Exploring low clozapine C/D ratios, inverted clozapine–norclozapine ratios and undetectable concentrations as measures of non-adherence in clozapine patients

Article *in* Schizophrenia Research · July 2023

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This is the preprint (first submitted version to the journal) that was finally published as: Ruan CJ, Olmos I, Ricciardi C, Schoretsanitis G, Vincent PD, Anıl Yağcıoğlu AE, Eap CB, Baptista T, Clark SR, Fernandez-Egea E, Kim SH, Lane HY, Leung J, Maroñas Amigo O, Motuca M, Every-Palmer S, Procyshyn RM, Rohde C, Suhas S, Schulte PFJ, Spina E, Takeuchi H, Verdoux H, Correll CU, Molden E, De Las Cuevas C, de Leon J. Exploring low clozapine C/D ratios, inverted clozapine-norclozapine ratios and undetectable concentrations as measures of non-adherence in clozapine patients: A literature review and a case series of 17 patients from 3 studies. Schizophr Res. 2023 Jul 22:S0920-9964(23)00228-1. doi: 10.1016/j.schres.2023.07.002. Epub ahead of print. PMID: 37487869. The final published version is available in the journal webpage 10.1016/j.schres.2023.07.002. or by requesting from the last author for private use. The final Word version will be available here after 12 months from initial publication. Please be aware that this version was modified after peer-reviewed; the words increased from 2027 to 3860 and the references from 44 to 55; the Supplementary Boxes from 7 to 8 and the Supplementary Table from 13 to 15.

Words in text: 2027

Words in abstract: 250

References: 44

Table: 1 Box: 1

Supplementary Material: 13 Supplementary Tables and 7 Supplementary Boxes

FULL-LENGTH ARTICLE

Exploring low clozapine C/D ratios, inverted clozapine-norclozapine ratios and undetectable concentrations as measures of non-adherence in clozapine patients.

A literature review and a case series of 15 patients from 3 studies

Running title: Clozapine non-adherence

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Abstract

Background: Up to 1/3 to 1/2 of outpatients prescribed clozapine may be partially or fully non-adherent, based on therapeutic drug monitoring (TDM). Three indices for measuring partial or full non-adherence are proposed: 1) a clozapine concentration/dose (C/D) ratio which drops to half or more of what is expected in the patient; 2) a clozapine/norclozapine ratio that becomes inverted; and 3) a clozapine concentration that becomes non-detectable. **Methods:** These 3 proposed indices are based on reviews of the literature and 3 samples which provided 15 cases of possible non-adherence. The three samples include: 1) an inpatient study in a Chinese hospital, 2) an inpatient randomized clinical trial in a United States hospital, and 3) and an outpatient study in Uruguay. **Results:** Partial non-adherence in average patients can be suspected when the clozapine C/D ratio is less than half the ratio corresponding to the patient's specific ancestry group and sex-smoking subgroup. Knowing the minimum therapeutic dose of the patient based on repeated TDM makes it much easier to establish non-adherence. Inverted clozapine/norclozapine ratios in the absence of alternative explanations may be a sign of non-adherence. By using half-lives, the chronology of the 3 indices of non-adherence was modeled in two patients: 1) the clozapine C/D ratio dropped to half or more of what is expected from the patient (around day 2); 2) the clozapine/norclozapine ratio becomes inverted (around day 3); and 3) the clozapine concentration became undetectable by the laboratory (around days 9-11). **Conclusion:** Future prospective studies should explore these proposed indices.

Keywords: clozapine/blood; clozapine/metabolism; clozapine/therapeutic use; drug interaction; medication adherence; schizophrenia

This brief article first reviews non-adherence and clozapine, clozapine metabolism, and clozapine therapeutic drug monitoring (TDM) and then proposes that partial non-adherence can be identified by low concentration-to-dose (C/D) ratio, an inverted clozapine/norclozapine ratio or, even better, by the combination of both. When clozapine concentration becomes non-detectable despite therapeutic dosing, it may be a sign of full non-adherence.

1. Introduction

1. Non-adherence and clozapine

Supplementary Box S1 explains that up to one-third to one-half of outpatients with schizophrenia or prescribed clozapine who are considered by their clinicians to have treatment-resistant schizophrenia (TRS) are actually rather "pseudo-resistant", as they are partially or fully non-adherent, based on TDM (McCutcheon et al., 2018; De las Cuevas and de Leon, 2020; Kyllesø et al., 2020; Takeuchi et al., 2020; De Las Cuevas et al., 2021; Brodeur et al., 2022).

1.2. A focused review of clozapine metabolism

Supplementary Box S2 (Schaber et al., 1998; Bowskill et al., 2012; Loi et al. 2013; Schoretsanitis et al., 2019; Kyllesø et al., 2022; de Leon, 2023; Reeves et al., 2023) explains 1) the main metabolic pathway for the parent compound, clozapine, the cytochrome P450 1A2 (CYP1A2); 2) the importance of renal elimination for the main metabolite, norclozapine; and 3) thus, the importance of CYP1A2 and renal elimination for clozapine clearance. Norclozapine does not appear to have antipsychotic activity but may contribute to some adverse drug reactions (ADRs).

1.3. A focused review of clozapine TDM

1.3.1. Variability of clozapine TDM across individuals

The therapeutic range of plasma clozapine concentrations is 350-600 ng/mL (Hiemke et al., 2018; Northwood et al., 2023). Although some patients may respond to lower concentrations (Schulte, 2003), the lower range of 350 ng/mL allows standardization by calculating the minimum therapeutic clozapine

dosage across the variations of clozapine metabolism in individuals and ancestry groups (Ruan et al., 2019; de Leon et al., 2022).

1.3.2. Variability of clozapine TDM within the same individual

A patient's TDM values vary from day to day, even with the same dose; thus, a mean of several TDM values better represents his/her metabolism. In a stabilized patient, in which clozapine TDM is measured under standard conditions (trough and steady-state), the speed at which clozapine decreases after a patient becomes partially or fully non-adherent varies according to his/her metabolic capacity (called drug clearance). Thus, the ability to detect non-adherence is tightly related to the ability to understand the measure of metabolic capacity of that specific patient, presuming stability of comorbidities, comedication, clozapine brand and dosing regimen.

1.4. Clozapine TDM and setting

Two basic characteristics should govern the interpretation of clozapine TDM regarding non-adherence: 1) single vs. repeated measures and 2) inpatient vs. outpatient setting. Thus, the way to approach non-adherence varies across clozapine-treated patients: 1) inpatients with many repeated clozapine TDMs or TDMs that can easily be repeated, 2) outpatients with repeated clozapine TDMs, or 3) outpatients without prior clozapine TDM. Inpatient treatment increases the possibility that the patient is completely adherent and that repeated measures facilitate the interpretation of a single TDM within a pattern considering the variability within that patient.

1.5. Basic assumptions needed to interpret concentrations when exploring adherence

Using a trough steady-state clozapine concentration to assess for adherence assumes that two basic concepts are true: 1) clozapine metabolism follows linear kinetics (Supplementary Box S3), and 2) the patient is an average metabolizer.

Estimating minimum therapeutic doses is based on the principle of linear kinetics, meaning that without changes in external circumstances, the concentration-to-dose (C/D) ratio is a constant in each

individual. Although not well-studied, linear kinetics may not occur until the plasma clozapine concentration has reached 150 ng/mL (de Leon et al., 2020; Schoretsanitis et al., 2024).

Supplementary Box S4 (Spina et al., 2016; 2020; Stout et al., 2021; Schoretsanitis et al., 2024) describes non-average patients, who are called poor metabolizers (PMs) and ultrarapid metabolizers (UMs).

We propose three indices for measuring partial or full non-adherence: the first is a clozapine concentration/dose (C/D) ratio which drops to half or more of what is expected in the patient; the second, a clozapine/norclozapine ratio that becomes inverted; and the third is a clozapine concentration that becomes non-detectable.

2. Methods

These 3 proposed indices are based on our reviews of the literature and our experience with 3 samples which provided 15 cases of possible non-adherence. Box 1 describes the three samples: 1) an inpatient study in a Chinese hospital (Ruan et al., 2019;2020), 2) an inpatient RCT in a US hospital (Simpson et al., 1999; Diaz et al., 2018), and 3) and an outpatient study in Uruguay (Olmos et al., 2019; Schoretsanitis et al., 2021). These cases come from published studies in articles that described the ethics committees that approved the studies.

3. Results

3.1. Using low clozapine C/D ratio to identify poor adherence

Unexpectedly low clozapine C/D ratios can be explained if an inducer was added or an inhibitor was discontinued, so these explanations need to be ruled out before considering low clozapine C/D ratio to be a sign of non-adherence (Supplementary Box S4).

3.1.1. Low clozapine C/D ratio based on one's ancestry and smoking status

Supplementary Table S1 describes the typical ranges of clozapine C/D ratios using trough steady-state conditions during linear kinetics within sex-smoking subgroups and within 3 ancestry groups. Partial

non-adherence in patients who are not clozapine PMs or UMs can be suspected when the clozapine C/D ratio is less than half the ratio corresponding to the patient's specific ancestry group and sex-smoking subgroup.

3.1.2. Low clozapine C/D ratio based on one's own C/D ratio

Knowing the minimum therapeutic dose of the patient makes it much easier to establish non-adherence. From the clozapine TDM study in 131 Chinese inpatients (Ruan et al., 2020), Case 1 was identified as having 3 definitive short-lived episodes of poor adherence (Supplementary Table S2). Table 1 shows that based on 23 clozapine C/D ratios she had a mean minimum therapeutic dose of 318 mg/day, but in these 3 episodes the minimum therapeutic doses were 552 to 602 mg/day.

3.2. Using inverted clozapine/norclozapine ratio to estimate non-adherence

Supplementary Box S5 explains the history of the use of the clozapine/norclozapine ratio; when the clozapine concentration is lower than the norclozapine concentration, it is called an inverted ratio (Centorrino et al., 1994; Carrillo et al., 1998; Rostami-Hodjegan et al., 2004; Schoretsanitis et al., 2019; Flanagan et al., 2023).

3.2.1. Inverted clozapine-norclozapine ratio

Supplementary Box S6 describes the limited published information indicating that plasma norclozapine has a half-life that is 1.32 times longer than that of clozapine, which means that after clozapine cessation the norclozapine concentration takes longer to decrease than the clozapine concentration (de Leon et al., 1996; Renwick et al., 2000; Wang et al., 2004; Tamminga et al., 2006; Golden and Honigfeld, 2010; Schoretsanitis et al., 2019). After repeated measures in trough and steady-state conditions, the apparition of an inverted ratio is compatible with missing clozapine doses for several days when the clozapine C/D ratio is low.

3.2.2. Gemfibrozil can explain inverted clozapine C/D ratios and normal clozapine C/D ratios

Supplementary Box S7 explains how co-prescription with gemfibrozil, a possible inhibitor of the renal elimination of norclozapine, can explain inverted clozapine/norclozapine ratios with normal clozapine C/D ratios (Alfaro et al. 2001; de Leon and Diaz, 2003; Golden and Honigfeld, 2008; Varma et al., 2015; Tornio et al, 2017; Barclay et al., 2019; Schoretsanitis et al., 2019).

3.2.3. Prevalence of an inverted clozapine-norclozapine ratio in 3 samples

3.2.3.1. TDM inpatient study in a Beijing Hospital

The first panel of Box 1 describes a TDM study in a Beijing hospital (Ruan et al., 2019; 2020) with 3,928 TDMs from 536 inpatients. The frequency of clozapine/norclozapine inverted ratios was 0.8% (95% CI 0.5 to 1.1%); 0.2% occurred for no known reason (95% CI 0.1 to 0.4%), leading the first and last authors to identify 5 cases of non-adherent inpatients. Thus, definitive non-adherence was possibly around 1% (5/536=0.9%) which is very low and was probably explained by the inpatient setting where nurses carefully supervise the intake of clozapine in each patient. Cases 2 to 6 are described in detail in Supplementary Tables S3 to S7 and briefly summarized in Table 1. They had 3 indices of low adherence in: 1) 5 inpatients with at least 1 inverted clozapine/norclozapine ratio accompanied by a low clozapine C/D ratio leading to a very high minimum therapeutic dose, 2) 3 inpatients with at least another low clozapine C/D ratio leading to a very high minimum therapeutic dose without an inverted clozapine/norclozapine ratio, and 3) 2 inpatients with undetectable clozapine and norclozapine concentrations even with high clozapine doses.

3.2.3.2. TDM inpatient study in Philadelphia using a double-blind randomized design

The second panel of Box 1 describes a United States (US) double-blind randomized controlled trial (RCT) with comprehensive TDM (Simpson et al., 1999; Diaz et al., 2018). The frequency of clozapine/norclozapine inverted ratios was 10.3% (95% CI 8.2 to 12.7%) and 1.4% for those with no known reason (95% CI 0.7 to 2.5%). The first and last authors identified 3 cases of non-adherent inpatients among 47 inpatients. Thus, definitive non-adherence was possibly around 1% (3/47=0.6%) which is very low and was probably explained by the inpatient setting of a research unit where nurses

were trained to carefully supervise the intake of clozapine for each patient in the RCT. Cases 7 to 9, described in detail in Supplementary Tables S8 to S10, are briefly summarized in Table 1. All 3 inpatients had 2 indices of low adherence: 1) at least 1 inverted clozapine/norclozapine ratio accompanied by a low clozapine C/D ratio leading to a very high minimum therapeutic dose, and 2) at least another low clozapine C/D ratio leading to a very high minimum therapeutic dose without an inverted ratio.

3.2.3.3. TDM outpatient study in Uruguay

A Uruguayan study providing 166 trough and steady-state TDMs with completed data from 94 outpatients having 1 or 2 TDMs using trademarked and/or generic clozapine formulations is described in the third panel of Box 1 (Olmos et al., 2019; Schoretsanitis et al., 2021; de Leon et al. 2022b).

The frequency of clozapine/norclozapine inverted ratios was 24.7% (95% CI 18.1 to 31.3%) and all appear to be explained by lack of adherence. Lack of adherence was probably present in 30 of 94 outpatients, providing a frequency of 31.9% (95% CI 22.3 to 41.5%). Only 6 obvious cases are presented; these are Cases 10 to 15 (Supplementary Table S11). The table describes these 6 outpatients with definitive non-adherence as demonstrated by 2 indices in: 1) 6 outpatients with at least 1 inverted clozapine/norclozapine ratio accompanied by a low clozapine C/D ratio leading to a very high minimum therapeutic dose, and 2) 1 outpatient with an undetectable clozapine concentration after a high clozapine dose.

3.3. Using half-lives to model complete non-adherence in Cases 2 and 11

Supplementary Tables S12 and S13 model how non-adherence would influence TDM in Cases 2 and 11, based on the standard half-lives of clozapine and norclozapine. Among the chronology of the 3 indices of non-adherence: 1) the clozapine C/D ratio, which drops to half or more of what is expected from the patient (day 2 for Cases 2 and 11); 2) the clozapine/norclozapine ratio, which becomes inverted (day 3 for Case 2 and day 3 for Case 11); and 3) the clozapine concentration, which becomes undetectable by the laboratory (day 9 for Case 2 and day 7 for Case 11). The time needed to reach these indices of non-adherence will be shorter in clozapine UMs and longer in clozapine PMs.

4. Conclusion

An accompanying article in this issue proposes a definition of clozapine UM (Schoretsanitis et al., 2024), which needs to be distinguished from non-adherence. The same article described two possible examples of non-adherence in clozapine UMs (Days 218 to 258 of Case 4, Supplementary Table S5 and Days 235 to 330 and Days 347 to 358 of Case 5, Supplementary Table S6).

Future prospective studies need to explore our proposal that 1) partial non-adherence may be identified by using low clozapine C/D ratios and inverted clozapine/norclozapine ratios and 2) full non-adherence by non-detectable plasma clozapine concentrations. Careful follow-up studies of clozapine UMs and PMs are needed to explore these 3 indices of non-adherence in them.

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Box 1. Three studies used to obtain Cases 1 to 15 described in this study

1. Large TDM database from an inpatient naturalistic study in a Chinese hospital

- 3,928 TDMs in a Beijing hospital[a] during 2016 to 2019 included 536 inpatients.
- Inverted clozapine/norclozapine ratio: 32 of 3928 TDMs were inverted, providing a frequency of 0.8% (95% CI 0.5 to 1.1%).[b] As gemfibrozil is not prescribed, 2 overlapping explanations were:
  - No steady-state C: 50% (16/32)
  - Clozapine Cs< 150 ng/mL: 50% (16/32).
- Inverted clozapine/norclozapine ratios with no explanation: 9 TDMs from 7 different patients providing a frequency of 0.2% (95% CI 0.1 to 0.4%).[b]
- These 9 Cs with unexplained inverted clozapine/norclozapine ratio from 7 patients:
  - 1 patient had a clozapine/norclozapine ratio=0.97 with no clear signs of non-adherence.
  - 6 patients in which the first and last authors agreed were non-adherent (Cases 2-7).
- A detailed review of TDM of 131 patients. The first and last authors carefully reviewed all 1,384 trough steady-state clozapine TDMs from 131 patients to study infection effects (Ruan et al., 2020). They agreed that 1 case with intermittent low clozapine C/D ratio (Case 1) was non-adherent (See Supplementary Table S2).

2. TDM database from an inpatient RCT in a US hospital

- 691 TDMs from 47 inpatients in a US double-blind randomized trial.[c]
- Inverted clozapine/norclozapine ratio: 71 of 691 TDMs were inverted, providing a frequency of 10.3% (95% CI 8.2 to 12.7%),[b] including following overlapping explanations:
  - No steady-state C: 21% (15/71)
  - Clozapine Cs< 150 ng/mL: 54% (38/71)
  - On gemfibrozil: 69% (49/71).
- Inverted clozapine/norclozapine ratios with no explanation: 10 TDMs from 4 different patients had inverted clozapine/norclozapine ratios with no explanation, providing a frequency of 1.4% (95% CI 0.7 to 2.5%).[b]
- These 10 Cs with unexplained inverted clozapine/norclozapine ratio: came from 4 patients:
  - 1 patient had a clozapine/norclozapine ratio=0.97 with no clear signs of non-adherence.
  - 3 patients whom the first and last authors agreed were non-adherent (Cases 8-10).

3. Small TDM database from Uruguayan outpatients taking 2 clozapine brands

- There were 166 trough and steady-state TDMs with completed data from 94 patients.[d]
- Inverted clozapine/norclozapine ratio: 41 of 166 TDMs were inverted, providing a frequency of 24.7% (95% CI 18.1 to 31.3%).[b] Gemfibrozil was not prescribed and all trough Cs were supposed to be steady state since patients were on the same dose for several months. There were:
  - Clozapine Cs< 150 ng/ml: 34% (14/41). These 14 low Cs in 13 patients appear to reflect a lack of adherence, since most of the doses were supposed to provide >350 ng/ml and 12 patients had doses 200-500 mg/day. The other patient had a low dose of 150 mg/day but with an inverted clozapine/norclozapine ratio suggestive of lack of adherence.
- Considerable problem of non-adherence: Of 94 patients, 30 had TDMs in which 1 or 2 values had a clozapine/norclozapine inverted ratio and low clozapine C/D ratios compatible with lack of adherence. If this is correct, lack of adherence was present with a frequency of 31.9% (95% CI 22.3 to 41.5%).[b] Six obvious cases were described as Cases 11-16 (Supplementary Tables S12 to S17). The patients were supervised by psychiatrists and there were no obvious psychotic exacerbations.

C: concentration; C/D: concentration-to-dose; CI: confidence interval; D: dose; RCT: randomized clinical trial; US: United States.

[a]It included 536 inpatients (134 genotyped and well-studied and 402 not so well studied) (Ruan et al., 2019; 2020).

[b]Using a bootstrap for percentage based on 1000 bootstrap samples to estimate the 95% CIs.

[c]It included 50 patients of which 47 provided at least 1 C who were included in a US double-blind randomized trial (Simpson et al., 1999; Diaz et al., 2018). The researchers were blind to both clozapine Ds and Cs. The study had comprehensive TDM and 3 study doses (100, 300 and 600 mg/day) during 16 weeks each and then the possibility of open clozapine treatment.

[d]The original sample from Uruguay had 98 adult outpatients stabilized on a clozapine dosage who were included in a study comparing a generic form of clozapine with a trademarked formulation (Olmos et al. 2019), leading to 81 patients with trough steady-state concentrations in both formulations. As this was a clinical sample it was confounded by co-medications (including valproate and omeprazole), obesity and undiagnosed inflammation (Schoretsanitis et al., 2021).

Table 1 Summary of Cases 1 to 15 with low clozapine C/D ratios and sometimes inverted clozapine/norclozapine ratios

			D for 350			**Non-adherence**			
			TDM N[a]	Found vs exp	Day	CLO CD	High D For 350 ng/ml	C<25	CLO/NCLO ratio
Chinese inpatient	1	♀ NS	23	318 vs 166					
					93	0.63	552		Not inverted (1.74)
					196	0.58	602		Not inverted (2.09)
					225	0.62	563		Not inverted (1.50)
Chinese inpatient	2	♂ NS	21	202 vs 205					
					43			On 400	
					92		573		0.94
					293		365		Not inverted (2.78)
Chinese inpatient	3	♀ NS	3	unable vs 166					
Non-adherent by chart					20	0.51	685		0.90
					31	0.41	864		0.82
					425	0.58	607		0.75
Chinese inpatient	4	♂ NS	17	372 vs 205					
					182	0.60	585		Not inverted (1.33)
					190	0.66	534		Not inverted (1.52)
					225	0.59	598		0.91
					265	0.42	840		Not inverted (1.26)
Chinese inpatient	5	♂ NS	0	unable[b] vs 205					
Non-adherent by chart			(46)[b]		183	0.45	771		0.98
Close to obese					358			On 425	
Chinese inpatient	6	♀ NS	1	216 vs 166					
					11	0.85	413		0.97
US inpatient	7	♀ NS	3	172 vs 150					
European ancestry					303	1.24	282		0.83
Obese					331	1.38	253		0.98
US inpatient	8	♂ NS	0	unable[b] vs 175					
European ancestry			(32)[b]		43	0.87	402		0.88
Obese					57	0.77	453		0.79
					71	0.84	416		Not inverted (1.25)
					100	1.20	292		0.94
					113	0.74	473		0.75
					134	0.76	459		Not inverted (1.31)
					162	0.39	890		0.77

US inpatient	9	♀ S	1	161 vs 150					
European ancestry					57	0.45	784		0.35
Obese					159	0.84	418		0.65
					187	0.84	418		0.68
					291	0.65	540		0.53
Uruguayan outpatient	10	♂ S	1	324 vs 368					
European ancestry					Brand	0.35	1000		0.42
					Generic	1.08	324		Not inverted (1.94)
Uruguayan outpatient	11	♂ S	1	443 vs 368					
European ancestry					Brand	0.42	833		0.26
					Generic	0.79	443		Not inverted (1.07)
Uruguayan outpatient	12	♂ S	1	134[c] vs 368					
European ancestry					Brand	0.85	412		0.42
					Generic	2.61	134[c]		Not inverted (1.15)
Uruguayan outpatient	13	♀ S	0	unable vs 357					
European ancestry					Brand	0.28	1250		0.26
					Generic	0.25	1400		0.71
Uruguayan outpatient	14	♂ S	0	unable vs 368					
European ancestry					Brand	0.32	1094		0.38
					Generic	0.21	1667		0.69
Uruguayan outpatient	15	♂ NS	0[d]	unable[d] vs 150					
European ancestry					Brand			On 500	<0.56
Obese					Generic	1.36	257[d]		0.69

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; exp: exposed; N: number of TDM samples; NCLO: norclozapine; NS: non-smoker; S: smoker; TDM: therapeutic drug monitoring; US: United States.

[a]N of tough steady-state TDM samples used to estimate the minimum therapeutic D needed to reach 350 ng/ml.

[b]As the patient appeared to be always partially non-adherent we were unable to estimate the minimum therapeutic D. Thus, the number of TDMs is described as 0 as there was no TDM not confounded by adherence. In parentheses is the number of TDMs available that were confounded by partial non-adherence.

[c]This minimum therapeutic D is too low for a ♂ S of European ancestry. That TDM may be contaminated by inflammation or the patient could have been a genetic poor metabolizer.

[d]The minimum therapeutic D =257 mg/day obtained in a generic compound is not likely to be correct, since the patient probably was non-adherent because the clozapine/norclozapine ratio was inverted.

Supplementary Material

Exploring low clozapine C/D ratios, inverted clozapine-norclozapine ratios and undetectable concentrations as measures of non-adherence in clozapine patients. A literature review and a case series of 15 patients from 3 studies

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Supplementary Box S1. Non-adherence in clozapine patients

1. Non-adherence in treatment-resistant schizophrenia (TRS)

- All schizophrenia guidelines describe the impact of poor adherence and guidelines for TRS insist on ruling out non-adherence before diagnosing treatment-resistance (De las Cuevas and de Leon, 2020) since studies using TDM are demonstrating that from only 1/3 to ½ of schizophrenia patients considered TRS by their psychiatrists are merely non-adherent (McCutcheon et al., 2018; Kyllesø et al., 2020).

2. Non-adherence to antipsychotics and the treatment setting

- Addressing non-adherence to clozapine treatment is difficult to establish with TDM; consideration of variations over time within each patient must be made, and there are major differences between acute inpatient treatment and long-term maintenance outpatient treatment (De Las Cuevas and de Leon, 2020).
- The most refractory non-adherent patients are those who do not attend outpatient treatment. Even among patients prescribed clozapine who regularly follow outpatient treatment, there is no guarantee that they are taking their prescribed treatments.
- Most clozapine-treated patients are receiving clozapine in the context of psychiatric polypharmacy and many patients receive some or all of these medications irregularly; when outpatients who come regularly for treatment are asked, they report that they can be very selective in their non-adherence taking some drugs but not others (De Las Cuevas et al., 2021).

3. Limited studies of lack of adherence in clozapine patients

- The limited studies which have tried to specifically focus on clozapine adherence have used indirect measures of adherence (Takeuchi et al., 2020; Brodeur et al., 2022).
- A study focused on a well-known clinical sample provided the sobering result that only 1/3 of clozapine patients of that sample were consistently adherent (Takeuchi et al., 2020).

4. Two major TDM categories of non-adherence in clozapine patients

- Non-detectable C. Current well-validated laboratory methods typically can quantify C which vary from laboratories but are as low as 50-1 ng/mL.[a] Thus, when a patient is taking doses that are therapeutic in an average patient but there is no detectable plasma C, the patient is obviously non-adherent and could not respond to clozapine.
- Detectable C but <350 ng/mL. It is much more complicated to establish how to diagnose non-adherence based on concentrations of clozapine that are lower than 350 ng/ml but are quantifiable (De Las Cuevas and de Leon, 2020). Moreover, one cannot rely on one single TDM value to assess metabolism and/or adherence.

5. Lack of adherence had major confounding effects on outpatient clozapine TDM data

- Based on a UK TDM database, Rostami-Hodjegan et al. (2004), recommended minimum therapeutic doses of 265 mg/day in female non-smokers to 525 mg/day in male smokers, but this study was probably was contaminated by 30-50% non-adherence. Taylor et al. (2021), in a UK textbook, used these dose recommendations.
- After paying more attention to adherence in the same database, Reeves et al. (2023) recommended much smaller minimum therapeutic doses for non-geriatric patients:
  - Instead of 265 mg/day for female nonsmokers, the recommended doses per ancestry were 175 mg/day (0.66 of prior dose) in Asians, 225 mg/day (0.85 of prior dose) in Europeans[b] and 250 mg/day (0.94 of prior dose) in Africans.[b]
  - Instead of 525 mg/day for male smokers, the recommended doses were 250 mg/day (0.48 of prior dose) in Asians, 375 mg/day (0.71 of prior dose) in Europeans[b] and 400 mg/day (0.76 of prior dose) in Africans.[b]

C: concentration; TDM: therapeutic drug monitoring; TRS: treatment-resistant schizophrenia.
[a]Ten to 20 years ago in wealthy countries limit of quantification typically were 50 or 25 ng/ml; more recently due to the use of more sophisticated techniques limit of quantification of 5 or 1 ng/ml are frequently available.
[b]In the original article the patients of European ancestry were called “Whites” and those of African ancestry “Afro-Caribbeans”.

Supplementary Box S2. A focused review of clozapine metabolism

1. Metabolism of clozapine (the parent compound)
• There are three metabolic pathways for clozapine. From most to least important they are: 1) demethylation to norclozapine, 2) oxidation to clozapine-N-oxide, and 3) glucuronidation (Schoretsanitis et al., 2019). • The main metabolic pathway for demethylation is CYP1A2 but other minor demethylation pathways are CY2C19, CYP3A4, and CYP2D6. • CYP1A2 activity is influenced by sex-smoking subgroups. Female non-smokers need the lowest minimum therapeutic dose (estrogens inhibit CYP1A2). Male smokers have the highest activity since tobacco smoking is a CYP1A2 inducer. CYP1A2 activity varies across ancestries; it is lowest in patients of Asian (defined by ancestries ranging from Pakistan to Japan) or American ancestry (Indigenous Americans), intermediate in those of European ancestry and highest in those of African ancestry (de Leon, 2023; Reeves et al., 2023). • The minimum therapeutic dose required for reaching 350 ng/ml can be used to compare individuals and groups according to their capability to metabolize clozapine, which pharmacologists call clozapine clearance. In patients of Asian or American ancestry, the average clozapine minimum therapeutic dose ranges from 166 mg/day (in female non-smokers) to 270 mg/day (in male smokers) while in those of European ancestry it ranges from 236 to 368 mg/day. In patients of African ancestry, the limited data suggest that US doses (300 to 600 mg/day) may be a reasonable range (de Leon et al., 2022).
2. Renal elimination of norclozapine
• Loi et al. (2013) proposed that 5/6th of norclozapine is eliminated by the kidneys. • In a small intensive study in patients taking clozapine, Schaber et al. (1998) estimated that 71% of free norclozapine was renally eliminated. • Norclozapine can also be eliminated by glucuronidation (Kyllesø et al., 2022); these metabolites are eliminated by the kidney and/or the gut.
3. Clozapine clearance
• Individuals have varying ability to eliminate clozapine from their bodies; pharmacologists have a precise word to describe it: "clozapine clearance." It is mainly dependent on CYP1A2 activity but renal clearance can become relevant, too. • In normal circumstances with normal renal function, clozapine clearance is mainly dependent on CYP1A2 activity. • When there is massive renal impairment, it may ↓ the clearance of clozapine metabolites but this has not been well studied (Schoretsanitis et al., 2019). • A large TDM study proposed that geriatric age led to progressive ↓ renal clozapine clearance (Bowskill et al., 2012). More recently, the same group, using a population pharmacokinetic model in the same database, proposed that patients 80 years of age should be treated with lower clozapine doses than 40-year-olds after correcting for ancestry group and sex-smoking subgroup. Most of the dose estimations for 80-year-olds corresponds to lower doses by a correction factor of 0.60-0.70 (Reeves et al., 2023).

C: concentration; CYP1A2: cytochrome P450 1A2; CYP2C19: cytochrome P450 2C19; CYP2D6: cytochrome P450 2D6; CYP3A4: cytochrome P450 3A4; TDM: therapeutic drug monitoring.

Supplementary Box S3. Linear kinetics

1. Definition
• Estimating minimum therapeutic doses is based on the principle of linear kinetics. • In therapeutic Ds, a specific patient whose important environmental variables related to clozapine metabolism remain stable, the relationship between C and D is linear and stable; thus, the C/D ratio is a constant.
2. When Ds are too low to reach the threshold of linear kinetics <150 ng/ml
• However, in Cs <150 ng/mL, clozapine may not follow linear kinetics and has substantial metabolic variability; therefore, Cs <150 ng/mL cannot be used to estimate the minimum therapeutic dose of clozapine (de Leon et al., 2022). As a matter of fact, in Cs <150 ng/mL there is great variability in the minimum therapeutic Ds in each patient (Schoretsanitis et al., 2024). • This threshold for interpretation of 150 ng/ml may be compared to the 350-ng/ml threshold of response. Thus, the 150-ng/ml interpretation threshold is 43% of the 350 ng/mL threshold of response. This range of Cs from 150 to 350 ng/mL allows better estimation of the minimum therapeutic D, but for the clozapine D in each patient to reach this range is dependent on the clozapine clearance of that patient.

C: concentration; C/D: concentration-to-dose; D: dose.

Supplementary Box S4. Clozapine PMs and UMs

1. Clozapine PMs
• Genetic PMs are probably explained by rare genetic mutations on CYP1A2 alleles that vary according to ancestry groups. The combination of multiple rare genetic mutations may explain around 5-10% of clozapine patients (Ruan and de Leon, 2020). • Causes of non-genetic PMs are 1) co-prescription of clinically relevant inhibitors (e.g., oral contraceptives), 2) obesity and/or 3) the presence of chronic inflammation. • The prevalence of combining all kinds of clozapine PMs (genetic and non-genetic) varies from sample to sample but may be 1/3 of clozapine-treated patients (Ruan and de Leon, 2020; de Leon, 2023). • PMs have minimum therapeutic doses of approximately half that of the patients of the same ancestry group and sex-smoking subgroup.
2. Clozapine UMs
• Clozapine UMs tend to be less frequent (<5% of clozapine patients). • Carbamazepine, phenobarbital and phenytoin are moderate inducers (Stout et al., 2021) and require multiplying the clozapine daily dose by ≤ 2. It is possible that rifampicin has even greater effects and may be a strong inducer (Spina et al., 2016; 2020). • Weak inducers include tobacco smoking and omeprazole, whereas the inducing effects of valproate are more complex. Some males exposed to weak inducers such as smoking and/or valproate can, on rare occasions, behave as clozapine UMs. Their minimum therapeutic dose is > 900 mg/day in patients of European ancestry and > 600 mg/day in patients of Asian ancestry. Our current limited estimation is that 1-2% of patients of European ancestry and <1% of those of Asian ancestry may be clozapine UMs during weak induction (Schoretsanitis et al., 2024).
3. To explore non-adherence: rule out changes from NM to UM or from PM to NM

• Unexpected low clozapine C/D ratios can be explained by:
  o adding an inducer (converting the patient from a NM to an UM) or
  o discontinuing an inhibitor (converting the patient from a PM to a NM)
• Clinicians need to rule out these two explanations before considering low clozapine C/D ratio as a sign of non-adherence.

C/D: concentration-to-dose; NM: normal metabolizer is referred here to briefly define patients who are not PMs and are not UMs; PM: poor metabolizer; UM: ultrarapid metabolizer

Supplementary Box S5. An inverted clozapine/norclozapine ratio may be a sign of non-adherence

1. Clozapine/norclozapine ratio

- In 1994, Centorrino et al. used the clozapine-to-norclozapine ratio in plasma for the first time in a published article.
- In 1998, in a study correlating plasma clozapine Cs with indexing of caffeine metabolism urine, Carrillo et al., proposed that the norclozapine/clozapine ratio may be an index of CYP1A2 activity. Most of the published articles have used the clozapine/norclozapine ratio (Schoretsanitis et al., 2019).
- In 2004, a widely quoted British TDM study established the fame of the clozapine/norclozapine ratio as a metabolic ratio for clozapine (Rostami-Hodjegan et al., 2004).
- Unfortunately, a 2019 systematic review of the clozapine-norclozapine ratio (Schoretsanitis et al., 2019) demonstrated that this ratio:
  - is not an index of CYP1A2 activity,
  - stability within individuals had not been studied,
  - had a weighted mean of 1.73 in 2,317 adult patients from 19 studies, and
  - ranged widely in the studies from 1.19 to 3.37.

2. An inverted clozapine/norclozapine ratio is proposed as a sign of non-adherence

- In 2010 the same British research group, using the same TDM database (Couchman et al., 2010) that the 2004 study used, proposed that a clozapine/norclozapine ratio:
  - < 0.5 "suggest either poor adherence within the last 24 hours or so, or that alterations in dose schedule might be beneficial. For example, if a young male smoker was given a relatively high dose, predominantly at night, weighting the dose such that more was given during the day could be helpful."
  - >3 "suggests that (1) absorption of clozapine from the last dose may not have been completed at the time the sample was obtained, or (2) that clozapine metabolism is saturated either because of the dose prescribed or because of inhibition of clozapine metabolism by a co-administered drug such as fluvoxamine or ciprofloxacin."
- Unfortunately, no data is presented to support this classification and their TDM data is contaminated by including concentrations that were not trough or steady-state and because multiple measures of the same individual were analyzed as independent samples (Schoretsanitis et al., 2019).
- In a more recent review (Flanagan et al., 2023), this British research group proposed a clozapine/norclozapine ratio:
  - ≤ 0.5 "suggest either (a) that the patient is a very fast metaboliser of clozapine (e.g. a young, male smoker) or (b) that clozapine has not been taken for day or two, possibly longer, prior to sampling."
  - ≥ 3, "but the plasma clozapine is 0.2–0.3 mg/L or so, this suggests that the patient may not have been taking clozapine regularly"
  - ≥ 3 "but the plasma clozapine is 1 mg/L or so, this suggests (a) inhibition of clozapine N-demethylation by a drug such as fluvoxamine, (b) possible downregulation of clozapine N-demethylation as a result of infection/inflammation or (c) that the sample was taken before absorption/distribution of the last dose was complete."
- This is a modification of the prior classification but no data is presented to support this modified classification. Moreover, non-adherence has been a major confounder in all published results of this TDM database.

C: concentration; TDM: therapeutic drug monitoring

Supplementary Box S6. Norclozapine half-life is longer than the half-life of the parent compound

1. Studies by the pharmaceutical company were not published
• The trial of norclozapine as an antipsychotic failed and the company published no articles (Schoretsanitis et al., 2019) but an abstract from a scientific meeting described "repeated dose studies suggest that steady-state is established after 6-7 days with an estimated half-life 15-35 hours" (Tamminga et al., 2006).
2. After sudden discontinuation, norclozapine Cs ↓ more slowly (de Leon et al., 1996)
• In Patient 2 after 7 days of discontinuation the clozapine C decreased to 1.1% (from 1279 to 15 ng/ml) and the norclozapine C decreased to 16.7% (from 739 to 30 ng/ml). The slower decrease indicated that norclozapine has a longer half-life. After 14 days both Cs were undetectable. • In Patient 3 after 7 days of discontinuation the clozapine C decreased to 3.7% (from 485 to 18 ng/ml) and the norclozapine C decreased to 4.6% (from 298 to 14 ng/ml) The slower decrease indicated that norclozapine has a longer half-life. After 14 days both Cs were undetectable.
3. Estimations of half-lives after overdose (Renwick et al., 2000)
• After an overdose of 3 g of clozapine, the terminal elimination half-lives were estimated to be 16.9 hours for clozapine and 22.5 hours for norclozapine (1.33 times higher, 22.5/16.9=1.33).
4. Half-lives after a single dose in 9 healthy Chinese ♂ volunteers (Wang et al., 2004)
• A single 10-mg dose study in 9 healthy Chinese ♂ volunteers. • In the early sampling times (1 to 6 hours) the plasma clozapine C was higher than norclozapine C (clozapine-to-norclozapine ratio >1). • In the late sampling times (12, 24 and 48 hours) the mean plasma clozapine C became progressively lower than the mean norclozapine C and the clozapine-to-norclozapine ratio became inverted and decreased in value (0.82, 0.54 and 0.06, respectively). This finding indicates that norclozapine has a much longer half-life. At 48 hours almost all clozapine C disappeared and most of the remaining compound in plasma was norclozapine.
5. Estimations of half-lives during brand comparisons (Golden and Honigfeld, 2008)
• This US study was a randomized, open-label, multiple-dose study in which a new clozapine form (with similar half-life) was compared to the clozapine brand. • 30 of 36 patients were included in the steady-state analyses. In the brand compound, the half-life was estimated to be 17 hours for clozapine and 22.5 hours for norclozapine (1.32 times higher, 22.5/17=1.32).
6. Summary of limited data: norclozapine's half-life is 1.32 times greater than clozapine's half-life

C: concentration.

Supplementary Box S7. Gemfibrozil: inverted clozapine/norclozapine ratios with normal clozapine C/D ratios

1. Gemfibrozil pharmacology
• Tornio et al. (2017) described gemfibrozil as an inhibitor of some renal transporters including the organic anion transporting polypeptide 1B1 (OATP1B1) and the organic anion transporter 3 (OAT3). • Currently it is not known which renal transporter may contribute to eliminating norclozapine (Schoretsanitis et al., 2019).
2. Gemfibrozil may invert clozapine/norclozapine ratio
• An African-American female was described in which gemfibrozil inverted the clozapine/norclozapine ratio with a normal clozapine C/D ratio. The patient was followed for 8 years (Barclay et al., 2019): o No gemfibrozil: 7 clozapine-to-norclozapine ratios ranging from 1.16-1.84 (mean=1.60). o On gemfibrozil: 2 clozapine-to-norclozapine ratios ranging from 0.41-0.63 (mean=0.52). o Gemfibrozil did not appear to change the clozapine C/D ratio of this patient. • Alfaro et al. (2001) described a male smoker who did not reach 350 ng/ml on 900 mg/day and was considered to metabolize clozapine faster than usual. He was taking gemfibrozil and his clozapine/norclozapine ratios ranged from 0.71 to 0.33.
3. Valproate may eliminate the effect of gemfibrozil ↓ norclozapine elimination
• In vitro data suggest that gemfibrozil inhibitory properties may be explained by one of its glucuronidation metabolites (Varma et al., 2015). • As valproate is an inhibitor of glucuronidation, this may explain that in a clozapine patient taking valproate, gemfibrozil did not invert the clozapine-norclozapine ratio (de Leon and Diaz, 2003).

C/D: concentration-to-dose

Supplementary Table S1. Current recommendations for low C/D ratios compatible with non-adherence. They assumed that the patient is not a CLO PM and CLO Cs are in the range that follows linear kinetics. These values may need to be modified as new data is collected.

	CLO C/D ratios		Minimum therapeutic D		D for 150 ng/ml		Range from
	Non-adherence	Expected	Calculated	Rounded	Calculated	Rounded	150 to 350 ng/ml
Ancestry from Asia or Indigenous Americans							
♀ NS	<1.06	2.12	166 (350/2.12)	175	71 (150/2.12)	75	75-175
♂ NS	<0.83	1.67	210 (350/1.67)	225	90 (150/1.67)	100	100-225
♀ S	<0.65	1.30	270 (350/1.30)	300	115 (150/1.30)	125	125-300
♂ S	<0.65	1.30	270 (350/1.30)	300	115 (150/1.30)	125	125-300
Ancestry from Europe (or Western Asia)							
♀ NS	<0.74	1.48	236 (350/1.48)	250	101 (150/1.48)	125	125-250
♂ NS	<0.59	1.18	256 (350/1.18)	275	127 (150/1.18)	150	150-275
♀ S	<0.49	0.99	357 (350/0.99)	375	152 (150/0.99)	175	175-375
♂ S	<0.47	0.95	368 (350/0.95)	375	158 (150/0.95)	175	175-375
Ancestry from SubSaharan Africa (not well-studied but based on doses recommended in the US)							
♀ NS	<0.75	1.17 (350/300)		300	128 (150/1.17)	150	150-300
♂ S	<0.29	0.58 (350/600)		600	259 (150/0.58)	275	275-600

C, concentration; C/D, concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; NS: non-smoking; S: smoking; US, United States.

Red font indicates which value of clozapine C/D ratio is suggestive of partial non-adherence in each ancestry group and sex-smoking subgroup.

Supplementary Table S2. Case 1: A 54-yo female non-smoker from an inpatient study in a Chinese hospital (no inverted CLO/NCLO ratio but 3 low clozapine C/D ratios)

Day	D	CLO/NCLO ratio	C[b] NCLO	C[b] CLO	C/D ratio	Estimated D for 350 ng/mL
1	500	1.56	291	455	0.91	385
15	500	2.08	241	501	1.00	349
30	500	1.85	250	461	0.92	379
44	500	1.32	297	391	0.78	448
70	500	2.06	259	534	1.07	328
44	500	1.32	296	391	0.78	448
70	500	2.06	259	534	1.07	328
80	500	1.56	328	512	1.02	342
93	500	1.74	182	317	0.63	552 abnormal for Chinese ♀
107	500	1.70	271	460	0.92	380
120	500	1.74	346	602	1.20	291
128	500	1.57	231	363	0.73	482
142	500	1.88	309	581	1.16	301
155	500	1.89	288	543	1.09	322
169	500	1.54	398	613	1.23	286
183	500	1.87	268	500	1.00	350
190	500	2.82	228	642	1.28	273
196	500	2.09	139	291	0.58	602 abnormal for Chinese ♀
204	500	2.08	300	625	1.25	280
211	500	2.12	267	570	1.14	307
218	450					
225	450	1.50	187	280	0.62	563 abnormal for Chinese ♀
238	450	2.05	244	499	1.11	315
241	400					
267	400	1.44	256	369	0.92	380
280	400	1.85	224	414	1.03	338
286	400	2.02	242	489	1.22	287
294	400	2.04	275	560	1.40	250
301	400	2.32	277	642	1.61	218
309	400	1.20	332	397	0.99	352
331	400	1.82	278	504	1.26	278
Mean of 23 possibly adherent CLO C/D ratios					**1.10**	**318**

C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; yo: years old.

Blue font indicates concentrations that are not in SS.

Red font indicates concentrations that were considered likely to be partially adherent by agreement of the first and the last authors.

Supplementary Table S3. Case 2: A 44-yo male non-smoker from an inpatient study in a Chinese hospital (1 inverted CLO/NCLO ratio with low clozapine C/D ratios, 1 other low clozapine C/D ratio and 1 non-quantifiable CLO C)

Day	D	CLO/NCLO ratio	C[b]		C/D ratio	Estimated D for 350 ng/mL
			NCLO	CLO		
1	375	1.93	241	567	1.51	231
12	400					
14	400	1.55	425	660	1.65	212
21	400	1.34	358	479	1.20	292
28	400	1.91	396	757	1.89	185
40	400	2.91	159	461	1.15	304
43	400		<25[a]	<25[a]		
47	400	1.39	503	698	1.75	201
53	375					
56	375	2.12	317	672	1.79	195
64	375	2.04	253	517	1.38	254
75	375	1.99	336	668	1.88	197
92	375	0.94	245	229	0.61	573 abnormal for Chinese ♂
99	400					
102	400	1.86	368	684	1.71	205
104	400	1.65	411	678	1.69	207
112	325	1.28	448	573	1.76	199
118	400	1.81	350	632	1.58	221
126	400	1.86	438	816	2.04	172
133	450					
137	450	1.78	409	726	1.61	217
139	400					
151	400	1.66	436	723	1.81	194
152	400	1.77	413	732	1.83	191
173	400	1.57	290	456	1.14	307
186	400	1.94	459	892	2.23	157
204	400	1.63	477	781	1.95	179
223	400	1.54	357	549	1.37	255
251	400	1.88	375	705	1.76	199
266	400	2.37	382	903	2.26	155
293	400	2.78	138	383	0.96	365
308	400	2.45	325	795	1.99	176
312	375					
316	350					
319	375					
321	450	1.94	319	618	1.65	212
341	375	1.61	372	600	1.60	219
354	375	1.65	371	612	1.63	214
Mean of 21 possibly adherent CLO C/D ratios					**1.73**	**202**

C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; yo: years old.

[a]Below quantification limit.

Blue font indicates concentrations that are not in SS.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S4. Case 3: A 46-yo female non-smoker with BMI=26.9 from an inpatient study in a Chinese hospital (three inverted CLO/NCLO ratios with low CLO C/D ratios)

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL	
			NCLO	CLO			
1	225	1.50	66	99	0.44	799	Non-adherent before[a]
4	250						
9	225	0.93	123	114	0.51	699	Not SS/non-adherent
10	275						
12	325						
15	350						
20	350	0.90	200	179	0.51	685	Non-adherent
22	375						
25	400						
31	400	0.82	197	162	0.41	864	Non-adherent
33	200						
53	250						
54	300						
58	300	1.28	158	202	0.67	521	Not SS/non-adherent
64	325						
66	350	1.08	229	248	0.71	494	Not SS/non-adherent
67	375						
68	425						
73	425	0.75	326	245	0.58	607	Non-adherent

Unable to calculate mean CLO C/D ratios; all appear to indicate non-adherence

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; yo: years old.

[a]This C reflects that she was probably non-adherent with outpatient treatment before being admitted.

Blue font indicates concentrations that are not in SS.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S5. Case 4: A 20-yo male non-smoker with BMI=21.6 from an inpatient study in a Chinese hospital (1 inverted CLO/NCLO ratio with low clozapine C/D ratios and 3 other low clozapine C/D ratios)

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL	
			NCLO	CLO			
1	100	1.11	71	79	0.79	445	Non-adherent before[a]
11	125						
20	125	1.47	89	131	1.04	335	C<150 ng/ml
21	100						
26	125						
28	175						
34	175	1.64	115	188	1.08	325	
35	225						
38	250						
41	275	1.58	147	232	0.84	414	C<150 ng/ml
43	300						
45	300	1.20	178	213	0.71	493	Not SS
48	325						
51	325	1.46	195	286	0.88	398	Not SS
55	350						
58	375	1.42	208	296	0.79	444	Not SS
62	375	1.39	203	282	0.75	466	
63	400						
65	400	1.32	205	270	0.67	518	Not SS
66	425						
71	425	1.41	282	398	0.94	373	
72	400						
73	350						
77	350	1.37	213	292	0.83	419	Not SS
78	300						
79	250						
98	200	1.38	104	143	0.72	490	Not SS
105	200	1.45	84	120	0.60	580	C<150 ng/ml likely non-adherent
113	200	1.47	155	227	1.13	309	
120	250	1.39	105	146	0.58	599	Not SS C<150 ng/ml
121	300						
124	350						
126	350	1.43	211	303	0.86	405	Not SS
127	400						
129	450						
133	450	1.24	275	340	0.76	463	
138	500						
140	500	1.42	264	373	0.75	469	Not SS
141	550						
143	600						
147	550	1.47	315	463	0.78	416	Not SS
148	450						
149	400						
154	400	1.34	208	279	0.70	502	
161	400	1.62	199	323	0.81	434	

168	400	1.23	209	257	0.64	544	
170	350						
171	300						
175	300	1.32	191	252	0.84	418	
182	300	1.33	135	179	0.60	585	
190	300	1.52	129	197	0.66	534	
196	300	1.44	162	233	0.78	450	
198	350						
203	350	1.60	197	316	0.90	388	
204	400						
208	450						
210	450	1.64	247	406	0.90	388	Not SS
217	450	1.82	301	548	1.22	288	
225	450	0.91	290	263	0.59	598	
232	450	1.44	378	545	1.21	289	
238	450	1.24	408	503	1.12	313	
256	200	1.16	176	204	1.02	343	Not SS
258	250						
265	250	1.26	83	194	0.42	840	
276	300	1.44	143	205	0.68	512	Not SS
279	200						
288	250						
293	250	1.60	147	236	0.94	370	
296	225	1.44	227	329	1.46	240	Not SS
309	225	1.53	134	205	0.91	384	
310	200						
318	200	1.20	164	196	0.98	358	
328	175	1.14	147	167	0.96	366	Not SS
331	150						
335	150	1.39	138	182	1.28	274	Not SS
336	125						
339	125	1.03	109	113	0.90	389	Not SS
Mean of 17 possibly adherent CLO C/D ratios					**0.94**	**372**	

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; yo: years old

Blue font indicates concentrations that are not in SS.

Purple font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and last authors but had C<150 ng/ml.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

[a]This C indicates that he was probably non-adherent with outpatient treatment before being admitted.

Supplementary Table S6. Case 5: A 39-yo male non-smoker with BMI=29.1 from an inpatient study in a Chinese hospital (1 inverted CLO/NCLO ratio with low clozapine C/D ratios and 1 non-quantifiable CLO C)

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL	
			NCLO	CLO			
1	125	1.86	52	97	0.77	452	Non-adherent before
7	150						
8	150	1.37	74	102	0.68	517	Not SS C<150 ng/ml
15	150	1.46	101	148	0.98	356	C<150 ng/ml
23	150	1.38	66	92	0.61	572	C<150 ng/ml
29	150	1.13	89	101	0.67	522	C<150 ng/ml
42	175						
43	175	1.34	76	101	0.58	606	C<150 ng/ml
44	200						
50	200	1.15	131	149.6	0.75	468	C<150 ng/ml
64	200	1.37	98	134	0.67	523	C<150 ng/ml
66	225						
71	250	1.34	123	164	0.66	534	Not SS
78	250	1.01	160	161	0.64	544	
85	250	1.09	126	138	0.55	632	C<150 ng/ml
98	275						
99	275	1.12	127	143	0.52	674	Not SS C<150 ng/ml
105	275	1.00	114	114	0.41	845	C<150 ng/ml
113	275	1.05	164	173	0.63	558	
120	300	1.08	117	127	0.42	830	Not SS C<150 ng/ml
127	325	1.10	136	149.7	0.46	760	Not SS C<150 ng/ml
134	325	1.20	131	157	0.48	725	
141	325	1.07	133	142	0.44	804	C<150 ng/ml
148	325	2.27	78	178	0.55	639	C<150 ng/ml
155	325	1.29	120	154	0.47	739	
161	350						
162	350	1.42	164	233	0.67	536	Not SS
169	350	1.22	156	191	0.55	642	
176	375	1.40	159	222	0.59	592	Not SS
183	375	0.98	173	170	0.45	771	
189	400						
190	400	0.97	149	143	0.36	976	Not SS
205	425	1.07	123	131	0.31	1132	Not SS
211	425	1.04	144	149.7	0.35	994	C<150 ng/ml
218	425	1.13	164	185	0.43	806	
225	425	1.11	174	194	0.46	769	
235	425	1.21	96	117	0.28	1271	C<150 ng/ml
246	425	1.07	82	88	0.21	1700	C<150 ng/ml
253	425	1.07	82	88	0.21	1700	C<150 ng/ml
260	425	1.05	135	141	0.33	1053	C<150 ng/ml
267	425	1.73	110	191	0.45	781	
281	425	1.10	113	124	0.29	1196	C<150 ng/ml
291	425	0.89	142	127	0.30	1172	C<150 ng/ml
301	425	1.26	114	143	0.34	1039	C<150 ng/ml
309	425	1.13	125	141	0.33	1108	C<150 ng/ml

330	425	1.10	105	116	0.27	1288	C<150 ng/ml
337	425	1.21	142	172	0.41	863	
347	425	1.06	122	129	0.30	1150	C<150 ng/ml
358	425		<25	<25	<0.06	>5950	C<150 ng/ml

Mean[a] was not calculated; it is possible that all Cs were confounded by lack of adherence

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; yo: years old

[a]According to a prior study mean C/D ratio in male smokers is 1.71, providing a D to reach 350 ng/ml of 205 mg/day.

Blue font indicates concentrations that are not in SS.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S7. Case 6: A 62-yo female non-smoker with BMI=21.6 from an inpatient study in a Chinese hospital (1 inverted CLO/NCLO ratio with low clozapine C/D ratio)

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL	
			NCLO	CLO			
1	200	1.59	121	191	0.96	366	Non-adherent before
4	125						
7	250						
11	250	0.97	218	212	0.85	413	
19	275						
22	300	0.99	287	286	0.95	367	Not SS
27	200						
40	200	1.47	220	324	1.62	216	
Last probably NOT confounded by lack of adherence					**1.62**	**216**	

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; yo: years old

Blue font indicates concentrations that are not in SS.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S8. Case 7: A 58-yo female non-smoker of European ancestry with BMI=31.1 from an inpatient study in a US hospital (2 inverted CLO/NCLO ratios with low clozapine C/D ratios).

Day	D	CLO/NCLO ratio	C[b] NCLO	C[b] CLO	C/D ratio	Estimated D for 350 ng/mL	Comment
1	50						
4	100						
8	100	1.52	73	111	1.11	315	Not SS and C<150
16	50	2.47	161	398	3.98	88	Not SS/Infection
17	100						
22	100	1.91	113	216	2.16	162	Not SS
29	100	1.45	83	120	1.20	292	Not SS and C<150
43	100	1.17	84	98	0.98	357	C<150
57	100	1.47	51	75	0.75	467	C<150
72	100	1.43	53	76	0.76	461	C<150
85	100	2.14	44	94	0.94	372	C<150
99	100	1.41	58	82	0.82	427	C<150
113	100	1.32	65	86	0.86	407	C<150
117	150						
120	200						
124	250						
127	300						
135	300	1.78	344	613	2.04	171	Not SS
162	300	1.62	420	679	2.16	155	
197	300	1.53	387	592	1.97	177	
218	300	1.44	384	552	1.84	190	
229	400						
232	450						
236	500						
239	600						
246	600	1.29	694	892	1.49	235	Not SS
275	600	1.09	790	862	1.44	244	Probably non-adherent
303	600	0.83	903	746	1.24	282	Non-adherent
331	600	0.98	844	830	1.38	253	Non-adherent
Mean from 3 possibly adherent CLO C/D ratios					**2.03**	**172**	

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; US: United States; yo: years old.

Blue font indicates concentrations that are not in SS.

Dark green font indicates concentrations that are not in SS but were contaminated by an infection.

Orange font indicates concentrations that were considered probably non-adherent by agreement of the first and the last authors.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S9. Case 8: A 32-yo male non-smoker[a] of European ancestry with BMI=32.8 from an inpatient study in a US hospital (5 inverted CLO/NCLO ratios with low clozapine C/D ratios and 2 other low clozapine C/D ratios)

Day	D	CLO/NCLO ratio	C[b] NCLO	C[b] CLO	C/D ratio	Estimated D for 350 ng/mL	Comment
1	50						
4	100						
8	150						
11	200						
15	250						
18	300						
22	400						
25	500						
29	600	1.71	321	549	1.10	318	Not SS/Myoclonus
43	600	0.88	591	523	0.87	402	Non-adherent
57	600	0.79	588	464	0.77	453	Non-adherent
71	600	1.25	405	505	0.84	416	Non-adherent
85	600	1.09	391	428	0.71	491	Infection
100	600	0.94	770	720	1.20	292	Non-adherent
113	600	0.75	598	448	0.74	473	Non-adherent
113	500						
117	400						
120	300						
134	300	1.31	175	229	0.76	459	Non-adherent
141	300						Myoclonus
162	300	0.77	154	118	0.39	890	Non-adherent
176	study terminated						

Mean was not calculated; it is possible that all Cs were confounded by lack of adherence

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; US: United States; yo: years old.

[a]Although the patient occasionally smoked cigarettes, all 6 plasma cotinine concentrations were below the limit of quantification.

Blue font indicates concentrations that are not in SS.

Dark green font indicates concentrations that are not in SS but were contaminated by an infection.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S10. Case 9: A 58-yo female smoker (20 cig/day) of European ancestry with BMI 30.8 from an inpatient study in a US hospital (4 inverted CLO/NCLO ratios with low clozapine C/D ratios).

Day	D	CLO/NCLO ratio	C[b] NCLO	C[b] CLO	C/D ratio	Estimated D for 350 ng/mL	Comment
1	50						
4	100						
9	150	2.09	87	182	1.21	288	Not SS and C<150
15	250	1.95	128	249	1.00	351	Not SS/Infection
22	300	2.87	222	637	2.12	165	Not SS/Infection
37	300	1.78	324	578	1.93	182	
43	300	2.37	210	497	1.66	211	
57	300	0.35	380	134	0.45	784	Non-adherent
71	300	2.30	329	756	2.52	139	
86	300	2.12	336	712	2.37	147	
99	300	1.77	412	730	2.43	144	
117	400						
120	450						
124	500						
127	600						
134	600	0.95	701	664	1.11	316	Not SS
159	600	0.65	773	502	0.84	418	Non-adherent
187	600	0.68	733	502	0.84	418	Non-adherent
219	600	0.53	730	389	0.65	540	Non-adherent/Infection
226	500						
230	400						
233	300						
236	250						
240	200						
244	150						
246	100	1.38	146	202	2.02	173	Not SS and C<150
274	100	1.67	81	135	1.35	259	C<150
309	100	1.67	95	159	1.59	220	C<150
330	100	1.40	87	122	1.22	287	C<150
Mean from 3 possible adherent CLO C/D ratios					**2.18**	**161**	

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; SS: steady state; US: United States; yo: years old

Blue font indicates concentrations that are not in SS.

Dark green font indicates concentrations that are not in SS but were contaminated by an infection.

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S11. Cases 10-15: of European ancestry from an outpatient study in Uruguay with two TDMs, the first on brand-name CLO and the second on generic CLO

	D	CLO/NCLO ratio	C NCLO	C CLO	C/D ratio	Estimated D for 350 ng/mL	Comment
Case 10: 34-yo ♂ smoker with BMI=27.7							
Brand	500	0.42	417	177	0.35	1000	
Generic	500	1.94	278	540	1.08	324	
Case 11: 32-yo ♂ smoker and taking omeprazole with BMI=22.3							
Brand	200	0.26	315	83	0.42	833	
Generic	200	1.07	148	157	0.79	443	
Case 12: 28-yo ♂ smoker with BMI=25.6							
Brand	300	0.42	611	256	0.85	412	
Generic	300	1.15	678	782	2.61	134	
Case 13: 43-yo ♀ smoker with BMI=24.9 (mean minimum therapeutic D=357 mg/day)[a]							
Brand	350	0.26	366	97	0.28	1250	
Generic	350	0.71	126	89	0.25	1400	
Case 14: 39-yo ♂ smoker with BMI=25.5 (mean minimum therapeutic D=368 mg/day)[b]							
Brand	400	0.38	337	129	0.32	1094	
Generic	400	0.69	120	82	0.21	1667	
Case 15: 36-yo ♂ nonsmoker with BMI=30.0 (mean minimum therapeutic D=150 mg/day)[c]							
Brand	500	<0.56	45	<25	<0.05	>7000	
Generic	500	0.70	978	682	1.36	257	estimation may not be correct

BMI: body mass index; C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; yo: years old

[a]Of 269 ♀ smokers of European ancestry, mean D was 357 mg/day (Schoretsanitis et al., 2021).

[b]Of 546 ♂ smokers of European ancestry, mean D was 368 mg/day (Schoretsanitis et al., 2021).

[c]The patient is a ♂ non-smoker of European ancestry, but is probably a poor metabolizer due to obesity and his minimum therapeutic dose may be around 150 mg/day (de Leon et al., 2022b).

Red font indicates concentrations that were considered likely to be partially non-adherent by agreement of the first and the last authors.

Supplementary Table S12. Modeling total non-adherence starting in day 1 in Case 2 (based on CLO half-life of 24 hours and NCLO half-life of 32 hours[a])

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL
			NCLO	CLO		
1	400	1.83	378[b]	692[b]	1.73	202
2	0 (400)[c]	1.22	284	346	0.87	402
3	0 (400)[c]	0.81	213	173	0.43	814
4	0 (400)[c]	0.54	160	87	0.22	1591
5	0 (400)[c]	0.37	120	44	0.11	3182
6	0 (400)[c]	<0.28	90	<25[d]	<0.06	>5,833
7	0 (400)[c]	<0.37	68	<25[d]	<0.06	>5,833
8	0 (400)[c]	<0.49	51	<25[d]		
9	0 (400)[c]		38	ND[e]		
10	0 (400)[c]		29	ND[e]		
11	0 (400)[c]		<25[d]	ND[e]		
12	0 (400)[c]		<25[d]	ND[e]		
13	0 (400)[c]		<25[d]	ND[e]		
14	0 (400)[c]		ND[e]	ND[e]		

C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; ND: non-detectible.

[a]For CLO C we assume that a half-life of 24 hours means that after 1 day of not taking any CLO D the CLO C will be half of the C of the prior day. For NCLO C we assume that clearance was constant and that half-life of 32 hours means that in 24 hours approximately 0.75 (24/32=0.75) of the NCLO C will be eliminated.

[b]Based on 21 adherent TDMs the mean CLO C/D ratio was 1.73 ng/ml per mg/day and the mean CLO/NCLO ratio was 1.83. For 400 mg/day, the expected CLO C was 692 ng/ml (400 x 1.73=692) and the NCLO C was 378 ng/ml (692/1.83=378).

[c]We assumed Case 2 had been taking every dose of his prescribed 400 mg/day but after TDM was collected on day 1, he stopped taking any more clozapine.

[d]We assumed that the limit of quantification is 25 ng/ml for CLO and NCLO.

[e]We assumed that the limit of detection is 5 ng/ml for CLO and NCLO.

Blue font indicates Day 3 when the CLO/NCLO ratio becomes inverted suggesting non-adherence.

Light green font indicates Days 9 and 14 when CLO and NCLO Cs becomes ND by the laboratory.

Red font indicates Day 2 when the CLO C/D ratio drops in half and suggests partial non-adherence.

Supplementary Table S13. Modeling total non-adherence starting on day 1 in Case 11 (based on CLO half-life of 24 hours and NCLO half-life of 32 hours[a])

Day	D	CLO/NCLO ratio	C		C/D ratio	Estimated D for 350 ng/mL
			NCLO	CLO		
1	200	1.06	148[b]	157[b]	0.79	443
2	0 (200)[c]	0.72	111	79	0.40	875
3	0 (200)[c]	0.48	83	40	0.20	1,750
4	0 (200)[c]	<0.40	62	<25[d]	<0.13	>2,692
5	0 (200)[c]	<0.53	47	<25[d]	<0.13	>2,692
6	0 (200)[c]	<0.71	35	<25[d]	<0.06	>5,833
7	0 (200)[c]		26	ND[e]		
8	0 (200)[c]		<25[d]	ND[e]		
9	0 (200)[c]		<25[d]	ND[e]		
10	0 (200)[c]		<25[d]	ND[e]		
11	0 (200)[c]		<25[d]	ND[e]		
12	0 (200)[c]		ND[e]	ND[e]		

C: concentration; C/D: concentration-to-dose; CLO: clozapine; D: dose; NCLO: norclozapine; ND: non-detectible.

[a]For CLO C we assume that a half-life of 24 hours means that after 1 day of not taking any CLO D the CLO C will be half of the C of the prior day. For NCLO C we assume that clearance was constant and that half-life of 32 hours means that in 24 hours approximately 0.75 (24/32=0.75) of the NCLO C will be eliminated.

[b]Based on 1 adherent TDM that had a CLO C=157 and NLO C=148.

[c]We assumed Case 11 had been taking every dose of his prescribed 200 mg/day, but after TDM was collected on day 1, he stopped taking any more clozapine.

[d]We assumed that the limit of quantification is 25 ng/ml for CLO and NCLO.

[e]We assumed that the limit of detection is 5 ng/ml for CLO and NCLO.

Blue font indicates Day 2 when CLO/NCLO ratio became inverted, suggesting non-adherence.

Light green font indicates Days 9 and 14 when CLO and NCLO Cs became ND by the laboratory.

Red font indicates Day 2 when the CLO C/D ratio drops in half and suggests partial non-adherence.