Association between blood cell count, neutrophil fluorescence and treatment outcome in clozapine users with treatment-resistant schizophrenia

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Abstract (NL)

Introductie

Verandering van het bloedbeeld is een veel voorkomende bijwerking van clozapine. Er wordt gesuggereerd dat deze immunomodulatoire effecten ook een rol kunnen spelen in de unieke effectiviteit van clozapine. Het doel van deze studie was het onderzoeken van associaties tussen het bloedbeeld en fluorescentie van neutrofielen en de behandeluitkomst bij clozapine gebruikers met therapieresistente schizofrenie. Zo kan deze studie bijdragen aan het vinden van voorspellers van de behandeluitkomst van clozapine, waardoor de behandeling van deze patiënten verbeterd en gepersonaliseerd kan worden.

Methode

In deze studie is gebruik gemaakt van geanonimiseerde data van patiënten die deelgenomen hebben aan de Clozapine International studie of aan de Genetic Risk and Outcome Psychosis cohort studie. Met behulp van meervoudige lineaire regressie en ANCOVA is de associatie tussen het bloedbeeld (het aantal witte bloedcellen, neutrofielen, eosinofielen, rode bloedcellen en fluorescentie van neutrofielen) en de behandeluitkomst van clozapine, zowel de behandelrespons als het totaal aantal bijwerkingen, geanalyseerd.

Resultaten

In totaal zijn er 32 patiënten geïncludeerd in deze studie. Er zijn geen significante associaties gevonden tussen het bloedbeeld of fluorescentie van neutrofielen en de behandelrespons. Daarnaast was er geen significant verschil in bloedbeeld of fluorescentie van neutrofielen tussen patiënten met een hoge en patiënten met een lage symptoomernst. Uit de resultaten blijkt wel dat er een negatieve associatie is tussen het aantal neutrofielen en het totaal aantal bijwerkingen.

Discussie

De resultaten van deze studie wijzen er niet op dat de immunomodulatoire effecten van clozapine gerelateerd zijn aan de unieke effectiviteit. Echter, om vast te stellen of veranderingen in het bloedbeeld geassocieerd zijn met de behandeluitkomst bij clozapine gebruikers met therapieresistente schizofrenie wordt verder onderzoek met voldoende power aangeraden.

Abstract (EN)

Introduction

Hematological changes are common side-effects of clozapine, and it has been suggested that these immunomodulatory effects may play a role in clozapine's unique efficacy. Therefore, the objective of this study was to investigate the association between blood cell count, neutrophil fluorescence and treatment outcome in clozapine users with treatment-resistant schizophrenia. Gaining more insight into potential predictors of clozapine treatment outcome contributes to the improvement of treatment of these patients and provides possibilities for personalized medicine.

Methods

This study used anonymized data of patients that participated in the Clozapine International study or the Genetic Risk and Outcome Psychosis cohort study. We used multiple linear regression and ANCOVA to analyze associations between blood cell count (i.e. white blood cell count, neutrophilic granulocyte absolute count, eosinophilic granulocyte absolute count, red blood cell count, and neutrophil fluorescence) and clozapine treatment outcome, including treatment response and the total number of side-effects experienced by patients.

Results

In total, 32 patients were included in this study. We did not find significant associations between blood cell count or neutrophil fluorescence and treatment response. Furthermore, blood cell count and neutrophil fluorescence was not significantly different between patients with high and low symptom severity. The results show a negative association between neutrophilic granulocyte absolute count and the total number of side-effects experienced by patients.

Discussion

Our findings do not support the hypothesis that the immunomodulatory effects of clozapine are associated with its unique efficacy. However, further research with sufficient power is required to establish if hematological changes are associated with treatment outcome of clozapine users with treatment-resistant schizophrenia.

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List of abbreviations

BMI	Body mass index
CGI-S	Clinical Global Impression-Severity scale
CGI-I	Clinical Global Impression-Improvement scale
CIA	Clozapine-induced agranulocytosis
CLOZIN	Clozapine International study
GROUP	Genetic Risk and Outcome in Psychosis cohort study
PANSS	Positive and Negative Syndrome Scale
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SD	Standard deviation
TRS	Treatment-resistant schizophrenia

Introduction

Background

Treatment-resistant schizophrenia (TRS) occurs in approximately 30% of patients diagnosed with schizophrenia (1). TRS is defined as nonresponse to at least 2 antipsychotic trials of adequate dose, duration, and adherence. In such cases, clozapine therapy is indicated. Clozapine is the most effective antipsychotic agent for patients with TRS, and is superior to all other antipsychotics (2). However, due to its severe side-effects, clozapine is still prescribed to a limited extent and initiation is frequently delayed (2, 3). Previous research suggests that a delay in clozapine initiation in patients with TRS is associated with poor clinical outcomes (4). Finding predictors of clozapine treatment outcome may contribute to the reduction of the delay in initiation of clozapine and can be used to personalize treatment of individual patients.

Hematological abnormalities are common side-effects of clozapine, including neutropenia, leukocytosis, eosinophilia, and thrombocytosis. These changes mainly occur in the first weeks after starting clozapine and in most cases are transient and stabilize after a few weeks to months. Reported incidence rates vary between studies and depend on type of blood cell (5-7).
However, clozapine-induced agranulocytosis (CIA) is a very serious condition with potentially fatal outcomes. It has an incidence rate ranging from 0.38% to 0.7% and generally occurs in the first three months after clozapine initiation (8, 9). To reduce associated mortality rates, weekly blood monitoring is mandatory in the first 18 weeks of clozapine treatment (8).
Underlying mechanisms that cause such hematological changes in clozapine users are not fully understood, and different mechanisms have been proposed. There is evidence that toxic effects of clozapine on neutrophils lead to a reduction of neutrophil count (10, 11). On the other hand, immunological effects of clozapine, such as elevation of cytokine levels, can cause several alterations in blood cell count (7, 11). It is suggested that these immunomodulatory effects of clozapine also play a role in the unique efficacy of clozapine in patients with TRS (3, 11).

Several studies investigated the association between hematological changes and clozapine response. Blackman et al (2021) did not find any significant associations between changes in white blood cell count, neutrophil count or platelet count and treatment response. Treatment response was measured by the Clinical Global Impression-Improvement (CGI-I) scale. Therefore, positive and negative symptoms could not be assessed separately.
In contrast, a study of Mauri et al (2003) did find an association between lower neutrophil counts and the reduction of positive symptoms measured by the Scale for the Assessment of Positive Symptoms (SAPS) in patients diagnosed with schizophrenia. In addition, they found that patients who responded well to clozapine treatment measured by the Scale for the Assessment of Negative Symptoms (SANS) had significantly less reduction of WBC counts compared to non-responders (12). Fabrazzo et al (2017) found an association between positive clinical outcomes and the occurrence of persistent neutrophilia and eosinophilia, and a higher probability of a poor clinical response in patients with persistent anemia (6).
Furthermore, a case report suggests that leukocytosis is associated with loss to treatment response in two patients with schizophrenia (13).
In addition to blood cell count, recent studies have shown that there is an association between clozapine use and elevated neutrophil fluorescence. A dose-response relationship has been observed, but further research is needed to investigate the underlying mechanisms. However, authors suggest that neutrophil fluorescence is a potential biomarker for treatment adherence. To our knowledge, no studies have investigated the association between neutrophil fluorescence and treatment outcome in clozapine users (14-16).
In conclusion, the majority of previous studies of hematological effects of clozapine focus on associated incidence rates, or mainly discuss agranulocytosis and neutropenia. Only a few studies investigated the association between such hematological changes of clozapine and treatment outcome.

Objectives

The primary aim of this study was to investigate the association between changes in blood cell count, FL3-fluorescence and clozapine treatment outcome in patients with TRS. We focused on white blood cell count, neutrophil count, eosinophil count, red blood cell count, and neutrophils fluorescence, since previous studies suggest that changes in these hematological factors may be associated with treatment response. The Clinical Global Impression-Severity (CGI-S) scale and the Positive and Negative Syndrome Scale (PANSS) were used as

measures of treatment response. By using PANSS, we were able to assess positive and negative symptoms independently as well. In this way, the results of this study are of added value to most previous studies, which do not distinguish between positive and negative symptoms. The secondary outcome of this study was the total number of side-effects experienced by patients.
The results of this study contribute to improved knowledge of the immunomodulatory effects of clozapine and the hypothesis whether this is related to clozapine's unique efficacy. Furthermore, gaining more insight into potential biomarkers of clozapine treatment outcome, including treatment response and the occurrence of side-effects, results in a more effective treatment of patients diagnosed with TRS and will provide possibilities for personalized medicine.

Methods

Study design

This study used anonymized data of patients that participated in the Clozapine International (CLOZIN) study or the Genetic Risk and Outcome in Psychosis (GROUP) cohort study. Inclusion criteria that were used to select participants include: (1) use of clozapine during the study period; (2) diagnosis of schizophrenia spectrum disorder, and (3) availability of hematological measurements within 30 days before or after a study visit.

Patients were divided in two groups based on the number of study visits that have been conducted. A distinction was made between patients for whom longitudinal data were available and patients for whom only cross-sectional data were available. Patients with cross-sectional data had one study visit, without a limitation on the duration of clozapine treatment. In contrast, patients with longitudinal data had multiple study visits, part of which have had a study visit prior to clozapine initiation or within 10 days after initiation.

Data extraction

Patient characteristics and clinical data were retrieved from the CLOZIN and GROUP databases. Patient characteristics included age, sex, body mass index (BMI), smoking status, concomitant medication, duration of disease, and age of disease onset. Furthermore, the following clinical data was extracted: CGI-S and CGI-I scores, PANSS scores (negative, positive, general and total scores), and the total number of reported side-effects. Furthermore, data of clozapine dose, blood levels, and hematological variables were extracted.

Hematological variables

We focused on several hematological variables, including white blood cell, neutrophilic granulocyte absolute count, eosinophilic granulocyte absolute count, and red blood cell count. In addition to neutrophil count, we assessed FL3-fluorescence for neutrophil cell viability.
Initially, for patients with cross-sectional data we used the hematological measurements closest to the date of the study visit. However, for additional analyses we used the mean of all hematological measurements within 30 days before or after the study visit. Changes in hematological variables after clozapine initiation and during clozapine treatment were evaluated using longitudinal data of patients who have had multiple hematological measurements within 30 days before or after a study visit. All hematological values were compared to reference values in order to explore hematological abnormalities.

Primary outcome

The primary outcome of this study was treatment response, measured by the PANSS or CGI-S scale during study visits. The PANSS scale consists of a positive scale and a negative scale (ranging from 7 to 49), but also includes a general psychopathology scale (ranging from 16 to 112). These three scales and the total PANSS score were assessed separately. The CGI-S scale ranges from 1 ("normal, not at all ill") to 7 ("among the most extremely ill patients") and was used as measure for treatment response as well. In addition, in case PANSS scores were missing, CGI-S scores were converted to PANSS total scores and vice versa, using a converting table provided by Leucht et al (2006) (17).
Patients with longitudinal data were defined as treatment responders when there was a reduction of 25% or more in PANSS scores relative to baseline scores. Patients with less than 25% reduction in PANSS scores were classified as non-responders. Regarding patients with cross-sectional data, a classification was made based on symptom severity. Low symptom severity corresponds with PANSS total scores of 30 to 74, and high symptom severity to PANSS total scores of 75 or higher (17, 18).

Secondary outcome

The secondary outcome of this study was the total number of side-effects experienced by patients, which are questioned during study visits. Side-effects that were included in this study are more sleep necessity, drowsiness, constipation, hypersalivation, changes in weight, and reduced sex drive.

Data analysis

Descriptive statistics, including frequencies, mean and standard deviation, were calculated to summarize demographic and clinical characteristics.
Analysis of cross-sectional data was conducted in two different ways. To determine which hematological variables can be used to predict clozapine treatment outcome, we analyzed the association between absolute blood cell count and PANSS scores, CGI-S scores, or total number of side-effects using multiple linear regression. The independent variables included absolute leukocyte, neutrophil, eosinophil, red blood cell count and FL3-fluorescence of neutrophils. Dependent variables were PANSS scores, including negative, positive, and total scores, CGI-S scores, and total number of side-effects. All variables were log-transformed, with the exception of total number of side-effects. Second, patients with cross-sectional data were divided in two groups based on symptom severity (see paragraph 'Primary outcome') to assess differences in blood cell count between these two groups. We used ANCOVA to compare absolute blood cell counts, including hematological variables as dependent variables and symptom severity as independent variable. All hematological variables were log-transformed.
We adjusted for several confounders, such as gender, age, clozapine dose, and smoking (yes/no). These factors may affect blood count and are based on previous studies of hematologic changes in clozapine users (3, 6). The significance level for all statistical test was set to 0.05, so P-values of ≤ 0.05 were considered statistically significant. Data analysis was performed using SPSS, version 26.

Patients with longitudinal data were divided in two groups based on treatment response (see paragraph 'Primary outcome'). Unfortunately, we were not able to assess differences in blood cell count and FL3-fluorescence of neutrophils between treatment responders and non-responders, due to limited data. Consequently, it was not possible to conduct within-person analyses using linear mixed models to analyze associations between changes in absolute blood cell count or FL3-fluorescence of neutrophils and changes in PANSS or CGI-S scores.

Results

Patient characteristics

In total, 32 patients were included in this study. There was cross-sectional data available of 32 patients, i.e. one study visit and one or more hematological measurements within 30 days before or after the study visit. Longitudinal data, i.e. two study visits and one or more hematological measurements within 30 days before or after each study visit, was only available for 8 patients.

Clinical and hematological characteristics of the 32 patients with cross-sectional data are shown in table 1. The mean age of these patients was 30.81 years (SD = 8.14) and 25 patients (78.1%) were male. The mean PANSS total score, PANSS positive score and PANSS negative score was 75.6 (SD = 19.8), 19.1 (SD = 7.34), and 15.7 (SD = 5.65) respectively. 17 patients had a PANSS total score of 75 or higher, and therefore are classified as patients with high symptom severity. 13 patients had a PANSS total score of 74 or less, and are classified as patients with low symptom severity. 1 patient could not be classified due to a missing PANSS total score, and is therefore excluded in the ANCOVA analysis.

Table 1: Patient characteristics of patients with cross-sectional data, including differences between patients with high and low symptom severity. BMI = body mass index, SD = standard deviation.

	All patients (N = 32)	Patients with high symptom severity (N = 18)	Patients with low symptom severity (N = 13)	P-values
Sex, n (%)				0.092
Female	7 (21.9)	6 (33.3)	1 (7.7)	
Male	25 (78.1)	12 (66.7)	12 (92.3)	
Age, years (SD)	30.81 (8.14)	30.3 (8.6)	32.0 (7.91)	0.573
Smoking, n (%)				0.880
Yes	22 (68.8)	12 (66.7)	9 (69.3)	
No	10 (31.3)	6 (33.3)	4 (30.8)	
BMI, kg/m² (SD)	27.1 (6.40)	28.0 (6.86)	25.3 (5.49)	0.191
Clozapine dose, mg/day (SD)	283.0 (207.5)	253.3 (205.7)	318.8 (221.9)	0.435
Dose-adjusted clozapine level (SD)	2.01 (1.38)	2.04 (1.63)	1.92 (0.81)	0.907
Age of disease onset, years (SD)	16.3 (9.65)	14.4 (9.54)	18.8 (9.99)	0.232
Duration of disease, years (SD)	11.8 (8.82)	12.6 (8.87)	8.38 (8.84)	0.206
Use of other antipsychotics, n (%)				0.722
Yes	10 (31.3)	6 (33.3)	4 (30.8)	
No	18 (56.3)	9 (50.0)	8 (61.5)	
NA	4 (12.5)	3 (16.7)	1 (7.7)	
Use of mood stabilizers, n (%)				
Yes	0 (0)	0 (0)	0 (0)	
No	28 (87.5)	15 (83.3)	12 (92.3)	
NA	4 (12.5)	3 (16.7)	1 (7.7)	
Use of antidepressants, n (%)				0.095
Yes	7 (21.9)	2 (11.1)	5 (38.5)	
No	21 (65.6)	13 (72.2)	7 (53.8)	
NA	4 (12.5)	3 (16.7)	1 (7.7)	
Use of benzodiazepines, n (%)				0.547
Yes	13 (40.6)	8 (44.4)	5 (38.5)	
No	15 (46.9)	7 (38.9)	7 (53.8)	
NA	4 (12.5)	3 (16.7)	1 (7.7)	
PANSS scores (SD)				
Total score	75.6 (19.8)	88.3 (14.3)	57.9 (10.5)	<0.001
General score	35.2 (10.3)	45.3 (6.44)	28.1 (5.17)	<0.001
Negative score	19.1 (7.34)	23.4 (4.96)	17.1 (7.25)	0.064
Positive score	15.7 (5.65)	20.4 (4.39)	12.6 (4.30)	0.002
CGI-S score (SD)	4.07 (1.18)	4.73 (0.759)	3.16 (1.05)	<0.001
Total number of side-effects, n (SD)	4.07 (1.64)	4.00 (1.41)	4.25 (2.36)	0.803
White blood cell count, 10^9/L (SD)	7.38 (2.12)	7.57 (2.20)	7.14 (2.16)	0.592
Neutrophilic granulocyte absolute count, 10^9/L (SD)	4.16 (1.58)	4.21 (1.56)	4.13 (1.73)	0.893
Eosinophilic granulocyte absolute count, 10^9/L (SD)	0.221 (0.291)	0.276 (0.358)	0.13 (0.15)	0.317
Red blood cell count by Impedance, 10^{12}/L (SD)	4.83 (0.382)	4.76 (0.361)	4.92 (0.42)	0.254
FL3-fluorescence of neutrophils	91.9 (13.5)	89.5 (14.5)	94.9 (12.0)	0.354
Deviations from reference values, n (%)				
White blood cell count	5 (15.6)	2 (11.1)	3 (23.1)	0.371
Neutrophilic granulocyte absolute count	1 (3.13)	0 (0)	1 (7.69)	0.232
Eosinophilic granulocyte absolute count	4 (12.5)	3 (16.7)	0 (0)	0.121
Red blood cell count by Impedance	2 (6.25)	1 (5.56)	1 (7.69)	0.811

The baseline characteristics of 8 patients with longitudinal data are shown in table 2. The mean age was 35.3 years (SD = 14.4) and 6 out of 8 patients (75%) were male. The mean PANSS total score, PANSS negative score, and PANSS positive score was 74.4 (SD = 19.3), 20.0 (SD = 6.97), and 18.0 (SD = 5.26) respectively. 6 patients were treatment non-responders, corresponding to a mean change in PANSS total score of 1.95% (range -23.10% – +22.64%, SD = 16.0) over a period of approximately 6 months to 4 years. Only 1 patient was defined as treatment responder, based on a reduction of 54.9% in PANSS total score over a period of 6 months. 1 patient could not be classified due to a missing PANSS total score.

Table 2: Baseline characteristics of patients with longitudinal data, including differences between treatment responders and treatment non-responders. BMI = body mass index, SD = standard deviation.

	All patients (N = 8)	**Treatment non-responders (N = 6)**	**Treatment responders (N = 1)**
Sex, n (%)			
Female	2 (25)	4 (66.7)	0 (0)
Male	6 (75)	2 (33.3)	1 (100)
Age, years (SD)	35.3 (14.4)	33.7 (12.1)	22
Smoking, n (%)			
Yes	2 (25)	2 (33.3)	0 (0)
No	6 (75)	4 (66.7)	1 (100)
BMI, kg/m^2 (SD)	26.7 (5.53)	23.1 (1.01)	29.10
Clozapine dose, mg/day (SD)	217 (173)	206 (200)	300
Dose-adjusted clozapine level	9.13 (12.7)	11.1 (13.7)	1.04
Age of disease onset, years (SD)	21.1 (5.08)	20.7 (5.50)	19
Duration of disease, years (SD)	14.1 (10.8)	13.0 (8.53)	3
Use of other antipsychotics, n (%)			
Yes	2 (25)	2 (33.3)	0 (0)
No	6 (75)	4 (66.7)	1 (100)
Use of mood stabilizers, n (%)			
Yes	1 (12.5)	0 (0)	1 (100)
No	7 (87.5)	6 (100)	0 (0)
Use of antidepressants, n (%)			
Yes	2 (25)	2 (33.3)	0 (0)
No	6 (75)	4 (66.7)	1 (100)
Use of benzodiazepines, n (%)			
Yes	6 (75)	4 (66.7)	1 (100)
No	2 (25)	2 (33.3)	0 (0)
PANSS scores (SD)			
Total score	74.4 (19.3)	68.7 (18.1)	82
General score	36.4 (10.2)	34.3 (11.2)	41
Negative score	20.0 (6.97)	17.5 (3.94)	20
Positive score	18.0 (5.26)	16.8 (5.67)	21
CGI-S score (SD)	4.46 (1.40)	4.11 (1.44)	6.0
Total number of side-effects, n (SD)	3.80 (0.84)	4.00 (1.00)	3
White blood cell count, 10^9/L (SD)	7.29 (1.03)	7.51 (0.92)	5.64
Neutrophilic granulocyte absolute count, 10^9/L (SD)	4.47 (0.89)	4.77 (0.69)	2.88
Eosinophilic granulocyte absolute count, 10^9/L (SD)	0.162 (0.104)	0.13 (0.10)	0.28
Red blood cell count by Impedance, 10^{12}/L (SD)	4.76 (0.571)	4.65 (0.64)	5.11
FL3-fluorescence of neutrophils	83.0 (13.3)	81.7 (15.4)	94.1
Deviations from reference values, n (%)			
White blood cell count	0 (0)	0 (0)	0 (0)
Neutrophilic granulocyte absolute count	0 (0)	0 (0)	0 (0)
Eosinophilic granulocyte absolute count	0 (0)	0 (0)	0 (0)
Red blood cell count by Impedance	2 (25)	2 (33.3)	0 (0)

Hematological changes after clozapine initiation

9 patients were visited prior to clozapine initiation or within 10 days after clozapine initiation. In addition, multiple hematological measurements have been conducted within 30 days before or after the study visit. Figure 1 to 5 show hematological changes during this period for each patient individually, including hematological reference ranges defined by the Central Diagnostic Laboratory of UMC Utrecht.

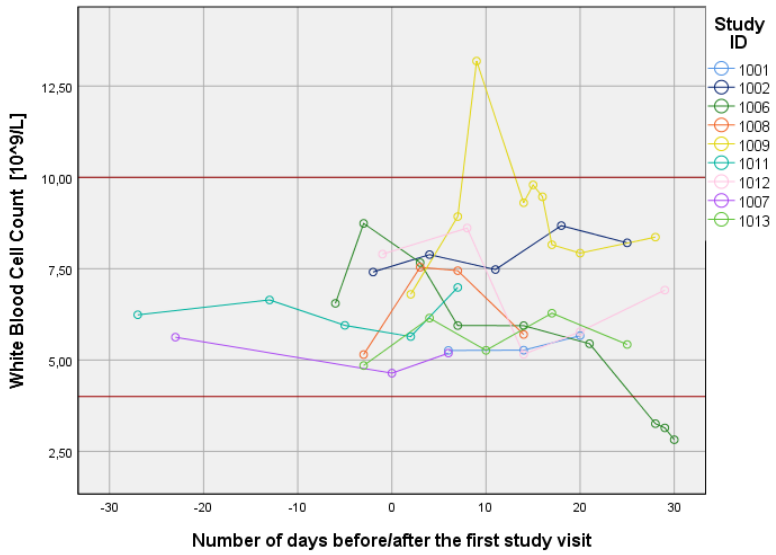

Figure 1: Changes in white blood cell count after clozapine initiation.

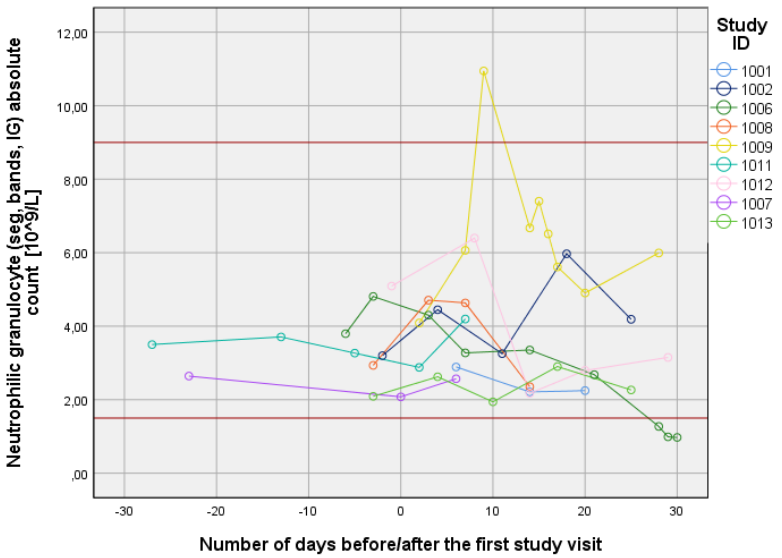

Figure 2: Changes in neutrophilic granulocyte absolute count after clozapine initiation.

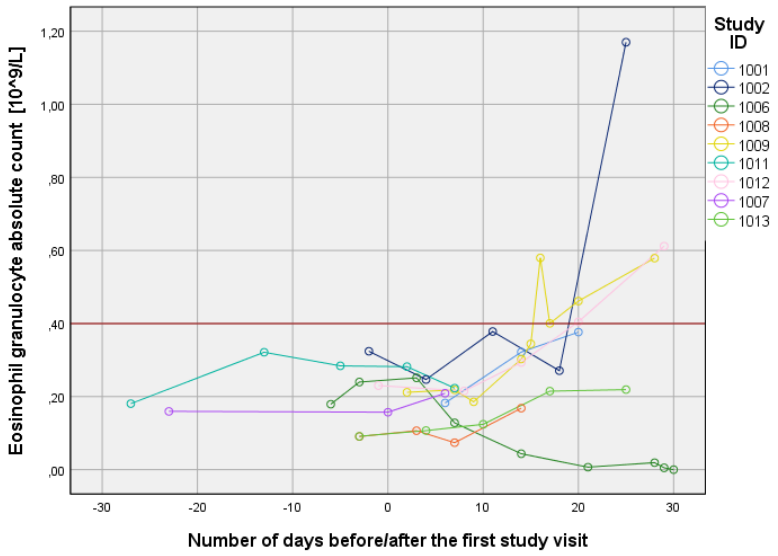

Figure 3: Changes in eosinophilic granulocyte absolute count after clozapine initiation.

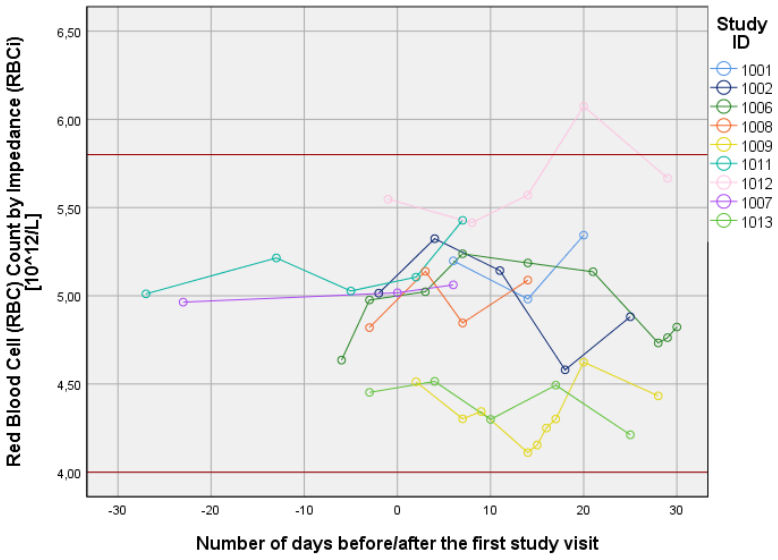

Figure 4: Changes in red blood cell count after clozapine initiation.

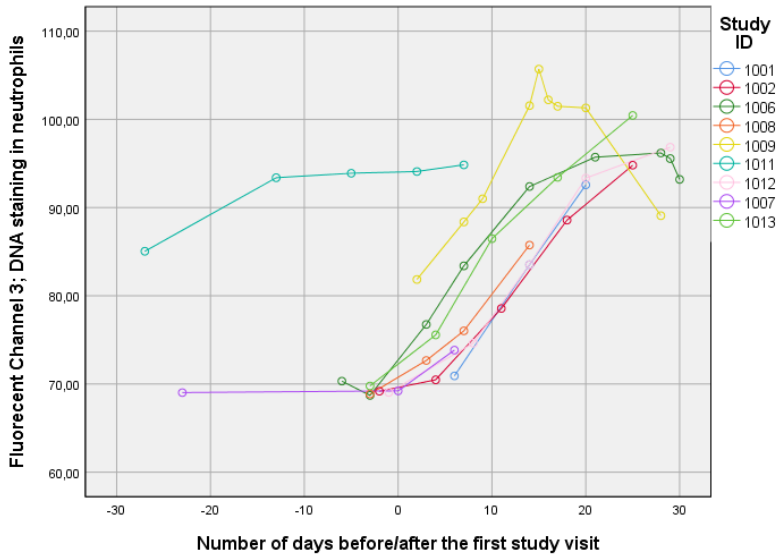

Figure 5: Changes in FL3-fluorescence of neutrophils after clozapine initiation.

Hematological changes during clozapine treatment

Furthermore, there were multiple hematological measurements available of 17 patients, within 30 days before or after study visits at a random time during clozapine treatment. Changes in blood cell count and FL3-fluorescence of neutrophils are shown in figure 6 to 10 for each patient individually, including hematological reference ranges defined by the Central Diagnostic Laboratory of UMC Utrecht.

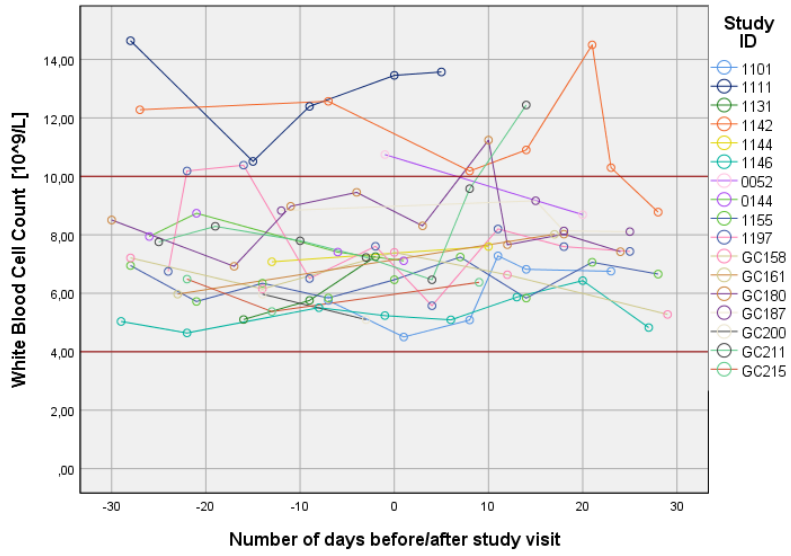

Figure 6: Changes in white blood cell count during clozapine treatment.

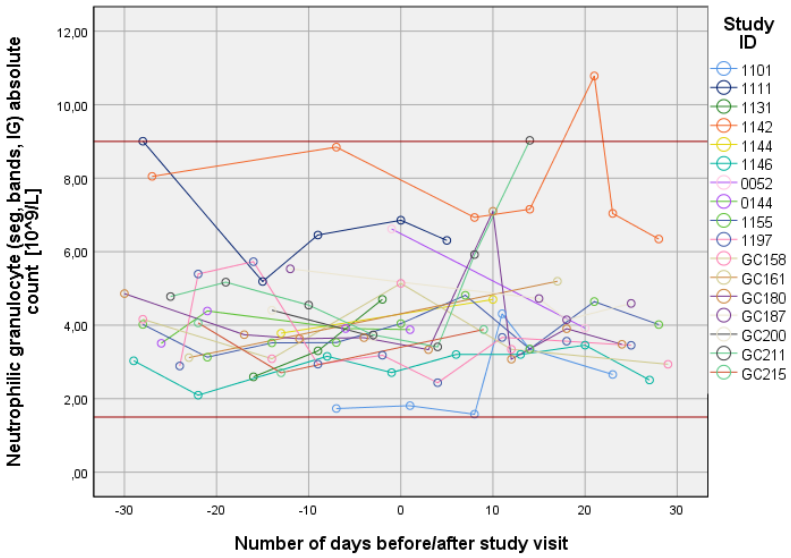

Figure 7: Changes in neutrophilic granulocyte absolute count during clozapine treatment.

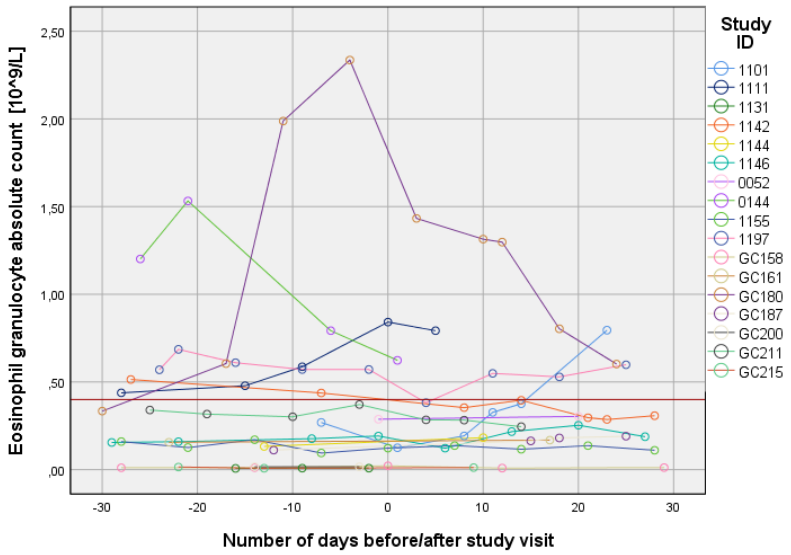

Figure 8: Changes in eosinophilic granulocyte absolute count during clozapine treatment.

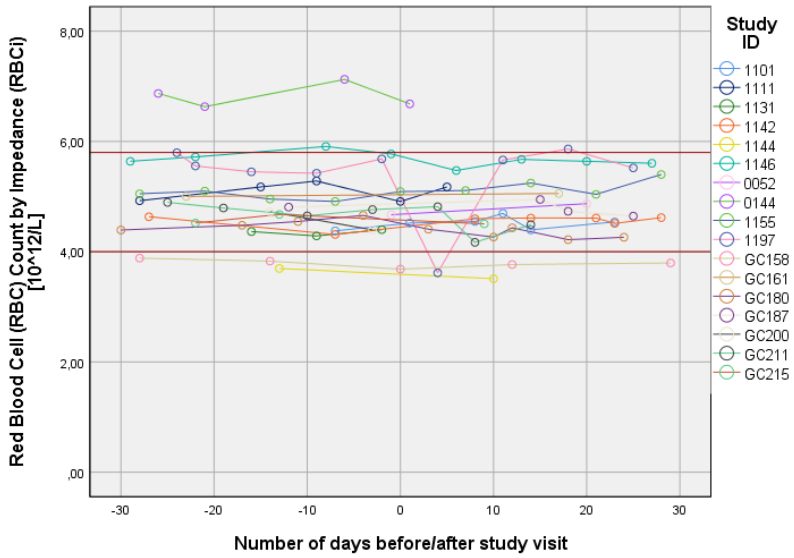

Figure 9: Changes in red blood cell count during clozapine treatment.

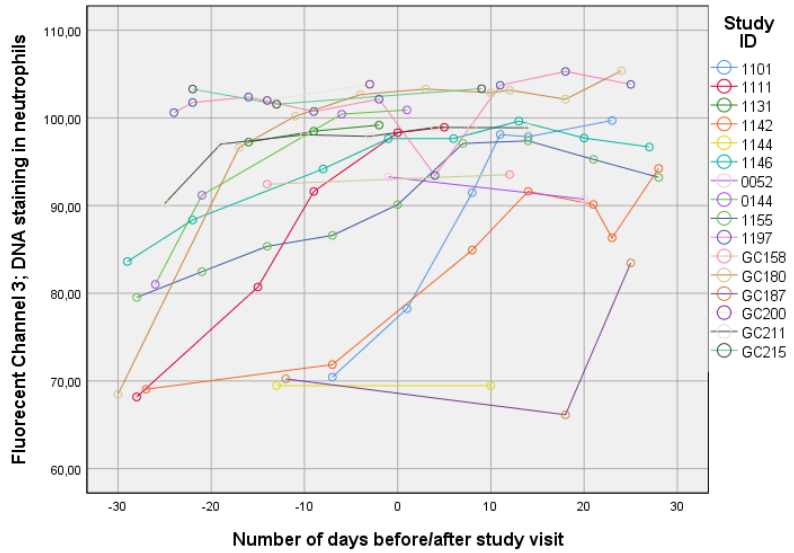

Figure 10: Changes in FL3-fluorescence of neutrophils during clozapine treatment.

Association between blood cell count and treatment outcome

Multiple linear regression models were used to study if white blood cell count, neutrophilic granulocyte absolute count, eosinophilic granulocyte absolute count, red blood cell count or FL3-fluorescence of neutrophils were able to predict clozapine treatment outcome. The results of the regression models including PANSS total score, PANSS negative score, or PANSS positive score as dependent variable are shown in Supplementary table 1. After adjusting for sex, age, smoking, and clozapine dose, we did not find any statistically significant associations between hematological variables and PANSS scores. Supplementary table 2 presents an overview of the regression models including CGI-S score as dependent variable. No statistically significant associations between hematological variables and CGI-S score were found.

In addition, we used multivariate regression to explore if specific combinations of hematological variables were able to predict PANSS scores. After adjusting for sex and age, we found that eosinophilic granulocyte absolute count and red blood cell count significantly predicted PANSS positive score ($R^2 = 0.650$, $p = 0.006$). However, after adjusting for sex, age, smoking, and clozapine dose, no significant results were found.

Since the majority of patients had multiple hematological measurements within 30 days before or after the study visit, we used the mean values of these measurements instead of the value measured closest to the study visit in additional analyses (Supplementary table 3 and 4). After adjusting for sex and age, eosinophilic granulocyte absolute count significantly predicted CGI-S score ($R^2 = 0.320$, $p = 0.014$). In line with the aforementioned analyses, the regression model did not remain statistically significant after adjusting for sex, age, smoking, and clozapine dose.

Our secondary outcome was the total number of side-effects experienced by patients. To explore associations between blood cell count and FL3-fluorescence of neutrophils and the occurrence of side-effects, we used multiple linear regression models. Only 16 patients of the CLOZIN study were included in this analysis, since these data was not available of patients of the GROUP cohort study.
As shown in Supplementary table 5, neutrophilic granulocyte absolute count was negatively associated with the total number of side-effects ($R^2 = 0.695$, $p = 0.032$).

Comparison between patients with high symptom severity and low symptom severity

ANCOVA was used to compare differences in mean blood cell count and FL3-fluorescence of neutrophils between patients with high symptom severity and low symptom severity. As shown in Supplementary table 6, no statistically significant differences were found between the two groups after adjusting for sex and age.

Discussion

The objective of this study was to investigate the association between blood cell count, FL3-fluorescence of neutrophils and treatment outcome of clozapine users diagnosed with TRS. We aimed to get better understanding of the immunomodulatory effects of clozapine and to explore whether hematological changes are potential biomarkers for clozapine treatment outcome, including treatment response and the occurrence of side-effects.
The findings of this study do not show significant associations between white blood cell count, neutrophilic granulocyte absolute count, eosinophilic granulocyte count, red blood cell count or FL3-fluorescence of neutrophils and PANSS scores or CGI-S scores. The results are in line with a previous study of Blackman et al (2021), in which no significant associations between changes in white blood cell count, neutrophilic count, or platelet count and CGI-I scores were found (3). However, Fabrazzo et al (2017) did find an association between persistent neutrophilia and eosinophilia and positive clinical outcomes. Furthermore, a poor clinical outcome was associated with persistent anemia (6). Our findings do not support these results. This difference may be explained by the fact that Fabrazzo et al (2017) focused on hematological abnormalities during the first 18 weeks after clozapine initiation, and this study mainly included patients with hematological values within the reference ranges at varying times during clozapine treatment.
We did not find significant associations between positive or negative symptoms and blood cell count, after adjusting for sex, age, smoking, and clozapine dose. This differs from a study of Mauri et al (2003), which shows a positive association between reduction neutrophil counts and reduction of positive symptoms, and a negative association between reduction in white blood cell count and reduction of negative symptoms (12). This can also be due to the fact that Mauri et al (2003) studied the association between hematological changes and treatment response during the first 8 weeks of clozapine treatment, in contrast to this study in which cross-sectional data was used.
Recent studies show that the use of clozapine is positively associated with FL3-fluorescence of neutrophils and suggest that this might be a possible biomarker for treatment adherence (16, 19, 20). In addition, we investigated the association between FL3-fluorescence of neutrophils and clozapine treatment outcome. Overall, the results of this study suggest that there is no association between FL3-fluorescence of neutrophils and clozapine treatment outcome.
In addition to treatment response, we investigated the relationship between total number of side-effects and blood cell count. The results show a negative association between neutrophilic granulocyte absolute count and total number of side-effects ($R^2 = 0.695$, $p = 0.032$). So, these findings suggest that changes in neutrophilic granulocyte absolute count are associated with the occurrence of side-effects in clozapine users, which, as far as we know, has not yet been described in previous research. However, it is important to note that we only focused on the total number of side-effects, regarding the following six side-effects of clozapine: more sleep necessity, drowsiness, constipation, hypersalivation, changes in weight, and reduced sex drive.

Therefore, future studies which assess all side-effects independently and include additional side-effects of clozapine will be of added value.

One of the strengths of this study is that we used PANSS scores and CGI-S scores as measures for clozapine treatment response. Positive and negative symptoms of patients with TRS were assessed separately using PANSS positive and negative scores, in contrast to most previous studies (3, 6). Furthermore, to our knowledge, this was the first study to investigate the association between FL3-fluorescence of neutrophils and treatment outcome in clozapine users.
However, this study also has several limitations. First, a possible explanation of why no significant associations were found in this study is the fact that we were not able to assess changes in hematological variables and PANSS or CGI-S scores over time, due to limited longitudinal data. Further studies on changes over time after clozapine initiation will provide a more complete picture of the effects of clozapine on blood cell count and associations with treatment response or the occurrence of side-effects, and therefore are recommended. Another important limitation of this study is the small sample size (N = 32). For this reason, the power of the study is reduced. Post hoc power analysis was conducted using G*Power version 3.9.1.4, which shows that the power for detecting a medium effect size ($f^2 = 0.15$) at a significance level of 0.05 was 0.29 for multiple linear regression (21). So, this could explain why no statistically significant results were found. Last, we adjusted for several confounders, including sex, age, clozapine dose, and smoking. However, use of concomitant medication, such as other antipsychotics, mood stabilizers, antidepressants and benzodiazepines, or age of onset of schizophrenia spectrum disorder may affect the results as well (3, 6).

Conclusion

In conclusion, our findings do not suggest that there is an association between blood cell count or FL3-fluorescence of neutrophils and treatment response in clozapine users with TRS. White blood cell count, neutrophilic granulocyte absolute count, eosinophilic granulocyte absolute count, red blood cell count, and FL3-fluorescence of neutrophils did not predict PANSS scores or CGI-S scores. Therefore, the results of this study do not support the hypothesis that the immunomodulatory effects of clozapine play a role in clozapine's unique efficacy. However, future studies, which have sufficient power and preferably include longitudinal data analysis, are required to establish if changes in blood cell count induced by clozapine are associated with treatment response and the occurrence of side-effects in patients with TRS.

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Supplementary material

Supplementary table 1: Multiple linear regression model, including PANSS scores as dependent variable and the hematological variables as independent variable. Regarding the hematological variables, hematological measurements closest to the study visit was used. All variables were log-transformed.

	R square	Adjusted R square	Sig. model	Constant	B	Beta	95% CI for B	Sig. beta
White Blood Cell Count								
PANSS total score	0.003	-0.031	0.768	1.908	-0.053	-0.055	-0.413 – 0.308	0.768
PANSS negative score	0.026	-0.035	0.524	1.453	-0.243	-0.161	-1.033 – 0.547	0.524
PANSS positive score	0.075	0.017	0.272	0.862	0.359	0.273	-0.310 – 1.029	0.272
After adjusting for sex and age								
PANSS total score	0.026	-0.082	0.869	1.892	-0.064	-0.068	-0.447 – 0.318	0.733
PANSS negative score	0.066	-0.134	0.803	1.351	-0.322	-0.213	-1.186 – 0.542	0.438
PANSS positive score	0.199	0.027	0.360	0.813	0.322	0.245	-0.374 – 1.018	0.338
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.071	-0.150	0.894	1.936	-0.032	-0.034	-0.471 – 0.408	0.881
PANSS negative score	0.157	-0.370	0.901	1.535	-0.362	-0.240	-1.557 – 0.833	0.505
PANSS positive score	0.207	-0.289	0.824	0.820	0.346	0.264	-0.662 – 1.355	0.451
Neutrophilic granulocyte absolute count								
PANSS total score	0.027	-0.006	0.374	1.925	-0.106	-0.165	-0.346 – 0.134	0.374
PANSS negative score	0.160	0.107	0.100	1.485	-0.407	-0.400	-0.902 – 0.087	0.100
PANSS positive score	0.007	-0.055	0.743	1.124	0.074	0.083	-0.394 – 0.542	0.743
After adjusting for sex and age								
PANSS total score	0.056	-0.048	0.660	1.892	-0.124	-0.194	-0.380 – 0.131	0.327
PANSS negative score	0.234	0.070	0.278	1.301	-0.485	-0.476	-1.016 – 0.046	0.070
PANSS positive score	0.143	-0.040	0.524	1.035	0.022	0.025	-0.467 – 0.510	0.925
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.097	-0.118	0.808	1.951	-0.110	-0.171	-0.400 – 0.180	0.439
PANSS negative score	0.320	-0.104	0.606	1.451	-0.495	-0.486	-1.213 – 0.222	0.150
PANSS positive score	0.146	-0.388	0.916	1.054	0.027	0.030	-0.673 – 0.726	0.932
Eosinophilic granulocyte absolute count								
PANSS total score	0.143	0.113	0.036	1.931	0.069	0.378	0.005 – 0.133	0.036
PANSS negative score	0.048	-0.011	0.382	1.311	0.063	0.219	-0.086 – 0.213	0.382
PANSS positive score	0.189	0.138	0.071	1.279	0.109	0.435	-0.011 – 0.229	0.071
After adjusting for sex and age								
PANSS total score	0.175	0.083	0.153	1.898	0.072	0.394	0.006 – 0.138	0.034
PANSS negative score	0.080	-0.117	0.751	1.170	0.069	0.239	-0.091 – 0.229	0.371
PANSS positive score	0.368	0.232	0.085	1.129	0.120	0.477	0.005– 0.236	0.043
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.200	0.010	0.414	1.918	0.070	0.383	-0.009 – 0.148	0.079
PANSS negative score	0.131	-0.413	0.933	1.284	0.049	0.169	-0.184 – 0.282	0.642
PANSS positive score	0.383	-0.003	0.478	1.071	0.130	0.518	-0.041 – 0.301	0.117
Red Blood Cell Count by Impedance								
PANSS total score	0.036	0.003	0.306	2.313	-0.660	-0.190	-1.953 – 0.634	0.306
PANSS negative score	0.002	-0.061	0.873	1.093	0.224	0.041	-2.694 – 3.143	0.873
PANSS positive score	0.348	0.307	0.010	3.096	-2.826	-0.590	-4.878 – -0.774	0.010
After adjusting for sex and age								
PANSS total score	0.043	-0.063	0.749	2.272	-0.583	-0.168	-2.111 – 0.945	0.441
PANSS negative score	0.034	-0.173	0.919	0.657	0.634	0.115	-2.906 – 4.175	0.707
PANSS positive score	0.366	0.231	0.086	2.932	-2.584	-0.539	-5.079 – -0.090	0.043
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.083	-0.135	0.855	2.258	-0.472	-0.136	-2.258 – 1.314	0.588
PANSS negative score	0.111	-0.445	0.954	0.937	0.471	0.085	-4.550 – 5.491	0.834
PANSS positive score	0.379	-0.008	0.485	3.075	-2.751	-0.574	-6.398 – -0.896	0.120
FL3-fluorescence of neutrophils								
PANSS total score	0.075	0.038	0.167	2.794	-0.474	-0.274	-1.163 – 0.213	0.167
PANSS negative score	0.229	0.159	0.098	3.813	-1.320	-0.478	-2.928 – 0.288	0.098
PANSS positive score	0.002	-0.089	0.883	1.380	-0.109	-0,045	-1.700 – 1.482	0.883

After adjusting for sex and age								
PANSS total score	0.094	-0.024	0.509	2.786	-0.471	-0.271	-1.190 – 0.248	0.188
PANSS negative score	0.238	-0.016	0.462	3.690	-1.288	-0.467	-3.188 – 0.543	0.146
PANSS positive score	0.143	-0.143	0.691	1.129	-0.043	-0.018	-1.732 – 1.645	0.955
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.098	-0.117	0.804	2.692	-0.421	-0.243	-1.511 – 0.668	0.430
PANSS negative score	0.321	-0.164	0.664	4.729	-1.866	-0.676	-4.823 – 1.091	0.179
PANSS positive score	0.145	-0.465	0.933	0.931	0.072	0.030	-2.814 – 2.958	0.955

Supplementary table 2: Multiple linear regression models using CGI-S scores as dependent variable and the hematological variables as independent variable. Regarding the hematological variables, the hematological measurements closest to the study visit was used. All variables were log-transformed.

	R square	Adjusted R square	Sig. model	Constant	B	Beta	95% CI for B	Sig. beta
White Blood Cell Count	0.010	-0.024	0.589	0.685	-0.111	-0.101	-0.527– 0.305	0.589
After adjusting for sex and age	0.029	-0.079	0.848	0.677	-0.117	-0.106	-0.559 – 0.325	0.592
After adjusting for sex, age, smoking, and clozapine dose	0.125	-0.083	0.700	0.761	-0.068	-0.061	-0.561 – 0.426	0.779
Neutrophilic granulocyte absolute count	0.045	0.012	0.252	0.682	-0.157	-0.212	-0.433 – 0.118	0.252
After adjusting for sex and age	0.069	-0.035	0.581	0.657	-0.173	-0.233	-0.467 – 0.121	0.237
After adjusting for sex, age, smoking, and clozapine dose	0.159	-0.042	0.567	0.765	-0.149	-0.201	-0.473 – 0.174	0.348
Eosinophilic granulocyte absolute count	0.164	0.136	0.024	0.677	0.085	0.406	0.012 – 0.159	0.024
After adjusting for sex and age	0.191	0.101	0.121	0.656	0.088	0.418	0.013 – 0.163	0.024
After adjusting for sex, age, smoking, and clozapine dose	0.248	0.070	0.269	0.718	0.080	0.378	-0.008 – 0.168	0.061
Red blood cell count by Impedance	0.013	-0.021	0.543	0.902	-0.457	-0.114	-1.972 – 1.059	0.543
After adjusting for sex and age	0.024	-0.084	0.879	0.852	-0.354	-0.088	-2.140 – 1.432	0.687
After adjusting for sex, age, smoking, and clozapine dose	0.123	-0.086	0.710	0.821	-0.147	-0.037	-2.169 – 1.875	0.881
FL3-fluorescence of neutrophils	0.154	0.120	0.043	2.136	-0.789	-0.393	-1.551 – -0.028	0.043
After adjusting for sex and age	0.173	0.066	0.215	2.185	-0.798	-0.397	-1.593 – -0.003	0.049
After adjusting for sex, age, smoking, and clozapine dose	0.179	-0.016	0.490	2.007	-0.700	-0.348	-1.903 – 0.503	0.240

Supplementary table 3: Multiple linear regression models using PANSS scores as dependent variable and the hematological variables as independent variable. Regarding the hematological variables, mean hematological values were used. All variables were log-transformed.

	R square	Adjusted R square	Sig. model	Constant	B	Beta	95% CI for B	Sig. beta
White Blood Cell Count								
PANSS total score	0.005	-0.029	0.694	1.928	-0.075	-0.074	-0.464 – 0.313	0.694
PANSS negative score	0.026	-0.035	0.441	1.514	-0.314	-0.194	-1.157 – 0.529	0.441
PANSS positive score	0.050	-0.010	0.373	0.897	0.316	0.223	-0.416 – 1.048	0.373
After adjusting for sex and age								
PANSS total score	0.029	-0.079	0.847	1.913	-0.092	-0.090	-0.507 – 0.322	0.652
PANSS negative score	0.084	-0.112	0.736	1.416	-0.412	-0.254	-1.336 – 0.512	0.355
PANSS positive score	0.175	-0.001	0.425	0.851	0.266	0.188	-0.500 – 1.032	0.469
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.074	-0.147	0.887	1.959	-0.063	-0.062	-0.539 – 0.412	0.784
PANSS negative score	0.180	-0.333	0.868	1.618	-0.470	-0.290	-1.742 – 0.802	0.419
PANSS positive score	0.181	-0.331	0.866	0.857	0.286	0.202	-0.824 – 1.397	0.568
Neutrophilic granulocyte absolute count								
PANSS total score	0.038	0.005	0.293	1.942	-0.133	-0.195	-0.387 – 0.121	0.293
PANSS negative score	0.153	0.101	0.108	1.496	-0.422	-0.392	-0.948 – 0.103	0.108
PANSS positive score	0.032	-0.028	0.475	1.067	0.169	0.180	-0.322 – 0.661	0.475
After adjusting for sex and age								
PANSS total score	0.069	-0.035	0.582	1.905	-0.154	-0.227	-0.462 – 0.117	0.253
PANSS negative score	0.231	0.066	0.284	1.341	-0.511	-0.474	-1.077 – 0.054	0.073
PANSS positive score	0.159	-0.021	0.474	0.996	0.125	0.133	-0.392 – 0.642	0.612

After adjusting for sex, age, smoking, and clozapine dose	0.105	-0.109	0.779	1.958	-0.134	-0.196	-0.444 – 0.176	0.379
PANSS total score	0.312	-0.117	0.622	1.453	-0.519	-0.481	-1.291 – 0.252	0.159
PANSS negative score	0.163	-0.360	0.892	1.018	0.135	0.144	-0.608 – 0.879	0.686
PANSS positive score								
Eosinophilic granulocyte absolute count								
PANSS total score	0.118	0.087	0.059	1.902	0.045	0.343	-0.002 – 0.092	0.059
PANSS negative score	0.116	0.060	0.167	1.306	0.071	0.340	-0.033 – 0.174	0.071
PANSS positive score	0.116	0.060	0.496	1.194	0.031	0.171	-0.064 – 0.126	0.496
After adjusting for sex and age								
PANSS total score	0.153	0.059	0.206	1.861	0.048	0.368	0.000 – 0.097	0.050
PANSS negative score	0.159	-0.022	0.475	1.143	0.077	0.371	-0.033 – 0.188	0.157
PANSS positive score	0.193	0.020	0.376	1.056	0.041	0.227	-0.053– 0.136	0.366
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.161	-0.039	0.559	1.874	0.045	0.339	-0.017 – 0.106	0.147
PANSS negative score	0.200	-0.300	0.836	1.216	0.072	0.347	-0.099 – 0.243	0.359
PANSS positive score	0.197	-0,305	0.841	1.022	0.047	0.258	-0.103 – 0.196	0.492
Red Blood Cell Count by Impedance								
PANSS total score	0.002	-0.032	0.797	1.875	-0.016	-0.048	-0.140 – 0.108	0.797
PANSS negative score	0.024	-0.037	0.542	1.186	0.080	0.154	-0.191 – 0.351	0.542
PANSS positive score	0.189	0.138	0.072	1.314	-0.197	-0.434	-0.412 – -0.019	0.072
After adjusting for sex and age								
PANSS total score	0.023	-0.085	0.886	1.860	-0.014	-0.041	-0.144 – 0.116	0.832
PANSS negative score	0.059	-0.143	0.831	1.018	0.098	0.189	-0.195 – 0.391	0.485
PANSS positive score	0.306	0.157	0.152	1.235	-0.186	-0.411	-0.406 – -0.034	0.091
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.082	-0.136	0.859	1.968	-0.039	-0.118	-0.192 – 0.114	0.605
PANSS negative score	0.119	-0.432	0.946	1.189	0.065	0.125	-0.362 – 0.491	0.736
PANSS positive score	0.339	-0.074	0.567	1.364	-0.214	-0.474	-0.537 – -0.108	0.163
FL3-fluorescence of neutrophils								
PANSS total score	0.014	-0.025	0.551	2.272	-0.210	-0.120	-0.924 – 0.504	0.551
PANSS negative score	0.078	-0.006	0.356	2.749	-0.771	-0.279	-2.530 – 0.989	0.356
PANSS positive score	0.023	-0.066	0.619	1.886	-0.368	-0.153	-1.950 – 1.214	0.619
After adjusting for sex and age								
PANSS total score	0.038	-0.087	0.823	2.284	-0.225	-0.129	-0.964 – 0.515	0.536
PANSS negative score	0.102	-0.197	0.796	2.628	-0.733	-0.280	-2.749 – 1.202	0.399
PANSS positive score	0.172	-0.103	0.617	1.855	-0.416	-0.173	-2.074 – 1.241	0.584
After adjusting for sex, age, smoking, and clozapine dose								
PANSS total score	0.073	-0.148	0.889	2.097	-0.099	-0.057	-0.961 – 0.762	0.812
PANSS negative score	0.139	-0.477	0.940	2.331	-0.569	-0.206	-3.153 – 2.016	0.619
PANSS positive score	0.175	-0.414	0.900	1.937	-0.473	-0.196	-2.683 – 1.738	0.955

Supplementary table 4: Multiple linear regression models using CGI-S scores as dependent variable and the hematological variables as independent variable. Regarding the hematological variables, mean hematological values were used. All variables were log-transformed.

	R square	**Adjusted R square**	**Sig. model**	**Constant**	**B**	**Beta**	**95% CI for B**	**Sig. beta**
White Blood Cell Count	0.013	-0.021	0.547	0.704	-0.134	-0.113	-0.581– 0.314	0.589
After adjusting for sex and age	0.032	-0.076	0.828	0.696	-0.143	-0.121	-0.622 – 0.335	0.544
After adjusting for sex, age, smoking, and clozapine dose	0.128	-0.080	0.688	0.785	-0.100	-0.085	-0.635 – 0.434	0.700
Neutrophilic granulocyte absolute count	0.062	0.029	0.177	0.707	-0.196	-0.249	-0.487 – 0.094	0.177
After adjusting for sex and age	0.087	-0.015	0.477	0.675	-0.215	-0.273	-0.526 – 0.095	0.167
After adjusting for sex, age, smoking, and clozapine dose	0.169	-0.029	0.529	0.774	-0.181	-0.229	-0.526 – 0.165	0.289
Eosinophilic granulocyte absolute count	0.284	0.259	0.002	0.660	0.081	0.533	0.032 – 0.130	0.002
After adjusting for sex and age	0.320	0.245	0.014	0.619	0.085	0.557	0.034 – 0.135	0.002
After adjusting for sex, age, smoking, and clozapine dose	0.331	0.172	0.108	0.644	0.079	0.517	0.015 – 0.142	0.018

Red blood cell count by Impedance	0.056	0.024	0.200	0.523	0.090	0.237	-0.050 – 0.230	0.200
After adjusting for sex and age	0.077	-0.026	0.532	0.496	0.093	0.247	-0.053 – 0.240	0.201
After adjusting for sex, age, smoking, and clozapine dose	0.146	-0.058	0.619	0.625	0.063	0.166	-0.108 – 0.234	0.452
FL3-fluorescence of neutrophils	0.154	0.120	0.300	1.407	-0.419	-0.207	-1.233 – -0.396	0.300
After adjusting for sex and age	0.065	-0.057	0.667	1.442	-0.435	-0.215	-1.280 – -0.005	0.297
After adjusting for sex, age, smoking, and clozapine dose	0.131	-0.076	0.676	1.125	-0.223	-0.110	-1.187 – 0.742	0.240

Supplementary table 5: Multiple linear regression using total number of side-effects as dependent variable and hematological variables as independent variables. All hematological variables were log-transformed.

	R square	Adjusted R square	Sig. model	Constant	B	Beta	95% CI for B	Sig. beta
White Blood Cell Count	0.228	0.169	0.072	9.387	-6.252	-0.478	-13.14 – 0.632	0.072
After adjusting for sex and age	0.332	0.150	0.201	10.52	-5.738	-0.411	-12.71 – 1.950	0.135
After adjusting for sex, age, smoking, and clozapine dose	0.628	0.421	0.071	11.07	-3.917	-0.299	-10.28 – 2.447	0.197
Neutrophilic granulocyte absolute count	0.301	0.248	0.034	6.901	-4.843	-0.549	-9.263 - -0.424	0.034
After adjusting for sex and age	0.385	0.217	0.135	8.235	-4.210	-0.477	-8.982 – 0.562	0.078
After adjusting for sex, age, smoking, and clozapine dose	0.695	0.525	0.032	9.536	-3.551	-0.402	-7.403 – 0.300	0.067
Eosinophilic granulocyte absolute count	0.001	-0.075	0.896	4.161	0.093	0.037	-1.407 – 1.592	0.896
After adjusting for sex and age	0.174	-0.051	0.533	6.651	-0.032	-0.013	-1.553 – 1.490	0.964
After adjusting for sex, age, smoking, and clozapine dose	0.556	0.309	0.137	8.302	-0.247	-0.098	-1.582 – 1.089	0.686
Red blood cell count by Impedance	0.075	0.004	0.323	-4.865	13.089	0.274	-14.42 – 40.60	0.323
After adjusting for sex and age	0.183	-0.039	0.508	2.816	5.278	0.111	-27.37 – 37.92	0.729
After adjusting for sex, age, smoking, and clozapine dose	0.609	0.392	0.085	-1.982	14.09	0.295	-12.56 – 40.74	0.262
FL3-fluorescence of neutrophils	0.202	0.140	0.093	25.10	-10.74	-0.449	-23.53 – 2.055	0.093
After adjusting for sex and age	0.426	0.270	0.095	30.81	-12.10	-0.506	-24.21 – 0.010	0.050
After adjusting for sex, age, smoking, and clozapine dose	0.594	0.368	0.098	22.19	-7.508	-0.314	-22.22 – 9.205	0.336

Supplementary table 6: Comparison of blood cell count and FL3-fluorescence of neutrophils between patients with high and low symptom severity, adjusted for sex and age. All hematological variables were log-transformed. SD = standard deviation.

Dependent variable	Estimated marginal means high symptom severity	Estimated marginal means low symptom severity	Mean difference	F	Sig.
White blood cell count, 10^9/L	0.862	0.835	0.026	0.282	0.600
Neutrophilic granulocyte absolute count, 10^9/L	0.589	0.581	0.007	0.010	0.920
Eosinophilic granulocyte absolute count, 10^9/L	-0.874	-1.269	0.395	2.432	0.131
Red blood cell count by Impedance, 10^{12}/L	0.680	0.685	0.005	0.174	0.680
FL3-fluorescence of neutrophils	1.946	1.974	0.029	0.982	0.332