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Immunological and neuroimaging correlates of bipolar disorder

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Sommario

ABSTRACT

Bipolar disorder (BD) is a chronic and recurring psychiatric condition. The neuroimmunology of BD is recently gaining attention but only few articles directly studied the state of T cell lines in these patients; furthermore, no study has investigated the potential correlations between gray matter cortical thickness (CT) and alterations of T-cells immunophenotype.

Methods: We enrolled 43 BD patients (24 in depressed, 19 in manic phase) and 24 healthy controls (HC). For each participant we collected blood samples and performed anatomical T1-weighted MRI with a 1.5-T GE scanner.

We investigated: (1) plasma levels of cytokines and c-reactive protein (CRP), (2) circulating T-cell subpopulations frequencies through immunofluorescence analysis by flow cytometry, (3) CT and (4) the correlation between CT and immunological data.

Results: We found (1) an increase of IL-6 in manic patients and CPR in all BD subjects; a significant (2) increase in CD4 T-cells in BD as well as a significant decrease in some T CD8 subpopulation ("effector memory", "terminal effector memory" and CD8 IFNγ+) in mania. Concerning MRI analysis, we found (3) CT diffusely reduced in BD compared to HC. Finally, in BD patients we found (4) a negative correlation between peripheral levels of T CD4 "central memory" and CT in the left Fusiform Gyrus (lFG) and the right Precuneus (rPC).

Conclusions: We replicated literature findings of a pro-inflammatory state (increased IL-6 and CRP) and a diffusely reduced CT in BD. Moreover, we found

specific alterations in T-cell subpopulations, and an association between increased T CD4 “central memory” and reduced CT in the lFG and rPC. We speculate that in BD increased pro-inflammatory cytokines and T CD4 cells (especially CD4 “central memory”) could support the cerebral infiltration by activated T CD8. This could lead to a neuroinflammatory condition possibly linked with the CT reduction.

INTRODUCTION

BIPOLAR DISORDER

Bipolar Disorder (BD) is a psychiatric condition characterized by pathological fluctuations in mood, psychomotricity and thought. [1]

History

From an historical perspective we can find the roots of the bipolarity concept back to the ancient Greeks and specifically in Hippocrates (460-377 BC) works. [2]

He described the two pathological extremes of the human mood: Melancholia (melas=black and chole= bile) and mania, respectively resulting from an excess of black and yellow bile. [3] Indeed, according to the theories of pre-Hippocratic Greek physicians the pathophysiology of mental disorders was explained by the interaction between body liquids and the brain. [2]

Later, in the second century AD, Aretaeus of Cappadocia[4] was the first to explicitly consider mania and melancholia to have the same aetiology and to be different states of a single pathological condition. [2]

However, to see the modern concept of "bipolar disorder" recognised as an independent entity we have to wait until the fifties of the XIX century when the French psychiatry Jean-Pierre Falret, based on longitudinal observations, coined

the term “folie circulaire” (circular madness). [5] In the same years Jules Baillarger in one of his publication[6] defined it as “folie à double forme” (double shape madness).

In the late ‘800 Emil Kraepelin unified all mood disorders, single and recurrent manic or depressed episodes, under the new category of “manic–depressive insanity”; despite its heterogeneity, this group was contrasted to the other big Kraepelin’s category of “dementia praecox” that encompassed all chronic psychotic disorders. [7] However, during the same period other authors kept on maintaining the distinction between depression, mania and manic-depressive disorder. [8] [9]

In 1952 the American psychiatric association, with the first edition of the Diagnostic and Statistics Manual of mental disorders (DSM-I)[10], officially broke Kraepelin’s singular category in three different groups of mood alteration disorders: depression, mania and other. In DSM-II[11] the term “other” was changed into “circular” which was defined as “at least one attack of both a depressive episode and a manic episode”. Finally in the third DSM [12] edition, for the first time BD was introduced with this name and separated from depressive syndromes.

Diagnosis

In the Diagnostic and Statistics Manual of mental disorders (DSM-5) [13] Bipolar and related disorders are presented after the chapter of schizophrenia and other

psychotic disorders and before the one of depression as a bridge between the two classes.

This DSM-5 chapter includes different diagnostic subgroups: BD type I, BD type II, cyclothymic disorder, substance/medication-induced bipolar and related disorder, bipolar and related disorder due to another medical condition, other specified bipolar and related disorder, and unspecified bipolar and related disorder.

BD type I is characterized at least by one manic episode (Table A) not better explained by other schizophrenic/psychotic spectrum disorders.

BD type II is characterized at least by one hypomanic episode (Table B) and one major depressive episode (Table C).

Cyclothymic disorder is characterized by at least two years (one year in children and adolescents) of numerous periods with hypomanic symptoms that do not meet criteria for a hypomanic episode and numerous periods with depressive symptoms that do not meet criteria for a major depressive episode. During these two years the symptoms should be present for at least half the time and the individual shouldn’t have been without the symptoms for more than 2 months at a time.

For all these three categories, symptoms shouldn’t be better explained by other schizophrenic/psychotic spectrum disorders and should cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Table A
Manic Episode

A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased goal-directed activity or energy, lasting at least 1 week and present most of the day, nearly every day (or any duration if hospitalization is necessary).

B. During the period of mood disturbance and increased energy or activity, three (or more) of the following symptoms (four if the mood is only irritable) are present to a significant degree and represent a noticeable change from usual behavior:

1. Inflated self-esteem or grandiosity.
2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep).
3. More talkative than usual or pressure to keep talking.
4. Flight of ideas or subjective experience that thoughts are racing.
5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), as reported or observed.
6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation (i.e., purposeless non-goal-directed activity).
7. Excessive involvement in activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments).

C. The mood disturbance is sufficiently severe to cause marked impairment in social or occupational functioning or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.

D. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or to another medical condition.

Note: A full manic episode that emerges during antidepressant treatment (e.g., medication, electroconvulsive therapy) but persists at a fully syndromal level beyond the physiological effect of that treatment is sufficient evidence for a manic episode and, therefore, a bipolar I diagnosis.

Note: Criteria A–D constitute a manic episode. At least one lifetime manic episode is required for the diagnosis of bipolar I disorder.

Table B
Hypomanic Episode

A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy, lasting at least 4 consecutive days and present most of the day, nearly every day.

B. During the period of mood disturbance and increased energy and activity, three (or more) of the following symptoms (four if the mood is only irritable) have persisted, represent a noticeable change from usual behavior, and have been present to a significant degree:

1. Inflated self-esteem or grandiosity.
2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep).
3. More talkative than usual or pressure to keep talking.
4. Flight of ideas or subjective experience that thoughts are racing.
5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), as reported or observed.
6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation.
7. Excessive involvement in activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments).

C. The episode is associated with an unequivocal change in functioning that is uncharacteristic of the individual when not symptomatic.

D. The disturbance in mood and the change in functioning are observable by others.

E. The episode is not severe enough to cause marked impairment in social or occupational functioning or to necessitate hospitalization. If there are psychotic features, the episode is, by definition, manic.

F. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment).

Note: A full hypomanic episode that emerges during antidepressant treatment (e.g., medication, electroconvulsive therapy) but persists at a fully syndromal level beyond the physiological effect of that treatment is sufficient evidence for a hypomanic episode diagnosis. However, caution is indicated so that one or two symptoms (particularly increased irritability, edginess, or agitation following antidepressant use) are not taken as sufficient for diagnosis of a hypomanic episode, nor necessarily indicative of a bipolar diathesis.

Note: Criteria A–F constitute a hypomanic episode. Hypomanic episodes are common in bipolar I disorder but are not required for the diagnosis of bipolar I disorder.

Table A, B: *diagnostic criteria for manic (A) and hypomanic (B) episode from DSM-5* [13]

Table C
Major Depressive Episode

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
Note: Do not include symptoms that are clearly attributable to another medical condition.
1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, or hopeless) or observation made by others (e.g., appears tearful). (**Note:** In children and adolescents, can be irritable mood.)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (**Note:** In children, consider failure to make expected weight gain.)
4. Insomnia or hypersomnia nearly every day.
5. Psychomotor agitation or retardation nearly every day (observable by others; not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nearly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C. The episode is not attributable to the physiological effects of a substance or another medical condition.
Note: Criteria A–C constitute a major depressive episode. Major depressive episodes are common in bipolar I disorder but are not required for the diagnosis of bipolar I disorder.
Note: Responses to a significant loss (e.g., bereavement, financial ruin, losses from a natural disaster, a serious medical illness or disability) may include the feelings of intense sadness, rumination about the loss, insomnia, poor appetite, and weight loss noted in Criterion
A, which may resemble a depressive episode. Although such symptoms may be understandable or considered appropriate to the loss, the presence of a major depressive episode in addition to the normal response to a significant loss should also be carefully considered. This decision inevitably requires the exercise of clinical judgment based on the individual's history and the cultural norms for the expression of distress in the context of loss.

***Table C**. diagnostic criteria for manic major depressive episode from DSM-5* [13]

Prevalence

According to a 2013 study, the lifetime prevalence was 0.6% for BD type I, 0.4% for BD type II, 1.4% for sub-threshold BD, and 2.4% for bipolar disorder spectrum (BPS). [14] However new studies made after DSM-5 publication suggest a decreasing trend in prevalence of BD diagnosis. [15]

The prevalence did not depend on nationality, ethnic origin, or socioeconomic status. [1]

Genetic and pathophysiology

BD is one of the most heritable psychiatric condition. [16]

A large Swedish family-based study[17] showed that first degree relatives of BD probands had a risk 5.8-7.9 times higher than people without a BD patient in the family, and that the risk is progressively decreased with genetic distance.

However, genetic transmission patterns are highly heterogeneous, with a complex polygenic genetic. [18] The most useful strategy to find specific genetic variants associated with BD appears to be Genome-wide association studies (GWASs). These analyses identified some common polymorphism such as variants of the genes CACNA1C (encoding the alpha subunit of the L-type calcium channel), ODZ4 (encoding teneurin transmembrane protein 4) and NCAN (encoding for neurocan, a chondroitin sulfate proteoglycan component of the extracellular matrix of the central nervous system[19]). [20]

GWASs have also shown a genetic overlap between BD and other psychiatric disorders. Furthermore, it's also important to consider the interaction with the environment and the childhood history [21]. The pathophysiology is not yet clear, and the research is moving in different directions. Some study focused on impairments in neurotransmission [22] (see in "BD and inflammation" paragraph) with particular interest for the dopaminergic system [23]; however other more consistent findings point to alterations in synaptic modulation and neural plasticity, neuroinflammation (see below), mitochondrial dysfunction and epigenetic changes. [22]

Furthermore, according to the neurobiological progression hypothesis BD evolution seems to be characterized by progressive neurobiological changes depending on illness duration and the number of previous episodes. [24]

Therapy

Concerning the treatment is very important to confirm the diagnosis excluding for example unipolar depression or other psychotic conditions and to develop since the beginning a good therapeutic alliance that promotes the collaboration and the adherence to therapy.[25]

For the acute management it's indicated to use mood stabilizers and antipsychotic[26]; The usage of antidepressants is still controversial, and associated with possible hypo(manic) switch, especially in BD type I. [27] The use of mood-stabilizing therapy is essential for long-term management, with concordant

evidence of effectiveness for lithium, particularly in combination with valproate[28] or antipsychotic drugs such as quetiapine. [2]

The prevention of relapse during euthymia include the combination between psychotherapy with pharmacotherapy. [30]

Bipolar disorder and Magnetic Resonance Imaging (MRI)

Over the past two decades lots of studies have investigated BD patients' brains with different neuroimaging techniques finding some interesting alterations in both Grey Matter (GM) and White Matter (WM).

The most used techniques have been structural and functional MRI. With the first one it is possible to study GM and WM.; indeed, from T1-weighted images we can measure GM density[31] (with Voxel Based Morphometry, VBM) and thickness[32], while from DTI (diffusion tensor imaging) we can get WM Voxel Wise Integrity (with Tract-based spatial statistics, TBSS) and tractography.[33] [34]

On the other hand, functional MRI (fMRI) shows neuronal activity based on the blood oxygen level-dependent (BOLD) signal [35] while the subject is doing a specific task or resting.

sMRI

Structural Magnetic Resonance Imaging (sMRI) has been the most used technique because of its ability to precisely detect anatomical differences in the brain.

Starting from GM thickness, according to a systematic review from 2016 [32] various studies reported in BD patients a decreased cortical thickness especially in the left anterior cingulate/paracingulate, left superior temporal gyrus and bilaterally in several prefrontal regions.

A huge study from 2018 [36] found that, compared to healthy controls, BD patients had extensive bilateral patterns of reduced cortical thickness in frontal, temporal and parietal regions more accentuated in adolescent and younger adults. In particular, the strongest reduction effect was present in the left pars opercularis, left fusiform gyrus and left rostral middle frontal cortex.

GM volumes reduction patterns in BD patients have been reported by two recent VBM meta-analysis:

- right insula, bilateral superior frontal gyri (SFG) in the Prefrontal Cortex (PFC), left anterior cingulate cortex (ACC) [37]
- right insula, bilateral medial orbitofrontal cortex (OFC) in the PFC, right thalamus [38]

All these regions are in different ways involved in automatic and voluntary emotion regulation and their alteration could explain some of the main symptoms and traits of mood disorders. [39] [40]

Insular cortex is gaining interest in psychiatric research for its role in emotional

processing and modulation [41] and the same goes for ACC and thalamus that are important components of attentional, cognitive, and emotional networks. [42]

PFC too is involved in the regulation of emotional behaviour [43] but also in other different cognitive functions, such as working memory, attention, reward appraisal, and decision-making. [44] [45] [46]

Wang et al. [37] also found phase-related differences between euthymic and depressed patients in particular in the right insula. Other differences were found in previous within-patients studies, where BD depressed subjects compared to BD euthymic subjects presented:

- Decreased GM density in the right dorsolateral and bilateral dorsomedial prefrontal cortex and portions of the left parietal lobe. Increased GM density in the left temporal lobe and right posterior cingulate cortex/parahippocampal gyrus. [47]
- Decreased GM density in the superior (SFG) and inferior (IFG) frontal gyri and ACC. Increased GM density in subgenual prefrontal cortex, parahippocampal gyrus, and inferior temporal gyri. [48]
- Decreased GM total volume of OFC. [49]

Some study pointed out also structural amygdala and hippocampal abnormalities but without a univocal direction [50] [51]. It could be useful to deepen our knowledge about amygdala's structural and functional (see below) alterations present in BD since this is a crucial and historically recognised region involved in emotional perception and arousal. [52] [53] [54]

Also WM alterations seems to be relevant for BD and other psychiatric disorders.[55 56 57]

Several DTI studies in BD showed differences in the corpus callosum integrity that could cause alterations in inter-hemispheric connectivity. [58]

For example, a study[59] showed lower fractional anisotropy (FA) all across corpus callosum, while in another WM volume was decreased in the posterior corpus callosum. [60]

Another study [61] reported in BD higher FA within the right and left frontal WM and lower FA within the left cerebellar white matter.

Interesting results come also from tractography studies that isolate and analyse WM single tracts.

One of these [61] showed white matter abnormalities along the pontine crossing tract, corticospinal/corticopontine tracts, and thalamic radiation fibers.

Furthermore, BD patients compared to control showed significantly decreased FA in the anterior thalamic radiation and uncinate fasciculus, and a trend towards lower FA in the superior longitudinal fasciculus and cingulum. [62]

fMRI

Lately also functional MRI (fMRI) is gaining importance in this field.

Some studies have investigated to find a correlation between structural and functional alterations. One research [63], conducted on euthymic BD patients compared to HC, showed reduced activation and cortical thickness in the IFG, SFG and cingulate cortex. Furthermore, they found that among BD patients there was a positive correlation between cortical thickness and functional activation in ACC (decreases in thickness correspond to decreases in activation) and a negative correlation between thickness and functional activation in IFG (decreases in thickness correspond to increased activity).

Besides from that, fMRI could be useful to study altered pattern of regional activation and functional connectivity representing an additional step to understand the pathophysiology of mood disorders, correlating organic analysis with behavioural outcomes [50].

A quantitative meta-analysis from 2011[64], collecting a great number of subjects, found abnormal frontal and limbic activation in BD patients compared to healthy controls. They highlighted a decreased activation of the IFG with both emotional and cognitive tasks and a limbic (basal ganglia, parahippocampal gyrus, hippocampus, and amygdala) overactivation with emotional tasks.

IFG has a role in cognitive control, impulsivity and emotional regulation [65] and a decreased activation of this area in the right hemisphere is reported also by

another meta-analysis. [66]

As mentioned in the previous paragraph, another interesting region could be amygdala. A certain grade of heterogeneity is still present also in fMRI findings, but some studies agree on a hyperactivation of the left amygdala during emotional tasks. [67] [68]

These studies, consistently with others, seems to point to a lateralized functional pattern in mania with a reduced activity of right ventromedial and ventrolateral prefrontal cortex and an increased activity of left amygdala, left ACC and left basal ganglia. [69] (FIG.1.)

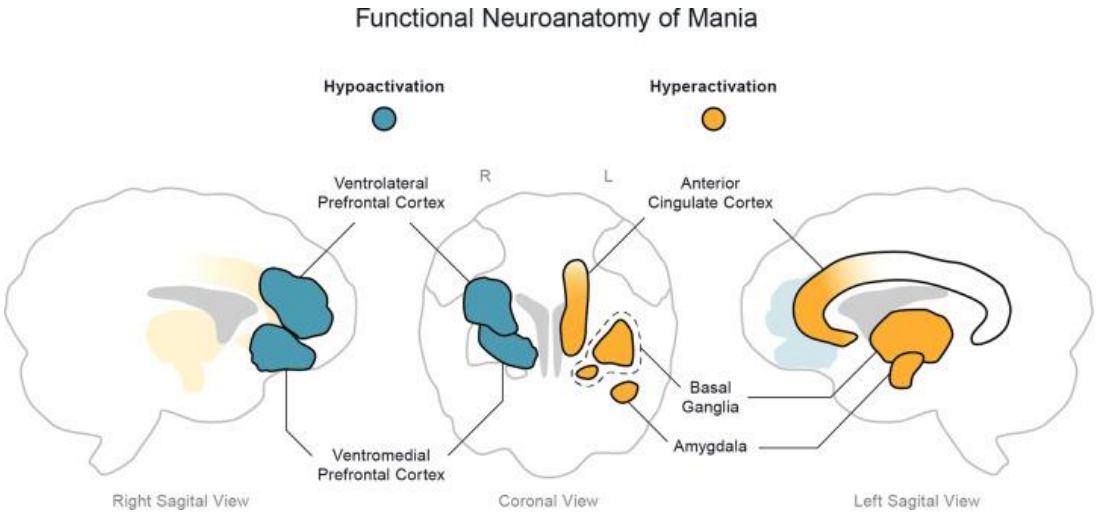


FIG.1. *functional lateralized alteration in mania*. [69]

Another study [70] showed a less intense activation of the right lateral OFC in BD patients using the Go-NoGo task.

OFC is a brain region involved in behavioural inhibition and its functional disruption could explain some of the impulsivity seen in mania.

Lots of other BD studies used the Go-NoGo task combined with fMRI to test the alterations in brain activation patterns underlying motor response inhibition.[71] [72] [73]

fMRI also offers the opportunity to analyse the functional connectivity (FC) between different brain areas.

A 2021 systematic review underlines the importance in BD to evaluate FC alterations in large-scales networks [74] finding disrupted intra and internetwork FC, especially involving the Default Mode Network [75].

Indeed, a recent interesting theory is the one trying to explain the opposite mood states present in BD through an altered balance between the DMN and the sensorimotor network (SMN). [76]

DMN connects areas from the ventral medial prefrontal cortex (VMPC), dorsal medial prefrontal cortex (DMPC), posterior cingulate cortex (PCC), precuneus and lateral parietal cortex.[77] Its activation is enhanced during RS fMRI compared to the task related, and it's believed to be involved in emotional processing (VMPC), self-referential mental activity (DMPC), and the recollection of prior experiences (PCC and precuneus).

On the other hand, SMN activation is related with psychomotor excitation, hyperactivity, logorrhoea.

In BD patients DMN/SMN balance was tilted toward the DMN in depression and

toward SMN in mania.

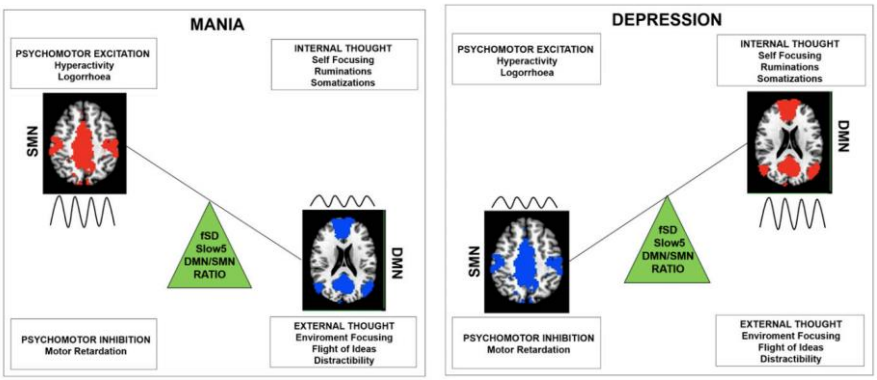


FIG.2*. DMN/SMN disbalance in depression and mania.*[76]

Besides from large scale networks BD patients showed lots of other alterations in FC.

Considering for example amygdala, BD patients showed a decreased resting-state functional connectivity (rsFC) between the amygdala and the left middle frontal cortex while a decreased rsFC between amygdala and right OFC was specific of manic state. [78]

Foland et al. [79] found a deficient modulation of amygdala response by prefrontal cortex in bipolar mania during a face matching paradigm. This task, elaborated by Hariri et al. in 2000[80], consists in three parts (FIG. 3); initially subjects have to match two faces that are expressing the same emotion showing thanks to fMRI scan the circuits involved in the emotional perception. In the second task subjects have to "label" the emotion matching a face with a linguistic description. The third task is just a control condition with geometrical figures. During the second task VLPFC normally displays an inhibitory effect on amygdala, but this seems to

be reduced in BD manic subjects.

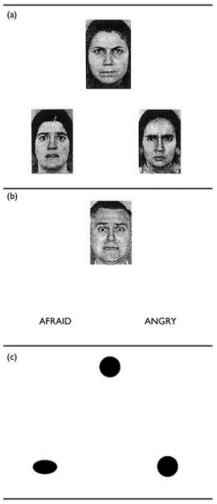


FIG.3. [80] *Faces matching paradigm (a) "perceive emotion" (b) "label emotion" (c) control.*

Another study showed a decreased rsFC between the left insula and mid-dorsolateral prefrontal cortex in BD compared to HC and subjects with major depression. [81]

Furthermore, a disrupted FC appeared between the right IFG, in particular the posterior ventral subregion, and other areas including the postcentral gyrus, the precentral gyrus, paracentral lobule, lingual Gyrus, fusiform and cerebellum posterior lobe. [65]

Recently also the cerebellum is gaining interest in psychiatric research especially after the theory proposed by Ito Masao; [82] he suggested a cognitive-emotional cerebellar function based on the same computational model that cerebellum uses

for its well-known role in motor learning and coordination.

Indeed, cerebellar alterations have been well documented in autism spectrum disorder[83], schizophrenia [84 85] and mood disorders [86].

According to a fMRI study BD type II patients compared to HC showed impaired patterns of FC with area of the DMN [87]. In detail, they noticed an increased FC between the right Crus I and bilateral precuneus and decreased FC between the left Crus II and bilateral medial prefrontal cortex and right medial frontal gyrus.

Bipolar disorder and inflammation

The theories that involve the immune system in the pathogenesis of BD dates to 1981, when Horrobin and Lieb [88] highlighted the similar paradoxical stabilizing action that lithium has on both immunity and mood regulation.

After this, other studies showed that the mechanism of action of mood stabilizers medications, such as Lithium, Carbamazepine and Valproic Acid may include cyclooxygenase-2 (COX-2) inhibition and reduction of pro-inflammatory cytokines. [89 90 91 92 93]

Nowadays mounting evidence support this hypothesis [94] starting for example from the association between BD and other inflammatory comorbidities such as metabolic and autoimmune-allergic diseases [95], Diabetes [96 97 98], psoriasis[99], rheumatoid arthritis [100], migraine [101]; The identified relationship has yet to be

established as causal; however, the available evidence suggests that it could be bidirectional. (FIG.4.)

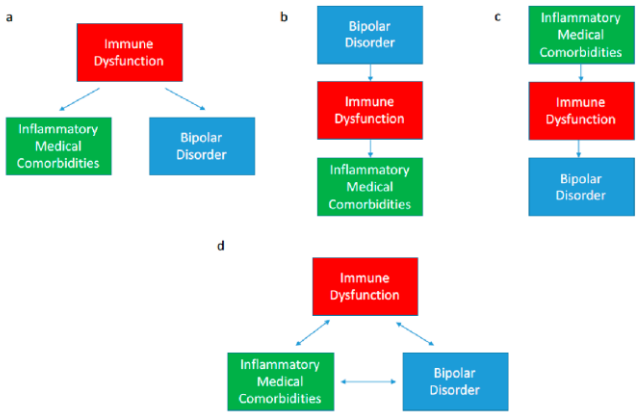


FIG.4. *"Potential interactions between BD, immune dysfunction and inflammatory comorbidities. (**a**) Immune dysfunction may be a common underlying cause of both BD and an inflammatory comorbidity; (**b**) BD may proceed the inflammatory condition or (**c**) vice versa. (d) Complex interaction by which immune dysfunction, BD and inflammatory comorbidities may be perpetuating each other."* [102]

Cytokines and C-Reactive protein

Furthermore, lots of studies found altered levels of cytokines and acute-phase proteins. [103] Both manic and depressive BD episodes are characterized by the activation of neuroinflammation pathways, shown by increased levels of pro-

inflammatory cytokines and acute-phase proteins such as haptoglobin, fibrinogen and C-reactive protein (CRP). [104] [105](FIG.5)

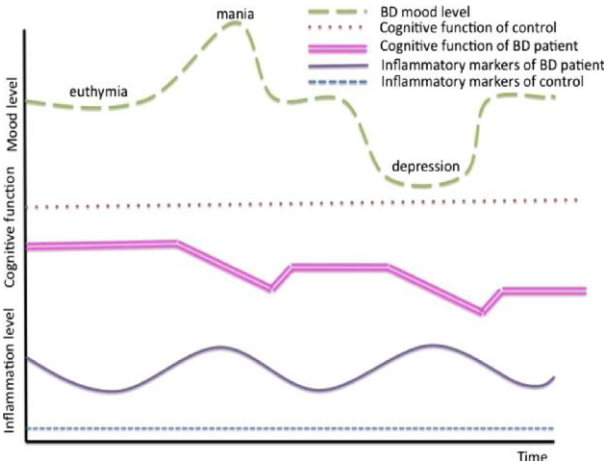


Fig.5*. "Hypothetical depiction of simultaneous changes in mood level, cognitive function, and inflammatory cytokine levels. Cytokine levels are elevated chronically and may increase during both manic and depressive episodes."* [106]

It has been also proposed that these increased levels of inflammatory molecules may also be associated with a decline in cognitive function. There is evidence of stable and lasting cognitive impairment in all phases of bipolar disorder. With resolution of mood episodes, some cognitive function may be restored; however, it seems clear that the "remitted" euthymic bipolar patients have still some impairments in executive function, verbal memory, psychomotor speed, and sustained attention. [107]

Supporting the prementioned possible relationship between inflammation and cognitive impairment, a study found an association between elevated levels of CRP and reduced cognitive functioning in BD. [108] They measured the cognitive functioning in BD patients with the Repeatable Battery for the Assessment of

Neuropsychological Status (RBANS) and they found there was an inverse relationship between CRP levels and performance on RBANS total (t=-2.48, p=.015), RBANS immediate memory (t=-2.16, p=.033), RBANS attention (t=-2.18, p=.032), RBANS language (t=-2.13, p=.036), Trail Making A (t=-2.39, p=.019).

C-Reactive Protein (CRP) is a homopentameric structured protein produced in the liver and secreted in the blood, which plays a central role in the inflammatory process. His expression is stimulated by pro-inflammatory cytokines, particularly IL-6, IL-1, and tumor necrosis factor. [109]

Its concentrations are increased in all BD stages, especially during manic episodes[110]. Moreover, excess of CRP can affect the blood-brain barrier (BBB) function increasing paracellular permeability[111] and this could permit easier diffusion across the barrier for other inflammatory molecules and finally explain one of CRP ways to impair the Central Nervous System (CNS).

About cytokines, they are signaling molecules of the immune system which may increase or decrease local and systemic inflammatory responses via complex networks [112]. Their levels are fluctuant and variable, but compared to healthy controls, BD patients have consistently shown higher levels of pro-inflammatory cytokines suggestive of chronic low-grade inflammation. [113] [114]

However, seen the heterogeneity of results in different studies, it's not easy to delineate an accurate cytokine profile characterizing each mood state. Below

reported in Fig. 6 and Table D two effective visual overview from a meta-analysis [114] and a review [105].

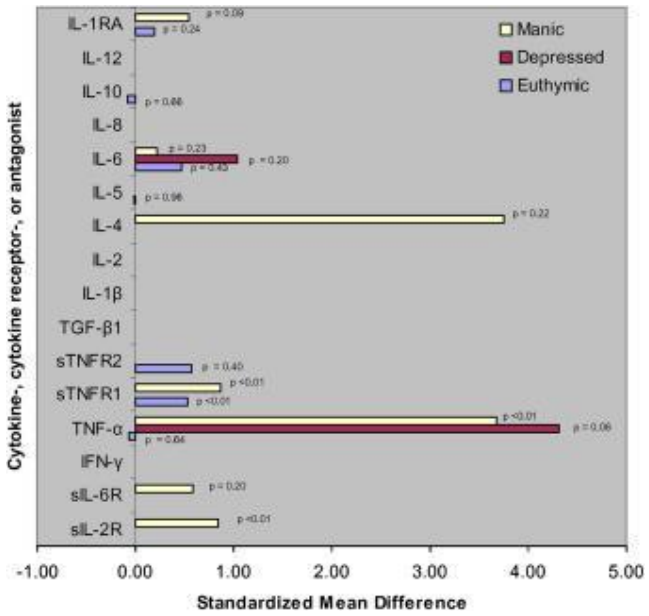


Fig.6*. Cytokine-, cytokine receptor-, or antagonist levels in bipolar disorder according to affective state. Standardized mean difference for individual cytokine parameters is presented for manic patients (yellow bars), depressed patients (red bars) and euthymic patients (blue bars) compared to healthy control subjects. Positive standardized mean differences, represented by bars going to the right, reflect higher levels of cytokines in bipolar patients compared with healthy control subjects, whereas negative standardized mean differences indicate higher levels of cytokines in healthy control subjects. IL (interleukin); IL1-Ra (IL-1 receptor antagonist); sTNF-R (soluble tumor necrosis factor receptor -1); sTNF-R2 (soluble tumor necrosis factor receptor-2); TNF-α (tumor necrosis factor-α); IFN-γ (interferon-γ); sIL-2R (soluble IL-2 receptor); sIL-6R (soluble IL-6 receptor); TGF-β1 (transforming growth factor-β1).* [114]

Mania	Depression	Euthymic
IL-1β	IL-1β	IL-1β
sIL-1R	IL-6 (Benedetti et al., 2002;	IL-4
IL-2	Ortiz-Dominguez et al.,	sTNF-R1
sIL-2R	2007; Prather et al., 2009)	
IL-4	IL-8	
IL-6	INF-γ	
IL-8	TNF-α	
INF-γ		
TNF-α		
sTNF-R1		

Table E. *Pro-inflammatory cytokines involved in mood switches of bipolar disorder patients* [105]

Microglia overactivation

An interesting hypothesis that can explain the link between inflammation, BD mood episodes and neurodegeneration is the one considering a pathological microglial overactivation in key brain regions subserving mood and cognition (prefrontal cortex, amygdala, hippocampus, insula, and anterior cingulate cortex) [115].

Microglial cells have been always considered as the CNS resident macrophages but according to new studies they have also a role in facilitating learning, modulating neuronal activity and social behaviour. [116]

In BD such as in other neurological pathologies (Alzheimer disease [117], multiple sclerosis [118], Amyotrophic lateral sclerosis [119], Parkinson's disease [120]), overactive microglia creates a positive feedforward loop: It releases cytokines, which further increase inflammation and enhance the excessive microglia activation.

According to this hypothesis (FIG.7) [105], manic and depressive recurring episodes constitute a chronic inflammatory status, leading to microglia hyperactivation. Inflammation and activated microglia induce kininogen synthesis and then its proteolytic cleavage into its degradation products. These lead to an up-regulation of the kinin-B1 receptor (B1BKR) and down-regulation of the kinin-B2 receptor (B2BKR) expression and activity. B1BKR up-regulation causes excitotoxicity via an increased release of glutamate, ROS levels and cytosolic Ca2+ concentration. Down-regulation of B2BKR results in GSK-3β activation, reducing brain-derived neurotrophic factor (BDNF) levels and increasing TNF-α expression and consequently an augmented release of pro-inflammatory cytokines. Finally, this toxic environment ends up with microglial cell senescence and/or death. With reduced microglia population the physiological balance is compromised, and this could contribute to neurodegeneration process and to stronger and more frequent mood switches.

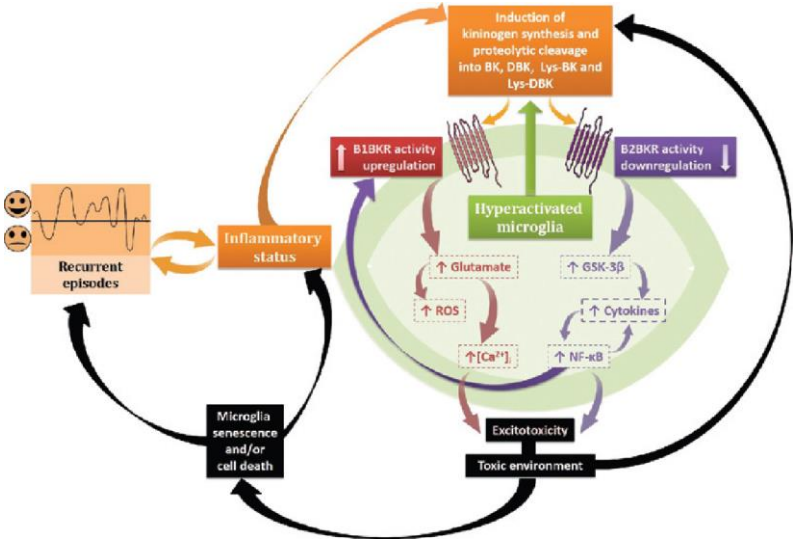


FIG.7 *[105] microglial hypothesis*

Oxidative stress

Moreover, in BD patients the cellular response to oxidative stress is believed to be compromised, and in particular the system activated by the transcription factor Nrf2 (Nuclear factor erythroid 2-related factor 2) [103]. Under oxidative stress conditions it detaches from Keap1, translocates to the nucleus, and turns on the transcription of genes encoding for antioxidant proteins[121]. It has also an important anti-inflammatory activity regulating anti-inflammatory gene expression and inhibiting the progression of inflammation [122].

Nrf2 increase the expression of the gene HO-1 that has been associated with the inhibition of the nuclear factor kB (NF-kB), a classical signaling pathway of inflammation. In addition, Nrf2 activity has been shown to attenuate lipopolysaccharide-induced transcriptional upregulation of proinflammatory cytokines, such as IL-16 and IL-1ß [123], and to downregulates the expression of

pro-inflammatory genes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)[124].

Post-mortem brain analysis showed lower levels of Nrf2 and Keap1 in the parietal cortex of BD patients compared with healthy controls [125]. In addition to that, alterations in oxidative balance may be associated with abnormal low levels of BDNF observed in individuals with BD in acute manic episodes. It is also interesting to notice that chronic treatment with lithium increases nuclear levels of Nrf2 in PC12 rat cells [126].

Steroids and neurotransmission

Another important mechanism by which inflammation could induce mood alterations in BD is hypothalamic–pituitary–adrenal axis dysregulation [127]. Indeed, it has been reported the possible induction of mania and depression via increased levels of steroids [128] [129] [130]. Pro-inflammatory cytokines stimulate cortisol secretion [131] and can affect glucocorticoid receptor function decreasing hypothalamic and pituitary sensitivity for the negative feedback [132].

There are also strong evidence supporting the relationship between inflammation and neurotransmitter systems[133], and this could be interesting especially considering the known alterations in the monoamines systems present in BD patients[134].

For example, high levels of noradrenaline and dopamine have been reported with mania and switching to mania[135] [23]. The serotonin system could be involved too; its influence in depression has been widely investigate, while it is still not so defined its role in mania stages [136]. Some evidence suggests that central serotonergic activity is reduced in the depressive and euthymic phase of BD. [137] Furthermore, pro-inflammatory cytokines can also modify glutamate [138] and GABA transmission, and both these systems, could take somehow part to BD pathogenesis [139] [140]. Magnetic Resonance Spectroscopy (MRS) studies in patients with unipolar depression have revealed the correlation between increased inflammation and increased glutamate levels in the basal ganglia, specifically associated with anhedonia and psychomotor slowing [141].

Lymphocytes populations

In the last few years, it's constantly gaining interest also the analysis of inflammatory cell populations. In a recent study[142] they found that neutrophil, platelet, monocyte counts and Neutrophil to Lymphocyte Ratio, Platelet to Lymphocyte Ratio, and Monocyte to Lymphocyte Ratio values were higher in the manic phase of BD compared to HC. However, the pathophysiological contribute and the possible clinical application of these results remain still uncertain.

Another study from 2019 [143] shows that BD type I patients had significantly higher percentages of total T cells, CD4+ T cells, activated B cells, and monocytes than healthy controls.

The altered count of activated T and B cells [144] [143] could be caused by an impaired function or number of the T regulatory (Treg) cells [145] [146] [147].

There are also other immune cells alterations in BD [148], such as a decreased neuronal calcium sensor-1 (NCS-1) expression in CD4+ T lymphocytes, CD19+ B lymphocytes and CD14+ monocytes [149] and a decreased immune suppressive effect of dexamethasone on T cells [150].

Furthermore, seen the high comorbidity with autoimmune disease is gaining interest the research of autoantibodies that can contribute to the pathogenesis of BD. [151]

Finally, it could be interesting to deepen the investigation into the involvement of gut microbiome alterations related to BD [152].

Only few papers investigated in BD the possible correlation between immunological abnormalities and alterations in the brain structure.

A study reported that levels of inflammatory cytokines were associated with lower integrity in large overlapping networks of WM fibers [153], while another paper found in manic patients a correlation between WM microstructure alterations and a reduction in blood levels of terminally differentiated CD8+ effector T cells. [154]

Three other studies found a negative correlation between inflammatory cytokines levels and GM volumes in different cortical regions. [155] [156] [157]

Aims of the study

The aim of our study (1) to analyse separately immunological and MRI data to find differences that could be characteristic for all BD patients or for a specific phase, and (2) to unify the two findings looking for a linear correlation between immunological variables and GM thickness.

Material and methods

Subjects

Sixty-seven subjects were enrolled (43 BD-I patients and 24 healthy controls) and for each one we collected blood samples and performed anatomical T1-weighted MRI with a 1.5-Tesla scanner.

Among BD patients there were 24 patients in the depressive phase and 19 in manic phase.

	n	gender	Age (min-max)	Age mean	Age SD
man	19	8 M 11 F	29-58	46.32	7.20
dep	24	10 M 14 F	23-59	45.83	9.15
HC	24	13 M 11 F	29-64	42.83	13.07

Table F. *Number (n), gender (M=males, F=females) and age data from the subjects of our study. Manic (man), depressed (dep), healthy controls (HC).*

The current mood state was clinically determined following the DSM-5 diagnostic criteria and supported by the score of two clinical scales:

The Hamilton Depression Scale (HAM-D)[158] and the Young Mania Rating Scale (YMRS)[159] assessing respectively the severity of depression and mania symptoms.

	YMRS mean	YMRS SD	HAMD mean	HAMD SD
man	18.32	6.91	5.32	4.73
dep	4.25	3.85	19.83	5.09

Table G. *Clinical scales data for our manic and depressed patients*

Immunology

Immunofluorescence analysis by flow cytometry

Immunofluorescence and flow cytometry were used to analyze cell expression of membrane antigens, necessary for achieving data relative to frequencies of circulating T cell subpopulations.

100 µl of fresh peripheral blood sample was incubated with specific fluorochrome-conjugated monoclonal Antibodies (mAbs) at 4 °C for 30 min in the dark and red cells were lysed with 2 ml of Beckton Dickinson fluorescence activated cell sorting (FACS) Lysing solution (Beckton Dickinson Biosciences, CA, USA) and resuspended in 200 µl of the same solution. The following mAbs were used: allophycocyanin (APC)-cyanin 7 (Cy7) conjugated anti-CD4 (Clone RPA-T4), phycoerythrin (PE)-conjugated anti-CD28 (Clone CD28.2), fluorescein isothiocyanate (FITC)-conjugated anti-CD45RA (Clone HI100), Brilliant Violet (BV) 421-conjugated anti-CD8 (Clone RPA-T8), and Horizon V500-conjugated anti-CD3 (Clone UCHT1).

The following gate strategy was used: identification of CD3+ T cells using CD3 versus SSC plot and definition of CD4+ and CD8+ populations in CD4 versus CD8 plot. After that, other analyses were performed to identify antigen exposure history and differentiation status. In particular, we got information on frequencies in the peripheral blood of total CD4+ or CD8+ T cells as well as the following T cell subpopulations: CD4+CD28+CD45RA+ (CD4+ naïve), CD4+CD28+CD45RA- (CD4+ central memory), CD4+CD28-CD45RA- (CD4+

effector memory), CD4+CD28-CD45RA+ (CD4+ terminal effector memory), CD8+CD28+CD45RA+ (CD8+ naïve), CD8+CD28+CD45RA- (CD8+ central memory), CD8+CD28-CD45RA- (CD8+ effector memory) and CD8+CD28-CD45RA+ (CD8+ terminal effector memory). [160] [161]

Samples were analyzed on a FACS Canto II flow cytometer by the FACS DIVA version 6.0 software.

The cytokine profile of peripheral CD4+ and CD8+ T lymphocytes, in terms of interferon (INF)γ, interleukin (IL)-17A, IL-4 and IL-10 production, was analyzed by intracellular staining and flow cytometry analyses. The samples were analyzed using a FACS Canto II flow cytometer by the FACS DIVA version 6.0 software.

All immunological analyses concerning T cell subpopulations are expressed as frequency referred to the total CD3+ population. FACS analyses were performed by two independent researchers and the mean of the two measures were considered. Laboratory analyses were performed blind to clinical information.

Analysis of cytokine concentrations

Plasma levels of IL-6 cytokine were analyzed by ELISA using a commercially available Human IL-6 ELISA Kit High Sensitivity (ABCAM, Cambridge Science Park, UK). The lower limit of quantification (LLOQ) was 0.8 pg/ml, range of sensitivity of the kit was 1.56 pg/ml - 50 pg/ml.

Plasma concentrations of INFγ, IL-10, IL-4, IL-17A, IL-1b, TNF-α and IL-6 cytokines were measured by a bead-based immunoassay (BDTM Cytometric

Bead Array, Beckton Dickinson) using the FACS Canto II cytometer equipped with the FACS DIVA version 6.0 software. Plasma cytokine concentration was expressed in picograms per milliliter by FlowCytomix Pro Software. LLOQs was 0.274 pg/ml for each cytokine and range of sensitivity was 0.274 pg/ml – 200 pg/ml.

Statistical analysis

We used the program "R" to perform the statistical analysis concerning the immunological part using gender and age as covariates. First, we verified the normal distribution of the data through the Shapiro test. Since the test failed, we opted for nonparametric models.

Group differences among our three groups (manic, depressed and HC) were tested with the ORL (ordinary logistic regression) model, using age and gender as covariate. Post hoc comparisons were run with the Wilcoxon rank sum test. P-values were corrected with the false discovery rate (FDR) method.

Neuroimaging

HC and BD patients underwent T1-weighted MRI analysis with 1,5 Tesla scanner.

- Anatomical data

3D T1-weighted MRI scans were converted to NIFTI format and resliced from sagittal to axial orientation. They were visually inspected, and their origin was set in correspondence with the anterior commissure. The following processes were

then carried out with the Computational Analysis Toolbox (CAT, version 12.6) within SPM12 using MATLAB (version 2017b). All images were normalized using an affine followed by non-linear registration, corrected for bias field inhomogeneity, and then segmented into GM, WM, and CSF components [162] The Diffeomorphic Anatomic Registration Through Exponentiated Lie (DARTEL) algebra algorithm normalizes the segmented scans into a standard MNI space using six iterations. Compared to the conventional algorithm, the DARTEL approach can provide more precise spatial normalization to the template. We performed a non-linear deformation on the normalized segmented images with the CAT12 toolbox as part of the modulation step. This modulation provides a comparison of the absolute amounts of tissue corrected for individual differences in brain size. All segmented, modulated, and normalized GM and WM images were smoothed using 8-mm full-width-half-maximum Gaussian smoothing.

- Cortical thickness analysis

The CT was evaluated according to the projection-based thickness method [163] The surface extraction pipeline used topology correction [164], spherical mapping [165], estimation of local surface complexity, and local gyrification [166]. Finally, cortex surfaces were smoothed (FWHM=15mm) and resampled to a 32k mesh compatible with the Human Connectome Project (HCP). Individual values of mean white (WM) and gray matter (GM) volumes were calculated in each ROI of the Neuromorphometrics atlas (labeled data provided by Neuromorphometrics Inc.). Mean CT values (mCT) within the ROIs defined in the a2000s atlas included in CAT were also calculated. In a parallel pipeline, following the canonical FSL anatomical one, the axial-reoriented T1 images underwent bias

field correction, skull-stripping, and non-linear co-registration to the standard MNI template. The resulting transformations were later used to normalize DTI and rs-fMRI data.

Results

Immunology

In our analysis, the main alterations comparing BD patients to HC, were detected during the manic phase.

Manic patients showed an augmented frequency of total CD4+ T cells (T CD4+ CD3+) and an augmented TCD4+/TCD8+ ratio.

Total CD8+ were not reduced but a significant decrease was present in the CD8+CD28- and in both their subgroups CD8+CD28-CD45RA- (CD8+ effector memory) and CD8+CD28-CD45RA+ (CD8+ terminal effector memory).

Compared to HC, manic patients also showed a decreased count of T CD8+ INFγ+.

Manic patients also showed an augmented IL-6 compared to HC and depressed patients.

		P value
T CD4+ CD3+	Man > HC	0.04464688
CD8+CD28-	Man < HC Man < Dep	0.002251278 0.025719517
CD8+CD28-CD45RA-	Man < HC	0.007044128
CD8+CD28-CD45RA+	Man < HC	0.003342061
T CD8+ INFγ+	man < HC	0.02099686
TCD4+/TCD8+ ratio	Man > HC	0.0140430
IL-6	Man > HC Man > Dep	0.01448703 0.02530201

Table H. *Results of immunological analysis between the three groups: manic(man), depressed (dep) and healthy controls (HC). The p value was corrected with false discovery rate (FDR) method.*

We also found a significant correlation between TCD8+ cell subpopulations and the score of the two clinical scales we used for all BD patients: YMRS and HAMD.

In detail we saw that there was a negative correlation between the score of the YMRS and the frequency of:

T CD8+CD28- (R=-0,34; p=0,028)

T CD8+CD28-CD45RA- (R= -0,38; p=0,012)

T CD8+CD28-CD45RA+ (R=-0,3; p= 0,053)

Furthermore, the same populations showed a positive correlation with the HAMD score:

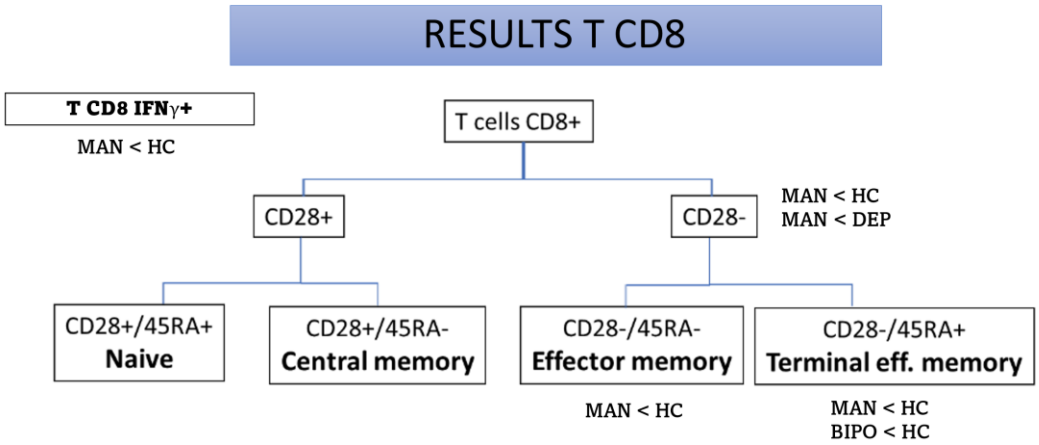
T CD8+CD28- R=0,49 p=0,00085

T CD8+CD28-CD45- R=0,32 p=0,034

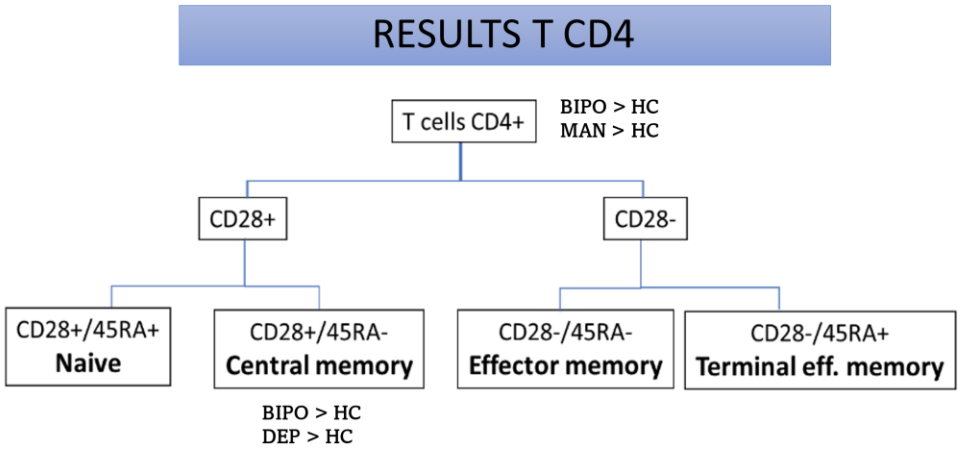
T CD8+ CD28-CD45+ R=0,37 p=0,014

IL-6 showed positive correlation with YMRS (R=0,31 p=0,065) and a negative correlation with HAMD (R=-0,38; p=0,021).

For the markers that didn't show significant differences between manic and depressed patients we took in consideration all BD patients making part of a single group and we compared it with the HC group with a T test. We could observe a significant increase in total T CD4+ (pvalue=0.049), T CD4+ CD28+ CD45RA- (pvalue=0.014) and PCR (pvalue=0.008) and a decrease of T CD8+ CD28- CD45RA- (pvalue=0.004).



(a)



(b)

FIG.8. *Schematic results of T cells analysis. (a)T CD8+CD28-, CD8 "effector memory", CD8 "terminal effector memory" and CD8 INFγ+ frequencies were reduced in manic patients (man) compared to Healthy controls (HC). (b)Total CD4+ frequencies were higher in bipolar patients (BIPO) and man compared to HC, while TC4 "central memory" were elevated in BIPO and depressed patients compared to HC.*

Neuroimaging

We found a diffuse reduction of GM cortical thickness in BD compared to HC high statistical significance.

Results in detail are reported in FIG.9.

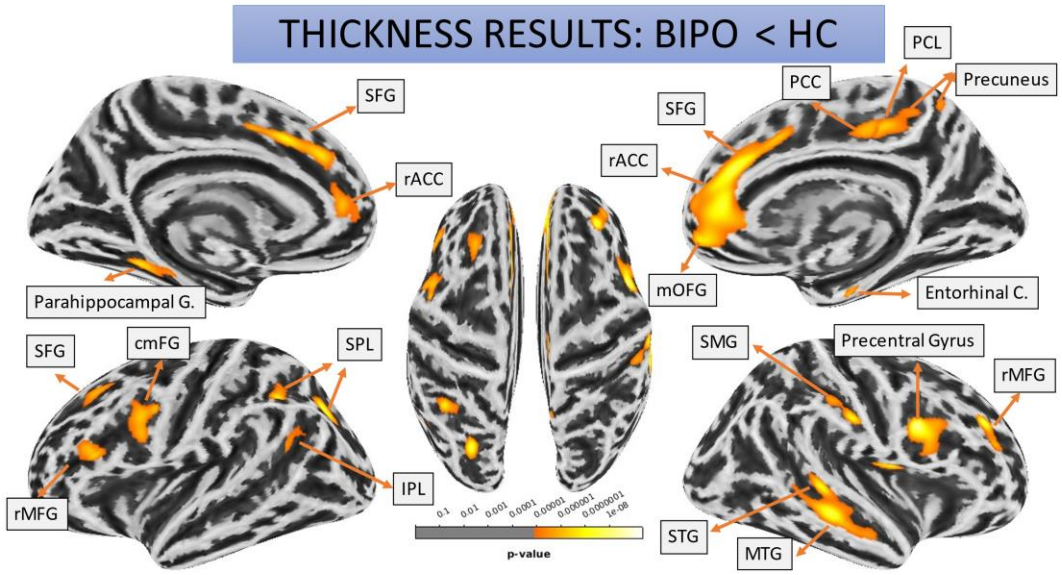


FIG.9. *Cortical areas with significant reduced thickness in BD patients (BIPO) compared with HC. Superior Frontal Gyrus (SFG), rostral Anterior Cingulate Cortex (rACC), parahippocampal Gyrus, caudal middle Frontal Gyrus (cmFG), Superior Parietal Lobule (SPL), rostral Middle Frontal Girus (rMFG), Inferior Parietal Lobule (IPL), Posterior Cingulate Cortex (PCC), Paracentral Lobulule (PCL), Precuneus, medial Orbitofrontal Gyrus (mOFG), Entorhinal Cortex, (SMG), Precentral Gyrus, Superior Temporal Gyrus (STG), Middle Temporal Gyrus (MTG).*

Correlation neuroimaging-immunophenotype

We performed a multiple regression between the lymphocyte populations and the imaging data in the different groups; in the group of all BD we found a negative correlation between frequency of TCD4+ CD28+ CD45RA- (central memory) and GM cortical thickness in the left Fusiform Gyrus (lFG) and in the right Precuneus.

This means that in BD patients increasing frequency of T CD4+ central memory correlates with a progressive reduction of the cortical thickness in these regions.

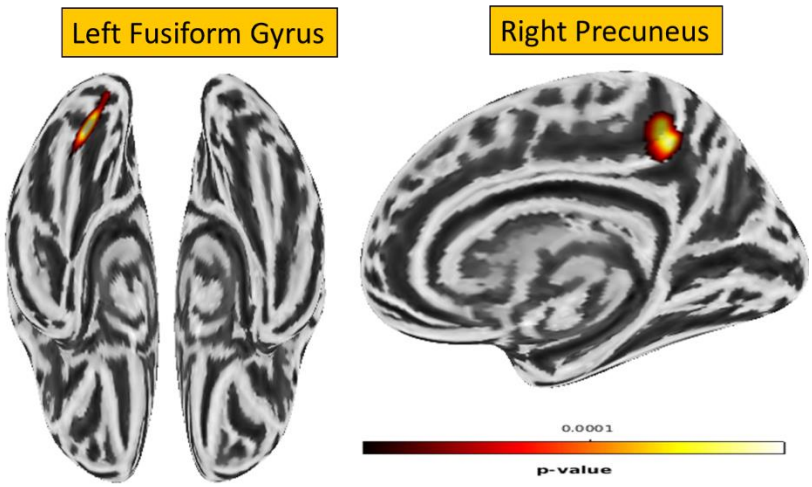


FIG.10. *cortical region where the thickness was negatively correlate with T CD4+ central memory frequency.*

Furthermore, in the depressed group we detected a negative correlation between the T CD4+ / TCD8+ ratio and the thickness of the cluster placed in the anterior division of the Superior Temporal Gyrus and of the Middle Temporal Gyrus.

Discussion

We based this study on a large amount of immunological and neuroradiological research findings described in BD population.

We replicated literature findings that reported a pro-inflammatory state (increased levels of IL6 and CRP) and a diffusely reduced CT in BD.

Concerning the immunology, our results from peripheral blood samples showed in manic patients a significant increase of the total T CD4+ and IL-6, and on the other hand a decrease of T CD8+ INFγ+, T CD8+ CD28- population and both of its subpopulations, T CD8+ effector memory and T CD8+ terminal effector memory.

Moreover, in the group of all BD patients we could observe a significant increase in total T CD4+, T CD4+ central memory and PCR and a decrease of T CD8+ terminal effector memory.

We suggest that BD patients, especially during manic phase, are characterized by an impaired inflammatory response with an increased count of T CD4+ secerning pro-inflammatory cytokines that support CD8+ infiltration and autoreactivity in the CNS and in wider terms a neuroinflammatory condition.

This migration could explain the decrease in blood levels of T CD8+ effector memory, terminal effector memory and CD8+ INFγ+ that are indeed the terminal differentiated lymphocytes prone to infiltrate tissues.

Notably, these alterations in CD8+ frequencies correlates in a linear way with the scores of the clinical scales we used; indeed, we notice that T CD8+ CD28- , T CD8+ effector memory and T CD8+ terminal effector memory frequencies had a negative linear correlation with the YMRS score and a positive correlation with the HAMD score.

The decrease of these lymphocytes' populations so could be specifically involved in the development and in the severity of manic symptoms.

At the same time, with our MRI analyses we replicated literature results showing a diffusely reduced cortical thickness in BD patients compared to HC.

In detail the regions with reduced thickness were:

Superior Frontal Gyrus (SFG), rostral Anterior Cingulate Cortex (rACC), parahippocampal Gyrus, caudal middle Frontal Gyrus (cmFG), Superior Parietal Lobule (SPL), rostral Middle Frontal Girus (rMFG), Inferior Parietal Lobule (IPL), Posterior Cingulate Cortex (PCC), Paracentral Lobulule (PCL), Precuneus, medial Orbitofrontal Gyrus (mOFG), Entorhinal Cortex, (SMG), Precentral Gyrus, Superior Temporal Gyrus (STG), Middle Temporal Gyrus (MTG).

Lots of these cortical regions were already found altered in previous BD imaging studies in terms of thickness or volumes.

The last aim of our study was to report if in BD the immunological phenotype correlates with alterations in the cortical thickness.

We found that there was a negative linear correlation between T CD4+ central memory and the thickness of two cortical areas; indeed, in BD patients this

population increase corresponds to a reduced thickness in the left Fusiform Gyrus (lFG) and right Precuneus cortex.

Both these two regions could have a relevant meaning in BD pathogenesis; a reduction in lFG cortical thickness appears to be a specific trademark of BD as reported by a huge study from the ENIGMA working group [36].

At the same time, Precuneus cortical alterations could be indicative of an impaired function of this area that is a core region of the DMN which is recently gaining interest in BD research (see "fMRI" paragraph of introduction).

It's necessary to underline how all of ours BD patients were in an acute phase of the disease, manic or depressive; it could be interesting to take in consideration also euthymic BD patients to understand which alteration is still present in absence of symptomatology.

Conclusions

We replicated literature findings of a pro-inflammatory state (increased IL-6 and CRP) and a diffusely reduced CT in BD. Moreover, we found specific alterations in T-cell subpopulations, and an association between increased T CD4 central memory and reduced CT in the lFG and rPC. We speculate that in BD increased pro-inflammatory cytokines and CD4 T cells (especially CD4 central memory) could support the cerebral infiltration by activated T CD8. This could lead to a neuroinflammatory condition possibly linked with the CT reduction.

It's necessary to say that this is one of the first studies exploring T cells populations frequencies and their correlation with structural abnormalities in BD.

A critical aspect we detected about lymphocytes in BD is that the literature is still very poor, and every paper used different ways to divide lymphocytes in subpopulations. Our results suggest that the method we used seems to show coherently explainable patterns of alterations and so should be further investigated.

Concerning the imaging part, it could be interesting to replicate the same kind of investigation using DTI and fMRI; indeed, neuroinflammation and immunological autoreactivity could have on white matter integrity and on functional activation even a stronger and faster effect.

We built our study to test the hypothesis that the abovementioned parameters are related but we were not able to find out the process by which immunological alterations impact on cerebral structure and function.

Future studies should analyse more directly CNS inflammatory state and look for T and B cells autoreactivity with specific cerebral antigens starting from immuno-histological examinations.

In conclusion we want to underline how a deeper knowledge of the pathophysiological process in BD can lead to new therapeutic strategies that in our specific case could be directed to modulate specific immunological alterations or more in general to contrast the low-level inflammation present in BD patients. Furthermore, from our perspective, we believe it's important to promote neurobiological studies in psychiatric conditions because consistent findings of organic impairments can bring these disorders on the same level of every other medical pathology, contributing to a reduction of the stigma that still afflicts psychiatric patients.

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