OPEN

Metabolite Profiling of Clozapine in Patients Switching Versus Maintaining Treatment

A Retrospective Pilot Study

Lennart Kyllesø, MSc,[1] Robert Løvsletten Smith, PhD,[1,3] Birgit M. Wollmann, MSc,[1] Øystein Karlstad, PhD,[2] Ole A. Andreassen, MD, PhD,[3] and Espen Molden, PhD[1,4]

Abstract:

Purpose/Background: Pharmacokinetics may be of relevance for the risk of clozapine discontinuation. We compared metabolite profiles, accounting for smoking habits, in patients switching versus maintaining clozapine treatment at therapeutic concentrations.

Methods/Procedures: Adult patients with clozapine serum levels above 1070 nmol/L (350 ng/mL) were retrospectively included from a Norwegian therapeutic drug monitoring service during 2018–2020. Inclusion criteria were (1) known smoking habits, (2) blood sample drawn within 10 to 30 hours after last clozapine intake, and (3) detectable levels of *N*-desmethylclozapine, clozapine-*N*-oxide, clozapine-5*N*-glucuronide, or clozapine-N^+-glucuronide. Patients comedicated with cytochrome P450 enzyme inducers, inhibitors, or valproic acid were excluded. The high-resolution mass spectrometry assay enabled detection of 21 clozapine metabolites. Metabolite profiles were compared between patients switching treatment (switchers), measured as clozapine being replaced by another antipsychotic drug in blood samples, versus maintaining clozapine treatment (nonswitchers) during the study period.

Findings/Results: Of the 84 patients fulfilling the study criteria, 7 patients (8.3%) were identified as clozapine switchers. After correcting for smoking habits, the clozapine-5*N*-glucuronide/clozapine ratio was 69% lower ($P < 0.001$), while the clozapine-N^+-glucuronide/clozapine-5*N*-glucuronide ratio was 143% higher ($P = 0.026$), respectively, in switchers versus nonswitchers. The other metabolite ratios did not significantly differ between switchers and nonswitchers.

Implications/Conclusions: The present study found a significantly reduced 5*N*-glucuronidation phenotype in patients switching from clozapine at therapeutic serum concentrations (>1070 nmol/L) to other antipsychotic drugs. This may indicate that glucuronidation, as a potential detoxification mechanism, is related to clozapine tolerability. However, the causality of this observation needs to be investigated in future studies with larger patient populations.

Key Words: clozapine, drug switch, pharmacokinetics, glucuronidation, therapeutic drug monitoring

(*J Clin Psychopharmacol* 2022;42: 470–474)

From the [1]Center for Psychopharmacology, Diakonhjemmet Hospital; [2]Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health; [3]NORMENT Centre, Division of Mental Health and Addiction, University of Oslo and Oslo University Hospital; and [4]Section for Pharmacology and Pharmaceutical Biosciences, Department of Pharmacy, University of Oslo, Oslo, Norway.

Received January 16, 2022; accepted after revision May 3, 2022.

Address correspondence to: Lennart Kyllesø, MSc, Center for Psychopharmacology, Diakonhjemmet Hospital, PO Box 85 Vinderen, 0319 Oslo, Norway (e-mail: lennart.kylleso@diakonsyk.no).

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.psychopharmacology.com).

ISSN: 0271-0749
DOI: 10.1097/JCP.0000000000001585

Schizophrenia is a severe mental disorder with major impact on the quality and length of life for approximately 0.7% of the world's population.[1] Antipsychotic medication is usually necessary for symptom control, but approximately a third of the patients develop treatment-resistant schizophrenia.[2] Treatment-resistant schizophrenia is defined as insufficient treatment response despite 2 or more antipsychotic trials of adequate duration, dosing, and adherence.[2]

Clozapine is the most effective drug in the treatment of schizophrenia[3,4] and is the only medication approved for treatment-resistant schizophrenia.[5] Still, clozapine remains underused because of the risk of major adverse effects.[6,7] Hematological monitoring is mandatory because of the risk of severe neutropenia (agranulocytosis), which occurs in approximately 1% of the patients.[8] Current clinical guidelines also recommend monitoring of clozapine serum levels to guide personalized dosing, optimize therapeutic response, and limit the risk of adverse effects.[5,9] There is a strong association between serum concentration and clinical effect of clozapine, and a concentration greater than 1070 nmol/L (350 ng/mL) is defined as the lower level for achieving optimal treatment response.[9,10]

Approximately 20% of all patients discontinue clozapine treatment.[6,11] Adverse drug reactions represents 60% of these cases, while nonadherence, the second most common reason for discontinuation, represents 15% to 20% of the events.[6,11] Only approximately 5% of the cases of clozapine discontinuation are caused by insufficient clinical effect.[6,11] There is a substantial risk of symptom relapse and hospitalizations after a failed clozapine trial.[4,12] Increased knowledge on mechanisms underlying the tolerability issues of clozapine is therefore necessary.

Clozapine is extensively metabolized, primarily via oxidative cytochrome P450 enzymes.[9,13] The formation of the major metabolite *N*-desmethylclozapine is mediated mainly by CYP1A2,[9,13] while the formation of clozapine-*N*-oxide probably involves CYP3A4 and flavin monooxygenase 3.[14] Clozapine is additionally glucuronidated by uridine 5'-diphospho glucuronosyltransferases (UGTs) to clozapine-5*N*-glucuronide and clozapine-N^+-glucuronide.[15] Among the major clozapine metabolites, *N*-desmethylclozapine has been linked to both favorable and unfavorable effects, for example, on cognition and granulocyte counts.[16,17]

Clozapine, *N*-desmethylclozapine, and clozapine-*N*-oxide are known to undergo bioactivation to highly reactive cytotoxic intermediates (ie, nitrenium ions) by myeloperoxidase 1 and CYP enzymes,[18,19] of which CYP3A4 and CYP2D6 are the most likely candidates.[14] These reactive metabolites are detoxified through glutathione (GSH) conjugation by glutathione S-transferases,[13] but other enzymes may also be relevant for "trapping" of the reactive nitrenium ions. Formation of nitrenium ions has been proposed as a possible mechanism underlying several adverse effects of clozapine.[18] There is a strong link between nitrenium ions and clozapine-induced agranulocytosis,[18] and clozapine-induced oxidative stress has also been associated with hypersalivation,[20] changes in lipid and glucose metabolism,[21] and to a lesser extent cardiotoxicity.[22]

Sex, age, drug-drug interactions, and pharmacogenetics are all shown to affect the metabolism of clozapine, but tobacco

smoking is probably the most important factor.[23] The frequency of smoking is 2 to 3 times higher in patients with schizophrenia than the general population.[24] Several enzymes, including CYP1A2, flavin monooxygenase 3, and UGTs are induced by polycyclic aromatic hydrocarbons formed by tobacco smoking.[25–27] Consequently, the serum concentrations of clozapine is decreased by approximately 30% in smokers.[28]

Recently, we reported that the absolute and dose-adjusted serum concentrations of clozapine were reduced in patients where clozapine was replaced by other antipsychotic drugs.[29] The aim of the present study is therefore to compare metabolite profiles in clozapine patients switching versus maintaining treatment by performing a comprehensive metabolite profiling of clozapine, accounting for smoking habits, which is the major factor determining the rate of clozapine metabolism.

MATERIALS AND METHODS

Study Design and Patient Inclusion

Adult patients (18–64 years) with clozapine serum concentrations above the lower therapeutic target level (>1070 nmol/L [350 ng/mL]) at the last TDM analysis were retrospectively included from the therapeutic drug monitoring (TDM) service at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, during the period January 2018 to December 2020. Inclusion criteria were (1) known smoking habits, (2) blood sample drawn within 10 to 30 hours after last intake of clozapine, and (3) detectable levels of *N*-desmethylclozapine, clozapine-*N*-oxide, clozapine-5*N*-glucuronide, and clozapine-N^+-glucuronide. Patients comedicated with the unselective CYP inducers carbamazepine, phenytoin, or phenobarbital, the CYP1A2 inhibitor fluvoxamine, or valproic acid, which has multiple effects on clozapine metabolism,[30,31] were excluded. For the nonswitchers (reference group), a stable trial of clozapine for at least 90 days between the first and last TDM analysis of clozapine in the database was required for inclusion. To avoid cases of dose titrations during treatment initiation or termination, TDM measurements at prescribed clozapine doses less than 100 mg/d were excluded. Furthermore, clozapine doses greater than 1000 mg/d were excluded to ensure dose-versus-concentration linearity. All the hereinabove information, as well as sex, age, and daily dosing, was retrieved from the patients' TDM requisition forms. Considering that smoking habits are of great importance for clozapine metabolism, such information provided on the TDM requisition form was confirmed by determining the nicotine metabolite cotinine[32] in the TDM samples. Patients with discrepancies between cotinine levels and reported smoking habits were excluded.

The study was approved by the Regional Committee for Medical and Health Research Ethics (#2014/1185) and the Hospital Investigational Review Board.

Defining Lower Therapeutic Serum Concentration and Discontinuation (Drug Switch) of Clozapine

Patients with clozapine serum concentrations greater than 1070 nmol/L at the last recorded TDM of clozapine were interpreted to have a clinically relevant treatment effect regardless of switch to other antipsychotic drugs. After reviewing the patients' longitudinal TDM records, they were subsequently split into switchers and nonswitchers. In this setting, switchers were defined as patients where another antipsychotic drug replaced clozapine in the blood samples after the last clozapine TDM. Clozapine-treated patients without replacement of another antipsychotic drug in TDM samples during the study period were defined as nonswitchers.

Assay for Analyzing Antipsychotic Drugs and Clozapine Metabolites

The ultra-performance liquid chromatography high-resolution mass spectrometry (HRMS) assay used for analyzing clozapine and metabolites is described in the Supplementary file, http://links.lww.com/JCP/A826. It includes analyses of all licensed antipsychotic drugs in Norway and their primary metabolites, as described earlier.[16] The assay is validated and certified for routine TDM according to the bioanalytical requirements.[33] Measurements of clozapine and *N*-desmethylclozapine were obtained directly from the TDM database. The serum levels of clozapine-*N*-oxide, clozapine-5*N*-glucuronide, and clozapine N^+-glucuronide were quantified by retrospective reprocessing of the HRMS data files, as described elsewhere for other compounds,[34] using the TraceFinder 4.1 software (Thermo Fisher Scientific, Waltham, Mass). Detection was confirmed using retention time and matched MS2 spectrum by analyzing reference standards purchased commercially (TLC Pharmaceutical Standards, Newmarket, Toronto, Canada). Supplementary Figure 1, http://links.lww.com/JCP/A826, shows extracted ion chromatograms corresponding to $(M + H)^+$ of clozapine and the respective primary metabolites. In addition, retrospective reprocessing of the HRMS data files was used for identification and semiquantitative measurements of clozapine metabolites where reference standards were unavailable. Representative ion chromatograms corresponding to $(M + H)^+$ of the metabolites are shown in Supplementary Figure 2, http://links.lww.com/JCP/A826.

Measures, Comparisons, and Statistics

As a measure of metabolism via different pathways, the metabolite-to-parent ratio (MR) of the respective metabolites was compared between clozapine switchers and nonswitchers. In addition, prescribed daily doses (in milligrams per day), absolute and dose-adjusted serum concentrations of clozapine, *N*-desmethylclozapine, clozapine-5*N*-glucuronide, clozapine-N^+-glucuronide, and clozapine-*N*-oxide (in nanomoles per liter) were compared between clozapine switchers and nonswitchers. Comparison of the log-transformed C/D ratios and metabolite ratios between switchers and nonswitchers were performed by multiple linear regression analyses. Smoking, sex, age, and sampling time within 10 to 30 hours after dose intake were included as covariates (independent variables).[23,25] Demographics were compared using Student *t* test or the Wilcoxon rank-sum test (continuous variables), whereas the Fisher exact test was used for dichotomous variables. Statistical analyses were carried out using STATA (v.16.1; StataCorp LP, College Station, Tex). The 2-sided statistical significance was set at a P value less than 0.05.

RESULTS

Of the 84 patients with registered TDM records of clozapine fulfilling the study criteria, 7 patients (8.3%) were identified as clozapine switchers. Among the remaining patients, defined as nonswitchers (n = 77, 91.7%), TDM of clozapine was not replaced by TDM of another antipsychotic drug during the study period. Patient characteristics are presented in Table 1. Age, sex, and smoking habits were not significantly different between switchers and nonswitchers ($P > 0.2$; Table 1). The observed mean daily dose was lower among switchers (−26%) than nonswitchers, but the difference was not statistically significant ($P = 0.091$; Table 1). The period between the first and last recorded TDM of clozapine in the database was significantly shorter among switchers than nonswitchers (9 vs 29 months, $P = 0.011$; Table 1).

Although there were no significant differences between switchers and nonswitchers in absolute or dose-adjusted serum concentrations of clozapine or any of the phase I metabolites

TABLE 1. Patient Characteristics, Dosing, and Time Between First and Last TDM Measurement of Clozapine (TDM Period) in Patients Defined as Nonswitchers or Switchers, Where the Latter Comprised Cases Where TDM of Clozapine Was Replaced by TDM of Another Antipsychotic Drug

Variables	Nonswitchers (n = 77)	Switchers (n = 7)	% Change	*P*
Age, median (IQR), y	42 (32–50)	35 (29–40)		0.20
Women, % (n)	32% (25)	43% (3)		0.68
Sampling time, mean (SD), h	13.3 (1.6)	12.8 (1.3)		0.46
Smokers, % (n)	71% (55)	57% (4)		0.42
Clozapine dose, mean (SD), mg/d	474 (186)	350 (146)	−26	0.091
TDM period, median (IQR), mo	29 (17–33)	9 (2–26)		0.011

Smokers were identified from TDM requisition forms and cotinine chromatographic peak areas.

Sampling time—time from ingested dose to blood drawn.

IQR, interquartile range; SD, standard deviation.

($P > 0.2$; Table 2), the absolute serum concentration of clozapine-5*N*-glucuronide was 70% lower among switchers compared with nonswitchers ($P < 0.001$; Table 2). After adjusting for covariates, the C/D ratio of clozapine-5*N*-glucuronide was also significantly lower among switchers than nonswitchers (−66%, $P = 0.003$; Table 2), showing altered formation of this metabolite in clozapine switchers. Accordingly, and after adjusting for covariates, the metabolite ratio for clozapine-5*N*-glucuronide/clozapine was 69% lower ($P < 0.001$; Table 2) in switchers compared with nonswitchers, which also remained significant when tested against a Bonferroni-adjusted α level of 0.0024 (0.05/21). Importantly, the parallel *N*-desmethylclozapine-5*N*-glucuronide/*N*-desmethylclozapine metabolite ratio differed in a similar pattern by being 78% lower in switchers versus nonswitchers ($P = 0.001$; Supplementary Table 1, http://links.lww.com/JCP/A826). Although there was no difference in the clozapine-N^+-glucuronide/clozapine metabolite ratio between the subgroups, the clozapine-N^+-glucuronide/clozapine-5*N*-glucuronide ratio was increased 2.4-fold in switchers ($P = 0.026$; Table 2). The regression analyses did not reveal any significant differences in the other metabolite ratios between switchers and nonswitchers.

DISCUSSION

In the present study, we found a significantly reduced 5*N*-glucuronidation phenotype of both clozapine and *N*-desmethylclozapine, that is, −69% and −78%, respectively, in patients switching versus maintaining clozapine treatment at therapeutic serum levels of clozapine (≥1070 nmol/L). In addition, the metabolite ratio of clozapine-N^+-glucuronide-to-clozapine-5*N*-glucuronide was increased 2.4-fold (ie, 143%) in switchers compared with nonswitchers. These findings suggest that the reduced 5*N*-glucuronidation phenotype might be an indicator of clozapine tolerability, although it is important to point out we lacked information on the

TABLE 2. Absolute Serum Concentrations (in nanomoles per liter), Dose-Adjusted Serum Concentrations (C/D Ratios, in nanomoles per liter over milligrams per day), and Metabolite Ratios ("Metabolite-to-Parent") of Clozapine and Metabolites Determined by Reference Standards, in Patients Defined as Nonswitchers or Switchers, Where the Latter Comprised Cases Where TDM of Clozapine Was Replaced by TDM of Another Antipsychotic Drug

Variables	Nonswitchers (n = 77)	Switchers (n = 7)	% Change	*P*
Absolute concentrations, median (IQR)				
Clozapine	1828 (1404–2245)	1537 (1185–2128)	−16	0.30
N-desmethylclozapine	1145 (893–1591)	870 (497–1550)	−24	0.19
Clozapine-5*N*-gluc	82 (50–136)	25 (8–48)	−70	**<0.001**
Clozapine-N^+-gluc	79 (39–152)	41 (37–94)	−48	0.27
Clozapine-*N*-oxide	149 (109–219)	136 (70–162)	−8.7	0.25
C/D ratios, mean (95% CI)				
Clozapine	4.2 (3.8–4.6)	4.6 (3.3–6.3)	9.4	0.61
N-desmethylclozapine	2.7 (2.5–3.0)	2.7 (2.0–3.8)	0.4	0.98
Clozapine-5*N*-gluc	0.18 (0.15–0.23)	0.06 (0.03–0.12)	−66	**0.003**
Clozapine-N^+-gluc	0.17 (0.14–0.21)	0.14 (0.07–0.28)	−17	0.62
Clozapine-*N*-oxide	0.35 (0.31–0.38)	0.32 (0.22–0.45)	−8.6	0.64
Metabolite ratios, mean (95% CI):				
N-desmethylclozapine /clozapine	0.65 (0.61–0.70)	0.60 (0.47–0.76)	−7.7	0.51
Clozapine-5*N*-gluc/clozapine	4.4 (3.7–5.2)*	1.4 (0.8–2.5)*	−69	**<0.001**
Clozapine-N^+-gluc /clozapine	4.0 (3.3–4.9)*	3.1 (1.6–6.0)*	−24	0.45
Clozapine-*N*-oxide/clozapine	8.2 (7.4–9.2)*	6.9 (4.8–10)*	−16	0.39
Clozapine-N^+-gluc/clozapine-5*N*-gluc	0.91 (0.73–1.13)	2.21 (1.05–4.64)	143	**0.026**

Bold font indicate statistical significance.

Absolute serum concentration comparisons were carried out using Wilcoxon rank-sum test. Mean C/D ratios and MRs in multiple linear regression analyses were adjusted for age, sex, sampling time, and smoking status.

P values are nominal. Clozapine conversion factor from nanomoles per liter to nanograms per milliliter 0.33.

*− $\times 10^{-2}$.

CI, confidence interval; IQR, interquartile range; gluc, glucuronide.

clinical basis for switching treatment. Thus, potential changes in metabolite spectra of clozapine should be further investigated in prospective studies in patients developing adverse effects versus those who do not.

The observed 2.4-fold increase in the clozapine-N^+-glucuronide-to-clozapine-5*N*-glucuronide ratio implies a change in glucuronidation phenotype being present among switchers. The impact of metabolic phenotypes has previously been demonstrated in studies on escitalopram and risperidone, where rapid and poor metabolizers of CYP2C19 and CYP2D6, respectively, were more likely to switch to other alternative medications.[35,36] Polymorphisms in different UGTs have been proposed as contributors to the wide interindividual variability of clozapine serum levels,[15] but there are no reports of clozapine toxicity being directly linked to the rate of glucuronidation. However, the fact that switchers and nonswitchers had similar concentrations above the lower threshold for a clinically relevant effect of clozapine may indicate that clozapine switch occurred because of reduced tolerability, and not reduced efficacy. Although the significantly reduced 5*N*-glucuronidation, without any change in clozapine levels, may lead one to expect increased metabolism via other pathways, we did not observe significantly increased formation rates among switchers for any of the included metabolites. Our data may thus indicate a shift among switchers, toward formation of other clozapine metabolites than those observed in the present study.

Oxidative metabolism produces highly reactive nitrenium ions, which are strongly associated with adverse effects and clozapine toxicity.[19] These nitrenium intermediates are usually rapidly trapped and neutralized through conjugation of GSH. However, the observed reduction in 5*N*-glucuronidation capacity among switchers may in effect have shifted metabolism toward oxidative pathways with subsequent depletion of GSH, accumulation of nitrenium-protein adducts, and adverse effects/intolerability. The nitrenium ions have very short half-lives (<1 minutes) and are therefore not detectable in vivo.[19] Additional studies are needed to clarify if the reduced 5*N*-glucuronidation phenotype in switchers is indicating that reduced tolerability precedes a clozapine switch, for example, by investigating levels of clozapine-GSH conjugates as a surrogate measure of nitrenium metabolites in switchers versus nonswitchers.

Methodological Considerations

The retrospective and naturalistic design of our study has some weaknesses. The limited number of included patients may have been insufficient to disclose differences with smaller effect sizes between switchers and nonswitchers. However, the large and significant difference in clozapine 5*N*-glucuronidation, despite the limited population size, suggests that this metabolic pathway is linked to clozapine tolerability. Because of the limited clinical information, it was not possible to study the reasons or the clinical assessments behind clozapine switch. However, all patients were above the established serum level for optimal treatment response at the last TDM of clozapine and the clinical superiority of clozapine renders intolerability a reasonable assumption for switch. No information was available on organ function, somatic diseases, infections, nutritional status, and body weight, which all may have varied during the study period. Furthermore, bias from potential interactions with glucuronidation inducing or inhibiting drugs not listed on the TDM forms, for example, undisclosed valproic acid use, could not be excluded.

There are several strengths with the methodology applied in the present study. When performing studies on clozapine metabolism, it is essential to be able to control for tobacco smoking. In the study described here, status as smoker or nonsmoker was retrieved from the TDM requisition forms and confirmed by analyses of cotinine in the serum samples. Overall, 70% of the patient population were smokers, a typical prevalence for a population of patients with schizophrenia.[24] The proportion of smokers among switchers and nonswitchers was not significantly different, and smoking was also included as a covariate in the regression analyses. Another methodological strength of the study was that clozapine switch was measured exactly in blood samples by replacement of TDM with another antipsychotic drug. Nonadherence, which may cause "pseudo" treatment failure and switch, was controlled for by using TDM data providing exact information on drug exposure. In addition, the study was restricted to patients with clozapine serum concentrations greater than 1070 nmol/L. Nonadherence was therefore not a substantial element in the risk of clozapine switch in the study population.

In conclusion, the present study found a significantly reduced 5*N*-glucuronidation phenotype in patients switching clozapine to other antipsychotic drugs at therapeutic serum concentrations. However, the causality behind this observation and the risk of clozapine intolerability is not clear and should be investigated further in prospective studies on patients with access to clinical data, where the formation of additional downstream metabolites of clozapine are assessed in relation to treatment switch.

AUTHOR DISCLOSURE INFORMATION

L.K., E.M., and R.L.S. have received grants (2018-007 and 2016-097) from the South-Eastern Norway Regional Health Authority. O.A.A., E.M., and R.L.S. have received support from the European Union's Horizon 2020 research and innovation program (964874/Realment). E.M. has received speaker's honoraria from Lundbeck, Lilly, and Otsuka. O.A.A. has received speaker's honoraria from Lundbeck and Sunovion and has been a consultant to HealthLytix. Ø.K. and B.M.W. declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

REFERENCES

1. McGrath J, Saha S, Chant D, et al. Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol Rev*. 2008;30:67–76.
2. Howes OD, McCutcheon R, Agid O, et al. Treatment-resistant schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) Working Group consensus guidelines on diagnosis and terminology. *Am J Psychiatry*. 2017;174:216–229.
3. Leucht S, Cipriani A, Spineli L, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet*. 2013;382:951–962.
4. Tiihonen J, Mittendorfer-Rutz E, Majak M, et al. Real-world effectiveness of antipsychotic treatments in a nationwide cohort of 29 823 patients with schizophrenia. *JAMA Psychiat*. 2017;74:686–693.
5. Nielsen J, Young C, Ifteni P, et al. Worldwide differences in regulations of clozapine use. *CNS Drugs*. 2016;30:149–161.
6. Legge SE, Hamshere M, Hayes RD, et al. Reasons for discontinuing clozapine: a cohort study of patients commencing treatment. *Schizophr Res*. 2016;174:113–119.
7. Nielsen J, Dahm M, Lublin H, et al. Psychiatrists' attitude towards and knowledge of clozapine treatment. *J Psychopharmacol*. 2010;24:965–971.
8. Mijovic A, MacCabe JH. Clozapine-induced agranulocytosis. *Ann Hematol*. 2020;99:2477–2482.

9. Hiemke C, Bergemann N, Clement HW, et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. *Pharmacopsychiatry*. 2018;51:9–62.
10. Siskind D, Sharma M, Pawar M, et al. Clozapine levels as a predictor for therapeutic response: a systematic review and meta-analysis. *Acta Psychiatr Scand*. 2021;144:422–432.
11. Ucok A, Yağcıoğlu EA, Yıldız M, et al. Reasons for clozapine discontinuation in patients with treatment-resistant schizophrenia. *Psychiatry Res*. 2019;275:149–154.
12. Masuda T, Misawa F, Takase M, et al. Association with hospitalization and all-cause discontinuation among patients with schizophrenia on clozapine vs other oral second-generation antipsychotics: a systematic review and meta-analysis of cohort studies. *JAMA Psychiat*. 2019;76: 1052–1062.
13. Dragovic S, Boerma JS, van Bergen L, et al. Role of human glutathione S-transferases in the inactivation of reactive metabolites of clozapine. *Chem Res Toxicol*. 2010;23:1467–1476.
14. Dragovic S, Gunness P, Ingelman-Sundberg M, et al. Characterization of human cytochrome P450s involved in the bioactivation of clozapine. *Drug Metab Dispos*. 2013;41:651–658.
15. Erickson-Ridout KK, Sun D, Lazarus P. Glucuronidation of the second-generation antipsychotic clozapine and its active metabolite *N*-desmethylclozapine. Potential importance of the UGT1A1 A(TA)$_7$TAA and UGT1A4 L48V polymorphisms. *Pharmacogenet Genomics*. 2012;22: 561–576.
16. Smith RL, Haslemo T, Andreassen OA, et al. Correlation between serum concentrations of *N*-desmethylclozapine and granulocyte levels in patients with schizophrenia: a retrospective observational study. *CNS Drugs*. 2017; 31:991–997.
17. Costa-Dookhan KA, Agarwal SM, Chintoh A, et al. The clozapine to norclozapine ratio: a narrative review of the clinical utility to minimize metabolic risk and enhance clozapine efficacy. *Expert Opin Drug Saf*. 2020;19:43–57.
18. Maggs JL, Williams D, Pirmohamed M, et al. The metabolic formation of reactive intermediates from clozapine, a drug associated with agranulocytosis in man. *J Pharmacol Exp Ther*. 1995;275:1463–1475.
19. Liu ZC, Uetrecht JP. Clozapine is oxidized by activated human neutrophils to a reactive nitrenium ion that irreversibly binds to the cells. *J Pharmacol Exp Ther*. 1995;275:1476–1483.
20. Uzun O, Bolu A, Celik C. Effect of N-acetylcysteine on clozapine-induced sialorrhea in schizophrenic patients: a case series. *Int Clin Psychopharmacol*. 2020;35:229–231.
21. Miljevic C, Nikolic M, Nikolic-Kokic A, et al. Lipid status, anti-oxidant enzyme defence and haemoglobin content in the blood of long-term clozapine-treated schizophrenic patients. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;34:303–307.
22. Arzuk E, Karakus F, Orhan H. Bioactivation of clozapine by mitochondria of the murine heart: possible cause of cardiotoxicity. *Toxicology*. 2021; 447:152628.
23. Rostami-Hodjegan A, Amin AM, Spencer EP, et al. Influence of dose, cigarette smoking, age, sex, and metabolic activity on plasma clozapine concentrations: a predictive model and nomograms to aid clozapine dose adjustment and to assess compliance in individual patients. *J Clin Psychopharmacol*. 2004;24:70–78.
24. de Leon J, Diaz FJ. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. *Schizophr Res*. 2005;76:135–157.
25. Thorn CF, Muller DJ, Altman RB, et al. PharmGKB summary: clozapine pathway, pharmacokinetics. *Pharmacogenet Genomics*. 2018;28:214–222.
26. Bloom AJ, Murphy SE, Martinez M, et al. Effects upon in-vivo nicotine metabolism reveal functional variation in FMO3 associated with cigarette consumption. *Pharmacogenet Genomics*. 2013;23:62–68.
27. Anderson GD, Chan LN. Pharmacokinetic drug interactions with tobacco, cannabinoids and smoking cessation products. *Clin Pharmacokinet*. 2016; 55:1353–1368.
28. Augustin M, Schoretsanitis G, Pfeifer P, et al. Effect of fluvoxamine augmentation and smoking on clozapine serum concentrations. *Schizophr Res*. 2019;210:143–148.
29. Kyllesø L, Smith RL, Karlstad Ø, et al. Absolute and dose-adjusted serum concentrations of clozapine in patients switching vs. maintaining treatment: an observational study of 1979 patients. *CNS Drugs*. 2021;35:999–1008.
30. Hommers L, Scharl M, Hefner G, et al. Comedication of valproic acid is associated with increased metabolism of clozapine. *J Clin Psychopharmacol*. 2018;38:188–192.
31. Smith RL, Wollmann BM, Kyllesø L, et al. Effect of valproic acid on the metabolic spectrum of clozapine in patients with schizophrenia. *J Clin Psychopharmacol*. 2022;42:43–50.
32. Kim S. Overview of cotinine cutoff values for smoking status classification. *Int J Environ Res Public Health*. 2016;13:1236.
33. US Food and Drug Administration. Bioanalytical method validation guidance for industry. 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed March 5, 2020.
34. Partridge E, Trobbiani S, Stockham P, et al. A case study involving U-47700, diclazepam and flubromazepam—application of retrospective analysis of HRMS data. *J Anal Toxicol*. 2018;42:655–660.
35. Jukić MM, Haslemo T, Molden E, et al. Impact of CYP2C19 genotype on escitalopram exposure and therapeutic failure: a retrospective study based on 2,087 patients. *Am J Psychiatry*. 2018;175:463–470.
36. Jukic MM, Smith RL, Haslemo T, et al. Effect of CYP2D6 genotype on exposure and efficacy of risperidone and aripiprazole: a retrospective, cohort study. *Lancet Psychiatry*. 2019;6:418–426.