UNIVERSITAT DE BARCELONA

Predictive value of white blood cell counts, ratios and C-reactive protein in schizophrenia spectrum disorder

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El trabajo sale
Vicente Llorca Llidó
(1958-2022)

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ABBREVIATIONS AND ACRONYMS

AHA: American Heart Association

AUC: Area Under the Curve

BCB: Blood-Cerebrospinal Fluid Barrier

BLR: Basophil-to-lymphocyte ratio

BMI: Body Mass Index

C4: Complement Component 4

CBC: Complete Blood Count

CDC: Centers for Disease Control and Prevention

CGI-S: Clinical Global Impression-Severity

CNS: Central Nervous System

CRP: C-Reactive Protein

CSF: Cerebrospinal Fluid

CPZ: Chlorpromazine

DNA: Deoxyribonucleic Acid

DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

ECT: Electroconvulsive Therapy

ELR: Eosinophil-to-lymphocyte ratio

FEP: First Episode of Psychosis

FES: First Episode of Schizophrenia

FGAs: First-Generation Antipsychotics

GAF: Global Assessment of Functioning

GWAS: Genome-wide association study

HLA: Human Leukocyte Antigens

IFN: Interferon

IL: Interleukin

LAI: Long-Acting Injectable

LLR: Leukocyte-to-lymphocyte ratio

MHC: Major Histocompatibility Complex

MLR: Monocyte-to-lymphocyte ratio

NfL: Neurofilament Light

NLR: Neutrophil-to-lymphocyte ratio

NMDA: *N*-methyl-D-aspartate

PANSS: Positive and Negative Syndrome Scale

PET: Positron Emission Tomography

PLR: Platelet-to-lymphocyte ratio

Qalb: CSF-to-serum albumin ratio

RSWG: Remission in Schizophrenia Working Group

SGAs: Second-Generation Antipsychotics

SMD: Standardized Mean Difference

TNF: Tumor Necrosis Factor

TRS: Treatment-Resistant Schizophrenia

TSPO: 18-kDa translocator protein

WBC: White Blood Cells

LIST OF ARTICLES IN THE THESIS

This doctoral thesis is presented as a compendium of articles, with five hypotheses and their respective objectives. To achieve these, nine articles have been included—eight published papers and one currently under review. All the articles have either been published in, or are currently under review by, scientific journals ranked in the first (Q1) or second quartile (Q2), with one of them ranked in the first decile (D1) according to the Journal Citation Reports (JCR) in the Psychiatry category. The articles are listed below, numbered from 1 to 9, following the different stages of the natural history of patients with schizophrenia.

I. Bioque M, Catarina Matias-Martins A, **Llorca-Bofí V**, Mezquida G, Cuesta MJ, Vieta E, et al. Neutrophil to Lymphocyte Ratio in Patients With a First Episode of Psychosis: A Two-Year Longitudinal Follow-up Study. *Schizophr Bull*. 2022;48(6):1327-1335.
IF 2022: 6.6 (Psychiatry Q1)

II. **Llorca-Bofí V**, Madero S, Amoretti S, Cuesta MJ, Moreno C, González-Pinto A, et al. Inflammatory blood cells and ratios at remission for psychosis relapse prediction: A three-year follow-up of a cohort of first episodes of schizophrenia. *Schizophr Res*.2024;267:24-31.
IF 2023: 3.6 (Psychiatry Q1)

III. **Llorca-Bofí V**, Bioque M, Pàmpols-Pérez S, Buil-Riné E, Adrados-Pérez M, Nicolau-Subires E, et al. Immune biomarkers and functional outcomes in acute schizophrenia: a retrospective cohort study. *Under Review*

IV. **Llorca-Bofí V**, Palacios-Garrán R, Rey Routo D, Buil-Reiné E, Adrados-Pérez M, Gich I, et al. High neutrophil-lymphocyte ratio upon admission is associated with better response in psychotic depression. *J Psychiatr Res*. 2021;143:38-42.
IF 2021: 5.2 (Psychiatry Q2)

V. **Llorca-Bofí V**, Bioque M, Madero S, Mallorquí A, Oliveira C, Garriga M, et al. Blood Cell Count Ratios at Baseline are Associated with Initial Clinical Response to Clozapine in Treatment-Resistant, Clozapine-Naïve, Schizophrenia-Spectrum Disorder. *Pharmacopsychiatry*. 2024;57(4):173-179.
IF 2023: 3.6 (Psychiatry Q1)

VI. **Llorca-Bofí V**, Petersen LV, Mortensen PB, Benros ME. White Blood Cell Counts, Ratios, and C-Reactive Protein Among Individuals with Schizophrenia Spectrum Disorder and Associations with Long-term Outcomes: A Population-Based Study. *Brain Behav Immun*. 2024;122:18-26.
doi:10.1016/j.bbi.2024.07.041.
IF 2023: 8.8 (Psychiatry D1)

VII. Bioque M[†], **Llorca-Bofí V**[†], Salmerón S, García-Bueno B, MacDowell KS, Moreno C, et al. Association between neutrophil to lymphocyte ratio and inflammatory biomarkers in patients with a first episode of psychosis. *J Psychiatr Res*. 2024;172:334-339.
IF 2023: 3.7 (Psychiatry Q1)

VIII. **Llorca-Bofí V**, Bioque M, Font M, Gich I, Mur M. Correlation between C-reactive protein and the inflammatory ratios in acute schizophrenia inpatients: are they associated*? J Psychiatr Res*. 2023;165:191-196.
IF 2023: 3.7 (Psychiatry Q1)

IX. **Llorca-Bofí V**, Mur M, Font M, Palacios-Garrán R, Sellart M, del Agua-Martínez E, et al. Differences in total and differential WBC counts and inflammatory parameters between psychiatric inpatients with and without recent consumption of cannabinoids, opioids, or cocaine: a retrospective single-center study. *Brain Behav Immun Health*. 2024;42:100898.
IF 2023: 3.7 (Psychiatry Q1)

Total Research Line Impact Factor: 35

SUMMARY

English

Introduction

Schizophrenia is a severe psychiatric disorder that affects approximately 0.5-1% of the population. It typically follows a chronic course characterized by multiple relapses, with up to 30% of patients experiencing treatment-resistant schizophrenia (TRS) and facing a reduced life expectancy. The causes of schizophrenia are complex and involve a combination of genetic and environmental risk factors, leading to various hypotheses about its origins. One prominent theory is the immune hypothesis, which suggests that immune system dysfunction plays a critical role in the disorder's etiopathophysiology. While numerous biomarkers have been investigated for their potential to indicate this dysfunction, the predictive value of cost-effective markers, such as white blood cell (WBC) counts, ratios, and C-reactive protein (CRP), has been largely understudied.

Hypotheses:

The main hypothesis of this thesis is that immune biomarkers, such as WBC counts, their ratios, and CRP levels, can predict clinical outcomes in patients with schizophrenia and other psychotic disorders across different phases of the illness, including treatment response, relapse, readmissions, and mortality.

Objectives

To investigate the previously stated hypotheses, five specific objectives are outlined, with nine articles addressing them. The first objective (**Articles I and II**) focuses on the early stages of the disease, evaluating the relationship between the neutrophil-to-lymphocyte ratio (NLR) and treatment response over three years in patients with a First Episode of Psychosis (FEP), and assessing whether WBC counts and ratios in stable patients after a First Episode of Schizophrenia (FES) can predict relapse. The second objective (**Articles III and IV**) examines acutely hospitalized patients with established psychotic disorders, exploring how WBC counts, ratios, and CRP levels can predict functional response, as measured by Global Assessment of Functioning (GAF) scores. The third objective (Article V) focuses on TRS, investigating whether WBC count ratios can predict response to clozapine initiation, assessed by Positive and Negative Syndrome Scale (PANSS)

scores. The fourth objective (**Article VI**) looks at long-term outcomes, exploring the association between WBC counts, ratios, and CRP levels in FEP patients, and their relationship to mortality, treatment response, and readmissions, using register-based proxies. The fifth objective (**Articles VII, VIII, and IX**) investigates the relationship between WBC count ratios and other immune markers in acutely hospitalized psychiatric patients, and examines how substance use (such as cannabis, opioids, or cocaine) impacts these markers.

Methods:

The nine articles included in this thesis encompass a variety of study designs, primarily focusing on longitudinal cohorts, both prospective and retrospective, while also incorporating cross-sectional studies. Data for these studies were drawn from three FEP multicentre cohorts in Spain: the PEPs cohort (**Article I**), the 2EPs cohort (**Article II**), and the FLAMM-PEPs cohort (**Article VII**). Additional data sources include registry databases from the Copenhagen Research Center for Mental Health in Denmark (**Article VI**), the outpatient clinic of the Barcelona Clinic Schizophrenia Unit (BCSU) in Barcelona, Spain (**Article V**), and the acute inpatient unit of the psychiatry department at Hospital Santa Maria in Lleida, Spain (**Article III, IV, VIII, and IX**).

Results:

In **Article I**, patients with a FEP who did not achieve remission by the end of the two-year follow-up had significantly higher NLR values compared to those who did. **Article II** found that, among patients who had remitted after a FES, elevated monocyte and basophil counts increased the risk of relapse, with AUCs of 0.661 and 0.752, respectively, limiting their clinical relevance. In **Article III**, for patients experiencing an acute relapse of schizophrenia requiring hospitalization, higher leukocyte counts increased the risk for a non-functional response, while a higher platelet-to-lymphocyte ratio (PLR) showed a protective effect, with AUCs of 0.520 and 0.532, respectively. Additionally, elevated lymphocyte and platelet counts were protective against non-functional remission, with AUCs of 0.617 and 0.589, although these AUC values indicated a poor level of discrimination and insufficient predictive power for clinical application. **Article IV** linked NLR values upon admission to greater clinical improvement during hospitalization in patients with an acute episode of psychotic depression. In **Article V**, in patients with TRS, pre-treatment NLR and monocyte-to-lymphocyte ratio (MLR) values predicted improvements in PANSS-positive symptoms at 8 weeks of follow-up, with AUCs of

0.714 and 0.712, respectively. While these values reflect acceptable discrimination, they fall below the threshold for strong clinical utility. **Article VI** reported that, in patients with FES, higher CRP levels had the highest predictive value for mortality (AUC of 0.84), indicating significant clinical utility. Elevated leukocyte, neutrophil, monocyte, leukocyte-to-lymphocyte ratio (LLR), NLR, and MLR values were associated with treatment resistance, while higher platelet counts were linked to a reduced risk of psychiatric readmissions. Conversely, higher LLR was associated with an increased risk of readmissions. In **Article VII**, NLR values in FEP patients were moderately positively correlated with levels of the prostaglandin E2. **Article VIII** showed that in acute schizophrenia patients, CRP was significantly, though moderately, associated with NLR, while its associations with PLR and MLR were weaker, and no association was found with the basophil-to-lymphocyte ratio (BLR). Finally, **Article IX** reported that recent cannabinoid use was associated with significantly higher levels of neutrophils in individuals with psychotic disorders. In contrast, recent cocaine use was linked to eosinophilia, while opioid use was associated with significantly lower MLR, regardless of the primary psychiatric diagnosis.

Conclusion

WBC counts, their ratios, and CRP levels were associated with various outcomes across the course of schizophrenia, reflecting both protective and risk factors. Elevated CRP levels at FES were strongly associated with an increased risk of long-term mortality, demonstrating high discriminatory power and potential clinical applicability. Higher NLR and MLR levels prior to treatment were linked to better clinical responses when initiating clozapine in TRS, with moderate discriminatory accuracy; however, larger studies are required to validate their clinical utility. Other biomarkers and scenarios demonstrated poor discriminatory ability, indicating limited clinical relevance.

Keywords (UNESCO Code)

Psychiatry (321100)

Immunology (241200)

Psychopharmacology (320909)

Mortality (520404).

Castellano

Título

Valor predictivo del recuento de leucocitos, sus ratios y la proteína C reactiva en los trastornos del espectro de la esquizofrenia

Introducción

La esquizofrenia es un trastorno psiquiátrico grave que afecta aproximadamente al 0.5-1% de la población. Suele seguir un curso crónico caracterizado por múltiples recaídas, hasta el 30% de los pacientes experimentan resistencia al tratamiento (TRS) y conlleva una esperanza de vida reducida. Las causas de la esquizofrenia son complejas e involucran una combinación de factores de riesgo genéticos y ambientales, lo que da lugar a diversas hipótesis sobre su origen. La hipótesis inmune es una de ellas y sugiere que la disfunción del sistema inmune juega un papel crucial en la etiopatofisiología del trastorno. Aunque se han investigado numerosos biomarcadores para caracterizar dicha disfunción, el valor predictivo de marcadores rentables, como los recuentos de leucocitos, sus ratios y la proteína C reactiva (PCR), ha sido poco estudiado.

Hipótesis

La hipótesis principal de esta tesis es que los biomarcadores inmunológicos, como los recuentos de leucocitos, sus ratios y los niveles de PCR, pueden predecir los resultados clínicos en pacientes con esquizofrenia y otros trastornos psicóticos en diferentes fases de la enfermedad, incluyendo la respuesta al tratamiento, las recaídas, los reingresos y la mortalidad.

Objetivos

Para investigar las hipótesis previamente planteadas, se han establecido cinco objetivos específicos, con nueve artículos que los abordan. El primer objetivo (**Artículos I y II**) se centra en las primeras etapas de la enfermedad, evaluando la relación entre la ratio neutrófilos-linfocitos (NLR) y la respuesta al tratamiento a lo largo de tres años en pacientes con un Primer Episodio de Psicosis (PEP), y evaluando si los recuentos y proporciones de leucocitos en pacientes estables tras un primer episodio de esquizofrenia (FES) pueden predecir las recaídas. El segundo objetivo (**Artículos III y IV**) se enfoca en pacientes con trastornos psicóticos establecidos que requirieron una hospitalización aguda, y explora cómo los recuentos de leucocitos, las proporciones y los niveles de CRP

pueden predecir la respuesta funcional, medida mediante las puntuaciones de la Evaluación Global del Funcionamiento (GAF). El tercer objetivo (**Artículo V**) se centra en la TRS, investigando si las proporciones de recuento de leucocitos pueden predecir la respuesta a la iniciación de clozapina, evaluada mediante las puntuaciones de la Escala de Síndromes Positivos y Negativos (PANSS). El cuarto objetivo (**Artículo VI**) analiza los resultados a largo plazo, explorando la asociación entre los recuentos de leucocitos, las proporciones y los niveles de CRP en pacientes con FEP, y su relación con la mortalidad, la respuesta al tratamiento y las readmisiones, utilizando medidas basadas en registros. El quinto objetivo (**Artículos VII, VIII y IX**) investiga la relación entre las proporciones de recuento de leucocitos y otros marcadores inmunitarios en pacientes psiquiátricos hospitalizados, y examina cómo el consumo de sustancias (como cannabis, opioides o cocaína) afecta estos marcadores.

Métodos

Los nueve artículos incluidos en esta tesis abarcan una variedad de diseños de estudio, centrados principalmente en cohortes longitudinales, tanto prospectivas como retrospectivas, e incorporando también estudios transversales. Los datos de estos estudios provienen de tres cohortes multicéntricas de PEP en España: la cohorte PEPs (**Artículo I**), la cohorte 2EPs (**Artículo II**) y la cohorte FLAMM-PEPs (**Artículo VII**). Fuentes adicionales de datos incluyen bases de datos registrales de Dinamarca (**Artículo VI**), la consulta externa de la Barcelona Clínic Schizophrenia Unit (BCSU) en Barcelona, España (**Artículo V**), y la unidad de hospitalización de agudos del departamento de psiquiatría del Hospital Santa María en Lleida, España (**Artículos III, IV, VIII** y **IX**).

Resultados

En el **Artículo I**, los pacientes con PEP que no alcanzaron la remisión al final de los dos años de seguimiento tuvieron valores significativamente más altos de NLR en comparación con aquellos que sí lo lograron. El **Artículo II** encontró que, entre los pacientes que habían remitido tras un primer episodio de esquizofrenia, los recuentos elevados de monocitos y basófilos aumentaron el riesgo de recaída, con AUC de 0.661 y 0.752, respectivamente, lo que limita su relevancia clínica. En el **Artículo III**, para los pacientes que experimentaron una recaída aguda de esquizofrenia que requirieron hospitalización, los recuentos elevados de leucocitos se asociaron con una mala respuesta funcional, mientras que una mayor ratio plaqueta-linfocito (PLR) se asoció con un efecto

protector, con AUC de 0.520 y 0.532, respectivamente. Además, los recuentos elevados de linfocitos y plaquetas fueron protectores contra la remisión no funcional, con AUC de 0.617 y 0.589, aunque estos valores de AUC indicaron un nivel de discriminación pobre y un poder predictivo insuficiente para la aplicación clínica. El **Artículo IV** vinculó los valores altos de NLR al ingreso con una mayor mejoría clínica durante la hospitalización en pacientes con un episodio agudo de depresión psicótica. En el **Artículo V**, en pacientes con TRS, los valores pretratamiento de NLR y la ratio monocito-linfocito (MLR) predijeron mejorías en los síntomas positivos de la PANSS a las 8 semanas de seguimiento, con AUC de 0.714 y 0.712, respectivamente. Aunque estos valores reflejan una discriminación aceptable, están por debajo del umbral para una utilidad clínica. El **Artículo VI** informó que, en pacientes con PEE, los niveles elevados de PCR tuvieron el mayor valor predictivo para la mortalidad (AUC de 0.84), lo que indica una utilidad clínica significativa. Los valores elevados de leucocitos, neutrófilos, monocitos, ratio leucocito-linfocito (LLR), NLR y MLR se asociaron con resistencia al tratamiento, mientras que los valores elevados de plaquetas se vincularon con un menor riesgo de reingresos psiquiátricos. En cambio, los valores elevados de LLR se asociaron con un mayor riesgo de reingresos. En el **Artículo VII**, los valores de NLR en pacientes con PEP estuvieron moderadamente correlacionados positivamente con los niveles de prostaglandina E2. El **Artículo VIII** mostró que, en pacientes con esquizofrenia aguda, la PCR se asoció significativamente, aunque moderadamente, con NLR, mientras que sus asociaciones con PLR y MLR fueron más débiles, y no se encontró ninguna asociación con la ratio basófilo-linfocito (BLR). Finalmente, el **Artículo IX** mostró que el uso reciente de cannabinoides se asoció con niveles significativamente más altos de neutrófilos en individuos con trastornos psicóticos. En cambio, el uso reciente de cocaína se vinculó con eosinofilia, mientras que el uso de opiáceos se asoció con una disminución significativa del MLR, independientemente del diagnóstico psiquiátrico primario.

Conclusión

Los recuentos de leucocitos, sus proporciones y los niveles de PCR estuvieron asociados con diversos resultados a lo largo del curso de la esquizofrenia, reflejando tanto factores protectores como de riesgo. Los niveles elevados de PCR en el primer episodio de esquizofrenia estuvieron fuertemente asociados con un mayor riesgo de mortalidad a largo plazo, demostrando un alto poder discriminatorio y una posible aplicabilidad clínica. Los niveles más altos de NLR y MLR antes del tratamiento estuvieron

relacionados con mejores respuestas clínicas al iniciar clozapina en la TRS, con una precisión discriminatoria moderada; sin embargo, se requieren estudios más grandes para validar su utilidad clínica. Otros biomarcadores y escenarios mostraron una baja capacidad discriminatoria, lo que indica una relevancia clínica limitada.

Palabras clave (Código UNESCO)

Psiquiatría (321100)

Inmunología (241200)

Psicofarmacología (320909)

Mortalidad (520404).

Català

Títol

Valor predictiu del recompte de leucòcits, les seves ràtios i la proteïna C reactiva en els trastorns de l'espectre de l'esquizofrènia

Introducció

L'esquizofrènia és un trastorn psiquiàtric greu que afecta aproximadament entre el 0,5% i l'1% de la població. Sol seguir un curs crònic caracteritzat per múltiples recaigudes; fins al 30% dels pacients experimenten resistència al tractament (TRS) i comporta una reducció de l'esperança de vida. Les causes de l'esquizofrènia són complexes i impliquen una combinació de factors de risc genètics i ambientals, fet que dona lloc a diverses hipòtesis sobre el seu origen. Una d'aquestes hipòtesis és la immunitària, que suggereix que la disfunció del sistema immunitari juga un paper crucial en l'etiopatogènesi del trastorn. Tot i que s'han investigat nombrosos biomarcadors per caracteritzar aquesta disfunció, el valor predictiu de marcadors assequibles, com els recomptes de leucòcits, les seves ràtios i la proteïna C reactiva (PCR), ha estat poc estudiat.

Hipòtesi

La hipòtesi principal d'aquesta tesi és que els biomarcadors immunològics, com els recomptes de leucòcits, les seves ràtios i els nivells de PCR, poden predir els resultats clínics en pacients amb esquizofrènia i altres trastorns psicòtics en diferents fases de la malaltia, incloent-hi la resposta al tractament, les recaigudes, els reingressos i la mortalitat.

Objectius

Per investigar les hipòtesis prèviament plantejades, s'han establert cinc objectius específics, abordats en nou articles. El primer objectiu (**Articles I i II**) se centra en les etapes primerenques de la malaltia, avaluant la relació entre la ràtio neutròfils-limfòcits (NLR) i la resposta al tractament al llarg de tres anys en pacients amb un Primer Episodi de Psicosi (PEP), i examinant si els recomptes i ràtio de leucòcits en pacients estables després d'un primer episodi d'esquizofrènia poden predir les recaigudes. El segon objectiu (**Articles III i IV**) es focalitza en pacients amb trastorns psicòtics establerts que han

requerit hospitalització aguda, i explora com els recomptes de leucòcits, les ràtio i els nivells de PCR poden predir la resposta funcional, mesurada mitjançant les puntuacions de l'Avaluació Global del Funcionament (GAF). El tercer objectiu (**Article V**) se centra en la TRS, investigant si les proporcions de recompte de leucòcits poden predir la resposta a la iniciació de clozapina, avaluada mitjançant les puntuacions de l'Escala de Síndromes Positius i Negatius (PANSS). El quart objectiu (**Article VI**) analitza els resultats a llarg termini, explorant l'associació entre els recomptes de leucòcits, les ràtio i els nivells de PCR en pacients amb PEP, i la seva relació amb la mortalitat, la resposta al tractament i les reingressos, utilitzant dades basades en registres. El cinquè objectiu (**Articles VII, VIII i IX**) investiga la relació entre les proporcions de recompte de leucòcits i altres marcadors immunitaris en pacients psiquiàtrics hospitalitzats, i examina com el consum de substàncies (com el cànnabis, els opiacis o la cocaïna) afecta aquests marcadors.

Mètodes

Els nou articles inclosos en aquesta tesi abasten una varietat de dissenys d'estudi, principalment de cohorts longitudinals, tant prospectives com retrospectives, i també estudis transversals. Les dades d'aquests estudis provenen de tres cohorts multicèntriques de PEP a Espanya: la cohort PEPs (**Article I**), la cohort 2EPs (**Article II**) i la cohort FLAMM-PEPs (**Article VII**). Altres fonts de dades inclouen bases de dades registrals de Dinamarca (Article VI), la consulta externa de la Barcelona Clínic Schizophrenia Unit (BCSU) a Barcelona, Espanya (**Article V**), i la unitat d'hospitalització d'aguts del departament de psiquiatria de l'Hospital Santa Maria a Lleida, Espanya (**Articles III, IV, VIII i IX**).

Resultats

A l'**Article I**, els pacients amb PEP que no van assolir la remissió al final dels dos anys de seguiment tenien valors significativament més alts de NLR en comparació amb aquells que sí que ho van aconseguir. L'**Article II** va trobar que, entre els pacients que havien assolit la remissió després d'un primer episodi d'esquizofrènia, els recomptes elevats de monòcits i basòfils augmentaven el risc de recaiguda, amb AUC de 0.661 i 0.752, respectivament, limitant la seva rellevància clínica. A l'**Article III**, els pacients que van experimentar una recaiguda aguda d'esquizofrènia que va requerir hospitalització, presentaven recomptes elevats de leucòcits associats a una mala resposta funcional,

mentre que una ràtio plaqueta-limfòcit (PLR) més alta es va associar amb un efecte protector (AUC de 0.520 i 0.532). L'**Article IV** va vincular els valors alts de NLR en l'ingrés amb una millor millora clínica durant l'hospitalització en pacients amb un episodi agut de depressió psicòtica. L'**Article V** va mostrar que, en pacients amb TRS, els valors pretractament de NLR i la ràtio monòcit-limfòcit (MLR) van predir millores en els símptomes positius de la PANSS a les 8 setmanes de seguiment, amb AUC de 0.714 i 0.712. L'**Article VI** va indicar que, en pacients amb PEP, els nivells elevats de PCR tenien el major valor predictiu per a la mortalitat (AUC de 0.84). L'**Article VII** va mostrar una correlació moderada entre els valors de NLR i els nivells de prostaglandina E2. L'Article VIII va indicar que la PCR es va associar significativament amb NLR, però menys amb PLR i MLR. L'**Article IX** va mostrar que el consum recent de cannabinoides es va associar amb nivells més alts de neutròfils en individus amb trastorns psicòtics, mentre que el consum de cocaïna es va relacionar amb eosinofília i el consum d'opiacis amb una disminució del MLR.

Conclusió

Els recomptes de leucòcits, les seves ràtio i els nivells de PCR es van associar amb diversos resultats al llarg del curs de l'esquizofrènia, reflectint factors tant protectors com de risc. Els nivells elevats de PCR en el primer episodi d'esquizofrènia es van associar fortament amb un major risc de mortalitat a llarg termini, demostrant un alt poder discriminatori i una possible aplicabilitat clínica. Altres biomarcadors i escenaris van mostrar una baixa capacitat discriminatòria, la qual cosa indica una rellevància clínica limitada.

Paraules clau (Codi UNESCO)

Psiquiatria (321100)

Immunologia (241200)

Psicofarmacologia (320909)

Mortalitat (520404).

1. INTRODUCTION

1.1. Schizophrenia, a brief overview

Schizophrenia is one of the most severe psychiatric disorders, with profound impacts on those affected. Recovery is often incomplete, and even those who improve face ongoing challenges such as social isolation, stigma, and limited relationship opportunities. Unemployment rates in Europe for individuals with schizophrenia are alarmingly high, between 70% and 90% (1). Additionally, life expectancy for people with schizophrenia is reduced by 13 to 15 years due to somatic comorbidities (2,3), and the suicide rate stands at approximately 5% to 10% (4). Consequently, schizophrenia is ranked among the top 15 causes of disability worldwide (5).

a. Epidemiology

The lifetime prevalence of schizophrenia is estimated to be around 0.5-1% of the global population (6), with slightly higher rates in the male population and in urban areas (7). It typically appears in late adolescence or early adulthood, with men often experiencing symptoms earlier than women (8). The disorder is seen across all cultures and socioeconomic groups, although factors like poverty and social adversity may increase risk (9).

b. Clinical features and diagnosis

schizophrenia presents with a mix of positive, negative, and cognitive symptoms. Positive symptoms include hallucinations, delusions, and disorganized thinking, while negative symptoms involve reduced emotional expression, social withdrawal, and lack of motivation. Cognitive impairments affect memory, attention, and executive function. The DSM-5 diagnosis requires at least two of the following: delusions, hallucinations, disorganized speech, disorganized or catatonic behaviour, and negative symptoms, with significant impairment in functioning. Symptoms must persist for at least six months, with active-phase symptoms for at least one month, or less if successfully treated (10).

c. Course and outcomes

schizophrenia is considered to begin before the first diagnosis, often marked by prodromic symptoms that gradually evolve into a psychotic episode, known as the First Episode of Psychosis (FEP). In about one-third of patients, these initial symptoms persist,

leading to a diagnosis of schizophrenia (11). The disorder usually follows a chronic, fluctuating course, with periods of exacerbation and remission (**Figure 1**). Unfortunately, over 80% of patients experience a relapse within the first five years of diagnosis, which can lead to worsening symptoms, cognitive decline, and a significant reduction in quality of life (12).

Both remission and relapse are defined based on clinical symptoms. The Remission in Schizophrenia Working Group (RSWG) defines remission as requiring mild severity (score of 3 or lower) in 8 specific items of the Positive and Negative Symptoms Scale (PANSS), with a minimum duration of 6 months during which these symptoms must be maintained (13). While various definitions of relapse have been used, including hospital admissions as a proxy for relapse in research, a recent evidence-based definition suggests that relapse is indicated by an increase of 12 or more points in the PANSS total score or a worsening of specific positive and disorganization symptoms (14).

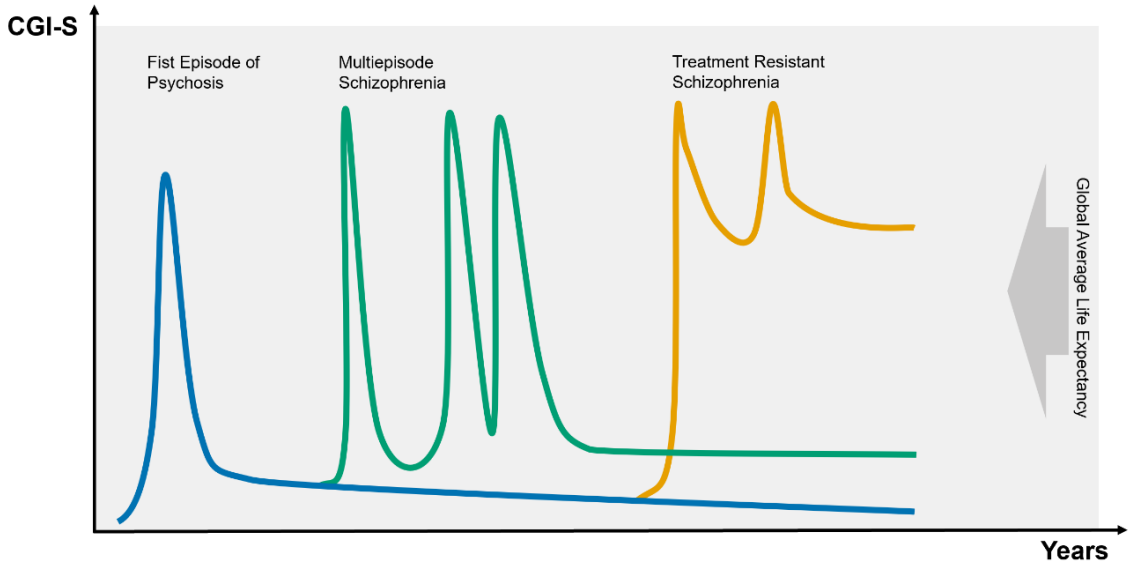

Figure 1. Clinical course of schizophrenia.

Multiple relapses characterize the illness course for most patients with schizophrenia. Pharmacological management focuses on controlling acute psychotic episodes and preventing relapses, but challenges often arise due to adverse effects and non-adherence. Despite advances in treatment, up to 30% of patients experience treatment-resistant schizophrenia (TRS), requiring more intensive therapeutic strategies. When compared to the general population, schizophrenia is associated with a reduced lifespan. Abbreviation: Clinical Global Impression-Severity (CGI-S).

While conventional treatment helps many individuals achieve partial or full remission, up to 30% continue to struggle with persistent symptoms, a condition known as Treatment-Resistant schizophrenia (TRS) (15,16), which requires a specialized

management approach (17). Long-term outcomes for individuals with schizophrenia often involve more severe functional decline than their peers, along with ongoing difficulties in social integration, employment, and independent living. Additionally, those with schizophrenia face a reduced life expectancy of 13 to 15 years, largely due to comorbid physical conditions and a heightened risk of suicide (2,18).

d. Causes

The causes of schizophrenia are intricate and involve a mix of genetic and environmental risk factors, along with their interactions (19,20). Most risk factors for the disease, such as a family history of mental illness, often reflect both genetic predispositions and environmental influences. Consequently, attempts to distinguish between these two types of factors may be spurious (21).

Several theories attempt to explain the origins of the disorder. The dopaminergic hypothesis suggests that schizophrenia arises from dysregulation in dopamine pathways (22). Specifically, hyperactivity in the mesolimbic pathway is associated with positive symptoms like hallucinations and delusions, while hypoactivity in the mesocortical pathway contributes to negative symptoms and cognitive deficits. Complementing this, the glutamatergic hypothesis posits that dysfunction in glutamate signalling, particularly through NMDA receptors hypofunction, disrupts neural circuits involved in cognition, emotion, and perception (23). Additionally, the immune hypothesis suggests that chronic inflammation may play a role in the onset and progression of schizophrenia (24), a topic that will be explored further in the next section.

These theories have collectively refined the original synaptic hypothesis of schizophrenia, first proposed by Irwin Feinberg in 1982, which suggested that faulty synaptic elimination during adolescence could be a causal factor (25). The updated version of this hypothesis proposes that a combination of genetic and environmental factors makes synapses more vulnerable to damage from glial cells, especially under stress, leading to disruptions in brain function that contribute to both cognitive and negative symptoms of schizophrenia, as well as increased dopamine activity linked to psychosis (26).

In addition to neurochemical abnormalities, schizophrenia is associated with structural and functional brain alterations. Neuroimaging studies have consistently shown abnormalities such as enlarged ventricles, reduced gray matter volume, and altered

connectivity in key brain regions, including the prefrontal cortex, hippocampus, and thalamus (27). These brain changes are believed to be linked to neurodevelopmental disruptions that occur early in life, possibly during prenatal or early postnatal development, with microglia and astrocytes being implicated (28). Environmental factors, such as prenatal infections, malnutrition, and stress, may interact with genetic predispositions to disrupt normal brain development, setting the stage for the later onset of schizophrenia.

Genetic factors play a significant role in the development of schizophrenia. The most recent genome-wide association study (GWAS) has identified over 287 loci associated with an increased risk of the disorder (29). These loci include both common variants with small effect sizes and rare variants with higher penetrance. Genomic research indicates that risk alleles are involved in various functions, including immune response, and there is an enrichment of common variant associations in genes related to neuronal function (30). In particular, gene sets linked to synaptic structure and function are highlighted (**Figure 2**), suggesting that schizophrenia may primarily be a disorder of neuronal, and particularly synaptic activity with widespread effects on multiple brain regions and functions.

Environmental factors are also relevant in the risk of developing schizophrenia. Key postnatal risks include urban living, migration, childhood trauma, and cannabis use (31). Immigrants, particularly those from disadvantaged backgrounds, often experience higher rates of schizophrenia, likely due to stress from reduced social status, while city living is associated with increased risk due to heightened psychosocial stress. Childhood trauma, such as abuse and parental loss, is a significant risk factor, and cannabis use, especially high-potency strains, is linked to a dose-dependent increase in risk (32). Prenatal factors, although more challenging to study, also contribute to schizophrenia risk. Advanced parental age, particularly paternal, is associated with higher risk, and the season of birth, along with prenatal exposure to infections like influenza and Toxoplasma gondii, has shown some correlation (33). Additionally, prenatal exposure to famine is a well-supported risk factor, as indicated by historical studies (34).

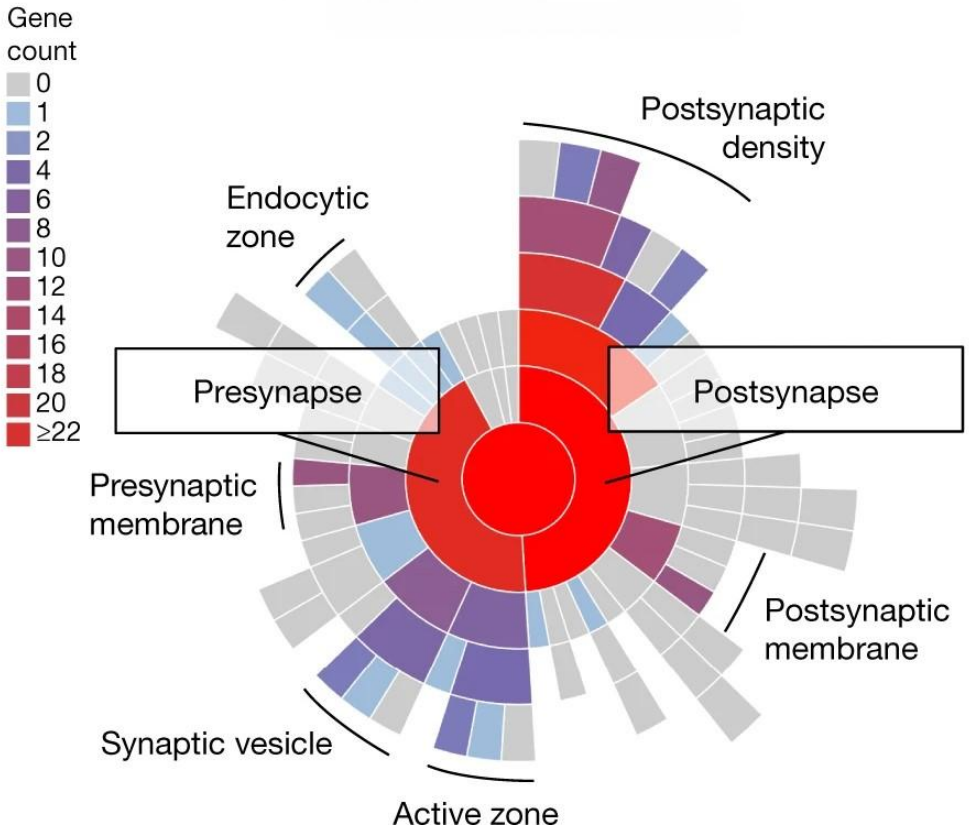

Figure 2. Sunburst plots showing where significant risk genes are located in the synapse.

The plots start with the synapse in the centre. The first ring shows pre- and postsynaptic locations, and each outer ring breaks these down into more specific categories. The number of genes in each category is represented by the colours shown in the legend. Adapted from Trubetskoy et al. (29) .

e. Treatment

Treatment for schizophrenia involves a combination of pharmacological and psychosocial approaches. The pharmacological management typically includes antipsychotic medications to control acute psychotic episodes and prevent relapses (35). After stabilization, long-term maintenance therapy is necessary to help sustain this stability. There are two main classes of antipsychotic drugs: first-generation (FGAs) and second-generation (SGAs). SGAs are generally preferred due to their lower risk of adverse effects, such as extrapyramidal symptoms, tardive dyskinesia, and relapses (36). However, managing schizophrenia with medication is challenging due to prevalent nonadherence, which significantly increases the risk of relapse and poor outcomes (37). To address this, long-acting injectable (LAI) versions of antipsychotics are widely used to improve adherence and reduce these risks (36).

Despite these efforts, up to 30% of patients have TRS and do not respond adequately to conventional antipsychotics (15,16). Several hypotheses for the neurobiological mechanisms underlying TRS include dopamine super sensitivity, glutamate and serotonin dysregulation, and immunity, highlighting the biological and clinical heterogeneity of TRS (38). For these individuals, clozapine is the only approved medication, and additional strategies like Electroconvulsive Therapy (ECT) have shown effectiveness (17). Alongside pharmacological treatments, psychosocial interventions, including cognitive-behavioral therapy, cognitive remediation therapy, social skills training, and supported employment, play a crucial role in enhancing patients' functioning and quality of life (39).

1.2. The immune system, a brief overview

The immune system is a complex network of cells, tissues, and organs that work in concert to safeguard the body from harmful invaders and maintain overall health. The immune system is generally divided into two main components: innate immunity and adaptive immunity (**Figure 3**) (40).

Innate immunity serves as the body's first line of defense. It is non-specific and responds rapidly to a broad range of pathogens. Key players in innate immunity include physical barriers like the skin and mucous membranes, as well as immune cells that recognize and attack pathogens using mechanisms that do not require prior exposure to the invaders.

Adaptive immunity provides a more targeted and specific response to pathogens. This component of the immune system is activated when innate defenses are insufficient. It involves specialized immune cells and also has a memory component, allowing the immune system to respond more effectively upon subsequent exposures to the same pathogen.

In addition to defending against infections, the immune system also plays critical roles in non-infectious contexts (41). It recognizes and neutralizes harmful substances from the environment, such as toxins and allergens, to prevent potential harm. Moreover, it is involved in detecting and combating abnormal cells, such as cancer cells, which may arise within the body.

The functionality of the immune system can be assessed through various biomarkers, each revealing different aspects of immune activity (42). This thesis focuses on white blood

cells and C-Reactive Protein due to their high accessibility in clinical practice. While we acknowledge the limitations in specificity of these biomarkers, which may hinder the study of unique immune pathways, their routine use provides significant potential for clinical application. These markers offer valuable insights into immune function and inflammation and can be easily integrated into everyday healthcare practices.

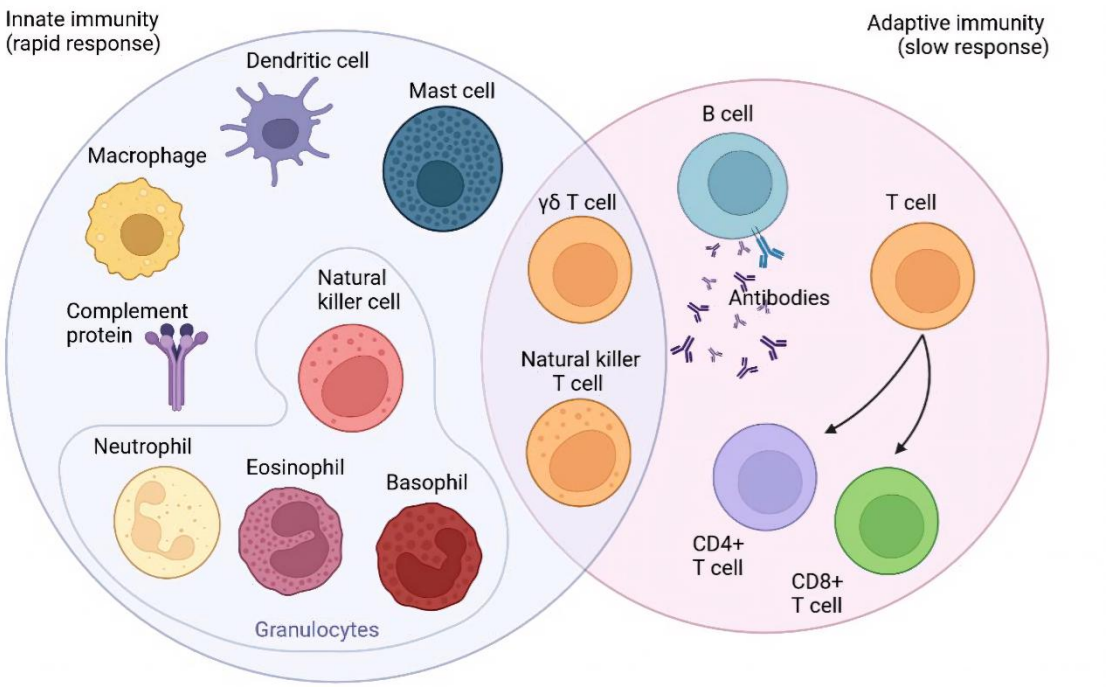

Figure 3. Components of the immune system.

Innate immunity consists of granulocytes (such as basophils, eosinophils, and neutrophils), mast cells, dendritic cells, macrophages, and natural killer (NK) cells. On the other hand, adaptive immunity involves T and B cells, which can further subdivide into specific subclasses. The intersectional (purple) cells connect the two systems, originating from T cell lineage but functioning in a way characteristic of the innate immune system. Adapted from Dranoff et al. (43) using Upscayl.

a. White blood cell counts

White blood cell (WBC) counts, or leukocytes, are essential components of the immune system, responsible for defending the body against infections, foreign invaders, and harmful substances. A WBC count measures the total number of these cells in the bloodstream, with normal levels ranging from 4,000 to 11,000 cells per microliter. WBCs are involved in the two key branches of the immune system: innate and adaptive immunity (**Figure 3**) (40).

Innate immunity involves several types of WBCs, including neutrophils, monocytes, eosinophils, and basophils, which quickly respond to infections and inflammation. In

contrast primarily relies on lymphocytes, such as B cells and T cells, to recognize and remember specific pathogens, produce antibodies, and mount a stronger defense upon re-exposure.

The five primary types of WBCs each play distinct roles:

- Neutrophils: The most abundant WBC, crucial to innate immunity, rapidly respond to and destroy pathogens.
- Lymphocytes: Key players in adaptive immunity, including B cells, which produce antibodies, and T cells, which directly attack infected cells.
- Monocytes: Differentiate into macrophages and dendritic cells to engulf pathogens and present antigens to lymphocytes, bridging innate and adaptive immunity.
- Eosinophils: Combat parasitic infections and are involved in allergic reactions.
- Basophils: Participate in allergic responses and inflammation by releasing histamine to combat invaders.

Furthermore, WBC counts provide key insights into immune function beyond overt inflammation, often reflecting subtle immune activity or "low-grade inflammation" (44). This refers to a persistent, mild activation of the immune system that can occur without obvious signs of infection or acute inflammation. WBC counts, particularly in specific cell types like neutrophils, lymphocytes, and monocytes, can indicate underlying immune system regulation, stress responses, or early signs of immune system dysregulation. This low-grade immune activation is linked to a variety of conditions, including metabolic disorders and chronic diseases, where immune activity plays a role in long-term health outcomes without manifesting as acute inflammation (45).

In clinical practice, WBC counts serve as a valuable marker of immune function and inflammation. A complete blood count (CBC) with differential is commonly used to measure WBC levels and provide a detailed breakdown of the different WBC types in circulation, helping to assess the body’s immune response.

b. WBC ratios

WBC ratios provide an alternative measure of immune function by comparing the levels of various types of white blood cells. These ratios help detect imbalances between innate immunity (represented by neutrophil, monocyte, eosinophil, or basophil counts) and

adaptive immunity (represented by lymphocyte counts) and have been proposed as a way to measure the body's response to stress (46). Commonly studied ratios include:

- Neutrophil-to-lymphocyte ratio (NLR)
- Basophil-to-lymphocyte ratio (BLR)
- Monocyte-to-lymphocyte ratio (MLR)
- Platelet-to-lymphocyte ratio (PLR)
- Eosinophil-to-lymphocyte ratio (ELR)
- Leukocyte-to-lymphocyte ratio (LLR)

Among these, the NLR is the most extensively studied (46). This ratio reflects two key aspects of the immune system: neutrophils, which are part of the innate immune response, and lymphocytes, which are involved in adaptive immunity. An increase in the NLR is often seen as a response to various stresses, such as infections, inflammation, or psychosocial stress. These stresses activate endogenous cortisol and catecholamines, leading to an increase in leukocytes, including neutrophils, and a decrease in lymphocytes (47). This imbalance results in an elevated NLR, as represented by the following equation:

$$\uparrow NLR = \frac{\uparrow Neutrophil\ count}{\downarrow Limphocyte\ count}$$

Although there is ongoing debate about the appropriate cut-off value for NLR, it has been identified as an independent prognostic factor for morbidity and mortality in the general population (48) and in several diseases (49–52). Its advantages include being a cost-effective and easily obtainable biomarker. However, factors such as age, cardiovascular comorbidities, and exogenous steroid use can falsely elevate the NLR, so these should be considered when interpreting the results (53).

Lastly, although platelets are not white blood cells, they play a role in inflammation and interact with immune cells, influencing immune responses. Including platelets in these ratios provides a broader perspective on the inflammatory and immune environment, which is why they are often incorporated into these measures.

c. C-Reactive Protein

C-reactive protein (CRP) is a peripheral biomarker of inflammation, produced by the liver as part of the innate immune response. In response to inflammatory signals such as cytokines like interleukin (IL) 6, CRP helps recognize and eliminate pathogens by activating the complement system (54) (see **Figure 4**). Beyond its immune defence role,

CRP is also involved in tissue repair, reflecting the body's healing efforts even when inflammation is not readily apparent (55).

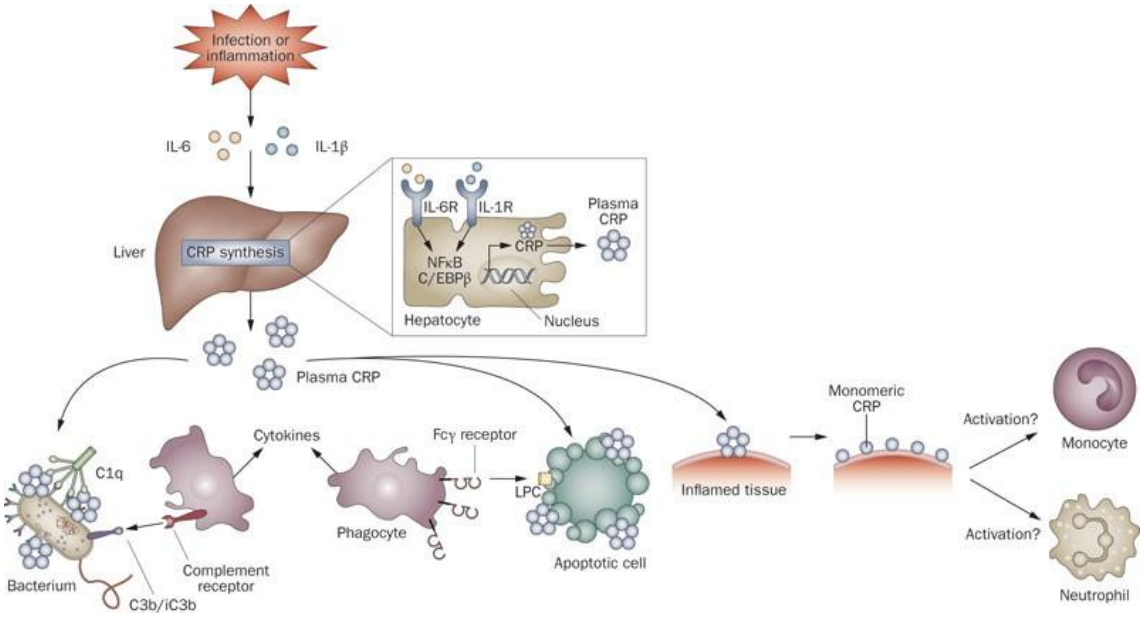

Figure 4: Functional CRP pathways

In response to cytokines like IL-6 and IL-1β, the liver significantly increases its production of CRP. Once in circulation, CRP binds to bacteria and dying cells, promoting their removal through the complement system and FcγR-mediated phagocytosis. The binding of CRP may also trigger phagocytic cells to release immunoregulatory cytokines such as IL-10. Growing evidence suggests that when plasma CRP deposits on inflamed tissues, it breaks into biologically active monomeric units, which are believed to have various proinflammatory effects. Abbreviations: CRP, C-reactive protein; LPC, lysophosphatidylcholine. Adapted from Rhodes et al. (54)

One of CRP's key advantages is its broad applicability as a non-specific marker of inflammation (55). Although it does not provide a specific diagnosis, CRP offers a general indication of the body's inflammatory status, making it useful for identifying individuals at risk for various conditions. Its low cost, ease of measurement, and quick response to inflammatory stimuli make CRP a practical biomarker for routine health assessments and disease management.

Elevated CRP levels are strongly associated with cardiovascular and metabolic disorders, signalling chronic inflammation that contributes to conditions like atherosclerosis, obesity, and type 2 diabetes. Lifestyle factors such as smoking and chronic stress can further raise CRP levels, indicating ongoing low-grade inflammation.

In the general population, CRP serves as a useful marker for predicting health risks. High CRP levels are linked to an increased risk of chronic diseases, including heart disease,

diabetes, and certain cancers, as well as higher mortality (56). In conditions like cancer, infections, and cardiovascular diseases, elevated CRP can also signal a poorer prognosis (57–60) .

CRP lab values are reported in either mg/dL or mg/L, depending on the context. In clinical practice, particularly when assessing acute inflammation caused by bacterial or viral infections, trauma, or autoimmune conditions, CRP levels are typically expressed in mg/dL. Common cut-off values include: less than 0.3 mg/dL as normal, 0.3 to 1.0 mg/dL as mildly elevated, 1.0 to 10.0 mg/dL as moderately elevated, more than 10.0 mg/dL as markedly elevated, and more than 50.0 mg/dL as severely elevated (55). For cardiac risk assessment, the U.S. Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) categorize CRP levels (reported in mg/L) as follows: less than 1 mg/L for low systemic inflammation, 1 to 2.9 mg/L for intermediate inflammation, 3 to 10 mg/L for high inflammation, and more than 10 mg/L for acute inflammation (61). This thesis will use the CDC/AHA guidelines, as schizophrenia is not primarily associated with acute inflammation.

1.3. The immune hypothesis in schizophrenia

Over the past few decades, the immune system has attracted increasing attention as a potential factor in the development of schizophrenia (62). Evidence from epidemiological, genetic, and biological research has led to the immune hypothesis, which suggests that immune system dysfunction plays a central role in the pathophysiology of schizophrenia. However, the connection between immunity and schizophrenia has been considered since the early days of the disorder's definition.

a. An historical perspective

In 1919, Emil Kraepelin coined the term "dementia praecox," later known as schizophrenia (63), suggesting it was hereditary and linked to brain self-poisoning (64). In the late 19th and early 20th centuries, neurosyphilis caused significant psychosis, contributing to a large proportion of psychiatric admissions (65). During this period, Austrian physician Julius Wagner-Jauregg used malaria fever therapy to treat it until penicillin became available after World War II (66).

After the 1918 influenza pandemic, researchers began exploring the link between infections and psychosis (67). However, in the mid-20th century, the dopamine hypothesis emerged (68)., and by the 1980s, the neurodevelopmental model suggested that altered brain development could contribute to schizophrenia. In the 1970s, interest in the connection between viral infections and schizophrenia resurfaced, with studies linking maternal infections, such as influenza, as well as factors like winter births and urban living, to an increased risk of developing the disorder (69–71).

The idea of schizophrenia as an autoimmune disorder emerged in the 1980s (72), with subsequent evidence showing that autoimmune diseases increase the risk of developing schizophrenia (73). However, schizophrenia is not currently considered an autoimmune disease due to the lack of consistent autoantibodies (74). The 2007 discovery of anti-NMDA receptor encephalitis, an autoimmune condition that mimics psychosis, emphasized the importance of distinguishing autoimmune causes of psychosis, requiring immunotherapy (75,76).

b. The epidemiological evidence

As previously noted, population-based epidemiological studies have been crucial in developing hypotheses about the immune theory of schizophrenia (77). Meta-analyses suggest that autoimmune diseases might act as risk factors for psychotic disorders, with several conditions positively linked to schizophrenia, though the strength and direction of these associations can vary by diagnosis. For instance, having a non-neurological autoimmune disease increases the risk of a psychotic disorder by 43%, including conditions such as celiac disease, autoimmune thyrotoxicosis, psoriasis, and pernicious anaemia (78). In contrast, rheumatoid arthritis and ankylosing spondylitis are negatively associated with schizophrenia. For autoimmune diseases affecting the nervous system, there is a 48% increased risk of schizophrenia, with multiple sclerosis identified as a risk factor, while Guillain-Barré syndrome is not (79). Additionally, schizophrenia patients have a 55% higher risk of developing autoimmune disorders compared to the general population, suggesting a potential bidirectional relationship (78).

schizophrenia is also linked to a higher prevalence of infections, including neurotropic viruses from the *Herpesviridae* family and the parasite *Toxoplasma gondii* (80). Longitudinal meta-analyses indicate that exposure to infections both during fetal development and in childhood is linked to an increased risk of developing schizophrenia in adulthood, although the strength of these associations varies (81,82). Additionally, a dose-response relationship has also been observed between childhood hospitalizations for serious infections and the risk of adult schizophrenia, with notable synergy between autoimmune diseases and infections, suggesting that the combined effect on schizophrenia risk is greater than expected under an additive model (**Figure 5**) (83). Despite these findings suggesting potential causal links, there is significant variability in pathogen types, affected body systems, infection timing, and severity, leading to no consensus on which specific infections contribute to later mental health issues. Furthermore, large-scale studies suggest that schizophrenia may also increase the risk of infections later in life, indicating a possible bidirectional relationship (83,84).

Overall, while the epidemiological evidence strongly supports a role for immunity in psychotic disorders, the underlying mechanisms remain to be fully established, underscoring the need for further research in this area.

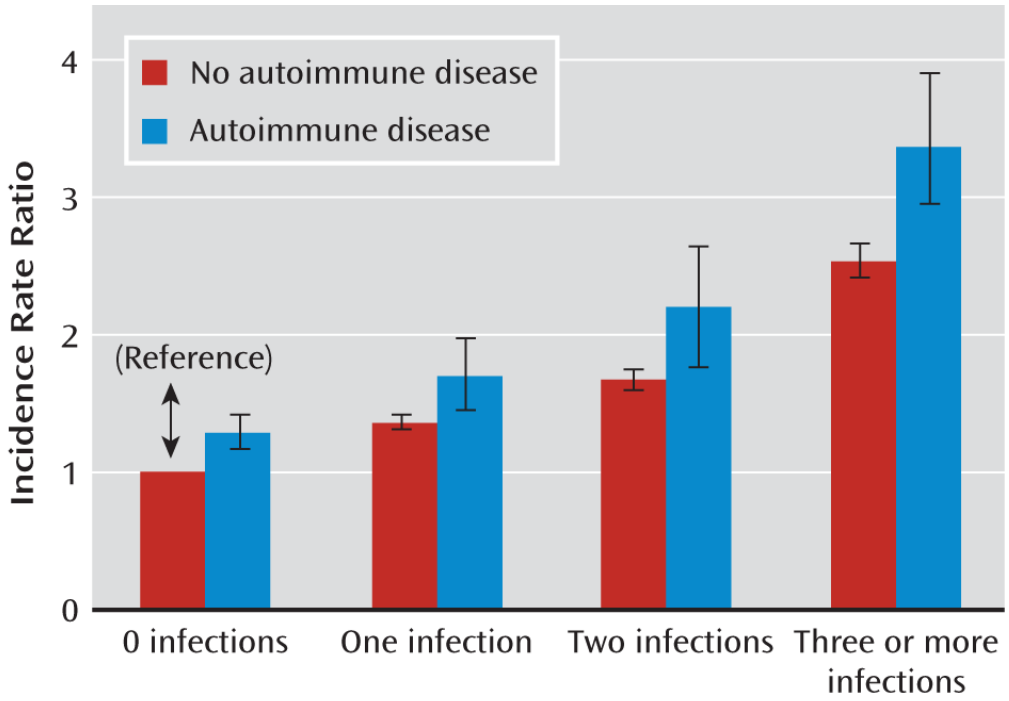

Figure 5: Incidence rate ratios of schizophrenia associated with prior diagnosis of autoimmune disease and infections. Adapted from Benros et al. (83).

c. The genetic evidence

Before genome sequencing, genetics relied heavily on twin studies to understand disease heritability, estimating schizophrenia's heritability at around 85% (85). A significant advance in the field came in 2014 from the schizophrenia Working Group of the Psychiatric Genomics Consortium, which analysed the DNA of nearly 37,000 schizophrenia patients and over 110,000 controls (86). This genome-wide association study (GWAS) identified 108 genetic loci linked to schizophrenia, with one of the strongest associations found at the locus encoding the major histocompatibility complex (MHC), or human leukocyte antigens (HLAs) (**Figure 6**). This finding supports the immune hypothesis, suggesting that the immune system may play a role in schizophrenia. Furthermore, a 2022 study by the same consortium replicated these findings in a much larger sample, reinforcing the association (29). Overall, these genetic studies indicate that schizophrenia is closely related to synapse biology and neurodevelopment (87).

As outlined in section 1.1.c, genetic findings have been linked to the synapse hypothesis of schizophrenia (26), which suggests that the disease is caused by excessive loss of neuronal connections, with a significant role for immune processes. A landmark study by Sekar et al. (88) identified complement component 4 (C4) genes within the MHC locus as potential drivers of genetic risk for schizophrenia. The complement system, which

helps mark cells for elimination and promotes inflammation, has been shown to be overactive in certain brain regions of individuals with schizophrenia (89). This overactivity is linked to microglial phagocytosis (90), which may lead to excessive synaptic pruning—a key feature of schizophrenia identified by Feinberg in 1982 (25). In cell models, increased C4A gene expression and TNF-α were associated with excessive pruning, which could be mitigated by pretreatment with minocycline, a drug that reduces inflammation (91). The updated synapsis hypothesis suggests that this abnormal complement-mediated pruning contributes to reduced brain volumes, cortical thinning, and cognitive symptoms in schizophrenia (26). However, not all patients have the harmful C4 gene variants, indicating that complement dysregulation may be more related to disease progression than initial cause (92).

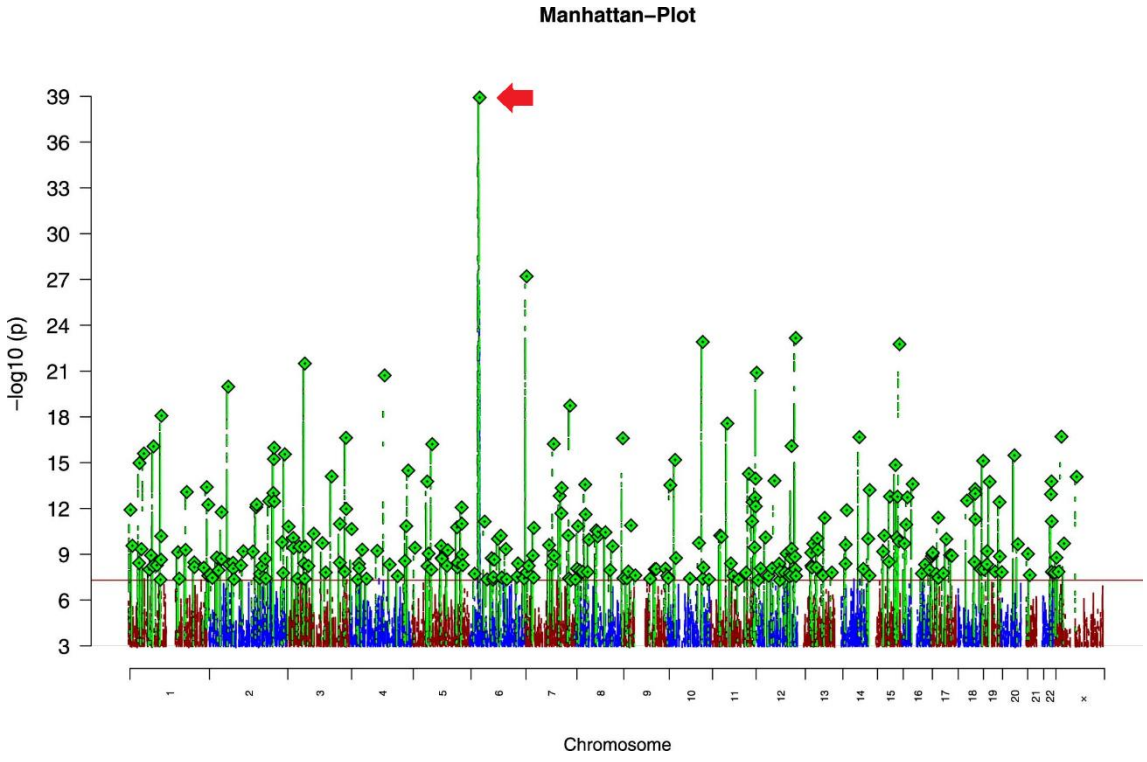

Figure 6. Manhattan plot of schizophrenia GWAS associations.

The x-axis represents chromosomal positions, and the y-axis shows the significance of association ($-\log_{10}(P)$). The red line indicates the genome-wide significance threshold (5×10^{-8}). Single nucleotide polymorphisms (SNPs) in green are in linkage disequilibrium (LD; $r^2 > 0.1$) with index SNPs (represented by diamonds), which indicate LD-independent, genome-wide significant associations. The red arrow marks the major histocompatibility complex (MHC) region, one of the strongest genetic loci associated with schizophrenia. Adapted from Trubetskoy et al. (29)

d. The blood-based biomarkers evidence

Individuals with schizophrenia exhibit altered peripheral biomarkers linked to dysregulated immune pathways and inflammatory mediators. This imbalance between pro- and anti-inflammatory mechanisms increases pro-inflammatory components, such as NFκB, iNOS, COX-2, and PGE2 [1], while reducing anti-inflammatory factors, including IκBα, 15dPGJ2, and PPARγ, ultimately activating oxidative and nitrosative stress, as indicated by TBARS (93,94). Recent meta-analyses highlight that these changes vary by specific biomarker and disease stage (95,96).

Regarding peripheral immune proteins, concentrations of IL-1β, IL-1 receptor antagonist, soluble IL-2 receptor, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, and CRP are consistently elevated in both acute and chronic schizophrenia compared to healthy controls (HC). In contrast, IL-2 and interferon (IFN)-γ are significantly elevated in acute schizophrenia, while IL-4, IL-12, and IFN-γ are significantly decreased in chronic schizophrenia (95). See **Table 1** for the effect sizes of each protein reported as standardized mean differences (SMD) at each stage.

Patients with schizophrenia have shown elevated levels of leukocytes, neutrophils, monocytes, and B lymphocytes in their circulating immune cells. Additionally, the NLR, MLR, and PLR are consistently increased (96). A recent GWAS study also identified shared genetic loci between schizophrenia and white blood cell counts, particularly lymphocytes, suggesting a genetic basis for systemic immune abnormalities (97). **Table 2** presents the effect sizes for WBC counts and ratios, reported as SMD. While immune protein research has been more extensively studied, fewer studies have focused on immune cells, and stage-specific data remain lacking, so these values are reported generally for schizophrenia.

[1] In blood, the protein expression levels of proinflammatory and anti-inflammatory mediators reflect dysregulated immune responses (188). The proinflammatory pathway includes components such as nuclear factor kappa B (NFκB), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and prostaglandin E2 (PGE2). In contrast, the anti-inflammatory pathway involves factors like inhibitor of κB alpha (IκBα) and 15-deoxy-Δ12,14-prostaglandin J2 (15dPGJ2). Additionally, oxidative and nitrosative stress is indicated by elevated levels of thiobarbituric acid reactive substances (TBARS).

Table 1. Standardized mean differences in peripheral immune proteins between schizophrenia patients and health controls by disease stage.

	Acute SCZ vs HC		Chronic SCZ vs HC		Acute SCZ vs Chronic SCZ	
	SMD (95% CI)	p-value	SMD (95% CI)	p-value	SMD (95% CI)	p-value
Consistent alteration patterns						
IL-1β	0.39 (0.17 to 0.61)	**<0.001***	0.53 (0.18 to 0.87)	**<0.001***	-0.13 (-0.53 to 0.26)	0.102
IL-1RA	0.48 (0.16 to 0.80)	**0.002***	0.52 (0.03 to 1.00)	**0.008***	-0.04 (-0.62 to 0.54)	0.305
sIL-2R	0.65 (0.44 to 0.87)	**<0.001***	0.35 (0.08 to 0.62)	**0.001***	0.30 (-0.04 to 0.065)	0.148
IL-6	0.79 (0.67 to 0.91)	**<0.001***	0.50 (0.36 to 0.64)	**0.001***	0.29 (0.11 to 0.47)	**0.001***
IL-8	0.27 (0.13 to 0.42)	**0.004***	0.25 (0.08 to 0.43)	**0.005***	0.02 (-0.20 to 0.24)	0.438
IL-10	0.18 (0.03 to 0.33)	**0.002***	0.33 (0.15 to 0.51)	**<0.001***	-0.15 (-0.38 to 0.08)	0.129
TNF-α	0.59 (0.41 to 0.97)	**<0.001***	0.53 (0.34 to 0.72)	**<0.001***	0.06 (-0.19 to 0.31)	0.311
CRP	0.69 (0.41 to 0.97)	**<0.001***	0.61 (0.30 to 0.92)	**<0.001***	0.08 (-0.33 to 0.49)	0.279
Inconsistent alteration patterns						
IL-2	0.48 (0.17 to 0.79)	**0.001***	0.26 (-0.15 to 0.67)	0.206	0.22 (-0.28 to 0.72)	0.430
IL-4	0.15 (0.00 to 0.29)	0.603	-0.88 (-1.13 to -0.64)	**<0.001***	1.03 (0.76 to 1.31)	**<0.001***
IL-12	0.13 (-0.03 to 0.29)	0.317	-0.43 (-0.64 to -0.22)	**<0.001***	0.56 (0.31 to 0.82)	**0.001***
IFN-γ	0.28 (0.13 to 0.42)	**0.001***	-0.32 (-0.52 to -0.12)	**<0.001***	0.60 (0.35 to 0.84)	**<0.001***

Proteins are analysed for statistical significance and effect size direction, comparing acute and chronic schizophrenia with HC. Markers with similar results in both comparisons are labelled as consistent, while those with differing results are labelled as inconsistent. Adapted from Halstead et al (95). **Abbreviations**: HC: health controls; SCZ: schizophrenia SMD: standardized mean difference; I^2: heterogeneity (values of 25%, 50%, and 75% correspond to low, moderate, and high heterogeneity, respectively); * denotes a statistically significant difference; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; CRP: c-reactive protein.

Table 2. Standardized mean differences in white blood cell counts and ratios between patients with schizophrenia and HC.

	SCZ vs HC SMD (95% CI)	p-value	I^2
WBC counts			
- Leukocytes	0.35 (0.24 to 0.46)	**<0.001***	73%
- Neutrophils	0.32 (0.11 to 0.54)	**<0.001***	92%
- Monocytes	0.40 (0.23 to 0.58)	**<0.001***	84%
- Basophils	-0.02 (-0.29 to 0.25)	0.88	71%
- Eosinophils	-0.14 (-0.39 to 0.11)	0.26	71%
- Lymphocytes	0.01 (-0.10 to 0.11)	0.88	67%
- Lymphocytes T	0.14 (-0.08 to 0.35)	0.21	49%
- Lymphocytes B	0.26 (0.04 to 0.48)	**0.020***	36%
WBC ratios			
- NLR	0.40 (0.19 to 0.60)	**<0.001***	91%
- MLR	0.31 (0.04 to 0.57)	**0.022***	88%
- PLR	0.23 (0.03 to 0.43)	**0.021***	63%

Adapted from Clausen et al (96). **Abbreviations:** WBC: white blood cells; SCZ: schizophrenia; SMD: standardized mean difference; I^2: heterogeneity (values of 25%, 50%, and 75% correspond to low, moderate, and high heterogeneity, respectively); * denotes a statistically significant difference; NLR: neutrophil-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio.

As discussed earlier, alterations in various biomarkers have been observed in patients with schizophrenia. However, most of these biomarkers are costly and not suitable for routine clinical use. In contrast, WBC counts, their ratios, and CRP are highly accessible and practical for clinical application. Therefore, this thesis will focus on these biomarkers.

The relationship between WBC ratios and the different components of the inflammatory cascade in patients with psychosis remains unclear. Specifically, it is uncertain whether alterations in WBC ratios represent a primary feature of the psychotic disorder itself, or if they are influenced by treatment, metabolic changes, or specific immune pathways (Karageorgiou et al., 2019). Additionally, several key factors such as age, obesity, infections, diabetes, and emotional stress can cause a "false" increase in WBC ratios (Buonacera et al., 2022). To explore this issue, in **Article VII**, we analyse the relationship between NLR and a panel of inflammatory and oxidative/nitrosative stress biomarkers, along with potential confounding factors, in a well-characterized cohort of 97 patients with FEP and 77 matched HC.

As previously mentioned, CRP is a biomarker of innate immune response, while WBC ratios reflect the balance between innate and adaptive immune pathways. However, it is still uncertain whether CRP levels are associated with WBC ratios in patients with schizophrenia, and if so, which specific ratio. In **Article VIII**, we explore this connection in greater detail in a cohort of 698 patients with acute schizophrenia.

Finally, approximately 40% of patients with schizophrenia also have a substance use disorder (SUD). Although the mechanisms by which these substances affect schizophrenia are not fully understood, they may involve modulation of the immune response, exerting either anti-inflammatory or pro-inflammatory effects depending on the substance and population studied (Tanasescu & Constantinescu, 2010; Bidwell et al., 2018; Zaparte et al., 2019; Henshaw et al., 2021). Nevertheless, the exclusion of patients with SUD or sporadic substance use from immunopsychiatry studies leaves a critical gap in the literature. To address this, in **Article IX**, we investigate the differences in WBC counts, their ratios, and CRP levels between 972 psychiatric inpatients, including those with psychotic disorders, who tested positive for cannabinoids, opioids, or cocaine, and those who tested negative.

In addition to the cross-sectional alterations in various biomarkers observed when comparing schizophrenia patients with HC, researchers have also explored the role of these biomarkers in predicting outcomes, particularly treatment response and mortality.

Treatment response

Various scenarios have been studied in relation to treatment outcomes in schizophrenia. Some studies have explored the predictive value of immunological biomarkers during the FEP for treatment outcomes in the following years. Elevated immune markers during FEP, including WBC counts, ratios, and CRP, have been associated with poorer treatment response at follow-up (98–103). However, inconsistencies between studies persist, and no specific biomarker pattern has been conclusively identified (104). To address these questions, in **Article I** we investigated NLR differences between a well-characterized cohort of 310 FEP patients and 215 health controls over a two-year follow-up.

When looking at the response to antipsychotics in acute schizophrenia patients, the predictive ability of immunological biomarkers on has been poorly investigated, with few studies focusing on this as a primary outcome. A recent review by Orbe and Benros highlights several limitations in current research, including underpowered sample sizes and the investigation of antipsychotics as a drug group (105). The largest study to date, which included 2,598 patients, examined the association between immunological biomarkers and acute clinical outcomes in schizophrenia, finding that higher leukocyte counts predicted poorer responses to antipsychotics, as measured by the PANSS total scores (106). However, specific WBC counts, ratios of CRP and particular antipsychotic treatments were not studied. To address these gaps, in **Article III** we conducted a retrospective study exploring the relationship between WBC counts, their ratios, and CRP levels at hospitalization and functional outcomes in a cohort of 354 patients with acute schizophrenia.

Researchers have also investigated patients with TRS, focusing on the association between immune cells and clinical response upon initiating clozapine, with mixed results. Two earlier studies reported contradictory findings regarding the ability of baseline leukocyte and neutrophil counts to predict the response to clozapine (107,108). In contrast, a more recent study by Jones et al. analysed the largest cohort to date, comprising 397 patients, and found that individuals with high-normal neutrophil counts were more likely to respond to clozapine over a longer-term period, as measured by the Clinical

Global Impression (CGI) scale (109). Additionally, using data from the United Kingdom's psychosis early intervention services, Osimo et al. developed a machine learning model to forecast the 8-year likelihood of clozapine use starting from FEP. This model integrated various analytical parameters, including WBC counts, achieving an area under the curve (AUC) of approximately 0.67 and highlighting the significant impact of lymphocytes on predictive ability (101). However, the influence of WBC ratios as an alternative measure of the imbalance between immune pathways has not yet been explored in this population.

To address these questions, we conducted two studies. In **Article V**, we performed a longitudinal study of 32 patients with TRS who initiated clozapine, examining the associations between WBC ratios before treatment initiation and the clinical response at 8 weeks. In **Article IV**, we conducted a longitudinal population-based register study including 6,845 patients with measurements of WBC counts, their ratios, and CRP during their first episode of schizophrenia. We followed them for up to 22 years, investigating associations with clozapine or ECT use during the follow-up period.

Mortality

Literature has demonstrated the predictive ability of WBC counts, ratios, and CRP for mortality in the general population (48,110–113) and in various medical conditions (114–116).

Given the known increased risk of premature mortality in schizophrenia patients compared to the general population —primarily due to cardiovascular causes (18)— researchers have investigated immune biomarkers to predict this mortality. A previous study using population-based data from Denmark found that higher CRP levels at the first diagnosis of schizophrenia are associated with increased mortality, particularly among individuals with somatic comorbidities (117). The researchers also included leukocyte counts but found no significant associations. However, specific WBC counts have not been thoroughly studied in schizophrenia, and the predictive ability of WBC counts and ratios has not been directly compared to that of CRP. To address these questions, in **Article IV** we conducted a longitudinal population-based register study including 6,845 patients with measurements of WBC counts, their ratios, and CRP during their first episode of schizophrenia. We followed them for up to 22 years and examined associations with all-cause mortality during follow-up.

e. The central nervous system evidence

While peripheral biomarkers are valuable for studying the immune hypothesis in schizophrenia due to the ease of sample collection, it is essential to examine alterations in the central CNS, as schizophrenia is primarily a brain-related disorder (118). Although there are fewer CNS-focused studies compared to peripheral investigations, recent advances have enhanced our understanding of immune dysfunction in schizophrenia. In this context, observations of altered cytokine levels and glial cell activity in the CNS have suggested a link between neuroinflammation and schizophrenia (119,120). However, this perspective often overlooks the non-inflammatory roles of cytokines and glial cells, such as their involvement in neural plasticity, immune regulation, and brain homeostasis (121).

In our group, we argue that significant misinterpretations exist, particularly regarding the use of the term "neuroinflammation" in the context of schizophrenia, as these changes do not fully align with the four classical criteria for neuroinflammation (122)—namely, increased cytokines, activated microglia, T-cell recruitment, and neurodegenerative tissue damage. Below, we provide a brief overview of the key areas relevant to the immune hypothesis in schizophrenia within the CNS.

Cytokines

Meta-analyses have shown elevated levels of pro-inflammatory cytokines IL-6 and IL-8 in the cerebrospinal fluid (CSF) of patients with psychotic disorders compared to HC, with medium effect sizes (SMD: ~0.5) (123,124). Earlier studies also reported increased IL-1β, though recent research has not consistently replicated these findings (125). However, the actual magnitude of cytokine elevations in schizophrenia is modest, far below levels seen in classical neuroinflammatory conditions like traumatic brain injury or bacterial meningitis. For instance, the average CSF IL-6 level in schizophrenia is around 2.82 pg/mL (123), which falls below the established normal cut-off of 7.5 pg/mL (126), remaining below the clinical threshold for concern.

Peripheral Cell Infiltration

Neuroinflammation in schizophrenia may involve the infiltration of peripheral immune cells into the brain, often considered harmful. Postmortem studies have reported increased densities of $CD3^+$ T lymphocytes and $CD20^+$ B lymphocytes in schizophrenia patients' brains, though results are inconsistent and based on small samples (127–129). A larger

study (40 patients) failed to replicate earlier findings, complicating efforts to draw definitive conclusions (130). Similarly, macrophage infiltration ($CD163^+$) does not show consistent increases in schizophrenia, though it may be present in patients with additional immune biomarkers (131,132).

Studies on CSF immune cells composition from living schizophrenia patients show trends toward higher white cell counts, but the differences are small and often not statistically significant (96,124,133). Regarding the actual magnitude of the reported increase, the combined mean value of CSF white cell count in patients with schizophrenia was 1.96 (±3.77), and only 3.1-3.6% of the patients showed values higher than the established normal range (>5 cells x 10^6/L) (124).

Microglia

Microglia activation, the main immune cells of the CNS, are frequently emphasized in discussions of neuroinflammation in schizophrenia. Inferring schizophrenia risk genes from known genetic risk variants primarily indicate neuronal cell types while there is no significant enrichment in microglia cells or in peripheral immune cells (29,134). This is in stark contrast to a prototypic neuroinflammatory disease such as e.g., multiple sclerosis (135). Contrary to initial expectations of heightened microglial activity, positron emission tomography (PET) studies using the 18-kDa translocator protein (TSPO)—a mitochondrial marker highly expressed in activated microglia—have revealed reduced TSPO levels in schizophrenia (136–138). This finding has led to the hypothesis that microglial maturation may be altered in schizophrenia, with more microglia remaining in a synaptic housekeeping state (139). However, the lack of specificity of TSPO, which also binds to astrocytes and endothelial cells, complicates these interpretations (140). More specific tracers are needed to investigate this hypothesis further.

Postmortem studies provide mixed evidence regarding microglial changes in schizophrenia. While some show increased microglial density and activation markers, recent meta-analyses suggest a shift in microglial phenotypes without a change in overall density (141). Variations in microglial function across different brain regions have also been reported, and emerging research highlights the diversity of microglial states beyond the traditional "resting" versus "activated" dichotomy. Notably, microglia can exhibit activated profiles even in the absence of inflammation, particularly during early brain development (142). Transcriptome data from schizophrenia patients further complicates

the picture, showing unchanged or downregulated microglial genes but upregulation of astrocyte genes, aligning with PET findings (143,144).

Astrocytes

Astrocytes, once thought to solely support neurons, are now recognized for their active roles in regulating synaptic neurotransmission, producing neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF) and contributing to neurovascular coupling to maintain the blood-brain barrier (145). Recent postmortem studies indicate a shift in focus from microglia to astrocytes in understanding immune dysfunction in schizophrenia; specifically, astrocytic gene expression appears to be upregulated, while microglial profiles may be downregulated (146). The authors suggest a potential connection between these findings, proposing that reduced markers of microglial activation in schizophrenia—observed in earlier PET studies—could result from excessive release of tumor growth factor-β by astrocytes. This release may reduce microglial activation and proinflammatory cytokine production, ultimately leading to increased synaptic elimination.

Neurodegenerative tissue damage

Neurodegeneration, characterized by the progressive loss of neuron structure and function, has long been associated with schizophrenia, initially described as "dementia praecox" over a century ago (147). However, contemporary understanding has shifted, recognizing schizophrenia more as a neurodevelopmental disorder (87). Meta-analyses indicate that common neurodegenerative markers, such as tauopathy and amyloid pathology, are not associated with schizophrenia (148,149), and studies show no significant differences in CSF S100β levels between schizophrenia patients and health controls (123). Furthermore, while lower Neurofilament Light (NfL) levels in schizophrenia have been reported, this remains controversial (150). Evidence suggests that neurodevelopmental factors, such as excessive dendritic and synaptic loss rather than primary neuron death, play a more significant role in schizophrenia pathology (26).

Post-mortem studies show lower synaptophysin levels, indicating reduced synaptic density in key brain regions of schizophrenia patients (151). Additionally, research involving induced pluripotent stem cells from schizophrenia patients shows compromised synaptic formation and increased synapse elimination (26). Neuroimaging studies reveal reductions in grey matter volume and cortical thickness in schizophrenia, particularly in

fronto-temporal areas, which are linked to synaptic and dendritic changes rather than neuronal death (27). This implies that neurodevelopmental predispositions, rather than classic neurodegenerative processes, underlie the dysfunction observed in schizophrenia, challenging the notion of using "neurodegeneration" as a basis for neuroinflammation in this context.

Blood-Cerebrospinal Fluid Barrier (BCB) [2]

Dysfunction of the blood-cerebrospinal fluid barrier (BCB) has been suggested in schizophrenia, supported by meta-analytic evidence of modest increases in the albumin CSF/serum ratio (Q_{alb}) (SMD ~ 0.4-0.55) and CSF total protein levels (SMD ~ 0.3) (124). However, these increases are mild and fall below clinical thresholds for concern, suggesting that any BCB disruption in schizophrenia is relatively minor compared to classical neuroinflammatory disorders. The mean Q_{alb} in schizophrenia patients was 5.53, well below the clinically significant cut-off of 9, indicating an intact barrier. Similarly, the mean CSF total protein level was 37.8 mg/dL, below the normal cut-off of 50-60 mg/dL (152). Finally, abnormal BCB integrity biomarkers are present in only a minority of schizophrenia patients (3-23%, depending on the parameter), and these abnormalities do not consistently correlate with symptom severity (153).

In summary, immune system alterations in the CNS are evident in schizophrenia compared to HC, but whether to label these changes as neuroinflammation is debated. Many findings, despite being upregulated, remain within clinically normal ranges and may not indicate neuroinflammation. Instead, they likely reflect neuroimmune mechanisms crucial to neurodevelopment, consistent with schizophrenia pathology involving exaggerated synaptic pruning (154). Therefore, we propose the term "altered neuroimmune activity" for schizophrenia instead of "neuroinflammation," though the debate remains unresolved.

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[2] The blood-brain barrier (BBB) consists of endothelial cells with tight junctions, supported by pericytes and astrocytes, and is found throughout the brain. In contrast, the blood-CSF barrier (BCB) is formed by epithelial cells in the choroid plexus and subarachnoid space. These structural differences affect how substances pass through each barrier.
In recent decades, the CSF-to-serum albumin ratio (Q_{alb}) has been widely, but incorrectly, used to assess BBB integrity, even though albumin is produced only in the liver. CSF is primarily produced in the choroid plexus, circulates around the brain and spinal cord, and only contacts the BBB in perivascular spaces. Since CSF from these spaces doesn't reach the lumbar region, an elevated Q_{alb} in lumbar CSF likely does not indicate BBB damage. Instead, Q_{alb} reflects BCB integrity, though factors like CSF flow and production are also relevant. For a deeper review, see Yakimov et al.(189).

f. The intervention evidence

The true test of the immune hypothesis in schizophrenia lies in assessing the effectiveness of treatments with known immune-modulating properties. Since the 2000s, several clinical trials have explored the efficacy of add-on anti-inflammatory agents in this population. A recent meta-analysis showed that drugs with primary or pleiotropic anti-inflammatory effects, when used alongside antipsychotic treatments, significantly reduced the severity of psychopathological symptoms compared to placebos, although the improvement was modest but statistically significant (SMD: -0.29) (**Figure 7**) (155). However, no advantage was observed for drugs that were exclusively anti-inflammatory, raising questions about the underlying mechanism of the observed effect. Additionally, a meta-regression analysis revealed that effect sizes for total psychopathology scores significantly decreased as the sample sizes in the studies increased, suggesting that smaller studies may have overestimated treatment effects.

Although several previous meta-analyses have examined the impact of such add-on treatments (156–160), none have explored whether baseline immune biomarkers can predict treatment outcomes. This is an important area for future research, as it could pave the way for the clinical use of immune-modulatory treatments (161).

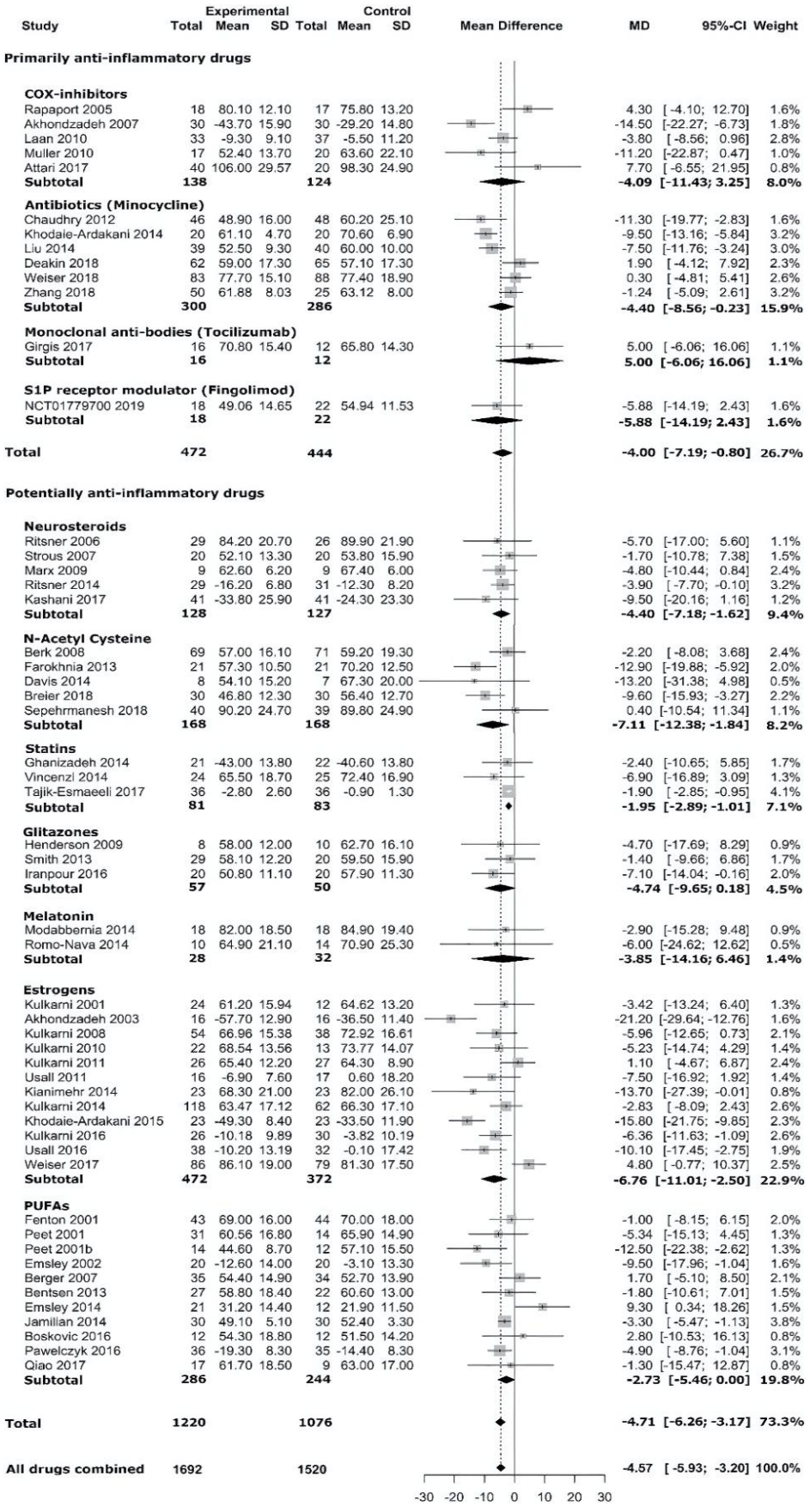

Figure 7. Mean difference in PANSS total psychopathology score for anti-inflammatory add-on RCTs. Adapted from Jeppesen et al. (155) using Upscayl.

2. HYPOTHESES

Main Hypothesis:

Immune biomarkers, including WBC counts, their ratios, and CRP, can predict clinical outcomes in patients with schizophrenia and other psychotic disorders across different phases of the illness.

Secondary Hypotheses:

Hypothesis 1: In patients in the early phases of schizophrenia, immune biomarkers can predict treatment response and the risk of relapse.

Hypothesis 2: In acutely hospitalized patients with psychotic disorders, immune biomarkers can predict functional outcomes.

Hypothesis 3: In patients with TRS, immune biomarkers can predict the response to the initiation of clozapine treatment.

Hypothesis 4: In patients experiencing a FES, immune biomarkers are associated with long-term outcomes, including treatment response, readmissions, and mortality.

Hypothesis 5: WBC counts, ratios are associated with specific immune pathways in psychotic disorders and are influenced by substance use.

3. OBJECTIVES

General Objective:

Investigate the role of immune biomarkers, including WBC counts, their ratios, and CRP, in predicting clinical outcomes in patients with schizophrenia and other psychotic disorders.

Specific Objectives:

Objective 1: To evaluate the relationship between the NLR and treatment response over three years in patients with a first episode of psychosis (FEP), and to assess whether WBC counts and ratios in stable patients after a first episode of schizophrenia (FES) can predict relapse. (**Articles I** and **II**)

Objective 2: Examine how WBC counts, ratios, and CRP levels can predict functional outcomes in acutely hospitalized patients with psychotic disorders, including schizophrenia and psychotic depression. **(Article III and IV)**

Objective 3: Study whether WBC count ratios can predict the response to the initiation of clozapine treatment in patients with TRS **(Article V)**

Objective 4: Evaluate how WBC counts, ratios, and PCR levels in patients with a FES are associated with long-term outcomes, including mortality, treatment response, and readmissions. **(Article VI)**

Objective 5: To determine the relationship between WBC count ratios and other immune markers in acutely hospitalized psychiatric patients, and to examine how substance use (such as cannabis, opioids, or cocaine) impacts these markers. (**Articles VII, VIII,** and **IX**)

4. MATERIAL, METHODS AND RESULTS

4.1. Article I

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Neutrophil to Lymphocyte Ratio in Patients With a First Episode of Psychosis: A Two-Year Longitudinal Follow-up Study

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***Background and Hypothesis*: A pro-inflammatory phenotype has been related to psychotic disorders. The neutrophil-lymphocyte ratio (NLR) is an accessible biomarker that could be helpful to characterize this systemic inflammation state. *Study Design*: This study evaluated the NLR in a cohort of 310 subjects with a first episode of psychosis (FEP) and a matched group of 215 healthy controls, recruited in 16 Spanish centers participating in the PEPs Project. We investigated the NLR measures over 2 years in a prospective, naturalistic study. *Study Results*: At baseline, the FEP group showed a significant higher mean NLR compared to the control group (1.96 ± 1.11 vs 1.72 ± 0.74, $P = 0.03$). These ratio differences between groups grew at the 24 months follow-up visit (2.04 ± 0.86 vs 1.65 ± 0.65, $P < 0.001$). Within the FEP group, there were no significant differences in NLR across the follow-up visits, between genders or diagnosis groups (affective vs nonaffective). NLR values did not correlate with the Positive and Negative Symptoms Scale scores. The group of patients who did not reach remission criteria at the end of the study showed a significant higher NLR than those who remitted (2.1896 ± 0.85 vs 1.95 ± 0.87, $P = 0.042$). A significant correlation between antipsychotic doses and NLR was found at the two-years follow-up visit ($r=0.461$, $P < 0.001$). *Conclusions*: Our results highlight the existence of an underlying predisposition of FEP patients to present an increased mean NLR. The use of NLR in clinical practice could be helpful to identify this inflammatory imbalance.**

Key words: antipsychotics/first episode/inflammation/neutrophil to lymphocyte ratio/NLR/psychosis/schizophrenia

Introduction

Psychotic disorders are among the most disabling mental disorders.[1] A first episode of psychosis (FEP) is characterized by the presence of positive symptoms (delusions, hallucinations, and bizarre behavior), usually accompanied by negative (apathy and alogia), affective and cognitive symptoms. Around 3% of the general population suffers a psychotic episode during their life, generally appearing in adolescence or early adulthood.[2]

The underlying pathophysiology of the FEP remains unclear, being considered multifactorial and based on genetic and environmental interaction.[1,3] In the last decade, several processes involving inflammatory pathways and consequent oxidative/nitrosative stress have been linked to schizophrenia and related psychotic disorders, suggesting both peripheral and central pro-inflammatory state.[4,5] Scientific evidence supporting the hypothesis that inflammatory changes may play an important role in psychotic disorders has mostly been described in the chronic stage of the disorder.[4] New data are supporting that low-grade proinflammatory changes may already be present from early phases.[6-8] Thus, it has been reported that, compared to healthy controls, patients with a FEP have a pro-inflammatory imbalance (ie, higher levels of homocysteine, interleukin-6, and tumoral necrosis factor alpha), and a reduced antioxidant capacity (ie, lower levels of docosahexaenoic acid (DHA).[8]

Neutrophil to lymphocyte ratio (NLR) has been identified as a marker of systemic inflammation in a vast spectrum of diseases, especially in different cancers, since it is associated with an increase in cytokines and C-reactive protein (CRP).[9] The use of NLR is interesting at the clinical practice since it is an inexpensive, easy to use marker of disease, calculated from the complete blood count dividing the number of neutrophils by the number of lymphocytes. In mental disorders, a recent study has proposed that NLR could be a predictor for bipolar depression.[10] Another recent study has showed that high NLR upon admission is associated with a better response in psychotic depression.[11] A systematic review and metanalysis showed increased NLR both in FEP and in multi-episodic schizophrenia, but it is not entirely clear whether this is a characteristic of the disease itself or whether treatment or metabolic changes could influence this value.[12] Another metanalysis has reported that in FEP compared with controls, neutrophils and monocytes were significantly increased.[13]

In this vein, the study of the population with a FEP is of great interest since it mitigates the effect of confounding variables. Cohort studies are crucial to identify biomarkers and predictors of outcome in this population.[14,15] Our group has previously reported that isolated neutrophil count is associated with reduced gray matter and enlarged ventricles in FEP.[16] The aim of the present study is to analyze the NLR differences between a well-characterized group of patients with a FEP and a control group during a two-year follow-up.

Subjects and Methods

Subjects

From April 2009 to April 2012, 16 Spanish centers participated in PEPs project ("Phenotype-genotype and environmental interaction. Application of a predictive model in first psychotic episodes").[15,17] 335 patients with a FEP and 253 healthy controls were recruited. The local ethics committee of each center approved the study, and it was obtained an informed consent from all participants or from parents/legal keeper in under 18-year-old subjects. The rationale and the complete clinical protocol used in the PEPs project were previously published.[17]

All patients included in this study were aged 7–35 years old, presented their first psychotic symptoms (positive symptoms or disorganization) for at least 1 week in the previous 12 months and spoke Spanish correctly. Patients with mental retardation according to the Diagnostic and Statistical Manual of mental disorders, 4th edition Text Revised (DSM-IV-TR) criteria,[18] history of head injury with loss of consciousness, and presence of an organic disease with mental repercussions were excluded. Those who met the inclusion criteria to this study were invited to participate, on either an inpatient or outpatient basis.

Healthy controls were matched by age, gender, and socio-economic status (measured by Hollingshead-Redlich scale [±1 level])[19] and they also had to speak Spanish correctly. The exclusion criteria of control subjects were the same as for patients plus having a personal antecedent of psychotic and/or major affective disorder and/or having a first-degree relative with history of psychotic disorders.

Diagnostic, Demographic, and Clinical Data Collection

In order to confirm each patient diagnosis, the Spanish translation of the Kiddie-SADS-Present and Lifetime Version (K-SADS-PL) was used to evaluate current and past psychopathology according to DSM-IV-TR criteria for children and adolescents,[20] and the Spanish translation of Structured Clinical Interview for DSM Disorders (SCID) parts I and II (SCID-I & II) for adults.[21]

A dichotomous categorization of affective vs nonaffective psychosis was used for some analyses. Affective psychosis includes DSM-IV-TR diagnosis of unipolar depression or bipolar disorder with psychotic features and schizoaffective disorder.[18] Psychotic symptoms were assessed using the validated Spanish version of the Positive and Negative Symptom Scale (PANSS).[22,23] To determine the rate of patients who achieve symptomatic remission during the last 6 months of the 2-year follow-up, we implemented the Remission in Schizophrenia Working Group (RSWG) criteria by Andreasen et al.[24]

As the PEPs Project was an observational naturalistic study, there were no specific guidelines for treatments, so the antipsychotic treatment was based on clinician's choice.[25] Thus, dosing, comedications, duration, or treatment changes were based on clinical need and registered in the common data base. To compare the different antipsychotics between them, the prescribed daily doses of antipsychotics were converted to an estimated equivalent amount of chlorpromazine (CPZ) following the international consensus.[26] Baseline polypharmacy was registered considering simultaneous treatment in the same patient with one antipsychotic together with an antidepressant, an anticholinergic drug, a mood stabilizer, a benzodiazepine, or another antipsychotic. A previous report gave a full description of the psychopharmacological treatment used in the PEPs project.[25]

Study Assessments

At baseline, a complete medical history was taken. Laboratory data of lymphocytes and neutrophils were assessed at baseline and at 2-, 6-, 12-, and 24-months follow-up visits in patients, and at baseline and at 24 months visit in controls. K2EDTA BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey) were used to collect blood samples which were stored at −20°C and sent to each site laboratory for analysis. The reference values at each site were recorded in a common database called GRIDSAM, where individual values were homogenized and included.[17,27]

Statistical Analysis

Continuous data are expressed as a mean ± standard deviation and categorical data are expressed as absolute values and percentages. A two-tailed Chi-square test was used to assess differences in categorical variables and a two-tailed *t*-test was used to assess differences on continuous variables with approximately normal distributions. The normality of continuous variables was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests, and the equality of the variance between groups was assessed using Levene's test. The Mann–Whitney U was used to assess nonparametric variables. Within the FEP group, a one-way repeated measures ANOVA was conducted to compare changes in patients' NLR measures between visits.

A mixed between-within subjects' analysis of variance was conducted to assess the impact of gender and diagnosis (affective vs nonaffective psychosis) on NLR measures. Wilks' Lambda was used to explore the relationship between gender and diagnostic and NLR measures at the 24 months of follow-up. Correlation of NLR with clinical scales scores was assessed by Spearman rank tests.

The relationship between the NLR outcomes and antipsychotic mean daily doses (in chlorpromazine equivalents) was investigated using Pearson correlation coefficient. A simple linear regression was used to evaluate the relationship between the antipsychotic doses and the NLR values.

Two-tailed *P*-values < .05 were considered to be of statistical significance. Statistical analyses were performed using IBM-SPSS v.25.[28]

Results

Baseline Characteristics and Study Drop-outs

From the 335 patients with a FEP and 253 healthy controls participants of the PEPs project, 25 patients, and 38 control subjects were excluded from the present study as NLR data were not available. Demographic, anthropometric, and diagnosis characteristics are presented in table 1. There were no differences between cases and controls in the matching variables, so the differences found do not result from an inadequate case-control group matching, or weight at baseline.

Of the total number of participants who started the study, it was possible to obtain the NLR data from 184 cases and 143 controls at two years. There were no baseline differences in gender, age, weight, or ethnicity among patients and controls who dropped out of follow-up, with the exception that a significant higher proportion of patients diagnosed with non-affective psychosis dropped out of the study early (43.2% vs 27.5%, χ^2=4.41, P = .036).

NLR Comparison Between FEP and Control Groups

At baseline, the FEP group showed a higher mean NLR compared to the control subjects (1.96 ± 1.11 vs 1.72 ± 0.74, P = .03). Mean NLR differences between cases and controls were even higher at the 24 months of follow-up (2.04 ± 0.86 vs 1.65 ± 0.65, P < .001). See table 2 for details.

There were no differences in the mean NLR values at baseline and 24 months within the control group (1.72 ± 0.84 vs 1.64 ± 0.65, F = 1.54, P = .28).

NLR Measures in the Follow-up Visits in Between FEP Group

Mean NLR values at baseline, 2, 6, 12, and 24 months within the FEP group are described in table 3. The one-way repeated measures ANOVA analyses did not detect statically significant increases in the NLR within the FEP group across the follow-up visits.

Within the FEP group, there were no differences at baseline NLR between women and men (2.07 ± 1.31 vs 1.91 ± 0.99; U = 10 348; P = .67). After 24 months of follow-up, there were still no differences (2.16 ± 0.96 vs 1.98 ± 0.81; P = .2). There were significant lower baseline NLR values in the underage (<18 years, n = 51)

Table 1. Demographic, Anthropometric, and Baseline Characteristics

	FEP (n = 310)	Control (n = 215)	Statistic	P-value
Age – years [mean (SD)]	23.6 ± 6	24.2 ± 6.4	t = −1.07	.29
Gender (male) – no. (%)	207 (66.8%)	140 (65.1%)	X^2 = 0.16	.69
Ethnic group – no. (%)			X^2 = 8.59	.28
Caucasian	266 (85.8%)	193 (89.8%)		
Gipsy	5 (1.6%)	0 (0)		
Maghrebian	7 (2.3%)	2 (0.9%)		
Sub-saharan	2 (0.6%)	0 (0)		
Asian	4 (1.3%)	1 (0.5%)		
Caribbean	7 (2.2%)	3 (1.4%)		
Hispanic	16 (5.2%)	12 (5.6%)		
Other	3 (1%)	4 (1.8%)		
Weight – (kg)	69.26 ± 14.06	69.23 ± 12.75	t = 0.023	.98
Tobacco use				
Active smoker (yes) – no. (%)	188 (60.6%)	70 (32.6%)	χ2 = 43.77	P < .001
Number of cigarettes per month	239.63 ± 263.65	68.45 ± 144.55		
Diagnosis				
Affective psychosis	51 (16.4%)	–		
Bipolar disorder	37 (11.9%)	–		
Major depressive disorder	7 (2.3%)	–		
Schizoaffective disorder	7 (2.3%)	–		
Non-affective psychosis	259 (83.6%)	–		
Psychotic disorder NOS	99 (31.9%)	–		
Schizophreniform disorder	62 (20%)	–		
Schizophrenia	48 (15.5%)	–		
Brief psychotic disorder	48 (15.5%)	–		
Delusional disorder	2 (0.6%)	–		
hospitalization				
Patients with hospitalization (%)	231 (74.5%)	–		
Duration (days) – mean (SD)	21.02 ± 22.48			
PANSS				
Positive subscale score – mean (SD)	18.69 (8.01)	–		
Negative subscale score – mean (SD)	18.6 (8.15)	–		
General subscale score – mean (SD)	37.8 (12.96)	–		
Total score – mean (SD)	75.1 (24.7)	–		

Note: FEP, First Episode of Psychosis; NOS, Not otherwise specified; PANSS, Positive and Negative Symptom Scale.

Table 2. NLR Comparison Between the Patients and Healthy Control Group at the Two-year Follow-up Visit

	FEP	Control	Statistic	P-value
NLR baseline – mean (SD)	1.96 ± 1.11 (n = 310)	1.72 ± 0.74 (n=215)	U = 29.637.50	**.03***
NLR 24 months – mean (SD)	2.04 ± 0.86 (n = 184)	1.65 ± 0.65 (n=143)	U = 19.883.00	**<.001***

Note: FEP, First Episode of Psychosis.
*P < .05 marked in bold.

Table 3. NLR Mean Measures for Patients Along All the Visits of the PEPs Study

	Baseline (n = 310)	2 Months (n = 140)	6 Months (n = 140)	12 Months (n = 140)	24 Months (n = 184)	Statistics	P-value	Partial Eta Squared[a]
NLR	1.96 ± 1.11	2.08 ± 1.11	1.99 ± 1.09	1.93 ± 0.91	2.04 ± 0.86	WL = 0.974 F = 0.895	.47	0.03

Note: WL, Wilk's Lambda.
[a]Partial Eta Squared value of effect size (0.01–0.05 = small, 0.06–0.13 = moderate, >0.14 = large).

cases: 1.62 ± 0.68 vs 2.03 ± 1.17, $U = 8048.5$, $P = .014$), but not at the end of the follow-up.

As expected, a greater proportion of patients smoked tobacco compared to controls at baseline (60.6% vs 32.6%, $\chi 2=43.77$, $P < .001$). However, both in the group patients and in controls, there were no differences in baseline NLR between smokers or non-smokers. NLR values also did not correlate with the number of cigarettes smoked daily ($r = 0.05$, $P = .21$).

There were no significant differences between diagnostic categories (nonaffective vs affective psychosis) neither at baseline (1.96 vs 2.00; $t = -0.251$, $P = .802$) nor at 24 months of follow-up (1.94 vs 2.06; WL = 0.94, $F = 2.14$, $P = .08$). No differences in NLR values were found between patients who had required hospital admission at baseline and those who did not, and the ratio was not correlated with the number of days hospitalized, both at baseline and at the end of the study. Nor was there a statistically significant correlation between NLR values and PANSS scores (positive, negative, general, and total) at both baseline and 24-month follow-up visits.

Of the 190 patients evaluated at the two-year follow-up visit, 123 (64.7%) met the RSWG remission criteria. The percentage of patients with affective psychosis who achieved remission at two years of follow-up was higher than the nonaffective psychosis group (80% vs 61.3%, $\chi^2=4.38$, $P = .036$). The group of patients who did not reach remission criteria at the end of the two-year follow-up showed a significantly higher NLR than the group in remission (2.19 ± 0.85 vs 1.95 ± 0.87, $U = 3896.5$, $P = .042$).

Psychopharmacological Treatment

Psychopharmacological treatment of the FEP cohort at baseline and at the 24-months follow-up is presented in table 4. At baseline, 285 patients were taking antipsychotics and 25 were antipsychotic naïve. When comparing the baseline NLR of these two groups there were no statistically significant differences (1.97 vs 1.85; $t = 0.548$, $P = .584$). There were not differences in baseline NRL between the groups who received mono and polytherapy (1.97 vs 1.99; $t = 0.117$, $P = .907$).

At baseline, we found a tendency of positive correlation between equivalent amount of CPZ doses and basal NLR ($r = 0.11$, $P = .056$). At 24 months of follow-up, this tendency was achieved, finding a statistically significant correlation between equivalent daily doses of CPZ and NLR ($r = 0.461$, $P < .001$) at 24 months of follow-up. A simple linear regression identified that CPZ equivalent doses explained the 21% of the variance in NLR values ($F = 25.13$, $P < .001$) at the end of the follow-up.

The group of patients who did not reach remission criteria at the end of the two-year follow-up showed a significantly higher mean antipsychotic doses than the group that reached remission (507 72 ± 310 84 vs 269 85 ± 243 73; $t = -4.17$; $P = .001$).

Table 4. Psychopharmacological Treatment in the Baseline and in the Two-year Follow-up Visit

	Baseline ($n = 310$)	24 Months ($n = 105$)
Antipsychotic treatment		
No antipsychotic therapy – no. (%)	25 (8.1%)	17 (5.5%)
Monotherapy – no. (%)	217 (70.0%)	77 (24.8%)
Polytherapy – no. (%)	68 (21.9%)	11 (3.5%)
2 antipsychotics – no. (%)	62 (20%)	10 (3.2)
3 antipsychotics – no. (%)	5 (1.6%)	1 (0.3%)
4 antipsychotics – no. (%)	1 (0.3%)	0
Chlorpromazine equivalent mean dose – mg/day (SD)	611.10 ± 449.91	346.30 ± 289.06
Subjects with other treatment		
Anticholinergics	39 (12.6%)	4 (1.3%)
Antidepressants	41 (13.2)	18 (5.8%)
Mood stabilizers	40 (12.9%)	27 (8.7%)
Benzodiazepines	123 (39.7%)	17 (5.5%)

The use of other psychopharmacological treatments (antidepressants, anticholinergics, mood stabilizers, or benzodiazepines) did not correlate with the NLR, neither at baseline nor in the two-year follow-up visit.

Discussion

A variety of mechanisms involving the immune system and an inflammatory activation has been related to the pathophysiology of schizophrenia and related psychosis.[4] Inflammatory ratios, especially NLR and monocyte/lymphocyte ratio (MLR), may be useful to detect this activation.[12,13,29] In this largest, longitudinal, case-control study analyzing NLR in FEP, we found: (1) Higher mean NLR values in a cohort of subjects with a FEP compared to a matched control group at baseline. These differences grew at the 24 months of follow-up; (2) Within the FEP group, there were no statistically significant differences in NLR across the study follow-up visits; (3) There was no significant correlation between NLR values and PANSS scores or hospitalization, but patients who reached remission criteria at the end of the two-year follow-up showed significantly lower mean NLR values than the non-remitted group; (4) significant lower NLR values at baseline in underage patients, compared to adults; (v) No significant differences in NLR values between genders, tobacco users/nonusers or affective vs non-affective psychosis; and (6) a significant positive correlation between antipsychotic equivalent daily doses of CPZ and NLR at the end of the study.

A major part of these findings are in line with previous studies.[12,30–32] Recent systematic reviews and metanalyses have concluded that NLR in schizophrenia patients is

increased, both in chronic disease and in first-episode psychosis.[12,13,29]

Steiner and collaborators found that positive symptoms correlated with neutrophil counts, describing a decreasing in NLR of FEP group after 6 weeks of follow-up of treatment, suggesting that these cells may act as a modulator of acute disease severity.[32] In our study, we didn't find a significant correlation between NLR and PANSS scores. These differences could be related, at least in part, to the fact the mean PANSS total score from our cohort was notably higher (more severe) at baseline than in the study from Steiner and collaborators (75.1 vs 31.0). In our study, we also did not find a correlation with another indirect marker of severity at baseline, such as the need for hospitalization and its duration. However, we found that the group of patients who did not reach remission criteria at the end of the two-year follow-up showed a significantly higher NLR than the group in remission. Similarly, Labonté et al. reported NLR decreases following treatment in the responsive group exclusively, but not in treatment resistant schizophrenia group.[33] Our results could be explained, at least in part, with the finding that nonremitted patients showed significantly higher mean antipsychotic doses than remitted at the two-year follow-up visit. These findings underscore the interest of studying NLR as a marker of severity in the evolution of psychotic disorders.

When analyzing by gender, there were no differences in both subgroups at baseline in the mean of NLR as it was observed in a similar study.[31] This observation was maintained during the 24-month follow-up. There was also no correlation between basal NLR values and smoking tobacco. On the other hand, we found significant lower NLR values at baseline in underage patients, compared to adults. This point could be related to a greater predisposition to present neutropenia in patients with an early onset of psychosis, which is especially relevant when starting certain antipsychotics, such as clozapine.[34] These differences, which were not maintained at 24 months, indicate the importance of considering age as a potential confounding factor to be considered when assessing the use of NLR in adolescent patients.

There is evidence of high neutrophil count and NLR in patients suffering from nonaffective psychosis and mood disorders compared to healthy controls.[29,35,36] Mazza and collaborators found in acute, multiepisodic patients that NLR was significantly more elevated in schizophrenia patients than in affective psychosis.[37] In our study, with patients in early phases, the affective psychosis subgroup showed comparable NLR values to the nonaffective psychosis subgroup, both at baseline and at 24 months of follow-up.

We did not find differences between the patients who were taking antipsychotics and antipsychotic naïve patients at the study entry. There is an interest in the field in clarifying the effects of antipsychotic treatment on NLR, having been proposed its increase after antipsychotic treatment.[12] However, there are studies that did not find significant differences in NLR between patients who had been receiving antipsychotic treatment and those who had not,[38-40] similarly to our results.

At baseline, we found a statistical trend for a positive correlation between equivalent amount of CPZ doses and NLR. This tendency was confirmed at the end of the 24 months of follow-up, when a statistically significant positive correlation between antipsychotic equivalent daily doses of CPZ and NLR was found. The previous study from Steiner and collaborators found that equivalent amount of CPZ doses was correlated negatively with the neutrophils count from baseline to follow-up.[32] The differences in antipsychotics patterns and psychotic symptoms severity between both studies could explain this contradictory results.

Another point we have focused on in our study was the effects of antipsychotic mono- and poly-therapy, which is a very common practice in everyday clinical practice.[25] At baseline, there were no differences in NLR between the patients who were in mono- and poly-therapy. The use of antidepressants and mood stabilizers did not affect the NLR value.

Some limitations should be considered at the moment of the analysis of these results. Firstly, patients could be included in the study being under antipsychotic treatment, which could be affecting the NLR in some cases. As the mean duration of the antipsychotic treatment at the study entry was of 54.08 days,[25] this effect could be mild. Correlation analyzes were performed to establish the relationship between antipsychotic doses and NLR results at both the baseline and final visit levels. Secondly, since the PEPs Project was a naturalistic study, treatments given during the follow-up were chosen by the clinicians according to clinical needs, so there was a certain level of heterogeneity of antipsychotic patterns.[25] In order to avoid these differences, a randomized controlled trial would be necessary. Thirdly, as most participating sites in this study are tertiary care centers linked to the Spanish network of translational research (CIBERSAM),[41] patient samples, and therapeutic strategies may differ from those used in other areas.[42] Besides, to better characterize the response to treatment and the clinical evolution during follow-up, we applied the RSWG remission criteria to the whole group of cases, despite the fact that a small percentage of patients had been diagnosed with an affective psychosis (16.4%), of which a greater proportion met these criteria (80% vs 61.3%, χ^2=4.38, P = .036). Finally, the relatively high number of drop-out during the follow-up period (40.6% of the cases and 33.5% of the controls) may have limited the capability to detect differences between groups in some analyses at the end of the study, although we did not find differences in demographic and baseline clinical variables between dropouts and completers.

This study had the advantage of having strict inclusion/exclusion criteria, a diagnostic evaluation done with a very comprehensive protocol, patients with a wide age of inclusion, trying to replicate the natural history of the diseases, which makes this sample as close as possible to the reality of the FEP population,[17] mitigating the effect of confounding variables. Besides, blood analyses were taken following a strict, unified protocol, which is especially relevant since leukocyte levels fluctuate throughout the day.[42]

Dysregulation of immunological and inflammatory processes has been repeatedly reported in peripheric samples from subjects with psychotic disorders,[4,43] and current research also suggests that schizophrenia patients may also present CSF abnormalities, including signs of blood-brain barrier impairment and inflammation.[44] However, despite numerous studies of the complex components of innate and adaptive immune processes, it still has not been clarified whether this imbalance occurs before psychosis (related to etiology), during its evolution (related to pathophysiology), or if it is an epiphenomenon accompanying it.[4,43] In this context, NLR values can be an accessible approach to know the inflammatory status in an individual, but the available evidence in FEP subjects does not rule out the possibility of alternative explanations to this association. Although NLR in peripheral blood can provide insights into the potential immunological contribution to psychosis, the central nervous system (CNS) is protected by the blood–brain-barrier. Thus, peripheral blood levels of NLR might not translate directly to changes within the central nervous system. In this line, Campana et al. have recently described no significant relationship between CSF alterations and peripheral inflammation measured with CRP, suggesting a FEP subgroup with an intrathecal inflammatory etiology of the disease.[45] NLR in blood and CSF at one time has been previously investigated in other fields like acute bacterial meningitis,[46] but studies in psychiatric patients are still scarce.[44,47] Future studies should focus on CSF measure to better understand the inflammatory process in the CNS in FEP.

Despite our attempt to control potential confounding factors (such as age, gender, tobacco use, or drug treatment), these variables and others (ie, primary and treatment-related metabolic differences, different health habits including nutrition and physical activity, gut dysbiosis, substance abuse, etc.) could be mediating in the differences we found between FEP and control groups.

It should also be noted that from other fields of medicine, for example in cancer, we have learned that the low diagnostic utility of unspecific inflammatory markers (such as C-reactive protein or erythrocyte sedimentation rate) is due to poor sensitivity.[48] In terms of NLR, oncological meta-analysis supported the relationship between elevated NLR and poor outcomes in cancer.[48] However, current evidence does not allow to determine whether the association is causal or due to confounding or reverse causation. Furthermore, the lack of a clinically relevant and accepted NLR cut-off hampers the applicability of the index in clinical practice. All these arguments are transferable to psychiatry, with fewer studies supporting causality and even less consensus on accurate cut-offs.[49] For all this, it is needed more evidence and clinical experience using NLR as a biomarker to determine neurobiological phenotypes or to be integrated in our treatment- decision algorithms (ie, choosing certain antipsychotic drug or adding co-adjuvant antiinflammatory drugs).

Overall, these findings support the existence of an underlying process in FEP patients to present an increased mean of NLR since the very beginning of the disorder, so its determination could be a useful tool to characterize this inflammatory imbalance, to define prognosis, and response to treatment in this population. As NLR is used in the scientific literature, once it starts to be used in the clinical practice, it would be interesting to describe its normal values in the general population. Future studies would also help to clarify the effects of antipsychotics over NLR and its correlation with clinical response, testing its utility as an accessible biomarker for treatment response.

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Author contribution

M.Bi. collected the clinical data, managed and analyzed the clinical data and wrote the first version of the paper; A.C.M.M. analyzed the clinical data and wrote the first version of the paper; G.M. collected the clinical data and managed the first version of the data base; M.Be. coordinated the PEPs study. All the authors contributed to the final version of the paper.

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4.2. Article II

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Inflammatory blood cells and ratios at remission for psychosis relapse prediction: A three-year follow-up of a cohort of first episodes of schizophrenia

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ABSTRACT

Background: The clinical course following a first episode of schizophrenia (FES) is often characterized by recurrent relapses, resulting in unfavorable clinical and functional outcomes. Inflammatory dysregulation has been implicated in relapse risk; however, the predictive value of inflammatory blood cells in clinically remitted patients after a FES has not been previously explored.
Methods: In this study, we closely monitored 111 patients in remission after a FES until relapse or a three-year follow-up endpoint. The participants were recruited from the multicenter 2EPS Project. Data on inflammatory blood cells and ratios were collected at baseline and at the time of relapse or after three years of follow-up.
Results: Monocyte counts (OR = 1.91; 95 % CI = 1.07–3.18; $p = 0.009$) and basophil counts (OR = 1.09; 95 % CI = 1.01–1.12; $p = 0.005$) at baseline were associated with an increased risk of relapse, while the platelet-lymphocyte ratio (OR = 0.98; 95 % CI = 0.97–0.99; $p = 0.019$) was identified as a protective factor. However, after adjusting for cannabis and tobacco use during the follow-up, only monocyte counts (OR = 1.73; 95 % CI = 1.03–2.29; $p = 0.027$) and basophil counts (OR = 1.08; 95 % CI = 1.01–1.14; $p = 0.008$) remained statistically significant. ROC curve analysis indicated that the optimal cut-off values for discriminating relapsers were 0.52 × 10^9/L (AUC: 0.66) for monocytes and 0.025 × 10^9/L (AUC: 0.75) for basophils. When considering baseline inflammatory levels, no significant differences were observed in the inflammatory biomarkers at the endpoint between relapsers and non-relapsers.
Conclusion: This study provides evidence that higher monocyte and basophil counts measured at remission after a FES are associated with an increased risk of relapse during a three-year follow-up period.

1. Introduction

Clinical course in schizophrenia (SCZ) is often characterized by recurrent relapses (Emsley et al., 2013), which are associated with adverse outcomes such as treatment-resistant symptoms, cognitive decline, and functional disability (Keepers et al., 2020; Takeuchi et al., 2018). Relapse occurs within 1 year for approximately 30 % (Brown et al., 2020) of individuals with SCZ and up to 50 % over 3 years (Bioque et al., 2022b). Several factors have been identified to increase the risk of relapse, including non-adherence to medication, persistent substance use and poorer premorbid adjustment (Alvarez-Jimenez et al., 2012; García et al., 2016; González-Pinto et al., 2011; Lauriello, 2020). Recent evidence has shown the positive impact of specific relapse prevention interventions on relapse rates (Abu Sabra and Hamdan-Mansour, 2022; Bighelli et al., 2021; Højlund et al., 2021; Rodolico et al., 2022). However, due to the limited real-life implementation and the modest effect of the interventions, relapse rates in SCZ remain high, increasing the personal, social, and financial burden of the disease (Pennington and McCrone, 2017; Pilon et al., 2021). Therefore, there is an urgent need to better understand the pathophysiology of relapse and identify endophenotypes with higher risk of relapse to develop and target efficient relapse-prevention interventions (Kapur et al., 2012; Rubio et al., 2021).

Inflammatory hypothesis in SCZ has gained increasing support (Benros et al., 2014; Steen et al., 2023). Total white blood cells (WBC) and differentials, including monocytes, lymphocytes, and neutrophils, are classified as immune blood cells due to their role in promoting systemic inflammation by releasing pro-inflammatory molecules. Elevated levels of total WBC, neutrophils, and monocytes have been observed in individuals with SCZ (Jackson and Miller, 2020; Mazza et al., 2020a) and have been linked to severity and treatment response (Steiner et al., 2020). By utilizing differential WBC counts, we can calculate inflammatory ratios that reflect the balance between innate immunity (indicated by neutrophil, monocyte, or platelet counts) and adaptive immunity (lymphocyte count) (Bhikram and Sandor, 2022). These ratios have been found to be increased in SCZ (Karageorgiou et al., 2019; Mazza et al., 2020b) and may offer enhanced predictive value when identifying imbalances between the innate and adaptive immune pathways (Llorca-Bofí et al., 2023). Higher inflammatory measures during the first episode of psychosis have been shown to predict worse treatment response at follow-up (Bioque et al., 2022a; Nettis et al., 2019; Osimo et al., 2021, 2023; Schwarz et al., 2012). However, the predictive value of immune blood cell counts for relapse in stable SCZ patients at early stages of the disease has not been previously studied. This raises the question: regardless of the underlying etiology, are inflammatory blood counts at remission predictive of relapse in SCZ? In our group, we have published longitudinal studies designed from the acute first episode of psychosis to clinical remission and assessing inflammatory factors (Bernardo et al., 2017; Bernardo and Bioque, 2014). Instead, in the 2EPs study we explore the opposite direction by analyzing the natural course of the disease: a longitudinal study to identify factors associated with a relapse within the years immediately following clinical remission of a first episode of schizophrenia (FES) (Bernardo et al., 2021). Using this design, we attempt to identify biomarkers for risk of relapse after FES and to move towards precision psychiatry (Vieta, 2015).

In this three-year follow-up study, we aimed to investigate (1) the potential value of inflammatory cells and ratios at baseline as biomarkers of relapse in a cohort of remitted FES patients with less than five years of evolution and (2) the itinerary of the inflammatory biomarkers over the course of the disease. We hypothesized that higher inflammation at baseline would be associated with higher risk of relapse during the follow-up.

2. Methods

2.1. Study setting, inclusion and exclusion criteria

The participants of this study came from the 2EPs Project. The background, rationale and study design have been previously presented elsewhere (Bernardo et al., 2021; Bioque et al., 2022b). The main aim of this project was to closely monitor the clinical course of clinical remitted

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FES patients and compare the subgroup of patients with a second episode to that which remains in remission. The sample was recruited from 15 clinical tertiary centers in Spain with experience of the preceding PEPs Study project (Bernardo et al., 2017) and affiliated with the Spanish Network of Translational Research in Mental Disorders (CIBERSAM) (Salagre et al., 2019).

The inclusion criteria for the 2EPs Project were: (1) age between 16 and 40 years; (2) met diagnostic criteria according to DSM-IV-TR for SCZ or schizophreniform disorder (American Psychiatric Association, 1994); (3) in remission from the first psychotic episode (which should have occurred within the last 5 years) according to the criteria set forth by the Remission in Schizophrenia Working Group (RSWG) (Andreasen et al., 2005). The exclusion criteria were: (1) intellectual disability defined by an estimated Intelligent Quotient (IQ) < 70, together with malfunctioning and difficulties with adaptive process, (2) history of head trauma with loss of consciousness and/or (3) presence of an organic disease with mental repercussions.

Out of the 223 patients initially included in the 2EPS cohort, only those who had completed the 3-year follow-up or had experienced a relapse during that period were considered in the present analysis. A total of 95 patients dropped out of the study, primarily due to their reluctance to attend demanding-time follow-up visits, and changes in their geographical locations affecting access to healthcare services (Bioque et al., 2022b). Additionally, we excluded nine patients whose diagnosis had changed during the follow-up period (seven to bipolar disorder, one to major depressive disorder with psychotic features, and another to substance-induced psychosis), as well as eight patients with incomplete biological data. Ultimately, the final analysis comprised 111 patients.

2.2. Clinical assessment and biological measures

Demographic data were collected for all patients through semi-structured interviews. Diagnoses were determined according to the DSM-IV-TR criteria (American Psychiatric Association, 1994). Clinical symptomatology was assessed using the Spanish validated version of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987; Peralta Martín and Cuesta Zorita, 1994). Anthropometrical measures, pharmacological treatment and substance abuse at baseline and follow-up were recorded. See Bernardo et al. (2021) for a complete description of the methodology used.

Laboratory data of white blood cells (WBC), neutrophils, monocytes, lymphocytes, basophils, eosinophils and platelet counts were assessed at baseline and, at relapse or 3-years follow-up. Inflammatory ratios where selected based on recent literature in SCZ (Bhikram and Sandor, 2022; Mazza et al., 2020b). The following ratios were calculated: neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), platelet-lymphocyte ratio (PLR) and basophil-lymphocyte ratio (BLR). K2EDTA BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA) were used to collect blood samples which were stored at −20 °C and sent to each site laboratory for analysis.

2.3. Relapse definition

Relapse was defined as when participants scored 4 or more on any of the following eight items of the PANSS Scale for at least one week during the follow-up: delusions, unusual thought content, hallucinatory behavior, mannerisms/posturing, blunted affect, social withdrawal, lack of spontaneity. Hospitalizations were also recorded during every follow-up visit and considered a relapse only when they were related to symptoms of schizophrenia (SCZ) and not due to other causes. Follow-up visits to detect relapses were scheduled every three months, where information was collected from the entire period between visits and both patients, family members or caregivers and clinical teams in charge of the clinical follow-up could notify the research team of the possible relapse of a participant.

2.4. Statistics

Data were analyzed using SPSS 23.0 (IBM-SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). Two-tailed p-values <0.05 were considered statistically significant. Continuous data were expressed as mean and standard deviation (SD), while categorical data were expressed as absolute values and percentages (%). The normality of continuous variables was tested using the Kolmogorov–Smirnov tests, and the equality of the variance between groups was assessed using Levene's test. Chi-square and t-student tests were used to compare those who did (relapsers) and those who did not experience a relapse (non-relapsers) after the three-year follow-up. As non-parametric alternatives, Fisher's exact and Mann-Whitney U tests were used when appropriate. Univariate logistic regressions analysis was used to explore whether inflammatory biomarkers at baseline were associated with relapse. We used two adjusted models: first, by including sex, age, duration of untreated psychosis (DUP), PANSS total score, body mass index (BMI) and antipsychotic treatment; and second, by adding cannabis and tobacco use during the follow-up to the first model. Fisher's exact test provided the significance, and the odds ratios (OR) and their 95 % confidence intervals (CI) provided the effect size. The relapse predictive properties of inflammatory biomarkers at baseline were tested using non-parametric ROC curve analysis. ANCOVA analysis using endpoint inflammatory levels as a dependent variable and baseline levels as covariates was used to compare inflammatory differences at endpoint between relapsers and non-relapsers. In order to account for multiple testing in our analysis, we applied the Benjamini & Hochberg method to the p-values.

3. Results

3.1. Demographic and baseline clinical characteristics

Demographic and baseline clinical characteristics of the cohort are shown in Table 1, differentiating relapsers and non-relapsers during the 3-years follow-up. In the univariate analyses, significant lower age and higher duration of untreated psychosis were found in relapsers (p = 0.039 and p = 0.046 respectively). During the follow-up, we found higher use of cannabis and lower use of tobacco in relapsers ($p < 0.001$ and $p < 0.001$ respectively). No statistically significant differences were found in the other variable.

3.2. Predictive value of inflammatory cells and ratios

At baseline, relapsers showed higher monocyte (1.93 ± 2.70 vs 0.53 ± 0.40; p = 0.003), lymphocyte (7.65 ± 11.25 vs 2.40 ± 2.11; $p < 0.001$), basophil (0.252 ± 0.619 vs 0.025 ± 0.046; $p < 0.001$) and eosinophil (0.84 ± 1.42 vs 0.17 ± 0.11; p = 0.004) counts. Furthermore, they showed lower PLR (80.29 ± 45.51 vs 111.3 ± 46.88; p = 0.001) and higher BLR (0.058 ± 0.155 vs 0.010 ± 0.020; $p < 0.001$) (Table 2).

In the univariate logistic regression for relapse monocyte (OR = 1.91; 95 % CI = 1.07 to 3.18, p = 0.009) and basophil (OR = 1.09; 95 % CI = 1.01 to 1.12; p = 0.005) counts increased the risk to relapse. In contrast, PLR (OR = 0.98; 95 % CI =0.97 to 0.99; p = 0.019) was a protective factor for relapse (Table 2). When adjusting for cannabis and tobacco use during the follow-up monocyte counts maintained the association (OR = 1.73; 95 % CI = 1.03 to 2.29; p = 0.027) but this was not significant after correcting the P-value for multiple testing. Basophil counts maintained the statistically significant increased risk of relapse (OR = 1.08; 95 % CI = 1.01 to 1.14; p = 0.008) (Table 2).

By using ROC curve analysis, we determined cut-off values of monocyte and basophil counts at baseline to predict relapse. ROC curve analysis suggested that the optimum cut-off values to discriminate patient's relapse were 0.52 × 10^9/L (sensitivity: 59.3 %; specificity: 73.7 %; AUC: 0.66; 95 % CI: 0.56–0.77; p = 0.002) for monocyte count, 0.025 × 10^9/L (sensitivity: 66.7 %; specificity: 77.2 %; AUC: 0.75; 95 % CI:

Table 1
Demographic and clinical baseline characteristics and univariate comparisons between relapse and non-relapse patients.

Mean ± SD	Relapse ($n = 54$)	No Relapse ($n = 57$)	Statistics	p-value
Female, n° (%)	24 (44.4)	32 (56.1)	$\chi^2 = 1.51$	0.218
Age at inclusion, years	24.1 ± 4.7	26.2 ± 5.9	$t = -2.08$	0.039
Duration of untreated psychosis, days	241.6 ± 395.6	122.2 ± 187.6	$t = 2.04$	0.046
PANSS				
- Positive	9.5 ± 3.1	9.3 ± 2.8	$t = 0.42$	0.670
- Negative	14.1 ± 5.7	14.5 ± 4.9	$t = -0.43$	0.666
- General	24.8 ± 7.0	24.7 ± 6.6	$t = 0.05$	0.954
- Total	48.4 ± 13.8	48.5 ± 12.8	$t = -0.04$	0.962
Antipsychotic treatment				
- Any use, n° (%)	51 (94.4)	49 (86.0)	$\chi^2 = 2.33$	0.239
- Clozapine use, n° (%)	3 (5.6)	8 (14.0)	$\chi^2 = 2.23$	0.204
- Chlorpromazine equivalent dose, mg/day	345.4 ± 337.8	246.1 ± 211.8	$t = 1.67$	0.096
BMI	24.8 ± 5.4	25.7 ± 4.9	$t = -0.08$	0.390
Substance use at baseline				
- Tobacco use (yes), n° (%)	31 (57.4)	28 (49.1)	$\chi^2 = 0.76$	0.382
- Cannabis use (yes), n° (%)	8 (15.1)	5 (8.9)	$\chi^2 = 0.98$	0.321
Substance use during follow-up				
- Tobacco use (yes), n° (%)	35 (64.5)	53 (93.0)	$\chi^2 = 13.393$	<0.001
- Cannabis use (yes), n° (%)	51 (94.4)	33 (57.9)	$\chi^2 = 20.124$	<0.001

Abbreviations: PANSS: Positive and Negative Syndrome Scale; CGI-S: Clinical Global Impression – Severity Scale; BMI: Body Mass Index. Significant values are highlighted in bold.

0.65–0.84; p < 0.001) for basophil count.

3.3. Differences in inflammatory cells and ratios at relapse or no relapse

By using ANCOVA analysis adjusted for baseline inflammatory levels and confounding factors (sex, age, DUP, PANSS total score, BMI and antipsychotic treatment), we found no significant differences in the inflammatory biomarkers at endpoint between those who relapse and those who did not (Fig. 1).

4. Discussion

Using routine blood counts form the 2EPs study cohort, we longitudinally examined the association of blood cell counts and ratios at remission after a FES and the risk of relapse. In this three-year follow-up study, we found: 1) At baseline, relapsers showed higher monocyte, lymphocyte, basophil and eosinophil counts, lower PLR and higher BLR; 2) Monocyte and basophil counts at baseline increased the risk to relapse; 3) when adjusting for established risk factors for relapse, such as cannabis or tobacco use, basophil count maintained its association, but monocyte counts lost significance after correcting for multiple testing probably due to the limited sample size; 4) ROC curve analysis suggested that the optimum cut-off values to discriminate relapsers were 0.52 × 10^9/L (AUC: 0.66) for monocytes and 0.025 × 10^9/L (AUC: 0.75) for basophils; 5) Considering baseline inflammatory levels, no significant differences were found in the inflammatory biomarkers at endpoint between relapsers and non-relapsers.

To our knowledge, this is the first study measuring longitudinal

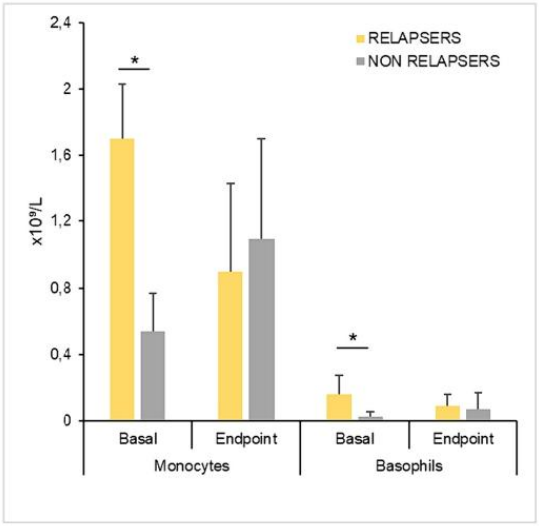

Fig. 1. Differences in monocyte and basophil counts between relapsers and non-relapsers at baseline and endpoint. *Significant results ($P < 0.05$).

Table 2
Univariate logistic regression for relapse using inflammatory blood cells and ratios at baseline.

	Relapse (n = 54)	No Relapse (n = 57)	Statistics	p-value	Adjustment 1[a]	Adjustment 2[b]
					OR univariate (CI 95 %)	OR univariate (CI 95 %)
WBC	7.25 ± 2.01	6.68 ± 1.96	U = 1251.0	0.131		
Neutrophils	12.01 ± 19.94	3.81 ± 1.62	U = 1276.1	0.121		
Monocytes	1.93 ± 2.70	0.53 ± 0.40	U = 1019.5	0.003*	1.91 (1.07 to 3.18); p = 0.009*	1.73 (1.03 to 2.99); p = 0.027
Lymphocytes	7.65 ± 11.25	2.40 ± 2.11	U = 948.5	<0.001*	1.13 (0.99 to 1.30); p = 0.055	1.09 (0.97 to 1.22); p = 0.112
Basophils	0.252 ± 0.619	0.025 ± 0.046	U = 768.5	<0.001*	1.09 (1.01 to 1.12); p = 0.005*	1.08 (1.01 to 1.14); p = 0.008*
Eosinophils	0.84 ± 1.42	0.17 ± 0.11	U = 1033.0	0.004*	1.02 (0.975 to 1.04); p = 0.110	1.01 (0.993 to 1.03); p = 168
Platelets	226.7 ± 62.2	216.9 ± 50.9	U = 1406.0	0.433		
NLR	1.62 ± 0.63	1.90 ± 1.04	U = 1342.5	0.246		
MLR	0.46 ± 0.95	0.23 ± 0.07	U = 1436.1	0.543		
PLR	80.29 ± 45.51	111.3 ± 46.88	t = 3.537	0.001*	0.98 (0.97 to 0.99); p = 0.019*	0.99 (0.98 to 1.01); p = 280
BLR	0.058 ± 0.155	0.010 ± 0.020	U = 857.5	<0.001*	1.23 (0.99 to 1.53); p = 0.061	1.29 (0.97 to 1.73); p = 0.076

Abbreviations: WBC: White Blood Cells, NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; PLR: platelet-lymphocyte ratio; BLR: basophil-lymphocyte ratio.

[a] Adjusted for Sex, Age, DUP, PANSS total score, BMI and AP treatment.

[b] Adjustment 1 + Cannabis and Tobacco use during follow-up. Significant values are highlighted in bold, significant values adjusted for multiple comparisons using the Benjamini and Hochberg method are marked with an asterisk.

associations between blood cell counts and ratios at remission after a FES and long-term relapse in a cohort of patients with a close follow-up. Our results suggest that monocyte and basophil cells may have a prognostic potential for patients at remission after a FES. Previous cross-sectional literature demonstrates immune blood cell alterations in first-episode psychosis (Jackson and Miller, 2020; Karageorgiou et al., 2019; Mazza et al., 2020a). Furthermore, higher inflammatory measures during the first episode of psychosis predict worse symptoms severity and treatment response at follow-up in longitudinal studies (Bioque et al., 2022a; Nettis et al., 2019; Osimo et al., 2021; Schwarz et al., 2012). However, studies analyzing the predictive value to relapse of peripheral inflammation in stable SCZ patients at early stages of the disease are scarce (Gonzalez-Blanco et al., 2018; Schwarz et al., 2012). In this study we were able to examine blood cell counts at remission after a FES to study associations with relapse in a cohort of 111 patients with a close 3-years follow-up.

Our findings of an association between inflammatory blood cells and longitudinal prognosis are in line with some studies but not with others. Using electronic health records of a naturalistic cohort of 749 patients with first-episode of psychosis, Osimo et al. (2021) showed that baseline monocyte, lymphocyte and platelet counts were associated with an unfavorable outcome (continued involvement of secondary mental health services) while baseline basophil and BLR ratios were associated with a favorable outcome (discharged to primary care with no onward referrals to community mental health teams) in a 3-yers follow-up study. In contrast, Horsdal et al. (2017) found no association between C-Reactive Protein and WBC levels and psychiatric readmission in a longitudinal population-based study in 690 patients with inflammatory biomarkers collected ±30 days from first diagnosis SCZ. In our study we found that monocyte and basophil count at baseline but not the other inflammatory blood cells increased the risk to relapse. Although we included a smaller sample than in the above-mentioned studies, it is important to note that in our study all patients were in remission after the first episode of SCZ and a close follow-up was scheduled every three months to detect relapse. This could explain some differences with the Osimo's study, which included patients in a first psychotic episode and unfavorable outcome was defined as involvement in secondary mental health services and not relapsing specifically. It highlights the importance of choosing the most relevant time to collect prognostic biomarkers in early stages of SCZ. Despite the differences in study design, the opposing results on basophil predictivity are surprising. Basophil has been associated with autoimmune diseases (Yang et al., 2017) and appears to be involved with insulin resistance (Lee et al., 2014). However, apart from the study by Osimo et al. (2021), research on basophils in SCZ is practically non-existent. We also found evidence of a protective association between PLR and relapse albeit with no significance after adjusting for substance use. This finding is new and need replication.

Remarkable, we found higher levels of innate (monocyte, basophil and eosinophil) and adaptative (lymphocyte) cell counts at baseline in relapsers; however, only innate cell counts at baseline (monocyte and basophil counts) increased the risk to relapse. These results are in line with previous literature showing activation of both innate and adaptative immunity in SCZ (Ermakov et al., 2022) and support the evidence that innate but not adaptative immunity may have a prognosis value in SCZ (Steiner et al., 2020). Furthermore, our results of monocyte implication in relapse support the hypothesis of the role of mononuclear phagocyte system in the pathophysiology of psychotic disorders and their shared genetics with cortical structure (Drexhage et al., 2010; Parker et al., 2024). Microglia is a monocyte-derived cell in the central nervous system and circulating monocyte count has been considered an accessible peripheral marker of microglia activation (Mazza et al., 2020a). As propose by Miller and Buckley (2012), abnormal homeostasis during remission after a FEP results in cellular activation and proinflammatory cytokine production, which in turn stimulates an inflammatory response. In the setting of increased blood brain barrier permeability, cytokine abnormalities and mononuclear phagocyte system may modulate microglial activation and dopaminergic neurotransmission resulting in acute psychosis relapse. In this line, despite being at remission after the FES, higher monocyte counts may have higher microglial activation (Bisht et al., 2016; Casquero-Veiga et al., 2019) resulting in an increased risk of relapse, as shown in our sample.

Substance use after the FEP has been linked to higher relapse rates (Weibell et al., 2017). More specifically, cannabis use is a well stablished independent risk factors for relapse (González-Pinto et al., 2011; Schoeler et al., 2016), and tobacco use has been associated with worse clinical and functional outcomes in SCZ (Quigley and MacCabe, 2019). In the entire 2EPs cohort, we were able to objectively monitor substance use during the follow-up after the FES and found that relapsers exhibited higher rates of cannabis use (93.2 % vs. 56.7 %, $p < 0.001$) during the follow-up (Bioque et al., 2022b). In the present study, to gain a deeper understanding of the influence of substance use on the relationship between inflammatory blood cells and the risk of relapse, we employed a second predictive model that included cannabis and tobacco use during the follow-up in addition to the first predictive model. Our findings support the association between certain inflammatory cells (monocyte and basophils) even when adjusting for established risk factor for relapse like cannabis or tobacco use. It's worth noting that while monocyte counts maintained their association, they lost significance after correcting for multiple testing, possibly due to the limited sample size.

Finally, ROC curve analysis was employed to assess the predictive effectiveness of baseline monocyte and basophil counts for relapse. The monocyte count exhibited an AUC of 0.66, indicating low discrimination, while the basophil count showed an AUC of 0.75, suggesting acceptable discrimination but likely of limited clinical relevance. Despite their association with an increased risk of relapse, both monocyte and basophil counts displayed low sensitivity and specificity, with AUC values falling below the 0.8 threshold proposed as minimally useful (Abi-Dargham et al., 2023). Therefore, inflammatory cell counts at remission after a FES may be involved in the pathophysiology of relapse but possess limited predictive power for relapse in clinical practice. Future studies with larger sample sizes and similar designs should seek to replicate these findings.

4.1. Strengths and limitations

The major strength of our study is that the sample included is much closer to the "real life" population with a FES, as the diagnostic evaluation was performed with a very comprehensive protocol, with strict inclusion-exclusion criteria (Bernardo et al., 2021). Being SCZ a heterogeneous clinical entity, the first episode subgroup is of great interest because it avoids the effect of confounding variables, such as prolonged antipsychotic treatment, medical comorbidities or chronicity (Bernardo and Bioque, 2014). In terms of biological measures, blood samples were collected following a strict and unified protocol, which is especially relevant since white blood cells levels fluctuate throughout the day (Sandberg et al., 2021; Villar et al., 2023). Other advantages of the study are the long period of follow-up and the deep characterization of the sample included allowing the detection of key variables closely related to the risk of presenting a relapse after having remitted from a FES.

Our study should, however, be considered in the context of several limitations. Firstly, the 2EPs Project was a naturalistic study, not a randomized controlled trial, which means that patients could change treatments during the follow-up period based on the clinician's discretion. Secondly, while most sociodemographic and clinical variables did not differ significantly between relapsers and non-relapsers, it's important to note that relapsers were younger and had a longer duration of untreated psychosis, both of which are associated with a higher risk of relapse (Bogers et al., 2022; Marshall et al., 2005). As a result, both variables were included as covariates in all the analyses. It's worth mentioning that older age has been linked to higher inflammatory ratios, but this significant increase typically occurs around the age of fifty and beyond (Brinn and Stone, 2020). Since our patients were in their

twenties and thirties (24.1 ± 4.7 for relapsers vs. 26.2 ± 5.9 for non-relapsers), age alone is unlikely to account for the observed differences. Thirdly, among all the patients enrolled in the 2EPs study, we encountered a dropout rate of 49.6 % during the follow-up, as reported by Bioque et al. (2022b), which reduced the sample size to 111 patients. This sample size could potentially affect statistical significance when adjusting for multiple comparisons and might lead to lower AUC values for the variables. Lastly, including information on medication adherence could have been beneficial in assessing the influence of continued oral antipsychotic medication on the likelihood of relapse.

5. Conclusion

In this 3-year longitudinal study of patients in remission after a first episode of psychosis (FES), we have observed that higher monocyte and basophil counts at baseline were linked to an increased risk of relapse during the follow-up period, even after adjusting for confounding factors such as cannabis and tobacco use. Nevertheless, it is important to note that the predictive capacity of these cell counts for relapse is limited. These findings should be validated through replication in larger samples with similar study designs.

CRediT authorship contribution statement

Vicent Llorca-Bofí: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Santiago Madero:** Writing – review & editing, Methodology. **Silvia Amoretti:** Writing – review & editing, Formal analysis. **Manuel J. Cuesta:** Writing – review & editing. **Carmen Moreno:** Writing – review & editing. **Ana González-Pinto:** Writing – review & editing. **Dani Bergé:** Writing – review & editing. **Roberto Rodriguez-Jimenez:** Writing – review & editing. **Alexandra Roldán:** Writing – review & editing. **María Ángeles García-León:** Writing – review & editing. **Angela Ibáñez:** Writing – review & editing. **Judith Usall:** Writing – review & editing. **Fernando Contreras:** Writing – review & editing. **Gisela Mezquida:** Writing – review & editing, Project administration, Data curation. **Clemente García-Rizo:** Writing – review & editing. **Esther Berrocoso:** Writing – review & editing. **Miquel Bioque:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

Dr. Bioque has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board of has received honoraria from talks and/or consultancy of Adamed, Angelini, Casen Recordati, Exeltis, Ferrer, Janssen, Lundbeck, Neuraxpharm, Otsuka, Pfizer and Sanofi, and grants from the Spanish Ministry of Health, Instituto de Salud Carlos III (PI20/01066) and Fundació La Marató de TV3 (202206-30-31).

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Dr.Saiz-Ruiz has been as speaker for and on the advisory boards of Adamed, Lundbeck, Servier, Medtronic, Casen Recordati, Neurofarmagen, Otsuka, Indivior, Lilly, Schwabe, Janssen and Pfizer, outside the submitted work.

Dr. Roldán has served as advisor or speaker for the companies Otsuka and Angelini.

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4.3. Article III

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Blood-based immune biomarkers and functional outcomes in acute schizophrenia: a retrospective cohort study

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ABSTRACT

Background: Schizophrenia is a chronic psychiatric disorder characterized by acute relapses and significant functional impairment. While antipsychotic medications are effective in managing acute episodes, many patients fail to achieve functional remission. Recent research suggests that immune mechanisms may influence treatment outcomes, yet studies focusing on the relationship between immune biomarkers and functional recovery are limited.

Objective: This study aims to evaluate whether blood-based immune biomarkers can predict functional response and remission in patients with acute schizophrenia.

Methods: A retrospective cohort study was conducted with 354 inpatients diagnosed with schizophrenia, admitted to an acute psychiatric unit between January 2010 and December 2020. Sociodemographic, clinical, and immune biomarker data were extracted from electronic records. Functional outcomes were measured using Global Assessment of Functioning (GAF) scores at admission and discharge. Immune biomarkers assessed included white blood cell counts, ratios and C-reactive protein. Functional response was defined as a GAF score improvement >40 points, while functional remission was defined as a GAF score ≥70 at discharge.

Results: Higher leukocyte counts increased the risk of non-functional response, while a higher platelet-lymphocyte ratio (PLR) was a protective factor. Additionally, Higher lymphocyte and platelet counts were protective against non-functional remission. However, the predictive performance for both functional response and remission was limited, with an AUC ranging from 0.53 to 0.61.

Conclusion: Immune biomarkers, particularly leukocyte counts, PLR, and lymphocyte counts, show significant associations with functional outcomes in acute schizophrenia. However, their predictive value for clinical practice remains limited.

Keywords: Schizophrenia, Immune Biomarkers, Functional Outcomes, Global Assessment of Functioning, Antipsychotic Treatment.

1. INTRODUCTION

Schizophrenia is a chronic disorder that affects approximately 1% of the global population, typically during an individual's most productive years [1]. The condition is characterized by acute relapses and a progressive decline in functionality. While various antipsychotic medications have demonstrated efficacy in managing acute episodes, up to two-thirds of patients fail to achieve symptomatic remission, and an even higher proportion remain functionally impaired [2–4].

The underlying pathophysiology of schizophrenia remains unclear, but there is growing evidence to suggest that immune mechanisms may play a role [5]. This is supported by epidemiological [6], genetic [7–9] and biomarker studies [10,11]. Additionally, dysregulation of inflammatory pathways has been hypothesized as a factor in treatment resistance, highlighting the need to identify immunological biomarkers for the early detection of resistance [12].

Meta-analyses have shown that patients with schizophrenia have increased levels of white blood cell (WBC) counts, ratios, and C-reactive protein (CRP) compared to healthy controls [10,11,13,14]. These elevated biomarker levels are evident from the early stages of the disease [15,16], persist during stable phases, and show further increases during acute episodes [10,17]. Additionally, they have been linked to poor outcomes in schizophrenia [18–21]. In terms of acute response, various baseline biomarkers have been studied to predict treatment response to antipsychotics. A recent narrative review identified 12 studies that explored this association, reporting significant but variable correlations depending on the specific antipsychotic used [22].

Despite these findings, studies focusing on the relationship between immune biomarkers and functional outcomes, rather than just symptomatic response, are limited and primarily focus on clinically stable patients [23–26]. Treatment guidelines emphasize that improving a patient's level of functioning during the acute phase of treatment should be a primary goal in schizophrenia management [27–29]. Patients often remain functionally impaired due to persistent negative, cognitive, and residual symptoms, as well as antipsychotic side effects [30–33].

In this study, we aimed to evaluate whether blood-based immune biomarkers can serve as predictors of functional response and remission in patients with acute schizophrenia, assess their discriminatory performance for clinical practice, and explore potential differences based on antipsychotic treatments.

2. METHODS

2.1. Study design and setting

This is a register-based retrospective cohort study of individuals with schizophrenia admitted to an acute psychiatric unit of Santa María University Hospital in Lleida, Spain. Sociodemographic, clinical and functioning data and immune peripheral biomarkers measures were extracted from electronic clinical records. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting cohort studies [34].

2.2. Eligibility criteria

Figure 1 represents the patient selection process for the study. Inclusion criteria were: (1) admission to the Inpatient Psychiatric Unit between January 1, 2010, and December 31, 2020; (2) age 18 years or older; (3) primary psychiatric diagnosis of schizophrenia (F20) according to the International Classification of Diseases, Tenth Edition, Clinical Modification (ICD-10-CM; https://eciemaps.mscbs.gob.es); (4) Availability of Global Assessment of Functioning (GAF) scores [35] at admission and discharge.

Exclusion criteria were: (1) incomplete data about blood levels of total and differential WBC counts, platelets or CRP; (2) presence of acute conditions that could influence immune or inflammatory parameters; (3) pregnant or breastfeeding women; (4) readmitted in less than 30 days in order to avoid duplications .

2.3. Variables

The following sociodemographic variables were collected: sex (male/female), age (years), type of episode (First Episode/Multi Episode schizophrenia), illness duration (years), cardiovascular risk factors (CVRF) (high blood pressure, dyslipidaemia, diabetes mellitus, obesity and metabolic syndrome using International Diabetes Federation criteria [36]), addictive behaviours including substance use (tobacco, alcohol, cannabinoids, cocaine, opioids, amphetamines and polysubstance use defined as the use of three or more substances excluding tobacco and methadone) and problematic gambling, and family history of severe mental disorders (yes/no).

The treatment at discharge was recorded, with particular attention to the type of antipsychotic prescribed. Antipsychotics were categorized into seven mutually exclusive groups: risperidone, paliperidone, aripiprazole, olanzapine, clozapine, other second-generation antipsychotics, and first-generation antipsychotics. The prescribed daily doses of antipsychotics were converted to their estimated chlorpromazine (CPZ) equivalent according to international consensus guidelines [37]. For patients on polypharmacy involving antipsychotics, classification was based on the antipsychotic with the highest equivalent dose. Other treatments were also documented, including antidepressants (yes/no), mood stabilizers (yes/no), and electroconvulsive therapy (yes/no).

Blood was collected by experienced nurses belonging to the Department of Psychiatry, during the first 24 h upon admission, between 8.00 and 10.00 a. m., and after an overnight fasting. Selected immune biomarkers included: total and differential WBC counts (i.e., leukocytes, neutrophils, basophils, eosinophils, monocytes, basophils and lymphocytes), platelet counts and CRP. The following ratios were calculated: neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), platelet-lymphocyte ratio (PLR) and basophil-lymphocyte ratio (BLR) [38]. Total and differential WBC counts were assessed by Flow Cytometry using a Sysmex XN analyzer, while CRP levels were assessed by an immunoturbidimetric assay on a Beackman Coulter automated analyzer.

2.4. Outcomes

Functionality was measured by using GAF scores at admission and discharge[39], with two distinct outcomes:

a) Functional Response, defined as a quantitative variable by the difference in GAF scores from admission to discharge, and as a qualitative variable by a GAF change > 40 points from admission to discharge. Although no standardized cutoffs exist for defining functional response during acute episodes, previous research has demonstrated a strong correlation between the GAF and the Positive and Negative Syndrome Scale (PANSS) [40]. Given that a 30% improvement in PANSS scores is commonly used to define clinical response [41], we applied equipercentile linking to translate this threshold to the GAF scale. Based on this method, a 40-point increase in GAF scores was determined as the functional response criterion in this study.

b) Functional Remission, defined as a GAF score ≥ 70 at discharge, reflecting at most mild impairment in social, occupational, or academic functioning [35].

2.5. Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as means with standard deviations (SD), while categorical variables are summarized as absolute values and percentages. Normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Due to deviations from normality in the WBC counts, ratios, and C-reactive protein (CRP) levels, these variables were log-transformed prior to correlation and regression analyses, following the methodology of Feng et al. (2013). Comparisons between responders and non-responders, as well as between remitters and non-remitters, were performed using chi-square and Student's t-tests. When assumptions for parametric tests were not met, Fisher's exact test and the Mann-Whitney U test were applied as non-parametric alternatives.

To evaluate functional response, a multiple generalized linear regression analysis was performed, using the change in GAF score from admission to discharge as the primary dependent variable. Immune biomarkers were included as independent variables. Additionally, a multiple logistic regression analysis was conducted to assess the role of immune biomarkers in predicting functional response, defined as a GAF improvement of more than 40 points. For functional remission, defined as a GAF score above 70 points at discharge, a separate multiple logistic regression analysis was used to determine the predictive value of immune biomarkers. All models were adjusted for variables known to affect immune parameters, such as age, sex, and metabolic syndrome, as well as for significant variables identified in the univariate analysis, such as the GAF score at admission. These included type of episode and polysubstance use for functional response, and opioid use for functional remission.

Results are reported as standardized Beta (β) coefficients with 95% confidence intervals (CI) for linear regression analyses, and as odds ratios (OR) with 95% CI for logistic regression analyses. The Benjamini-Hochberg method was applied to account for multiple comparisons and corrected p-values are reported as p_{corr} [43]. Significant associations were determined using a threshold of $p_{corr} < 0.05$.

ROC-AUC analysis was used to assess the discriminatory performance of immune biomarkers in identifying functional response and functional remission

3. RESULTS

3.1. Demographic and Baseline Clinical Characteristics

A total of 354 inpatients with acute schizophrenia (age= 40.6 ±12.6 years; 30.5% females; illness duration= 11.5 ±10.1 years) were included in the study. At discharge, 28.2% of patients achieved a functional response, and 24% achieved functional remission. Risperidone was the most commonly used antipsychotic (36.7%), followed by paliperidone (24.2%). Baseline demographics and treatment characteristics are detailed in **Tables 1**.

3.2. Immune Biomarkers and Functional Response

Patients who did not achieve a functional response at discharge had higher leukocyte counts (8.08 ± 2.52 vs 7.48 ± 2.1, p = 0.037) and lower platelet-to-lymphocyte ratio (PLR) (113.6 ± 28.9 vs 124.8 ± 34.5, p = 0.028) at baseline (**Table 2**).

In the linear regression model assessing the change in GAF score, leukocyte and neutrophil counts were inversely associated with GAF change (β = -0.139, CI95% = -1.063 to -0.143, p_{corr} = 0.0375; β = -0.118, CI95% = -1.177 to -0.066, p_{corr} = 0.0382 respectively), while PLR was positively associated (β = 0.172, CI95% = 0.012 to 0.055, p_{corr} = 0.015) (**Table 3,**).

In logistic regression analyses for non-response (**Table 3, Figure 2**), higher leukocyte counts were associated with increased risk (OR = 1.134, CI95% = 1.019 to 1.262, p_{corr} = 0.022), while higher PLR was associated with a protective effect (OR = 0.995, CI95% = 0.990 to 0.999, p_{corr} = 0.016). Neutrophil counts showed non-significant results.

When assessing the discriminatory performance of immune biomarkers in distinguishing functional responders from non-responders, the threshold for leukocyte count was approximately 7.32, with a sensitivity of 56.7%, specificity of

47.0%, and an AUC of 0.562. For the PLR, the threshold was around 114.8, with a sensitivity of 42.1%, specificity of 38.6%, and an AUC of 0.532 (**Table 3**).

3.3. Immune Biomarkers and Functional Remission

Patients who did not achieve functional remission at discharge had lower lymphocyte counts (2.20 ± 0.79 vs 2.51 ± 0.84, p_{corr} = 0.002) and platelets (230.6 ± 59.3 vs 146.4 ± 53.1, p_{corr} = 0.027) but higher MLR (0.322 ± 0.15 vs 0.285 ± 0.11) at baseline (**Table 2**).

In the logistic regression model (**Table 3, Figure 2**), higher lymphocyte and platelet counts were associated with a protective factor against non-functional remission (OR = 0.669, CI95% = 0.489 to 0.917, p_{corr} = 0.012; OR = 0.993, CI95% = 0.989 to 0.998, p_{corr} = 0.005 respectively).

When assessing the discriminatory performance of immune biomarkers in distinguishing functional remitters from non-remitters, the threshold for lymphocyte count was approximately 2.37, with a sensitivity of 56.5%, specificity of 36.4%, and an AUC of 0.617. For the platelet, the threshold was around 119.5, with a sensitivity of 87.1%, specificity of 68.3%, and an AUC of 0.589 (**Table 3**).

3.4. Impact of Antipsychotic on Immune Biomarkers and Functional Outcomes

An exploratory analysis was conducted to assess the association between different antipsychotic treatments, immune biomarkers, and functional outcomes. (**Supplementary Material**)

Functional Response

In patients treated with risperidone, PLR was positively associated with GAF change (β = 0.203, CI95% = 0.040 to 0.075, p_{corr} = 0.028). Among patients receiving paliperidone, leukocyte (β = -0.249, CI95% = -1.616 to -0.105, p_{corr} = 0.0382), neutrophil (β = -0.232, CI95% = -2.018 to -0.065, p_{corr} = 0.0427), and basophil counts (β = -0.330, CI95% = -199.99 to -43.73, p_{corr} = 0.015) were inversely associated with GAF change. In the first-generation antipsychotic group, platelet counts were positively associated with GAF change (β = 0.675, CI95% = 0.023 to 0.214, p_{corr} = 0.045). In patients treated with Aripiprazole, monocytes (β = -0.453, CI95% = -46.332 to -5.969, p_{corr} = 0.013) and eosinophils (β = -0.426, CI95% = -92.096 to -7.549, p_{corr} = 0.023) were inversely associated with GAF change. No other significant associations were found for the remaining antipsychotic groups. (**Table S1**)

In logistic regression analyses, within the risperidone group, higher PLR was associated with a lower risk of non-response (OR = 0.991, CI95% = 0.983 to 0.999, p_{corr} = 0.022). In the first-generation antipsychotic group, higher lymphocyte counts were associated with an increased risk of non-response (OR = 5.767, CI95% = 1.121 to 29.666, p_{corr} = 0.011), while higher PLR was associated with a lower risk (OR = 0.986, CI95% = 0.972 to 0.999, p_{corr} = 0.041). No other significant results were observed for the remaining antipsychotic groups. (**Table S2**)

Functional Remission

For functional remission, in the risperidone group, higher platelet counts were associated with a lower risk of non-remission (OR = 0.991, CI95% = 0.984 to 0.999, p_{corr} = 0.023). In the olanzapine group, higher neutrophil counts were associated with a lower risk of non-remission (OR = 0.186, CI95% = 0.051 to 0.687, p_{corr} = 0.012). No other significant results were found for the remaining antipsychotic groups. (**Table S3**)

4. DISCUSSION

This retrospective cohort study aimed to explore the relationship between blood-based immune biomarkers at admission and functional outcomes, measured by the GAF scale, in patients with acute schizophrenia. The findings reveal significant associations between specific immune biomarkers and both functional response and remission. Key results include: (1) higher leukocyte and neutrophil counts were linked to poorer functional response, while a higher PLR was associated with better functional response; (2) higher leukocyte counts increased the risk of non-functional response, while a higher PLR was a protective factor; (3) higher lymphocyte and platelet counts were protective against non-functional remission; (4) predictions for both functional response and remission showed poor to fair

discriminatory performance, with AUC ranging from 0.53 to 0.61; (5) the relationship between immune biomarkers and functional outcomes varied depending on the specific antipsychotic used.

Our findings support previous research linking elevated leukocyte counts and inflammation markers to worse clinical outcomes in schizophrenia [20,44,45]. However, our study expands on this by focusing on functional outcomes during an acute episode, rather than just symptomatic remission. Unlike past studies that primarily examined stable patients or predicted symptomatic response [22], our study delves deeper into the role of immune markers in predicting functional improvement—a key yet underexplored aspect of schizophrenia treatment.

The largest study to date investigating the association between immunological biomarkers and acute clinical outcomes in schizophrenia, which included 2598 patients, found that higher leukocyte counts predicted poorer response to antipsychotics, as measured by the PANSS total scores [46]. Although that study used clinical response as the primary outcome, our findings are consistent in that leukocyte levels predicted functional response. This is not surprising, as the PANSS and GAF scales have shown strong negative correlations [40], and the concept of acute response can be captured by changes in both clinical symptoms and functionality. We also found that neutrophil counts predicted functional response but with slightly smaller effect size than leukocytes, and this finding has not been previously reported in the literature. These results support the hypothesis that the immune system plays a role in the neurobiology of schizophrenia and that alterations in this system impact treatment response [47]. In fact, the latest meta-analysis exploring the effect of anti-inflammatory agents on psychotic disorders found small but significant effects [48]. However, researchers suggest that future studies should stratify patients based on immune alterations to better understand the potential benefits of these treatments. Biomarkers like those identified in our study could help in this classification process.

The protective role of PLR observed in our study is also noteworthy. While elevated PLR is generally associated with worse outcomes in various medical conditions [49], our findings suggest a more nuanced role for this marker in schizophrenia. The positive association between PLR and functional improvement, may reflect complex interactions between the immune system and antipsychotic response that warrant further investigation. This finding is consistent with a previous population-based study showing that higher PLR at initial diagnosis of schizophrenia was associated with lower mortality [20], and a previous longitudinal study in stable schizophrenia patients showing a protective association between PLR and relapse [50]. Despite focusing on different outcomes and designs, these studies collectively suggest that PLR may play a significant protective role in schizophrenia, warranting further in-depth exploration in the future.

Functional remission is a more demanding goal than merely achieving a clinical response [51]. While symptomatic remission generally leads to better functional outcomes, it does not always translate into full functional recovery. In our study, only 24% of patients achieved functional remission at discharge (GAF > 70), consistent with previous findings in similar populations [52,53]. Notably, the previously reported link with leukocyte counts was not observed for this outcome, but higher lymphocyte and platelet counts emerged as protective factors against non-functional remission.

Our findings expand on previous research linking immunological biomarkers, such as IL-2 and IL-12p70, to functional outcomes in schizophrenia, where higher IL-2 levels have been associated with worse prognosis [23,24].. However, while IL-2 stimulates T-lymphocyte proliferation, our results suggest that higher overall lymphocyte counts are protective, which may seem contradictory. This discrepancy could be explained by differences in study design—previous research focused on stable outpatients and specific cytokines, while our study examines acute schizophrenia and overall lymphocyte levels rather than subpopulations. These findings highlight the importance of studying immune markers across different disease stages to better understand their role in functional outcomes.

ROC curve analysis was used to assess the ability of immune biomarkers to distinguish functional outcomes. Both response and remission predictions showed poor to fair discriminatory performance, with AUC values ranging from 0.53 to 0.61—below the 0.8 threshold considered clinically relevant [54]. Moreover, the difference in leukocyte count between responders and non-responders was minimal (only 0.6×10^9/L), further limiting the clinical significance of this finding. These results suggest that while white blood cell counts and ratios at admission may be involved in the pathophysiology of acute schizophrenia, their predictive value for functional outcomes in clinical practice remains low.

Future studies with longitudinal biomarker data, detailed symptom profiling, and similar methodologies are needed to validate these findings.

Finally, our exploratory analysis suggests that different antipsychotics may influence the relationship between immune biomarkers and functional outcomes in distinct ways. While long-term antipsychotic use affects overall psychosocial functioning [55], the impact of individual drugs remains unclear [56,57]. The retrospective nature of our real-world cohort study limited the identification of monotherapy patients, and the small sample size for certain antipsychotics restricted individual drug analyses. Despite these limitations, we observed functional differences between treatments, suggesting that antipsychotic pharmacological profiles may differentially impact the immune system, potentially influencing their effects. However, this hypothesis should be interpreted with caution, as only a few patients in our sample received monotherapy. Future studies with larger monotherapy cohorts and immune biomarker assessments are needed to explore this relationship further [58].

Strengths and Limitations

This study's strengths include a relatively large sample size of 354 patients and a comprehensive assessment of immune biomarkers, conducted with standardized blood sampling procedures in a real-world clinical setting. The use of GAF scores to evaluate functional outcomes provides a practical measure of real-life functioning, a critical yet often underemphasized aspect of schizophrenia treatment.

However, several limitations must be acknowledged. First, the observational and retrospective design of this study introduces selection and information biases, limiting the ability to establish causality. Second, the immune biomarkers used—total and differential WBC counts, platelet counts, and CRP—lacked specificity, restricting our ability to analyse immunological pathways in depth. Future research should address this limitation by incorporating more specific markers, such as cytokine profiles or immune cell subtypes. Third, biomarkers were measured at a single time point, preventing the assessment of longitudinal relationships between immune markers and functional outcomes over time. Fourth, no symptom-based rating scales were available to evaluate specific symptoms in patients with schizophrenia, making it impossible to examine associations between biomarkers and particular symptom domains. Fifth, response was defined solely by functionality, measured using the GAF scale and linked to PANSS [40]. However, this definition lacks validation, which may affect its reliability and clinical relevance. Finally, stratifying by antipsychotic type significantly reduced the sample size, and patients receiving polypharmacy were categorized based on the antipsychotic with the highest equivalent dose, complicating the interpretation of results for individual medications. Therefore, findings from these sub-analyses should be interpreted with caution.

5. CONCLUSION

This is the first study to examine blood-based immune biomarkers at admission in relation to functional outcomes in patients with acute schizophrenia. The findings demonstrate an association between differential white blood cell counts, their ratios, and functional outcomes but highlight their limited discriminatory performance. Future research should validate these biomarkers in monotherapy cohorts and investigate immune changes over time to better understand their role in schizophrenia.

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Table 1: Sociodemographic, clinical and treatment characteristics of the sample

	Total Sample N=354	Functional Response (GAF change ≥ 40 points)		p-value	Functional Remission (GAF ≥ 70 at discharge)		p-value
		Non-Responders n=254 (71.8%)	Responders n=100 (28.2%)		Non-Remitters n=269 (76%)	Remitters n=85 (24%)	
Female, n (%)	108 (30.5)	80 (31.4)	28 (28)	0.520	89 (33.1)	19 (22.4)	0.061
Age (years), mean (SD)	40.6 (12.6)	41.4 (12.8)	38.7 (12.1)	0.067	41.3 (13.3)	38.6 (10)	0.096
First Episode of Schizophrenia, n (%)	36 (10.2)	18 (7)	18 (18)	**0.002***	26 (9.7)	10 (11.8)	0.577
Illness duration, mean (SD)	11.5 (10.1)	11.6 (9.6)	11.4 (11.5)	0.838	11.9 (10.8)	10.3 (7.8)	0.191
Cardiovascular Risk Factors, n (%)							
High Blood Pressure	124 (35)	92 (36.2)	32 (32)	0.454	94 (34.9)	30 (35.3)	0.953
Diabetes Mellitus	59 (16.7)	44 (17.3)	15 (15)	0.598	47 (17.5)	12 (14.1)	0.469
Dyslipidaemia	151 (42.7)	113 (44.4)	38 (38)	0.266	112 (41.6)	39 (45.9)	0.490
Obesity	112 (31.6)	86 (33.8)	26 (26)	0.152	90 (33.5)	22 (25.9)	0.191
Metabolic Syndrome	132 (37.3)	94 (37)	38 (38)	0.862	100 (37.2)	32 (37.6)	0.937
Addictive Behaviours, n (%)							
Tobacco	230 (65)	166 (65.3)	64 (64)	0.810	171 (63.6)	59 (69.4)	0.325
Alcohol	102 (28.8)	70 (27.5)	32 (32)	0.406	79 (29.4)	23 (27.1)	0.682
Cannabinoids	193 (54.5)	132 (51.9)	61 (61)	0.124	147 (54.6)	46 (54.1)	0.932
Cocaine	49 (13.8)	31 (12.2)	18 (18)	0.155	37 (13.8)	12 (14.1)	0.933
Opioids	21 (5.9)	15 (5.9)	6 (6)	0.973	21 (7.8)	0 (0)	**0.003***
Amphetamines	18 (5.1)	11 (4.3)	7 (7)	0.303	14 (5.2)	4 (4.7)	0.558
Polysubstance use (3 substance excluding tobacco and methadone)	36 (10.2)	20 (7.8)	16 (16)	**0.023***	27 (10)	9 (10.6)	0.884
Problematic Gambling	3 (0.8)	3 (1.1)	0 (0)	0.368	3 (1.1)	0 (0)	0.438
Family History of SMI, n (%)	132 (37.3)	89 (35)	43 (43)	0.163	97 (36.1)	35 (41.2)	0.395
Global Assessment of Functioning (GAF), mean (SD)							
GAF at admission	29.3 (10.1)	31.9 (9.5)	22.4 (7.9)	**<0.001***	27.1 (9.1)	36.0 (10)	**<0.001***
GAF at discharge	59.1 (11.2)	56.7 (11.3)	65.3 (8.4)	**<0.001***	54.9 (9.2)	72.7 (4)	**<0.001***
GAF change	29.8 (10.6)	24.8 (7.3)	42.9 (4.8)	**<0.001**	27.8 (9.9)	36.7 (9.9)	**<0.001***
Antipsychotics, n (%)							
Risperidone	130 (36.7)	98 (38.6)	32 (32)	0.247	99 (36.8)	31 (36.5)	0.956
Paliperidone	86 (24.2)	64 (25.2)	22 (22)	0.528	63 (23.4)	23 (27.1)	0.495
Aripiprazole	30 (8.4)	18 (7)	12 (12)	0.135	24 (8.9)	6 (7.1)	0.591
Other second-generation AP	74 (20.9)	48 (18.9)	26 (26)		54 (20.1)	20 (23.5)	
First-generation AP	34 (9.6)	26 (10.2)	8 (8)	0.520	29 (10.8)	5 (5.9)	0.388
Antidepressant, n (%)	43 (12.1)	36 (14.1)	7 (7)	0.063	33 (12.2)	10 (11.7)	0.902
Mood stabilizer, n (%)	39 (11)	29 (11.4)	10 (10)	0.701	34 (12.6)	5 (5.8)	0.083
ECT, n (%)	9 (2.5)	8 (3.1)	1 (1)	0.225	8 (3)	1 (1.2)	0.321

Bold values denote statistical significance after adjusting for multiple comparisons using the Benjamini-Hochberg method.
Abbreviations: Electroconvulsive Therapy (ECT), Serious Mental Illness (SMI)

Table 2. Immunological parameters by functional response and remission status

Immunological parameters	Total Sample N=354	Non-Responders n=254 (71.8%)	Responders n=100 (28.2%)	p-value	Non-Functional Remission n=269 (76%)	Functional Remission n=85 (24%)	p-value
Leukocytes (10x9/L)	7.92 (2.44)	8.08 (2.54)	7.48 (2.1)	**0.037***	7.83 (2.53)	8.17 (2.1)	0.264
Neutrophils (10x9/L)	4.76 (2.01)	4.88 (2.14)	4.43 (1.58)	0.056	4.75 (2.11)	4.76 (1.65)	0.951
Lymphocytes (10x9/L)	2.28 (0.81)	2.31 (0.8)	2.19 (0.84)	0.210	2.20 (0.79)	2.51 (0.84)	**0.002***
Monocytes (10x9/L)	0.66 (0.23)	0.65 (0.22)	0.65 (0.23)	0.837	0.65 (0.23)	0.66 (0.19)	0.715
Eosinophils (10x9/L)	0.18 (0.13)	0.18 (0.12)	0.17 (0.12)	0.252	0.18 (0.13)	0.18 (0.11)	0.983
Basophils (10x9/L)	0.04 (0.02)	0.042 (0.026)	0.038 (0.018)	0.079	0.042 (0.023)	0.040 (0.016)	0.977
Platelets (10x9/L)	234.4 (58.2)	234.4 (59.4)	234.3 (55.5)	0.992	230.6 (59.3)	246.4 (53.1)	**0.027***
NLR	2.36 (1.43)	2.37 (1.47)	2.34 (1.31)	0.836	2.43 (1.51)	2.14 (1.1)	0.097
MLR	0.313 (0.142)	0.307 (0.135)	0.328 (0.16)	0.218	0.322 (0.15)	0.285 (0.11)	**0.037***
PLR	116.8 (53.9)	113.6 (28.9)	124.8 (34.5)	**0.028***	118.1 (32.6)	112.5 (37.9)	0.408
BLR	0.019 (0.010)	0.019 (0.011)	0.018 (0.009)	0.441	0.019 (0.01)	0.017 (0.01)	0.153
CRP (mg/L)	7.3 (12.6)	7.7 (12.7)	6.1 (11.9)	0.280	7.3 (11.4)	7.4 (15.6)	0.941

Bold values denote statistical significance after adjusting for multiple comparisons using the Benjamini-Hochberg method.

Abbreviations: Neutrophil-to-Lymphocyte Ratio (NLR); Monocyte-to-Lymphocyte Ratio (MLR); Platelet-to-Lymphocyte Ratio (PLR); Basophil-to-Lymphocyte Ratio (BLR); C-Reactive Protein (CRP).

Table 3. Associations between blood-based immune biomarkers and functional outcomes in acute schizophrenia: regression coefficients, odds ratios, and discriminatory performance.

Immunological parameters	GAF change	Non-functional response (GAF change <40 points)		Non-functional remission (GAF at discharge < 70)	
	Beta (95% CI) [a]	OR (95% CI) [b]	AUC	OR (95% CI) [c]	AUC
Leukocytes (10x9/L)	**-0.139 (-1.063 to -0.143)***	**1.134 (1.019 to 1.262)***	0.562	0.961 (0.869 to 1.062)	-
Neutrophils (10x9/L)	**-0.118 (-1.177 to -0.066)***	1.132 (0.996 to 1.286)	-	1.001 (0.884 to 1.133)	-
Lymphocytes (10x9/L)	-0.096 (-2.669 to 0.198)	1.204 (0.900 to 1.611)	-	**0.669 (0.489 to 0.917)***	0.617
Monocytes (10x9/L)	-0.054 (-7.370 to 2.440)	1.113 (0.403 to 3.075)	-	1.010 (0.342 to 2.988)	-
Eosinophils (10x9/L)	-0.075 (-14.931 to 2.665)	3.032 (0.454 to 20.237)	-	1.544 (0.217 to 10.984)	-
Basophils (10x9/L)	-0.105 (-97.997 to 0.068)	16.932 (0.314 to 854.621)	-	0.3152 (0.001 to 18.765)	-
Platelets (10x9/L)	0.038 (-0.013 to 0.027)	1.000 (0.996 to 1.004)	-	**0.993 (0.989 to 0.998)***	0.589
NLR	0.011 (-0.709 to 0.875)	1.017 (0.864 to 1.199)	-	1.156 (0.939 to 1.421)	-
MLR	0.081 (-1.866 to 13.805)	0.381 (0.081 to 1.785)	-	8.414 (0.949 to 74.592)	-
PLR	**0.172 (0.012 to 0.055)***	**0.995 (0.990 to 0.999)***	0.532	1.000 (0.995 to 1.005)	-
BLR	-0.032 (-135.1 to 72.1)	4.113 (0.013 to 25.237)	-	4.113 (0.013 to 25.237)	-
CRP (mg/L)	-0.058 (-0.138 to 0.040)	1.012 (0.990 to 1.034)	-	0.997 (0.977 to 1.017)	-

[a] Adjusted for age, sex, metabolic syndrome and GAF at baseline.

[b] Adjusted for age, sex, metabolic syndrome, GAF at baseline, type of episode, and polysubstance use.

[c] Adjusted for age, sex, metabolic syndrome, GAF at baseline and opioid use.

* Denote statistical significance after adjusting for multiple comparisons using the Benjamini-Hochberg method.

Abbreviations: Neutrophil-to-Lymphocyte Ratio (NLR); Monocyte-to-Lymphocyte Ratio (MLR); Platelet-to-Lymphocyte Ratio (PLR); Basophil-to-Lymphocyte Ratio (BLR); C-Reactive Protein (CRP).

Figure 1. Patient selection flowchart for the study

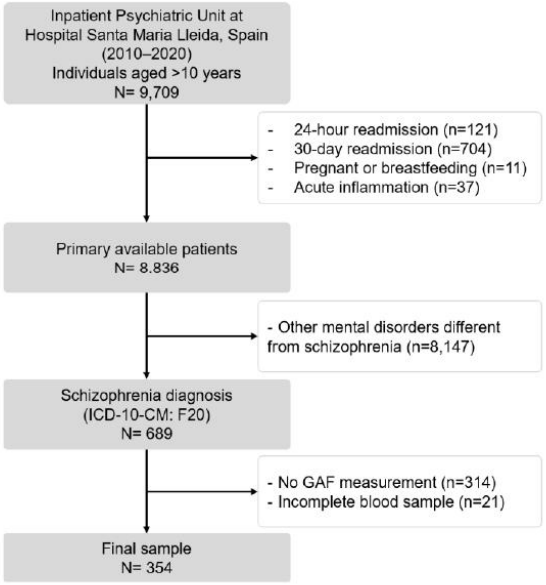
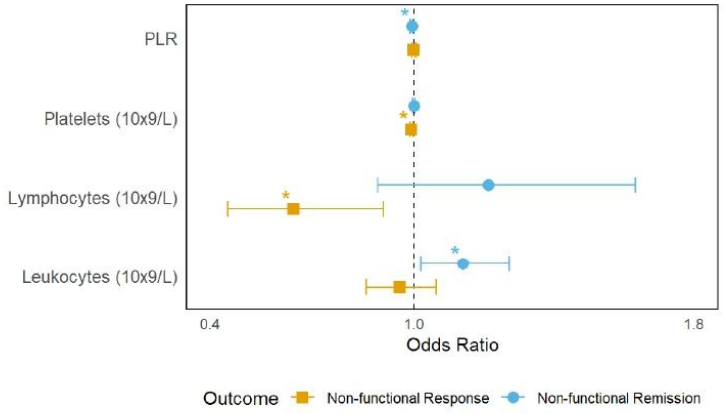


Figure 2. Forst-plot of associations between blood-based Immune biomarkers and functional outcomes in acute schizophrenia.



Note: *denotes statistical significance.

4.4. Article IV

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High neutrophil-lymphocyte ratio upon admission is associated with better response in psychotic depression

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ABSTRACT

Recent evidence relates the inflammatory system to the aetiology and evolution of mood disorders. The Neutrophil-Lymphocyte Ratio (NLR) is an affordable and reproducible biomarker of inflammation. The aim of the study is to retrospectively evaluate the association between NLR and response to treatment in 50 patients aged over 50 with a diagnosis of Psychotic Depression (PD) who were admitted to an acute psychiatric unit between 2010 and 2018. They were stratified according to sex and treatment received: antidepressants, antipsychotics and electroconvulsive therapy (ECT). The NLR was collected on admission and the clinical response was quantified by the Global Assessment of Functioning (GAF) scale. In the simple linear regression, high NLR upon admission was associated with better clinical response during hospitalization as measured by GAF. When stratifying the patients, this association was maintained in women, in patients who received antidepressant treatment with tricyclics and SNRIs, antipsychotic treatment with olanzapine/quetiapine and those who did not receive ECT. NLR is an accessible biomarker in clinical practice, and in PD patients it could guide the therapeutic strategy and be a predictor of response.

1. Introduction

Unipolar major depression with psychotic features (PD) is a serious and disabling disorder that affects approximately 0.4% of the general population, the incidence being much higher in patients over 50 years of age (Jääskeläinen et al., 2018). It presents a more severe clinical course, with greater psychomotor impairment, depressive feelings, and greater neuropsychological dysfunction than non-psychotic depression (Costa et al., 2020). It is also associated with an increased risk of recurrence and mortality (Jääskeläinen et al., 2018). Given the differential characteristics with non-psychotic major depression (MDD) it has been proposed that it be considered as a differentiated entity, but controversies continue to exist in this regard (Keller et al., 2007). While in some classifications it appears as a severe subtype of major depressive disorder (ICD-10), in others it appears independently of severity (DSM-5).

With regard to therapeutic management, both pharmacological treatment and electroconvulsive therapy (ECT) are accepted first-line strategies, although today it is still difficult to establish a single treatment of choice (Dubovsky et al., 2020). The most widely accepted pharmacological strategy is the combination of an antipsychotic with an antidepressant rather than either of the two treatments separately (Wijkstra et al., 2015). The use of ECT as monotherapy and its use as a first line treatment has been proposed in those patients with PD and high risk of suicide (Dubovsky et al., 2020). However, some clinical practice guidelines (CPG) suggest ECT as a first line treatment even without the presence of suicidal ideation or acute somatic deterioration due to its speed of action and high effectiveness in PD (SEPB, 2018).

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There is increasing evidence on the association between depression and inflammation (Miller and Raison, 2016). Numerous studies look into the response to pharmacological treatment or ECT depending on the underlying inflammation using CRP or interleukins as biomarkers of said inflammation (Liu et al., 2020; van Buel et al., 2015). Recently, the Neutrophil-Lymphocyte Ratio (NLR) has been proposed as affordable and reproducible biomarker of the systemic inflammatory response (Brinn and Stone, 2020). Higher NLR values have been found in depressed patients compared to healthy controls and its value has been correlated with the severity of MDD (Mazza et al., 2018). However, its association with response to the treatment of PD has not been previously studied.

The aim of this study is to analyse the association between NLR and clinical improvement in patients admitted with PD depending on the antidepressant, antipsychotic and ECT treatment received.

2. Material and methods

A retrospective study of patients hospitalized in the Psychiatry Service of Santa Maria University Hospital (Lleida) was carried out from January 1, 2010 to December 31, 2018. The local ethics committee approved the study.

All patients aged over 50 with a diagnosis of major depressive disorder, single episode, severe, specified with psychotic features (296.24 and F32.3) and major depressive disorder, recurrent episode, severe, specified with psychotic features (296.34 and F33.3) according to ICD 9 and 10 were included. Patients with a diagnosis of bipolar disorder, autoimmune diseases, anti-inflammatory or immunosuppressant treatments and/or laboratory abnormalities (anaemia, leucocytosis or elevated C-reactive protein (CRP)) on admission were excluded. NLR was calculated as the ratio of neutrophil count to lymphocyte count at the time of admission. The presence of cardiovascular risk factors (hypertension, hyperlipidemia and/or diabetes mellitus) and smoking at admission was assessed to consider possible confounding factors related to NLR. Of the 102 patients who met the inclusion criteria, 2 were eliminated due to incomplete data in their clinical history, 9 due to presenting autoimmune diseases and 41 due to laboratory abnormalities on admission. A total of 50 patients were included. All patients were evaluated at admission and discharge using the Global Assessment of Functioning (GAF) scale (American Psychiatric Association, 2013) to quantify clinical improvement.

Patients were classified according to the treatment given upon admission: antidepressants (tricyclics, serotonin reuptake inhibitors (SSRIs) or dual antidepressants (SNRI)), antipsychotics (risperidone, olanzapine/quetiapine or aripiprazole) and ECT. For all pharmacological treatments, standardized equivalent doses were calculated (Hayasaka et al., 2015; Leucht et al., 2016) upon discharge. Patients who received treatment with drugs from two different groups were classified according to the drug that provided the most standardized equivalent dose. When stratifying patients according to antipsychotic treatment, 4 patients did not receive antipsychotic treatment and only 2 patients received aripiprazole treatment, so they were not included in the sub-analysis.

Statistical analyses were performed using IBM-SPSS v.23. Continuous data were expressed as a mean ± standard deviation while categorical data were expressed as absolute values and percentages. Normal distribution was evaluated using the Shapiro-Wilk test. Chi-square, student's t-tests and analysis of variance (ANOVA) were used for continuous data. As non-parametric alternative, the Mann–Whitney U and the Kruskal-Wallis test were used as appropriate. Correlation analysis was performed using the Pearson or Spearman correlation test. A simple linear regression stratified by each of the subgroups was used to evaluate the NLR as a predictor of change in GAF. Type I error was fixed at the usual value of 5% (alpha = 0.05), bilateral approximation.

3. Results

NLR was correlated with the change in GAF (r = 0.41; p = 0.003). The simple linear regression showed that higher NLR levels were associated with a greater change in GAF during hospitalization (β = 0.41, p = 0.003). When stratifying patients by sex, the association was maintained in women (β = 0.45, p = 0.011) but not in men (β = 0.28, p = 0.247) (Table 1). When stratifying patients by possible confounding factors, the association was maintained in those without cardiovascular risk factors (β = 0.63, p = 0.007) and those who did not smoke (β = 0.41, p = 0.010).

3.1. Antidepressant treatment

The table shows the characteristics of the patients included in the study according to the antidepressant treatment received. The three groups were similar in all characteristics, except in the equivalent dosage of fluoxetine received, which was lower in the SSRI group (p = 0.002) and the percentage of ECT received, which was higher in the group of tricyclics (p = 0.039).

In stratifying patients according to antidepressant treatment, for those who received tricyclics or SNRI, NLR continued to be a significant predictor of the change in GAF (β = 0.58, p = 0.046; β = 0.41, p = 0.023 respectively), but not for those who received SSRI (β = 0.33, p = 0.411) (Table 1, Fig. 1A).

3.2. Antipsychotic treatment

In stratifying patients according to the antipsychotic treatment received, both groups were equal in every characteristic studied, except for the percentage of women, which was higher in the group of risperidone (p = 0.039) (Table 2).

Simple linear regression showed that NLR was a significant predictor of change in GAF during hospitalization in patients treated with olanzapine/quetiapine (β = 0.51, p = 0.023), but not in those treated with risperidone (β = 0.29, p = 0.214) (Table 1, Fig. 1B).

3.3. Electroconvulsive therapy

When stratifying the patients according to whether or not they

Table 1
Association between NLR and difference in GAF in each group.

NLR and difference in GAF	Simple linear regression		
	Coefficient β	sr^2	p-value
Global (N = 50)	0.41	0.15	0.003*
SEX			
Women (N = 32)	0.45	0.17	0.011*
Men (N = 18)	0.28	0.025	0.247
CARDIOVASCULAR RISK FACTORS			
No (N = 17)	0.632	0.35	0.007*
Yes (N = 33)	0.297	0.09	0.093
TOBACCO CONSUMPTION			
No (N = 39)	0.41	0.14	0.010*
Yes (N = 11)	0.39	0.05	0.238
ANTIDEPRESSANTS			
Tricyclics (N = 12)	0.58	0.27	0.046*
SSRI (N = 20)	0.33	−0.03	0.411
SNRI (N = 30)	0.41	0.14	0.023*
ANTIPSYCHOTICS			
Olanzapine/Quetiapine (N = 24)	0.51	0.23	0.010*
Risperidone (N = 20)	0.29	0.03	0.214
ECT			
No (N = 28)	0.44	0.17	0.017*
Yes (N = 22)	0.35	0.08	0.101

Abbreviations: NLR: Neutrophil-Lymphocyte Ratio; GAF: Global Assessment of Functioning scale; SSRI: Selective Serotonin Reuptake Inhibitors; SNRI: Serotonin and Noradrenaline Reuptake Inhibitors. (*p < 0.05).

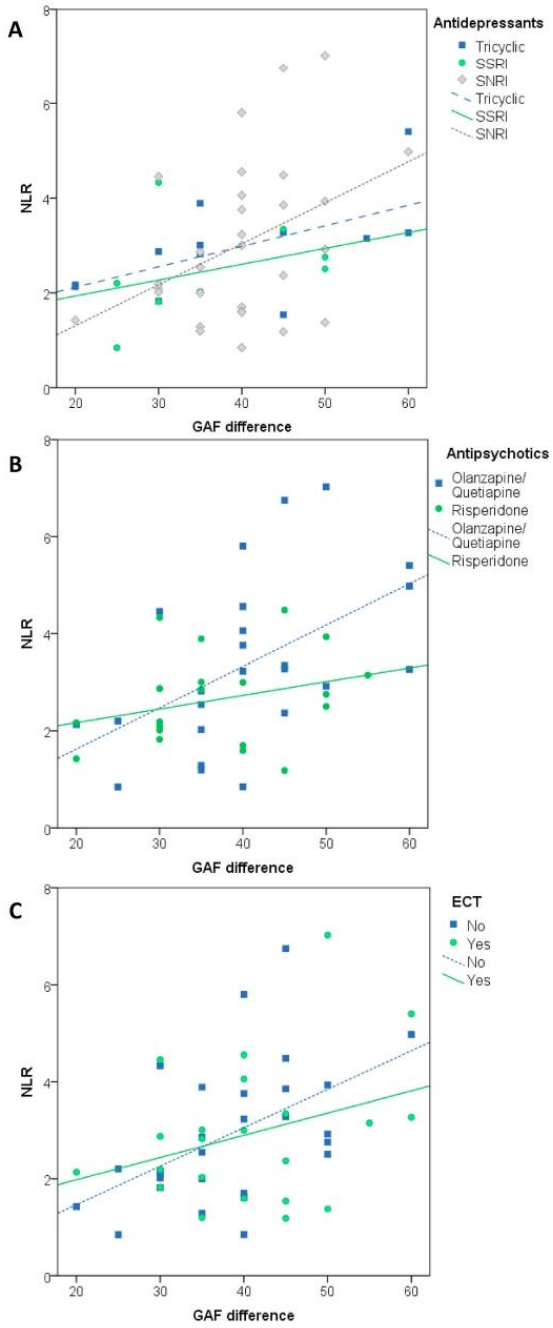

Fig. 1. Scatter chart showing the association between NLR and difference in GAF. A: according to antidepressant treatment; B: according to antipsychotic treatment. C: according to ECT treatment.

received ECT, the two groups were comparable in every characteristic studied except for the doses of benzodiazepines, which were lower in the ECT group ($p = 0.041$) (Table 2).

Simple linear regression showed that NLR was a significant predictor of the change in GAF during hospitalization in patients who did not receive ECT ($\beta = 0.44$, $p = 0.017$) but not in those who received ECT ($\beta = 0.35$; $p = 0.101$) (Table 1, Fig. 1C).

4. Discussion

High NLR values upon admission are associated with greater clinical improvement in PD evaluated by the change in GAF during hospitalization. When stratifying patients, this association was maintained in women, in those who received antidepressant treatment with tricyclics and SNRIs, antipsychotic treatment with olanzapine/quetiapine and those who did not receive ECT. There is no literature studying the association between inflammation measured by NLR and response to treatment in patients admitted with PD.

Some research has shown a positive correlation between NLR and depression severity, and an NLR greater than 1.57 has been proposed as an independent predictor of severe or very severe depression (Sunbul et al., 2016). However, Kayhan et al. (2017) found no differences in NLR between depressed patients with or without psychotic symptoms. According to our results, the mean NLR was 3.09 (±1.66) which corroborates the severity of the sample and an association is found between inflammation and response to treatment, especially in women.

It is important to note that NLR can be influenced by other baseline conditions that involve underlying inflammation such as cardiovascular risk factors or tobacco use (Howard et al., 2019). In this regard, we studied the impact of these factors on the relationship between NLR and clinical response and found that the association was maintained in those without cardiovascular risk factors and those who did not smoke. Nevertheless, no differences were found between the treatment subgroups when studying the distribution of these factors. These results highlight the importance of baseline inflammation in the response to treatment and support the validity of our subgroup results.

Regarding antidepressant treatment, both SSRI and SNRI have been proposed without any specific preference, as there is no clinical trial comparing both antidepressants in PD (Dubovsky et al., 2020). In contrast to our results, some studies indicate that elevated levels of baseline inflammation are associated with an attenuated response to antidepressant treatments (Strawbridge et al., 2015). However, the meta-analysis by Liu et al. (2020) points out that most of the associations found did not reach statistical significance and that only low levels of circulating IL-8 were associated with a better response to antidepressant treatment. This disparity in results suggests the possibility that certain antidepressant treatments may depend in part on inflammation, or that different subtypes of depression (inflammatory or non-inflammatory) may respond to one treatment approach but not to another. However, that meta-analysis does not include any study that measures NLR.

Recently, Arteaga-Henríquez et al. (2019) demonstrated that the presence of low-grade inflammation in patients with MDD is associated with a worse response to serotonergic antidepressant treatment but a better response to treatments that add a noradrenergic, dopaminergic or glutamatergic action. These findings support the validity of our results, since the positive association between the degree of inflammation and response to treatment is maintained in those patients who received tricyclic or dual antidepressants, but not in those who received SSRIs, thus supporting the possible anti-inflammatory effect of adding a second action mechanism to the serotonergic.

Regarding antipsychotic treatment, the CPGs recommend adding an atypical antipsychotic to the antidepressant, but do not specify the preference of one family of antipsychotics over another (Dubovsky et al., 2020). However, the three clinical trials of drug therapy combined with atypical antipsychotics and antidepressants in PD use drugs from the dibenzodiazepine family (olanzapine and quetiapine) (Dubovsky et al.,

Table 2
Characteristics of patients according the antidepressant, antipsychotic or ECT treatment.

Characteristics	Global (n = 50)	Antidepressant treatment				Antipsychotic treatment			Electroconvulsive therapy		
		Tricyclics (n = 12)	SSRI (n = 8)	SNRI (n = 30)	p-value	Olanzapine/ Quetiapine (n = 24)	Risperidone (n = 20)	p-value	No ECT (n = 28)	ECT (n = 22)	p-value
Women (%)	32 (64.2)	7 (58.3)	6 (75.0)	19 (63.3)	0.442	12 (50.0)	16 (80.0)	0.039*	19 (67.9)	13 (59.1)	0.526
Age (years)	68 (±9)	69 (±9)	68 (±12)	68 (±9)	0.839	67 (±8)	68 (±10)	0.741	67 (±10)	70 (±8)	0.296
Cardiovascular risk factors (%)	33 (66.0)	6 (50.0)	5 (62.5)	22 (73.3)	0.344	14 (58.3)	14 (70.0)	0.423	17 (60.7)	16 (72.7)	0.373
Tobacco consumption (%)	11 (22.0)	2 (16.6)	1 (12.5)	8 (26.6)	0.241	6 (25.0)	3 (15.0)	0.413	7 (25.0)	4 (18.2)	0.563
Standardized doses											
Chlorpromazine (mg/day)	258.1 (±473.4)	131.6 (±122.8)	626.5 (±1058.4)	219.5 (±280.1)	0.069	242.3 (±271.8)	285.7 (±664.04)	0.062	332.3 (±613)	169.5 (±197)	0.150
Fluoxetine (mg/day)	51.7 (±26)	66.8 (±38.2)	25.9 (±12.1)	51.2 (±17.0)	0.002*	57.6 (±29.0)	43.5 (±21.9)	0.177	49.6 (±26)	52.1 (±27)	0.919
Diazepam (mg/day)	26.5 (±12.2)	22.7 (±4.2)	30.90 (±24.7)	25.3 (±18.2)	0.079	29.8 (±10.1)	21.4 (±16.1)	0.189	29.3 (±12.2)	18.7 (±11.5)	0.041*
Antidepressant polypharmacy (%)	10 (20.0)	4 (33.3)	2 (25.0)	4 (13.3)	0.318	4 (16.7)	5 (25.0)	0.495	8 (28.6)	2 (9.1)	0.071
Antipsychotic polypharmacy (%)	6 (12.0)	0 (0.0)	2 (25.0)	4 (13.3)	0.186	2 (8.3)	4 (20.0)	0.444	4 (16.0)	2 (9.1)	0.516
Electroconvulsive therapy (%)	22 (44.0)	9 (75.0)	2 (25.0)	11 (36.7)	0.039*	13 (54.2)	7 (35.0)	0.204	13 (46.4)	10 (45.5)	0.946
Severity											
GAF upon admission	28 (±9)	31 (±7)	33 (±10)	26 (±9)	0.191	29 (±9)	25 (±6)	0.212	27 (±7)	29 (±10)	0.449
GAF Variation	39 (±11)	38 (±15)	36 (±11)	40 (±8)	0.593	41 (±11)	37 (±10)	0.257	38 (±10)	40 (±11)	0.391
NLR	3.09 (±1.66)	2.95 (±1.03)	2.48 (±1.04)	3.04 (±1.67)	0.633	3.38 (±1.74)	2.65 (±0.96)	0.131	2.73 (±1.28)	3.18 (±1.62)	0.289

Abbreviations: NLR: Neutrophil-Lymphocyte Ratio; GAF: Global Assessment of Functioning scale; SSRI: Selective Serotonin Reuptake Inhibitors; SNRI: Serotonin and Noradrenaline Reuptake Inhibitors; ECT: Electroconvulsive Therapy.

2020). Both drugs have shown an antidepressant effect that could be explained by the 5HT2C, 5HT7 and α1 antagonist properties of olanzapine and the 5HT2C antagonist properties, 5HT1A partial agonism, as well as the inhibition of the norepinephrine transporter of quetiapine at doses of approximately 300 mg (Grinchii and Dremencov, 2020).

There is evidence that anti-inflammatory actions are attributed to antipsychotic drugs by mitigating cell-mediated immune activation, as well as oxidative and nitrous stress. In particular, atypical antipsychotics appear to stimulate the production of anti-inflammatory cytokines such as IL-4, IL-12, and IL-17 (Pandurangi and Buckley, 2020). However, when it comes to narrowing down which alterations characterize a specific family of antipsychotics, studies show disparate and even contradictory results due to their different approaches (Pandurangi and Buckley, 2020).

Our results show that in the olanzapine/quetiapine group, a higher NLR was significantly associated with a greater change in GAF during hospitalization, which would indicate that the effect of these drugs could mediate their anti-inflammatory action. However, this association is not supported by previous studies that, although having been carried out mainly in patients with schizophrenia, have not demonstrated a differential anti-inflammatory action among atypical antipsychotics (Pandurangi and Buckley, 2020).

In relation to the ECT response, a neuroimmune and neurotrophic mechanism has been postulated given the rapid changes in inflammatory markers and the increase in the volume of the hippocampus and other brain regions (Singh and Kar, 2017).

Kruse et al. (2018) identified high levels of IL-6 as a potential predictor of improvement in patients with resistant MDD treated with ECT. Furthermore, low baseline IL-8 levels and subsequent IL-8 increase during the course of ECT were associated with a therapeutic response in women but not in men (Kruse et al., 2020). Likewise, the MODECT study (Carlier et al., 2019), which analysed patients with MDD over 55 years of age like in our sample, found better remission rates in those with moderately high CRP levels. Recently Bioque et al. (2019) have described how the good response to ECT could be explained by the modulation of cellular damage caused by inflammation (Bioque et al., 2019).

Most of the studies into NLR in ECT treatment have focused on demonstrating that the technique does not modify the rate (Canan et al., 2015). Our results show that NLR was associated with response to treatment in those who received pharmacological treatment, but not in those who also received ECT. The latter presented a trend towards a greater initial severity in GAF and higher NLR, but did not reach statistical significance.

The strength of this study is the good characterization of patients, including only those with unipolar PD and excluding those with laboratory abnormalities or infections and autoimmune diseases upon admission. Nevertheless, a few limitations of the study need to be acknowledged. First, a specific instrument measuring the severity was not used for depression, so the interpretation of the response to treatment may be difficult, but a global functioning scale for mental illness was used that showed to what extent the symptoms of a depression affect people in their daily lives. Second, the small sample size makes it difficult to interpret the sub-analysis of the SSRI group, although there are probably few PD patients treated with this family of first-line antidepressants. Third, the NLR value at the end of hospitalization was not available and we were unable to study NLR variation during hospitalization, hence future studies should include both values to examine this relationship. Finally, given the retrospective nature of the study, cause-effect relationships beyond that of association should be avoided because confounding factors such as body mass index have not been controlled. Therefore, prospective studies would be interesting.

In conclusion, this study shows that high values of baseline inflammation measured by NLR upon admission are associated with better clinical response in PD in a sample of hospitalized patients aged over 50. The NLR measurement in PD patients could guide the therapeutic strategy and be a response marker with clinical and health care applicability. The search for biomarkers accessible to clinical practice is of great interest for the treatment and prognosis of patients with PD.

Contributors

VLB and RPG conceived the study, obtained the data, conducted the statistical analyses and drafted the first version of the manuscript. DRS, EBR, MAP, MB and MM revised the manuscript and provided a substantial conceptual contribution. IG conducted the statistical analyses and revised the manuscript. All authors proofread and approved the final draft of the manuscript.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of interestDOCI

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4.5. Article V

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Original Paper

Thieme

Blood Cell Count Ratios at Baseline are Associated with Initial Clinical Response to Clozapine in Treatment-Resistant, Clozapine-Naïve, Schizophrenia-Spectrum Disorder

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ABSTRACT

Background Clozapine is the recommended treatment for managing treatment-resistant schizophrenia (TRS), and immunological mechanisms may be involved in its unique antipsychotic efficacy. This study investigated whether baseline immune abnormalities measured with blood cell count ratios can predict the clinical response after initiating treatment with clozapine in patients with clozapine naïve TRS.

Methods A longitudinal design was developed, involving 32 patients diagnosed with treatment-resistant, clozapine-naïve schizophrenia-spectrum disorder. Patients were evaluated at baseline before clozapine starting and 8 weeks of follow-up. Psychopathological status and immune abnormalities (blood cell count ratios: neutrophil-lymphocyte ratio [NLR], monocyte-lymphocyte ratio [MLR], platelet-lymphocyte ratio [PLR] and basophil-lymphocyte ratio [BLR]) were evaluated in each visit.

Results Baseline NLR ($b = -0.364$; $p = 0.041$) and MLR ($b = -0.400$; $p = 0.023$) predicted the change in positive symptoms over the 8-week period. Patients who exhibited a clinical response showed higher baseline NLR (2.38 ± 0.96 vs. 1.75 ± 0.83; $p = 0.040$) and MLR (0.21 ± 0.06 vs. 0.17 ± 0.02; $p = 0.044$) compared to non-responders. In the ROC analysis, the threshold points to distinguish between responders and non-responders were approximately 1.62 for NLR and 0.144 for MLR, yielding AUC values of 0.714 and 0.712, respectively. No statistically significant differences were observed in the blood cell count ratios from baseline to the 8-week follow-up.

Conclusion Our study emphasizes the potential clinical significance of baseline NLR and MLR levels as predictors of initial clozapine treatment response in patients with TRS. Future studies with larger sample sizes and longer follow-up periods should replicate our findings.

Original Paper

Thieme

Introduction

The management of treatment-resistant schizophrenia-spectrum disorder (TRS) presents a significant challenge in psychiatric care [1], demanding the exploration of novel predictors of treatment outcomes [2]. Clozapine is the gold standard for the treatment of TRS [3,4]. However, a substantial proportion of patients do not exhibit the desired therapeutic response even upon clozapine administration [5], and some of the potential adverse events associated with clozapine use can be very severe [6]. This highlights the importance of identifying dependable indicators that can aid in predicting how individuals will respond to clozapine treatment, thereby mitigating the risk of severe adverse events in those with poor response indicators.

There is evidence suggesting that immune dysfunction plays a significant role in the pathophysiology of schizophrenia (SCZ). Elevated cytokine levels have consistently been observed in both peripheral blood [7] and cerebrospinal fluid [8] of individuals with SCZ. Recent analyses suggest genetic links between SCZ and immune regulatory aspects [9, 10]. Moreover, aberrations in immune-inflammatory pathways have been found to play a role in TRS [11] with specific signatures in this population [12]. In this line, immunological mechanisms may also contribute to treatment response to antipsychotics [13], and clozapine may exert its superior efficacy via immune mechanisms [14].

Within this context, numerous peripheral biomarkers have been studied to assess immune dysfunction in mental health. Blood cell count ratios, such as the neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), platelet-lymphocyte ratio (PLR), and basophil-lymphocyte ratio (BLR), are among these biomarkers. They can be easily calculated from differential white blood cell counts and reflect the balance between innate immunity (indicated by neutrophil, monocyte, or platelet counts) and adaptive immunity (lymphocyte count). These ratios are elevated in individuals with SCZ [15, 16], including those experiencing their first episode of psychosis who are antipsychotic-naïve [17] or minimally treated [18]. Furthermore, these ratios may provide improved predictive value when identifying imbalances between innate and adaptive immune pathways [19]. Previous studies have linked persistent increase in blood cell count ratios with TRS [20]. However, the relationship between baseline blood cell count ratios and clinical responses to clozapine in patients new to clozapine treatment (clozapine-naïve) has not been previously studied.

The objective of this study was to examine whether baseline blood cell count ratios can predict the clinical response at 8 weeks of follow-up in individuals with TRS after starting treatment with clozapine. We hypothesized that higher baseline blood cell count ratios would be associated with greater clinical improvement.

Methods

Study design and participants

This study employed a longitudinal design, involving 32 patients diagnosed with treatment-resistant, clozapine-naïve schizophrenia-spectrum disorder. The baseline assessment took place when the patients were classified as having TRS following TRRIP Consensus [21]: they failed to respond to two antipsychotic trials, other than clozapine, with adequate dose and duration of treatment. Subsequently, these patients commenced pharmacological treatment with clozapine and underwent prospective follow-ups at 8 weeks post-baseline to assess their clinical progress. Patient recruitment spanned from 2015 to 2019 and was conducted at the outpatient facility of the Barcelona Clínic Schizophrenia Unit (BCSU), overseen by a senior psychiatrist. Although data for a one-year follow-up of the project have been published [22, 23], in this instance, we opted to utilize only the first 8 weeks of follow-up to preserve the sample size and guarantee the reliability of clinical assessments and biological biomarkers.

Inclusion criteria were: (i) individuals aged between 18 and 50 years, (ii) patients diagnosed with schizophrenia and other psychotic-related disorders, following DSM-5 criteria [24], and (iii) a diagnosis of TRS, defined as nonresponse to at least two sequential antipsychotic trials of sufficient dose, duration, and adherence. Exclusion criteria included: (i) any history of traumatic brain injury, (ii) presence of intellectual disabilities, (iii) acute or chronic infections (including human immunodeficiency virus, hepatitis B, and hepatitis C), and (iv) autoimmune diseases. Each participant received comprehensive information regarding the study's objectives, procedures, and potential risks before granting informed consent through a consent form. The study protocol obtained approval from the local Ethical Committee and was conducted in adherence with the principles set forth in the Declaration of Helsinki.

Intervention and clinical data collection

Clozapine was initiated at the baseline visit in accordance with current guidelines [25]. Psychopathological assessments using the Positive and Negative Syndrome Scale (PANSS) [26] were conducted at baseline and weeks 8. According to the recommendations for treatment-resistant patients [27], participants were categorized as responders if they achieved a > 25 % change in PANSS scores (PANSS total, PANSS factor score for positive symptoms [PANSS-FSPS] or PANSS factor score for negative symptoms [PANSS-FSNS]) at 8 weeks of follow-up.

The number of antipsychotic treatments prior to the first use of clozapine, tobacco use, and anthropometric parameters were collected at baseline. Weight was measured in kilograms, with participants in their underwear, barefoot, standing in the center of the scale with their arms by their side, and the weight distributed on both feet. Height was measured in meters, with participants standing barefoot and with their feet together. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters (BMI = weight [kg] / height^2 [m^2]).

Biological samples

Following current guidelines [25], routine weekly blood tests were performed. However, for the present study, only samples from two time points were used: baseline, prior to initiating clozapine, and at 8 weeks. The blood sampling procedure involved venipuncture conducted by skilled nurses, following an overnight fast of 8 to 10 hours. Flow Cytometry, utilizing a Sysmex XN analyzer, was employed to determine total and differential white blood cell (WBC) counts, and platelet counts. The specific differentials encompassed neutrophils, basophils, eosinophils, monocytes, and lymphocytes. From these measurements, several ratios were derived, including the NLR, MLR, PLR, and BLR. Due to the absence of currently de-

fined cutoffs for blood cell count ratios in psychiatric patients, we adhered to the published methodology [28] and categorized them into ascending tertiles (T1 or lower tertile, T2, and T3 or upper tertile) based on our data.

Statistical analysis

Continuous data are expressed as a median and interquartile range (IQR), while categorical data are expressed as absolute values and percentages. Shapiro-Wilk test was used to test the normality of blood cell count ratios. The correlation of blood cell count ratios with clinical scale scores was assessed by Spearman rank tests. A linear regression model was used to assess the ability of baseline blood cell count ratios in predicting clinical responses at 8 weeks. Considering the limited participant count [29], only the WBC and BMI at baseline were chosen for covariate inclusion in the regression model. A one-way ANOVA was employed to investigate differences in PANSS change at 8 weeks based on blood cell count ratio tertiles. The Mann–Whitney U was used to assess nonparametric variables and compare responders vs. non-responders. Receiver Operating Characteristic (ROC) curve analyses to test the ability of baseline blood cell count ratios to correctly differentiate non-responders from responders. The Wilcoxon Signed-Ranks test was used to detect differences in paired blood cell count ratio measurements at baseline and 8 weeks. Two-tailed P-values < 0.05 were considered statistically significant. Statistical analyses were performed using IBM-SPSS v.23.

Results

Baseline characteristics

The study included 32 patients, out of which 14 (43.8 %) were female. Among them, 81.2 % were diagnosed with schizophrenia, and 18.7 % were diagnosed with schizoaffective disorders. Clinical response was attained by 56.2 % of the patients based on PANSS total score, 71.8 % on PANSS-FSPS, and 50 % on PANSS-FSNS (▶ **Table 1**).

Blood cell count ratios at baseline and baseline psychopathology

Higher NLR values were associated with higher PANSS-FSPS at baseline (Spearman r = 0.314; p = 0.040). However, no associations were observed for the other blood cell count ratios or the other psychopathology measures.

Blood cell count ratios at baseline and symptoms change during the follow-up

At baseline, both NLR (b = −0.364; p = 0.041) and MLR (b = −0.400; p = 0.023) were predictive of the change in PANSS-FSPS scores at 8 weeks, with patients having the highest levels of NLR and MLR showing the greatest reduction in symptoms. No associations were found for PANSS-PSNS nor PANSS total score (▶ **Table 2**, ▶ **Fig. 1**).

When analyzing changes in symptoms based on blood cell count ratios tertials at baseline, we found that patients in the higher tertile showed a greater change in PANSS-PSPS than those in the first tertile for NLR (p = 0.024) and MLR (p = 0.035) (▶ **Fig. 2**).

▶ **Table 1** Sample characteristics.

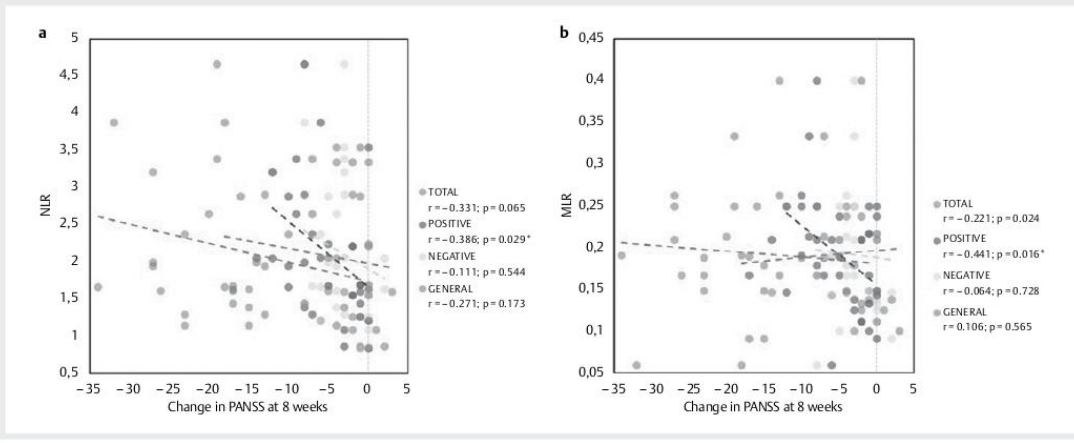
Characteristics	Sample (n = 32)
Age, years, median (IQR)	36 (24–42.2)
Female, nº (%)	14 (43.8)
Diagnosis, nº (%)	
Schizophrenia	26 (81.3)
Schizoaffective disorder	6 (18.7)
Tobacco use, nº (%)	11 (34.4)
BMI, mean (SD)	26.6 (5.5)
Nº of previous treatment, median (IQR)	2 (1–3.5)
PANSS at baseline, median (IQR)	
Positive scale	20 (14.5–24.7)
Negative scale	21.5 (16.2–27)
General psychopathology scale	35.5 (30.2–45.7)
Total score	76.5 (65–93)
Blood cell count ratios at baseline, median (IQR)	
NLR	1.8 (1.4–2.8)
MLR	0.188 (0.143–0.238)
PLR	108.8 (90–155.6)
BLR	0.001 (0.000–0.006)
Clinical response, nº (%) PANSS	
PANSS-FSPS	18 (56.3)
PANSS-FSNS	10 (31.3)
PANSS total	17 (53.1)

Abbreviations: PANSS: Positive and Negative Syndrome Scale; BMI: body mass index; NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; PLR: platelet-lymphocyte ratio; BLR: basophil-lymphocyte ratio; Coef.: coefficient β; FSPS: factor score for positive symptoms; FSNS: factor score for negative symptoms.

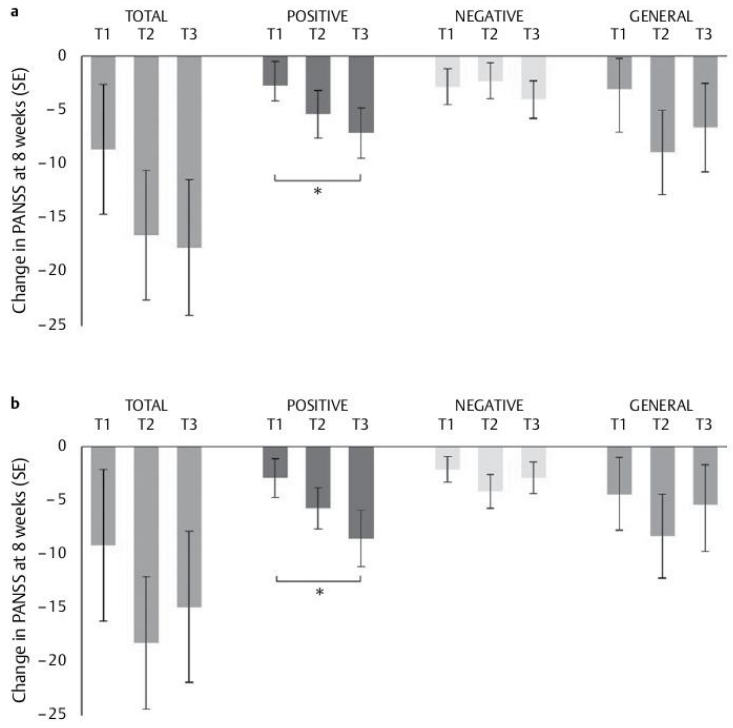
▶ **Table 2** Results of the linear regression model showing associations between baseline blood cell count ratios and change in psychopathology scores after 8 weeks of treatment.

	Change in PANSS after 8 weeks							
	POSITIVE		NEGATIVE		GENERAL		TOTAL	
	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value
NLR	**−0.364**	**0.041***	−0.188	0.312	−0.142	0.439	−0.278	0.123
MLR	**−0.400**	**0.023***	−0.052	0.784	0.0.83	0.653	−0.113	0.540
PLR	0.016	0.932	0.010	0.325	0.026	0.425	−0.041	0.826
BLR	0.054	0.769	−0.117	0.524	−0.016	0.932	−0.020	0.912

Abbreviations: PANSS: Positive and Negative Syndrome Scale; NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; PLR: platelet-lymphocyte ratio; BLR: basophil-lymphocyte ratio; Coef.: coefficient β.



▶ **Fig. 1** Association between NLR (**a**) and MLR (**b**) at baseline and change in PANSS subscales at 8 weeks. NLR, neutrophil-lymphocyte ratio; MLR, monocyte-lymphocyte ratio; PANSS, Positive and Negative Syndrome Scale.



▶ **Fig. 2** Change in PANSS Punctuation at 8 Weeks Based on Tertiles (T1, T2 and T3) of NLR (**a**) and MLR (**b**). NLR, neutrophil-lymphocyte ratio; MLR, monocyte-lymphocyte ratio; PANSS, Positive and Negative Syndrome Scale.

▶ **Table 3** Blood cell count ratios measured at baseline and 8 weeks of follow-up, expressed as median and IQR.

	Baseline	8 weeks	Statistics	p-value
NLR	1.8 (1.4–2.8)	1.9 (1.4–2.5)	−0.804	0.421
MLR	0.188 (0.143–0.238)	0.181 (0.154–0.234)	−0.964	0.340
PLR	108.8 (90–155.6)	125.9 (92.9–153.6)	−1.515	0.130
BLR	0.001 (0.000–0.006)	0.001 (0.000–0.004)	−1.521	0.128

Abbreviations: NLR: neutrophil-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; PLR: platelet-lymphocyte ratio; BLR: basophil-lymphocyte ratio.

Differences in blood cell count ratios between responders and non-responders

PANSS-FSPS responders showed higher baseline NLR (2.05 (1.65–2.97) vs. 1.57 (1.18–2.21); p = 0.040) and MLR (0.20 (0.16–0.25) vs. 0.14 (0.12–0.22); p = 0.044) than non-responders. No significant differences were found for the other blood cell count ratios or the other psychopathology measures.

When investigating the ability of baseline blood cell count ratios to distinguish PANSS-FSPS responders from non-responders, the threshold points were around 1.62 (sensitivity: 77.8 %; specificity: 42.9 %; AUC: 0.714) for NLR and 0.144 (sensitivity: 99.4 %; specificity: 57.1 %; AUC: 0.712) for MLR.

Change of blood cell count ratios during follow-up

Mean blood cell count ratios values at baseline and 8 weeks are described in ▶ **Table 3**. The Wilcoxon Signed-Ranks Test for paired measures did not detect statically significant changes in the NLR, MLR, PLR or BLR from baseline to follow-up. At 8 weeks, patients who achieved PANSS-FSPS response maintained higher NLR (2.40 (1.88–3.07) vs. 1.53 (1.25–2.03); p = 0.013) and MLR (0.21 (0.17–0.26) vs. 0.15 (0.14–0.19); p = 0.011) than non-responders.

Discussion

The study examined the ability of blood cell count ratios at baseline to predict clinical response at 8 weeks of follow-up after initiating treatment with clozapine in patients with a treatment-resistant, clozapine-naïve, schizophrenia-spectrum disorder. We found that NLR and MLR values before clozapine initiation predict improvement in PANSS-positive symptoms. Patients with the highest levels of NLR and MLR show higher symptoms response. Furthermore, clinical responders have higher NLR and MLR at baseline, and the threshold points to distinguish between responders and non-responders could be around 1.62 for NLR and 0.144 for MLR with an AUC of 0.714 and 0.712, respectively.

Clozapine remains the only medication approved for TRS [3]. Clinical trials have consistently shown its superiority in effectively reducing psychotic symptoms [30, 31]. Although its comprehensive mechanism of action is not completely understood, it acts on multiple neurotransmitter systems, including dopamine, serotonin, glutamate, and others. This broad spectrum of activity may make it more effective in cases where other medications have failed. It has also been suggested that its immunomodulatory properties could play a role in this unique efficacy [32].

Previous research has investigated whether there is an association between immune cells and the clinical response when initiating clozapine, yielding mixed results. Mauri et al. [33], for instance, examined leukocyte and neutrophil counts in a small sample but found no baseline differences between responders and non-responders. Blackman et al. [34], in a larger study, did not find any association between neutrophil counts and clinical global impression (CGI). However, in a more recent study, Jones et al. [14] analyzed the largest cohort to date, comprising 397 patients, and observed that individuals with high-normal neutrophil count, were more likely to respond to clozapine over a longer-term period as measured by CGI.

In contrast, we employed blood cell count ratios that are suggested to be more appropriate when identifying imbalances between innate and adaptive immune pathways [19, 35]. We found that patients with the highest baseline NLR and MLR values exhibited more substantial improvements in positive symptomatology, but PLR and BLR did not show the same pattern. Furthermore, we identified that clinical responders, individuals experiencing significant reductions in positive symptoms, exhibited higher NLR and MLR levels at baseline.

Contrary to our results, Labonté et al. [20] found lower PLR in patients with a poor response to clozapine and no differences in NLR and MLR. These differences could be explained by design discrepancies because, although they analyzed a considerable sample size (n = 156), they used a retrospective study with a cross-sectional design and a proxy definition of clinical response based on clozapine use and augmentation strategies. Moreover, our findings align with the results reported by Jones et al. [14], which used a more similar design and found that patients with a high-normal neutrophil count were more likely to respond to clozapine. These findings, along with research showing that genes related to WBC counts are linked to the risk of SCZ [9, 10], suggest that immune mechanisms may play a role in certain groups of TRS patients who respond well to clozapine [36].

Finally, while some studies have linked BLR with longitudinal outcomes after a first episode of psychosis [37], no links have been described with TRS, as demonstrated in our study.

Importantly, we established threshold points for NLR (approximately 1.62) and MLR (approximately 0.144) to distinguish between responders and non-responders, with respective AUC values of 0.714 and 0.712. These values indicate acceptable discrimination but are likely of limited clinical relevance, as they fall below the proposed threshold of 0.8, considered clinically useful [38]. However, because of the limited sample size of our study, higher AUC values of blood cell count ratios regarding response to positive symptoms cannot be excluded. Further studies are needed to investigate this important issue, as it may have a significant impact on clinical practice.

The main limitation of the study is the sample size, which increases the likelihood of committing type II errors, presents difficulties in controlling for confounders, and reduces the statistical power for ROC analysis. Another limitation is the relatively short follow-up period, which limits the probability of obtaining results related to slower-to-improve aspects, such as negative symptoms and functionality.

Conclusion

In conclusion, our study underscores the potential clinical significance of baseline NLR and MLR levels as predictors of clozapine treatment response in patients with TRS. If replicated, these findings could hold implications for personalized treatment strategies, enabling clinicians to more effectively identify individuals likely to benefit from clozapine therapy and potentially enhancing outcomes for this challenging-to-treat population. Further investigation is warranted to validate our findings and elucidate the underlying mechanisms linking immunity to the therapeutic effects of clozapine.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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4.6. Article VI

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Full-length Article

White blood cell counts, ratios, and C-reactive protein among individuals with schizophrenia spectrum disorder and associations with long-term outcomes: a population-based study

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ABSTRACT

Background: Immune mechanisms are associated with adverse outcomes in schizophrenia; however, the predictive value of various peripheral immune biomarkers has not been collectively investigated in a large cohort before.
Objective: To investigate how white blood cell (WBC) counts, ratios, and C-Reactive Protein (CRP) levels influence the long-term outcomes of individuals with schizophrenia spectrum disorder (SSD).
Methods: We identified all adults in the Central Denmark Region during 1994–2013 with a measurement of WBC counts and/or CRP at first diagnosis of SSD. WBC ratios were calculated, and both WBC counts and ratios were quartile-categorized (Q4 upper quartile). We followed these individuals from first diagnosis until outcome of interest (death, treatment resistance and psychiatric readmissions), emigration or December 31, 2016, using Cox regression analysis to estimate adjusted hazard ratios (aHRs).
Results: Among 6,845 participants, 375 (5.5 %) died, 477 (6.9 %) exhibited treatment resistance, and 1470 (21.5 %) were readmitted during follow-up. Elevated baseline levels of leukocytes, neutrophils, monocytes, LLR, NLR, MLR, and CRP increased the risk of death, whereas higher levels of lymphocytes, platelets, and PLR were associated with lower risk. ROC analysis identified CRP as the strongest predictor for mortality (AUC=0.84). Moreover, elevated levels of leukocytes, neutrophils, monocytes, LLR, NLR and MLR were associated with treatment resistance. Lastly, higher platelet counts decreased the risk of psychiatric readmissions, while elevated LLR increased this risk.
Conclusions: Elevated levels of WBC counts, ratios, and CRP at the initial diagnosis of SSD are associated with mortality, with CRP demonstrating the highest predictive value. Additionally, certain WBC counts and ratios are associated with treatment resistance and psychiatric readmissions.

1. Introduction

Previous research has increasingly provided evidence of a connection between immune abnormalities and schizophrenia, supported by epidemiological (Benros et al., 2014; Cullen et al., 2019; Pedersen et al., 2020), genetic (Leung et al., 2024; Steen et al., 2023; Trubetskoy et al., 2022; Williams et al., 2022) and biomarker studies (Halstead et al., 2023; Misiak et al., 2021; Osimo et al., 2021a; Perry et al., 2021; Pillinger et al., 2019). A recent *meta*-analysis showed increased white blood cell (WBC) counts, including neutrophil, monocyte, and lymphocyte counts, in patients with schizophrenia compared to healthy controls (Clausen et al., 2024). Additionally, these counts have been linked to

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unfavourable outcomes in first-episode psychosis (FEP) (Núñez et al., 2019; Osimo et al., 2023, 2021b). WBC ratios, such as the neutrophil–lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR), reflect the balance between innate immunity (indicated by neutrophil, monocyte, or platelet counts) and adaptive immunity (lymphocyte count) (Mazza et al., 2020). These ratios are also higher in people with schizophrenia compared to healthy individuals (Clausen et al., 2024) and may provide alternative predictive value when assessing inflammation, especially in detecting imbalances between the innate and adaptive immune pathways (Bhikram and Sandor, 2022; Llorca-Bofí et al., 2023). Finally, elevated levels of C-Reactive Protein (CRP) have been reported during both the acute and chronic stages of schizophrenia (Halstead et al., 2023; Yuan et al., 2019), and these levels have been linked to poorer clinical outcomes (Horsdal et al., 2017a; Kose et al., 2021; Nettis et al., 2019; Osimo et al., 2021a).

WBC counts, NLR, and CRP have all shown a significant association with overall mortality in the general population (Fest et al., 2019; Park et al., 2023; Proctor et al., 2015; Song et al., 2021). They have also been linked to outcomes and can predict disease progression in patients with various medical conditions (Guo and Zou, 2023; de Jager et al., 2010; Liu et al., 2020; Ni et al., 2020; Park et al., 2018; Templeton et al., 2014). In patients with mental disorders treated in secondary mental health services, elevated NLR has been associated with increased mortality but not with hospital admissions, as reported in a cross-sectional study without specific information about disorder-specific mortality (Brinn and Stone, 2020). Additionally, in individuals with schizophrenia, higher levels of CRP have been linked to increased mortality (Horsdal et al., 2017a), and various immune system markers may affect treatment response (Fond et al., 2020; Kose et al., 2021; Llorca-Bofí et al., 2024a; Orbe and Benros, 2023). However, to our knowledge, there is a lack of large-scale longitudinal studies that investigate different inflammatory biomarkers and long-term outcomes among patients with schizophrenia. Therefore, it is crucial to examine the association between WBC counts, ratios, and CRP assessed at the time of diagnosis and subsequent prognosis in schizophrenia (Bhikram and Sandor, 2022). Such investigations may provide valuable insights for developing prediction models suitable for routine use and for enabling more personalized treatment early in the course of the disorder. This is particularly important because individuals with schizophrenia experience an 11–13-year shorter life expectancy (Laursen et al., 2019; Llorca-Bofí et al., 2024c).

In this first large-scale population-based study of inflammatory biomarkers at first diagnosis of schizophrenia spectrum disorder (SSD), we investigated the associations between WBC counts and ratios, as well as CRP, with three long-term outcomes: 1) all-cause mortality, 2) treatment resistance, and 3) psychiatric readmissions.

2. Methods

2.1. Registers

This study was based on the linking of Danish health care registries and databases covering the entire Danish population by using the unique civil registration number assigned to every Danish citizen in *The Danish Civil Registration Register* since 1968 (Pedersen, 2011). For a more detailed description of the registers see **Supplementary Material**.

2.2. Study population

Diagnoses of SSD were obtained from *The Danish Psychiatric Central Research Register*. The diagnoses are coded according to the International Classification of Disease (8th revision [ICD-8] until the end of 1993, and 10th revision [ICD-10] thereafter). We identified individuals with a first main diagnosis of SSD (see **Table S1** for ICD codes) in an in- or outpatient setting (index date being first admission date for inpatients or first contact date for outpatients) between January 1, 1994, and December 30, 2013. The final study population included individuals who were born in Denmark after January 1, 1955, aged 18 years or older at the diagnosis, and were living in Central Denmark Region at the time of diagnosis identified by linkage to *The Danish Civil Registration System*. A flowchart illustrating the inclusion and exclusion criteria is shown in **Figure S1**.

2.3. Exposure: assessment of WBC counts, ratios and CRP

We used the *LABKA* research database (Grann et al., 2011) to identify all measurements for leukocyte count, neutrophil count, monocyte count, platelet count, lymphocyte count and CRP within ± 30 days from index date and used the measurement closest to index date as the baseline measurement (see **Table S2** for blood component codes). We computed WBC ratios as follows: leukocyte to lymphocyte counts ratio for LLR; neutrophil to lymphocyte counts ratio for NLR; monocyte to lymphocyte counts ratio for MLR; platelet to lymphocyte counts ratio for PLR. As there are no currently defined cut-off values for WBC ratios in psychiatric patients (Bhikram and Sandor, 2022), we divided them into four groups based on quartiles in our dataset: Q1 (lower quartile), Q2, Q3, and Q4 (upper quartile) at baseline. A composite index was defined as having ≥ 2 indexes in Q3 or Q4. We adhered to the recommendations outlined by the U.S. Centers for Disease Control and Prevention and the American Heart Association (Myers et al., 2004) to classify CRP levels at baseline into the following groups: low level of systemic inflammation (<1 mg/L); intermediate level of systemic inflammation (1–2.9 mg/L); high level of systemic inflammation (3–10 mg/L); and acute inflammation (>10 mg/L). In the analyses, we employed a time-dependent exposure approach. Measurements of WBC counts, ratios and CRP taken later than 30 days after baseline were grouped according to the cut-off at baseline and included in the analysis from the time of their measurement. Therefore, patients with additional assessments during follow-up had their measurements updated accordingly during the follow-up period.

2.4. Outcomes: assessment of all-cause mortality, psychiatric readmissions, and treatment resistance

Three outcomes where defined:

a) All-cause mortality: it was ascertained from *The Danish Civil Registration System* (Pedersen, 2011).
b) Treatment resistance: we identified any subsequent use of clozapine or electroconvulsive therapy (ECT) that occurred more than 14 days after the first contact (discharge date for inpatients and contact date for outpatients). We used *The Danish National Prescription Registry* (Wallach Kildemoes et al., 2011) to identify clozapine prescription, the *LABKA* (Grann et al., 2011) research database to identify blood test for clozapine levels, and *The Danish National Patient Registry* to identify ECT procedures (**Table S3** for clozapine and ECT codes).
c) Psychiatric readmissions: we identified any subsequent admission in an inpatient setting at a psychiatric hospital that occurred more than 14 days after the first contact (discharge date for inpatients and contact date for outpatients) using data from *The Danish Psychiatric Central Research Register* (Mors et al., 2011).

2.5. Assessment of baseline characteristics

We obtained information from multiple Danish national registers:
The Danish National Patient Register was used to sum the number of contacts (in- and outpatient and emergency room contacts) at the somatic hospitals within 1 year prior to index date, including the number of days hospitalized (below or above 10 days). We also assessed cardiovascular risk factors defined as the presence of hypertension, diabetes mellitus, dyslipidemia, and/or obesity (see **Table S4** for ICD codes). Finally, we identified individuals with autoimmune disorders or severe

infections diagnosed within one month prior to the blood test and onwards (see Table S5-S6 for ICD codes).

The Psychiatric Central Research Register was used to assess comorbid substance use disorders (see Table S4 for ICD codes).

The Danish Education Registers (Jensen and Rasmussen, 2011) were used to assess highest completed education level (primary school vs. a combined group of higher-level education.

The Danish National Prescription Registry (Wallach Kildemoes et al., 2011) was used to trace anti-inflammatory prescriptions (anti-inflammatory drugs, corticosteroids, and analgesics) filled in the month before the index date (see Table S4 for anti-inflammatory ATC-codes).

2.6. Statistical analysis

Baseline characteristics, including WBC counts, ratios, and CRP levels among different SSDs, were analysed. Categorical variables were compared using Chi-square tests, while continuous variables were assessed using Kruskal-Wallis tests (Wilcoxon rank-sum test). Regarding the risk of the outcome, follow-up began on the index date or the date of the alternate LABKA measure and ended on the date of inpatient admission, treatment resistance (incident use of clozapine or ECT), death, emigration, or December 31, 2016. For individuals hospitalized at their first diagnosis who were only at risk of admission after discharge, their follow-up began on the date of discharge when examining admissions. We utilized Cox proportional hazards regression analysis to compute hazard ratios (HRs). Three adjusted models were used sequentially in an additive manner. The first model included gender, age, and calendar period. The second model added family history of psychiatric disorders and education level of the parents. The third, or fully adjusted model, added cardiovascular risk factors, comorbid substance use disorder, and prior use of anti-inflammatory drugs. The fully adjusted model was used for the reported data except when specified otherwise. Receiver operating characteristic (ROC) curve analysis was used to evaluate the performance of WBC counts, ratios and CRP for mortality. Statistical analyses were conducted using Stata version 13.1 (StataCorp LP, College Station, Texas, USA), and ROC analysis was performed using the *pROC* package in R (Robin et al., 2011). The statistical significance level was set at 0.05 in all analyses.

2.7. Sensitivity analysis

We carried out two sensitivity analyses. First, to reduce misdiagnosis with other psychotic disorders, we restricted analyses to patients with schizophrenia (ICD-10: F20). Secondly, to minimize confounding factors that could influence measured biomarkers at the time of the blood test, we restricted the analysis by excluding patients diagnosed with autoimmune diseases or severe infections within one month prior to the blood test and onwards.

3. Results

3.1. Population characteristics

During the study period, a total of 33.887 individuals with incident SSD were identified. Among all individuals, a total of 6.845 (20.1 %) had a first-time SSD diagnosis of schizophrenia (n = 2.636) or other psychotic disorders (n = 4.209) and a complete WBC counts and/or CRP measurement at baseline. Baseline characteristics among individuals with SSD and baseline measurements are shown in Table 1. Individuals diagnosed with schizophrenia differed from those diagnosed with other psychotic disorders in terms of gender, age at the initial diagnosis, inpatient status at the initial diagnosis, number of somatic and psychiatric contacts in the previous year, use of clozapine, and use of anti-inflammatory treatment in the preceding month (all P<0.05).

Table 1
Baseline characteristics for individuals with schizophrenia spectrum disorder included in the study.

	Total Schizophrenia Spectrum Disorder (N=6845)	Schizophrenia (N=2636)	Other psychotic disorders (N=4209)	p-value
Female, n° (%)	3166 (46.3)	1138 (43.2)	2028 (48.2)	0.0001
Age at index diagnosis, median (IQR), y	26.3 (21.2–34.6)	24.8 (21.2–31.8)	27.9 (21.2–36.3)	0.0001
Primary education as the highest completed level, no. (%)	3348 (48.9)	1288 (48.9)	2060 (48.9)	0.95
Inpatient at index diagnosis, no. (%)	2640 (38.6)	975 (37.0)	1666 (39.6)	0.03
Calendar year at index diagnosis, no. (%)				
1994–1999	1746 (25.5)	700 (26.6)	1046 (24.9)	ref
2000–2004	1776 (26.0)	689 (26.1)	1087 (25.8)	0.43
2005–2009	1927 (28.2)	714 (27.1)	1213 (28.8)	0.06
2010–2016	1396 (20.4)	533 (20.2)	863 (20.5)	0.27
Somatic contacts in the previous year				
– Any contact, no. (%)	1942 (28.4)	1246 (47.3)	696 (16.5)	0.0001
– Admitted more than 10 days, no. (%)	594 (8.7)	367 (13.9)	227 (5.4)	0.0001
Cardiovascular Risk Factors	544 (8.0)	195 (7.4)	349 (8.3)	0.18
– Hypertension disease, no. (%)	128 (1.9)	37 (1.4)	91 (2.2)	0.02
– Diabetes Mellitus, no. (%)	154 (2.3)	54 (2.0)	100 (2.4)	0.37
– Dyslipidaemia, no. (%)	63 (0.9)	18 (0.7)	45 (1.1)	0.10
– Obesity, no. (%)	340 (5.0)	131 (5.0)	209 (5.0)	0.99
Family history of psychiatric disorders				
– None, no. (%)	5594 (81.7)	2166 (82.3)	3425 (81.4)	Ref
– Any, no. (%)	1037 (15.2)	378 (14.3)	659 (15.7)	0.16
– F2, no. (%)	214 (3.1)	89 (3.4)	125 (3.0)	0.41
Psychiatric contacts in the previous year				
– Any contact, no. (%)	2087 (30.5)	1006 (38.2)	1081 (25.7)	0.0001
– Admitted more than 30 days, no. (%)	1433 (20.9)	665 (25.2)	768 (18.3)	0.0001
Substance use disorder, no (%)	2052 (30.0)	775 (29.4)	1277 (30.3)	0.41
Autoimmune disorder and/or severe infection the previous month and onwards, no (%)	3357 (49.0)	1242 (47.1)	2115 (50.2)	0.01
Treatment resistance during follow-up (clozapine or ECT), no. (%)	568 (8.3)	349 (13.2)	219 (5.2)	0.0001
Anti-inflammatory drugs, corticosteroids or analgesics the previous month and onwards, no (%)	188 (2.8)	122 (4.6)	66 (1.6)	0.0001

3.2. WBC counts, ratios and CRP at baseline

Baseline characteristics across WBC counts, ratios and CRP are shown in **Table S7-S16**. At first diagnosis, the median values of WBC counts were 7.2 (interquartile range [IQR]: 6.2–9.9) for leukocytes, 4.1 (IQR: 3.5–6.5) for neutrophils, 0.55 (IQR: 0.42–0.71) for monocytes, 2.1 (IQR: 1.7–2.6) for lymphocytes and 250 (IQR: 220–300) for platelets. The median values of WBC ratios were 3.3 (IQR: 2.7–4.2) for LLR, 1.9 (IQR: 1.4–2.9) for NLR, 0.25 (IQR: 0.19–0.34) for MLR and 122 (IQR: 94–154) for PLR. The median CRP level was 7.0 mg/L (IQR: 2.0–29). No significant differences were observed between schizophrenia and other psychotic disorders in terms of WBC counts, ratios, and CRP levels.

3.3. All-cause mortality

Among the 6.845 individuals with WBC counts and/or CRP measured at baseline, 375 (5.5 %) died during the follow-up period. Rates and hazard ratios (HRs) of all-cause mortality for individuals with SSD are shown in Table 2, **Table S17** and Fig. 1.

Overall, elevated baseline levels of various white blood cell (WBC) counts and ratios were significantly associated with all-cause mortality. Individuals with Q4 levels of leukocyte, neutrophil, and monocyte counts had increased mortality risks, with adjusted hazard ratios (aHRs) of 2.45 (95 % CI: 1.67–3.59), 2.86 (95 % CI: 1.82–4.49), and 2.51 (95 % CI: 1.78–3.54), respectively. Conversely, higher lymphocyte counts were linked to reduced mortality, with aHRs of 0.46 (95 % CI: 0.33–0.63), 0.45 (95 % CI: 0.33–0.62), and 0.51 (95 % CI: 0.39–0.68) for Q2, Q3, and Q4, respectively. Elevated platelet counts in Q2 and Q3 were also associated with lower mortality (aHRs 0.62, 95 % CI: 0.45–0.85 and 0.52, 95 % CI: 0.38–0.72). Higher levels of LLR, NLR and MLR in Q4 indicated increased mortality, with aHRs of 3.61 (95 % CI: 2.39–5.44), 3.28 (95 % CI: 2.19–4.92), and 2.54 (95 % CI: 1.85–3.50), respectively. PLR in Q3 was associated with reduced mortality (aHR 0.55, 95 % CI: 0.37–0.81). Additionally, a composite index of $\geq$ 2 biomarkers in Q3 or Q4 increased mortality risk (aHR 1.94, 95 % CI: 1.48–2.55), and CRP levels > 10 mg/L were linked to higher mortality (aHR 2.32, 95 % CI: 1.59–3.39).

3.3.1. Receiver-operating characteristic (ROC) curve for mortality

Receiver-operating characteristic curve analyses were performed to assess the predictive capabilities of WBC counts, ratios and CRP for mortality in SSD. According to the ROC analysis, the area under the curve (AUC) was as follows in decreasing order: 0.84 (95 % CI 0.82–0.86; Sensitivity 0.76, Specificity 0.78) for CRP; 0.79 (95 % CI 0.77–0.81; Sensitivity 0.76, Specificity 0.69) for leukocytes; 0.79 (95 % CI 0.77–0.82; Sensitivity 0.78, Specificity 0.69) for LLR; 0.78 (95 % CI 0.75–0.81; Sensitivity 0.69, Specificity 0.75) for neutrophils; 0.78 (95 % CI 0.75–0.80; Sensitivity 0.71, Specificity 0.71) for monocytes; 0.78 (95 % CI 0.75–0.81; Sensitivity 0.65, Specificity 0.78) for NLR; 0.78 (95 % CI 0.75–0.80; Sensitivity 0.70, Specificity 0.73) for MLR; 0.78 (95 % CI 0.73–0.80; Sensitivity 0.78, Specificity 0.66) for PLR; 0.77 (95 % CI 0.75–0.80; Sensitivity 0.80, Specificity 0.63) for platelets; and 0.77 (95 % CI 0.75–0.80; Sensitivity 0.80, Specificity 0.62) for lymphocytes (Table 3 and Fig. 2).

3.4. Treatment resistance

Among the 6.845 individuals with WBC counts and/or CRP measured at baseline, 477 (6.9 %) exhibited treatment resistance during the follow-up period. Rates and HRs of treatment resistance for individuals with SSD are shown in Table 2 and **Table S18**.

Elevated baseline leukocyte counts in Q3 and Q4 showed significant associations with increased treatment resistance, with aHRs of 1.58 (95 % CI: 1.18–2.12) and 1.48 (95 % CI: 1.10–1.99), respectively. Neutrophil counts in Q2, Q3 and Q4 were also significantly associated with higher resistance, with aHRs of 1.64 (95 % CI: 1.15–2.32), 1.46 (95 % CI:1.03–2.08), and 1.64 (95 % CI: 1.16–2.32), respectively. Monocyte counts in Q2, Q3, and Q4 also demonstrated associations with aHRs of 1.43 (95 % CI: 1.06–1.93), 1.63 (95 % CI: 1.22–2.18), and 1.50 (95 % CI: 1.11–2.03), respectively. Among WBC ratios, LLR in Q2, Q3 and Q4 were significantly associated with higher treatment resistance, with aHRs of 1.71 (95 % CI: 1.22–2.40), 2.11 (95 % CI: 1.50–2.96) and 1.59 (95 % CI: 1.11–2.26), respectively. NLR and MLR showed a significant association in Q3, with aHRs of 1.49, (95 % CI: 1.07–2.07) and 1.74 (95 % CI: 1.30–2.33), respectively. The composite index of $\geq$ 2 biomarkers in Q3 or Q4 was associated with increased resistance (aHR 1.33, 95 % CI: 1.07–1.67). Non-significant associations were observed for lymphocyte and platelet counts, PLR and CRP.

3.5. Psychiatric readmissions

Among the 6.845 individuals with WBC counts and/or CRP measured at baseline, 1470 (21.5 %) were admitted to a psychiatric hospital during the follow-up period. Rates and HRs of psychiatric readmissions for individuals with SSD are shown in Table 2 and **Table s19**.

Individuals with baseline Q4 levels of platelet counts had a lower risk of psychiatric readmissions, with an aHR of 0.76 (95 % CI: 0.63–0.90). In contrast, LLR in Q4 increased the risk of psychiatric readmission, with an aHR of 1.32 (95 % CI: 1.08–1.61). Non-significant associations were observed for the other WBC counts, ratios, or CRP levels.

3.6. Sensitivity analyses

Sensitivity analyses focusing exclusively on schizophrenia patients (F20) yielded comparable results for mortality, but differences emerged in terms of psychiatric readmissions and treatment resistance (**Table S20**). Regarding mortality, baseline Q2 NLR levels emerged as a significant association (aHR: 2.75, 95 % CI: 1.16–6.50), while Q3 PLR lost its association (aHR: 0.67, 95 % CI: 0.35–1.26). The remaining WBC counts, ratios, and CRP showed similar mortality results to those found in the complete analyses. Concerning treatment resistance, Q4 leukocytes (aHR: 1.49, 95 % CI: 0.99–2.23), Q3 neutrophils (aHR: 1.49, 95 % CI: 0.94–2.38), and Q3 and Q4 monocytes (aHR: 1.49, 95 % CI: 0.99–2.23; aHR: 1.49, 95 % CI: 0.98–2.26, respectively) lost their significant associations. In terms of psychiatric readmissions, Q2 lymphocytes emerged as a significant association (aHR: 0.76, 95 % CI: 0.59–0.99), but Q4 platelets and LLR lost their associations (aHR: 0.77, 95 % CI: 0.58–1.00; aHR: 0.77, 95 % CI: 0.91–1.66, respectively).

When conducting sensitivity analyses for individuals with autoimmune disease or severe infection, we excluded/censored a small number of cases, which prevented us from reporting specific findings regarding this subgroup.

4. Discussion

We conducted the largest longitudinal population-based study to date, assessing WBC counts, ratios, and CRP at the time of the first diagnosis of SSD, and investigated their associations with all-cause mortality, treatment resistance, and psychiatric readmissions. Our findings were as follows: (1) higher baseline leukocytes, neutrophils, monocytes, LLR, NLR, MLR, and CRP were associated with increased all-cause mortality, while lymphocytes, platelets, and PLR decreased the risk; (2) CRP demonstrated the highest predictive value for mortality with a ROC-AUC of 0.84; (3) elevated levels of leukocytes, neutrophils, monocytes, LLR, NLR, and MLR were associated with treatment resistance; (4) higher platelets decreased the risk of psychiatric readmissions, while LLR increased this risk. Our results underscore the need for a greater focus on somatic states among individuals with SSD to prevent premature mortality and highlight the potential of WBC counts, ratios, and CRP in assessing longitudinal prognosis in this population.

Inflammatory activation is associated with higher mortality in the

Table 2
Associations of baseline WBC counts, ratios, and CRP with mortality, treatment resistance, and readmissions in schizophrenia spectrum disorder.

	Mortality			Treatment resistance			Psychiatric readmissions		
	N	Rate[a]	HR[b] (CI)	N	Rate[a]	HR[b] (CI)	N	Rate[a]	HR[b] (CI)
Baseline Leukocyte count									
– Q1	33	0.12	1 (ref)	70	0.28	1 (ref)	310	3.13	1 (ref)
– Q2	53	0.16	1.22 (0.79–1.89)	95	0.34	1.26 (0.92–1.72)	328	3.12	0.99 (0.84–1.15)
– Q3	76	0.21	1.36 (0.90–2.05)	137	0.44	**1.58 (1.18–2.12)**	335	3.06	0.97 (0.83–1.14)
– Q4	177	0.43	**2.45 (1.67–3.59)**	135	0.39	**1.48 (1.10–1.99)**	373	3.20	1.03 (0.89–1.21)
Baseline Neutrophil count									
– Q1	23	0.11	1 (ref)	52	0.29	1 (ref)	179	2.86	1 (ref)
– Q2	39	0.17	1.36 (0.81–2.29)	85	0.43	**1.64 (1.15–2.32)**	202	2.77	1.11 (0.91–1.36)
– Q3	74	0.26	**1.89 (1.18–3.03)**	87	0.37	**1.46 (1.03–2.08)**	212	2.88	1.19 (0.97–1.46)
– Q4	134	0.45	**2.86 (1.82–4.49)**	100	0.42	**1.64 (1.16–2.32)**	229	3.06	1.16 (0.94–1.42)
Baseline Monocyte count									
– Q1	45	0.15	1 (ref)	80	0.31	1 (ref)	264	2.61	1 (ref)
– Q2	52	0.19	1.19 (0.80–1.78)	93	0.40	**1.43 (1.06–1.93)**	251	2.98	1.09 (0.91–1.29)
– Q3	69	0.24	1.37 (0.94–2.01)	114	0.47	**1.63 (1.22–2.18)**	260	3.20	1.15 (0.96–1.36)
– Q4	148	0.52	**2.51 (1.78–3.54)**	98	0.42	**1.50 (1.11–2.03)**	259	4.40	1.15 (0.96–1.37)
Baseline Lymphocyte count									
– Q1	117	0.44	1 (ref)	81	0.35	1 (ref)	270	3.16	1 (ref)
– Q2	55	0.19	**0.46 (0.33–0.63)**	88	0.36	1.01 (0.74–1.36)	266	2.84	0.86 (0.73–1.02)
– Q3	62	0.20	**0.45 (0.33–0.62)**	105	0.40	1.17 (0.87–1.56)	269	2.93	0.87 (0.73–1.03)
– Q4	87	0.26	**0.51 (0.39–0.68)**	119	0.41	1.21 (0.91–1.61)	282	3.17	0.94 (0.79–1.11)
Baseline Platelet count									
– Q1	120	0.38	1 (ref)	100	0.38	1 (ref)	298	3.29	1 (ref)
– Q2	58	0.21	**0.62 (0.45–0.85)**	103	0.43	1.13 (0.86–1.49)	263	2.98	0.90 (0.76–1.07)
– Q3	54	0.17	**0.52 (0.38–0.72)**	99	0.36	0.99 (0.75–1.31)	302	3.05	0.92 (0.78–1.08)
– Q4	96	0.28	0.82 (0.62–1.08)	106	0.36	1.01 (0.76–1.34)	263	2.48	**0.76 (0.63–0.90)**
Baseline LLR									
– Q1	27	0.13	1 (ref)	49	0.26	1 (ref)	174	2.70	1 (ref)
– Q2	50	0.17	1.31 (0.82–2.10)	105	0.42	**1.71 (1.22–2.40)**	257	2.91	1.16 (0.96–1.41)
– Q3	46	0.17	1.21 (0.75–1.95)	110	0.49	**2.11 (1.50–2.96)**	211	2.73	1.18 (0.96–1.45)
– Q4	163	0.56	**3.61 (2.39–5.44)**	91	0.38	**1.59 (1.11–2.26)**	257	3.13	**1.32 (1.08–1.61)**
Baseline NLR									
– Q1	29	0.14	1 (ref)	59	0.32	1 (ref)	176	2.91	1 (ref)
– Q2	48	0.18	1.38 (0.87–2.19)	86	0.39	1.26 (0.90–1.76)	213	2.77	1.01 (0.83–1.24)
– Q3	53	0.20	1.38 (0.88–2.18)	94	0.43	**1.49 (1.07–2.07)**	190	2.63	1.04 (0.84–1.28)
– Q4	136	0.53	**3.28 (2.19–4.92)**	79	0.38	1.24 (0.88–1.74)	228	3.16	1.18 (0.97–1.45)
Baseline MLR									
– Q1	55	0.19	1 (ref)	82	0.32	1 (ref)	257	2.90	1 (ref)
– Q2	54	0.18	0.94 (0.64–1.37)	91	0.36	1.21 (0.90–1.64)	254	2.71	0.94 (0.79–1.12)
– Q3	51	0.20	0.99 (0.68–1.46)	108	0.50	**1.74 (1.30–2.33)**	243	3.12	1.07 (0.90–1.28)
– Q4	141	0.63	**2.54 (1.85–3.50)**	75	0.39	1.33 (0.97–1.83)	229	3.55	1.14 (0.95–1.37)
Baseline PLR									
– Q1	100	0.38	1 (ref)	78	0.37	1 (ref)	209	3.38	1 (ref)
– Q2	51	0.21	0.72 (0.51–1.01)	89	0.45	1.11 (0.82–1.51)	204	2.86	0.92 (0.75–1.11)
– Q3	34	0.16	**0.55 (0.37–0.81)**	78	0.44	1.08 (0.79–1.49)	164	2.54	0.81 (0.66–1.00)
– Q4	91	0.38	1.23 (0.92–1.65)	77	0.37	0.95 (0.69–1.31)	245	3.19	1.10 (0.91–1.33)
Composite index									
<2 indexes Q3 or Q4	72	0.16	1 (ref)	130	0.34	1 (ref)	389	2.96	1 (ref)
≥2 indexes Q3 or Q4	205	0.36	**1.94 (1.48–2.55)**	202	0.43	**1.33 (1.07–1.67)**	479	2.94	1.10 (0.96–1.27)
Baseline CPR									
<1 mg/L	35	0.15	1 (ref)	11	0.19	1 (ref)	72	3.46	1 (ref)
1- <3 mg/L				49	0.32	1.76 (0.91–3.40)	170	3.23	1.05 (0.79–1.39)
3–10 mg/L	35	0.17	1.03 (0.66–1.61)	79	0.32	1.47 (0.77–2.80)	240	3.03	0.93 (0.71–1.23)
>10 mg/L	245	0.40	**2.32 (1.59–3.39)**	177	0.34	1.25 (0.66–2.37)	486	2.84	0.83 (0.63–1.09)

Bold values denote statistical significance.
Abbreviations: LLR=Leukocyte-to-Lymphocyte ratio; NLR=Neutrophil-to-lymphocyte ratio; MLR=Monocyte-to-Lymphocyte ratio; PLR=Platelet-to-Lymphocyte ratio; CRP=C-Reactive Protein; Q=quartiles (Q1 or lower quartile, Q4 or upper quartile); HR=hazard ratio; CI=confidence interval.
[a] Rate /10000 person-years;
[b] Adjusted Model 3: sex, age, calendar period, family history of psychiatric disorders, education level of the parents, cardiovascular risk factors, comorbid substance use disorder, and prior use of anti-inflammatory drugs.

general population (Proctor et al., 2015). Some markers of the inflammatory response have demonstrated independent prognostic value in patients with a variety of medical conditions. In SSD, there is a higher risk of premature mortality compared to the general population, mainly due to cardiovascular causes (Plana-Ripoll et al., 2022). We previously demonstrated that higher CRP levels at first diagnosis of schizophrenia are associated with increased mortality, particularly among individuals with somatic comorbidity (Horsdal et al., 2017a). In this new study, we analysed WBC counts and ratios and found that leukocytes, neutrophils, monocytes, LLR, NLR and MLR are also associated with all-cause mortality. These findings align with previous studies using WBC counts and ratios in the general population (Abete et al., 2019; Chan et al., 2022; Fest et al., 2019; Park et al., 2023; Shah et al., 2017; (Song et al., 2021)) and other medical conditions (de Jager et al., 2010; Lattanzi et al., 2019; Templeton et al., 2014). Additionally, we evaluated the predictive abilities of WBC counts, ratios and CRP for mortality. Our findings indicate that CRP exhibits the highest predictive accuracy for mortality, with an AUC of 0.84. Consequently, we affirm the independent association between inflammation and mortality and highlight CRP as a predictor when assessing this relationship in SSD. Interestingly, we observed that lymphocytes, platelets, and PLR were associated with a lower risk of mortality. There are inconsistencies in the literature

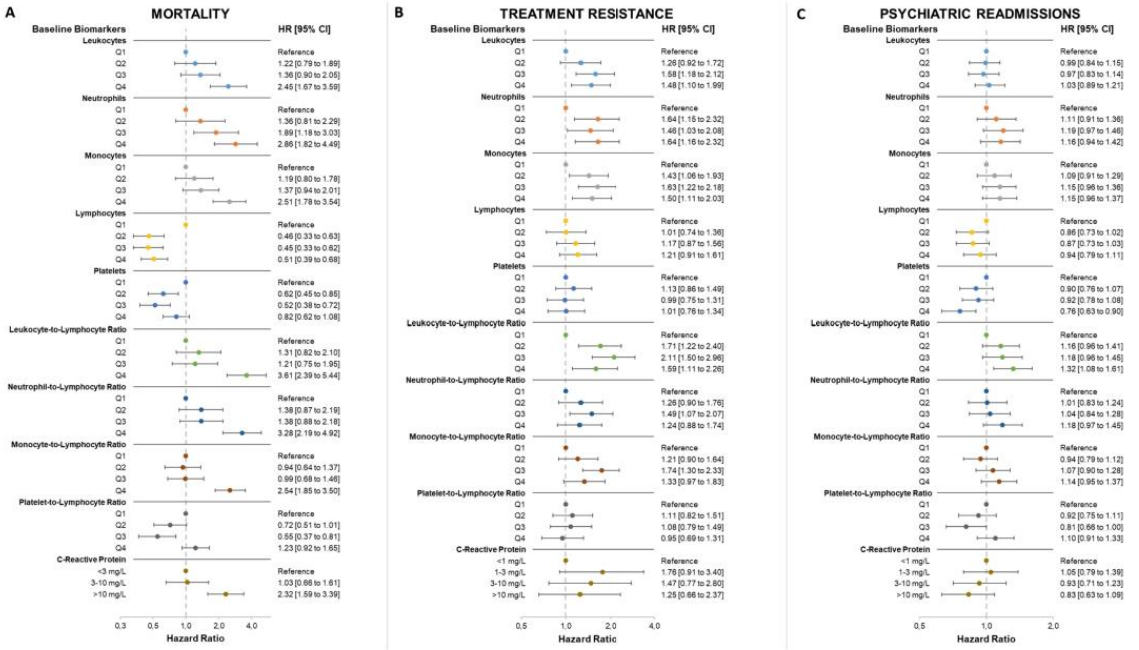

Fig. 1. Adjusted hazard ratios for mortality (A), treatment resistance (B) and psychiatric readmissions.

Table 3
Receiver-operating characteristic (ROC) curve of WBC counts, ratios and CRP for mortality in schizophrenia spectrum disorder.

Variables	AUC	95 % CI	Threshold	Sensitivity	Specificity	Precision
Leukocyte count	0.79	(0.77–0.81)	0.05	0.76	0.69	0.12
Neutrophil count	0.78	(0.75–0.81)	0.07	0.69	0.75	0.16
Monocyte count	0.78	(0.75–0.80)	0.06	0.71	0.71	0.12
Thrombocyte count	0.77	(0.75–0.80)	0.05	0.80	0.63	0.11
Lymphocyte count	0.77	(0.75–0.80)	0.05	0.80	0.62	0.11
LLR	0.79	(0.77–0.82)	0.05	0.78	0.69	0.11
NLR	0.78	(0.75–0.81)	0.08	0.65	0.78	0.16
MLR	0.78	(0.75–0.80)	0.06	0.70	0.73	0.13
PLR	0.78	(0.73–0.80)	0.05	0.78	0.66	0.12
CRP	0.84	(0.82–0.86)	0.07	0.76	0.78	0.18

Abbreviations: LLR=Leukocyte-to-Lymphocyte ratio; NLR=Neutrophil-to-lymphocyte ratio; PLR=Platelet-to-Lymphocyte ratio; MLR=Monocyte-to-Lymphocyte ratio; CRP=C-Reactive Protein; AUC=Area Under the Curve.

regarding their predictive value. Some studies report that higher lymphocyte counts are linked to increased mortality (Abete et al., 2019), while others suggest a connection with lower lymphocyte counts (Izaks et al., 2003). Similarly, the association between higher platelet counts and mortality has been noted in some studies within the general population, but not all studies replicate this finding (Izzi et al., 2018). However, our findings are novel because these associations have not been previously investigated in schizophrenia, suggesting a need for further investigation in the field.

Treatment resistance is not a homogeneous subgroup within SSD, and different clinical and neurobiological pathways may be involved in the condition (Potkin et al., 2020). Hypotheses suggest that inflammation at an early age followed by chronic low-grade activation contributes to treatment resistance (Ioannou et al., 2021). Osimo and collaborators showed that elevated monocyte and lymphocyte counts at FEP were linked to poor clinical outcomes, defined as continued involvement in secondary mental health services, even after adjusting for four confounders (Osimo et al., 2021b). Additionally, they developed a machine-learning model to forecast the 8-year likelihood of treatment resistance (defined by clozapine use) starting from FEP. This model integrated various analytical parameters, including neutrophils and lymphocytes, achieving an AUC of approximately 0.67 (Osimo et al., 2023). In our study, lymphocytes were not associated with treatment resistance, but we found associations with a high number of immune cells and ratios (leukocytes, neutrophils, monocytes, LLR, NLR, and MLR), reinforcing the idea of an involvement of the immune system in treatment resistance. The observed differences in lymphocyte levels might be due to varying distributions of inflammatory markers among ethnic groups and the impact of comorbidities (Coates et al., 2020; Lang et al., 2021). A combined measure of inflammatory markers has been suggested to better explain these findings and WBC ratios may be a good choice to detect imbalances between the innate and adaptive immune pathways. In this line, Bioque and collaborators found that in FEP psychosis patients, those who did not meet remission criteria at the two-year follow-up had significantly higher NLR than the group in remission (Bioque et al., 2022). Our results align with this, indicating that not only NLR,

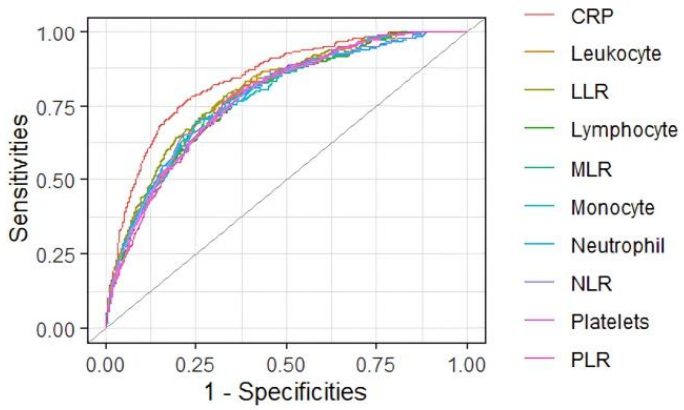

Fig. 2. ROC curve of WBC counts, ratios and CRP for mortality in schizophrenia spectrum disorder. **Abbreviations:** LLR=Leukocyte-to-Lymphocyte ratio; NLR=Neutrophil-to-lymphocyte ratio; MLR=Monocyte-to-Lymphocyte ratio; PLR=Platelet-to-Lymphocyte ratio; CRP=C-Reactive Protein.

but also LLR and MLR, are associated with long-term treatment resistance. In terms of CRP, Nettis and collaborators found that higher CRP in combination with other metabolic parameters were associated with poor clinical response at 1-year follow-up after a FEP (Nettis et al., 2019), and Fond and collaborators showed that CRP levels were associated with ultra-resistant schizophrenia (Fond et al., 2019). However, these findings were not replicated in our group when we analysed first-episode schizophrenia patients who later developed treatment resistance (Horsdal et al., 2017b). In the current study, which includes a larger sample size, we again found no association, replicating our previous results.

Several factors have been reported to contribute to readmission in SSD (Mi et al., 2020). However, no single inflammatory biomarker has demonstrated a consistent association with psychiatric readmissions (Brinn and Stone, 2020; (Horsdal et al., 2017a,b; Llorca-Bofí et al., 2024)). Surprisingly, in our study, higher platelet levels decreased the risk of psychiatric readmissions, while lymphocyte-to-leukocyte ratio (LLR) increased this risk in the whole SSD population; however, these results were not maintained when analysing schizophrenia patients only. Our results on platelet associations contrast with those reported by Osimo and collaborators (2021b), who found that higher platelet values were associated with worse psychiatric outcomes in a cohort of FEP patients, although this association was lost after adjusting for confounders. In contrast, our study shows that higher platelet levels lower the risk of psychiatric readmission. This discrepancy may be due to different study populations (established SSD vs. FEP) and outcome definitions (readmission vs. involvement in secondary mental health services). Future investigations should replicate these results and further explore the role of platelets in SSD (Asor and Ben-Shachar, 2012). Finally, LLR has not been previously investigated in relation to longitudinal outcomes in SSD (Mazza et al., 2020), and therefore, this result has not been previously reported. It is essential to replicate this association, as it may be a chance finding.

Strengths and limitations

A major strength of this study is its size and comprehensiveness: it is a population-based register study of up to 22 years' follow-up of all adult and incident SSD patients in a whole region of Denmark, with very little missing data. The use of these registries minimized the risk of both selection and information bias with a high validity of the diagnoses used to identify the study population (Uggerby et al., 2013). Another advantage is the ability to adjust for a range of possible confounding variables collected independently of the blood tests through linking Danish health care registries and databases (Jensen and Rasmussen, 2011; Lynge et al., 2011; Mors et al., 2011; Pedersen, 2011; Petersson et al., 2011; Wallach Kildemoes et al., 2011).

Some limitations should be considered when interpreting our results. First, a potential weakness of our study is that it was not possible to include a healthy control group since everyone in the clinical laboratory information system had been in contact with a hospital or general practitioner due to suspected illness. Second, we only included individuals who had a WBC counts and/or CRP measurement within ± 30 days of the initial SSD diagnosis, whereby we may have introduced selection bias as there might be indications for the blood testing beyond monitoring in SSD, thus limiting generalizability. To further understand the potential impact of selection bias, we explored the differences between patients included and not included in the study (**Table S21**). We found higher proportions of female patients, individuals with higher education levels, as well as higher somatic and psychiatric contacts in the previous year among those included. No differences were found in terms of age, cardiovascular risk factors, or diagnosis. Third, due to the lack of information on symptom severity, we could not use the TRIPP criteria for the definition of treatment resistance (Howes et al., 2017). Notwithstanding, we defined treatment resistance as the use of clozapine and/or ECT, which are two of the strategies proposed by the TRIPP consensus for this type of patient (Kane et al., 2019). Fourth, due to the register-based origin of the blood sample data, factors related with the blood sampling that are known to modify immune cells such as previous fasting, sleep disturbance or timing of the procedure were not available (Sandberg et al., 2021; Villar et al., 2023). Fifth, blood tests were not analysed at the same laboratory. Therefore, despite regular quality control and calibration for high precision in all laboratories, potential inter-laboratory differences in the measurements cannot be ruled out. Finally, we did not have information on factors such as smoking, weight, nutrition, and physical activity, which can influence inflammatory processes in the body (Howard et al., 2019).

5. Conclusions

Elevated levels of WBC counts, ratios, and CRP at the first diagnosis of SSD increased the risk of long-term mortality, except for lymphocytes, platelets, and PLR, which reduce the risk. CRP exhibited the highest predictive value for mortality. Treatment resistance was associated with elevated levels of leukocytes, neutrophils, monocytes, LLR, NLR, and MLR. Higher levels of platelets decreased the risk of psychiatric readmissions, while LLR increased this risk. Consequently, WBC counts, ratios, and CRP at the first diagnosis of SSD demonstrated long-term predictive potential.

CRediT authorship contribution statement

Vicent Llorca-Bofí: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Liselotte Vogdrup Petersen:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Preben Bo Mortensen:** Writing – review & editing, Supervision. **Michael E Benros:** .

Declaration of Competing Interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.07.041.

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4.7. Article VII

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Association between neutrophil to lymphocyte ratio and inflammatory biomarkers in patients with a first episode of psychosis

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ABSTRACT

Neutrophil to lymphocyte ratio (NLR) has been proposed as an emerging marker of the immune system alterations in psychotic disorders. However, it is not entirely clear whether NLR elevation is a characteristic of the psychotic disorder itself, which inflammatory pathways activation is detecting, or which possible confounding variables could alter its interpretation.

We aimed to analyze the relationship of NLR values with a panel of inflammatory and oxidative/nitrosative stress biomarkers and main potential confounding factors in a well-characterized cohort of 97 patients with a first episode of psychosis (FEP) and 77 matched healthy controls (HC).

In the FEP group, NLR values presented a moderate, positive correlation with the pro-inflammatory mediator Prostaglandin E_2 levels ($r = 0.36$, $p < 0.001$) and a small but significant, positive correlation with cannabis use ($r = 0.25$, $p = 0.017$). After controlling for cannabis use, the association between NLR and PGE_2 remained significant (beta = 0.31, $p = 0.012$). In the HC group, NLR values negatively correlated with body mass index (BMI, $r = -0.24$, $p = 0.035$) and positively correlated with tobacco use ($r = 0.25$, $p = 0.031$).

These findings support a relationship between the elevation of NLR values and an elevated expression of proinflammatory pathways related to stress response in patients with a FEP. In addition, our study highlights the importance of considering variables such as cannabis or tobacco consumption, and BMI when interpreting the results of studies aimed to establish a clinical use of NLR. These considerations may help future research to use NLR as a reliable biomarker to determine immune system status in this population.

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1. Introduction

Despite significant advances in the field, many aspects of the pathophysiology of schizophrenia and related psychotic disorders remain unknown (Jauhar et al., 2022). An accumulation of scientific evidence supports the hypothesis that several processes involving inflammatory pathways and consequent oxidative/nitrosative stress are implicated, pointing to a persistent low-grade, pro-inflammatory state (Jauhar et al., 2022; Leza et al., 2015). This state appears both centrally and peripherally, starting from the initial phases of psychotic disorders (Fraguas et al., 2019; García-Bueno et al., 2014).

Neutrophil to lymphocyte ratio (NLR), calculated as a simple ratio between the neutrophil and lymphocyte counts measured in peripheral blood, has emerged as a potential marker of the intricate interplay between the immune system and a vast spectrum of diseases, including cancer, infections, and cardiovascular diseases (Buonacera et al., 2022; Li et al., 2018). Its accessibility and low cost make it an attractive option. Furthermore, it conjugates two faces of the immune system: the innate immune response, mainly due to neutrophils, and adaptive immunity, supported by lymphocytes (Buonacera et al., 2022; Song et al., 2021).

Two metanalysis have showed increased NLR values both in first episode of psychosis (FEP) and in multi-episodic schizophrenia (Karageorgiou et al., 2019; Mazza et al., 2020). Our group replicated these results in a FEP cohort, revealing that NLR differences between cases and control subjects increased over a 24-month follow-up period (Bioque et al., 2022). Notably, higher NLR values were associated to not achieving remission by the end of the study. A different metanalysis reported that neutrophils and monocytes were significantly increased in subjects with a FEP compared with controls (Jackson and Miller, 2020). Interestingly, we have also reported a connection between this systemic parameter and structural brain changes, associating isolated neutrophil count with reduced grey matter and enlarged ventricles in FEP (Núñez et al., 2019).

However, it is not entirely clear whether NLR elevation represents a primary characteristic of the psychotic disorder itself, or if treatment or metabolic changes could influence this value, or its relation with specific inflammatory pathways (Karageorgiou et al., 2019). Moreover, several major variables and conditions could determine a "false" increase in NLR values, including age, obesity, infections, diabetes, and emotional stress among others (Buonacera et al., 2022). Thus, the FEP population is of great interest since it could mitigate the impact of some of these confounding variables (Bernardo et al., 2013).

In this context, the current study aims to analyze the relationship of NLR values with a panel of inflammatory and oxidative/nitrosative stress biomarkers and main potential confounding factors in a well-characterized cohort of patients with a FEP and a matched control group.

2. Subjects and methods

2.1. Subjects

Participants in this study came from the Flamm-PEPs project, a multicentre study performed within the Spanish Network for Mental Health Research (CIBERSAM). The patient inclusion/exclusion criteria and the clinical protocol were published previously (Bernardo et al., 2013; García-Bueno et al., 2014). Briefly, patients were aged 9–35 years old, presented a FEP of at least 1 week of duration in the previous 12 months and spoke Spanish correctly. Patients with mental retardation, history of head injury with loss of consciousness or ongoing infections, fever, allergies, or the presence of other serious medical conditions (autoimmune, cardiac, pulmonary, endocrine, chronic infectious diseases, cancer) were excluded.

Healthy controls (HC) were matched by age (±10%), sex and parental socio-economic status, measured by the Hollingshead-Redlich scale (±1 level) (Hollingshead and Redlich, 1958). The exclusion criteria of control subjects were the same as for patients plus having a personal history of psychotic and/or major affective disorder and/or having a first-degree relative with history of psychotic disorders.

The local ethics committee of each site approved the study, and it was obtained an informed consent from all participants or from parents/legal keeper in under 18-year-old subjects.

2.2. Diagnostic, demographic and clinical data collection

Diagnostics were confirmed using the Spanish translation of the Kiddie-SADS-Present and Lifetime Version for children and adolescents (Kaufman et al., 1997), and the Spanish translation of Structured Clinical Interview for DSM Disorders (SCID-I & II) for adults (First et al., 1996). Psychotic symptoms were assessed using the validated Spanish version of the Positive and Negative Symptom Scale (PANSS) (Peralta and Cuesta, 1994).

Clinical assessment included a complete medical history and physical examination, with body mass index (BMI = weight in kg/height in m^2) determination. Cannabis and tobacco use was individually evaluated with the European Adaptation of a Multidimensional Assessment Instrument for Drug and Alcohol Dependence (Kokkevi and Hartgers, 1995). A systematic recording of drug misuse habits was performed, also interviewing the families and caregivers, consulting medical records and urine toxicology, when available.

The number of traumatic experiences of participants was self-reported from the list of events that appear in the Traumatic Experiences in Psychiatric Outpatients Questionary (TQ) (Davidson and Smith, 1990).

To compare antipsychotic doses in the statistical analyses, the prescribed daily doses were converted to an equivalent amount of chlorpromazine (CPZ) following the international consensus (Gardner et al., 2010).

2.3. Specimen collection and preparation

After overnight fasting, 10 ml of venous blood samples were collected between e8:00 and 10:00 a.m. in K2EDTA BD Vacutainer® EDTA tubes (Becton Dickinson®). Samples were sent to each site routine laboratory for analysis. Blood cell counts and glucose levels were directly analyzed by enzymatic procedures with an automatic chemical analyser in each participating site. The reference values at each site were recorded in a common database called GRIDSAM, where individual values were homogenized (Bioque et al., 2018).

Part of the blood was centrifuged at 641 g for 10 min at 4 °C and the resulting plasma was stored at −80 °C until use. The rest of the sample was diluted in culture medium (RPMI 1640, Invitrogen®), and a gradient with Ficoll-Paque® (GE Healthcare®) was used to isolate peripheral blood mononuclear cells (PBMC) by centrifugation (800 g × 40 min). PBMC layer was aspired and resuspended in RPMI and centrifuged (1116 g × 10 min). The supernatant was removed, and the mononuclear cell enriched pellet was also stored at −80 °C.

2.3.1. Biochemical determinations methods in plasma

- Prostaglandins: Cyclooxygenase (COX) by-products PGE_2 and 15 d-PGJ_2 were determined by enzyme immunoassay (Cayman Chemicals® and DRG® Diagnostics, respectively).
- Nitrites (NO_2^-), the final and stable product of NO, were determined by using the Griess method.
- Lipid peroxidation was determined by Thiobarbituric Acid Reactive Substances (TBARS) assay (Cayman Chemicals®).
- Homocysteine was quantified using an ELISA kit (Cloud-Clone®) following the manufacturer's instructions. Absorbance was measured at 450 nm (Synergy 2, Biotek®).

2.3.2. Biochemical determinations methods in PBMC

PBMC samples were first fractionated in cytosolic and nuclear extracts. Determination of pro-inflammatory p65 NFκB subunit and anti-inflammatory PPARγ respective transcriptional activities were carried out in nuclear extracts from PMBC:

- Nuclear factor kappa B (NFκB) activity: Activation of NFκB occurs by enzymatic degradation of the bound inhibitory protein (IκBα), allowing movement from cytoplasm to the nucleus where they bind to consensus κB sequences in DNA. The presence of p65 subunit in cell nuclei is considered an index of activity measured with a transcription factor assay (Cayman Chemicals®).
- PPARγ Transcription Factor activity was determined using ELISA-based kits (Cayman Chemicals®).
- Western Blot (WB) Analysis: protein levels of IκBα, COX2 and iNOS were quantified by WB in cytosolic extracts from PBMC. Protein levels of PPARγ were quantified in nuclear extracts. The house keeping gene β-actin was used as loading control. Nuclear factor SP1 was used as loading control for PPARγ.

2.4. Statistical analysis

Continuous data are expressed as a mean ± standard deviation and categorical data are expressed as absolute values and percentages. The normality of continuous variables was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests, and the equality of the variance between groups was assessed using Levene's test. A two-tailed Chi-square test was used to assess differences in categorical variables and a two-tailed *t*-test was used to assess differences on continuous variables with approximately normal distributions. The Mann-Whitney U was used to assess non-parametric variables.

The relationship between NLR with inflammatory biomarkers, possible confounders (age, BMI, fasting glucose, tobacco use, cannabis use, antipsychotic dose), and symptomatology (measured by means of the PANSS total score and the PANSS anxiety item score) were investigated using a Pearson product moment correlation coefficient. Hierarchical multiple regression was used to assess the ability of correlated biomarkers levels to predict NLR values, after controlling for potential significant confounding factors.

Being these analyses preliminary and exploratory, corrections were not made for multiple comparisons (Bender and Lange, 2001).

Two-tailed p-values <0.05 were considered to be of statistical significance. Statistical analyses were performed using IBM-SPSS v.29®.

3. Results

Ninety-seven subjects with a FEP and seventy-seven HC were included in the present analysis. We excluded 20 cases and 29 HC from the original sample with no blood cell count data available. Demographic, clinical characteristics and biomarkers determinations are presented in Table 1.

There were no differences between cases and HC in the matching variables or BMI, so the differences found did not result from an inadequate case-control group matching.

As expected from previous analysis, there were significant differences between cases and controls in NLR, increased proinflammatory and oxidative/nitrosative stress markers determinations (NFκB, PGE_2, iNOS, COX2, and TBARS) and tobacco use, and decreased production of various components of antiinflammatory pathway (IκBα, $15dPGJ_2$ and PPARγ).

In the correlation analyses between NLR and different biomarkers, we found a moderate, positive correlation between NLR and PGE_2 levels ($r = 0.36$, $n = 83$, $p < 0.001$) in FEP participants. In the HC group, no correlation was found between NLR values, and the biomarkers analyzed.

The relationship between NLR and possible confounders and symptomatology was also investigated using the same method. In the group of cases, we found a small but significant, positive correlation between cannabis use and NLR ($r = 0.25$, $n = 89$, $p = 0.017$) with more cannabis use associated with high NLR values. This relationship was not found in the control group. However, NLR values in the HC group were found to be negatively correlated with BMI ($-r = 0.24$, $n = 74$, $p = 0.035$) and positively correlated with tobacco use ($r = 0.25$, $n = 76$, $p = 0.031$).

Age was not correlated with any of the biomarkers analyzed, nor were differences found in mean NLR values between underage patients vs. adults ($t = -1.27$, $p = 0.21$). No significant correlation was neither found between the number of traumatic experiences and the

Table 1
Demographic, clinical characteristics, and biological markers.

	FEP (n = 97)	CONTROL (n = 77)	Statistic	p-value
Gender (female) – no. (%)	30 (31%)	29 (38%)	X^2 = 0.87	0.35
Age (years) - mean (sd)	24 ± 6,03	25.39 ± 6.87	t = −1.42	0.15
Body mass index (kg/m^2) - mean (sd)	24.02 ± 3.8	23.10 ± 3.21	t = 1.67	0.09
Glucose (mg/dL) - mean (sd)	84.11 ± 9.89	84.1 ± 7.86	U = 3868	0.59
Tobacco use (cigarettes per month)	**211.38 ± 256.37**	**47.95 ± 122.94**	U = 2283	< **0.001**[a]
Cannabis use (cigarettes per month)	6.01 ± 23.34	1.13 ± 6.64	U = 3545.5	0.42
Traumatic experiences - mean (sd)	1.63 ± 2.62	1.28 ± 2.09	t = 0.93	0.36
Antipsychotic Chlorpromazine equivalent dose (mg/d) – mean (sd)	581.23 ± 386.55	–	–	–
PANSS Total score	66.44 ± 26.39	–	–	–
PANSS anxiety item score	2.53 ± 1.38	–	–	–
NLR	**2.06 ± 1.44**	**1.68 ± 0.6**	t = 2.23	**0.028**[a]
NFκB -act-	**13.31 ± 23.72**	**4.17 ± 3.25**	U = 423	**0.004**[a]
iNOS -WBc-	**130.85 ± 51.9**	**94.45 ± 37.97**	U = 1311	< **0.001**[a]
COX2 -WBc-	**139.11 ± 100.24**	**106.03 ± 66.67**	U = 1611	**0.006**[a]
NO^-_2 -sol-	14.37 ± 6.16	12.73 ± 4.77	U = 919	0,28
PGE_2 -sol-	**527.24 ± 775.38**	**296.46 ± 226.96**	U = 2734.5	**0.028**[a]
TBARS -sol-	**3.41 ± 3.71**	**2.27 ± 2.47**	U = 2535.5	**0.026**[a]
Hcy -sol-	17.05 ± 18.42	9.33 ± 5.58	U = 842	0.056
IκBα -WBc-	**83.73 ± 49.74**	**105.89 ± 50.83**	U = 2936	**0.003**[a]
$15dPGJ_2$ -sol-	**589.97 ± 158.01**	**650.21 ± 160.76**	U = 3915.5	**0.040**[a]
PPARy – WBn-	**77.84 ± 34.24**	**106.18 ± 20.82**	t = −2.39	**0.026**[a]
PPARy -act-	**1.31 ± 0.99**	**1.67 ± 1.09**	U = 2389.5	**0.019**[a]

15 d-PGJ_2: Prostaglandin 15-deoxy-PGJ_2; COX2: Cyclooxygenase type 2; IκBα: Inhibitor of nuclear factor kappa-B kinase, subunit alpha; iNOS: Inducible nitric oxide synthase; NLR: Neutrophil to lymphocyte ratio; NFκB: Nuclear transcription factor κB; NO_2^-: Nitrogen dioxide; PANSS: Positive and Negative Syndrome Scale; PGE_2: Prostaglandin E_2; PPARγ: Peroxisome proliferator activated receptors, gamma isoform; TBARS: Thiobarbituric Acid Reactive Substances (TBARS); WB: Western-Blott.
Analyses carried out in WB: protein expression, determined by western blot in PBMC (WBc: in cytoplasmatic fraction; WBn: in nuclear fraction); sol: plasma levels of soluble compounds; act: activity assay in nuclear extracts.
[a] p-value <0,05. The **bold values** in the table represent the values reaching statistical significance (**p-value < 0,05**).

inflammatory biomarkers analyzed.

3.1. NLR predictive model in FEP subjects

A hierarchical multiple regression assessed the ability of PGE_2 levels to predict NLR levels in the group of FEP patients, after controlling for cannabis use. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. No major deviations from normality were detected in the Normal Probability Plot (P–P) of the regression standardized residual. The scatterplot of the standardized residuals showed an adequate distribution to dismiss a violation of the linearity and homoscedasticity assumption, also allowing to discard the presence of outliers. Multicollinearity was checked through a Pearson correlation test between the dependent variable (NLR values) and the independent variables (PGE2 levels, $r = 0.36$, $p < 0.001$; cannabis use, $r = 0.25$, $p = 0.017$), showing that bivariate correlation was in all cases <0.7.

Monthly cannabis use was entered at Step 1, explaining 6% of the variance in NLR. After entry of the PGE_2 levels at Step 2, the total variance explained by the model was 13.5%, $F(2,80) = 6.23$, $p = 0.003$. PGE_2 measures explained an additional 7% of the variance in NLR values, after controlling for cannabis use, R squared change = 0.071, F change $(1,80) = 6.58$, $p = 0.012$. In the final model, only PGE_2 levels were statistically significant (beta = 0.31, $p = 0.012$).

4. Discussion

In this case-control study in FEP patients analyzing the relationship between NLR values with a panel of inflammatory and oxidative/nitrosative stress biomarkers and main potential confounding factors, we found: (i) In the FEP group, NLR values presented a moderate, positive correlation with the levels of the pro-inflammatory prostaglandin PGE_2, and a small but significant, positive correlation with cannabis use; (ii) After controlling for cannabis use, the association between NLR and PGE_2 remained significant; and (iii) In the HC group, NLR values negatively correlated with BMI and positively correlated with tobacco use.

NLR is a cheap and easy-to-obtain biomarker that mirrors the balance between innate and adaptive immunity (Buonacera et al., 2022). Although no fixed cut-off values are available, changes in NLR over time are considered a sign of immune system dysregulation in several medical conditions, either acute or chronic. To incorporate NLR determinations into clinical practice, it is necessary to understand which processes of the inflammatory and/or immunological response can be reflected by this parameter. This would allow us, for example, to identify which patients or at what stages of their psychotic disorder could benefit from interventions on the immune system or inflammatory response (i.e. coadjuvant antiinflammatory treatment, use of certain antipsychotics). In this sense, the positive correlation between the values of NLR and PGE_2 found in our study reflects an activation of a nuclear proinflammatory pathway of stress response in the subjects suffering a FEP (García-Bueno et al., 2014). PGE_2 contributes to the inflammatory response to stress binding to G-coupled protein receptors and, in terms of immunity, prostaglandins regulate lymphocyte function (Furuyashiki and Narumiya, 2011). Moreover, most studies suggest that PGE_2 overexpression impairs the function of innate phagocytes such as macrophages and neutrophils and limits Th1 immune responses (Martínez-Colón and Moore, 2018). Besides, it is worth considering the preliminary nature of these results, as well as the fact that the total variance explained by the model was 13.5%, a moderate proportion when analysing the relationships between biomarkers.

Interestingly, cannabis use was also associated with an increase in NLR values, indicating the role of cannabis and the dysregulation of the endocannabinoid system as a mediator of the inflammatory response in patients with a FEP (Bioque et al., 2013).

Another relevant conclusion of our results, with implications for interpreting NLR values in future studies of patients with psychosis and controls, is the importance of controlling for possible confounding variables, such as BMI and tobacco consumption. These factors, which had already been identified in previous studies with the general population (Buonacera et al., 2022), are highly prevalent in the population with psychotic disorders, especially in chronic phases.

These findings must be seen considering some limitations. Firstly, all these biomarkers were determined from peripheral blood sample, which might not translate directly to changes within the central nervous system. Secondly, although the inflammatory biomarkers analyzed in this study reflect the signal of a canonical stress response pathway, association with other more widely used inflammatory biomarkers (for example, certain interleukins or c-reactive protein) would have also been very informative (Llorca-Bofí et al., 2023). Thirdly, patients could be included in the study being under antipsychotic treatment, which could be affecting the expression of certain biomarkers in some cases. For those reasons, correlation analyses were controlled for antipsychotic doses. Finally, subjects in whom the main diagnosis was that of a substance-induced psychotic episode were not included in the study. For this reason, the percentage of patients who had consumed substances other than cannabis and/or nicotine was very low (less than 5%), not allowing us to include these variables in the statistical plan designed for this study. Studies with much larger samples are needed to help answer the question of how other abuse substances influence inflammatory biomarkers.

On the other hand, this study also has several strengths: (i) strict inclusion/exclusion criteria and case-control matching variables were established; (ii) diagnostic evaluations were made with a very comprehensive protocol, including participants with a wide age range, mirroring the natural history of psychotic disorders; (iii) the inclusion of FEP participants mitigate the impact of confounding variables such as comorbidities, long treatment exposure or chronicity; and (iv) blood analyses were taken following a strict, unified protocol, which is especially relevant to avoid leukocyte levels fluctuations throughout the day.

In conclusion, our findings underscore a significant association between the elevation of NLR values and an elevated expression of proinflammatory pathways related to stress response in patients with a FEP. In addition, our study highlights the importance of considering variables such as cannabis consumption, smoking, and BMI when interpreting the results of studies that seek to establish a clinical utility of NLR. These considerations may help future research establish NLR as a valuable biomarker for assessing the immune system status and inflammatory response in this population.

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CRediT authorship contribution statement

Miquel Bioque: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Vicent Llorca-Bofí:** Writing – review & editing. **Sergi Salmerón:** Writing – review & editing. **Borja García-Bueno:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Karina S. MacDowell:** Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Carmen Moreno:** Writing – review & editing. **Pilar A. Sáiz:** Writing – review & editing. **Ana González-Pinto:** Writing – review & editing. **María Hidalgo-Figueroa:** Writing – review & editing. **María Fe Barcones:** Writing – review & editing. **Roberto Rodriguez-Jimenez:** Writing – review & editing. **Miquel Bernardo:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing –

review & editing. **Juan Carlos Leza:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

M Bioque has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board of has received honoraria from talks and/or consultancy of Adamed, Angelini, Casen-Recordati, Exeltis, Ferrer, Janssen, Lundbeck, Neuraxpharm, Otsuka, Pfizer, Rovi and Sanofi, and grants from Spanish Ministry of Health, Instituto de Salud Carlos III (PI20/01066), Fundació La Marató de TV3 (202,206-30-31) and Pons-Bartran legacy (FCRB_IPB1_2023).

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The rest of authors declare no conflict of interest.

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4.8. Article VIII

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Correlation between C-reactive protein and the inflammatory ratios in acute schizophrenia inpatients: are they associated?

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ABSTRACT

C-reactive protein (CRP) and inflammatory ratios have been proposed to study immune dysregulation in schizophrenia. Nevertheless, links between CRP and inflammatory ratios in acute SCZ inpatients have been understudied. This study investigated the relationship between CRP and inflammatory ratios (Neutrophil-Lymphocyte Ratio [NRL], Platelet-Lymphocyte Ratio [PLR], Monocyte-Lymphocyte ratio [MLR] and Basophil-Lymphocyte Ratio [BLR]) in a total of 698 acute SCZ inpatients; and analysed how this relationship is affected by sex and type of episode. CRP correlated with NLR ($r_s = 0.338$, $p < 0.001$), PLR ($r_s = 0.271$, $p < 0.001$) and MLR ($r_s = 0.148$, $p < 0.001$) but not with BLR ($r_s = 0.059$, $p = 0.121$). Multiple lineal regression analysis showed that high levels of NLR, MLR and PLR but not BLR were independently associated with high CRP levels. No sex-related variations were found. Significant associations were maintained for NLR and MLR in first-episode and multiepisode SCZ, although the strength of the association was stronger in multiepisode SCZ. Again, no sex-related differences were found in these associations. In conclusion, inflammatory ratios were low to moderately associated with CRP in acute SCZ inpatients. NLR and multiepisode SCZ showed the highest associations with CRP. Future studies should consider inflammatory ratios not as a substitute for CRP but as a complementary biomarker.

1. Background

There is a rise of evidence supporting the existence of immune system abnormalities in schizophrenia (SCZ) (Leza et al., 2015; Miller and Goldsmith, 2020; Ermakov et al., 2022). Both peripheral and central pro-inflammatory state has been described in a subgroup of SCZ patients with higher severity of symptoms and treatment resistance (Mondelli et al., 2017; Kose et al., 2021). And there is evidence for a dose-dependent effect of inflammation on psychopathology in patients with SCZ (Barnett et al., 2019; Fond et al., 2021). Numerous inflammatory biomarkers have been suggested to hold potential as predictors of worse clinical outcomes (Goldsmith et al., 2016; Miller et al., 2013; Upthegrove et al., 2014; Pillinger et al., 2019; Orsolini et al., 2018; Dunleavy et al., 2022). However, finding the best approach to predict clinical response to existing treatment is essential to improve treatment strategies in this subgroup of patients (Sun et al., 2022).

C- reactive protein (CRP) is one of the peripheral inflammatory biomarkers studied in patients with SCZ. It is an acute phase protein playing a role in the innate humoral response that has been used a proxy for systemic inflammation. Cross-sectional evidence reports that moderately elevated CRP levels are present in approximately one third of SCZ (Ermakov et al., 2022; Pillinger et al., 2019). CRP levels have been associated with a risk of increased positive symptoms, cognitive impairment and long-term mortality in subjects with SCZ (Kose et al., 2021; Fond et al., 2015; Horsdal et al., 2017). Furthermore, a sex-specific modulation of the mentioned associations has been described with an inverse association between CRP and cognition in female but not in male patients (Dal Santo et al., 2020). Inflammatory

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ratios (Neutrophil-Lymphocyte Ratio [NRL], Platelet-Lymphocyte Ratio [PLR], Monocyte-Lymphocyte Ratio [MLR] and Basophil-Lymphocyte Ratio [BLR]) are another inflammatory biomarker studied in SCZ. They are calculated using complete white blood cell counts and reflect the balance between the innate and adaptive immunity. A systematic review and meta-analysis showed increased NLR in both first-episode psychosis (FE) and multiepisode (ME) SCZ (Karageorgiou et al., 2019), and a reduction of NLR has been associated with clinical remission (Bhikram and Sandor, 2022; Bioque et al., 2022).

It has been hypothesized that inflammatory ratios may be more predictive when assessing inflammation, and particularly in detecting imbalances between the innate and adaptive immune pathways, than using general inflammation biomarkers (Bhikram and Sandor, 2022). At the same time, researchers call for an increasing need for combinations of different biomarkers to improve the predictive ability of isolated biomarkers and move towards a personalized medicine in SCZ (Fond et al., 2015). Nevertheless, links between CRP and inflammatory ratios in acute SCZ inpatients have been understudied. In this context, a better understanding of the relationship between blood biomarkers could help us to better define the inflammatory state in SCZ patients and guide the use to improve the prediction of clinical outcome.

This large cross-sectional study aimed to investigate (1) whether inflammatory ratios are related with CRP in acute SCZ inpatients at time of hospitalisation and (2) how this relationship is affected by sex and type of episode.

2. Methods

2.1. Study setting, population, and design

For this retrospective study, all electronic medical records of patients admitted to the Inpatient Psychiatric Unit (Santa María University Hospital, Lleida, Spain) between January 1st, 2010, and December 31st, 2020, were extensively reviewed by an experienced clinician (i.e., psychiatrist, clinical psychologist). Included patients were aged 18 or older, with a primary psychiatric diagnosis of Schizophrenia (F20) according to the International Classification of Diseases (tenth edition) Clinical Modification (ICD-10-CM; https://eciemaps.mscbs.gob.es). Excluded patients were those (1) with incomplete data about blood levels of total and differential white blood cell (WBC) counts, platelets or CRP; (2) suffering from any acute and/or chronic medical condition that could influence the levels of the immune/inflammatory parameters assessed; (3) pregnant or breastfeeding women; (4) readmitted in less than 48 h in order to avoid duplications. The following sociodemographic variables where collected: sex (male/female), age (years), cardiovascular risk factors (CVRF) (hypertension, dyslipidaemia and diabetes mellitus), tobacco use (yes/no), urine tested drugs of abuse (cannabinoids, opioids and/or cocaine) (yes/no) and type of episode (First Episode (FE)/Multi Episode (ME) SCZ). The study protocol was approved by the Local Ethics Committee belonging to the Santa María University Hospital (Lleida, Spain) (CEIC-2341).

2.2. Measurements

Blood was collected by experienced nurses belonging to the Department of Psychiatry at Santa María University Hospital (Lleida, Spain), during the first 24 h upon admission, between 8.00 and 10.00 a.m., and after an overnight fasting. Selected markers included: total and differential white blood cell (WBC) counts (i.e., neutrophils, basophils, eosinophils, monocytes, basophils, lymphocytes), platelet counts and C-reactive protein (CRP). The following ratios were calculated: NLR, MLR, PLR and BLR. Total and differential WBC counts were assessed by Flow Cytometry using a Sysmex XN analyzer, while CRP levels were assessed by an immunoturbidimetric assay on a Beackman Coulter automated analyzer. The lower limit of detection for CRP, as determined by the assay manufacturer, was set on 2 mg/L. In addition to the continuous values, CRP was trichotomized into low level of systemic inflammation (<3 mg/L), high level of systemic inflammation (3–10 mg/L), and acute inflammation (>10 mg/L) following the American Heart Association and Center for Disease Control and Prevention recommendations (Myers et al., 2004).

2.3. Statistical analysis

Statistical analyses were performed using IBM-SPSS v.23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). Continuous data were expressed as mean and standard deviation (SD), while categorical data were expressed as absolute values and percentages (%). Data were tested for normal distribution by the Kolmogorov-Smirnov test ($n \geq 30$). Because of the normality violation for inflammatory ratios and CRP, we log-transformed these variables for correlation and regression analysis (Feng et al., 2013). Associations between CRP and inflammatory ratios were estimated by Spearman correlation (r). By using a sample size of 698 patients and assuming a bilateral $\alpha \leq 0.05$, the statistical power to detect a significant Spearman correlation (>0.3) is greater than 99%. A multiple generalized linear regression was used to evaluate inflammatory ratios as a predictor of change in CRP. CRP levels were used as the primary dependent variable and inflammatory ratios were used as the independent variables. The following covariates were included in our model: age, sex, CVRF, tobacco use, urine tested drugs of abuse and type of episode. Associations are reported as standardized Beta (β) and 95% confidence interval (CI) values. To further investigate the dose-response relationship, CRP levels were trichotomized and a one-way ANCOVA was conducted to compare the inflammatory ratios in the three groups whilst controlling for age, sex and CVRF. Pairwise comparisons were performed to identify which groups are different using estimated marginal means. The Bonferroni multiple testing correction was applied. All hypotheses were tested with $\alpha \leq 0.05$ (two-sided).

3. Results

A total of 698 inpatients with acute SCZ were included in the study. Demographic, cardiovascular risk factors and biomarkers characteristics are presented in Table 1.

Table 1
Sample characteristics.

Characteristics	Schizophrenia (n = 698)
Age, years (SD)	42.1 (13.2)
Gender (female), n° (%)	312 (44.8)
Cardiovascular risk factors, n° (%)	
Hypertension	105 (15.1)
Dyslipidaemia	153 (22.0)
Diabetes Mellitus	67 (9.6)
>2	128 (18.3)
Tobacco use, n° (%)	496 (71.1)
Urine tested drugs of abuse, n° (%)	129 (18.5)
Type of episode	
First episode schizophrenia	149 (21.4)
Chronic schizophrenia	547 (78.6)
C-Reactive Protein,	
mean (SD)	7.9 (13.1)
<3 mg/L, n° (%)	335 (48.1)
3–10 mg/L, n° (%)	226 (32.5)
>10 mg/L, n° (%)	135 (19.4)
Inflammatory Ratios	
NLR, mean (SD)	2.310 (1.269)
MLR, mean (SD)	0.314 (0.129)
PLR, mean (SD)	117.9 (52.8)
BLR, mean (SD)	0.019 (0.011)

Abbreviations: NLR: Neutrophil-Lymphocyte Ratio; MLR: Monocyte-Lymphocyte Ratio; PLR: Platelet-Lymphocyte Ratio; BLR: Basophil-Lymphocyte Ratio.

3.1. General associations

CRP showed significant positive correlations with NLR ($r_s = 0.338$, $p < 0.001$), PLR ($r_s = 0.271$, $p < 0.001$) and MLR ($r_s = 0.148$, $p < 0.001$) but not with BLR ($r_s = 0.059$, $p = 0.121$). Multiple lineal regression analysis showed that high levels of NLR, MLR and PLR but not BLR were independently associated with high CRP levels (Table 3).

3.2. Sex-related associations

When stratifying by sex, no differences between subgroups were found for CRP or inflammatory ratios levels (Table 2). Both female and male patients showed significant associations between CRP and NLR and MLR. PLR showed significant associations in males and a significant correlation but not regression in females. BLR maintained non-significant associations in both female and male patients (Table 3).

3.3. Episode-related associations

When stratifying by type of episode, higher levels of CRP and inflammatory ratios were found in ME SCZ (Table 2). In FE patients, CRP showed significant associations with NLR and MLR but not with PLR nor BLR. In ME patients, CRP showed significant associations with NLR, MLR and PLR but not with BLR (Table 3).

3.4. CRP categorizations analysis

In the trichotomized CRP analysis, NLR ($F(2,693) = 35.317$, $p < 0.001$, $\eta2 = 0.092$), MLR ($F(2,693) = 23.744$, $p < 0.001$, $\eta2 = 0.064$) and PLR ($F(2,693) = 6.492$, $p = 0.002$, $\eta2 = 0.018$) were significant related with CRP. There was no significant effect of BLR on CRP ($F(2,685) = 1.015$, $p = 0.363$, $\eta2 = 0.003$) (Fig. 1).

4. Discussion

The evidence of increased immune activation in at least a subgroup of patients with SCZ has suggested a role of the immune system in the pathophysiology of the disease (Leza et al., 2015; Mondelli et al., 2017; Pillinger et al., 2019). CRP and inflammatory ratios are two of the peripheral biomarkers proposed to study this immune dysregulation in SCZ (Pillinger et al., 2019; Karageorgiou et al., 2019). Nevertheless, links between these biomarkers in SCZ have been scarcely studied. In this retrospective study analyzing the association between CRP and inflammatory ratios in acute SCZ inpatients, we found: (1) CRP is significantly but moderately associated with NRL, the association with PLR and MLR is small and there is no association with BLR; (2) no sex-related variations were found in the associations; (3) significant associations were maintained for NLR and MLR in FE and ME SCZ, although the strength of the association was stronger in ME SCZ; PLR was significant only in ME SCZ and BLR remained non-significant in both groups.

Inflammation is a cornerstone of the immune response to infection and physiological injury. While acute inflammation is crucial for cellular and tissue homeostasis after a pathogenic or physical insult, chronic inflammation is maladaptive and can lead to gradual tissue degradation, and in turn, disease onset (Furman et al., 2019). Such low grade, persistent inflammation is present in at least a subgroup of SCZ patients (Benros et al., 2011; Yuan et al., 2019; Fišar, 2023) and has been related with the activation of microglia accelerating the dendritic pruning in these patients (Leza et al., 2015; Parellada and Gassó, 2021). There is a growing body of research to disentangle the best biomarker in assessing such low-grade inflammation in SCZ (Sun et al., 2022). On the one hand, CRP is an acute-phase reactant protein widely used as a measure of acute systemic inflammation but has also been validated as a low-grade inflammation biomarker (Nehring et al., 2022). The American Heart Association and Center for Disease Control and Prevention have proposed clear CRP thresholds as indicators of inflammation levels (<1 = "low", 1–3 = "medium", >3 mg/L = "high", >10 mg/L = "acute infection") (Myers et al., 2004). CRP is mainly produced by hepatocytes and is directly modulated by IL-6 and IL-1β, both of which appear to be increased particularly during exacerbations of psychotic status (Goldsmith et al., 2016; Pillinger et al., 2019). On the other hand, inflammatory ratios are calculated using complete white blood cell counts and reflect the balance between the innate immunity (as indicated by the

Table 2
Differences in CRP and inflammatory ratios according to sex and type of episode.

Sex-related groups			
	Female (n = 312)	Male (n = 386)	p-value
CRP	6.5 (9.1)	8.4 (15.9)	0.057
NLR	2.283 (1.289)	2.332 (1.254)	0.616
MLR	0.310 (0.138)	0.314 (0.122)	0.922
PLR	115.4 (53.7)	119.9 (52.1)	0.266
BLR	0.021 (0.010)	0.021 (0.012)	0.902
Episode-related groups			
	FEP (n = 149)	Chronic SCZ (n = 547)	p-value
CRP	4.0 (4.9)	8.4 (14.6)	**<0.001**
NLR	1.913 (1.071)	2.419 (1.298)	**<0.001**
MLR	0.276 (0.107)	0.324 (0.133)	**<0.001**
PLR	105.7 (45.7)	121.2 (54.2)	**0.001**
BLR	0.016 (0.009)	0.021 (0.011)	**<0.001**

Abbreviations: CRP: C-Reactive Protein; NLR: Neutrophil-Lymphocyte ratio; MLR: Monocyte.Lymphocyte Ratio; PLR: Platelet-Lymphocyte Ratio; BLR: Basophil-Lymphocyte Ratio; Bold p-values indicate $p < 0.05$.

Table 3
Association between CRP and inflammatory ratios.

Outcome: CRP	Correlation analysis		Mixed linear regression analyses		
	r_s	p-value	R^2	β (95% CI)	p-value
Total Sample (N = 698)					
NLR	0.338	**<0.001**	0.127	0.323 (0.556–0.899)	**<0.001**
MLR	0.271	**<0.001**	0.095	0.262 (0.561–1.030)	**<0.001**
PLR	0.148	**<0.001**	0.054	0.125 (0.122–0.561)	**0.002**
BLR	0.059	0.121	0.036	0.005 (−0.151 to 0.170)	0.907
Female sample (n = 312)					
NLR	0.327	**<0.001**	0.072	0.272 (0.340–0.829)	**<0.001**
MLR	0.291	**<0.001**	0.081	0.243 (0.354–1.023)	**<0.001**
PLR	0.155	**0.006**	0.053	0.117 (−0.006 to 0.602)	0.055
BLR	0.059	0.281	0.042	0.026 (−0.180 to 0.283)	0.663
Male sample (n = 384)					
NLR	0.351	**<0.001**	0.174	0.365 (0.611–1.092)	**<0.001**
MLR	0.256	**<0.001**	0.127	0.274 (0.544–1.205)	**<0.001**
PLR	0.148	**0.004**	0.049	0.132 (0.066–0.697)	**0.018**
BLR	0.025	0.634	0.040	−0.007 (−0.239 to 0.211)	0.902
First episode schizophrenia (n = 149)					
NLR	0.256	**0.002**	0.042	0.236 (0.137–0.818)	**0.006**
MLR	0.184	**0.025**	0.051	0.195 (0.073–0.996)	**0.023**
PLR	0.101	0.221	0.014	0.136 (−0.089 to 0.803)	0.115
BLR	−0.102	0.215	0.008	−0.103 (−0.463 to 0.113)	0.232
Chronic schizophrenia (n = 547)					
NLR	0.337	**<0.001**	0.124	0.344 (0.600–0.998)	**<0.001**
MLR	0.269	**<0.001**	0.096	0.276 (0.591–1.136)	**<0.001**
PLR	0.144	**<0.001**	0.033	0.123 (0.084–0.591)	**0.009**
BLR	0.071	0.099	0.023	0.031 (−0.125 to 0.258)	0.496

Abbreviations: r_s: Spearman's rank correlation coefficient; R^2: coefficient of determination; CRP: C-Reactive Protein; NLR: Neutrophil-Lymphocyte Ratio; MLR: Monocyte-Lymphocyte ratio; PLR: Platelet-Lymphocyte Ratio; BLR: Basophil-Lymphocyte Ratio; Bold p-values indicate $p < 0.05$.

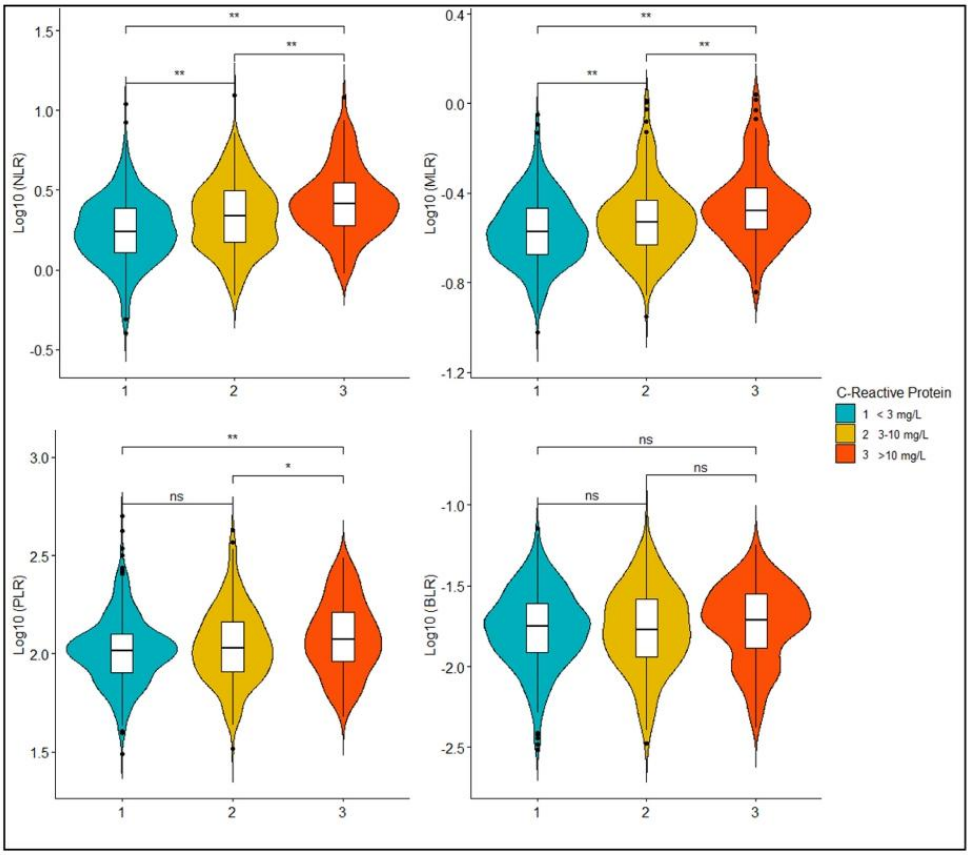

Fig. 1. Tricotomized C-Reactive Protein analysis.
Abbreviations: NLR: Neutrophil-Lymphocyte Ratio; MLR: Monocyte-Lymphocyte Ratio; PLR: Platelet-Lymphocyte ratio; BLR: Basophil-Lymphocyte Ratio. *p < 0.05, **p < 0.001, ns = non-significant.

neutrophil, monocyte, or platelet counts) and adaptive immunity (lymphocyte count). Increased inflammatory ratios in SCZ suggest that there is an imbalance in favor of innate immunity compared to adaptive immunity (Mazza et al., 2020). Although there are currently no standard cut-off values for inflammatory ratios, it has been hypothesized that they may be more predictive in assessing inflammation than the use of general biomarkers of inflammation (Bhikram and Sandor, 2022).

In our study we found a moderate association between NLR and CRP ($r_S = 0.338$, $p < 0.001$) and small association of PLR and MLR with CRP ($r_S = 0.271$, $p < 0.001$; $r_S = 0.148$, $p < 0.001$). Previous research has shown similar results for NLR in non-clinical population (Lin et al., 2016) and other medical conditions (Oh et al., 2013; Malhotra et al., 2015; Xu et al., 2016; Ljungström et al., 2017). However, contrary to our results, Yüksel et al. (2018) found that CRP and NLR levels were not associated in a relatively small sample of acute SCZ inpatients. Both CRP and inflammatory ratios are general markers of systemic inflammation and not specific for SCZ. Therefore, there is no reason to believe that the association between the two biomarkers behaves differently in patients with SCZ. Our results agree with those of Oh et al. (Lin et al., 2016) showing low to moderate associations and arguing that inflammatory ratios are no replacements for CRP but should be used in addition to each other.

Recently, Osimo et al. (2021) found an inverse association between BLR and a worse clinical course in FE psychosis. Studies about BLR are scarce, but a decrease in BLR has been observed in some autoimmune disease (Yang et al., 2017). In our study we found no association between BLR and CRP in either FE or ME SCZ patients.

Previous studies have shown that higher CRP levels are associated with symptoms severity at baseline and worse clinical outcomes in SCZ (Kose et al., 2021; Lestra et al., 2022). However, findings of NLR associations with psychopathological severity in SCZ are less consistent among the studies (Bhikram and Sandor, 2022). It seems clear however, that NRL is elevated during the acute FE psychosis and remains elevated throughout the course of the disorder, with reductions occurring at clinical remission or after long-term treatment with antipsychotics (Bhikram and Sandor, 2022; Bioque et al., 2022). In our study we did not collect psychopathological data, but the aforementioned studies support the hypothesis that inflammatory ratios might measure inflammation from a different point of view than CRP. Thus, future works should explore whether inflammatory ratios in comparison with CRP could be of clinical value in both short and long-term outcomes in SCZ.

When analysing by gender, neither inflammatory ratios nor CRP showed differences between subgroups as it was observed in similar studies (Bioque et al., 2022; Zhou et al., 2020). The associations remained significant in both sexes for NLR, MLR and PLR suggesting that there is no sex-related association between CRP and inflammatory ratios.

Considering the stage of the disease, immune dysfunctions have been

shown in both FE and ME SCZ patients. A recent meta-analysis by Fernandes et al. (2016) found elevated serum levels of CRP in both stages but higher levels in chronic SCZ (Bolu et al., 2019; Steiner et al., 2020). At the same time, recent meta-analyses show increased NLR both in FE and ME SCZ (Karageorgiou et al., 2019; Mazza et al., 2020) and, again, with a trend towards higher levels of NRL in ME SCZ (Mazza et al., 2020; Steiner et al., 2020). Acute episodes in SCZ result in increased levels of stress that induce the inflammatory response (Upthegrove and Khandaker, 2020). This is characteristic of acute psychopathological worsening occurring both in the FE or in a relapse after a stable phase. Antipsychotic treatment appears to have some anti-inflammatory effect with decreased CRP (Lestra et al., 2022) and inflammatory ratios (Sandberg et al., 2021) levels. However, symptomatic relapses requiring hospitalisation are most often triggered by non-adherence or treatment discontinuation (Higashi et al., 2013; Tiihonen et al., 2018). Therefore, many of the ME patients in the acute phase may have lost the anti-inflammatory effect of the antipsychotic treatment. In addition, patients in more advanced stages of the disease tend to be older, with a higher body mass index and a higher prevalence of cardiovascular risk factors contributing to increased persistent low-grade inflammation (Osimo et al., 2018). This would explain why ME patients in the acute phase have higher levels of biomarker levels than FE patients. Our results are in line with previous literature showing higher levels of CRP and inflammatory ratios in ME SCZ. Furthermore, we found stronger associations in ME than in FE SCZ ($r_s = 337$ vs $r_s = 256$ for NLR; $r_s = 267$ vs $r_s = 184$ for MLR respectively).

4.1. Strengths and limitations

The strengths of our study include its large sample size, the homogeneity of the sample by including only patients with a diagnosis of SCZ from a single centre whose psychiatrists share common clinical criteria and the description of the clinical stage of the disease including FE and ME SCZ. Another advantage is the standardised blood sampling procedure in all included patients, which diminishes potential technical and biological bias (Sandberg et al., 2021).

Our study should be however considered in light of several limitations. First, potential confounding variables with an impact on the collected biomarkers, such as body mass index (BMI), (Timpson et al., 2011) and or psychotropic medication (Öztürk et al., 2019) were not available. However, sample size was large enough to be able to control for other potential confounding variables not frequently addressed in clinical studies, such as the presence of CVRFs, tobacco use, drugs of abuse and/or type of episode. Second, studied biomarkers were measured at a single time point; thus, we were unable to assess the longitudinal relationship between CRP and inflammatory ratios over time. Third, no rating scales were used to assess the clinical severity of patients with SCZ and thus it is not possible to study the relationship of biomarkers with specific symptoms. However, we used acute hospitalisation as a criterion for severity (Addington et al., 2012) and all patients were selected from a single hospital whose psychiatrists share common clinical criteria. Fourth, we found that type of episode impacts the association but information about disease course (duration of untreated psychosis, years since onset) was not available. Fifth, no sex and aged matched healthy controls were included in the study to examine the particularities of the biomarkers. However, the purpose of our study was not to explore the specificity of the inflammatory biomarkers but to examine their associations in this population. Finally, the cross-sectional nature of our study limits inferences that can be drawn about causation, although it provides important insights to future studies.

5. Conclusions

Overall, these findings support the existence of a relationship between CRP and inflammatory ratios, especially with NLR in multiepisode SCZ, with no sex-related differences observed. However, due to the low/moderate strength of the association, we propose that inflammatory ratios are no replacements for CRP but should be used in conjunctionto each other. Future studies should study both biomarkers together to assess their clinical utility in SCZ, potentially determining prognosis and guiding treatment for these patients.

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Author statement

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Declaration of competing interest

None.

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4.9. Article IX

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Differences in total and differential white blood cell counts and in inflammatory parameters between psychiatric inpatients with and without recent consumption of cannabinoids, opioids, or cocaine: A retrospective single-center study

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ABSTRACT

Several drugs of abuse may exert their action by modulating the immune system. Despite this, individuals using substances of abuse are often excluded from immunopsychiatry studies. We conducted a retrospective, single-center study to examine differences in circulating immune/inflammatory parameters (i.e., total and differential white blood cell (WBC) counts, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte (MLR) ratio, platelet-to-lymphocyte ratio, and C-reactive protein) between psychiatric inpatients with a positive urine test to cannabinoids, opioids, or cocaine, and those with negative toxicology. A total of 927 inpatients were included. Patients with positive toxicology (n = 208) had significantly higher WBC counts ($P < 0.001$, $\eta^2p = 0.02$), as well as increased neutrophils ($P = 0.002$, $\eta^2p = 0.01$), monocytes ($P < 0.001$, $\eta^2p = 0.02$), lymphocytes ($P < 0.001$, $\eta^2p = 0.02$), and eosinophils ($P = 0.01$, $\eta^2p = 0.01$) compared to those with negative toxicology (n = 719). The increase in neutrophil counts was particularly evident in patients who tested positive for cannabinoids (n = 168; $P < 0.001$, $\eta^2p = 0.02$). In contrast, eosinophil counts were particularly increased in the cocaine-positive subgroup (n = 27; $P = 0.004$, $\eta^2p = 0.01$). Patients with a positive urine test to opioids (n = 13) were characterized by a significantly lower MLR ($P = 0.03$, $\eta^2p = 0.005$). The type of psychiatric diagnosis moderated the differences in neutrophil counts between patients with a positive and negative toxicology to cannabinoids. Notably, significantly higher neutrophil counts were found only in patients diagnosed with a psychotic disorder ($P < 0.001$, $\eta^2p = 0.03$). Taken together, our findings suggest that drugs of abuse may differently impact the immune/inflammatory response system in individuals diagnosed with psychiatric conditions. Specifically, recent cannabinoids use may be associated with an acute activation of the inflammatory response system, particularly in individuals with a psychotic disorder, while cocaine and opioid use may be associated with eosinophilia and a decrease in the MLR, respectively, regardless of the primary psychiatric diagnosis.

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1. Introduction

Accumulating evidence suggests that immunological and/or inflammatory changes may underly several psychiatric conditions, such as schizophrenia, major depressive disorder, bipolar disorder, autism spectrum disorder and/or personality disorders. An increased expression of different pro-inflammatory genes, abnormalities in the number and/or function of several immune cells (e.g., monocytes, lymphocytes), and increased blood and/or cerebrospinal fluid levels of pro-inflammatory compounds (e.g., cytokines, chemokine and/or C-reactive protein (CRP)) have been repeatedly reported in individuals diagnosed with these conditions (Goldsmith et al., 2016; Vogels et al., 2017; Becking et al., 2018; Garcia-Rizo et al., 2019; Jackson and Miller, 2020; Simon et al., 2023; Bioque et al., 2022; Arteaga-Henríquez et al., 2022; López-Villatoro et al., 2023; Sørensen et al., 2023). The relevance of these findings lies in their potential use as biological diagnostic and therapeutic markers in individuals with psychiatric symptoms (personalized psychiatry) (Drexhage et al., 2010; Benedetti et al., 2017; Becking et al., 2018; Arteaga-Henríquez et al., 2019; Ioannou et al., 2024; Llorca-Bofí et al., 2024). For a better understanding of the whole picture, it is important to investigate how several factors, such as age (Grosse et al., 2015), previous viral infections (Simon et al., 2023), childhood trauma (Schiweck et al., 2020) and/or obesity (Milaneschi et al., 2020; Arteaga-Henriquez et al., 2021) may influence the immune/inflammatory changes found in individuals diagnosed with psychiatric conditions (Pillinger et al., 2019).

Substance abuse is highly comorbid in individuals diagnosed with psychiatric disorders (Ross and Peselow, 2012; Toftdahl et al., 2016), i.e., about 10–50% of the subjects diagnosed with a psychiatric disorder will also experience a comorbid substance use disorder (SUD) at some point in their lives (Ross and Peselow, 2012; Toftdahl et al., 2016). Accumulating research suggests that individuals who use substances of abuse may be at a higher risk of treatment resistance and suicidal behavior. On the contrary, other reports also suggest the potential beneficial effect of substances like cannabinoids in the management of anxiety (Schott, 2019). The mechanisms by which these agents impact mood and behavior are not fully understood but may be related to their capability to impact the immune/inflammatory response system exerting, depending on the agent and probably, on the target population, and anti-inflammatory and/or pro-inflammatory action (Tanasescu and Constantinescu, 2010; Bidwell et al., 2020; Henshaw et al., 2021). Despite this, patients meeting criteria for a SUD or under sporadic substance of abuse use are frequently excluded from Immunopsychiatry studies representing an important gap in the literature.

The aim of this retrospective study was to investigate the differences in relation to the levels of a set of immune/inflammatory parameters (i.e., total and differential white blood cell (WBC) counts, platelet counts, neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and CRP), between patients with a psychiatric diagnosis and a positive toxicology to cannabinoids, opioids or cocaine and those with a negative toxicology.

2. Material and methods

2.1. Study participants

For this retrospective study, electronic medical records of all patients admitted to the Inpatient Psychiatric Unit (Santa María University Hospital, Lleida, Spain) between January 1, 2010, and December 31, 2020, were extensively reviewed by two experienced psychiatrists. In order to avoid duplications (and also, to indirectly control for other factors, such as chronicity and/or polypharmacy), we only included data referred to the first time of admission.

Included were acutely ill psychiatric inpatients aged 18 or over, from whom a blood and urine sample was collected at admission (i.e., within the first 24 h). Excluded were subjects diagnosed with a psychiatric disorder secondary to a known medical condition or with a non-specified psychiatric disorder. Patients admitted after a suicide attempt, pregnant or breastfeeding women, as well as individuals diagnosed with a comorbid autoimmune and/or acute/chronic inflammatory, metabolic, cardiovascular, or neurological (including neurocognitive) condition were excluded, too. Subjects testing positive for other agents of abuse than benzodiazepines (BZD), cannabinoids, opioids and/or cocaine (i.e., those testing positive for amphetamines/amphetamine derivatives) were also not included. Given the described association between alcohol consumption and immune system/inflammatory dysfunction (Calleja-Conde et al., 2021), subjects under an alcohol substance use disorder and/or under alcohol use were excluded.

The study protocol was approved by the Local Ethics Committee belonging to the Santa María's University Hospital (Lleida, Spain).

2.2. Study procedures

Blood and urine were collected between 8.00 and 10.00 a.m. by an experienced nurse belonging to the Department of Psychiatry at Santa María's University Hospital (Lleida, Spain) after an overnight fasting. Selected blood markers included: total and differential WBC counts (i.e., basophils, eosinophils, neutrophils, monocytes, lymphocytes), platelet counts and CRP levels. In addition, the following indexes were calculated: NLR, MLR, and PLR. Total and differential WBC counts were assessed by flow cytometry using a Sysmex XN analyzer; the detection range, as determined by the assay manufacturer was set at 0-440 $\times$ 10^9/L. Platelet counts were assessed by impedance also by a Sysmex XN analyzer; the detection range was in this case set at 0-5000 $\times$ 10^9/L. CRP levels were assessed by an immunoturbidimetric assay on a Beckman Coulter automated analyzer; the lower limit of detection, as determined by the assay manufacturer was set on 2 mg/L. Positivity in urine (no/yes) was determined by immunochromatography (i.e., Multi-line Drug Screen Test Device (MONLAB)), and established according to both Substance Abuse Mental Health Administration (SAMHSA) (Verstraete and Pierce, 2001), and United Nations International Drug Control Program criteria (SAMHSA, 2004). The cut-off limits for considering a patient as "positive" were 50 ng/mL for cannabinoids, 300 ng/mL for opioids and 300 ng/mL for cocaine (Verstraete and Pierce, 2001).

2.3. Statistical analyses

Statistical analyses were performed using IBM-SPSS v.23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). Continuous data were expressed as mean $\pm$ standard deviation (SD), while categorical data were expressed as absolute values and percentages (%). Data were tested for normal distribution by the Kolmogorov-Smirnov test ($n \geq 30$). Group comparisons of sample characteristics were analyzed by using Mann-Whitney *U* tests (i.e., continuous data), or Pearson's chi-square (χ^2) tests (i.e., categorical data). A univariate analysis of covariance (ANCOVA) model was used to compare mean immune/inflammatory parameter levels between patients with a negative vs. those with a positive toxicology. Age (years), consumption of BZD (no/yes) and the type of primary psychiatric diagnosis were introduced as covariables. Sensitivity subgroup analyses were additionally performed, in order to explore the effect of the type of agent of use, as well as the type of primary psychiatric diagnosis on immune/inflammatory parameters. Effect sizes are reported as partial eta-squared (η^2p). All tests were two-tailed, with *P*-values equal or less than 0.05 being considered as of statistical significance.

3. Results

3.1. Baseline characteristics of study participants

Baseline characteristics of the 927 patients included are reported in Table 1. For a complete description of the diagnoses included, see

Table 1
Sample characteristics of study participants.

	Negative n = 719	Positive n = 208	Negative vs. Positive:	Cannabinoids n = 168	Negative vs. Positive:	Opioids n = 13	Negative vs. Positive:	Cocaine n = 27	Negative vs. Positive:
Age (years); mean (SD)	44.93 (16.16)	35.11 (10.75)	**<0.001**	33.89 (10.25)	**<0.001**	42.95 (15.12)	0.66	38.93 (9.22)	0.06
Females; n (%)	366 (51%)	111 (53%)	0.53	91 (54%)	0.45	9 (69%)	0.19	11 (41%)	0.30
Agent of use, n (%)									
- BZD	396 (55%)	136 (66%)	**0.01**	105 (63%)	0.08	9 (69%)	0.32	22 (81%)	**0.005**
- Cannabinoids	0 (0%)	168 (81%)							
- Opioids	0 (0%)	13 (6%)							
- Cocaine	0 (0%)	27 (13%)							
Primary Psychiatric; n (%)									
- SUD	0 (0%)	46 (22%)	**<0.001**	29 (17%)	**<0.001**	5 (38%)	**<0.001**	12 (44%)	**<0.001**
- Psychotic Disorder	312 (43%)	81 (39%)	0.25	73 (43%)	0.99	1 (8%)	**0.01**	7 (26%)	0.07
- Depressive Disorder	108 (15%)	7 (3%)	**<0.001**	4 (2%)	**<0.001**	3 (23%)	0.31	0 (0%)	**0.01**
- Bipolar Disorder	130 (18%)	26 (12%)	0.06	23 (14%)	0.17	2 (15%)	0.57	1 (4%)	**0.03**
- Adjustment Disorder	85 (12%)	14 (7%)	**0.02**	10 (6%)	**0.03**	2 (15%)	0.47	2 (7%)	0.48
- PD	46 (6%)	23 (11%)	**0.02**	18 (11%)	**0.05**	0 (0%)	0.43	5 (18%)	**0.03**
- NDV	7 (1%)	1 (1%)	0.43	1 (1%)	0.64	0 (0%)	0.88	0 (0%)	0.77
- OCD	7 (1%)	0 (0%)	0.17	0 (0%)	0.23	0 (0%)	0.88	0 (0%)	0.77
- Eating Disorder	17 (2%)	1 (1%)	0.06	1 (1%)	0.12	0 (0%)	0.73	0 (0%)	0.53
- Conduct Disorder	7 (1%)	9 (4%)	**0.001**	9 (5%)	**0.001**	0 (0%)	0.88	0 (0%)	0.77

Abbreviations: BZD: benzodiazepines; NDV: Neurodevelopmental Disorder; OCD: Obsessive-Compulsive Disorder; PD: Personality Disorder; SD: standard deviation; SUD: Substance Use Disorder. *P*-values were based on Mann Whitney *U* tests (continuous variables), and on χ^2–Squared test (categorical variables). Significant values are highlighted in bold.

Table S1.

In total, 719 (78%) of patients had a negative toxicology; 208 (22%) had a positive toxicology (i.e., cannabinoids (n = 168, 18%), opioids (n = 13, 1%), cocaine (n = 27, 3%)). Among the total of patients with a positive toxicology, 46 (22%) were diagnosed with a SUD.

Patients with a positive toxicology were statistically significantly younger ($P < 0.001$) and showed a statistically significantly higher consumption of BZD ($P = 0.01$) compared to those with a negative urine test. In addition, subjects with a positive toxicology were diagnosed with a conduct (i.e., 9% vs 4%; $P = 0.001$) or a personality disorder (i.e., 11% vs. 6%; $P = 0.02$) at a statistically significantly higher proportion; and with a depressive (i.e., 3% vs. 15%; $P < 0.001$) or an adjustment disorder (7% vs. 12%; $P = 0.02$) at a statistically significantly lower proportion, (Table 1).

Significant differences were not found between patients testing negative and those testing positive in relation to sex or to the proportion of psychotic, bipolar, neurodevelopmental, obsessive-compulsive or eating disorder diagnoses (Table 1).

Table 2
Blood levels of immune/inflammatory parameters in individuals with a negative urine test vs. individuals with a positive urine test.

	Negative n=714	Positive n=207	Negative vs. Positive		Direction of Difference
Blood parameter	Mean (SD)	Mean (SD)	*P*-value	η^2p	
WBC count ($10x^9$/L)	7.10 (2.17)	7.92 (2.42)	**<0.001**	0.02	↑
Basophil count ($10x^9$/L)	0.04 (0.02)	0.04 (0.02)	0.25	0.001	
Eosinophil count ($10x^9$/L)	0.17 (0.15)	0.21 (0.14)	**0.01**	0.01	↑
Neutrophil count ($10x^9$/L)	4.16 (1.83)	4.51 (2.08)	**0.002**	0.01	↑
Monocyte count ($10x^9$/L)	0.60 (0.21)	0.66 (0.25)	**<0.001**	0.02	↑
Lymphocyte count ($10x^9$/L)	2.12 (0.73)	2.49 (0.83)	**<0.001**	0.02	↑
Platelet count ($10x^9$/L)	237.62 (61.54)	242.81 (51.01)	0.17	0.00	
CRP (mg/L)	6.24 (15.47)	6.69 (16.59)	0.26	0.00	
Inflammatory ratios	Mean (SD)	Mean (SD)	*P*-value	η^2p	
NLR	2.23 (1.38)	2.02 (1.27)	0.83	0.00	
MLR	0.31 (0.14)	0.29 (0.14)	0.91	0.00	
PLR	124.72 (54.65)	107.61 (37.96)	**0.01**	0.01	↓

Abbreviations: CRP: C-reactive protein; MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; WBC: white blood cell count. Analyses were based on an analysis of covariance (ANCOVA) model with the corresponding blood parameter as the dependent variable, group (negative/positive) as fixed effect variable, and age, consumption of BZD (yes/no), and the type of primary psychiatric diagnosis, as covariates. Significant *P*-values are highlighted in bold and marked with an asterisk (i.e., ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05).

3.2. Blood levels of immune/inflammatory parameters in individuals with a negative urine test vs. individuals with a positive urine test

Overall, patients with a positive toxicology were characterized by a statistically significantly higher WBC count ($P < 0.001$, $\eta^2p = 0.02$) and by statistically significantly higher blood levels of neutrophils ($P = 0.002$, $\eta^2p = 0.01$), monocytes ($P < 0.001$, $\eta^2p = 0.02$), lymphocytes ($P < 0.001$, $\eta^2p = 0.02$) and eosinophils ($P = 0.01$, $\eta^2p = 0.01$) compared to those with a negative toxicology (Table 2). Patients with a positive toxicology were on the contrary characterized by a statistically significantly lower PLR ($P = 0.01$, $\eta^2p = 0.01$) compared to those testing negative (Table 2). Significant differences were not found between patients with a positive toxicology and those with a negative toxicology in relation to any of the other immune/inflammatory parameters assessed (Table 2).

3.3. Blood levels of immune/inflammatory parameters in individuals testing negative vs. those testing positive, stratified by the agent of use

As a sensitivity analysis, we stratified patients with a positive urine test according to their agent of use and then compared them to those with a negative urine test (Figs. 1 and 2, Table S2).

By doing so, we found that patients with a positive toxicology to cannabinoids were characterized by a statistically significantly higher WBC count ($P < 0.001$, $\eta^2p = 0.03$) and by statistically significantly higher neutrophil ($P < 0.001$, $\eta^2p = 0.02$), monocyte ($P < 0.001$, $\eta^2p = 0.02$) and lymphocyte ($P = 0.002$, $\eta^2p = 0.01$) counts compared to those with a negative toxicology (Fig. 1A, B, C, D, Table S2). Patients testing positive in urine to cannabinoids were also characterized by a statistically significantly lower PLR ($P = 0.02$, $\eta^2p = 0.01$) compared to those with a negative toxicology (Fig. 2D–Table S2). A moderator effect for age and for the type of primary psychiatric diagnosis was suggested for the differences in neutrophil counts ($P = 0.02$, $\eta^2p = 0.01$ and $P = 0.02$, $\eta^2p = 0.01$, respectively). Age also seemed to moderate the findings for the differences in the PLR ($P < 0.001$, $\eta^2p = 0.02$) and in lymphocyte counts ($P < 0.001$, $\eta^2p = 0.004$); a moderator effect of BZD consumption was additionally suggested for the differences in lymphocyte counts ($P = 0.05$, $\eta^2p = 0.004$).

In relation to patients under opioids use, a statistically significantly lower MLR and a statistically significantly higher lymphocyte counts were found when compared to patients with a negative toxicology ($P = 0.03$, $\eta^2p = 0.005$ and $P < 0.001$, $\eta^2p = 0.01$, respectively) (Figs. 1C and 2C, Table S2). Age seemed to moderate the findings for the differences in the MLR and in lymphocyte counts ($P < 0.01$, $\eta^2p = 0.02$ and $P < 0.01$, $\eta^2p = 0.03$, respectively); a moderator effect of BZD consumption was also suggested for the differences in lymphocyte counts ($P = 0.04$, $\eta^2p = 0.004$).

In addition, patients with a positive toxicology to cocaine were characterized by statistically significantly higher eosinophil counts compared to those testing negative ($P = 0.004$, $\eta^2p = 0.01$) (Fig. 1E–Table S2). Again, age seemed to moderate the findings for the differences in eosinophil counts ($P = 0.01$, $\eta^2p = 0.01$).

Statistically significant differences were only found for the NLR and for neutrophil and eosinophil counts when comparing patients with a positive toxicology to cannabinoids, opioids or cocaine among themselves. Specifically, patients testing positive to cannabinoids showed the highest NLR and neutrophil counts, and those testing positive to cocaine showed the highest eosinophil counts (Figs. 1 and 2, Table S2).

3.4. Blood levels of immune/inflammatory parameters in individuals testing negative vs. those testing positive to cannabinoids, stratified by primary psychiatric diagnosis

A moderator effect of the type of primary psychiatric diagnosis was suggested for the differences in neutrophil counts between patients with a positive toxicology to cannabinoids and those with a negative urine test (see 3.3). This may indicate that neutrophil counts may vary depending on the primary psychiatric diagnosis. Accordingly, we investigated the existence of differences in the levels of neutrophil counts between patients with a negative toxicology to cannabinoids and those with a positive toxicology, after stratifying them according to their primary psychiatric diagnosis (Table 3). Since a statistically significant correlation was found between neutrophil counts and the WBC ($rs = 0.88$, $P < 0.001$), monocyte counts ($rs = 0.55$, $P < 0.001$), basophil counts ($rs = 0.22$, $P < 0.001$) and platelet counts ($rs = 0.24$, $P < 0.001$), as well as between neutrophil counts and CRP levels ($rs = 0.29$, $P < 0.001$), the NLR ($rs = 0.74$, $P < 0.001$), MLR ($rs = 0.44$, $P < 0.001$) and PLR ($rs = 0.13$, $P < 0.001$), the existence of differences in these parameters were also investigated. Due to a low sample size (see Table 1), patients with a depressive disorder and an adjustment disorder were grouped into one diagnostic category, and patients with a personality disorder and a those with a conduct disorder, into another one. Analyses on patients with a neurodevelopmental disorder, an eating disorder, and an obsessive-compulsive disorder could unfortunately not be performed.

In the subgroup of patients diagnosed with a psychotic disorder, patients testing positive in urine to cannabinoids showed a statistically significantly higher WBC ($P < 0.001$, $\eta^2p = 0.04$) and statistically significantly higher neutrophil ($P < 0.001$, $\eta^2p = 0.03$) and monocyte ($P = 0.002$, $\eta^2p = 0.02$) counts compared to those testing negative (Table 3).

Patients with a positive toxicology to cannabinoids and a diagnosis of either bipolar disorder, depressive/adjustment disorder, personality/conduct disorder or SUD did not statistically significantly differ in relation to any of the immune/inflammatory parameters assessed when compared with those with a negative toxicology except for the PLR, with patients with a bipolar disorder diagnosis and a positive urine test showing a statistically significantly PLR compared to those testing negative (Table 3).

Statistically significant differences in relation to the WBC, neutrophil, monocyte and platelet counts were not found when comparing patients with a psychotic disorder, bipolar disorder, depressive/adjustment disorder, personality/conduct disorder or SUD among themselves (data not shown).

4. Discussion

To the best of our knowledge, this is the first study to date aimed at investigating differences in a set of immune/inflammatory parameters between psychiatric inpatients with a positive urine test to cannabinoids, opioids or cocaine, and those with a negative urine test.

Overall, patients with a positive toxicology were characterized by a significantly higher WBC counts, as well as significantly higher blood levels of neutrophils, monocytes, lymphocytes and eosinophils compared to those with a negative one. The increase in eosinophil counts was particularly evident in the subgroup of patients who tested positive for cocaine. In contrast, patients who tested positive for cannabinoids were characterized by significantly higher WBC, neutrophil and monocyte counts compared to those with a negative toxicology. In addition, a statistically significantly lower MLR was found in the subgroup of patients testing positive in urine to opioids when compared to those with a negative toxicology. These findings are supported by previous existing human studies where an association between cocaine consumption and an increase in eosinophil counts (Berman et al., 2016; Reyes et al., 2018; Gill et al., 2023), opioids consumption and a lower MLR (Banerjee and Sarkar, 1994; Orum et al., 2018), and cannabis use and higher neutrophil counts (Lorenz et al., 2017; Alshaarawy, 2019; Romeo et al., 2023) has been reported, suggesting that cannabinoids, opioids and cocaine may differently impact the immune/inflammatory response system in individuals with and without psychiatric conditions.

Something which makes our study novel is the fact that analyses were not restricted to patients diagnosed with a specific psychiatric disorder, i.e., patients with different psychiatric conditions were

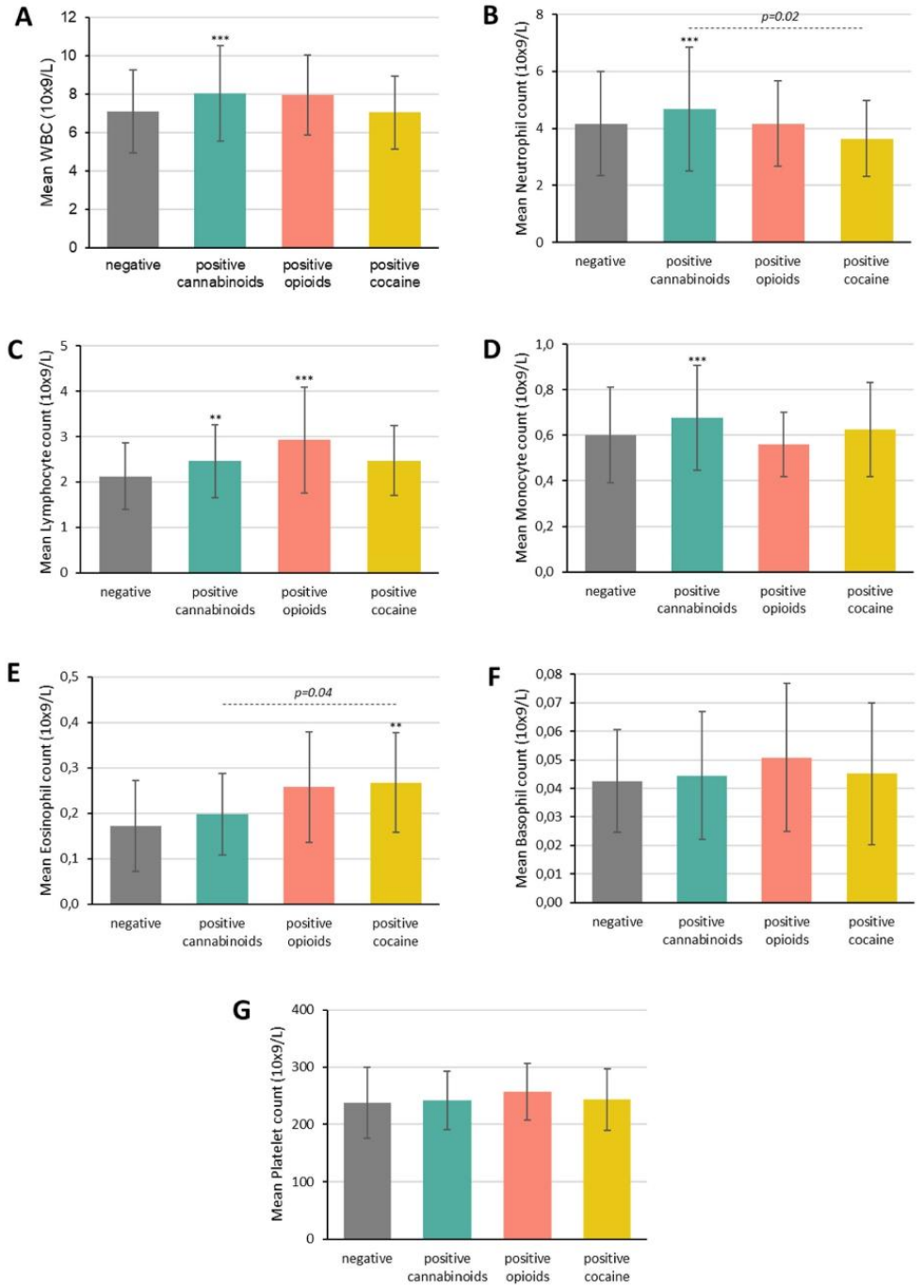

Fig. 1. Blood levels of immune parameters in individuals with a negative urine test vs. individuals with positive urine test (stratified by agent of consumption; cannabinoids, opioids, or cocaine).
Abbreviations: WBC: white blood cell count. Analyses were based on an analysis of covariance (ANCOVA) model with the corresponding blood parameter as the dependent variable, group as fixed effect variable, and age, consumption of BZD, and type of primary psychiatric diagnosis, as covariates. Significant values are marked with an asterisk (i.e., ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05) when comparing negative vs. specific positive urine tests, and with the corresponding p-value (—) when comparing the positive urine tests among themselves.

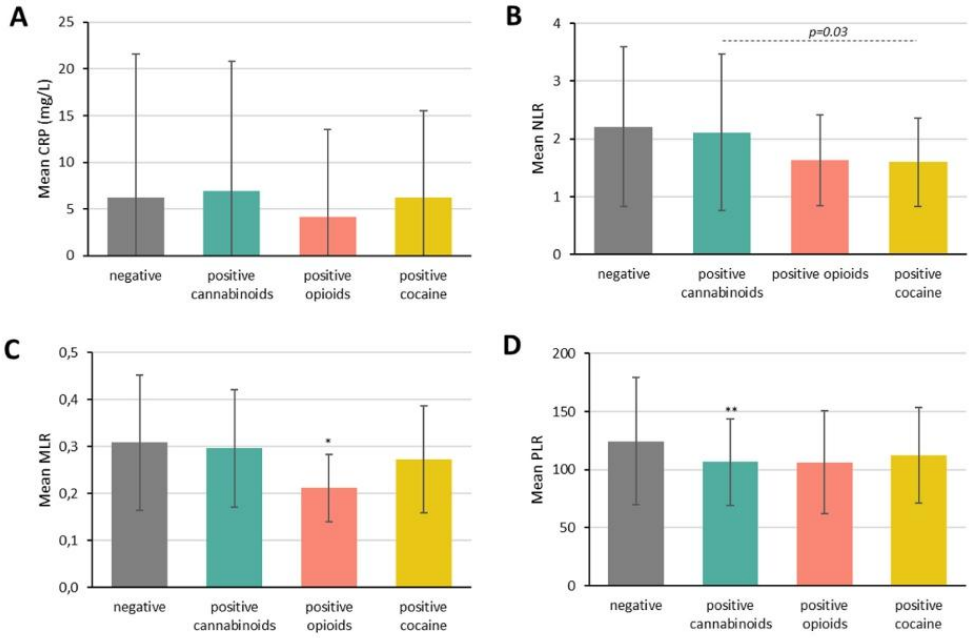

Fig. 2. Blood levels of inflammatory parameters in individuals with a negative urine test vs. individuals with positive urine test (stratified by agent of consumption; cannabinoids, opioids, or cocaine).
Abbreviations: CRP: C-reactive protein; MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio. Analyses were based on an analysis of covariance (ANCOVA) model with the corresponding blood parameter as the dependent variable, group as fixed effect variable, and age, consumption of BZD, and type of primary psychiatric diagnosis, as covariates. Significant values are marked with an asterisk (i.e., ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05) when comparing negative vs. specific positive urine tests, and with the corresponding p-value (—) when comparing the positive urine tests among themselves.

Table 3
Blood levels of immune/inflammatory parameters in individuals with a negative urine test vs. those with positive urine test (stratified by primary psychiatric diagnosis).

	Psychotic Disorder (1)		Bipolar Disorder (2)		Depressive + Adjustment Disorder (3)		Personality + Conduct Disorder (4)		SUD (5)	
	Negative n = 312	Positive n = 73	Negative n = 130	Positive n = 23	Negative n = 193	Positive n = 14	Negative n = 53	Positive n = 27	Negative n = 17	Positive n = 29
Blood parameter	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
WBC count ($10x^9$/L)	7.16 (2.14)	**8.38 (2.83)****	7.23 (2.51)	8.35 (2.11)	6.95 (1.94)	7.61 (2.17)	7.47 (2.40)	7.88 (2.41)	7.68 (1.39)	7.45 (2.07)
Basophil count ($10x^9$/L)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.04 (0.03)	0.04 (0.02)	0.05 (0.02)	0.05 (0.03)	0.04 (0.02)
Neutrophil count ($10x^9$/L)	4.22 (1.83)	**5.08 (2.59)****	4.29 (2.16)	4.82 (1.75)	4.04 (1.61)	4.43 (1.59)	4.34 (1.77)	4.23 (1.93)	3.94 (1.29)	4.17 (1.69)
Monocyte count ($10x^9$/L)	0.61 (0.22)	**0.71 (0.28)****	0.59 (0.22)	0.66 (0.21)	0.58 (0.17)	0.59 (0.19)	0.64 (0.25)	0.72 (0.31)	0.63 (0.18)	0.61 (0.15)
Platelet count ($10x^9$/L)	236.48 (63.26)	241.18 (47.39)	240.30 (59.48)	247.08 (41.25)	241.82 (59.72)	238.14 (49.33)	235.67 (61.45)	239.18 (59.24)	149.23 (55.62)	236.38 (55.62)
CRP (mg/L)	5.40 (11.26)	7.45 (19.84)	5.67 (9.53)	8.30 (22.73)	6.74 (15.42)	2.31 (1.55)	12.10 (37.08)	5.40 (7.81)	9.26 (12.62)	7.04 (16.59)
Inflammatory ratios	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
NLR	2.30 (1.47)	2.28 (1.55)	2.27 (1.38)	2.06 (1.38)	2.14 (1.35)	1.94 (0.59)	2.19 (1.08)	1.72 (0.95)	1.64 (0.94)	1.99 (1.11)
MLR	0.32 (0.16)	0.31 (0.15)	0.30 (0.27)	0.27 (0.15)	0.29 (0.13)	0.28 (0.13)	0.32 (0.14)	0.28 (0.11)	0.26 (0.12)	0.28 (0.14)
PLR	126.51 (56.72)	107.90 (34.46)	124.49 (44.45)	**97.36 (27.98)***	124.66 (57.39)	112.85 (56.46)	122.44 (62.50)	101.55 (42.35)	100.24 (27.18)	109.42 (43.16)

Abbreviations: CRP: C-reactive protein; MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; WBC: white blood cell count. Analyses were based on an analysis of covariance (ANCOVA) model with the corresponding blood parameter as the dependent variable, group (negative/positive), as fixed effect variable, and age, consumption of BZD (yes/no), and agent of use (cannabinoids/opioids/cocaine), as covariates. Significant *P*-values are highlighted in bold and marked with an asterisk (i.e., ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$).

included. Thereby, we were able to investigate the influence of the type of primary psychiatric diagnosis on the differences found between patients with a positive toxicology to any of the agents of abuse assessed, and those with a negative toxicology. Interestingly, a moderator effect for the type of psychiatric diagnosis was found for the differences in neutrophil counts in individuals under cannabinoids use, suggesting a differential effect of cannabinoids on neutrophil counts, depending on the primary psychiatric diagnosis. More specific, we found that only among patients with a psychotic disorder diagnosis did cannabinoid users have statistically significantly higher neutrophil counts compared to non-users. Psychotic patients under cannabinoids use were also the only ones characterized by a statistically significantly higher WBC and monocyte counts when compared to non-users.

While the association between cannabis use and non-psychotic psychiatric conditions is still under debate, exogenous cannabis is considered as one of the major environmental risk factors for psychosis. Consumption of cannabis has been repeatedly associated with not only a worsening in psychotic symptoms and/or with an increased risk of psychotic relapse (Kuepper et al., 2011; Haney and Evins, 2016; Bioque et al., 2022) but also, with an increased risk of developing a psychotic disorder in the future (Moore et al., 2007; Di Forti et al., 2019).

The mechanisms for such associations are not fully understood. However, accumulating recent evidence suggests that cannabinoids may increase the risk of psychosis and/or worsen psychotic symptoms by impacting the immune/inflammatory response system of predisposed individuals (Gibson et al., 2020).

Individuals with a psychotic disorder and/or predisposed to are characterized by a dysregulated endocannabinoid system (ECS) (Bioque et al., 2013). The ECS constitutes a complex regulatory network of neurotransmitters, receptors and enzymes that plays a crucial role in maintain inflammatory balance in the human body. While the ECS is widely expressed throughout the body and can be found in almost all organs, the human nervous system and the immune system have interestingly been found to represent the highest expression levels of cannabinoid receptors (Lu and Mackie, 2021). In individuals with psychosis, a decreased expression of the cannabinoid receptor 2 (CB2) and both endocannabinoids synthesizing enzymes (*N*-acyl phosphatidylethanolamine phospholipase and diacylglycerol lipase) have been found when compared to healthy controls (Bioque et al., 2013).

Activation of CB2 receptors has been associated with the inhibition of microglial activation (Olabiyi et al., 2023) and with the blockade of neutrophil and/or monocyte migration (Gómez et al., 2021), that is, with a systemic deactivation of the inflammatory response system. Therefore, abnormalities in the expression and/or of function of CB2 receptors (which may occur in psychosis) may be associated with both microglial activation and with an activation of the inflammatory response system, something which has been repeatedly described in subjects with psychosis (Bioque et al., 2024; Chen et al., 2024).

In psychotic patients under cannabis use, dysregulation of the ECS may be particularly accentuated (Bioque et al., 2013). Therefore, these patients may exhibit heightened activation of their immune/inflammatory response system, as supported by our findings.

The WBC is nowadays considered as a reliable measure of the overall immune system activity (i.e., a high WBC count, or leukocytosis, suggests the existence of an activated immune response system) (Soehnlein et al., 2017). While acute inflammation is characterized by an increase in neutrophil levels, chronic inflammation is also associated with elevated levels of mononuclear cells, such as monocytes (Soehnlein et al., 2017).

Taken all this together, we hypothesize that (recent) cannabinoids use may be associated with a significantly higher (acute) activation of the inflammatory response system in, particularly, individuals diagnosed with a psychotic disorder.

This may have important potential therapeutic implications. An association between an activated inflammatory response system and treatment-resistance has been reported in patients diagnosed with psychotic disorders (Llorca-Bofí et al., 2021; Bioque et al., 2022; Howes and Onwordi, 2023). Interestingly clozapine (i.e., an antipsychotic agent indicated for the management of treatment-resistant schizophrenia) may exert tits action by, among other mechanisms, modulating neutrophil levels (Jones et al., 2023; Llorca-Bofí et al., 2024). Indeed, one of the most serious adverse effects of clozapine is neutropenia, a potentially serious and mortal side effect characterized by a significant reduction in neutrophil counts, which are crucial for, for example, combating infections (Silva et al., 2020). Therefore, we hypothesize modulation of neutrophil counts as a novel treatment strategy in individuals with psychosis under cannabinoids use. Interestingly, accumulating research has suggested clozapine as the first-line treatment for the management of patients with schizophrenia and a history of cannabis use (Brzozowska et al., 2017; Tang et al., 2017).

Although encouraging, our findings must be considered in light of several important limitations. First, immune and/or inflammatory parameters were assessed in the blood, and findings may thus differ from those in the brain. Data about a healthy control group and/or about potential confounding factors, such as body mass index (Pangrazzi et al., 2020), childhood maltreatment and/or the use of psychotropic medication were also not available and therefore, not included in our analyses. However, we controlled for other potential confounding factors, such as type of primary psychiatric diagnosis, illness chronicity, BZD use and/or the presence of comorbid somatic conditions and have made an effort to review the existing literature on the topic, finding a concordance between previous findings, and ours.

Second, information about consumption periodicity was not collected. Thereby, and since measures in urine inform about recent use, findings may vary in patients with a history of chronic use. Unfortunately, immune/inflammatory parameters were assessed at a single timepoint, preventing us from assessing the effects of drug abstinence on these markers over time. In line with this, in a recent study performed by Romeo et al. (2023), tetrahydrocannabinol (THC) cessation was interestingly associated with an increase in inflammatory markers, including WBC count, as well as lymphocyte and monocyte levels, all this correlating with symptomatology of patients with psychosis. However, and as a strength of our study, information about drug consumption relied on quantitative (objective) information, rather than solely on self-reported substance patterns, and a standardized blood sampling procedure in all included patients was implemented, reducing the potential for technical and biological biases (Raouf et al., 2018). Third, data about cannabinoids composition were not collected. *Cannabis sativa* contains not only delta-9-tetrahydrocannabinol (Δ^9-THC) and/or cannabidiol (CBD), but more than 70 different cannabinoids that are also found in the cannabis products available on the market (Elsohly and Slade, 2005). This may be of relevance, since different animal (Malfait et al., 2000) and human studies have repeatedly suggested that Δ^9-THC and CBD may exert opposing immunomodulatory effects (Bidwell et al., 2020). Exogenous cannabinoids containing a high percentage of CBD may exert an immunosuppressor and/or anti-inflammatory action, decreasing the numbers of different leukocyte subpopulations, such as monocytes (McDougle et al., 2017; Henshaw et al., 2021).

Both the type and/or concentrations of cannabinoids may differ greatly by, among other factors, the place of origin (Potter et al., 2008; Mehmedic et al., 2010). Specifically in Spain, THC content in the cannabis usually consumed on the streets is between 15 and 28% on average. On the contrary, the mean CBD content has been set at 5% on average (Santos-Álvarez et al., 2021). Accordingly, we assumed that our patients were under consumption of exogenous cannabinoids containing a high percentage of Δ^9-THC, but unfortunately, we cannot ensure this, something which could have biased our findings and may be responsible of the inconsistencies found in relation to previous existing literature.

Also, the possibility of a type II error (i.e., false negative) should be taken into account when interpreting our findings. Despite including a relatively large total sample size (n = 927), sensitivity subgroup analyses based on the agent of use or on the primary psychiatric diagnosis

led to small sample sizes. This could contribute to failing to detect statistically significant differences when actually present. Finally, the retrospective nature of our study, coupled with the relatively small sample size, limited the inferences that could be drawn about causation.

5. Conclusions

Overall, our findings suggest that recent cannabinoids use may be associated with a significantly higher activation of the immune/inflammatory response system, particularly in individuals diagnosed with a psychotic disorder. On the contrary, recent cocaine and opioid use may be respectively associated with eosinophilia and with a significantly lower MLR, regardless of the primary psychiatric diagnosis.

We believe our findings may provide important novel valuable insights into how cannabinoids, opioids and cocaine may impact the inflammatory response system of patients diagnosed with different psychiatric conditions and may thus have important clinical and therapeutic implications. For example, modulation of neutrophil counts may represent a novel treatment strategy in individuals with a psychotic disorder and a comorbid cannabis use. However, larger prospective studies, including data about symptoms severity, consumption periodicity and/or about cannabinoids composition are warranted to confirm our findings.

CRediT authorship contribution statement

Vicent Llorca-Bofí: Writing – original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Maria Mur:** Writing – review & editing, Supervision, Funding acquisition. **Maria Font:** Writing – review & editing, Project administration, Methodology, Investigation. **Roberto Palacios-Garrán:** Writing – review & editing. **Maite Sellart:** Writing – review & editing. **Enrique del Agua-Martínez:** Writing – review & editing. **Miquel Bioque:** Writing – review & editing, Supervision. **Gara Arteaga-Henríquez:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Submission declaration

The present work has not been published previously, has not been submitted to another journal while under consideration for Brain, Behavior, and Immunity-Health, and will not be published elsewhere upon acceptance. The manuscript in its current form has been approved by all co-authors.

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Declaration of competing interest

VL-B reported receiving financial support for continuing medical education from Adamed, Advanz Pharma, Angelini, Casen Recordati, Exeltis, Janssen, Lundbeck, Neurocrine Biosciences and Rovi outside the submitted work. GA-H reported receiving personal fees from Janssen outside the submitted work.

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100898.

Data availability

Data will be made available on request.

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5. DISCUSSION

5.1. The rationale of the samples included in the thesis

This thesis is based on research involving patients with schizophrenia from various locations where I have had the opportunity to work, including single-centre, multi-centre, and population-based samples in Spain and Denmark. The nine articles included encompass a range of study designs, primarily focusing on clinical longitudinal cohorts, both prospective and retrospective, while also incorporating cross-sectional studies (see **Table 3**). In total, this thesis includes a total of 8,826 primary individuals (FEP: 310; FES: 6,956; schizophrenia: 730; other psychiatric diagnoses: 615; health controls: 215).

The main hypothesis of this thesis was that immune biomarkers, including WBC counts, their ratios, and CRP, can predict clinical outcomes in patients with schizophrenia and other psychotic disorders. To test this hypothesis, I conducted a series of studies that examined the relationships between these immune biomarkers and various clinical outcomes, such as treatment response, relapse rates, readmissions, and mortality across different stages of the psychotic illness. Each study was designed to address specific aspects of the hypothesis following the natural history of the disease including the FEP, the characteristic multiepisode of schizophrenia, the special group of patients with poor response to conventional antipsychotics and finally the shorter life expectancy in schizophrenia (**Figure 8**).

In the first two articles, I explore biomarkers in the early stages of the disease using data from two multicentre cohorts in Spain. **Article I** focuses on the PEPs cohort, which tracks patients from acute FEP over a two-year follow-up period. We monitored the NLR to investigate its relationship with treatment response and its potential as a predictive marker. In contrast, **Article II** takes the opposite approach, using data from the 2EPS cohort to study patients who have remitted after a FES and follows them over a three-year period to identify factors linked to relapse. My research specifically examines whether WBC counts and ratios can predict relapse.

The next two articles focus on exploring how immune biomarkers can predict treatment response in acutely hospitalized patients, as well as differences in response based on the type of treatment. Both studies use data from the acute hospitalization unit at Hospital Universitari Santa Maria in Lleida. **Article III** examines patients with schizophrenia

experiencing an acute relapse, investigating whether WBC counts, ratios, and CRP levels at admission can predict functional outcomes at discharge. In **Article IV**, I focus on patients with acute psychotic depression, assessing the potential of the NLR to predict treatment response.

Next, I focused on patients who exhibit a poor response to conventional antipsychotics, known as TRS. In **Article V**, I analysed data from a cohort of patients at Hospital Clínic de Barcelona who began clozapine treatment and were followed for eight weeks. Specifically, I explored the predictive value of WBC ratios for assessing the response to the initiation of clozapine.

In addition to examining short-term outcomes, I investigated the long-term outcomes for patients with FES. In **Article VI**, I utilized population-based registry data from Denmark to analyse the relationship between immune biomarkers present during the first episode—specifically WBC counts, ratios, and CRP—and long-term outcomes. These outcomes include mortality, treatment resistance indicated by the use of clozapine or ECT, and rates of readmission. I also compared the predictive ability of these biomarkers for mortality.

Finally, I conducted three cross-sectional studies to explore the relationship between WBC ratios and other immune biomarkers, while considering the impact of confounding factors such as substance use. In **Article VII**, using data from the multicentre FLAMM-PEPs cohort, I examined the association between the NLR and a panel of inflammatory and oxidative/nitrosative stress biomarkers in patients with FEP and HC. In **Articles VIII** and **IX**, I analysed data from the acute hospitalization unit at Hospital Universitari Santa Maria in Lleida. The first study investigated the relationship between WBC ratios and CRP in a cohort of schizophrenia patients, while the second examined the impact of substance use on immune biomarkers in acutely hospitalized psychiatric patients, including those with schizophrenia.

Across the different studies included in this thesis, apart from the significant associations, when possible, I used predictive performance metrics for maximizing generalizability. Area under the curve (AUC) in receiver-operator curves is one of the common metrics used for this purpose, with higher AUC values indicating improved discriminative ability to identify true positives without excessive false positives. In this thesis, as a general reference, the American Psychiatric Association Work Group on Neuroimaging Markers of Psychiatric Disorders suggestion of an AUC >0.8 as a minimally useful threshold

(162) is used as recommended by experts (163). AUC levels are classified by level of discrimination and potential clinical use. [3]

Through these studies, I aimed to provide comprehensive evidence supporting the hypothesis that easily accessible immune biomarkers are valuable predictors of clinical outcomes in patients with schizophrenia and other psychotic disorders across different scenarios. In the following sections, I will discuss the five secondary hypotheses outlined earlier.

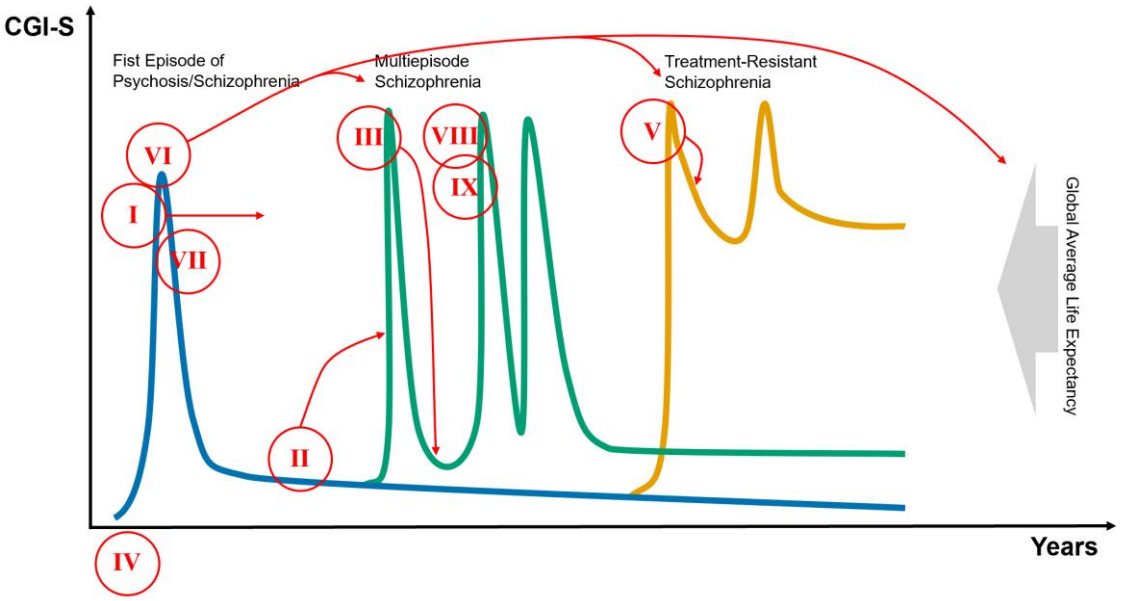

Figure 8. Schematic representation of the articles included in the thesis exploring different scenarios across the clinical course of schizophrenia.

Articles are represented by Roman numerals and positioned along the x-axis according to the disease stage they examine, and along the y-axis based on the severity of the studied sample. Longitudinal studies, both prospective and retrospective, are marked with arrows indicating the outcomes they measure (Articles I, II, V, VI), while cross-sectional studies are shown without arrows (Articles VII, VIII, IX). Article IV is placed outside the graph because it analyses a different population—those with psychotic depression. *Abbreviation*: Clinical Global Impression-Severity (CGI-S).

[3] AUC Interpretation based on de Hold et al.(190) :

- 0.5: No discrimination capability. The test is ineffective for predicting the outcome.
- 0.5 < AUC < 0.7: Poor to fair level of discrimination; the model can distinguish between positive and negative classes, but not reliably for clinical practice.
- 0.7 ≤ AUC < 0.8: Moderate level of discrimination; the model is considered acceptable for predicting outcomes and provides some useful information, but is minimally useful for clinical application.
- 0.8 ≤ AUC < 0.9: Good level of discrimination; the model is highly effective at distinguishing between positive and negative classes, making it useful for clinical application.
- ≥ 0.9: Excellent level of discrimination; the model is very effective, indicating a strong predictive ability and readiness for clinical application.

Table 3: Overview of the articles included in the thesis

Article	Journal	Year of publication	Impact	Sample origin	Population and sample size	Design	Measures	Outcomes	Ref.
I	*Schizophr Bull* PMID : 35876785	2022	IF 2022: 6.6 Psychiatry Q1	Multicentre cohort (PEPs), Spain	FEP: 310 HC: 215	Prospective cohort 2-years follow-up	NLR	Clinical Response (PANSS)	(164)
II	*Schizophr Res* PMID: 38513331	2024	IF 2023: 3.6 Psychiatry Q1	Multicentre cohort (2EPs), Spain	FES: 111	Prospective cohort 3-years follow-up	WBC counts	Relapse (RSWG)	(165)
III	*Brain Behav Immun Health* PMID: 39634076	2024	IF 2023: 3.7 Psychiatry Q1	Single centre (HUSM-Lleida), Spain	SCZ: 354 Other dx: 573	Retrospective cohort Acute hospitalization	WBC counts WBC ratios CRP	Functionality (GAF)	(166)
IV	*J Psychiatr Res* PMID: 34438202	2021	IF 2021: 5.2 Psychiatry Q2	Single centre (HUSM-Lleida), Spain	PD: 50	Retrospective cohort Acute hospitalization	NLR	Functionality (GAF)	(167)
V	*Pharmacopsychiatry* PMID: 38621701	2024	IF 2023: 3.6 Psychiatry Q1	Single centre (HCB), Spain	TRS: 32	Prospective cohort 8-week follow-up	WBC ratios	Clinical Response (PANSS)	(168)
VI	*Brain Behav Immun* PMID: 39097201	2024	IF 2023: 8.8 Psychiatry D1	Population-based register study, Denmark	FES: 6.845	Retrospective cohort 22-years follow-up	WBC counts WBC ratios CRP	Mortality Treatment Resistance Readmission	(169)
VII	*J Psychiatr Res* PMID: 38437766	2024	IF 2023: 3.7 Psychiatry Q1	Multicentre cohort (FLAMM-PEPs), Spain	FEP: 97 HC: 77	Cross-sectional Acute FEP	NLR Immune biomarkers panel	Associations	(170)
VIII	*J Psychiatr Res* PMID: 37515951	2023	IF 2023: 3.7 Psychiatry Q1	Single centre (HUSM-Lleida), Spain	SCZ: 698	Cross-sectional Acute hospitalization	WBC ratios CRP	Associations	(171)
IX	Under Review	-	-	Single centre (HUSM-Lleida), Spain	SCZ: 312 Other: 615	Cross-sectional Acute hospitalization	WBC counts WBC ratios CRP	Associations	

The impact factor and quartile are based on the Journal Citation Report (JCR) for the year of publication. If not available, the immediately preceding year is used. *Abbreviations*: PMID: PubMed reference number; IF: Impact Factor; HUSM-Lleida: Hospital Universitari Santa Maria-Lleida; HCB: Hospital Clínic de Barcelona; FEP: First Episode of Psychosis; FES: First Episode of Schizophrenia; SCZ: Schizophrenia; PD: Psychotic Depression; TRS: Treatment-Resistant Schizophrenia; Dx: Diagnosis; NLR: Neutrophil-to-lymphocyte ratio; WBC: White Blood Cell; CRP: C-Reactive Protein; GAF: Global Assessment of Functioning (172); RSWG: Remission in Schizophrenia Working Group (13). PANSS: Positive and Negative Syndrome Scale (173).

5.2. The predictive value in first stages of the disease (Hypothesis 1)

Studying the early stages of schizophrenia is crucial for understanding its pathophysiology and avoiding confounding factors associated with chronicity and long-term antipsychotic treatment. In our sample from **Article I**, we found that FEP patients had higher mean NLR values compared to matched healthy controls. Although we did not observe a significant correlation between NLR values and PANSS scores or hospitalization, patients who met the RSWG remission criteria at the end of the two-year follow-up had significantly lower mean NLR values than those who did not remit. Additionally, we identified a significant positive correlation between the daily equivalent doses of chlorpromazine (CPZ) and NLR at the end of the study.

Our finding of a non-significant association between NLR and PANSS scores contrasts with the results reported by Steiner et al (98). These discrepancies may partially stem from the fact that the mean PANSS total score in our cohort was notably higher (indicating more severe symptoms) at baseline compared to Steiner's study (75.1 vs. 31.0). We also found no correlation with other indirect markers of severity at baseline, such as the need for hospitalization and its duration.

Perhaps the most significant result of our study is that patients who did not achieve remission at the end of the two-year follow-up exhibited a significantly higher NLR than those who did. Similarly, Labonté et al. (174) reported that NLR decreased following treatment exclusively in the responsive group, but not in those with treatment-resistant schizophrenia. This finding supports the idea that immune system dysfunction is related to treatment response in psychosis (175). Furthermore, in light of the results from Article VII (see Section 5.6), it suggests a potential involvement of stress response pathways in this population.

It is important to note that non-remitted patients had significantly higher mean antipsychotic doses than the remitted group at the two-year follow-up, and a significant correlation was found between antipsychotic dose and NLR. This could potentially confound the results. Despite this, the findings emphasize the importance of studying NLR as a potential marker of severity in the progression of psychotic disorders.

In **Article II**, we found that patients who relapsed within three years after remission from a FES had higher baseline counts of monocytes, lymphocytes, basophils, and eosinophils, along with lower PLR and higher BLR. In our predictive model, elevated monocyte and

basophil counts were associated with an increased risk of relapse, with AUC values of 0.661 and 0.752, respectively.

Our results align with some previous studies, although differences in study design, particularly the timing of sample collection, may account for inconsistencies with others. Our study is unique in that we focused on remitted patients following a FES, so comparisons should be made with caution.

Notably, we observed higher baseline levels of both innate (monocytes, basophils, eosinophils) and adaptive (lymphocytes) immune cells in patients who relapsed. However, only the innate cell counts (monocytes and basophils) were significant predictors of relapse risk. These findings are consistent with literature suggesting activation of both innate and adaptive immunity in schizophrenia (176), but they also support the idea that innate immunity, rather than adaptive immunity, may have greater prognostic value (98).

Despite this, the ROC curve analysis revealed AUC values of 0.66 for monocytes and 0.75 for basophils, indicating poor to moderate discrimination. Both cell types showed low sensitivity and specificity, with AUC values falling below the 0.8 threshold considered minimally useful for clinical application (163). Therefore, while we demonstrated that immune cell counts at remission may play a role in the pathophysiology of relapse, their predictive value in clinical practice remains limited.

5.3. The predictive value in acute hospitalization (Hypothesis 2)

The progression of illness in most patients with schizophrenia is marked by repeated relapses. In our sample from **Article III**, among patients experiencing an acute relapse of schizophrenia requiring hospitalization, higher leukocyte counts were identified as risk factors for non-functional response (GAF change < 40), while a higher PLR was associated with a protective effect, with AUC values of 0.520 and 0.532, respectively. Additionally, higher lymphocyte and platelet counts were identified as protective factors against non-functional remission (GAF < 70 at discharge), with AUC values of 0.617 and 0.589, respectively. However, WBC ratios did not show any significant associations with functional outcomes.

The largest study to date involving 2,598 patients found that higher leukocyte counts predicted poorer responses to antipsychotics, measured by PANSS total scores (Zhang et al., 2024). Our findings align, as leukocyte levels also predicted functional response. This is expected since PANSS and GAF scales are strongly negatively correlated (177), allowing acute responses to be reflected in both symptom and functional changes.

We also identified significant associations for lymphocytes, platelets, and the PLR, all of which were linked to better clinical outcomes. This novel finding has not been previously reported, but it aligns with the results from **Article VI**, where a protective effect of platelets and PLR, along with a trend toward significance for lymphocytes in relation to treatment readmissions, was observed. The mechanisms behind these protective associations are still unclear, but they suggest that these cells may have unique effects. This points to the possibility that immune pathways influence the neurobiology of schizophrenia and its treatment response in distinct ways.

Additionally, we found variations in how specific antipsychotic treatments affected functional outcomes. However, the small sample sizes—10 patients in the clozapine group and 16 in the first-generation group—limit our conclusions about individual medications. Future studies should examine immune biomarkers in patients on monotherapy with larger sample sizes (178).

Finally, it should be noted that the predictive associations showed AUC values around 0.5 to 0.6, indicating a poor level of discrimination and insufficient predictive capability for clinical application (163).

In **Article IV**, we found that high NLR values upon admission are associated with greater clinical improvement, as measured by changes in the GAF during hospitalization, in patients experiencing an acute episode of psychotic disorder. When stratifying the patients, this association was maintained in women, those who received antidepressant treatment with tricyclics and SNRIs, those treated with antipsychotics such as olanzapine or quetiapine, and those who did not receive ECT. This study expands our understanding of the immune system's role in psychotic disorders beyond schizophrenia and suggests that specific psychopharmacological treatments may influence immune responses.

5.4. The predictive value in TRS (Hypothesis 3)

Up to 30% of patients with schizophrenia develop TRS, for which clozapine is the only approved medication. In our sample from **Article V**, we found that pre-treatment values of the NLR and MLR predicted improvement in PANSS-positive symptoms at 8 weeks of follow-up in patients with TRS. However, PLR and BLR did not show significant predictive value. Patients with the highest NLR and MLR levels demonstrated a greater symptom response. Furthermore, clinical responders exhibited higher baseline NLR and MLR, with threshold values of 1.62 for NLR and 0.144 for MLR, achieving AUC of 0.714 and 0.712, respectively, to distinguish between responders and non-responders.

Previous research exploring the association between immune cell counts and clinical response to clozapine initiation has produced mixed results (107,108,174). However, the largest study to dates (n=397) found that individuals with high-normal neutrophil counts were more likely to respond to clozapine over a longer period, as measured by CGI (109). In contrast to previous studies, we utilized WBC ratios, which are believed to better reflect imbalances between innate and adaptive immune pathways (179). Our results align with those of Jones, supporting the role of neutrophils in the response to clozapine.

Furthermore, our findings, in conjunction with GWAS linking genes associated with WBC counts to schizophrenia risk (29,97), suggest that immune mechanisms may play a role in certain subgroups of TRS patients who respond well to clozapine (180).

Importantly, we identified threshold values of approximately 1.62 for NLR and 0.144 for MLR to differentiate between responders and non-responders, with AUC values of 0.714 and 0.712, respectively. While these values indicate acceptable discrimination, they fall below the clinical utility threshold of 0.8 (163). Due to the limited sample size of our study, higher AUC values for blood cell count ratios in predicting response to positive symptoms cannot be ruled out. Further research with larger samples is necessary to confirm these findings, as they could have significant implications for clinical practice.

5.5. The prediction of long-term outcomes (Hypothesis 4)

Predicting long-term outcomes during a FES is a challenging task, and immunological biomarkers may aid in this process. In **Article VI**, we found that higher baseline levels of leukocytes, neutrophils, monocytes, the LLR, NLR, MLR, and CRP were associated with increased all-cause mortality. Conversely, higher levels of lymphocytes, platelets, and the PLR were linked to a decreased risk of mortality. Among these markers, CRP demonstrated the highest predictive value for mortality, with an AUC of 0.84. Furthermore, elevated levels of leukocytes, neutrophils, monocytes, LLR, NLR, and MLR were associated with treatment resistance. Finally, higher platelet counts were associated with a reduced risk of psychiatric readmissions, while LLR was linked to an increased risk of readmissions.

Our finding that CRP has a predictive value for mortality in schizophrenia, with an AUC greater than 0.8, could have significant public health implications. Patients with schizophrenia typically experience a reduced life expectancy of 13-15 years, primarily due to cardiovascular comorbidities (18). Based on these results, screening for CRP levels at the first episode of schizophrenia may help identify patients at risk for premature mortality, enabling targeted interventions to prevent fatal outcomes.

Treatment resistance in schizophrenia is not uniform, as it involves various clinical and neurobiological pathways (38). In **Article V**, we demonstrated that the NLR and MLR predicted improvement upon initiating clozapine. However, in **Article VI**, we examined the ability of immune biomarkers at FES to predict the development of TRS, and found a significant signal for both NLR and MLR. These findings suggest that these ratios may not only help identify patients at risk for treatment resistance but also those who could respond better to clozapine. Nonetheless, to confirm this, the results from **Article V** need to be replicated in a larger sample.

Several factors contribute to readmission in schizophrenia, yet no single biomarker consistently predicts psychiatric readmissions. The protective effect of platelets on readmissions, as noted in Section 5.3 and in **Article III**, contrasts with recent reporting higher platelet counts associated with worse psychiatric outcomes in FEP patients (100). To resolve this discrepancy, further research is urgently needed to better understand the role of platelets in the pathophysiology of psychotic disorders.

5.6. Associations between biomarkers and confounders (Hypothesis 5)

To better understand the prognostic value of immune biomarkers, it is crucial to examine their relationships within established inflammatory pathways. In **Article VII**, we found that NLR values exhibited a moderate positive correlation with the levels of the pro-inflammatory prostaglandin PGE2 and a small but significant positive correlation with cannabis use in patients with acute FEP. In the health control group, NLR values negatively correlated with body mass index (BMI) and positively correlated with tobacco use.

These associations suggest the activation of a nuclear pro-inflammatory stress response pathway in individuals experiencing FEP. PGE2 is the major product of inducible cyclooxygenase-2 (COX-2) and contributes to the stress response by binding to G-protein-coupled receptors and plays a key role in regulating lymphocytes, macrophages, and neutrophils (181). As discussed in **Article I**, higher NLR levels are linked to non-remission after a FEP, indicating that this ratio reflects dysfunction in the stress response, which appears to be involved in clinical remission outcomes.

In **Article VIII**, we found that CRP is significantly, though moderately, associated with NLR, while its associations with PLR and MLR are smaller, and there is no association with BLR. These relationships showed no variation by sex. CRP is primarily produced by hepatocytes and is directly regulated by IL-6 and IL-1β, both of which tend to increase during psychotic exacerbations (see Section 1.3.b). Meanwhile, WBC ratios reflect the balance between innate and adaptive immunity. Given the modest strength of these associations, we propose that WBC ratios should not replace CRP but rather be used alongside it. Future studies should evaluate both biomarkers together to better determine their clinical utility in schizophrenia, potentially aiding in prognosis and treatment decisions.

In **Article IX**, which included a cohort of psychiatric inpatients, we found that recent cannabinoid use was associated with significantly higher levels of leukocytes, neutrophils, and monocytes. When stratified by diagnosis, this association was only maintained in individuals with psychotic disorders. In contrast, recent cocaine use was linked to eosinophilia, while opioid use was associated with significantly lower MLR, regardless of the primary psychiatric diagnosis.

The unique effect of cannabinoids on the immune system in psychotic disorders aligns with the current understanding of the endocannabinoid system in this population (182). It is known to be dysregulated, leading to heightened immune and inflammatory responses, as supported by our findings. Although no differences in NLR were found in this article, the results regarding neutrophil implications align with those reported in Article VII, reinforcing the impact of cannabis on these cells.

5.7. Limitations

When interpreting the results of this thesis, it is important to consider the limitations of the included studies. The analysis includes 8,826 individuals, with some overlap between the cross-sectional and cohort studies. However, this overlap accounts for less than 4% of the total sample and does not affect the cohort studies, which assess the biomarkers' predictive abilities. Furthermore, the observational designs (prospective, retrospective, and cross-sectional cohorts) inherently limit the ability to draw causal conclusions due to confounding variables, lack of experimental manipulation, and potential biases. Although temporal associations are identified and provide a basis for future research, they do not meet key criteria for establishing causality. Further details on these limitations are provided in Section 4 of each article, with the key issues summarized below:

Articles I and II

These articles use data from the PEPs and 2EPs cohorts, both of which share a prospective cohort design. Despite being well-characterized cohorts, they have intrinsic limitations, including vulnerability to residual confounding variables (e.g., physical activity, diet, social contact, and socio-economic status) due to the lack of randomization and participant loss during follow-up. Specific limitations include the naturalistic design of the cohorts, where treatment decisions during follow-up were made by clinicians based on individual needs, resulting in heterogeneity in antipsychotic treatment patterns. High dropout rates during follow-up (40.6% in the PEPs cohort and 49.6% in the 2EPs cohort) likely reduced the ability to detect differences between groups and may have affected statistical significance. Furthermore, data on medication adherence were unavailable, potentially impacting outcomes. Finally, in the PEPs cohort, some patients were already receiving antipsychotic treatment (mean duration: 54.1 days) at study entry, which may have influenced NLR values.

Articles III and IV

These articles follow a retrospective cohort design, which introduces limitations such as reliance on pre-existing data, potential misclassification, and limited control over confounding variables. No symptom-based rating scales were available to assess individual symptoms, restricting the analysis to functionality as measured by the GAF scale. Stratifying by psychopharmacological treatment significantly reduced the sample size, and in Article III, patients on polypharmacy were classified by the antipsychotic with the highest equivalent dose, complicating interpretation for specific drugs. Biomarkers were measured only once, at hospitalization, preventing longitudinal assessments of their relationship with functional outcomes. Lastly, the retrospective design may introduce selection bias, and its observational nature limits causal inference.

Article V

This article employs a prospective cohort design with its intrinsic limitations, including vulnerability to confounding variables and participant loss during follow-up. The small sample size increased the likelihood of type II errors, complicated confounder control, and reduced statistical power for ROC analysis. Additionally, the short follow-up period limited the ability to capture outcomes related to slower-to-improve factors, such as negative symptoms and functionality.

Article VI

This is a retrospective cohort study based on population registers. Its limitations include reliance on secondary data, residual confounding factors, and limited generalizability due to missing individual-level details. Specifically, it was not possible to include a healthy control group, as all individuals in the clinical laboratory information system had prior hospital or primary care contact due to suspected illness. Selection bias was introduced by including only individuals with WBC counts and/or CRP measurements within ±30 days of their schizophrenia diagnosis. Furthermore, data on factors influencing immune cells (e.g., fasting, sleep disturbances, and procedure timing) were unavailable, and potential inter-laboratory differences in measurements cannot be ruled out. Lastly, the study lacked data on smoking, weight, nutrition, and physical activity, all of which could affect immune processes and influence findings.

Article VII

This study follows a cross-sectional design with intrinsic limitations, including an inability to establish causality, susceptibility to recall and selection biases, and a lack of temporal data. Biomarkers were derived from peripheral blood samples, which may not accurately reflect CNS changes. While the biomarkers analysed are part of a canonical stress response pathway, incorporating additional immune markers (e.g., interleukins or CRP) could have provided deeper insights. Lastly, excluding individuals with substance-induced psychotic episodes prevented analysis of the impact of substances beyond cannabis and tobacco.

Article VIII

This study also follows a cross-sectional design with its previously stated intrinsic limitations. Data on key confounding variables, such as BMI and psychotropic medication use, were unavailable. However, the large sample size allowed for control of other confounders, including cardiovascular risk factors, tobacco use, drug abuse, and episode type. No clinical rating scales were used to assess schizophrenia severity, limiting the ability to analyse biomarker-symptom relationships. Additionally, information on disease course variables, such as the duration of untreated psychosis and years since onset, was not collected.

Article IX

This last study is also a cross-sectional study with similar intrinsic limitations. Data on healthy controls and key confounding factors (e.g., BMI, childhood maltreatment, and psychotropic medication use) were unavailable. The study also lacked information on drug consumption frequency, as urine tests reflected only recent use. Immune parameters were assessed at a single time point, preventing analysis of abstinence effects over time. Furthermore, the absence of cannabinoid composition data may have introduced bias. While the total sample size was relatively large (n = 927), subgroup analyses resulted in smaller sample sizes, increasing the risk of type II errors and necessitating cautious interpretation of results.

5.8. Future research lines

Future research on the role of immune biomarkers in the prognostic assessment and treatment of patients with schizophrenia should concentrate on the results that demonstrate the highest predictive performance in this thesis to enhance the clinical utility of subsequent studies.

First, among all the predictive values reported in this thesis, CRP demonstrated the highest predictability, specifically for long-term mortality in patients with FES, achieving an AUC of 0.84, which indicates significant clinical implications. While cardiovascular issues are the leading cause of death in individuals with schizophrenia, it is crucial to investigate the specific causes of death in those with elevated CRP levels compared to a control population, and I am currently analysing this data. Furthermore, although the findings from the Nordic population are noteworthy due to the high quality of their health records, their external validity may be limited for Southern European populations, especially for those under clozapine treatment. Therefore, validating these results in our population is a critical task that I am also working on. If replicated, these findings could support the implementation of routine CRP screenings for patients with FES, guiding targeted cardiovascular prevention programs and testing specific interventions.

Second, pre-treatment NLR and MLR demonstrated AUC values of 0.714 and 0.712, respectively, for predicting clozapine response in TRS. Although these values fall below the clinical utility threshold of 0.8, they still indicate acceptable discrimination. Given that AUC values are significantly influenced by sample size—and our study involved a small sample of only 32 patients—these results could potentially be clinically relevant if tested in a larger and more appropriate cohort. The development of such biomarkers could enhance clinical applicability, especially since there are currently no reliable markers to guide clinical responses to clozapine. I am currently collaborating with the CLOZIN study (https://www.clozinstudy.com/), an international consortium dedicated to sharing data about clozapine treatment and aiming to replicate these findings.

Third, this thesis investigates various WBC ratios—NLR, MLR, ELR, BLR, PLR, and LLR—as potential predictors of longitudinal outcomes, proposing specific cut-off values for each. Despite the potential utility of these ratios, standardized cut-offs to distinguish normal from abnormal levels remain debated, limiting their consistent application and comparability across studies and disorders. Among these biomarkers, NLR has been the

most extensively studied. In healthy populations, the mean NLR is approximately 1.7 (112,183,184), while meta-analyses suggest that values of 5 or higher are associated with poor prognosis in cardiovascular and respiratory conditions (185,186), and values above 10 are often linked to systemic infections or sepsis (46). In psychiatry, establishing NLR cut-offs has been challenging due to small sample sizes and limited control for confounding factors (179). Standardizing NLR levels would enhance its utility as an immune marker, enabling more consistent comparisons across psychiatric and other medical studies. To address this need, I am collaborating with the UK Biobank to access a large sample of patients with schizophrenia, accompanied by detailed comorbidity data. The aim is to determine whether WBC ratios have an independent effect on schizophrenia beyond their association with known risk factors. Additionally, this research seeks to refine WBC ratio cut-offs, enhance their clinical applicability, and promote their adoption as reliable markers across various health domains.

Fourth, our study found a poor level of discrimination for the biomarkers in predicting the response to conventional antipsychotics during acute episodes of schizophrenia (schizophrenia). However, significant methodological problems hindered our results. Therefore, it would be beneficial to investigate this association in a better-characterized sample, ideally under monotherapy conditions. In this context, I am conducting a meta-analysis of individual data using clinical trial data for paliperidone available in The YODA Project (https://yoda.yale.edu/) to test this hypothesis in a controlled population.

Finally, the results of this thesis are based on blood-based biomarkers that exhibit immunological features in the periphery. It is expected that these peripheral immune alterations mirror changes in the CNS and thus affect the neurobiology of schizophrenia. However, this expectation needs to be validated by data. A recent meta-analysis that included 1,679 paired CNS and peripheral samples found a weak but significant correlation between IL-6 and TNF-α, though not for other biomarkers (187). Importantly, samples from psychiatric conditions are underrepresented in the meta-analysis, with neurological cohorts predominating, which limits the ability to draw direct conclusions about schizophrenia. Therefore, studying CNS biomarkers in schizophrenia patients is crucial for understanding the impact of immune dysfunction on the disorder's neurobiology. In this context, I am currently collaborating on a project funded by the Instituto de Salud Carlos III, which focuses on biomarkers of synaptic loss in FEP (PI20/01066; PI: Miquel Bioque).

6. CONCLUSSIONS

Based on the hypotheses outlined in this thesis, we can draw the following conclusions:

1. Elevated white blood cell counts, their ratios, and C-reactive protein levels were associated with various clinical outcomes in schizophrenia spectrum disorders, suggesting their potential as predictive biomarkers.
2. Persistently higher neutrophil-to-lymphocyte ratio values were observed in first-episode psychosis patients who failed to achieve remission over two years, indicating a potential role in early disease monitoring.
3. During acute first-episode psychosis, the neutrophil-to-lymphocyte ratio was linked to proinflammatory pathways associated with the stress response.
4. In remitted first-episode schizophrenia patients, elevated monocyte and basophil counts increased relapse risk but had limited clinical relevance.
5. In acute schizophrenia requiring hospitalization, leukocyte counts, platelet-to-lymphocyte ratio, and lymphocyte and platelet counts were associated with functional outcomes but showed insufficient predictive capability.
6. In treatment-resistant schizophrenia, baseline elevations in neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratios moderately predicted improvements in positive symptoms after clozapine initiation, requiring further validation.
7. Higher C-reactive protein levels at first-episode schizophrenia were significantly associated with long-term mortality risk, demonstrating strong clinical utility for risk stratification.
8. Relationships between immune markers, such as C-reactive protein and white blood cell ratios, varied across clinical contexts and disease stages, highlighting immune dysfunction heterogeneity in schizophrenia spectrum disorders.
9. Substance use, including cannabis, cocaine, and opioids, was linked to specific alterations in immune biomarkers, reflecting immune modulation dependent on substance type and diagnosis.
10. While white blood cell counts and ratios are accessible biomarkers, their predictive accuracy for clinical outcomes, remains limited, requiring further research for refinement in personalized psychiatry.
11. These findings support the immune hypothesis in schizophrenia, demonstrating systemic immune dysregulation's impact on clinical outcomes, though deeper investigation is needed to guide interventions.

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