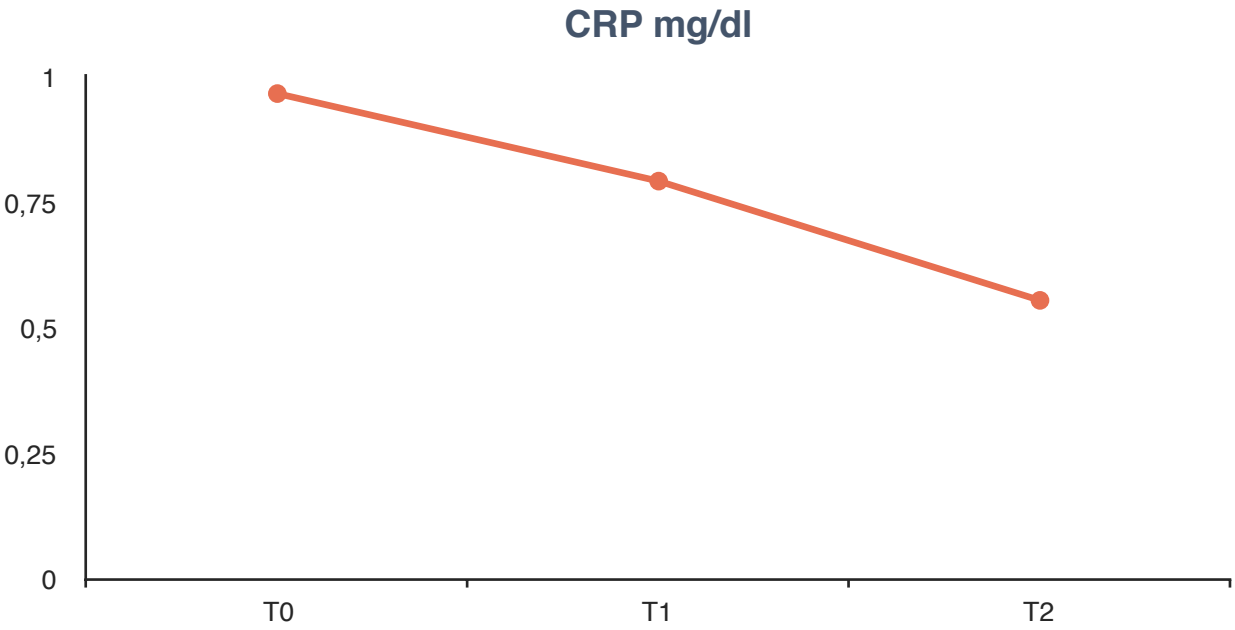
We decided to evaluate the performance of the CRP of the whole sample over time from admission (T0), 7 ± 4 days from admission (T1) and to the first possible control in day hospital (T2). We also observed the impact of therapy with mood stabilizers between the acute and post-acute phase of the disease on CRP.

A statistically significant difference (p=0.029) between CRP and time factor (T0, T1, T2), however, the interaction between these and the variable "drug therapy" does not seem statistically significant (Table 3).

CRP		SUM OF TYPE III SQUARES	DF	AVERAGE OF SQUARES	F	Sig.
TIME	Sphericity	0,609	2	0,305	4,030	0,022
	Greenhouse-Geisser	0,609	1,681	0,362	4,030	0,029
	Huynh-Feldt	0,609	1,800	0,338	4,030	0,026
	Lower limit	0,609	1,000	0,609	4,030	0,052
THERAPY WITH MOOD STABILIZERS	Sphericity	0,277	2	0,139	1,835	0,167
	Greenhouse-Geisser	0,277	1,681	0,165	1,835	0,174
	Huynh-Feldt	0,277	1,800	0,154	1,835	0,171
	Lower limit	0,277	1,000	0,277	1,835	0,184

Table 3. ANOVA analysis results in repeated measures, by CRP in relation to the time factor and therapy with mood stabilizers, on the whole sample

We divided the sample into two groups based on initial normal or altered CRP value at the admission (T0): 82 patients made up the CRP-***α*** group and had normal biomarker values (<0.5 mg/dl), 22 patients made up the CRP-***β*** group and had altered biomarker values (≥ 0.5 mg / dl). The CRP-***β*** group had mean values of CRP at T0 of 0.97 mg/dl (DS ± 0.48), at T1 of 0.79 mg/dl (DS ± 0.31) and at T2, where n=12, of 0.56 mg/dl (DS ± 0.44). (Graph 4)



Graph 4. Mean C-reactive protein value in the CRP-***β*** group (≥0.5 mg/dl) over the three study periods (T0, T1, T2)

Through the repeated measures ANOVA analysis, which evaluates the differences in the CRP values in relation to the elapsed time, was possible to find a statistically significant difference (*p=0.036*). We also applied the ANOVA analysis to repeated measures, correlating the CRP values to the time factor and to therapy with mood stabilizers, but no statistically significant difference emerged (Table 4).

CRP-B		**SUM OF TYPE III SQUARES**	**DF**	**AVERAGE OF SQUARES**	**F**	**Sig.**
TIME	Sphericity	1,649	2	0,824	5,019	0,017
	Greenhouse-Geisser	1,649	1,285	1,283	5,019	0,036
	Huynh-Feldt	1,649	1,539	1,071	5,019	0,028
	Lower limit	1,649	1,000	1,649	5,019	0,049
THERAPY WITH MOOD STABILIZERS	Sphericity	0,296	2	0,148	0,902	0,422
	Greenhouse-Geisser	0,296	1,285	0,231	0,902	0,386
	Huynh-Feldt	0,296	1,539	0,193	0,902	0,401
	Lower limit	0,296	1,000	0,164	0,902	0,365

Table 4. ANOVA analysis results in repeated measures, for CRP in relation to the time factor and therapy with mood stabilizers, on the CRP-β group

Analysis of the difference in Clinical Global Impression - Severity of Illness (CGI-S) in the time predicted by the study, in the group of patients with impaired CRP (CRP-***β***), demonstrated a statistically significant (*p 0.000*) (Table 5).

CGI-S		SUM OF TYPE III SQUARES	DF	AVERAGE OF SQUARES	F	Sig.
TIME	Assumendo la sfericità	32,333	2	16,167	54,826	0,000
	Greenhouse-Geisser	32,333	1,246	25,942	54,826	0,000
	Huynh-Feldt	32,333	1,314	24,499	54,826	0,000
	Limite inferiore	32,333	1,000	32,333	54,826	0,000

Table 5. ANOVA repeated measures results, for CGI-S in relation to the time factor on the CRP-β group

5.6 Leucocytes biomarkers

The trend of total leukocytes of the whole sample over time from admission (T0), 7 ± 4 days from admission (T1) and to the first possible checkup in day hospital (T2) were analyzed. We also observed the impact of therapy with mood stabilizers between the acute and post-acute phase of the disease on leukocyte values. With the repeated measures ANOVA analysis, was evaluated the differences in the values in relation to the elapsed time and the therapeutic strategy with mood stabilizing drugs: there was no statistically significant correlation regarding the time factor (T0, T1, T2), nor between total leukocytes, time factor and therapy with mood stabilizers (Table 6).

LEUCOCYTES		SUM OF TYPE III SQUARES	DF	AVERAGE OF SQUARES	F	Sig.
TIME	Assumendo la sfericità	0,570	2	0,285	0,259	0,773
	Greenhouse-Geisser	0,570	1,994	0,286	0,259	0,772
	Huynh-Feldt	0,570	2,000	0,285	0,259	0,773
	Limite inferiore	0,570	1,000	0,285	0,259	0,613
THERAPY WITH MOOD STABILIZERS	Assumendo la sfericità	1,459	2	0,570	0,662	0,518
	Greenhouse-Geisser	1,459	1,994	0,730	0,662	0,517
	Huynh-Feldt	1,459	2,000	0,732	0,662	0,518
	Limite inferiore	1,459	1,000	0,730	0,662	0,419

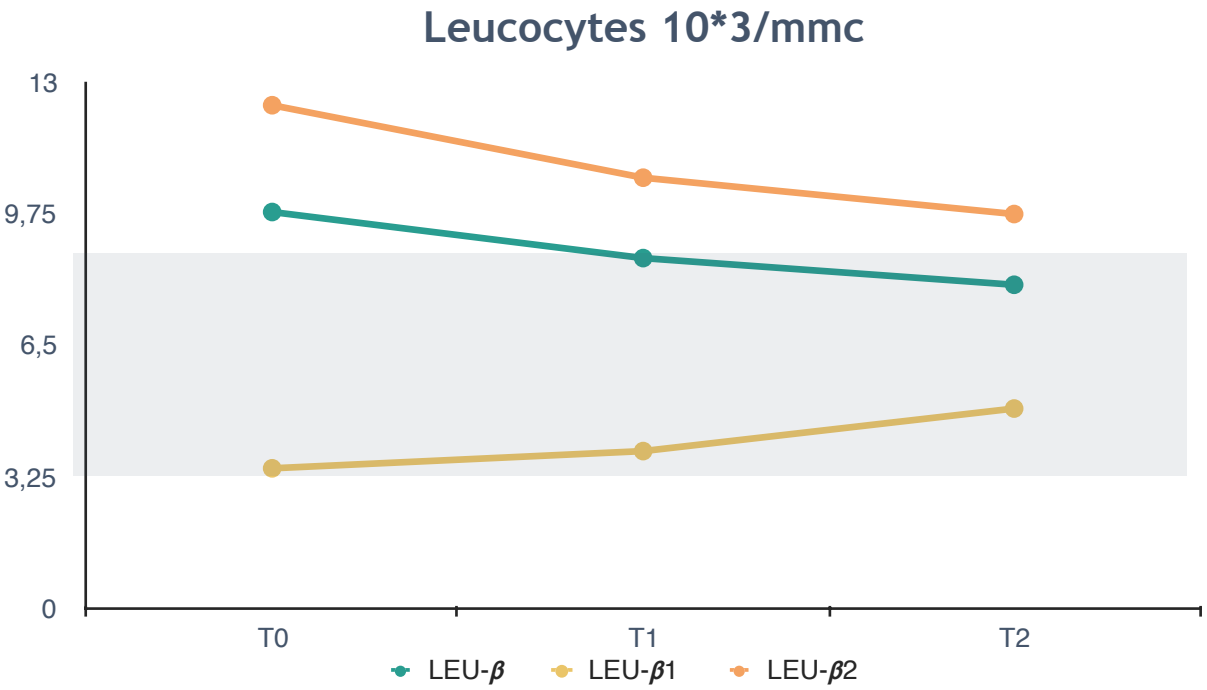
Table 6. ANOVA analysis results in repeated measures, for total leukocytes in relation to the time factor and therapy with mood stabilizers, on the whole sample

The sample was divided into two groups based on normal or altered value of leukocytes at the admission (T0): 84 patients constituted the LEU-***α*** group and had normal values of the bio-marker (4-10 10*3/mmc), 20 patients constituted the LEU-***β*** group and had altered biomarker values (> or <4-10 10*3/mmc). A further distinction in the case of total leukocytes, was made between those who had reduced or increased values compared to the normal range: LEU- ***β***1 subgroups with leukocytes <4 10 * 3/mmc (n=6) and LEU- ***β***2 with leukocytes>10 10*3/mmc (n=12).

The LEU-***β*** group had mean leukocyte values at T0 of 9.79 10 * 3/mmc (DS ± 4.56), at T1 of 8.65 mg/dl 10 * 3 /mmc (DS ± 3.82) and at T2, where n = 11, of 7.99 mg / dl 10*3/mmc (DS ± 2.89).

The LEU-***β***1 subgroup reported mean leukocyte values of respectively: 3.45 10 * 3/mmc (SD ± 0.17) at T0; 3.87 10 * 3/mmc (DS ± 0.46) at T1; 4.93 10 * 3/mmc (DS ± 0.91) at T2.

The LEU-***β***2 subgroup reported mean leukocyte values of respectively: 12.43 10 * 3/mmc (SD ± 2.08) at T0; 10.64 10 * 3/mmc (SD ± 2.55) at T1; 9.74 10 * 3/mmc (DS ± 2.04) at T2 (Graph 5).



Graph 5. Mean leukocyte values in the LEU-***β*** (<or> 4-10 10 3/mmc), LEU-***β***1 (<4 10 * 3/ mmc) and LEU-***β***2 (> 4 10 * 3/mmc) group in the three times provided by the study (T0, T1, T2)

Using the repeated measures ANOVA analysis, which evaluates the differences in Leukocyte values in relation to the elapsed time, it is possible to find a statistically significant difference for both subgroups: LEU-***β***1 (*p 0.030*), LEU-***β***2 (*p 0.036*).

LEU-*β*		**SUM OF TYPE III SQUARES**	**DF**	**AVERAGE OF SQUARES**	**F**	**Sig.**
LEU-*β*1 TIME	Sphericity	4,654	2	2,327	8,063	0,039
	Greenhouse-Geisser	4,654	1,268	3,670	8,063	0,080
	Huynh-Feldt	4,654	2,000	2,327	8,063	0,039
	Lower limit	4,654	1,000	4,654	8,063	0,105
LEU-*β*2 TIME	Sphericity	16,844	2	8,422	4,458	0,036
	Greenhouse-Geisser	16,844	1,794	9,392	4,458	0,042

LEU-β2 TIME - MOOD STABILIZERS	Huynh-Feldt	16,844	2,000	8,422	4,458	0,036
	Lower limit	16,844	1,000	16,844	4,458	0,079
	Sphericity	10,925	2	5,463	4,650	0,037
	Greenhouse-Geisser	10,925	1,850	5,904	4,650	0,042
	Huynh-Feldt	10,925	2,000	5,463	4,650	0,037
	Lower limit	10,925	1,000	10,925	4,650	0,084

Table 7. ANOVA analysis results in repeated measures, for LEU-***β***1 and LEU-***β***2 in relation to the time factor and therapy with mood stabilizers

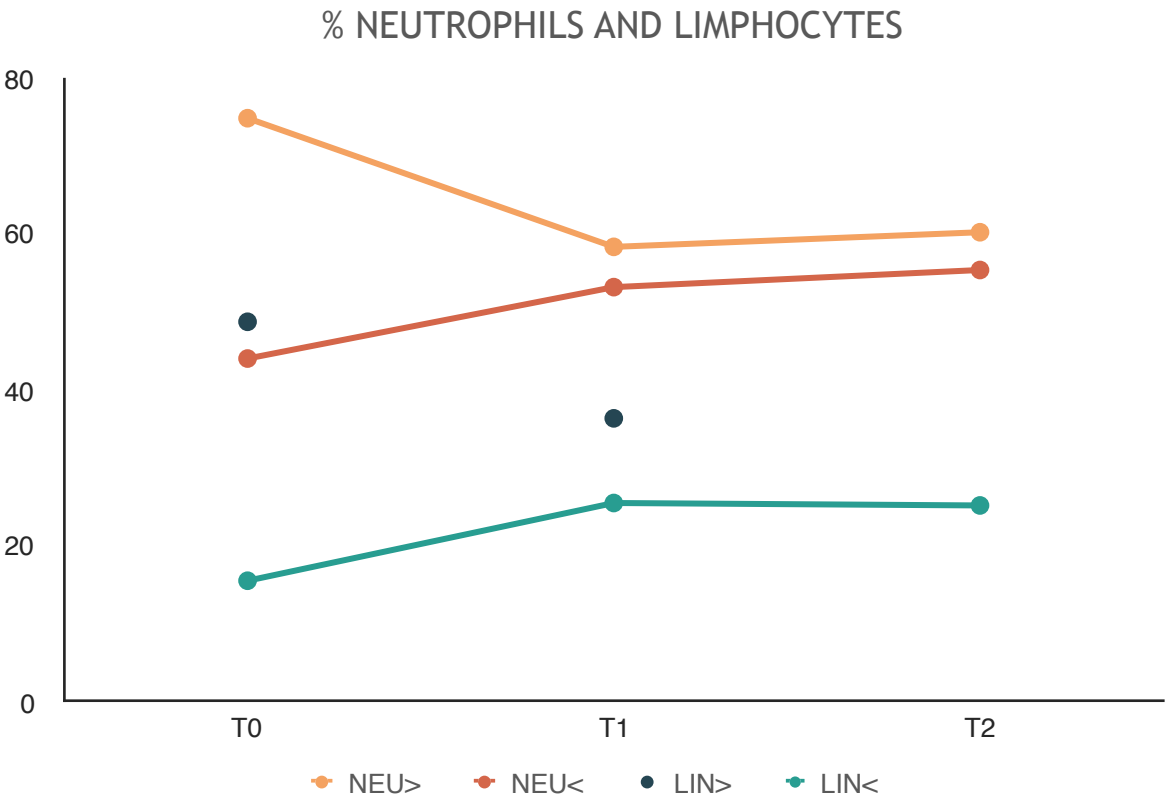
We then analyzed, using repeated measures ANOVA, the difference in Clinical Global Impression - Severity of Illness (CGI-S) in the time predicted by the study, in the group of patients with impaired leukocytes (LEU-***β***), and it was statistically significant (*p 0.000*). (Table 8)

CGI-S		**SUM OF TYPE III SQUARES**	**DF**	**AVERAGE OF SQUARES**	**F**	**Sig.**
TIME	Sphericity	13,818	2	6,909	15,616	0,000
	Greenhouse-Geisser	13,818	1,462	9,454	15,616	0,001
	Huynh-Feldt	13,818	1,649	8,381	15,616	0,000
	Lower limit	13,818	1,000	13,818	15,616	0,003

Table 8. ANOVA analysis results at repeated measures, for CGI-S in relation to the time factor on the LEU-β group

Focusing on the group of patients with altered leukocytes, was explored the leukocyte formula and in particular the possible alterations in the percentage of neutrophils and lymphocytes. 76.47% of patients with altered leukocytes had an altered percentage of neutrophils (normal range 55-70%): 9 patients had an increase in the percentage, with a mean of 74.94% (DS ± 14.50) at T0, 58.42% (DS ± 8.26) at T1 and 60.3% (DS ± 8.98) at T2; 5 patients had a percentage reduction with a mean of 44.08% (DS ± 6.51) at T0 53.25% (DS ± 7.65) at T1 and

55.45% (DS ± 3.18) at T2. Seventy (59%) of patients with altered leukocytes had an altered percentage of lymphocytes (25-48%): except one patient who had an increased percentage of lymphocytes (48.8% at T0, which normalized at T1 with value of 36.4%), 11 patients showed a reduction in the percentage of lymphocytes with a mean of 15.55 (DS ± 5.56) at T0, 25.53 (DS ± 9.29) at T1 and 25.21 (DS ± 7.85) at T2. (Graph 6)



Graph 6. Average values of the percentage of Neutrophils and Lymphocytes in the group with altered total leukocytes in the three times foreseen by the study (T0, T1, T2)

6. Discussion

The main hypothesis underlying our study proposed the existence of a group of patients with bipolar disorder who had an increased CRP levels, an alteration in the white blood cell count and the relative number of neutrophils and lymphocytes during the acute phase of the disease. Therefore, we analyzed inflammatory biomarkers at the admission (T0), in which patients were in the acute phase of the disease, 7 ± 4 days from the start of hospitalization (T1), at which time a reduction in acute symptomatology, and on the first day of the possible continuation of the therapeutic project in the day hospital (T2), which we can define as the post-acute phase.

Our study allowed us to detect elevated serum CRP values (>0.5 mg/dl) in 22% of patients; leukocytes outside the normal range (4-10 10 * 3/mmc) in 17% of patients; and among the patients with leukocyte alteration, 76.47% had an altered percentage of neutrophils and 70.59% of lymphocytes, upon admission, or in full acute phase.

These results are in according with the initial hypothesis of the existence of an inflammatory state in a group of patients suffering by bipolar disorder, as previously described by Ângelo B. Cunha et al. (58): in a study of 80 patients diagnosed with bipolar disorder emerged evidences of significant increase in serum protein C, particularly evident in patients in the acute phase of mania compared to the depressive and/or euthymic phase (78) and the same evidence was later confirmed by Fernandes et al. in 2016 (53).

The finding of a group of patients with serum values of leukocytes and neutrophils slightly out of the norm is in agreement with the studies conducted by Mayda et al. (79), Ivkovic et al. (74), Cakir et al. (71) and Munkholm (54) who find an increase in the leukocyte and neutrophil counts in bipolar patients, without show a specific correlation with the disease phase or the severity of symptoms.

Analyzing the variables that could impact on the values of the haematochemical parameters, we observed the influence of first-line drug therapy in bipolar disorder with mood stabilizers (valproic acid, lithium, carbamazepine) on protein levels CRP on admission (T0).

We then measured and correlated the average biomarker values in patients who were already using mood stabilizers and in those not yet treated to this drug therapy. This research led us to a relevant result of our study: set as variable the average value of CRP at admission with respect to the presence or absence of therapy with mood stabilizers, was possible to detect lower serum CRP levels (CRP = 0.22 mg / dl; DS ± 0.32) in patients already receiving one or more mood stabilizers than in the control group. This evidence highlights a protective role of pharmacotherapy with mood stabilizers on the inflammatory state, in accordance with the results obtained by Fernandes et al. (53) who, by the systematic review and the metanalytical studies, have shown that CRP levels tend to be higher in patients suffering from a manic or depressive episode not on stabilizer therapy, compared to treated patients.

In agreement with our study, also in the review by van den Ameele et al. (62), emerges that patients with BD without drug therapy have disease-related inflammatory cytokine alterations, while patients in euthymia and on drug therapy with mood stabilizers, and in particular lithium, have values similar to healthy controls; these results suggest a normalizing role of the immune system by lithium which, as in our study, seems to emerge after prolonged use of the drug. In addition, it is interesting to note that valproate has also shown anti-inflammatory properties in preclinical models, modulating both systemic and central nervous system responses (63).

The trend of the blood parameters object of the study over time (T0, T1, T2) and the trend of the same in relation to the therapy with mood stabilizing drugs adopted during the recovery was evaluated. It was not possible to detect, by repeated measures ANOVA analysis, a significant difference in the mean values of CRP and leukocytes in correlation with the effects of therapy with stabilizers, nor of total leukocytes with the time variable, however a significant difference emerged between the trend of the CRP value and the follow-up times. To focus the analysis on patients who had altered blood parameter values at the admission (T0), we divided the sample into groups based on the presence or absence of biomarker alterations.

This subdivision allowed us to highlight a trend in reduction of the average levels of the inflammatory marker CRP, in those patients who had shown an alteration of CRP (CRP-β

group≥0.5 mg/dl), and it is interesting to note that this difference was shown to be statistically significant (*p 0.036*). Similarly, we highlighted a normalization of the total leukocytes during the different time of study, obtaining a significant difference both for the group that presented leukocytes below the normal range (*p 0.039*) and for the group with values above the limit (*p 0.036*). For the latter subgroup, there was also a significant impact of therapy with mood stabilizers (*p 0.037*).

Moreover, emerges that among patients with leukocytes alteration it was possible to highlight a corresponding alteration in the percentage of neutrophils and lymphocytes, of 76.47% and 70% respectively, which tends to normalize moving away from the acute phase of the disease. Finally, by comparing the total values obtained at the CGI-S in the same period of time under examination, in the two groups with haematochemical alterations (CRP-β and LEU-β) a significant difference (*p 0.001*) can be noted, indicating a clear improvement in state of health of patients and a regression of symptoms. Therefore, in conclusion, it can be stated that although the data on the entire sample do not show a unique statistical significance for all the biomarkers studied, the identification of groups in which inflammation plays a key role in bipolar disorder shows promising results. Furthermore, it would seem that the use of mood stabilizers brings a protective effect on the inflammatory state of patients with bipolar disorder following prolonged therapy and that this role cannot be highlighted in the acute and post-acute phase. Therefore it would be useful to deepen this aspect, extending the follow up time of the patients under examination.

7. Conclusions

Several studies in recent years investigate the role of neuroinflammation in the pathogenesis of psychiatric disorders. Bipolar disorder has a significant impact on quality of life of patients for its influence on mood and cognitive status, but also for its frequent association with clinical co-morbidities such as diabetes, endocrine, autoimmune and disease cardiovascular. This frequent association also reinforces the thesis that bipolar disorder is a systemic disease with an inflammatory and immune component, explaining at least in part the risk factors common to bipolar disorder and its co-morbidities.

This study emphasizes the existence of sub-groups of patients with bipolar disorder who have high baseline values of serum CRP and an altered leukocyte, lymphocyte and neutrophil count in relationship with an acute manic or depressive episode. Our results suggest a trend of normalization of the biomarkers studied between the acute and post-acute phase of the disease with a substantial attenuation of the inflammatory state. This improvement coincides with the reduction in symptoms, described by the decrease in the total mean scores of the psychometric scales. Finally, the study found a positive impact of drug therapy with mood stabilizers (Lithium, Valproic Acid and Carbamazepine) on serum CRP levels in patients already being treated with these drugs.

Limitations of our study are: firts of all, the shortness of the duration of patient follow-up, which does not allow us to observe the trend of biomarkers during the euthymia period; secondly, it should be emphasized that the basal serum C-reactive protein concentration shows a great inter-individual variability. Therefore, for the purpose of a more in-depth analysis, a control analysis could be useful that compares the values of the basal biomarkers measured during the acute phase of the disease with the values of the basal ones in the euthymic and well-being phases of the patients under study. Furthermore, the psychometric scales, while representing very useful and reliable tools in clinical practice, are subject to operator dependent variability. Finally, the possibility of a confounding effect on the analysis of serum C reactive protein values caused by minor co-morbidities, the use of non-psychiatric drugs or the undeclared substance abuse must not be excluded.

This study is part of a field of scientific research in continuous evolution and in the process of exploration, and in which it is possible to find partial results and conflicting evidence. Through our results, was possible to the evidence supporting a central role of the neuroinflammato-

ry system in mood disorders and in particular in bipolar disorder, and about the possibility of using inflammatory biomarkers as predictors of disease severity.

In conclusion, the trend of the inflammatory indices could on the one hand express the causal relationship between the inflammatory state and the development of the disease or the recurrence of a mood; on the other hand, it could reflect the existence of a low-grade inflammatory epiphenomenon related to this condition, its risk factors or its co-morbidities.

We therefore propose to focus our attention on further inflammatory markers that may have an important role in bipolar disorder; prolong the follow-up period of markers until complete regression or prolonged stabilization of symptoms, in order to obtain a more accurate assessment of both the correlation between the patient's inflammatory state and symptoms, and of the possible protective influence on the inflammatory state of the pharmacotherapy. This could open the way for the improvement of diagnosis, clinical management and new therapeutic strategies for the patient with bipolar disorder, offering a personalized therapeutic strategy.

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