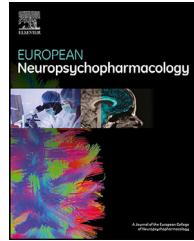




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# Higher lymphocyte count associated with larger hippocampal volume and fewer depressive symptoms in drug-naïve first-episode psychosis

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Depressive  
symptoms

## Abstract

Circulating white blood cells (leucocytes), which form the peripheral immune system, are crucial in inflammatory processes but their role in brain structural change in schizophrenia has been scarcely studied. With this study we want to determine how and which type of white blood cells are associated with hippocampal volume (as a key structure in schizophrenia etiopathology) in first episode psychosis (FEP) patients. Moreover, to determine the association between white blood cells and clinical symptomatology, including positive and negative symptoms, cognition and depression. For this purpose fifty drug-naïve FEP were included in this study. All patients underwent an assessment at baseline and at 1 year follow-up, including sociodemographic and clinical variables (substance use, DUP, PANSS, GAF and CDSS). Fasting blood samples were obtained before administering any medication at baseline. Structural T1 MRI was performed at baseline and brain volumes were quantified. In the present study, higher lymphocyte count was associated with larger right hippocampal volume at baseline in FEP drug-naïve patients. Higher lymphocyte count was associated with lower depressive symptomatology measured with CDSS and Marder depressive factor from PANSS at baseline and 1-year follow-up. These results suggest that lymphocytes may have a protective effect in hippocampal volume at

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baseline in antipsychotic naïve FEP and also, are associated with a better depressive course over follow up. These results open the door to identify new biomarkers and therapeutic targets for patients with schizophrenia.

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## 1. Introduction

Schizophrenia is a chronic, heterogeneous mental disorder associated with substantial disability (Harvey et al., 2019). Although the underlying pathophysiology remains unclear, it is most likely multifactorial, including both genetic and environmental factors. One of the most promising hypotheses for the etiology of schizophrenia is immune system involvement, either immunological alterations or autoimmune mechanisms (Radhakrishnan et al., 2017).

The relationship between the immune system and the central nervous system (CNS) is highly complex. Nevertheless, some authors have suggested that increased brain pro-inflammatory status, decreased neurotrophic function, and increased production of neurotoxic cytokines in the brain could play a key role in the pathophysiology of schizophrenia (Sommer et al., 2014). Circulating white blood cells (leucocytes), which are part of the peripheral immune system, play a crucial role in inflammatory processes, but have received scant attention in the context of immune activation and brain structural alterations in schizophrenia. Under certain pathological conditions, the blood-brain barrier (BBB) may be disrupted and allow peripheral blood cells to enter the brain (Prinz and Priller, 2017). Studies conducted in rats have shown an increase in BBB permeability after acute stress exposure (Sharma et al., 1991; Esposito et al., 2001). Some evidence suggests that the BBB may also be disrupted in schizophrenia (Kirkpatrick and Miller, 2013).

Several studies, both in mice and humans, have explored the effects of leucocytes in CNS injuries. One study in mice suggested that neutrophils may be involved in edema formation, as well as contributing to cell death and tissue loss in the brain (Kenne et al., 2012). Another study showed that neutrophils promote BBB disruption, cerebral edema, cellular injury, and neurologic impairment after ischemic stroke (Jickling et al., 2015). Another study in mice found that infiltrating monocytes promote brain inflammation and exacerbate neuronal damage (Varvel et al., 2016); however, those findings were contradicted by the results of another study, which suggested that post-stroke peripheral monocytes may be protective (Gliem et al., 2016).

Based on the currently available evidence, it is still not clear whether lymphocytes have a beneficial or detrimental effect on brain tissue (Brait et al., 2012). One study found accelerated progression of Alzheimer's disease in mice lacking lymphocytes (Marsh et al., 2016). By contrast, in a study involving immunodeficient (severe combined immunodeficiency [SCID] or nude) mice, reconstitution of the immune compartment with T cells improved recovery from CNS injury, suggesting that T cells play a role in neuroprotection (Kipnis et al., 2002).

Numerous studies have evaluated the role of lymphocytes in schizophrenia and first-episode psychosis (FEP). A meta-analysis of 16 studies concluded that absolute levels of total lymphocytes were significantly decreased in FEP patients (Miller et al., 2013). A more recent meta-analysis indicated that patients with non-affective psychosis had significantly higher neutrophil-lymphocyte (NLR) and monocyte-lymphocyte (MLR) ratios than healthy controls (Mazza et al., 2020). Another study in FEP patients found that higher neutrophil count was associated with reduced gray matter volume, but increased cerebrospinal fluid (CSF) volume (Núñez et al., 2019). In that study, a higher neutrophil count was associated with more severe hallucination and avolition symptoms.

Alterations in hippocampal volume have been consistently found in schizophrenia, thus supporting the possible association between structural brain abnormalities and schizophrenia. The hippocampus is a particularly interesting region of interest due to its involvement in memory and regulation of affect, both of which are compromised in patients with schizophrenia (van Erp et al., 2016). Anatomical studies have shown volume deficits and shape deformities associated with cognitive impairment (Gardner et al., 2010), negative symptoms (Makowski et al., 2017) and depressive symptoms (Barch et al., 2019). T lymphocytes may modulate behavior, cognition, and adult hippocampal neurogenesis through direct interaction with the CNS (Song et al., 2020). In both schizophrenia and FEP, progressive brain volume disruptions may be at least partially caused by the effects of peripheral white blood cells on the CNS (Núñez et al., 2019; Zhang et al., 2016). However, the role of immune parameters—particularly white blood cells—in hippocampal volumetric alterations is still unknown.

In short, the available data suggest that peripheral immune system alterations may be associated with brain volume alterations in patients with schizophrenia and/or FEP. To date, however, only one study has been conducted to assess the role of these alterations in patients with FEP (Núñez et al., 2019). In that study, decreased neutrophil count was significantly associated with decreased gray matter volume and worse symptomatology. However, to our knowledge, none of the studies conducted to date have specifically assessed the hippocampus as a specific Region of Interest (ROI) in FEP and schizophrenia.

Given this background, the aim of the present study was to determine the blood cell types associated with hippocampal volume in FEP patients and the nature of the association. A second aim was to assess the association between blood cells and clinical symptomatology (positive, negative, cognitive, and depressive symptoms).

We hypothesized that hippocampal volume in FEP patients would be associated with the blood cells count

(specifically neutrophils and/or lymphocytes) and, furthermore, white blood cells count (specifically neutrophils and/or lymphocytes) would be associated with clinical symptomatology.

## 2. Experimental procedures

### 2.1. Study population

We included 50 consecutive drug-naïve FEP patients treated between April 2013 and July 2017 within the Study and Treatment of First-Episode Psychosis Program (*Estudi i Tractament de Primers Episodis Psicòtics*; ETEP) at the Hospital del Mar (Barcelona, Spain). ETEP is a specialized, early intervention unit for young adults (age 18 to 35 years) with FEP. The program consists of a multimodal intervention, including extensive assessment with intensive medical and psychosocial treatment. The ETEP has been extensively described in detail elsewhere (Bergé et al., 2016).

The study inclusion criteria were: (1) age 18–35 years; (2) fulfillment of DSM-IV-TR criteria for any of the following: brief psychotic disorder, schizophriform disorder, schizophrenia with < one year of symptoms, or unspecified psychosis; (3) no previous history of severe neurological medical conditions or severe traumatic brain injury; (4) presumed IQ level > 80 based on clinical records (evidence from past IQ assessments or suggested by the patient's educational or employment level), and (5) no substance abuse or dependence disorders except for cannabis and/or nicotine use. All patients were antipsychotic/ antidepressant-naïve. Treatment with benzodiazepines was allowed.

The local ethics committee approved this study. All participants provided written informed consent.

### 2.2. Clinical assessment and demographic data

All patients underwent a comprehensive assessment by two experienced psychiatrists (A.M., D.B.) at baseline and at the one-year follow-up. Sociodemographic variables were recorded. The clinical assessment included the following: structured clinical interview for DSM-IV- TR Axis I disorders for diagnosis; substance use assessment including tobacco (cigarettes/day) and cannabis use (user/nonuser); the Positive and Negative Syndrome Scale (PANSS) for psychotic-related symptoms (Kay, 1990); the Global Assessment of Function- ing (GAF) (Aas, 2011) for functionality; and the Calgary Depression Scale for Schizophrenia (CDSS) for depressive symptoms (Addington et al., 1990).

To better capture specific symptom dimensions, we calculated the five factor dimensions defined by Marder and colleagues (hostility, cognition, depression, negative and positive symptoms) from the PANSS scores: (Marder et al., 1997). The antipsychotic dose at the 12-month follow- up was obtained from medical records and these doses were converted into chlorpro- mazine equivalents (mg/day) to facilitate comparison (Gardner et al., 2010). Any antipsychotic treatments taken by patients at follow-up were included in the analyses.

### 2.3. Collection of blood samples

Fasting blood samples were obtained upon the patient's arrival at the hospital prior to administering any medication (except for benzodiazepines). All blood samples were obtained in the morning (between 8 a.m. and 12 p.m.). Blood samples were collected in glass K3-EDTA blood-drawing tubes for whole blood.

### 2.4. Image acquisition and processing

Baseline brain imaging was performed with a Philips Achieva 3.0 Tesla magnet resonance imaging (MRI) scanner (Philips Healthcare; Best, The Netherlands) equipped with an eight-channel phased-array head coil. The imaging protocol involved the acquisition of high-resolution anatomic three-dimensional (3D) images based on a T1-weighted fast spoiled gradient inversion recovery prepared sequence with the following parameters: repetition time 8.2 ms; echo time 3.8 ms; flip angle 8°; field of view 24 cm; 256 × 256- pixel matrix; in-plane resolution 0.94 × 0.94 mm<sup>2</sup>; and slice thickness 1 mm.

Anatomical images were visually inspected before analysis by a trained operator to detect any motion effect. No gross brain abnormalities were identified. The quality of the raw T1- weighted MRI scans was quantitatively assessed by using the automated weighted image quality rating (IQR), a measure of general image quality combining parameters of noise, inhomogeneities, and spatial resolution, provided by the CAT12 toolbox (<http://dbm.neuro.uni-jena.de/cat/>); scores > 60% were considered of sufficient quality for inclusion in subsequent analyses. All of the scans passed this threshold.

Volumetric segmentation was performed using the fully automated and validated segmentation software package FreeSurfer v6.0 (<http://surfer.nmr.mgh.harvard.edu>) with the default “recon-all” stream as described in previous studies (Haukvik et al., 2016; Sasabayashi et al., 2020; Fischl et al., 2002). Briefly, important preprocessing steps included removal of non-brain tissue, intensity normalization, automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures (Pujol et al., 2020). Segmentations of the hippocampus (left and right hemisphere) were visually inspected for accuracy by overlaying the segmentation label of each structure on the individual T1-weighted brain scan; specifically, the imaging quality control protocol for subcortical segmentations developed by the ENIGMA consortium (<http://enigma.ini.usc.edu/protocols/imaging-protocols/>) was used to generate a standard set of images from each participant's brain scan, displaying boundaries of segmented structures on a series of slices in axial, coronal, and sagittal planes for visual inspection of segmentation inaccuracies. The estimated left and right hemisphere hippocampal volumes, as well as estimated total intracranial volume, were extracted for each participant from the aseg.stats output files in FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/fswiki/asegstats2table>). Group-level means with standard deviations (SD) and histogram plots were used to identify statistical outliers (defined as measured volume > 2.698 SDs from the global mean) and to confirm the normal distribution of volumetric data.

The relative volume ratio for the anatomical regions of interest was calculated as the volume in native space (ml)/total intracranial volume (L). The MRI assessment was performed at least two weeks after the initial evaluation when patients were able to stand still (i.e., without high psychomotor agitation or disorganized behavior, thus allowing them to tolerate the MRI procedure).

### 2.5. Statistical analysis

The Kolmogorov-Smirnov test was used to test the normality of the data distribution. Univariate analyses were performed to compare baseline variables between completers and non-completers (lost to follow-up).

To evaluate the associations between relative left and right hippocampal volumes and white cell counts (leukocytes, neutrophils, lymphocytes, monocytes, basophils and eosinophils) a two-stage analysis plan was implemented. Firstly, exploratory univariate analyses were performed using a Pearson's correlation. Secondly, those

**Table 1** Sociodemographic, clinical, biochemical and volumetric characteristics of FEP patients at baseline and at one-year follow-up.

Variable	Baseline	One-year follow-up
Age, median (IQR)	26 (24 - 30.25)	
Sex, n (% female)	22 (44)	
DUP in days, median (IQR)	31 (8 - 115)	
Cannabis use, n (% users)	29 (58)	
Tobacco use, median (IQR)	4.5 (0 - 14)	
PANSS P score, m (sd)	24.88 (6.74)	12.69 (7.03)
PANSS N score, m (sd)	16.86 (6.65)	17.18 (5.89)
PANSS GP score, m (sd)	43.68 (8.27)	31.67 (9.22)
PANSS T score, m (sd)	85.24 (15.76)	62.51 (19.39)
CDSS score, m (sd)	1.22 (2.02)	0.64 (1.11)
GAF score, m (sd)	29.7 (8.89)	61.18 (17.68)
Leukocytes in $\times 10^3$ _u/mcL, m (sd)	11.89 (7.04)	
Neutrophils in $\times 10^3$ _u/mcL, m (sd)	8.99 (6.89)	
Lymphocytes in $\times 10^3$ _u/mcL, m (sd)	1.95 (0.76)	
Monocytes in $\times 10^3$ _u/mcL, m (sd)	0.81 (0.44)	
Basophils in $\times 10^3$ _u/mcL, m (sd)	0.13 (0.13)	
Eosinophils in $\times 10^3$ _u/mcL, m (sd)	0.03 (0.02)	
Relative left hippocampus in mL	2.64 (0.27)	
Relative right hippocampus in mL	2.78 (0.31)	

\*All values given as means with standard deviation, unless otherwise indicated.

Abbreviations: *N* = sample size; IQR = interquartile range; mL = millilitres; DUP = duration untreated psychosis; PANSS *P* = Positive and Negative Syndrome Scale positive; PANSS *N* = Positive and Negative Syndrome Scale negative; PANSS GP = Positive and Negative Syndrome Scale general pathology; PANSS *T* = Positive and Negative Syndrome Scale total; CDSS = Calgary Depression Scale for Schizophrenia; GAF = Global Assessment of Functioning.

white cell lines associated with hippocampal volumes at a priori specified probability value of 0.10 were included in subsequent analyses to assess the robustness of the association after adjusting for the effects of potential confounders. These potential confounders were: age at onset of psychosis, gender, benzodiazepine treatment, duration of untreated psychosis (DUP) and baseline cannabis use. DUP was specified as it has been consistently shown to be a predictor of many clinical outcomes of FEP (Harris et al., 2005). And it has been shown that hippocampus structure could be altered in psychotic patients due cannabis use (Batalla et al., 2013).

The same method was used to explore which variables were associated between symptomatology at baseline and at one-year follow-up (Marder 5-factors) and white cell count. As only Marder depression score showed significance, we decided to add a confirmatory analysis repeating the same procedure with CDSS, a scale specifically developed to assess depressive symptoms in patients with psychosis (Addington et al., 1990).

### 3. Results

#### 3.1. Characteristics of FEP patients

A total of 50 patients were included in this study. Sociodemographic, clinical, biochemical, and the hippocampal volumetric characteristics of the patients at baseline and at one-year are shown in Table 1. Eleven patients were lost to follow-up. However, there were no statistically significant differences between the completers and non-completers (Supplementary Table 1).

Most of the patients were treated with benzodiazepines at inclusion (86%), with a mean ( $\pm$  SD) dose of clonazepam

1 ( $\pm$  0.5) mg. At one-year follow-up, the mean ( $\pm$  SD) chlorpromazine equivalents dose was 295.61 ( $\pm$  157.76).

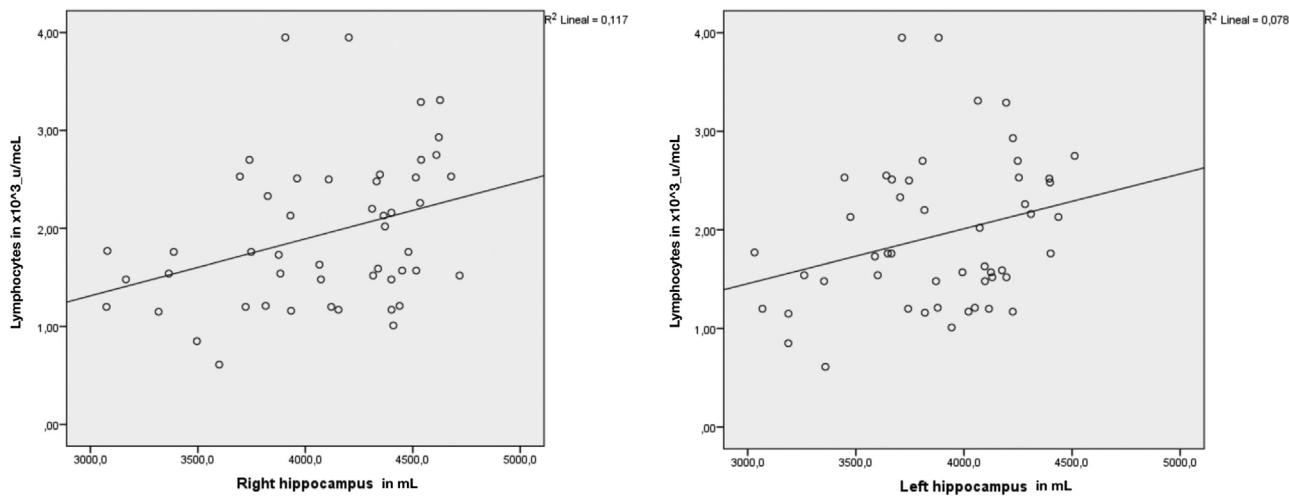
The mean ( $\pm$  SD) absolute hippocampal volume in this FEP cohort was 3.88 mL ( $\pm$  0.38) and 4.09 mL ( $\pm$  0.45) in the left and right hippocampus, respectively. Considering the absolute total intracranial volume, the relative volumes of these patients were significantly lower than healthy controls, according to our previous study (Toll et al., 2022). These data were comparable to previous studies in similar clinical populations using the same FreeSurfer method (Fan et al., 2019; Gardner et al., 2010; Haukvik et al., 2016; Sasabayashi et al., 2020; Pujol et al., 2020).

#### 3.2. White cells and brain volume

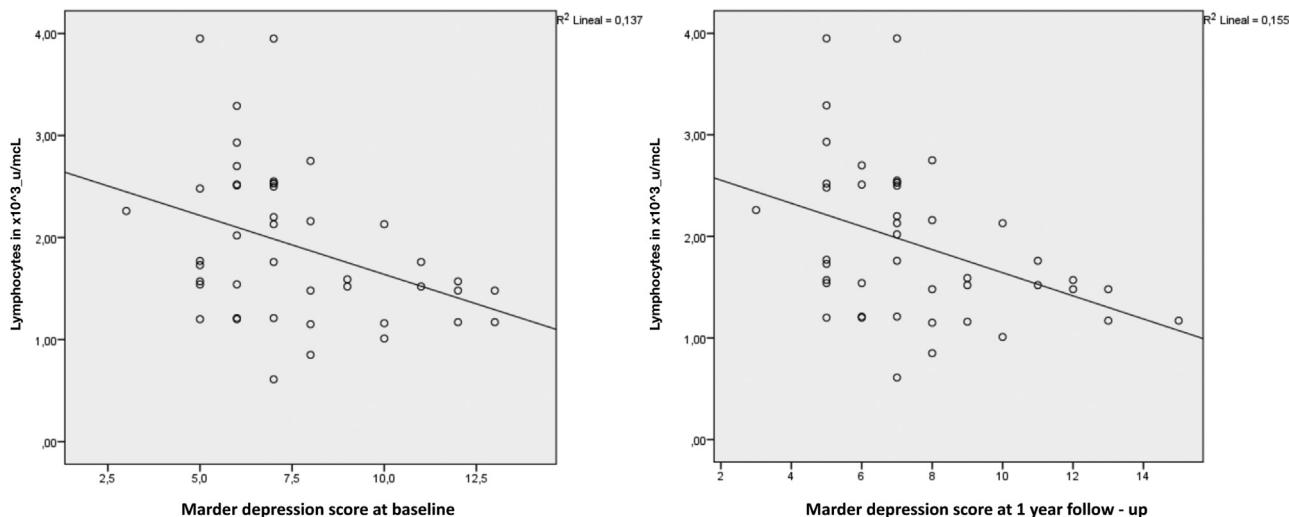
In this patient cohort, lymphocyte count was positively correlated with the right ( $r = 0.28, p = 0.049$ ) and left ( $r = 0.34, p = 0.015$ ) hippocampus at baseline (Fig. 1). After Bonferroni's correction, they lost the significance ( $0.049$  and  $0.015 > 0.013$ ).

The other white cell lines evaluated showed no correlation with either the left or right hippocampal volumes (Supplementary Table 2).

On the multivariate analysis, the model that best predicted left hippocampal volume was a single variable model (sex), with an adjusted  $R^2$  of 0.165. Thus, female sex ( $B = 0.22$ ; 95% confidence interval [CI]: 0.07 - 0.36;  $p = 0.005$ ) was significantly associated with higher left hippocampal volume in FEP patients at baseline. A bivariate (sex and lymphocyte count) model was the best predictor of right hippocampal volume, with an adjusted  $R^2$  of 0.391,



**Fig. 1** Correlation between lymphocytes and right and left hippocampus in FEP patients at baseline.



**Fig. 2** Correlation between lymphocytes and Marder depression score at baseline and one-year follow-up in FEP patients.

confirming that female sex ( $B = 0.19$ ; 95% CI: 0.01 - 0.37;  $p = 0.044$ ) and higher lymphocyte count ( $B = 0.08$ ; 95% CI: -0.05 - 0.21;  $p = 0.028$ ) were associated with greater right hippocampal volume in FEP patients at baseline.

### 3.3. White blood cells and Marder five-factor model scores

Lymphocytes were negatively correlated with the Marder depression score at baseline ( $r = -0.37$ ,  $p = 0.013$ ) and at the one-year follow-up ( $r = -0.39$ ,  $p = 0.008$ ) (Fig. 2). After Bonferroni's correction, they maintained the significance ( $p < 0.013$ ).

The other white cell lines were not correlated with the other Marder five factor model scores at the one-year follow-up (Supplementary Tables 3 and 4).

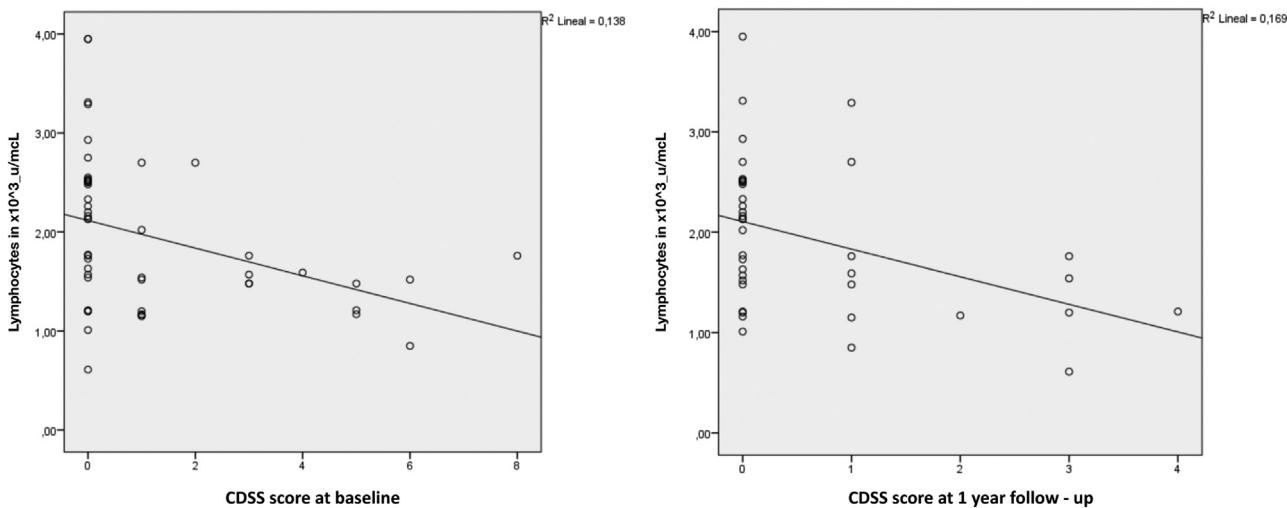
On the multivariate analysis, the model that best predicted the Marder depression score at baseline included a single variable (lymphocyte count), with an adjusted  $R^2$  of 0.137. Consequently, lower lymphocyte counts ( $B = -1.19$ ;

95% CI: -2.12 to -0.26;  $p = 0.013$ ) were significantly associated with increased depressive symptomatology in FEP patients at baseline. This same single-variable model was also the best predictive model for Marder depression score at one-year, with an adjusted  $R^2$  of 0.175, demonstrating that a lower lymphocyte count ( $B = -1.37$ ; 95% CI: -2.43 to -0.32;  $p = 0.012$ ) was significantly associated with higher depressive symptomatology in FEP patients at one year.

### 3.4. White blood cells and depressive symptomatology

Lymphocyte counts were negatively correlated with CDSS scores at baseline ( $r = -0.37$ ,  $p = 0.008$ ) and at one-year ( $r = -0.41$ ,  $p = 0.009$ ) (Fig. 3). After Bonferroni's correction, they maintained the significance ( $p < 0.013$ ).

None of the other white cell lines correlated with CDSS scores at baseline or at the one-year follow-up (Supplementary Table 5).



**Fig. 3** Correlation between lymphocytes and CDSS score in FEP patients at baseline and at one-year follow-up.

On the multivariate analysis, the model that best predicted baseline CDSS scores included only a single-variable (lymphocyte count), with an adjusted  $R^2$  of 0.138. This finding shows that lower lymphocyte count ( $B = -0.99$ ; 95% CI:  $-1.71$  to  $-0.27$ ;  $p = 0.008$ ) was associated with higher depressive symptomatology in FEP patients at baseline. The same single-variable model was also the best predictor of CDSS scores at one-year, with an adjusted  $R^2$  of 0.279. Consequently, lower lymphocyte count ( $B = -0.51$ ; 95% CI:  $-0.95$  to  $-0.07$ ;  $p = 0.026$ ) was significantly associated with higher depressive symptomatology in FEP patients at the one-year follow-up.

#### 4. Discussion

In the present study, a higher lymphocyte count was associated with larger right hippocampal volume at baseline in drug-naïve FEP patients. The presence of a higher lymphocyte count was also associated with less depressive symptomatology (as measured by CDSS and the Marder depressive factor from PANSS) at baseline and at the one-year follow-up.

A higher lymphocyte count was associated with a larger hippocampal volume. This finding is consistent with data from animal studies, which suggest that lymphocytes, particularly T-cells, have a protective effect on the brain (Marsh et al., 2016; Kipnis et al., 2002), stimulate neurogenesis (Ritzel et al., 2016), and have a beneficial effect on hippocampal-dependent learning (Radjavi et al., 2014). Postmortem studies of brain samples obtained from patients with schizophrenia (Busse et al., 2012) have described a significant increase in the number of T-lymphocytes in the superior temporal gyrus (Sneeboer et al., 2020) and in other cortical areas (Bogerts et al., 2018). More specifically, those studies found greater infiltration of T lymphocytes in the hippocampus of patients with residual schizophrenia compared to those diagnosed with paranoid schizophrenia. This increase in lymphocytes in schizophrenia patients versus controls would seem to contradict our findings regarding the suggested neuroprotective effect of lymphocytes. However,

the observed increase in lymphocytes in that study may be due to an adaptative response to an hyperinflammatory state (Moro-García et al., 2018). Inflammation and oxidative stress are essentially associated and, neural damage caused by oxidative stress, can activate inflammation and immune response (Lugrin et al., 2014). The inflammation process has been suggested to have a key role in the promotion of neurogenesis (Brait et al., 2012), mainly through the release of growth-related proteins and cytokines, potentially from T lymphocytes as well as other peripheral and resident immune cells. It has been suggested that this action is specifically due to CD4+ and not CD8+ T cells (Wolf et al., 2009), and it occurs through classical CNS antigen-dependent T-cell activation through neurotrophic factors increase (Ziv et al., 2006). However, if the inflammation process is dysregulated, it can cause neuronal damage (Mondelli et al., 2011). In these sense, a previous study in FEP patients found the expression of IL-6, to be independently an associated with smaller hippocampal volume.

In our cohort, a higher lymphocyte count was also associated with less depressive symptomatology over one-year follow-up. These findings are in line with animal studies that have found that nude mice repopulated with T lymphocyte cells exhibited less anxious and depressive-like behaviors and increased levels of hippocampal cell proliferation than controls (Upthegrove et al., 2014). Only a few studies have investigated the relationship between lymphocytes and specific clinical features in schizophrenia and FEP (Núñez et al., 2019). However, one study found that lymphopenia and a relative granulocytosis at baseline predicted poorer recovery after six months of antipsychotic treatment in neuroleptic-free naïve patients with schizophrenia and predominantly positive symptoms, but not in those with negative symptomatology (Zorrilla et al., 1998). However, that study did not evaluate the potential association between lymphocyte count and depressive symptomatology.

In our study, female sex was associated with greater right and left hippocampal volumes. Similar findings have been previously reported by other authors (Pruessner et al., 2014), suggesting that sex differences in schizophrenia could be a consequence of differences in brain development

between males and females (Goldstein et al., 2002). The putative neuroprotective effect of estrogen in women and its absence in men has been suggested as an explanation for these differences in hippocampal volume (Pruessner et al., 2014; Hu et al., 2013; Lord et al., 2008).

Although other studies have found an association between white cells and another symptomatology type in FEP patients, we were unable to replicate this finding. For example, Núñez et al. evaluated 137 patients with FEP, finding that higher neutrophil count was associated with higher total PANNS scores (including both positive and negative symptomatology) (Núñez et al., 2019). These results are consistent with the findings of another recent study, which showed that FEP patients with high neutrophil or monocyte counts had higher PANSS-P scores at baseline (Steiner et al., 2020). Another study showed that more severe negative and cognitive symptoms were associated with decreased relative numbers of CD4+ memory T cells in patients with treatment-resistant schizophrenia (Fernandez-Egea et al., 2016). The findings of those studies, which are inconsistent with our results, could potentially be explained by the limited sample size in our study or other methodological differences, such as the inclusion of antipsychotic treatment in other studies.

We believe that our findings are highly relevant given how inexpensive and convenient it is to measure lymphocyte counts, which can be quantified through a single blood sample. Furthermore, these results open the door to identify new therapeutic targets for psychosis and related disorders by modulating adaptative immune response. Nevertheless, more research is needed to assess whether this therapeutic approach could improve the course of psychotic disorders.

#### 4.1. Study strengths and limitations

This study has several limitations, most notably the observational design, which does not allow us to infer a cause-effect relationship. In addition, MRI acquisition and blood sampling were not always performed on the same day. By contrast, the study also has several strengths, most importantly the large number of antipsychotic-naïve patients with FEP. This clinical population is particularly difficult to recruit because antipsychotic treatment is generally prescribed shortly after the diagnosis of psychosis, and antipsychotics can alter lymphocyte counts (Dawidowski et al., 2021). Another strength is the longitudinal study design and high retention rate (78%). A final strength is that we controlled for numerous potential confounding factors (e.g., sex, age, body mass index, and tobacco and cannabis use). This approach strengthens our findings and provides a more accurate assessment of the true association between white cells, hippocampal volumes, and symptomatology.

#### 4.2. Conclusions

This study demonstrates that higher lymphocyte counts in antipsychotic-naïve FEP patients is associated with larger hippocampal volume at baseline and with a greater reduction in depressive symptoms over the course of follow-up. These findings may allow researchers to identify new

biomarkers and therapeutic targets for patients with schizophrenia.

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The present study does not have any funding sources.

#### Author contributions

A. Toll and A. Mané designed the study. D. Bergé acquired the data, which L. Blanco Hinojo and V. Pérez-Solà analyzed. A. Toll wrote the article, which all other authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

#### Declaration of Competing Interest

A. Mané and D. Bergé have received financial support to attend meetings, travel support, and served as a speaker from Otsuka and Janssen Cilag. The other authors of this manuscript do not have any conflicts of interest.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2023.01.002.

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