

Early neutrophil trajectory following clozapine may predict clozapine response - Results from an observational study using electronic health records

Jones, Rowena; Morales-Munoz, Isabel; Shields, Adrian; Blackman, Graham; Legge, Sophie E; Pritchard, Megan; Kornblum, Daisy; MacCabe, James H; Upthegrove, Rachel

DOI:

[10.1016/j.bbi.2023.07.012](https://doi.org/10.1016/j.bbi.2023.07.012)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Jones, R, Morales-Munoz, I, Shields, A, Blackman, G, Legge, SE, Pritchard, M, Kornblum, D, MacCabe, JH & Upthegrove, R 2023, 'Early neutrophil trajectory following clozapine may predict clozapine response - Results from an observational study using electronic health records', *Brain, Behavior, and Immunity*, vol. 113, pp. 267-274. <https://doi.org/10.1016/j.bbi.2023.07.012>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

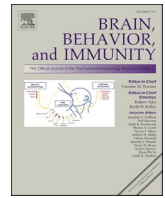
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.



Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Early neutrophil trajectory following clozapine may predict clozapine response – Results from an observational study using electronic health records

Rowena Jones^{a,b,*}, Isabel Morales-Munoz^a, Adrian Shields^c, Graham Blackman^{d,h}, Sophie E. Legge^e, Megan Pritchard^f, Daisy Kornblum^g, James H. MacCabe^{g,h,1}, Rachel Uptegrove^{a,i,1}

^a Institute for Mental Health, School of Psychology, University of Birmingham, UK^b Birmingham and Solihull Mental Health Foundation Trust, UK^c Clinical Immunology Service, University of Birmingham, UK^d Department of Psychiatry, University of Oxford, Warneford Hospital, OX3 7JX, UK^e Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, Cardiff, UK^f Norwich Medical School, University of East Anglia, UK^g King's College London (Institute of Psychiatry, Psychology and Neuroscience), London, UK^h Department of Psychosis Studies, King's College London, and South London and Maudsley NHS Foundation Trust, UKⁱ Early Intervention Service, Birmingham Women's and Children's NHS Trust, UK

ARTICLE INFO

Keywords:

Treatment resistant schizophrenia
Clozapine
Neutrophils
Immune response
Biomarkers

ABSTRACT

Background: Clozapine has unique effectiveness in treatment-resistant schizophrenia and is known to cause immunological side-effects. A transient spike in neutrophils commonly occurs in the first weeks of clozapine therapy. There is contradictory evidence in the literature as to whether neutrophil changes with clozapine are linked to treatment response.

Aims: The current study aims to further examine the neutrophil changes in response to clozapine and explore any association between neutrophil trajectory and treatment response.

Methods: A retrospective cohort study of patients undergoing their first treatment with clozapine and continuing for at least 2 years identified 425 patients (69% male/31% female). Neutrophil counts at baseline, 3 weeks and 1 month were obtained predominantly by linkage with data from the clozapine monitoring service. Clinical Global Impression-Severity (CGI-S) was rated from case notes at the time of clozapine initiation and at 2 years. Latent class growth analysis (LCGA) was performed to define distinct trajectories of neutrophil changes during the first month of treatment. Logistic regression was then conducted to investigate for association between the trajectory of neutrophil count changes in month 1 and clinical response at 2 years as well as between baseline neutrophil count and response.

Results: Of the original cohort, 397 (93%) patients had useable neutrophil data during the first 6 weeks of clozapine treatment. LCGA revealed significant differences in neutrophil trajectories with a three-class model being the most parsimonious. The classes had similar trajectory profiles but differed primarily on overall neutrophil count: with low, high-normal and high neutrophil classes, comprising 52%, 40% and 8% of the sample respectively. Membership of the high-normal group was associated with significantly increased odds of a positive response to clozapine, as compared to the low neutrophil group [Odds ratio (OR) = 2.10, p-value = 0.002; 95% confidence interval (95% CI) = 1.31–3.36]. Baseline neutrophil count was a predictor of response to clozapine at 2 years, with counts of $\geq 5 \times 10^9/l$ significantly associated with positive response (OR = 1.60, p-value = 0.03; 95% CI = 1.03–2.49).

Conclusions: Our data are consistent with the hypothesis that patients with low-level inflammation, reflected in a high-normal neutrophil count, are more likely to respond to clozapine, raising the possibility that clozapine exerts its superior efficacy via immune mechanisms.

* Corresponding author at: Mary Seacole House, Lodge Road, Birmingham B185SD, UK.

E-mail address: rowena.jones1@nhs.net (R. Jones).

¹ Authors contributed equally.

<https://doi.org/10.1016/j.bbi.2023.07.012>

Received 2 May 2023; Received in revised form 11 July 2023; Accepted 22 July 2023

Available online 24 July 2023

0889-1591/© 2023 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The pathogenesis of schizophrenia is far from fully understood, but it has become clearer in recent years that inflammation may play a significant role (Miller and Goldsmith, 2019). Until recently neutrophils have been considered primarily to be short-lived, non-specific cells which contribute to the innate immune response. However, interest in the role of neutrophils has intensified and they are now understood to carry out a wide range of functions in the immune system (Malech et al., 2014). A recent meta-analysis has shown that neutrophil counts are elevated in schizophrenia compared to controls (Jackson and Miller, 2020). Higher total white cell counts (of which neutrophils predominate) have been found to be associated with higher symptom levels in schizophrenia (Fan et al., 2010) and raised neutrophil counts have also been seen in first episode psychosis with an improvement of positive symptoms correlating with declining neutrophil scores (Steiner et al., 2019). Patients with persistent positive symptoms may show a more pronounced inflammatory process (Goldsmith et al., 2016). Treatment resistant schizophrenia (TRS) is defined as a failure to respond adequately to two adequate trials of antipsychotic medication (Meltzer, 1997). TRS may be categorically distinct from treatment responsive schizophrenia with abnormalities primarily in glutamate rather than in dopamine transmission (Gillespie et al., 2017), akin to more well-characterised neuro-immune disorders (Levite, 2017; Kayser and Dalmau, 2016; Sheldon and Robinson, 2007). Clozapine has superior efficacy to conventional antipsychotics in the management of positive symptoms in TRS (Siskind et al., 2016) and on initiation can result in a wide range of immunologically mediated effects (Røge et al., 2012; Siskind et al., 2020; Regen et al., 2017). Recent studies have shown that clozapine is associated with acquired immunoglobulin deficiency (Ponsford and Jolles, 2019; Lozano et al., 2016) which may explain why patients established on clozapine have higher rates of infections, particularly pneumonia (Leon et al., 2020; Schoretsanitis et al., 2021; Nielsen et al., 2009), although pneumonia may also be caused by other adverse effects such as sedation and sialorrhea (de Leon et al., 2020). Studies have consistently shown increases in pro-inflammatory cytokines, most notably interleukin (IL)-6, in patients prescribed clozapine (Löffler et al., 2010; Kluge et al., 2009; Maes et al., 1997; Pollmacher et al., 1996), with limited longitudinal data indicating changes in cytokine levels related to clozapine response (Lü et al., 2004).

Clozapine is known to cause a range of blood dyscrasias, most notably agranulocytosis (Mijovic and MacCabe, 2020). However, a transient increase in neutrophils is more common than a decrease (Hummer et al., 1994; Alvir et al., 1995; Deliliers, 2000; Al et al., 2012; Lee et al., 2015; Fabrazzo et al., 2017), albeit with reported rates of leucocytosis varying widely. This variation appears to be mainly due to differences in classification of leucocytosis. The largest study to date included 2,404 patients (Deliliers, 2000) and reported that 7.7% of patients had a total white cell count greater than $15 \times 10^9/l$. Other studies report rates closer to 20%, using a lower threshold of neutrophils greater than $7 \times 10^9/l$ (Al et al., 2012; Fabrazzo et al., 2017). In most studies, a spike in neutrophils occurs early in the course of treatment, typically after two to three weeks, however other evidence suggests this may occur over six weeks (Blackman et al., 2021; Deliliers, 2000; Al et al., 2012; Lee et al., 2015). A systematic review of neutrophilia with clozapine therapy (Paribello et al., 2021) concluded that the finding was likely an epiphenomenon and potentially related to smoking. However, it is also possible that an elevation in neutrophils may be directly related to treatment response. In a retrospective study (Fabrazzo et al., 2017), reviewed the weekly blood counts of a sample of 135 patients who had commenced clozapine and found that the development of neutrophilia greater than $7 \times 10^9/l$ was significantly associated with response to clozapine after 18 weeks of treatment (Fabrazzo et al., 2017). Another retrospective study by (Blackman et al., 2021) found no association between peak neutrophil count and treatment response at 12 weeks in a sample of 188 patients (Blackman et al., 2021). These conflicting

findings may be explained by different outcome measures and response rates in the two studies as well as by the relatively small sample sizes and differences in patient demographics. Given the clear evidence in support of clozapine causing an increase in neutrophil count in the early phase of treatment, which coincides with its clinical efficacy, further study of the potential role of neutrophils in clozapine response is warranted. Early neutrophil count would be a particularly useful biomarker for predicting response to clozapine, as it is already routinely monitored in clinical practice.

In summary, there is evidence that immune dysfunction may be relevant in some patients with schizophrenia, including involvement of innate actors such as neutrophils. The immunomodulatory effects of clozapine alongside its superior efficacy in TRS may indicate the presence of a subgroup of patients with immune dysregulation with reduced responsiveness to conventional antipsychotics. Neutrophil changes have been shown to occur in the first weeks of clozapine treatment and there is tentative evidence to suggest an association between its immunomodulatory effects and its clinical efficacy. As such, early neutrophil counts may be an accessible potential marker of clozapine response.

Using clinically representative data, we aimed to explore:

- 1) the early longitudinal neutrophil response to clozapine exposure.
- 2) the association between neutrophil trajectory and clinical outcome.

We hypothesised that early elevated neutrophil response would be associated with greater clinical improvement.

2. Methods

2.1. Participants

This study is based on a retrospective cohort study using data from the South London and Maudsley (subsequently referred to as ‘The Maudsley’) NHS Foundation Trust case register, which comprises complete anonymized patient electronic records from 1st January 2007 onwards. Data was accessed using the Clinical Records Interactive Search (CRIS) system, for which methodology has been published previously (Fernandes et al., 2013; Perera et al., 2016). The Maudsley is the largest mental health trust in the UK, serving a predominantly inner-city population of approximately 1.3 million people. Use of CRIS as an anonymised resource for secondary analysis has been approved by the Oxfordshire Research Ethics Committee (08/H0606/71). The current study was approved by the NIHR BRC CRIS oversight committee (application number 21–073).

Participants in this study came from a previously identified retrospective cohort of $n = 661$ patients who commenced clozapine over a 10-year period between 1st January 2007 and 31st December 2016. A full description of how the cohort was identified can be found elsewhere (2022). From the original cohort a sub-sample of patients were identified ($n = 425$) who were still taking clozapine 2 years later. Clinical Global Impression – severity (CGI-S) scores (Busner and Targum, 2007) were recorded at baseline and at 2 year follow up for this group. CGI-S scores were rated retrospectively by reviewing patient records. Ratings were carried out by an experienced consultant psychiatrist (RJ). Baseline and 2-year scores were rated separately, with records for outcome scores restricted to the period 6 months pre and 6 months post the 2-year end point.

The final cohort consisted of all patients who were still taking clozapine at 2 years, had sufficient clinical data available to reliably complete CGI scores pre clozapine and at two years, and who had useable neutrophil data available during the first 6 weeks of clozapine treatment (see flowchart Fig. 1).

2.2. Measures

Neutrophil counts for the cohort were primarily extracted using

linkage with Zaponex Treatment Access System (ZTAS) data, ZTAS being the clozapine monitoring service used by the Maudsley throughout the time period of the cohort (ZAPONEX; Teva UK, Harlow, United Kingdom). For patients for whom ZTAS data was not available, neutrophil counts were extracted from CRIS data using structured lab results data. As this was only available for patients from 2013 onwards, a manual review of free text data was conducted to extract neutrophil counts if data from ZTAS and structured lab results were unavailable.

Neutrophil counts were collected at three time points: at baseline (before clozapine was commenced), 3 weeks, and at 1 month post clozapine initiation, with tolerance of ± 1 week. These time points were chosen in accordance with the literature available on the timing of the peak neutrophil count following clozapine (Blackman et al., 2021).

The main outcome measure used for the study was response to clozapine at 2 years, defined as a reduction in CGI-S score by at least 2 points from the start of the study period. CGI-S uses a Likert scale from 1 to 7 to assess overall illness severity, with lower scores indicating lower

levels of symptoms.

Outcome data was collected as part of the earlier study, which took place prior to the linkage with ZTAS, therefore CGI-S ratings were conducted blind to neutrophil scores.

2.3. Covariates

Data for significant covariates, which could affect both neutrophil count and outcome scores, were also collected. Covariates included 1) age, 2) sex, 3) ethnicity (UK census categories collapsed into four groups - white, black Caribbean, black other, mixed/other – to reflect the demographics of the study catchment area) and 4) medical illness (number of medical admissions during the two-year study period; 0, 1 or more than 1).

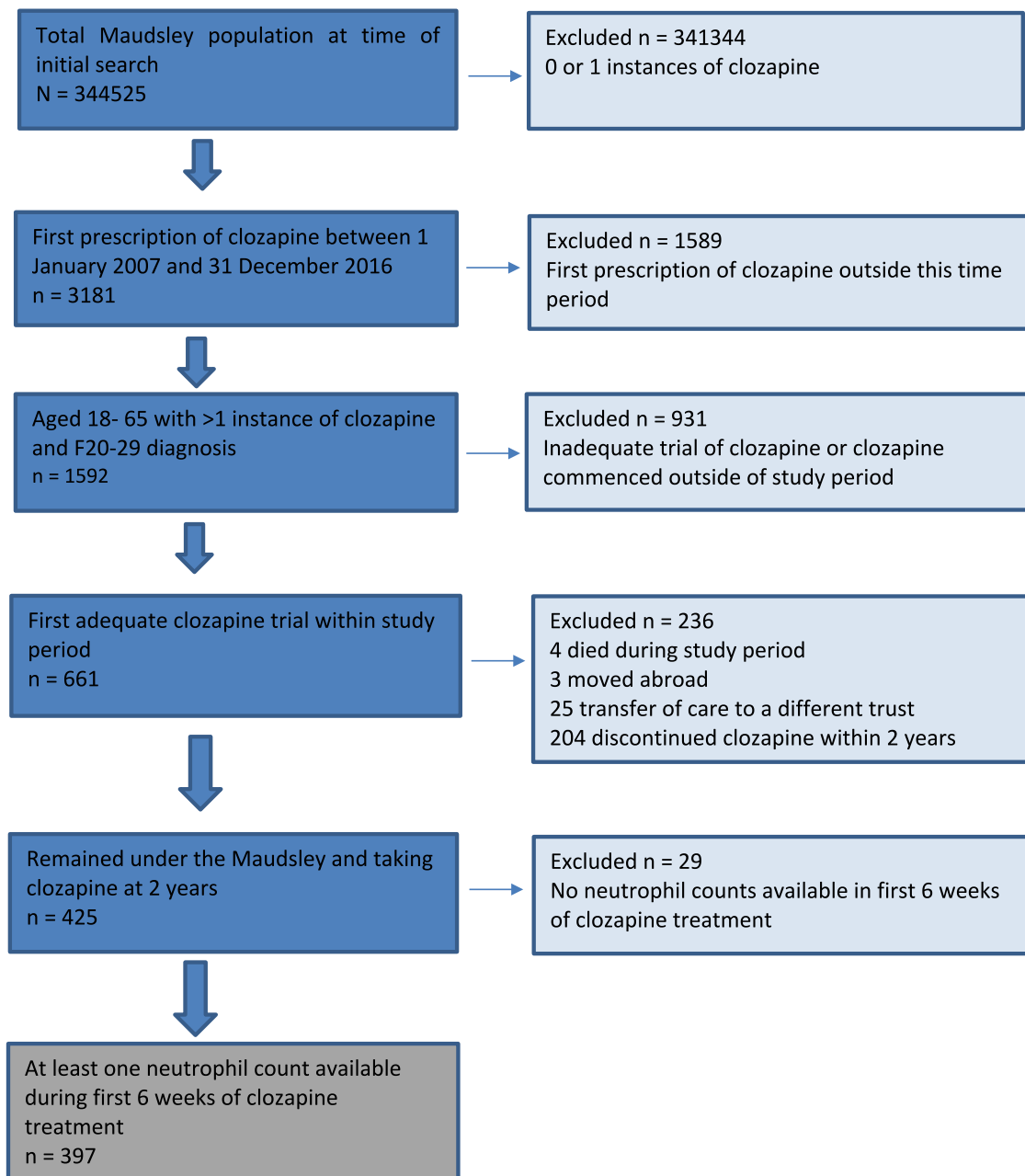


Fig. 1. Cohort identification.

2.4. Statistical analysis

First, Latent Class Growth Analyses (LCGA) (Jung and Wickrama, 2008) was conducted using M plus v8 (Muthén and Muthén, 1998), to detect trajectories of neutrophil response to clozapine across the time points (baseline, 3 weeks post clozapine and 1 month post clozapine). Five models were fitted, testing performance of two to six classes. The best fitting classification model was chosen according to fit indices (i.e., Bayesian Information Criteria [BIC] (Schwarz, 1978) and Vuong-Lo-Mendell-Rubin [VLMR] test) (Lo et al., 20011). Lower BIC values suggest a better model fit. A significant VLMR value ($p < 0.05$) suggests that a K-class model fits the data better than a (K-1) class model. Entropy, a measure of the degree of separation between classes (Ramaswamy et al., 1993), was also used to select the best model fit; entropy with values approaching 1 indicates clear delineation of classes. Finally, to decide the optimal class solution, an emphasis was placed on large enough group sizes (i.e., >2% of the sample) and clinically relevant and informative interpretation. Missing values due to attrition were handled by the Full Information Maximum Likelihood estimation method (Arbuckle et al., 1996).

Secondly, logistic regression was performed using STATA version 15 (StataCorp. StataCorp., 2017), to investigate the associations between neutrophil classes (obtained with LCGA-as the exposure) and treatment response (as the outcome), controlling for age, sex, ethnicity and medical comorbidity. Class 1 was used as the reference group to which the other two classes were compared.

Finally, logistic regression was performed to investigate for association between baseline neutrophil count (prior to clozapine initiation) and treatment response, adjusted for age, sex, ethnicity and medical comorbidity. Baseline neutrophil count was recorded as a dichotomous variable with higher or lower neutrophil groups, using a neutrophil count $\geq 5 \times 10^9/L$ as the cut-off, to include patients with high-normal counts in the higher group.

3. Results

Summary statistics for the sample are shown in Table 1.

3.1. Latent classes of neutrophils across time points

There was minimal missing data in the study (0.4%).

Table 2 shows VLMR, BIC, and entropy for all models assessed (2–6 classes), tested for neutrophil counts at baseline, 3 weeks and 1 month. The 3-class model was selected based on goodness of fit indices. The 2-class and 3-class models were the only models reporting significant VLMR p-values, which is one of the requirements for the selection of the model. In this case, a significant VLMR p-value for 3-class model indicated that the three-class model gives significant improvement in model fit over the 2-class model. Furthermore, the BIC value for the 3-class model was lower than the 2-class. Finally, all the classes from the 3-class model included a sample size greater than 2%. The selection of the 3-class model was reviewed by a clinical immunologist (AS), who corroborated its clinical validity.

The 3 derived classes of neutrophil counts from the 3-class model, and the sample size for each class are shown in Fig. 2. Briefly, Class 1 (blue) represented low to normal neutrophil counts and included 207 patients (52.1%), Class 2 (green) represented high-normal neutrophil counts of around $6 \times 10^9/L$ and included 158 patients (39.8%) and Class 3 (red) represented high neutrophil counts and included 32 patients (8.1%).

The demographics of the three classes are shown in Table 3. There were significant differences between the three classes in relation to age, ethnicity, medical co-morbidity and baseline CGI-S scores.

Table 1
Characteristics of the sample (n = 397).

Characteristic	Descriptor	Number (percent)
Sex	Male	273 (68.8)124
	Female	(31.2)
Ethnicity	White	153 (38.5)34
	Black	(8.6)144
	Caribbean	(36.3)66
	Black other	(16.6)
CGI-S score start	Mixed/other	
	1–4	42 (10.6)170
	5	(42.8)
	6	168 (42.3)17
	7	(4.3)
CGI-S score end	1	12 (3.0)40
	2	(10.1)141
	3	(35.5)141
	4	(35.5)55
	5	(13.9)8
	6–7	(2.0)
Medical admissions during study	0	278 (70.0)75
	1	(18.9)44
	>1	(11.1)
Baseline neutrophil count ($\times 10^9/L$)	≥ 5	139 (35.0)258
	<5	(65.0)
Characteristic	Descriptor	Mean (standard deviation/ range)
Age	Years	35.3 (10.8)
Neutrophil count	Pre-clozapine	4.6 (2.0/1.4–14.9)5.1
	3 weeks	(2.3/1.1–14.4)4.9
	1 month	(2.2/1.5–12.2)

CGI – S. Clinical Global Impression – Severity scale. 1 = normal, not at all ill; 2 = borderline mentally ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = among the most extremely ill patients.

Note Some cells have been collapsed due to CRIS rules which state that no cells <5 can be reported without special permission to avoid identification.

Table 2
Model section.

Model	BIC	VLMR-P	Entropy
2 classes	4899.712	<0.001	0.810
3 classes	4804.528	0.005	0.793
4 classes	4766.063	0.264	0.846
5 classes	4751.748	0.731	0.861
6 classes	4732.532	0.064	0.838

Bold print indicates preferred model.

BIC – Bayesian Information Criteria. VLMR-P – Vuong-Lo-Mendell-Rubin test.

3.2. Regression model of neutrophil classes and clozapine response

Logistic regression found that the odds of a positive response to clozapine was significantly increased in patients in class 2 (medium neutrophil group) compared to class 1 (low neutrophil group) [Odds ratio (OR) = 2.10, p-value = 0.002; 95% confidence interval (95% CI) = 1.31–3.36]. The odds of a positive response also increased for class 3 (high neutrophil group) compared to class 1, but the results were not significant (Table 4).

3.3. Regression model of baseline neutrophil count and clozapine response

Using baseline neutrophil count as a dichotomous variable (higher versus lower neutrophil count) with a cut off value of $\geq 5 \times 10^9/L$, logistic regression analysis showed that a higher neutrophil count prior to clozapine initiation was associated with greater clozapine response, adjusted for age, sex, ethnicity and medical admissions [Odds ratio (OR) = 1.68, p-value = 0.03; 95% confidence interval (95% CI) = 1.06–2.06]. Overall, 68% of the higher neutrophil group responded to clozapine compared to 56% in the lower neutrophil group.

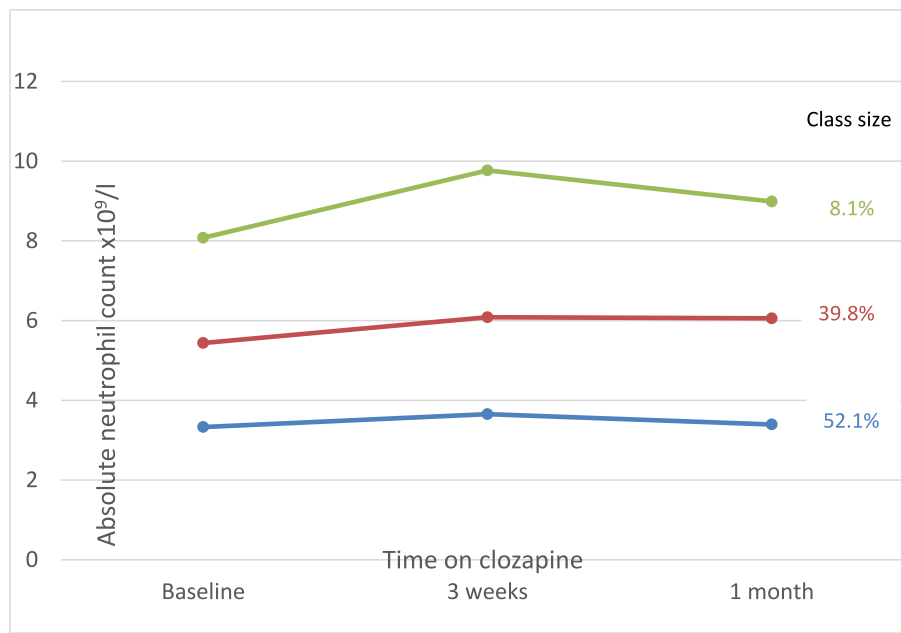


Fig. 2. Class sizes and mean neutrophil counts for each class for the three-class model, across time points.

Table 3

Characteristics of the three classes derived by LCGA (Latent Class Growth Analysis) Class 1 = low neutrophils. Class 2 = high-normal neutrophils Class 3 = high neutrophils.

	Class 1n = 207	Class 2n = 158	Class 3n = 32	F	p
Mean age	33.3	36.8	40.5	9.10	0.00
Male Sex	71%	66%	69%	0.56	0.57
White ethnicity	27.5%	51.3%	46.9%	11.74	0.00
1 or more medical admissions	25.1%	33.5%	43.7%	3.11	0.05
Baseline CGI-S score	5.30 (5.20–5.41)	5.51 (5.40–5.62)	5.4 (5.22–5.65)	3.28	0.04

Table 4

Logistic regression of neutrophil class on clozapine response (n = 397). Adjusted values are adjusted for age, sex, ethnicity and medical admissions.

	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Class 1				
2 versus 1	2.04 (1.32–3.16)	0	2.10 (1.31–3.36)	0
3 versus 1	1.11 (0.53–2.35)	0.78	1.42 (0.63–3.18)	0.4
Age	0.98 (0.96–1.00)	0.05	0.97 (0.95–0.99)	0.01
Sex	0.69 (0.43–1.04)	0.08	0.57 (0.35–0.91)	0.02
Ethnicity White				
Black	1 (0.46–2.17)	1	1.02 (0.45–2.30)	0.97
Caribbean			(0.40–1.11)	
Black other	0.66 (0.42–1.06)	0.08	0.88 (0.47–1.63)	0.12
Mixed other	0.46 (0.46–1.52)	0.56		0.68
Medical admissions 0				
1	1.03 (0.61–1.75)	0.9	0.95 (0.55–1.64)	0.86
>1	0.56 (0.30–1.07)	0.08	(0.30–1.19)	0.14

OR – odds ratio.

4. Discussion

The study examined neutrophil trajectories with clozapine exposure across the first month of treatment and their association with outcome. Our hypothesis was that neutrophil changes in response to clozapine

may be driving clinical outcome, however this was not upheld. Rather, using latent class growth analysis, we identified three stable classes of neutrophil counts detected across the time points. Neutrophil trajectory was divided into low, medium or high values, with differences present from baseline. Using class 1 (low neutrophils) as the reference group, the likelihood of a positive long-term response to clozapine, as measured by CGI-S score at 2 years, was significantly increased by membership of class 2 (high-normal neutrophils). A smaller effect was seen with class 3 (high neutrophils), and this effect was not significant. The effect size for class 2 was clinically significant with the odds of a positive response to clozapine more than doubled by membership of the high-normal group. Furthermore, baseline neutrophil counts alone were significantly associated with response to clozapine, with higher counts more likely to be associated with a positive response. However, response to clozapine was not limited to patients with higher baseline neutrophil counts, and therefore neutrophil count alone would not be sufficient to usefully predict outcome.

The improved response to clozapine in the high-normal compared to the low neutrophil group did not appear to be explained by a specific early spike in neutrophil count, as the rise in neutrophils in classes 2 was modest. This is in keeping with the study by Blackman et al. (2021), which found no association between early increases in neutrophil and subsequent treatment response. It should be noted that the sample in Blackman et al was a subsample of that reported in the present study, although Blackman et al studied baseline and peak neutrophil counts as opposed to trajectories as in this study. In our study the differences in neutrophil count between the classes appear to be set prior to clozapine initiation, consistent with the hypothesis that a proportion of TRS patients have a low-grade inflammation, reflected in high-normal neutrophil counts, and that these patients are especially responsive to clozapine. The lesser effect seen in the high neutrophil group, as compared to the high-normal group, may be due to the reduced precision of the estimate in this smaller group. Also, patients in this group were more likely to have co-morbid medical illnesses, and were older, both of which may have affected their response to clozapine. The early transient increase in neutrophils seen across all groups may represent mobilisation of neutrophils from the bone marrow as part of the immunological response to clozapine. The short half-life of neutrophils (hours to days) may explain the transient nature of this increase.

The precise role neutrophils may be playing in inflammation in TRS

is unclear, but there are a number of potential candidate mechanisms. For example, neutrophils are known to interact closely with the complement system, which is activated as part of the initial immune response. Complement activation triggers chemotaxis of neutrophils to the site of injury and promotes direct cell lysis. Stimulated neutrophils themselves release complement factors which further activate the cascade via the alternative and lectin pathways (Lubbers et al., 2017). The complement system has been shown to be activated in psychosis (Heurich et al., 2022), and (Susai et al., 2023), have shown that levels of complement factors are associated with response to antipsychotic treatment in first episode psychosis. Stimulated neutrophils also release pro and anti-inflammatory cytokines, including TNF α , IL-1 β , IL-1ra, IL-6 (Tamassia et al., 2018), several of which have been found to be elevated in psychosis (Miller and Goldsmith, 2019). In addition, there appears to be a complex interplay between neutrophils and T-cells and recent work has indicated that regulatory T-cells (Tregs), which are key to maintaining immune homeostasis, may be hypofunctional in psychosis (Corsi-Zuelli et al., 2022). Neutrophils also produce traps called NETs (neutrophil extra-cellular traps) which ensnare pathogens and activate antigen presenting cells, which promote the differentiation of T-helper cells (Malech et al., 2014). Recent animal models and clinical studies have implicated neutrophils in the pathophysiology of a number of neuro-immune conditions including multiple sclerosis (MS) (Woodberry et al., 2018), immune anti-NMDAR encephalitis (Zeng et al., 2019) and amyotrophic lateral sclerosis (ALS) (McGill et al., 2020; Murdock et al., 2016); all conditions with known glutamatergic dysfunction. Glutamate is the main excitatory neurotransmitter in the CNS and is also an important immunomodulator (Hansen and Caspi, 2010). The hypothesis that TRS is categorically distinct from treatment responsive illness and is driven by glutamatergic, rather than dopaminergic dysfunction (Gillespie et al., 2017), may indicate similarities between TRS and these illnesses and shed light on the neutrophil changes seen in TRS. For example, it has been suggested that neutrophils may play a role in the development of neuroinflammation in MS as a direct result of NETosis negatively impacting on the ability of the blood brain barrier to control the influx of immune cells into the brain (Woodberry et al., 2018). NETs have been directly shown to be present in a number of auto-immune conditions (Lee et al., 2017) and they have also recently been found in the plasma of patients with early schizophrenia (Corsi-Zuelli et al., 2022).

There may also be functional and/or phenotypic differences in neutrophils associated with disease severity or treatment responsiveness. This has been shown to be the case in both MS and ALS (Woodberry et al., 2018; McGill et al., 2020). Phenotypic studies in MS have shown that activated neutrophils (with enhanced ROS production compared to normal neutrophils) may be involved in MS immunopathology and that granulocytic myeloid derived suppressor cells, which are neutrophils with an immunosuppressive function, may participate in the recovery phase (Woodberry et al., 2018). In addition, current MS treatments have effects on neutrophils either by reducing their numbers or altering their functioning (Woodberry et al., 2018). Studies in ALS have shown that CD16 expression on neutrophils was increased in patients with more severe disease (McGill et al., 2020). Studies of neutrophil phenotypes as possible markers of disease activity and treatment responsiveness in TRS could help further understanding of the role of neutrophils in TRS pathology and treatment response.

The finding that neutrophil count is associated with treatment response may not be specific to clozapine, nor to TRS, as raised counts have been linked with symptom severity and treatment response in non-treatment resistant illness (Fan et al., 2010; Steiner et al., 2019). Whilst the unique efficacy of clozapine and its clear immunomodulatory properties, alongside the specific characteristics of TRS, suggest that clozapine may work differently to other antipsychotics in this patient group, studies looking at neutrophil counts and treatment response to alternative antipsychotics would be helpful.

Our study has several strengths. It is the largest study to our

knowledge to demonstrate a significant relationship between neutrophil count and response to clozapine. The methodology used (LCGA) fits well with a heterogenous condition such as TRS. Another strength is the generalisability of the study, being representative of a large epidemiological population. The use of CGI-S as an outcome measure has good validity and ratings were carried out by an experienced consultant psychiatrist. The main limitations of the study are its retrospective design and that patients who discontinued clozapine before two years were excluded. Another limitation is the lack of information regarding serum clozapine levels in the cohort; it is plausible that patients with lower neutrophils achieved lower clozapine doses, with sub-therapeutic clozapine levels, as clinicians were hesitant about more rapid increases, leading to reduced or delayed treatment effectiveness. The lower neutrophil group also had lower illness severity scores at baseline, which could mean that the effect size with clozapine was reduced in this group. It is also noteworthy that patients of black ethnicities were over-represented in the low neutrophil group, although we controlled for ethnicity in the analysis. This may indicate that the group included patients with benign ethnic neutropenia (BEN) (Atallah-Yunes et al., 2019), which occurs in people of African, Middle Eastern and West Indian ethnicities; the significance of which for the hypothesis being studied is unknown.

To conclude, our results are consistent with the hypothesis that patients with low-level inflammation, reflected in a high-normal neutrophil count, are more likely to respond to clozapine, raising the possibility that clozapine exerts its superior efficacy via immune mechanisms.

Ethics statement

Declaration of interest statement: RU reports grant funding from Medical Research Council (MR/S037675/1), National Institute for Health Research: Health Technology Assessment (NIHR 127700) and National Institute of Mental Health (1U01MH124631-01) and speaker fees from Sunovion, Springer Healthcare, Otuska and Vitaris outside the submitted work. RU holds unpaid officership with the British Association for Pharmacology- Honorary General Secretary 2021-2024 and is Deputy Editor, The British Journal of Psychiatry. JHM has received research funding from H Lundbeck A/S outside the present work.

Funding statement

This research utilised the Clinical Record Interactive Search (CRIS) platform funded and developed by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London.

This research is funded/supported by the NIHR Oxford Health Biomedical Research Centre. The views expressed are those of the author (s) and not necessarily those of the NIHR or the Department of Health and Social Care

The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

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 authors do not have permission to share data.

References

- Al, P., Undurraga, J., Al, B., Bernardo, M., 2012. Clozapine and agranulocytosis in Spain: Do we have a safer population? A 5-year haematologic follow-up. *Rev. Psiquiatr. Salud. Ment. Barc.* 5 (1), 37–42.
- Alvir, J.M., Lieberman, J., Safferman, A., 1995. Do white-cell count spikes predict agranulocytosis in clozapine recipients? *Psychopharmacol. Bull.* 31 (2), 311–314.
- Arbuckle, J., 1996. Full information estimation in presence of incomplete data. In: Marcoulides, G.A., Schumacker, R.E. (Eds.), *Advanced Structural Equation Modeling*. Taylor and Francis, pp. 243–277.
- Atallah-Yunes, S.A., Ready, A., Newburger, P.E., 2019. Benign ethnic neutropenia. *Blood Rev.* 37, 100586.
- Blackman, G., Lisshammer, J.E.L., Zafar, R., Pollak, T.A., Pritchard, M., Cullen, A.E., Rogers, J., Carter, B., Griffiths, K., Nour, M., David, A.S., McGuire, P., Stewart, R., MacCabe, J., 2021. Clozapine response in schizophrenia and hematological changes. *J. Clin. Psychopharmacol.* 41 (1), 19–24.
- Busner, J., Targum, S.D., 2007. The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry Edgmont Pa Townsh.* 4 (7), 28–37.
- Corsi-Zuelli, F., Schneider, A.H., Santos-Silva, T., Loureiro, C.M., Shuhama, R., Menezes, P.R., Guimarães, F.S., Gomes, F.V., Cunha, F.Q., Louzada-Junior, P., Del-Ben, C.M., 2022. Increased blood neutrophil extracellular traps (NETs) associated with early life stress: translational findings in recent-onset schizophrenia and rodent model. *Tranl. Psychiatry* 12 (1).
- de Leon, J., Ruan, C.-J., Verdoux, H., Wang, C., 2020. Clozapine is strongly associated with the risk of pneumonia and inflammation. *Gen Psychiatry.* 33 (2), e100183.
- Delliers, G., 2000. Blood dyscrasias in clozapine-treated patients in Italy. *Haematologica.* 85 (3), 233–237.
- Fabrazzo, M., Prisco, V., Sampogna, G., Perris, F., Catapano, F., Monteleone, A.M., Maj, M., 2017. Clozapine versus other antipsychotics during the first 18 weeks of treatment: a retrospective study on risk factor increase of blood dyscrasias. *Psychiatry Res.* 256, 275–282.
- Fan, X., Liu, E.Y., Freudenreich, O., Park, J.H., Liu, D., Wang, J., Yi, Z., Goff, D., Henderson, D.C., 2010. Higher white blood cell counts are associated with an increased risk for metabolic syndrome and more severe psychopathology in non-diabetic patients with schizophrenia. *Schizophr. Res.* 118 (1–3), 211–217.
- Fernandes, A.C., Cloete, D., Broadbent, M.T.M., Hayes, R.D., Chang, C.-K., Jackson, R.G., Roberts, A., Tsang, J., Soncul, M., Liebscher, J., Stewart, R., Callard, F., 2013. Development and evaluation of a de-identification procedure for a case register sourced from mental health electronic records. *BMC Med. Inform. Decis. Mak.* 13 (1), 71.
- Gillespie, A.L., Samanaite, R., Mill, J., Egerton, A., MacCabe, J.H., 2017. Is treatment-resistant schizophrenia categorically distinct from treatment responsive schizophrenia? A systematic review. *BMC Psychiatry* 17 (1).
- Goldsmith, D.R., Rapaport, M.H., Miller, B.J., 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry.* 21 (12), 1696–1709.
- Hansen, A.M., Caspi, R.R., 2010. Glutamate joins the ranks of immunomodulators. *Nat Med.* 16 (8), 856–858.
- Heurich, M., Föcking, M., Mongan, D., Cagney, G., Cotter, D.R., 2022. Dysregulation of complement and coagulation pathways: emerging mechanisms in the development of psychosis. *Mol. Psychiatry* 27 (1), 127–140.
- Hummer, M., Kurz, M., Barnas, C., Saria, A., Fleischhacker, W.W., 1994. Clozapine-induced transient white blood cell disorders. *J. Clin. Psychiatry* 55 (10), 429–432.
- Jackson, A.J., Miller, B.J., 2020. Meta-analysis of total and differential white blood cell counts in schizophrenia. *Acta Psychiatr. Scand.* 142 (1), 18–26.
- Jones, R., Upthegrove, R., Price, M.J., Pritchard, M., Chandan, J.S., Legge, S., et al., 2022. Duration of prior psychotic illness and clozapine response: a retrospective observational study using electronic health records. *Ther. Adv. Psychopharmacol.*
- Jung, T., Wickrama, K.A.S., 2008. An introduction to latent class growth analysis and growth mixture modeling: latent trajectory classes. *Soc. Personal Psychol. Compass.* 2 (1), 302–317.
- Kayser, M.S., Dalmau, J., 2016. Anti-NMDA receptor encephalitis, autoimmunity, and psychosis. *Schizophr. Res.* 176 (1), 36–40.
- Kluge, M., Schuld, A., Schacht, A., Himmerich, H., Dalal, M.A., Wehmeier, P.M., Hinze-Selch, D., Kraus, T., Dittmann, R.W., Pollmächer, T., 2009. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology* 34 (1), 118–128.
- Lee, K.H., Kronbichler, A., Park, D.-Y., Park, Y., Moon, H., Kim, H., Choi, J.H., Choi, Y., Shim, S., Lyu, I.S., Yun, B.H., Han, Y., Lee, D., Lee, S.Y., Yoo, B.H., Lee, K.H., Kim, T. L., Kim, H., Shim, J.S., Nam, W., So, H., Choi, S., Lee, S., Shin, J.I., 2017. Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. *Autoimmun. Rev.* 16 (11), 1160–1173.
- Lee, J., Takeuchi, H., Fervaha, G., Powell, V., Bhaloo, A., Bies, R., et al., 2015. The effect of clozapine on hematological indices: A 1-year follow-up study. *J. Clin. Psychopharmacol.* 35 (5), 510–516.
- Leon, J., Sanz, E.J., Norén, G.N., De las Cuevas, C., 2020. Pneumonia may be more frequent and have more fatal outcomes with clozapine than with other second-generation antipsychotics. *World Psychiatry* 19 (1), 120–121.
- Levite, M., 2017. Glutamate, T cells and multiple sclerosis. *J. Neural Transm.* 124 (7), 775–798.
- Lo, Y., Mendell, N., Rubin, D., 2001. Testing the number of components in a normal mixture. *Biometrika* 88 (3), 767–778.
- Löffler, S., Klimke, A., Kronenwett, R., Kobbe, G., Haas, R., Fehsel, K., 2010. Clozapine mobilizes CD34+ hematopoietic stem and progenitor cells and increases plasma concentration of interleukin 6 in patients with schizophrenia. *J. Clin. Psychopharmacol.* 30 (5), 591–595.
- Lozano, R., Marin, R., Santacruz, M.J., Pascual, A., 2016. Effect of clozapine on immunoglobulin M plasma levels. *Ther. Adv. Psychopharmacol.* 6 (1), 58–60.
- Lü L xian, Guo S qin, Chen W, Li Q, Cheng J, Guo J hua. [Effect of clozapine and risperidone on serum cytokine levels in patients with first-episode paranoid schizophrenia]. *1 Jun Yi Xue Xue Bao Acad J First Med Coll PLA.* 2004;24(11): 1251–4.
- Lubbers, R., van Essen, M.F., van Kooten, C., Trouw, L.A., 2017. Production of complement components by cells of the immune system. *Clin. Exp. Immunol.* 188 (2), 183–194.
- Maes, M., Bosmans, E., Kenis, G., De Jong, R., Smith, R.S., Meltzer, H.Y., 1997. In vivo immunomodulatory effects of clozapine in schizophrenia. *Schizophr. Res.* 26 (2–3), 221–225.
- Malech HL, DeLeo FR, Quinn MT. The Role of Neutrophils in the Immune System: An Overview. In: Quinn MT, DeLeo FR, editors. *Neutrophil Methods and Protocols* [Internet]. Totowa, NJ: Humana Press; 2014 [cited 2022 Nov 29]. p. 3–10. Available from: http://link.springer.com/10.1007/978-1-62703-845-4_1.
- McGill, R.B., Steyn, F.J., Ngo, S.T., Thorpe, K.A., Heggie, S., Ruitenberg, M.J., Henderson, R.D., McCombe, P.A., Woodruff, T.M., 2020. Monocytes and neutrophils are associated with clinical features in amyotrophic lateral sclerosis. *Brain Commun.* 2 (1).
- Meltzer, H.Y., 1997. Treatment-resistant schizophrenia - the role of clozapine. *Curr. Med. Res. Opin.* 14 (1), 1–20.
- Mijovic, A., MacCabe, J.H., 2020. Clozapine-induced agranulocytosis. *Ann. Hematol.* 99 (11), 2477–2482.
- Miller, B.J., Goldsmith, D.R., 2019. Inflammatory biomarkers in schizophrenia: implications for heterogeneity and neurobiology. *Biomark. Neuropsychiatry* 1, 100006.
- Murdock, B.J., Bender, D.E., Kashlan, S.R., Figueroa-Romero, C., Backus, C., Callaghan, B.C., Goutman, S.A., Feldman, E.L., 2016. Increased ratio of circulating neutrophils to monocytes in amyotrophic lateral sclerosis. *Neuroimmunol. Neuroinflammation* 3 (4), e242.
- Muthén, L., Muthén, B., 1998. *Mplus User's Guide*. Eighth.
- Nielsen, J., Foldager, L., Meyer, J.M., 2009. Increased use of antibiotics in patients treated with clozapine. *Eur. Neuropsychopharmacol.* 19 (7), 483–486.
- Paribello, P., Manchia, M., Zedda, M., Pinna, F., Carpiniello, B., 2021. Leukocytosis associated with clozapine treatment: a case series and systematic review of the literature. *Medicina (Mex)* 57 (8), 816.
- Perera, G., Broadbent, M., Callard, F., Chang, C.-K., Downs, J., Dutta, R., Fernandes, A., Hayes, R.D., Henderson, M., Jackson, R., Jewell, A., Kadra, G., Little, R., Pritchard, M., Shetty, H., Tulloch, A., Stewart, R., 2016. Cohort profile of the South London and Maudsley NHS Foundation Trust Biomedical Research Centre (SLaM BRC) Case Register: current status and recent enhancement of an Electronic Mental Health Record-derived data resource. *BMJ Open* 6 (3), e008721.
- Pollmacher, T., Hinze-Selch, D., Mullington, J., 1996. Effects of clozapine on plasma cytokine and soluble cytokine receptor levels. *J. Clin. Psychopharmacol.* 16 (5), 403–409.
- Ponsford, M.J., Jolles, S., 2019. Antibody deficiency in patients taking clozapine. *BMJ* 4, 1483.
- Ramaswamy, V., Desarbo, W., Reibstein, D., Robinson, W., 1993. An empirical pooling approach for estimating marketing mix elasticities with PIMS data. *Mark. Sci.* 12 (1), 103–123.
- Regen, F., Herzog, I., Hahn, E., Ruehl, C., Le Bret, N., Dettling, M., Heuser, I., Hellmann-Regen, J., 2017. Clozapine-induced agranulocytosis: Evidence for an immune-mediated mechanism from a patient-specific in-vitro approach. *Toxicol. Appl. Pharmacol.* 316, 10–16.
- Røge, R., Møller, B.K., Andersen, C.R., Correll, C.U., Nielsen, J., 2012. Immunomodulatory effects of clozapine and their clinical implications: What have we learned so far? *Schizophr. Res.* 140 (1–3), 204–213.
- Schoretsanitis, G., Ruan, C.-J., Rohde, C., Verdoux, H., De Las Cuevas, C., Spina, E., de Leon, J., 2021. An update on the complex relationship between clozapine and pneumonia. *Expert Rev. Clin. Pharmacol.* 14 (2), 145–149.
- Schwarz, G., 1978. The Bayesian information criterion. *Ann. Stat.* 6, 461–464.
- Sheldon, A.L., Robinson, M.B., 2007. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochem. Int.* 51 (6–7), 333–355.
- Siskind, D., McCartney, L., Goldschlager, R., Kisely, S., 2016. Clozapine v. first- and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. *Br. J. Psychiatry.* 209 (5), 385–392.
- Siskind, D., Sidhu, A., Cross, J., Chua, Y.-T., Myles, N., Cohen, D., Kisely, S., 2020. Systematic review and meta-analysis of rates of clozapine-associated myocarditis and cardiomyopathy. *Aust. N.Z. J. Psychiatry* 54 (5), 467–481.
- StataCorp. StataCorp. 2017. *Stata statistical software: Release 15*. College Station, TX: StataCorp.LLC. StataCorp.LLC; 2017.
- Steiner, J., Frodl, T., Schiltz, K., Dobrowolny, H., Jacobs, R., Fernandes, B.S., et al., 2019. Innate immune cells and c-reactive protein in acute first-episode psychosis and

- schizophrenia: relationship to psychopathology and treatment. *Schizophr. Bull.*, sbz068
- Susai, S.R., Föcking, M., Mongan, D., Heurich, M., Coutts, F., Egerton, A., et al., 2023. Association of complement and coagulation pathway proteins with treatment response in first-episode psychosis: A longitudinal analysis of the OPTiMiSE clinical trial. *Schizophr. Bull.* s0, bac201.
- Tamassia, N., Bianchetto-Aguilera, F., Arruda-Silva, F., Gardiman, E., Gasperini, S., Calzetti, F., et al., 2018. Cytokine production by human neutrophils: revisiting the “dark side of the moon. *Eur. J. Clin. Invest.* 48, e12952.
- Woodberry, T., Bouffler, S., Wilson, A., Buckland, R., Brüstle, A., 2018. The emerging role of neutrophil granulocytes in multiple sclerosis. *J Clin Med.* 7 (12), 511.
- Zeng, Z., Wang, C., Wang, B., Wang, N., Yang, Y., Guo, S., Du, Y., 2019. Prediction of neutrophil-to-lymphocyte ratio in the diagnosis and progression of autoimmune encephalitis. *Neurosci. Lett.* 694, 129–135.