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ORIGINAL RESEARCH

Potential Indicators of Bone Marrow Suppression in Patients with Schizophrenia Receiving Clozapine: Platelet-Large Cell Ratio and Immature Granulocytes

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Abstract

Objective: The use of clozapine is restricted due to its serious side effects, particularly bone marrow suppression, which occurs at an average rate of 1%. These side effects are markedly related to blood concentrations of clozapine and its metabolite nor-clozapine. Therefore, therapeutic drug monitoring is recommended for clozapine. Currently, laboratory monitoring of bone marrow suppression includes neutrophil count follow-up. However, using early-changing biomarkers may be more effective in detecting and preventing this side effect before neutropenia develops. Therefore, we aimed to evaluate serum clozapine and nor-clozapine levels and their relationship with early parameters reflecting bone marrow activity in complete blood count (CBC), immature granulocyte (IG) for neutrophils, mean platelet volume (MPV) and platelet-to-large cell ratio (P-LCR) for platelets in patients with schizophrenia receiving clozapine.

Methods: Fifty-one patients with schizophrenia receiving clozapine were included in the study. Of these, 49% (n=25) were on a low dose (<300 mg/day), 49% (n=25) on a middle dose (300-600 mg/day), and one patient was on a high dose (>600 mg/day) clozapine. CBC parameters—especially IG, MPV and P-LCR—and serum clozapine/nor-clozapine levels along with clozapine doses were recorded on the same day. The relationship between serum drug concentrations and CBC parameters was evaluated separately for the total patients and dose groups.

Results: There was no correlation between clozapine dose and serum clozapine or nor-clozapine concentrations. None of the patients had neutropenia or agranulocytosis. Serum clozapine and nor-clozapine levels negatively correlated with P-LCR (r=-0.402, p=0.006 and r=-0.465, p=0.001, respectively) and MPV (r=-0.294, p=0.036 and r=-0.397, p=0.004, respectively); positively correlated with neutrophil (r=0.381, p=0.011 and r=0.387, p=0.009, respectively) and IG counts (r=0.346, p=0.018 and r=0.335, p=0.023, respectively); but not with other CBC sub-parameters. Although the clozapine dosing differed between the two groups, the serum levels of clozapine and nor-clozapine were not significantly different (p=0.078 and p=0.058, respectively). Furthermore, in the middle dose group, serum clozapine and nor-clozapine levels were negatively correlated with P-LCR (rho=-0.547, p=0.007 and rho=-0.636, p=0.001, respectively), consistent with the total group.

Conclusion: Monitoring early biomarkers reflecting bone marrow activity, such as P-LCR for platelets and IG for neutrophils, alongside serum clozapine and nor-clozapine levels, is promising for predicting and preventing bone marrow suppression in patients receiving clozapine, thereby protecting against this serious side effect. However, our findings need to be supported by further research.

Keywords: Clozapine, Nor-clozapine, Therapeutic drug monitoring, Platelet large cell ratio, Immature granulocyte

INTRODUCTION

Clozapine is uniquely effective in reducing both positive and negative clinical symptoms in treatment-resistant schizophrenia (1-3). In addition, it is the most effective antipsychotic available in the treatment of affective disorders, some neurological disorders, aggression, and psychosis in dementia and parkinsonism (4).

Common side effects of clozapine are sedation, hypersalivation, tachycardia, hypotension, hypertension, tremor, tardive dyskinesia, weight gain, constipation, urinary incontinence, and fever. These are usually medically manageable and easily tolerated by patients. Serious side effects such as agranulocytosis, myocarditis,

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and neuroleptic malignant syndrome are fortunately rare (5). These side effects are correlated to blood concentrations of clozapine and its main metabolite nor-clozapine (6). It has been reported that clozapine and nor-clozapine are directly toxic to myeloid mitotic processes and maturation (7). Neutropenia (granulocyte count decreases to 500-1.500/mm^3) occurs in approximately 4% of patients and typically regresses within a few days after drug discontinuation. Agranulocytosis (granulocyte count below 500/mm^3) occurs in 1% of patients (8). Many countries have restricted the use of clozapine due to life-threatening agranulocytosis. Due to these serious side effects, the concerns of psychiatric specialists about clozapine have led to its use at significantly lower rates than necessary. This situation poses a significant public health issue, both globally and in Turkey. Clinically, clozapine offers superior efficacy and, due to its low cost, provides important pharmacoeconomic advantages. With appropriate therapeutic drug monitoring approaches, the side effects of clozapine can be managed to a significant extent (9).

To initiate clozapine treatment, the neutrophil count should be 1500/µL and above. The Food and Drug Administration (FDA) requires regular monitoring and recording of neutrophil counts (weekly for the first six months, biweekly for the second six months, and every four weeks thereafter) for all patients receiving clozapine. Pharmacies are also required to see and confirm the neutrophil count before giving the prescribed clozapine to the patient ("No blood, no medicine") (9, 10). If mild neutropenia (1000 to 1499/µL) develops during treatment, continue treatment, but increase the frequency of monitoring to three times a week. If moderate neutropenia (500 to 999/µL) develops, interrupt clozapine therapy (until 1000/µL). If severe neutropenia/agranulocytosis (<500/µL) develops, clozapine is discontinued. For this reason, complete blood count (CBC) monitoring is performed at regular intervals in patients using clozapine (9, 11). In recent years, mandatory CBC monitoring has significantly reduced both the incidence of agranulocytosis and its associated mortality (6).

In addition to traditional CBC parameters and five-part white blood cell (WBC) differential counts, since a while, the hematology analyzers provide immature granulocytes (IG), nucleated red blood cells (NRBCs), reticulocytes, detailed additional information on platelets (platelet-large cell ratio, P-LCR; mean platelet volume, MPV), and blasts and atypical leukocytes as auto analyzer flags (12). IG, less than 1% of leukocytes in healthy individuals, increases in the early-stage response of the bone marrow to stimuli, including infectious and inflammatory processes, and initial response to compensate for bone marrow suppression (13). MPV reflects the mean size of platelets and ranges from 7.5 fL to 10.5 fL. P-LCR reflects the proportion of platelets greater than 12 fL and less than 30% of the platelet count. In general, increased P-LCR occurs in platelet hyperdestruction, while decreased P-LCR is an indicator of platelet hypoproduction from the bone marrow. Therefore, a decrease in P-LCR is expected in bone marrow suppression (14).

The side effects of clozapine are related to blood concentrations of clozapine and its main metabolite nor-clozapine; clozapine drug monitoring is critical for newly drug-administered patients to observe side effects or drug efficiency (6).

For these reasons, we hypothesized that bone marrow damage in clozapine-treated patients would correlate with serum levels of clozapine and nor-clozapine. Additionally, we posited that WBC and platelet sub-parameters reflecting bone marrow activity in the CBC would exhibit changes earlier, potentially serving as early warning indicators.

We evaluated serum clozapine and nor-clozapine levels in patients with schizophrenia receiving clozapine treatment, and their relationship with drug dose, WBC, red blood cell (RBC), platelet counts, hemoglobin concentrations, and the parameters reflecting bone marrow activity, P-LCR, MPV and IG. In addition, patients were grouped based on the classification by the Psychopharmacology Institute: (1) Patients receiving below 300 mg/day clozapine, low dose group; (2) patients receiving 300-600 mg/day clozapine, middle dose group; (3) patients receiving above 600 mg/day clozapine, high dose group. Serum clozapine/nor-clozapine levels and hematologic parameters were compared between these groups and the relationships were re-evaluated separately in each group.

METHODS

Study Design

This study was designed as a cross-sectional, observational analysis conducted at a Community Mental Health Center from February 2017 to October 2017.

Patients who met the following criteria were included into the study: (1) aged 18-65 years, (2) diagnosed with

schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), (3) receiving clozapine treatment for at least 8 weeks, (4) having requested serum clozapine/nor-clozapine levels and CBC. Patients were excluded based on the following criteria to minimize confounding factors: (1) presence of additional chronic diseases other than schizophrenia, (2) presence of active infection, or (3) current hospitalization.

All blood samples were collected from the included patients before their morning medication doses and analyzed for CBC and serum clozapine/nor-clozapine levels. Potential correlations between hematological parameters and serum levels of clozapine and nor-clozapine were assessed.

The study population was grouped as follow: (1) Patients receiving below 300 mg/day clozapine, low dose group; (2) patients receiving 300-600 mg/day clozapine, middle dose group; (3) patients receiving above 600 mg/day clozapine, high dose group. Serum clozapine/nor-clozapine levels and hematologic parameters were compared between these groups and the relationships were re-evaluated separately in each group.

Laboratory Analysis

The blood samples were taken from the patients just before the morning dose in tubes containing dipotassium ethylene diamine tetra acetic acid (K_2EDTA) (3.2 mL, Vacuette; Greiner Bio-One, Kremsmünster, Austria) and serum tubes without gel separator (8 mL, Vacuette; Greiner Bio-One, Kremsmünster, Austria).

CBC analyses in EDTA whole blood samples were performed on the XN 9000 Hematology Analyzer (Sysmex Corporation). WBC, RBC, platelet counts, hemoglobin concentrations, IG counts and percentages, MPV, and P-LCR values of all patients were evaluated and recorded.

The serum tubes with gel separator were centrifuged at 2000xg for 10 minutes and serum was immediately separated to prevent absorption of clozapine by the separator gel. Serum clozapine and nor-clozapine levels were measured by high-performance liquid chromatography (HPLC) with the Thermo Scientific™ Dionex™ UltiMate™ 3000 rapid separation LC, including a UV detector. Thermo Scientific Accucore PFP column (50 × 2.1 mm, 2.6 µm particle size) was used with the chromsystems-mobile phase, and after the solid phase extraction (SPE) for sample pre-treatment, 50 µL of serum sample was directly injected into the HPLC column. The UV detector was recorded at 220 nm. Measurement was based on isocratic elution and the flow rate was maintained at 0.6 mL/min and the run time was 22 minutes. The method was linear for clozapine and nor-clozapine from 10 ng/mL (limit of quantitation) to 1000 ng/mL, covering the therapeutic range of clozapine (350-600 ng/mL). In addition, the intra-day and inter-day coefficients of variation (CV) were lower than 15% for both analytes.

Statistical Analysis

A power analysis was conducted to determine the required sample size for the study. Given a power of 0.95 (beta = 0.05) and an alpha level of 0.05, with an expected effect size (r) of 0.5, the necessary sample size was estimated to be 46 participants.

The data was analyzed on SPSS 21.0 statistical software (IBM Corp., Armonk, New York, United States). Normal distribution of the data was evaluated with histograms and Q-Q plots.

Summary statistics were presented as frequency (%) for categorical variables, mean ± standard deviation or median (IQR) for continuous variables according to normal distribution. Independent sample *t* test or Mann-Whitney U test according to normal distribution was used to compare continuous variables, chi-square test was used to compare categorical variables between groups and two-tailed *p* values were presented. Since clozapine dose, serum clozapine and nor-clozapine levels did not conform to normal distribution, the relationship between these variables and CBC parameters was evaluated by nonparametric Spearman correlation analysis and correlation coefficient (rho) value and two-tailed *p* values are presented. A *p*-value of below 0.05 was accepted as statistically significant. According to the rho value, the strength of the relationship was interpreted as follows: 0.299 and below, weak; 0.300-0.599, moderate; 0.600-0.900, strong; 0.900 and above, very strong.

Ethical Approval

The local ethics committee approved this study under the Declaration of Helsinki (Decision no: 23.08.2017-07) and informed consent forms were obtained from all participants.

RESULTS

The study population consisted of a total of 51 patients with schizophrenia, 37 males (72.5%) and 14 females (27.5%) with a mean age of 41.1+8.9 years (26-61) (Table 1).

The median daily clozapine dose was 300 mg (min-max, 25-700). While 49% (*n*=25) of the patients received low dose clozapine (<300 mg/day), 49% (*n*=25) received middle dose clozapine (300-600 mg/day) and one patient received high dose clozapine (>600 mg/day) with 700 mg/day (Table 1 and Table 2). While 27.4% (*n*=14) of the patients were taking clozapine monotherapy, 72.6% (*n*=37) were taking at least one antipsychotic in addition to clozapine: 49% quetiapine (*n*=25), 27.5% paliperidone (*n*=14), 13.7% olanzapine (*n*=7) and 7.8% risperidone (*n*=4).

The median serum clozapine level was 282 (113-555) ng/mL (therapeutic range, 350-600 ng/mL), and the median nor-clozapine level was 109.0 (50-188) ng/mL in total patients. 58.8% (*n*=30) of the patients were below the lower limit of serum clozapine therapeutic window of 350 ng/mL.

Three patients had a WBC count below the lower reference limit (3.91x10^3/µL, 3.91x10^3/µL, and 4.32x10^3/µL, reference range: 4.50-10.00x10^3/µL) and a patient had a platelet count below the lower reference limit (143 x10^3/µL, reference range: 150-400x10^3/µL). However, none of the patients had neutropenia or agranulocytosis (Table 1).

Table 1. Clozapine doses, serum clozapine / nor-clozapine levels, and CBC parameters of the total patients.

Parameters	Mean ± SD or Median (IQR)	Min	Max	Reference Range
Age (years)	41.1±8.9	26	61	-
Gender, (male, *n* (%))	37 (72.5%)	-	-	-
Clozapine dose (mg/day)	300 (150-400)	25	700	-
Clozapine (ng/mL)	282 (113-555)	7	2007	350-600
Nor-clozapine (ng/mL)	109.0 (50-188)	2	697	-
WBC (10^3/µL)	7.45 ± 2.17	3.91	12.61	4.5-10
Neutrophil (10^3/µL)	4.64 ± 1.72	2.41	9.08	1.8-7.5
Lymphocyte (10^3/µL)	2.16 ± 0.611	1.13	3.53	0.8-3.4
IG (/µL)	0.034 ± 0.023	0.00	0.09	0-0.1
IG (%)	0.421 ± 0.258	0.00	1.10	0-0.5
RBC (10^6/µL)	5.21 ± 0.47	4.27	6.11	3.7-5.5
Hemoglobin (g/dL)	15.1 ± 1.37	12.5	17.4	12-17
Platelet (10^3/µL)	242 ± 56.3	143	393	150-450
MPV (fL)	10.8 ± 1.12	8.7	13.8	9-12
P-LCR (%)	31.4 ± 9.28	15.1	55.7	15-35

Summary data are presented as median (interquartile range, IQR) and mean ± standard deviation (SD) according to normal distribution.
WBC, white blood cell; IG, immature granulocyte; RBC, red blood cell; MPV, mean platelet volume; P-LCR, platelet large cell ratio.

In the low dose group (*n*=25), 36% (*n*=9) of the patients were taking clozapine monotherapy, 64% (*n*=16) were taking at least one antipsychotic in addition to clozapine: 40% quetiapine (*n*=10), 20% paliperidone (n=5), 12% olanzapine (*n*=3), and 4% risperidone (*n*=1). In the middle dose group (*n*=25), 20% (*n*=5) of the patients were taking clozapine monotherapy, 80% (*n*=20) were taking at least one antipsychotic in addition to clozapine: 56% quetiapine (*n*=14), 36% paliperidone (*n*=9), 16% olanzapine (*n*=4), and 12% risperidone (*n*=3).

Table 2. Clozapine doses, serum clozapine / nor-clozapine levels, and CBC parameters of the dose groups.

Parameters	Low Dose n=25	Middle Dose n=25	High Dose n=1	p value*
Age (years)	41.2 ± 7.6	40.7 ± 10.4	47	0.851
Gender, (male, *n* (%))	15 (60%)	21 (84%)	1 (100%)	0.059
Clozapine dose (mg/day)	160 (100-200)	400 (300-400)	700	**<0.001**
Clozapine (ng/mL)	273 (113.5-422)	302 (111.5-750)	777	0.078
Nor-clozapine (ng/mL)	108 (32-154)	153 (56-285)	407	0.058
WBC (10^3/µL)	7.00 ± 2.24	7.85 ± 2.09	8.52	0.171
Neutrophil (10^3/µL)	4.28 ± 1.50	4.94 ± 1.90	6.26	0.211
Lymphocyte (10^3/µL)	2.03 ± 0.590	2.31 ± 0.621	1.70	0.143
IG (/µL)	0.029 ± 0.017	0.037 ± 0.027	0.080	0.309
IG (%)	0.391 ± 0.204	0.430 ± 0.291	0.900	0.603
RBC (10^6/µL)	5.14 ± 0.490	5.27 ± 0.463	5.18	0.329
Hemoglobin (g/dL)	14.9 ± 1.39	15.2 ± 1.38	16.2	0.519
Platelet (10^3/µL)	240 ± 56.7	223 ± 51.6	269	**0.020**
MPV (fL)	10.71 ± 1.00	10.96 ± 1.23	9.5	0.433
P-LCR (%)	30.1 ± 8.19	33.11 ± 10.16	21.70	0.293

Summary data are presented as median (interquartile range, IQR) and mean ± standard deviation (SD) according to normal distribution.
WBC, white blood cell; IG, immature granulocyte; RBC, red blood cell; MPV, mean platelet volume; P-LCR, platelet large cell ratio.
Low dose: <300 mg/day; Middle dose: 300-600 mg/day; High dose: >600 mg/day
**Comparison between low and middle dose groups. High dose group was not included in the analysis as there was only one patient. Statistical significance is indicated in bold.*

A comparative analysis was performed between the low (*n*=25) and middle (*n*=25) dose groups. A patient in the high dose group was using quetiapine in addition to clozapine. Unfortunately, since there was only one patient in the high dose group, it could not be included in the analysis. Although the clozapine dosing differed between the two groups, the serum levels of clozapine

and nor-clozapine were not significantly different (p=0.078 and p=0.058, respectively). 64% (n=16) in the low dose group, and 56% (n=14) in the middle dose group were below the lower limit of serum clozapine therapeutic window of 350 ng/mL. In terms of CBC parameters, platelet count was significantly lower in the middle dose group (p=0.020) (Table 2).

Correlation analysis conducted in the total patient group revealed that clozapine dose was not significantly correlated with serum clozapine or nor-clozapine levels, nor with most CBC parameters, except for platelet count (rho = – 0.304, p=0.030). Serum clozapine and nor-clozapine levels were not related to platelet counts; however, they were negatively correlated with platelet indices: mean platelet volume (MPV) (rho = – 0.294, p=0.036 and rho = – 0.397, p=0.004, respectively) and platelet large cell ratio (P-LCR) (rho = – 0.402, p=0.006 and rho = – 0.465, p=0.001, respectively). Both serum levels were positively correlated with neutrophil counts (rho = 0.381, p=0.011 and rho = 0.387, p=0.009, respectively), immature granulocyte (IG) counts (rho = 0.346, p=0.018 and rho = 0.335, p=0.023, respectively), and IG percentage (rho = 0.328, p=0.026 and rho = 0.284, p=0.055, respectively). No significant correlations were found between other CBC parameters and serum clozapine or nor-clozapine levels (Table 3).

Table 3. Correlation analysis results between clozapine doses, serum clozapine, serum nor-clozapine and CBC parameters in total patients.

Parameters	Clozapine dose		Clozapine		Nor-clozapine	
	rho	*p*	rho	*p*	rho	*p*
Clozapine (ng/mL)	0.232	0.101	1.000	-	**0.937**	**<0.001**
Nor-clozapine (ng/mL)	0.269	0.056	**0.937**	**<0.001**	1.000	-
WBC (10^3/µL)	0.121	0.397	0.219	0.123	0.257	0.068
Neutrophil (10^3/µL)	0.173	0.261	**0.381**	**0.011**	**0.387**	**0.009**
Lymphocyte (10^3/µL)	0.058	0.707	0.206	0.230	0.254	0.096
IG (/µL)	0.156	0.299	**0.346**	**0.018**	**0.335**	**0.023**
IG (%)	0.174	0.247	**0.328**	**0.026**	**0.284**	**0.055**
RBC (10^6/µL)	0.026	0.856	0.128	0.369	0.179	0.208
Hemoglobin (g/dL)	0.050	0.729	0.058	0.686	0.125	0.381
Platelet (10^3/µL)	**-0.304**	**0.030**	0.244	0.084	0.260	0.065
MPV (fL)	0.067	0.640	**-0.294**	**0.036**	**-0.397**	**0.004**
P-LCR (%)	0.048	0.750	**-0.402**	**0.006**	**-0.465**	**0.001**

WBC, white blood cell; IG, immature granulocyte; RBC, red blood cell; MPV, mean platelet volume; P-LCR, platelet large cell ratio.Statistical significance is indicated in bold.

Table 4. Correlation analysis results between clozapine doses, serum clozapine, serum nor-clozapine and CBC parameters in dose groups.

Parameters	Clozapine dose		Clozapine		Nor-clozapine	
	rho	*p*	rho	*p*	rho	*p*
Low dose group (*n*=25)						
Clozapine (ng/mL)	**0.565**	**0.003**	-	-	**0.897**	**<0.001**
Nor-clozapine (ng/mL)	**0.477**	**0.016**	**0.897**	**<0.001**	-	-
WBC (10^3/µL)	-0.237	0.255	0.154	0.462	0.281	0.173
Neutrophil (10^3/µL)	-0.063	0.781	0.379	0.082	0.417	0.054
Lymphocyte (10^3/µL)	-0.320	0.146	0.168	0.454	0.202	0.367
IG (/µL)	0.026	0.908	0.134	0.551	0.242	0.278
IG (%)	0.216	0.333	0.144	0.523	0.207	0.351
RBC (10^6/µL)	-0.176	0.399	0.158	0.452	0.234	0.260
Hemoglobin (g/dL)	-0.217	0.297	0.096	0.647	0.204	0.327
Platelet (10^3/µL)	0.080	0.704	0.173	0.408	0.307	0.136
MPV (fL)	-0.126	0.549	-0.274	0.185	**-0.413**	**0.040**
P-LCR (%)	-0.313	0.156	-0.281	0.206	-0.342	0.120
Middle dose group (*n*=25)						
Clozapine (ng/mL)	-0.239	0.250	-	-	**0.944**	**<0.001**
Nor-clozapine (ng/mL)	-0.231	0.267	**0.944**	**<0.001**	-	-
WBC (10^3/µL)	-0.104	0.620	0.187	0.371	0.164	0.434
Neutrophil (10^3/µL)	-0.108	0.641	0.208	0.366	0.214	0.352
Lymphocyte (10^3/µL)	-0.048	0.836	0.296	0.192	0.281	0.218
IG (/µL)	0.029	0.897	0.407	0.054	0.345	0.107
IG (%)	0.132	0.548	0.348	0.103	0.269	0.214
RBC (10^6/µL)	-0.128	0.542	0.048	0.818	-0.059	0.778
Hemoglobin (g/dL)	0.008	0.971	-0.025	0.904	-0.003	0.990
Platelet (10^3/µL)	**-0.453**	**0.023**	0.357	0.079	0.372	0.067
MPV (fL)	0.233	0.262	-0.303	0.141	-0.378	0.063
P-LCR (%)	0.186	0.395	**-0.547**	**0.007**	**-0.636**	**0.001**

WBC, white blood cell; IG, immature granulocyte; RBC, red blood cell; MPV, mean platelet volume; P-LCR, platelet large cell ratio.
Statistical significance is indicated in bold.
Low dose: <300 mg/day; Middle dose: 300-600 mg/day. Analysis was not applicable as there was only one patient in the high dose group (>600 mg/day).

When the correlation analyses were repeated according to the dose groups, there was a correlation between clozapine dose and serum clozapine and nor-clozapine levels in the low dose group, while there was no correlation between drug dose and drug levels in the

middle dose group. However, serum clozapine and nor-clozapine levels were strongly correlated in both groups. When the relationship with CBC parameters was evaluated, in the low dose group, only a negative correlation was observed between serum nor-clozapine concentrations and MPV (rho=-0.413 and p=0.040). In the middle dose group, there was a negative correlation between clozapine dose and platelet count (rho=-0.453, p=0.023). Both serum clozapine and nor-clozapine levels were negatively correlated with P-LCR (rho=-0.547, p=0.007 and rho=-0.636, p=0.001, respectively).

DISCUSSION

In this study, we evaluated the relation of serum clozapine and nor-clozapine concentrations with CBC parameters, including IG, MPV and P-LCR.

In the literature, there are many studies investigating the effect of clozapine on WBC and platelet counts, but to our knowledge, this is the first study to evaluate the relationship of clozapine with the early markers of bone marrow activity; IG for neutrophils and P-LCR for platelets. In addition, unlike most other studies, this study evaluated the relationship of CBC parameters not only with clozapine dose but also their association with serum clozapine and its metabolite nor-clozapine levels.

There are cases of clozapine-related thrombocytopenia reported in the literature. It is recommended to discontinue clozapine if the platelet counts fall below $100x10^3$/µL and continue treatment when they return to the reference range ($150\text{-}450x10^3$/µL). If thrombocytopenia recurs, clozapine should be permanently discontinued (15). Rudolf et al. reported that clozapine-related agranulocytosis and thrombocytopenia developed in a patient and neutropenia was successfully treated with G-CSF, thrombocytopenia continued and spontaneously resolved after 14 days. They concluded that the bone marrow toxicity of clozapine is not limited to granulocyte maturation but may also impair megakaryopoiesis, a process by which megakaryocytes and eventually platelets are produced from progenitor cells (16). Kate et al. reported that in a case who developed thrombocytopenia after clozapine treatment, it persisted for 24 weeks after the dose reduction and resolved only with discontinuation (17). Therefore, clozapine-induced thrombocytopenia is known to be transient, but Gonzales et al. reported one case that persisted for 40 months after discontinuation. In addition, they observed increased platelet serotonin release in vitro and thought that clozapine-related thrombocytopenia may be due to an immune-based mechanism (18).

In addition, there are studies evaluating the effect of clozapine on MPV, one of the platelet indices. Semiz et al. reported that MPV was elevated in patients with schizophrenia receiving atypical antipsychotics (19). Gharab et al. reported that platelets count and MPV values were significantly lower in clozapine-treated individuals compared to healthy volunteers (20). Lee et al. compared MPV and platelet counts before and one year after the initiation of clozapine in patients with schizophrenia and related disorders. Contrary to Semiz et al. and Gharab et al., they showed that MPV and platelet counts did not change significantly. They speculated that this may be due to MPV already being high prior to the initiation of clozapine or that clozapine had no effect on megakaryopoiesis, although they did not mention the reason for high MPV values before the treatment (21). In this study, we found that platelet counts were significantly lower in patients receiving middle-dose clozapine compared to those on low-dose clozapine. Both clozapine and nor-clozapine levels showed a negative correlation with P-LCR and MPV. Notably, in patients on standard-dose clozapine (corresponding to the middle dose group), the negative correlation with P-LCR was particularly pronounced. These findings suggest that clozapine may suppress platelet production in the bone marrow and the evaluation of P-LCR may be a better marker than MPV in the monitoring of platelet series suppression.

There are three main mechanisms of drug-induced bone marrow toxicity: (1) direct toxicity by the drug, (2) toxicity associated with metabolic products of the drug, and (3) the most common mechanism, toxicity associated with drug-induced immunogenicity (22). It is known that clozapine and its main metabolite nor-clozapine are directly toxic to the myeloid mitotic process and maturation (6). In this study, there was no significant suppression in myelopoietic and megakaryocytic series in patients, but there was a negative correlation between MPV and P-LCR levels and serum drug concentrations. P-LCR reflects the proportion of platelets greater than 12 fL and decreases in bone marrow suppression (14). The decreasing P-LCR with increasing serum drug levels suggests that clozapine suppresses platelet production in the bone marrow. Therefore, it can be concluded that clozapine may sometimes suppress platelet production more than granulocyte production, or it may be an early sign of hematotoxicity before granulocyte suppression and the development of neutropenia or agranulocytosis. These parameters are promising as a predictive marker

for clozapine-induced bone marrow suppression and should be evaluated in further studies. Possibly, with a cut-off value to be determined, risky patients can be detected before the development of agranulocytosis and significant reductions in mortality by reducing or discontinuing the drug dose.

In the study of Delieu et al., subclinical abnormality of neutrophil populations in patients with schizophrenia treated with chlorpromazine or clozapine was evaluated using cellular immaturity. The neutrophil maturity of patients and controls was compared by determining mean nuclear lobularity in peripheral blood smears. While WBC and neutrophil counts did not differ significantly between patients and controls, the mean nuclear lobularity of patients, who typically had immature neutrophils, differed significantly from controls (23). In our study, although there was no correlation with WBC counts, neutrophil and IG was positively correlated with serum clozapine and nor-clozapine levels. We think that this finding may be related to a defense mechanism of the bone marrow to prevent agranulocytosis. Blackman et al. showed that in patients receiving clozapine, firstly the WBC count fluctuated up and down, and then returned to pre-drug levels around the 12th week (24). Probably, their this finding could support our hypothesis. The increase in IG can be an early predictor of clozapine innocence and should be investigated with larger and more comprehensive studies.

Finally, another important finding of this study was that clozapine dose was not correlated with serum clozapine or nor-clozapine concentrations and there was no significant difference in serum drug concentrations between dose groups. The median serum drug concentrations in both groups were below the lower limit of the therapeutic window (350 ng/mL). Individual metabolic differences can significantly influence blood clozapine concentrations. Drug and food interactions may alter these levels by affecting clozapine absorption and/or metabolism through CYP1A2 activity, the major metabolizer of clozapine. In fact, in our study, most of the patients (72.6%) were using other antipsychotic drugs in addition to clozapine. Additionally, inadequate absorption due to gastrointestinal issues or irregular drug intake resulting from patient non-compliance can also lead to low serum drug levels. Genotype differences in CYP1A2 significantly affect clozapine efficacy and blood concentrations. Bayer et al. reported that carriers of the CYP1A2*1F*1F genotype were less responsive to clozapine compared to those with at least one wild-type allele (*1*1 or *1*1F). Furthermore, smoking reduced the response rate by 15% (25). Although Bayer et al. did not assess blood drug levels, there are studies indicating that both clozapine and nor-clozapine concentrations are lower in smokers compared to non-smokers (26, 27). Unfortunately, in this study, we were not able to obtain information about the smoking status of the patients and we could not evaluate the CYP1A2 polymorphism.

In addition, none of the P-LCR, MPV and IG, which were associated with serum clozapine and/or nor-clozapine levels, were correlated with the clozapine dose. These findings draw attention to the importance of blood clozapine and nor-clozapine monitoring in terms of following the therapeutic range, drug efficacy, and risk of side effects in patients receiving clozapine.

This study had several other limitations: (1) The sample size was relatively small. (2) The pre – and post-clozapine CBC results were not compared. (3) The patients were not newly diagnosed and followed longitudinally; they were only evaluated cross-sectionally. (4) Serum drug concentrations were lower than the therapeutic range. (5) Cytochrome polymorphisms affecting clozapine metabolism could not be assessed. (6) External validity may be limited as samples could not be obtained from other treatment centers.

For future studies, it is recommended to include a larger sample size, incorporate newly diagnosed patients, conduct pre – and post-treatment comparisons, and longitudinal follow-up including clinical and sociodemographic characteristics to better assess the relationship between clozapine/nor-clozapine levels and P-LCR and IG.

In conclusion, monitoring early biomarkers reflecting bone marrow activity, such as P-LCR for platelets and IG for neutrophils, alongside serum clozapine and nor-clozapine levels, is promising for predicting and preventing bone marrow suppression in patients receiving clozapine, thereby protecting against this serious side effect. However, our findings need to be supported by further research.

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