Reviewer #1:
Scientific Quality: Grade A (Excellent)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (High priority)
Specific Comments to Authors: I congratulate the researchers for their efforts. They have done a good job. The article is mostly well-written and the tables and figures are well developed. In my view the research idea is of great interest and can be a candidate for publication in World Journal of Psychiatry. However, I suggest that the manuscript is proofread by a native English speaker or Editing service. So please make sure there are no English errors.

Thank you for your compliments and support. We have now also improved the language and hope that there are now no English errors.

Reviewer #2:
Scientific Quality: Grade D (Fair)
Language Quality: Grade A (Priority publishing)
Conclusion: Major revision
Specific Comments to Authors: In my view there are some issues with validity and internal consistency which needs to be further addressed. I summarised the appraisal as strengths and weakness. Hope this will help you to improve the manuscript. Please the file.

Thank you for your comments addressing crucial points of validity and internal consistency. We have responded to all of your concerns point by point:

#1 Galectin-3 mediated risk of inflammation in stable schizophrenia, with only possible secondary consequences for cognition
Strength of the study

1. ***Good design to test the hypothesis.***
2. ***Diagnosis done with valid measures (chance of less bias in selection of patients)***
3. ***Clear inclusion and exclusion criteria Ethical approval mentioned (improved internal validity)***
4. ***Assessments were conducted by trained personnel and used valid measures***
5. ***Statistical analysis were appropriate***
6. ***Researchers followed strobe checklist***
7. ***References are appropriate and adequate***
8. ***Introduction and review is adequate***
9. ***Figures are appropriate and well placed***
10. ***Table 1 is appropriate***

Thank you very much for pointing out all the strengths of our study.

#2 Weakness

1. ***The authors need to clearly state how they arrived at the sample size. A power calculation is not done in this study which remains a limitation.***

   In the initial stages of study preparation, we performed a power calculation using G-Power, which is an integral part of the first author's PhD thesis. It has now been included in the methodology and supported with appropriate references (page 7, last paragraph of the *Participant* section).

2. ***PANSS cognitive factors usually measures learned verbal skills rather than cognitive domains.***

   At your suggestion, this aspect is now clarified in the *Clinical Assessment* section and in the last paragraph of the Discussion, taking into account the limitations of the study.

3. ***MOCA is mainly a screening tool. MOCA has been validated for 55-85 year olds and not below that age. Again interpretation based on this is likely to cause significant bias. It is a flaw is this study.***

   We have already stated that the MoCA is primarily a screening tool. We have now additionally stated that it can be used not only in mild cognitive impairment and dementia in the elderly, but also in patients with long-term psychosis, and substantiated this with a recent reference. For our study population, there are not many validated and brief instruments to assess cognitive impairment in schizophrenia. These aspects are now presented in more detail in *the Clinical Assessment* section and in the last paragraph of the Discussion, considering the limitations of the study.

4. ***Authors have not mentioned educational status of participants which determines the MOCA score. Usually below 12 years of education MOCA requires adjustment. This can also affects the results of the study.***

   We have now included the explanation of the educational status of the participants in the text and adjustment for MoCA is not needed.

5. ***It is not clear whether they had an a priori hypothesis in this study. I felt that post-hoc analysis, and results made the authors to look at various possibilities including hypothesis generation which caused some interpretation bias.***

   We wanted to go further in exploring Gal-3 interactions, well beyond measuring serum levels in schizophrenia stabilization. We have now included this statement in the aim of the study in the Introduction.

6. ***A conclusion on cognitive functioning may be done carefully given the study used MOCA which is mainly a screening tool for dementia and PANSS cognitive/disorganisation factors (practically limited number of factors). The PANSS cognitive factor may reflect verbal ability and memory, but is not sufficiently comprehensive to be considered as a replacement for direct assessment of cognitive functioning. (Ehman et al 2004).***

   As stated above, we have now additionally explained that MoCA could be used not only in mild cognitive impairment and dementia in an elderly population but also in patients with long-term psychosis and supported this with a recent reference. We also explained that cognitive function is adequately assessed using the cognitive factor of the PANSS. For our study population, there are not many validated and brief instruments to detect cognitive impairment in schizophrenia. All these aspects are now presented more precisely in the *Clinical Assessment* section and in the last paragraph of the Discussion, considering the limitations of the study.

7. ***I feel that the authors deviated from its main aim(Aim 1) of the study (to look at the relationship of cognitive functions to GL 3.) They finally made a conclusion that GL3 indirectly affects cognitive without substantiating the cognition well. Instead authors went on to talk about new findings and its correlation. They made a last paragraph passive mentioning of negative result.***

   Thank you for your valuable comment and for recognizing the major point of this manuscript. Although some recent studies have established the link between Gal-3 and cognition, we wanted to publish these negative results and conclude that this link might be more indirect and take into account immunometabolic changes in patients with schizophrenia.

   We have now moved the paragraph with the negative results to the beginning of the Discussion, and this now corresponds to the order of presentation in the Abstract.

8. ***Authors mentioned that the patients are on antipsychotic medication and hypnotics/anxiolytics . Potentially these medications can impair cognitive functions. In the design and analysis, nowhere it is mentioned about how these confounders were addressed.***

   Thank you for this observation. We have now addressed this in the limitation of the study, in the last paragraph of the Discussion.

9. ***Authors may have mentioned about the comorbidities of patients in their study. Comorbidities can significantly influence the both the inflammatory markers and the white blood cell count. This can also be a confounder in the study which is not addressed in the study or in analysis.***

   Thank you for this observation. We have now addressed this in the limitation of the study, in the last paragraph of the Discussion.

10. ***Authors did not mention about limitations of their study. The authors may put another paragraph on limitations of the study in additions to its strength.***

    We have now formulated a new paragraph that summarizes all the limitations of this study at the end of the Discussion.

11. ***Overall validity is bit compromised in the study.***

    We hope that this aspect will now be improved once we have incorporated all the necessary changes.

12. ***Suggest a balanced approach in writing the discussion particularly depends on the aims of the study and concluding accordingly.***

    At the suggestion of the reviewer, we have now toned down some statements in line with all the changes we have made in the manuscript.

13. ***Abstract may require minor revision. The main focus in abstract appears to be exploring the correlations of GL3 with cognition and other two factors. But in the main paper focus is more on the other two factors. This might need a minor revision.***

    We have tried to balance the content of the manuscript by organizing it in the way presented in the Abstract.

Reviewer #3:
Scientific Quality: Grade D (Fair)
Language Quality: Grade A (Priority publishing)
Conclusion: Rejection
Thank you for the opportunity to review this interesting paper.
The authors examine the correlation between Gal-3 and with cognition, serum cytokines, and white blood cell count in three-month stably treated schizophrenia patients. I think Gal-3 is a very important indicator, and it might play a role in schizophrenia patients to some extent. However, I have some concerns about the content of the paper.
Considering all your specific comments and those given by two other reviewers and editors we are hoping that we have substantially improved this manuscript and would very much appreciate it if you reconsider your decision.

Specific Comments to Authors:
#1. In result, the author said that "The serum levels of Gal-3, IL-33, sST2, TNF-α, IL-4, IL-6, IL-17, IL-23, IL-1β and TGF-β were examined in SC and HC groups." It is will be clearer to display these results in form or figure.
#2. In this study, there are statistical differences between the groups other indicators except TGF-β? Please clarify.
We have now stated that all these cytokines were measured in the same patient sample, as a part of the same project, and these data have been partially already published (Borovcanin et al., 2018 & Borovcanin et al., 2020), except IL-4, IL-23, TGF-β and IL-1β, which were now additionally measured and important data presented in the figure, to altogether be further included in more complex analyses. Now it is clarified that there are statistically significant differences in serum levels of Gal-3 and TNF-α that are elevated and TGF-β that is lower in these patients.

#3. In the binary logistic model, I don't know what confounders have been adjusted; in addition, the ORs value is close to "1" for TGF-β and Gal-3 in Figure B, but the P value is very small, please check.
We have now given a more detailed explanation for the binary logistic model, which included the presence of illness as a dependent variable and all measured cytokine serum levels as covariates in a stepwise Backward-Wald method, highlighting the particular role of Gal-3 and TGF-β in schizophrenia, also commented on the OR, and we state all this in the Results section. We have now checked the data presented in the Figure 1B.

#4. In linear regression analysis, what confounders have been adjusted? Please clarify.
The linear regression analysis was not adjusted for confounders, and we have now stated this as a limitation of this study.

#5. Do the author correct the impact of other confounders when doing correlation analysis?
We did not correct the impact of other confounders when doing correlation analysis, and we have now stated this as a limitation of this study.

#6. There is no meaningful to only explore the correlation of serum Gal-3 levels with cognition, serum cytokines, and white blood cell count, in contrast, I wonder that Gal-3 levels how to affect these indicators.

We have now given a rationale to explore the correlation of serum Gal-3 levels with cognition, referring to a recent study on Alzheimer's disease. Gal-3 as a conjunction of cognitive functioning and inflammation has not yet been studied in schizophrenia. We think that is necessary to explore further these issues in a larger sample with a much more thorough analysis of confounding factors that have not been in the scope of this manuscript, but these results are valuable to direct us in the future.

Science editor:

In paper aimed Exploring the correlation of serum Gal-3 levels with cognition, serum cytokines, and white blood cell count in three-month stably treated schizophrenia patients. Strength and weakness of the study needs to be addressed in the manuscript. In addition, i suggest that the manuscript is proofread by a native English speaker or Editing service. If possible, authors should redo the native speaker proofreading. This manuscript can publish in World Journal of Psychiatry.
Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade C (Good)

Thank you for your suggestions, we have now addressed the limitations of the study at the end of the Discussion section. We have now redone the native speaker proofreading and done a professional editing service.

Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Psychiatry, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English

language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

Thank you for your suggestion for the improvement of our manuscript. We have now corrected the figure, also redone the native speaker proofreading and done a professional editing service.

All changes are marked in yellow.

Manuscript Type: *Original Article*

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Case-Control Study

Galectin-3 mediated risk of inflammation in stable schizophrenia, with only possible secondary consequences for cognition

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Authors' contributions: Minic Janicijevic S and Borovcanin MM presented the design of this project, recruited the participants, performed the psychological and somatic assessment, collected the samples for laboratory measurements, structured the manuscript and incorporated

all parts of the manuscript. Jovanovic IP, Gajovic NM, and Arsenijevic NN performed the cytokine measurements. Jurisevic MM and Borovcanin MM did the statistical analysis and prepared tables and figures. All authors, especially Debnath M, additionally searched the literature and provided new insights into specific areas of their expertise, made a final revision of the manuscript, and corrected the figures. All authors read, discussed, and approved the final version of the manuscript.

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Abstract

BACKGROUND

Evidence suggests that cytokines cause immune disturbances, shape immunological sequelae later in life, and modulate the risk of schizophrenia. Galectin-3 (Gal-3), a multifaceted molecule of the glycan family, is involved in the formation of the immunological synapse and modulates the signalling pathway and effector functions of T lymphocytes, which are major producers of cytokines. We have previously reported elevated serum Gal-3 levels in stable schizophrenia patients. However, Gal-3 as a link between cognitive functioning and inflammation has not yet been investigated in schizophrenia.

AIM

To investigate the relationship between serum Gal-3 levels and cognitive performance, serum cytokines, and white blood cell count in three-month stably treated schizophrenia patients.

METHODS

Twenty-seven patients with schizophrenia (SC) in remission and 18 healthy volunteers participated in this case-control and correlational study. Clinical assessment was performed using the Positive and Negative Syndrome Scale (PANSS) and the Montreal-Cognitive Assessment (MoCA). The results of previously measured serum levels of Gal-3, interleukin (IL)-33, soluble suppression of tumorigenicity 2 (sST2), tumor necrosis factor-alpha (TNF-α), IL-6 and IL-17 were used for further statistical analyses, and IL-4, IL-23, IL-1β and transforming growth factor-beta (TGF-β) were now additionally measured with a sensitive enzyme-linked immunosorbent assay (ELISA). The number

of leukocytes in the blood and the percentage of neutrophils, lymphocytes, and monocytes were determined with a standardized routine measurement procedure (Sysmex Technology). Statistical analyses were performed using SPSS 20.0 software.

RESULTS

We found no correlation between serum Gal-3 levels and cognitive functioning in schizophrenia patients. A positive correlation was found between the levels of Gal-3 and TNF-α (r = 0.476; p = 0.012), Gal-3 and IL-23 (r = 0.417; p = 0.031), and Gal-3 and sST2 (r = 0.402; p = 0.038). The binary logistic model, which included all nine cytokines measured in this patient sample, indicated the particular role of Gal-3 and TGF-β in the duration of schizophrenia. In the stabilization phase of schizophrenia, we observed a moderate and negative correlation between serum Gal-3 levels and leukocytes (r = -0.449; p < 0.019). Additional linear regression analysis showed a positive correlation between Gal-3 expression and risperidone dose (F 4.467; p < 0.045; r^2 0.396).

CONCLUSION

The combined activity of Gal-3 and proinflammatory cytokines, TGF-β downregulation and lower counts of leukocytes influence the schizophrenia duration. Gal-3 likely manifests indirect immunometabolic regulation of cognition in schizophrenia.

Keywords: schizophrenia, galectin-3, cytokines, leukocytes, antipsychotics

Core tip: In clinical sampling, there is an urge to place the results of biological measurements in a much broader context. Elevated serum Gal-3 levels in schizophrenia have not been studied in relation to other peripheral biomarkers and subsequent neuroinflammation. We found

that Gal-3 contributes to ongoing peripheral systemic inflammation and disease duration in patients with schizophrenia. All of this may be an underlying indirect immunometabolic mechanism for cognitive performance in patients with schizophrenia.

INTRODUCTION

Immune dysregulations during prenatal and postnatal life are increasingly associated with neurodevelopmental disorders and have also recently been shown to be an important etiological construct of schizophrenia [1,2]. Multiple post-mortem brain and neuroimaging studies have also provided evidence for neuroinflammation in schizophrenia [3,4]. One of the best-known hypotheses, proposed by Bechter, links schizophrenia to mild and localized encephalitis [5]. There is strong evidence that cytokines cause these immune disturbances, shape immunological sequelae later in life, and modulate schizophrenia risk. In particular, T lymphocytes are one of the major producers of cytokines, and it has been reported that blood levels of cytokines derived from various lineages of T lymphocytes such as T helper 1 (Th1), Th2, Th17, and regulatory T cells (Treg) are altered in schizophrenia [6,7,8]. Studies have shown that patients with schizophrenia have increased serum concentrations of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) [9,10].

Studies have also shown that Gal-3, a multifaceted molecule in the glycan family, is directly involved in the formation of the immunological synapse and appears to play a pivotal role in modulating the signalling pathway and effector functions of T lymphocytes [11]. It is noteworthy that Gal-3 has both immune and non-immune functions in

the brain. Gal-3 appears to play a neuroprotective role in neuronal tissue and is involved in the reparative processes of brain lesions and ischemia. In contrast, Gal-3 may promote microglia-mediated neuroinflammation and contribute to neuroprogression [12]. Gal-3 increases the secretion of pro-inflammatory cytokines from microglia and astrocytes [13] and is also required for leukocyte recruitment during an acute inflammatory response [14].

Biomarkers that can be conveniently measured in blood may also reflect changes in the central nervous system and dysfunction of the blood-brain barrier (BBB). There is evidence of BBB dysfunction in brain disorders, including schizophrenia. Brain microvascular endothelial cells (BMECs) are a key element of the microvasculature that forms the BBB and shields the brain from toxins and reactive immune cells. However, it is not known whether BMECs themselves are functionally compromised and lead to BBB dysfunction in brain disorders [15]. An increased ratio of cerebrospinal fluid to serum albumin in patients with schizophrenia suggests increased permeability of the BBB [16]. Given the important role of galectins in cell adhesion, migration, polarity, and chemotaxis, it is likely that modulation of galectin levels in BMECs that form the BBB could compromise BBB integrity and consequently contribute to neuroinflammation [17]. Plasma levels of Gal-3 have been shown to be increased after aneurysmal subarachnoid hemorrhage (SAH), and a Gal-3 inhibitor could potentially prevent post-SAH BBB disruption by inhibiting Gal-3 [18].

We have previously reported elevated serum Gal-3 levels in patients with schizophrenia who received stable 3-month antipsychotic therapy [19]. We wanted to go further in exploring Gal-3 interactions and not only measure serum levels during stabilisation of schizophrenia. Recently, such an association between Gal-3 and cognition was found in Alzheimer's disease [20]. In this additional analysis, we tested the hypothesis that serum Gal-3 levels in patients with stable schizophrenia might be related to cognitive functioning and different white blood cell counts and types of cytokines in stable schizophrenia patients. In this

way, we aimed to investigate the possible involvement of this glycan in peripheral systemic inflammation and disease duration, but also its position as a link between cognitive functioning and inflammation, which has not yet been investigated in schizophrenia.

MATERIAL AND METHODS

Participants

Patients with schizophrenia in remission (SC in remission) were recruited in 2016 in the Psychiatric Day Hospital of the Kragujevac Clinical Centre. Participants were between 18 and 65 years old. Diagnoses were made using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) criteria [21] for schizophrenia (F20). The major inclusion criterion was stable mental functioning and adherence to three months of stable antipsychotic depot therapy with risperidone or paliperidone. Add-on therapy for patients included anxiolytics or hypnotics only. A complete medical history was obtained from each patient.

Exclusion criteria were current infections during the three-month remission period, allergies or autoimmune disorders, current anti-inflammatory or antiviral medications, or dual diagnoses of other mental illnesses. Healthy Controls (HCs) were recruited during blood donation at the Blood and Blood Products Service of the Kragujevac Clinical Centre, and controls with a family history of psychosis were excluded. All laboratory measurements and immunoassays were performed at the Centre for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac. The study was conducted after the Ethics Committee of the Kragujevac Clinical Centre gave its approval. Participants were able to give informed consent, and each patient signed the informed consent form before participating in the study.

The study sample was estimated considering the first type error (α) of 0.05 and the power of the study of 0.8 for the two-tailed t-test for two independent samples using the statistical softer G* Power 3.1.9.2.

Considering previous studies and similar methods for measuring serum cytokine levels [22], the minimum number of participants required in each group was estimated to be 14.

Clinical Assessment

Psychological assessment was performed by trained raters. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS) [23]. Cognition was assessed using the cognitive factor of the PANSS (consisting of items P2-N5-G11) [24], which primarily refers to sustained attention, and executive functioning such as mental flexibility and problem-solving as components of executive functioning [25]. In addition, cognitive impairment was assessed using the Montreal-Cognitive Assessment (MoCA) [26], a cognitive screening tool for older population with mild cognitive impairment and dementia that has also been shown to be useful in patients with psychosis [27]. The MoCA test assesses multiple cognitive domains including attention, concentration, executive functions, memory, language, visual-constructive skills, conceptualization, and orientation, with a maximum total score of 30 and a lower limit for normal cognition of 26.

Blood collection and cytokine measurements

Blood samples were taken in the morning (approximately 8 am) after overnight fasting. The blood clot was cut and then centrifuged. After separation, serum samples were stored at -20° until analysis. The results of previously measured serum levels of Gal-3, interleukin (IL)-33, soluble suppression of tumorigenicity-2 (sST2), tumor necrosis factor-alpha (TNF-α), IL-6 and IL-17 [19,28] were used for further statistical analyses, and IL-4, IL-23, IL-1β and transforming growth factor-beta (TGF-β) were now additionally measured using sensitive Enzyme-Linked ImmunoSorbent Assay (ELISA) kits specific for the human cytokines according to the manufacturer's instructions (R&D System, Minneapolis, MB). The procedure has been described in detail previously [19]. Briefly, 96-well plates coated with capture antibody and

incubated overnight were washed with wash buffer and incubated with blocking buffer for 1 hour at room temperature. Serum samples or standard recombinant IL-4/IL-23/IL-1β/TGF-β were added to the plates for 2 hours before a biotinylated detection antibody and streptavidin peroxidase were applied for 1 hour each at room temperature. The plates were developed with substrate reagent for 20 minutes, and the reaction was stopped by addition of 4 mol/L sulfuric acid. The absorbance was read at 495 nm using a microplate reader. The exact concentration of the above biomarkers was measured by interpolating a standard curve with a series of known concentrations according to the manufacturer's instructions. The values of the measured cytokines are expressed in pg/ml. Blood cell populations were determined using a standardized routine laboratory procedure (Sysmex Technology).

Statistical Analysis

Demographic and clinical data were presented descriptively. Various covariates were included in linear and multiple linear regression models to examine the effects of these variables on the results. Pearson's or Spearman's correlation analysis was used to examine the significance of the correlation between serum Gal-3 levels and blood cell counts, serum cytokine levels, and clinical scores and subscores of PANSS and MoCA. To determine the best prediction of serum cytokine levels for the presence of illness, binary logistic regression analysis was performed. A p-value of ≤ 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 20.0. Armonk, NY: IBM Corp.

RESULTS

Demographic and Clinical Characteristics

There were no statistically significant differences in age (p = 0.886) and sex (p = 0.851) between patients (n = 27) and healthy control subjects (n =

18). The demographic and clinical characteristics of the patients were the same as those presented previously [19,28] and are listed in **Table 1**. Among patients with schizophrenia, the duration of illness was 9.95 ± 7.71 years, with 2.18 ± 1.92 years as multiple previous hospitalizations. Most patients were individuals with high school education (n = 22). The mean PANSS total score and subscores, MoCA total score and subscores, and medications taken in the schizophrenia group are shown in **Table 1**.

Differentiation of serum cytokine levels between groups

In this study, lower TGF-β levels (272.09 ± 101.59 vs. 360.41 ± 45.13, p = 0.003) were observed in patients with schizophrenia (**Figure 1A**), with no difference in serum IL-4, IL-23 and IL-1β levels (data not shown). The binary logistic model, which included the presence of illness as a dependent variable and all measured cytokine serum levels as covariates in a stepwise Backward-Wald method, highlighted the particular role of Gal-3 and TGF-β in schizophrenia, both of which have an impact on disease presentation with an odds ratio for Gal-3 1.002 (95% C.I. 1.000-1.004; p = 0.022) and TGF-β 0.982 (95% C.I. 0.9968-0.997; p = 0.015) (**Figure 1B**), suggesting that higher Gal-3 levels are associated with stabilization in later phases of schizophrenia.

Serum Gal-3 levels correlate significantly with pro-inflammatory mediators and risperidone dosing

The correlation between Gal-3 serum levels and cognitive functioning considering MoCA total score, subscores, and PANSS Cog was not significant (data not shown). In addition, we now examined the relationship between systemic Gal-3 levels and cytokines with divergent immune properties. A positive and moderate correlation was observed between Gal-3 and TNF-α (r = 0.476; p = 0.012), Gal-3 and IL-23 (r = 0.417; p = 0.031), and Gal-3 and sST2 (r = 0.402; p = 0.038) levels (**Figure 2**).

Moreover, linear regression analysis revealed a positive correlation between Gal-3 and risperidone dose (F 4.467; $p < 0.045$; r^2 0.396).

Serum levels of Gal-3 inversely correlate with leukocyte count

We also examined the correlation between Gal-3 and the number of leukocytes (neutrophils, lymphocytes, and monocytes) involved in the immune response. A negative correlation was found between Gal-3 and total leukocyte count ($r = -0.449$, $p < 0.019$), with no other significant correlations with the percentages of specific populations.

DISCUSSION

The current study contains several new and interesting findings. One of the salient findings was a significant correlation between serum Gal-3 levels and levels of proinflammatory cytokines in a stable phase of schizophrenia. Serum Gal-3 correlated positively with TNF-α, IL-23, and soluble ST2 in schizophrenia in remission (**Figure 2**) and was associated with downregulation of the counterregulatory cytokine TGF-β and appears to play a role in disrupting leukocyte migration. In addition, the increase in Gal-3 might be influenced by risperidone dosing.

This study was the first to investigate a possible relationship between Gal-3 and cognitive functioning in schizophrenia patients. No correlation was found between serum Gal-3 levels and cognitive performance, suggesting a more indirect immunometabolic regulation of cognition in schizophrenia, as we have recently discussed [12]. It has been demonstrated that pro-inflammatory cytokines and mediators of oxidative stress could influence serum Gal-3 levels, and a reciprocal role of Gal-3 in these cascades could not be excluded [29]. Recently, Dal Lin et al. (2020) [30] pointed out the close relationship and regulatory effect of cognitive functioning on some molecular processes in the human body, including acute attenuation of oxidative stress and inflammation, which inversely affect Gal-3 levels. Based on these findings, Gal-3 may prove to be a potential therapeutic target in schizophrenia.

Currently, there are no studies on the correlation between Gal-3 and pro-inflammatory cytokine levels in schizophrenia patients. In our previous study on the same cohort, we found higher systemic Gal-3 levels [19] and TNF-α [24]. In addition to our study, Kajitani et al. (2017) [31] also reported elevated serum Gal-3 levels in a stable phase of schizophrenia. In one study, Gal-3 was tested for its capacity to induce pro-inflammatory cytokines such as TNF-α and IL-6 from plasmacytoid dendritic and form myeloid dendritic cells isolated from blood. This lectin was found to activate both, TNF-α and IL-6 [32]. In addition, a preclinical model of intracerebral haemorrhage (ICH) also demonstrated increased expression of Gal-3 in perihematomal brain regions after ICH and Gal-3-induced release of IL-6, suggesting a role for Gal-3 in inflammatory responses after ICH [33]. These findings suggest the hypothesis that neuronal damage could be followed by inflammation involving Gal-3. The elevated serum Gal-3 levels observed in schizophrenia patients in the current study could lead to BBB disruption and contribute to the persistence of mild chronic neuroinflammation suspected in schizophrenia.

In particular, somatic comorbidities common in schizophrenia, such as obesity, hyperlipidaemia, dyslipidaemia and type 2 diabetes, could be monitored by measuring Gal-3 [34]. Gal-3 correlates positively with obesity and inflammation, as measured by the inflammatory markers IL-6 and C-reactive protein (CRP) [35]. Contrary to this finding, the IL-6 axis was not active in this phase and in the specific subpopulation of patients, but rather overweighted type-1 immune response with representative TNF-α. Taken together, these findings suggest potential systemic inflammatory properties of Gal-3 through its interactions with proinflammatory markers in schizophrenia that contribute to immunometabolic processes in schizophrenia.

The association of Gal-3 and sST2 and their changes at follow-up with the development of heart failure in patients with ST-segment elevation myocardial infarction showed that the levels of Gal-3 and sST2 were significantly increased at one-year follow-up [36]. Interestingly, the

increased serum Gal-3 concentration correlated with the production of IL-17 and exhibited a significant correlation with neutrophil/lymphocyte ratio, white blood cell count, and CRP, but inversely correlated with the production of IL-10 and IL-12 in patients with untreated colorectal cancer [37]. Some findings suggest that Gal-3 is required to efficiently recruit leukocytes during an acute inflammatory response [38]. These findings may indicate the diverse role of Gal-3 in this schizophrenia chronic inflammation, as we have previously discussed that Gal-3 plays a predominant role in the resolution of inflammation [12]. In chronic SC, our studies have shown that serum Gal-3 levels are elevated and that Gal-3 is negatively correlated with leukocyte count. This lower leukocyte count may be related to the decline in immunity of patients with SC in later stable phases and their greater susceptibility to infection.

Although the Gal-3 signalling pathway is not well understood, Gal-3 can be secreted into the extracellular space, where it can interact with different structures such as cell surface and extracellular matrix glycoproteins [39]. In autoimmune neuroinflammation, endogenous Gal-3 may potentiate its severity by decreasing the frequency of regulatory T cells, controlling IL-10 production, and modulating Notch activation [40]. The Notch and TGF-β signalling crosstalk, which plays an important role in regulating endothelial and neural development [41], could also be influenced by Gal-3. Our findings might shed important light on the Notch- TGF-β axis in schizophrenia (**Figure 1B**). As for TGF-β, our previous data indicate that serum levels of TGF-β are significantly increased in patients with schizophrenia in relapse and first-episode psychosis compared to healthy subjects [42,43]. However, in the current study, significantly lower TGF-β levels were observed in SC patients in remission compared to a group of healthy control subjects (**Figure 1A**), suggesting that TGF-β levels vary during the course of schizophrenia.

Regarding the possible influence of antipsychotics, a recent *in vitro* study reported that the atypical antipsychotic risperidone reduced the production of pro-inflammatory cytokines by lipopolysaccharide-stimulated glial cells but had no effect on IL-10 [44]. However,

paliperidone increased TGF-β and IL-10 during acute stress and during prolonged chronic stress [45]. Our recent hypotheses about the involvement of antipsychotics in the processes of glycosylation can be explained by the effects of their higher doses on serum Gal-3 levels. The findings of the current study suggest that higher doses of prescribed risperidone may lead to an increase in Gal-3 levels. Whole-serum proteins show increased glycosylation after antipsychotic use, indicating the usefulness of these processes for understanding the pathogenesis and monitoring the treatment of patients with schizophrenia [46,34].

A higher percentage of Gal-3-expressing innate and adaptive immune cells in the lamina propria was observed in patients with comorbid ulcerative colitis and metabolic syndrome [47]; this encouraged us to explore other immune biomarkers in patients with schizophrenia. N-acetylcysteine (NAC) has been proposed for the adjunctive treatment of schizophrenia and ulcerative colitis [48]. Oral intake of NAC was shown to lower inflammatory biomarkers, CRP and Gal-3 in patients with acute myocardial infarction receiving fibrinolytic therapy [49]. Preliminary results indicated the usefulness of NAC in improving all domains of schizophrenia functioning [50].

As a limitation of our study in terms of cognitive assessment, we must consider that only specific domains of cognitive functioning were assessed, using available validated and brief instruments to detect cognitive impairment in schizophrenia in our population. Although we tried to exclude all somatic states, we should be aware that comorbidity and psychotropic medication could influence the results of both cognitive functioning and serum measurements. We believe that it is necessary to investigate these issues further in a larger sample with a much more thorough analysis of confounding factors, which has not been done within the scope of this manuscript, but these results are valuable to guide us in the future.

CONCLUSION

In clinical sampling, there is an urge to place the results of biological measurements into a much wider concept. Higher serum levels of Gal-3 in schizophrenia have not been explored in interaction with other peripheral biomarkers reflecting possible inflammatory changes. We observed that Gal-3 contributes to ongoing peripheral systemic inflammation and disease duration in patients with schizophrenia. Moreover, its influence on BBB permeability and consequent neuroinflammation should be explored.

Our data revealed some new complex roles of Gal-3, such as its possible involvement in neuroinflammation and cognitive processing, contributing to a better understanding of the specific immune profile in patients with schizophrenia. Inflammation also appears to be the potential pathway by which Gal-3 may affect cognitive functioning in schizophrenia. The efficacy of antipsychotics could be improved and their adverse effects corrected if the role of Gal-3 in glycosylation processes were considered. These findings provide a rationale for further strategies targeting Gal-3 for therapeutic intervention in schizophrenia.

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Figure 1. Transforming growth factor-beta and galectin-3 levels impact the illness (A and B).

Figure 1 Legend. Lower transforming growth factor-beta (TGF-β) levels (272,09 ± 101,59 vs. 360,41 ± 45,13 pg/ml, p = 0.003) were measured in patients (**A**). These parameters of serum concentrations of galectin-3 (Gal-3) and TGF-β both had an impact on disease presentation (**B**).

Figure 2. Correlations of serum concentrations of galectin-3 with proinflammatory mediators.