ORIGINAL RESEARCH ARTICLE

Absolute and Dose-Adjusted Serum Concentrations of Clozapine in Patients Switching vs. Maintaining Treatment: An Observational Study of 1979 Patients

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Abstract

Background Clozapine is an effective drug for the management of schizophrenia that has not responded to other agents, but some patients experience insufficient or adverse effects and discontinue treatment.

Objective We investigated a potential association between clozapine serum concentrations and switching to other antipsychotics in a large real-world patient population from a therapeutic drug monitoring service.

Methods Absolute and dose-adjusted serum concentrations (concentration-to-dose ratios [C/D ratios]) of clozapine during dosing between 100 and 1000 mg/day were measured in 1979 Norwegian patients during the period 2005–2019. These variables were compared in patients switching to other antipsychotic drugs versus maintaining clozapine treatment using linear mixed models. Smoking habits were known for 49% of the patients. To prevent potential nonadherence affecting clozapine switching, only patients with serum concentrations above 50% of the lower reference range were included.

Results In total, 190 patients (9.6%) switched from clozapine to another antipsychotic drug during the study period, whereas the remaining patients were not detected as switchers and were interpreted as maintaining treatment. Patients switching treatment had 23.5% lower absolute concentrations (954 vs. 1245 nmol/L; $p < 0.001$) and 15.7% lower daily doses (305 vs. 362 mg/day; $p < 0.001$) of clozapine than did nonswitchers, making the clozapine C/D ratio 9.7% lower in switchers than in nonswitchers after correcting for smoking habits (2.80 vs. 3.10 nmol/L/mg/day; $p = 0.032$).

Conclusions The present study suggests that decreased absolute and dose-adjusted serum concentrations of clozapine were associated with clozapine discontinuation. The significantly reduced clozapine concentrations regardless of prescribed dose in switchers versus nonswitchers may indicate a pharmacokinetic mechanism underlying the risk of clozapine discontinuation.

Key Points

This study suggests an association between lower serum concentrations of clozapine and treatment discontinuation.

Increased metabolism/higher clearance may underlie the discontinuation of clozapine.

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1 Introduction

Schizophrenia is chronic psychiatric illness characterized by severe symptoms such as hallucinations, delusions, lack of motivation and social withdrawal [1–3]. Among patients with schizophrenia, antipsychotic medication is generally required to obtain symptom control, but there is a substantial variability in treatment response. About one-third of patients with schizophrenia using antipsychotics are classified as having treatment-resistant schizophrenia (TRS) [4].

The atypical antipsychotic drug clozapine is currently the only drug licenced for TRS [5, 6]. Several meta-analyses

have indicated that clozapine is superior to all other oral antipsychotics in terms of both symptom improvement and risk of all-cause mortality [7–10]. Discontinuation rates are also lower for clozapine than for other second-generation antipsychotics [11]. However, clozapine therapy may commonly lead to several burdensome adverse effects, including sedation, weight gain, constipation and hypersalivation [12–14]. Clozapine use is also associated with severe neutropenia (agranulocytosis), which is why guidelines require regular monitoring of granulocyte counts during treatment [15]. Also, some patients do not respond to clozapine treatment. It is estimated that up to 40% of patients with TRS may have clozapine-refractory schizophrenia [16], defined as inadequate treatment response for 12 weeks despite stable daily doses of a minimum 500 mg of clozapine [4]. Thus, clozapine discontinuation may occur as a consequence of either insufficient treatment effect or serious adverse effects. However, the potential pharmacological mechanism(s) underlying poor response or intolerability of clozapine are unclear.

Studies have shown an association between the serum concentration of and treatment response to clozapine, and concentrations above 1070 nmol/L (350 ng/mL) are generally required to obtain sufficient therapeutic effect [17–19]. This is incorporated into current clinical guidelines, which recommend dosing to a serum concentration above this threshold to achieve an optimal treatment response to clozapine [19–21]. However, because of substantial individual pharmacokinetic variability and concentration-dependent adverse effects, many patients do not reach this target level within the labelled daily dose of 200–900 mg/day [22].

The metabolism of clozapine is very complex and involves many enzymes, including several cytochrome P450 (CYP) enzymes, flavin monooxygenase 3 (FMO3) and UDP glucuronosyltransferase 1A4 (UGT1A4) [23–28]. Clozapine is converted to the major metabolite *N*-desmethylclozapine via CYP1A2 [29]. A more than tenfold interindividual variability in CYP1A2 activity has been reported [30], which is likely one of the most important enzymes for the pharmacokinetic differences of clozapine.

Because of the pharmacokinetic variability and evident association between concentration and clinical response, use of therapeutic drug monitoring (TDM) during clozapine treatment is 'strongly recommended' in the *Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology* established by the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) expert group [19]. According to the AGNP guidelines, which have been adapted for use in many European countries, TDM of clozapine should be performed during dose titration and on a routine basis in clinical practice to prevent treatment failure [19].

Monitoring of clozapine levels is also recommended by national guidelines, including those of the UK National Institute for Health and Care Excellence [6, 31]. In addition, to titrate dosing and prevent treatment failure, TDM is used as a tool to disclose possible treatment nonadherence, which is a substantial problem in the treatment of schizophrenia [19, 32, 33].

Multiple factors are associated with the pharmacokinetic variability of clozapine. Cigarette smoking is probably the most important factor, but drug–drug interactions, age and sex are also relevant [34–36]. Cigarette smoking is two to three times more frequent in patients with schizophrenia than in the general population. Polycyclic aromatic hydrocarbons released during tobacco smoking induce several drug-metabolizing enzymes involved in clozapine metabolism, with CYP1A2 the most important [26]. Consequently, the serum concentrations of clozapine are decreased by approximately 30% in smokers [37].

Individual variability in clozapine pharmacokinetics, e.g. different metabolism, is measured by the dose-adjusted serum concentrations ('concentration-to-dose' ratios [C/D ratios]) [38–40]. Together with the administered dose, pharmacokinetic differences determine the absolute serum concentration, which is the clinically relevant measure. Although the absolute serum concentration of clozapine has been shown to correlate with clinical response [17, 18], the role of individual pharmacokinetic variability in the treatment outcome is unknown. The aim of the current study was therefore to investigate the absolute and dose-adjusted serum concentrations in patients discontinuing clozapine treatment, defined as switching treatment to other antipsychotic drugs, compared with in patients maintaining (not switching) clozapine treatment in a large real-world population.

2 Materials and Methods

2.1 Study Setting

Included patients were from the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway. This TDM service analyses more than 40,000 patient blood samples annually and has been operating as a national service institution for TDM analyses of psychotropic drugs for more than 20 years. TDM analyses of clozapine and other psychiatric drugs are ordered by treatment-responsible psychiatrists or general practitioners. Approximately 75% of the samples of antipsychotic drugs submitted for TDM analyses are from outpatient settings [32], and the remainder represent blood samples drawn from patients during hospitalization.

2.2 Inclusion of Therapeutic Drug Monitoring Measurements

Adult patients (aged 18–64 years) with longitudinal TDM records of clozapine were included in this study during the period January 2005 through September 2019. All included patients in the present study had at least one TDM event for clozapine requested by their physicians, fulfilling the criteria for inclusion in the pharmacokinetic assessment. Additional TDM sample inclusion criteria were (1) steady-state trough concentrations, i.e. sampling window set at 10–30 h since last intake of clozapine dose, and (2) information on prescribed daily dose of clozapine on the TDM requisition form. To avoid cases of dose titrations during treatment initiation or termination, TDM measurements at prescribed clozapine doses below 100 mg/day were excluded. Furthermore, clozapine doses above 1000 mg/day were excluded to ensure dose-versus-concentration linearity. Serum concentration measurements below 150 nmol/L (< 50% of the lower boundary of the laboratory reference range for clozapine, 300 nmol/L) were excluded to limit the impact of potential nonadherence on clozapine discontinuation. For the included patients, all TDM measurements compliant with the predefined criteria were used for statistical analyses. We interpreted TDM of clozapine as a proxy for TRS. Clozapine may also be prescribed in patients with psychiatric disorders in Parkinson's disease but in recommended doses far below the study inclusion criteria [41]. As patients aged > 64 years were also excluded, all included patients were therefore likely diagnosed with schizophrenia.

Information about comedications were drawn from the TDM requisition forms. Patients with recorded comedication of the CYP enzyme inducers carbamazepine, phenytoin or phenobarbital or the CYP enzyme inhibitor fluvoxamine were excluded. Information about current smoking habits was also drawn from the clozapine TDM requisition forms, although this information was not included for all patients. Information on the number of cigarettes smoked per day was unavailable, but this has been shown to be of limited importance for the degree of induction on clozapine metabolism [42]. Patient status as 'smoker' or 'nonsmoker' was registered when explicit data were present. Sex, age (at latest recorded clozapine measurement), clozapine and *N*-desmethylclozapine serum concentrations, sampling time within 10–30 h after last drug intake, time between the first and last TDM event of clozapine, antipsychotic and antiepileptic comedication and prescribed daily doses of clozapine were retrieved from the patients' TDM records/requisition forms and used in the current analyses.

To ensure the best possible quality of the data and limit potential bias of concentration-modulating factors not registered in the data file, the requisition forms of patients with observations of C/D ratios deviating extensively from the subgroup estimates were subjected to additional review. Patients/outliers were excluded when identified, e.g. co-prescription of interacting drugs not registered in the electronic database, erroneous information on prescribed dose or special conditions mentioned on the forms (infections, organ failure, indications of intoxications, etc.).

The study was approved by the Regional Committee for Medical and Health Research Ethics and the Hospital Investigational Review Board.

2.3 Defining Treatment Discontinuation

Treatment discontinuation was defined as switch from clozapine to another antipsychotic drug, measured as replacement of clozapine TDM with TDM of another antipsychotic drug(s). The antipsychotics potentially replacing clozapine were one or more of the following agents: amisulpride, aripiprazole (oral or intramuscular), flupenthixol, haloperidol, olanzapine (oral or intramuscular), paliperidone (oral or intramuscular), perphenazine, quetiapine, risperidone (oral or intramuscular), sertindole, ziprasidone and zuclopenthixol (oral or intramuscular). Clozapine-treated patients without TDM replacement by another antipsychotic drug during the study period were defined as maintaining treatment ('nonswitchers').

The study design did not allow for classifying whether clozapine switch was because of insufficient clinical effect or side effects/intolerability. However, prescribed doses are generally in the higher end of the recommended dose range when the clinical effect is insufficient, whereas the opposite is present in cases of intolerability. Therefore, the prescribed doses in switchers versus nonswitchers were used as a surrogate measure to indicate whether insufficient clinical effect or intolerability was the main reason for discontinuing clozapine treatment.

2.4 Serum Concentration Analyses of Antipsychotic Drugs and Metabolites

The liquid chromatography tandem mass spectrometry (LC MS/MS) assays used for serum concentration determination of all licenced antipsychotics in Norway were validated and certified for routine TDM according to the bioanalytical requirements of the US FDA. All the serum concentration analyses were performed by the same laboratory, including determination of the major metabolites for clozapine (*N*-desmethylclozapine), aripiprazole (dehydroaripiprazole), quetiapine (desalkyl quetiapine) and risperidone (9-hydroxyrisperidone). During the time course of the retrospective data collection, the analytical assays were slightly modified because the analytical instrumentation was renewed, but all modifications were cross-validated according to standard criteria defined by the FDA. In the most recent

method, all drugs and metabolites were determined in the same assay by ultra-performance LC MS/MS (UPLC MS/MS) as described earlier [43]. Briefly, chromatographic separation was obtained on an Acquity UPLC BEG shield RP18 column (1.7 μm, 1.0 × 100 mm; Waters, Milford, MA, USA) coupled to a Q Exactive Orbitrap UHMR (ultra-high mass range) mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), operated in positive ionization mode acquiring full scan data at a resolution of 70,000 within the 100–1500 Da scan range. The lower limit of quantification was 20 nmol/L (nM) or lower for all the analytes. The validation parameters show inter- and intra-run inaccuracy and imprecision < 5%.

2.5 Comparisons and Statistics

The C/D ratio was calculated by dividing the measured serum concentrations in nmol/L by the prescribed daily clozapine dose (mg/day) and compared between patients switching TDM from clozapine to other antipsychotic drugs ('switchers') and those maintaining clozapine TDM during the study period ('nonswitchers'). Absolute serum concentrations of clozapine and *N*-desmethylclozapine (nmol/L), i.e. not adjusted for dose, the *N*-desmethylclozapine-to-clozapine metabolic ratio (MR) and the prescribed daily doses were also compared between switchers and nonswitchers. In the study, molar concentrations of clozapine (nmol/L) were applied instead of mass concentrations (ng/mL) (conversion factor from nmol/L to ng/mL: 1/3.06) [19].

To obtain maximum statistical power weighting for multiple within-patient TDM measurements, we used a random intercept linear mixed model with restricted maximum likelihood for statistical comparisons of absolute concentrations and C/D ratios between clozapine switchers and nonswitchers. In the multivariate statistical analyses, all TDM measurements compliant with the predefined criteria were included. Although the absolute serum concentration of clozapine followed a normal distribution, this was not the case for the C/D ratios. Thus, natural log-transformation was employed to ensure normal distribution of the clozapine C/D ratios.

In the main statistical analyses, all patient measurements were included regardless of smoking habits. The prescribed daily doses were compared between switchers and nonswitchers without adjusting for any covariates. Sex, age and sampling time within 10–30 h after dose intake are known to have an impact on the serum concentration of clozapine [26, 35, 44] and were included as covariates when comparing the absolute serum concentrations, C/D ratios and MRs between the subgroups.

In subsequent comparisons between switchers and nonswitchers, patients with known smoking habits were separated, and similar statistical analyses as described were performed. Patients who changed smoking status during the study period, according to information registered on the TDM requisition forms, were excluded from the latter analysis. Furthermore, among patients with known smoking habits, we also compared the outcome measures between switchers and nonswitchers in relation to the prescribed dose, i.e. 100–499 versus 500–1000 mg/day. When assessing the absolute serum concentrations of clozapine in relation to treatment failure, we applied the lower target concentration (1070 nmol/L [350 ng/mL]) recommended by the AGNP guidelines [19]. We therefore compared the patient proportions with serum concentrations below 1070 nmol/L in switchers versus nonswitchers.

For comparisons of the demographic variables, Student's *t*-test or the Mann–Whitney test were applied for continuous variables, whereas Fisher's exact test was used for dichotomous variables. The latter test was also used for the comparison of clozapine subtherapeutic concentrations between switchers and nonswitchers.

Statistical analyses were carried out using STATA (v.16.1; StataCorp LP, College Station, TX, USA). The two-sided statistical significance was set at $p < 0.05$.

3 Results

In total, 2704 patients (51,533 TDM measurements) were considered for inclusion in the study. Of these, 1979 fulfilled the predefined criteria for valid clozapine serum concentrations, together representing 23,987 TDM measurements extracted from the database. The majority of the included patients (92.2%) had multiple serum concentration measurements.

Within the included population, 190 patients (9.6%) switched from TDM of clozapine to other antipsychotic drugs during the study period. Among the remaining patients ($n = 1789$ [90.4%]), clozapine TDM was not replaced by TDM of another antipsychotic drug during the study period. Table 1 provides an overview of patient characteristics and TDM statistics in clozapine switchers versus nonswitchers. The sex distribution was similar between the two subgroups, whereas the age was lower in switchers than in nonswitchers ($p < 0.001$; Table 1). The TDM frequency was higher in switchers than in nonswitchers ($p < 0.001$, Table 1). A higher proportion of switchers (77%) than nonswitchers (47%) did not reach therapeutic serum concentrations on the final registered TDM of clozapine ($p = 0.001$; Table 1). There was no significant difference between switchers and nonswitchers regarding time from clozapine intake to time of blood sampling ($p = 0.13$; Table 1). The most common drugs in monotherapy replacing clozapine were quetiapine (13%), olanzapine (12%) and risperidone (9%), and other

Table 1 Characteristics of the included patients, classified as switchers ($n = 190$) or nonswitchers ($n = 1789$) of clozapine treatment to other antipsychotic drugs

Demographics	Switchers	Nonswitchers	p value
Women	71 (37.4)	674 (37.6)	0.530
Age, years[a]	39 (37–41)	43 (42–44)	0.002
Patients with reported smoking habits	140 (73.7)	831 (46.5)	< 0.001
Smokers[b]	105 of 140 (75.0)	547 of 831 (65.8)	0.038
Number of CLZ TDM samples per year, median (IQR)[c]	5.6 (9.6)	3.6 (4.9)	< 0.001
Number of antipsychotic comedications	1.3 ± 1.1	0.7 ± 1.0	< 0.001
Patients with subtherapeutic serum levels at final registered CLZ TDM[d]	146 (77)	841 (46)	0.001
Sampling time, h, median (IQR) [c, e]	12.6 (1.5)	13.0 (2.2)	0.130

Switchers—replacement of clozapine TDM with TDM of other antipsychotic drugs during the study period

Data are presented as n (%), mean ± standard deviation or mean (95% confidence interval) unless otherwise indicated

CLZ clozapine, *IQR* interquartile range, *TDM* therapeutic drug monitoring

[a]Age at latest recorded CLZ measurement

[b]Smoker proportions (%) were calculated based on patients with known smoking habits

[c]Mann–Whitney test

[d]Serum levels < 1070 nmol/L

[e]Time between last CLZ intake and blood sampling

antipsychotics and numerous drug combinations made up the remaining cases.

In the multivariate mixed-model analysis including all the valid TDM measurements, the mean daily dose of clozapine was 15.7% lower (305 vs. 362 mg; $p < 0.001$) in switchers than in nonswitchers (Table 2). Further, the mean absolute serum concentration of clozapine was 23.5% lower (954 vs. 1245 nmol/L; $p < 0.001$) in switchers than in nonswitchers (Table 2). Accordingly, the mean C/D ratio of clozapine was 10.3% lower in switchers than

in nonswitchers (2.86 vs. 3.19 nmol/L/mg/day; $p < 0.01$) (Table 2). For the metabolite *N*-desmethylclozapine, the C/D ratio followed that of clozapine and was 10.6% lower (1.86 vs. 2.08 nmol/L/mg/day; $p = 0.003$) in switchers than in nonswitchers (Table 2); hence, the *N*-desmethyl-clozapine-to-clozapine MR was not significantly different in switchers and nonswitchers ($p = 0.83$; Table 2, Fig. 1a).

For patients prescribed doses of 100–499 mg, the C/D ratio was 12.3% lower in switchers than in nonswitchers (3.00 vs. 3.42 nmol/L/mg/day; $p = 0.011$). For patients

Table 2 Daily dosing and absolute and dose-adjusted serum concentrations of clozapine and *N*-desmethylclozapine and associated metabolic ratio in patients switching ($n = 190$) or not switching ($n = 1789$) from clozapine treatment to other antipsychotic drugs regardless of smoking habits

Variable	Switchers ($n = 190$) Mean (95% CI)	Nonswitchers ($n = 1789$) Mean (95% CI)	Switchers vs. nonswitchers	
			p value	Change, %
Dose CLZ, mg/day	305 (283–328)	362 (355–370)	<0.001	−15.7
Absolute serum concentration CLZ[a]	954 (858–1049)	1245 (1215–1275)	<0.001	−23.5
C/D ratio CLZ[b]	2.86 (2.64–3.10)	3.19 (3.10–3.29)	0.009	−10.3
C/D ratio N-CLZ[b]	1.86 (1.73–1.99)	2.08 (2.03–2.14)	0.003	−10.6
Metabolic ratio	0.68 (0.65–0.71)	0.68 (0.67–0.69)	0.828	–

Linear mixed-model analyses were used, accounting for multiple therapeutic drug monitoring events per patient. Values represent geometric means adjusting for sampling time (time between last CLZ intake and blood sampling), age and sex as covariates. Conversion factor from nmol/L to ng/mL: 1/3.06

C/D ratio dose-adjusted serum concentration, *CI* confidence interval, *CLZ* clozapine, *N-CLZ* N-desmethylclozapine

[a]nmol/L

[b]nmol/L/mg/day
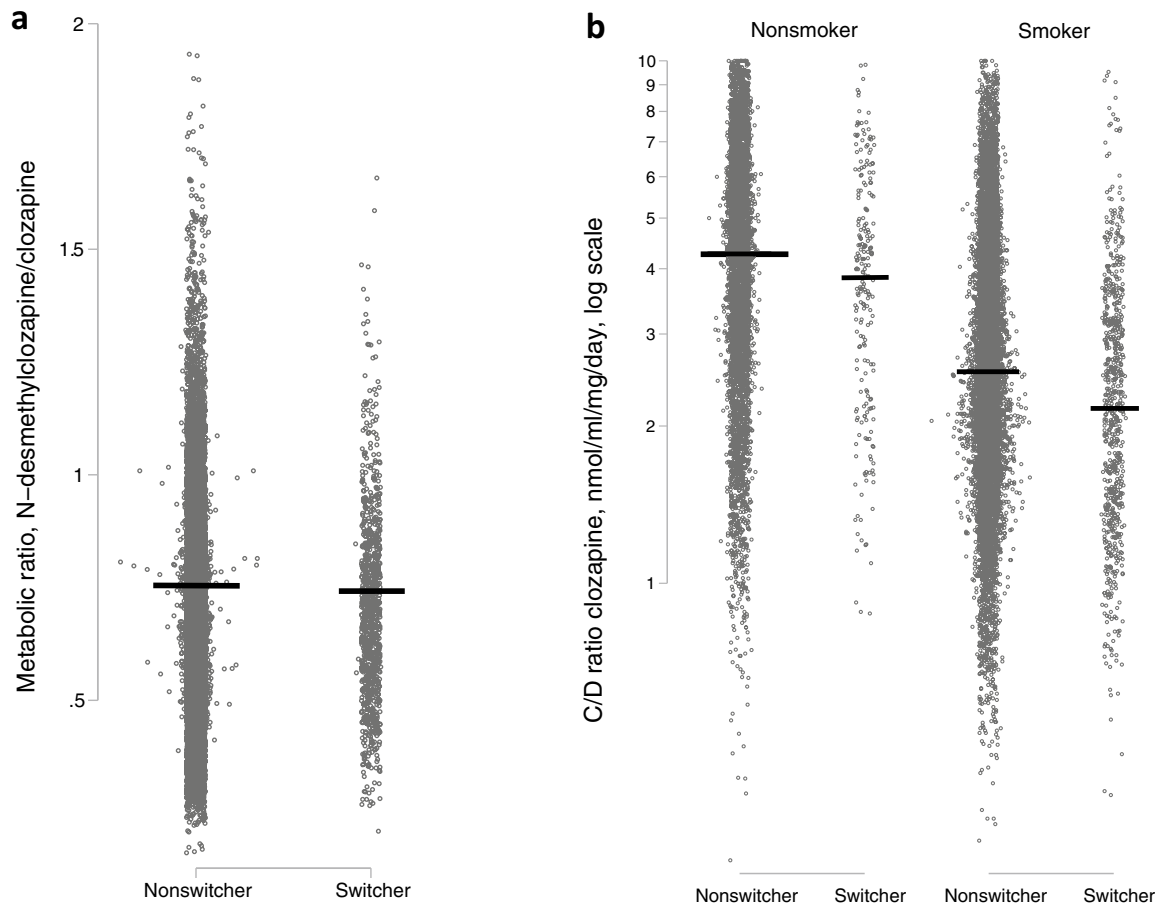
Fig. 1 Individual observations of **a** *N*-desmethyl-to-clozapine metabolic ratios among patients switching vs. not switching clozapine treatment, respectively, and **b** dose-adjusted serum concentrations of clozapine among patients switching vs. not switching treatment in nonsmokers and smokers. *Horizontal lines* indicate the geometric mean values in linear mixed-model analyses. In **b**, measurements with C/D ratios > 10 nmol/L/mg/day are not shown. Conversion factor from nmol/L to ng/mL: 1/3.06. *C/D ratio* concentration-to-dose ratio

using higher clozapine doses of 500–1000 mg/day, the C/D ratio was 17.2% lower in switchers than in nonswitchers (2.12 vs. 2.56 nmol/L/mg/day; 0.028). This illustrates the consistency of the C/D ratio differences between switchers and nonswitchers across the whole clozapine dose range of 100–1000 mg/day.

The subpopulation with known smoking habits comprised 971 patients (49%) with a total number of 13,647 clozapine TDM measurements. Within this subpopulation, the patient proportion confirmed to be smokers was slightly higher among switchers (75.0%) than among nonswitchers (65.8%; $p = 0.038$, Table 1). After adjusting for smoking habits in the subpopulation with known smoking habits, the relative differences in C/D ratios and the other outcome variables were the same between switchers and nonswitchers (Table 3, Fig. 1b) as for the whole study population (Table 2). For the clozapine C/D ratio, this was 9.7% lower in switchers than in nonswitchers in patients with known smoking habits ($p = 0.032$; Table 3).

4 Discussion

The present study shows that patients switching treatment from clozapine to another antipsychotic drug(s) had significantly lower (1) daily dosing (−15.7%), (2) absolute serum concentrations (−23.5%) and (3) dose-adjusted serum concentrations (−10.3%) of clozapine than did patients maintaining clozapine treatment. In total, 190 patients (9.6%) switched from clozapine to another antipsychotic drug during the study period. The findings suggest that the reduced absolute serum concentration, which is the clinically relevant measure, is associated with both lower dosing and different pharmacokinetics (higher clearance) in patients switching treatment to other antipsychotic drugs. Although the study design limited any causal interpretation, this association may provide new insight regarding clozapine treatment discontinuation because of insufficient clinical effect or intolerability.

Table 3 Daily dosing and absolute and dose-adjusted serum concentrations of clozapine and *N*-desmethylclozapine and the associated metabolic ratio in patients switching (*n* = 140) or not switching (*n* = 831) from clozapine to other antipsychotic drugs in patients with known smoking habits (*n* = 971)

Variable	Switchers (*n* = 140)	Nonswitchers (*n* = 831)	Switchers vs. nonswitchers	
	Mean (95% CI)	Mean (95% CI)	*p* value	Change, %
Dose CLZ, mg/day	309 (282–335)	378 (368–389)	<0.001	−18.5
Absolute serum concentration CLZ[a]	959 (852–1066)	1265 (1224–1306)	<0.001	−24.2
C/D ratio CLZ[b]	2.80 (2.56–3.01)	3.10 (3.00–3.19)	0.032	−9.7
C/D ratio N-CLZ[b]	1.82 (1.68–1.97)	1.99 (1.93–2.05)	0.049	−8.5
Metabolic ratio	0.68 (0.65–0.71)	0.67 (0.66–0.68)	0.628	–

Data are from patients with known smoking habits only. To account for multiple therapeutic drug monitoring events per patient, the values represent geometric means in linear mixed-model analyses adjusting for smoking habits, sampling time (time between last CLZ intake and blood sampling), age and sex as covariates. Conversion factor from nmol/L to ng/mL: 1/3.06

C/D ratio dose-adjusted serum concentration, *CI* confidence interval, *CLZ* clozapine, *N-CLZ* *N*-desmethylclozapine

[a]nmol/L

[b]nmol/L/mg/day

Potential nonadherence to treatment is challenging in any chronic illness, including schizophrenia [45], and may be a non-pharmacokinetic reason for interindividual variability in C/D ratios. The nonadherence rate for clozapine is significantly lower than for other antipsychotics [33], which may be because of the frequent monitoring of granulocyte counts and serum concentrations during clozapine therapy. Furthermore, in the present study, we excluded patients with clozapine serum concentrations < 150 nmol/L, i.e. a defined cut-off for partial nonadherence. In line with this, the fact that the C/D ratio of clozapine was significantly lower in switchers than in nonswitchers regardless of dosing suggests that pharmacokinetic variability is associated with the risk of clozapine discontinuation.

The underlying pharmacokinetic mechanism(s) of lower C/D ratios in patients discontinuing treatment is unclear, but a possible explanation is increased metabolism in switchers compared with nonswitchers. Metabolism via *N*-desmethylation is a major pathway in the elimination of clozapine, but the unchanged *N*-desmethylclozapine-to-clozapine ratio between the subgroups demonstrates that increased *N*-desmethylation is not the mechanism behind the reduced clozapine C/D ratio of clozapine within the former subgroup. The metabolism of clozapine is very complex [23], and it is challenging to hypothesize a specific metabolic pathway(s) that may be increased among switchers. However, the rate of this metabolic pathway is unlikely affected by smoking, because of the similar differences in clozapine C/D ratio between switchers and nonswitchers regardless of smoking habits. Further studies should therefore investigate potential metabolic profiles associated with discontinuing clozapine. Of particular interest is the enzymatic pathway mediating the formation of reactive nitrenium metabolites, which has been associated with reduced tolerability and reduction in granulocyte levels during clozapine treatment [46–48].

Interestingly, patients switching treatment from clozapine to other antipsychotics in the present study were prescribed 15–20% lower doses than the nonswitching patients. In addition, the observed mean serum concentration in the subgroup of clozapine-switching patients was below the established therapeutic threshold, i.e. < 1070 nmol/L (350 ng/mL) [17–19]. From a clinical point of view, it is unclear why the physicians did not increase the clozapine dose after TDM to reach therapeutic concentrations in the patients who subsequently switched to other drugs. One may speculate that TDM in the switching subgroup was requested to check whether the concentrations were high because of side effects, hence providing a basis for dose reductions. However, when revealing low concentrations, the patient may have switched from clozapine to other antipsychotic drugs instead. The latter is supported by guidelines recommending that dose should be titrated until therapeutic serum concentrations are reached [31, 39, 41] and also agrees with a study showing that intolerability during low dosing is the main reason for discontinuing clozapine treatment [49]. Furthermore, a pragmatic point is the range of clinical studies showing that clozapine discontinuation is rarely caused by inadequate efficacy [12, 14, 50]. However, whether the higher proportion of switchers not reaching therapeutic clozapine serum concentrations indicates that reduced tolerability precedes a clozapine switch needs to be investigated in future studies.

The retrospective and naturalistic design of the present study is associated with several limitations. Number one is uncertainty over whether physicians provided accurate information about dosing, sampling time and

comedications on the requisition forms. Furthermore, the study used laboratory data without information on the clinical assessments underlying drug switch/discontinuation and whether this was because of side effects or insufficient symptom control. Another aspect is that no information was available on the physiological variables that potentially had an impact on clozapine metabolism, e.g. organ function, somatic diseases, infections and body weight, and that may have varied during the study period. We checked for the latter by reviewing the requisition forms among patients with outlying concentration measurements but still relied on the details written on the forms by the physicians. The study measured clozapine switch by replacement of TDM with another antipsychotic drug. This is a conservative and definite measure of switch but may also underestimate the true proportion of switchers in the study population, e.g. patients switching after the observation period. TDM is routinely performed during clozapine treatment in Norway. It is therefore unlikely that patients were misclassified as switchers if TDM of non-clozapine antipsychotics was requested without being accompanied by clozapine TDM. A strong methodological aspect of the study is that the use of TDM data allowed for the inclusion of a large patient population with exact information on drug exposure and disclosure of drug switch by detected replacement in blood samples. Finally, a favourable aspect of using TDM data is that nonadherence can be controlled for by neutral drug exposure assessments.

5 Conclusions

The present study shows that decreased absolute and dose-adjusted serum concentrations of clozapine are associated with clozapine treatment discontinuation, as defined by switch to other antipsychotic drugs. The significantly lower dose-adjusted serum concentration of clozapine among treatment-adherent patients switching versus not switching to other antipsychotic drugs suggests a pharmacokinetic mechanism, e.g. increased metabolism to toxic metabolites, may underlie termination of clozapine because of intolerability. This latter hypothesis should be investigated further in mechanistic studies with access to complete metabolic profiles in relation to treatment discontinuation.

Declarations

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