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Main document

The significance of sampling-time in therapeutic drug monitoring of clozapine

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Running title: Sampling-time and clozapine concentrations

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Abstract

Objective: Therapeutic drug monitoring (TDM) of clozapine is standardized to 12-hour post-dose samplings. In clinical settings, sampling-time often deviates from this time-point, although the importance of the deviation is unknown. To this end, serum concentrations (s-) of clozapine and its metabolite N-desmethyl-clozapine (norclozapine) were measured at 12-hours ± one and two hours post-dose.

Method: Forty-six patients with a diagnosis of schizophrenia, and on stable clozapine treatment, were enrolled for hourly, venous blood sampling at 10-14 hours post-dose.

Results: Minor changes in median percentage values were observed for both s-clozapine (-8.4%) and s-norclozapine (+1.2%) across the four-hour time-span. Maximum individual differences were 42.8% for s-clozapine and 38.4% for s-norclozapine. Compared to 12-hour values, maximum median differences were 8.4% for s-clozapine and 7.3% for s-norclozapine at deviations of ± two hours. Maximum individual differences were 52.6% for s-clozapine and 105.0% for s-norclozapine. The magnitude of s-clozapine differences was significantly associated with age, body-mass-index and the presence of chronic basophilia or monocytosis.

Conclusion: The impact of deviations in clozapine-TDM-sampling-time, within the time-span of 10-14 hours post-dose, seems of minor importance when looking at median percentage differences. However, substantial individual differences were observed, which implies a need to adhere to a fixed sampling-time.

Keywords: Clozapine; norclozapine; sampling-time; serum concentration; therapeutic drug monitoring

Significant outcomes:

- Both clozapine- and norclozapine concentrations differed significantly within the four-hour time-span, although median percentage differences were minor
- Some individuals showed substantial percentage differences, indicating that deviations in sampling time could influence the interpretation of individual TDM-concentrations
- The magnitude of percentage clozapine differences was significantly associated with increasing age, decreasing BMI and the presence of chronic monocytosis or basophilia

Limitations:

- The sample size was small (n = 46), which limits the generalizability of our findings
- Multiple variables were tested for sensitive-sub-group-hypothesis generation, increasing the risk of mass-significance (type 1 errors)

Introduction

Clozapine is a highly effective, second generation antipsychotic, with a documented superiority to other compounds, when treating patients with treatment-resistant schizophrenia (1).

However, clozapine is also associated with several potential life-threatening adverse effects, such as an increased risk of myocarditis, ileus and agranulocytosis (2), which is why treatment with clozapine is restricted to patients with insufficient response to at least two other antipsychotics (2). The risk of developing a variety of hematological side-effects, such as agranulocytosis (3), is also the reason for the implementation of mandatory hematological monitoring programs world-wide, e.g. in Europe weekly white blood cell (WBC) differential counts for the first 18 weeks of treatment, and monthly thereafter (2).

Most side-effects to clozapine treatment do not have an established relationship with dose or concentration levels (4), although the rate of side-effects seems to double with concentrations above 350 ng/ml (1071 nmol/l; conversion factor 3.06 (5)) (6). This is also the suggested lower threshold for therapeutic response, as adequate response is more likely to occur at concentrations > 350 ng/ml (1071 nmol/l) (7). Inefficiently low concentration levels will typically result in treatment failure with relapse or withdrawal symptoms and increased hospitalization (7, 8). An upper limit for clozapine levels has not reached consensus (9); however, an alert level of 1000 ng/ml (3060 nmol/l) has been suggested (5), addressing a safety threshold, above which the risk of fulminant toxicity and CNS related adverse reactions, such as seizures, increases (9). Individual concentration to dose (C/D) responses are somewhat unpredictable though (7), with an almost 50-fold reported variation in C/D ratios for patients on fixed doses (10). Blood levels alone therefore seem to be a poor indicator of response. This is due to the complex nature of clozapine pharmacokinetics: Clozapine is rapidly absorbed with a mean 2.1 hours time-to-peak plasma concentration (range 0.4-4.2 hours). Elimination is biphasic with an average terminal half-life of 12-hours (range 6-26 hours) (11). It is extensively metabolized in the liver by various cytochrome P-450 (CYP) enzymes, primarily by CYP1A2, with N-desmethyl-clozapine (norclozapine) and clozapine-N-oxide being the major metabolites (12). Norclozapine is an active metabolite with suggested antipsychotic

and toxic effects on its own (13, 14), and a reverse metabolism, back into the parent compound, have been shown for the metabolite clozapine-N-oxide (15).

The turnover through the various CYP systems is influenced by several factors: smoking (16), caffeine consumption (11, 17), genetics (18, 19), inflammation (20) and drug-drug interactions. Numerous drugs have an effect on the activity of the CYP systems e.g. some antibiotics, antidepressants, anticonvulsants, proton pump inhibitors (PPI) and hormonal contraceptives (HC) (5, 11, 21) have been shown to markedly alter clozapine levels. Other factors that have shown to affect clozapine levels are gender, age, and bodyweight (4, 22).

Consequently, large inter- as well as intra-individual variability in the C/D ratio has been reported for clozapine treated patients (7, 10). However, one study suggests sampling-time-error as an important source of the intra-individual variance in clozapine concentrations (23). The authors of the mentioned study argue that blood sampling for therapeutic drug monitoring (TDM) of clozapine should follow guidelines in order to be of relevance, since only around 15% of the 773 investigated clozapine samples were collected at the standard 12-hour time-point and that s-clozapine significantly correlated with time from last administration (23).

TDM is a valuable and strongly recommended clinical tool in the process of optimizing clozapine treatment, while reducing the risk of concentration dependent toxicity or relapse, especially subsequent to changes in the concentration-affecting parameters (4, 5). Although most hospitals have standardized TDM of clozapine to 12-hour samples, accepted deviations in sampling time have not been clearly defined. Consequently, sampling time can deviate several hours from the 12-hour time-point (23). The clinical impact of these deviations remains to be elucidated.

Aims of the study

The aims of this study were to describe the impact on serum (s-) clozapine- and norclozapine levels, as sampling time deviated ± one and two hours from the standard 12-hour time-point and to identify possible impact-associated variables.

Materials and methods

The study was approved by the local ethics committee of the Capital Region of Denmark and the Danish health authorities. The study was registered at ClinicalTrials.gov and the local Good Clinical Practice (GCP) unit monitored the study.

Participants

Participants were recruited from outpatient services in the Capital Region of Denmark. Patients were included from September 2015 to December 2015.
All participants provided written informed consent before entering the study, and all eligible patients were screened by interview before final inclusion.

Inclusion criteria were: 1) age 18-64, 2) diagnosis of schizophrenia according to the criteria of International Classification of Diseases, 10th revision (ICD10) (24) or the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) (25), 3) unchanged dose and dosing of clozapine 30 days prior to trial, 4) usual night administration of clozapine between 9 and 12 PM.
Exclusion criteria were: 1) non- or partial clozapine compliance the day before blood sampling, 2) clozapine ingestion in the morning of or during the trial, 3) non-response to telephone call at the scheduled time of evening clozapine ingestion, 4) significant change, defined as cessation, starting, doubling or halving in smoking- or caffeine habits within 30 days prior to trial, 5) altered use of other antipsychotics or medications known to affect s-clozapine (Table 1), 30 days prior to trial (seven days for hormone based contraceptives), 6) pregnancy or breastfeeding, 7) alcohol- or drug abuse that would affect participation of the trial (assessed per interview).

Study design

The study was conducted at the Psychiatric Centre of Copenhagen, Rigshospitalet. Eligible patients were scheduled for venous blood sampling at precisely 10, 11, 12, 13 and 14 hours after their night clozapine dosing. The drug was to be ingested at the usual time of administration the evening before blood sampling. For subjects with

more than one daily dosing of clozapine (e.g. dosing additional to their night dosing between 9 and 12 PM, see Table 1), an eventual morning dosing of clozapine was postponed until ended sampling-time. Status of in- and exclusion criteria, compliance and exact time of drug administration were verified by telephone contact with the patient at the time of last drug administration. Sampling time was scheduled based on the confirmed time of night clozapine ingestion. Only blood samples drawn within ± five minutes from schedule were accepted.

Primary endpoints were differences in clozapine- and norclozapine concentrations within the four-hour time span.

Medical charts were retrospectively reviewed for confirmation of in- and exclusion criteria and extraction of secondary endpoint variables.

Secondary endpoints served for hypothesis generation in terms of subgroup analysis for correlations between s-clozapine differences and selected variables. Secondary endpoint variables included the parameters gender, age (both as a continuous and a binary variable: ≥ 45 years of age vs. < 45 years), body-mass-index (BMI), smoking habits, daily caffeine consumption based on self-reported amounts of caffeinated beverages (26), C-reactive protein (CRP) levels at inclusion (≥ 5 mg/l vs. < 5mg/l), relevant co-administrations (defined as drug-groups with known affecters of clozapine concentration), clozapine doses (night and total daily dose of clozapine), number of clozapine administrations (one vs. multiple daily administrations), clozapine concentration to daily dose ratio (C/D), metabolic ratio for norclozapine:clozapine (MR) and the presence of chronic blood dyscrasia (see below).

WBC differential counts from the inclusion day, or the observation nearest to the inclusion day, and six months retrospectively, were collected. Normal WBC differential reference-ranges (for usual clinical evaluation) were set by the Department of Clinical Biochemistry, Rigshospitalet (27). Chronic blood dyscrasia was defined as a minimum of 50% of the retrospective observations, for a given cell line, located either under or above the normal reference range. Only subjects with

three or more cell counts, within the retrospective period, were included for chronic blood dyscrasia evaluation.

Laboratory analysis

Blood sampling was performed at fixed time points (10, 11, 12, 13 and 14 hours post-dose), and 6 ml of venous blood was collected at each time-point for the determination of drug concentrations. Blood was collected in tubes with clot-activator and left to coagulate at room temperature, until the end of sampling time.
Collected blood was immediately here after centrifuged at 4000 revolutions per minute, and serum was extracted and stored at -20° C until analysis. Serum from the first blood sample was also used for measuring of baseline CRP.
S-clozapine and s-norclozapine assays were performed at the Section of Forensic Chemistry, University of Copenhagen.
Serum concentrations were measured from each blood sample by a slightly modified solid phase extraction and ultra-performance liquid chromatography, combined with tandem mass spectrometry (UPLC-MS-MS), using isotope standards as internal controls for each compound (28, 29).
A highly sensitive analysis for C-reactive protein (hsCRP) was performed using a validated high sensitivity luminescence-immunoassay (LIA) at the Department of Clinical Biochemistry, Rigshospitalet.

Statistical analyses

SAS Enterprise Guide 7.1 was used for the analyses, and only two-tailed *P* values < 0.05 were considered statistically significant.

For descriptive analyses, continuous outcomes are presented as medians and ranges between minimum and maximum values, or means and standard deviations (±SD), when applicable. Categorical outcomes are reported as numbers and proportions (n (%)).
In case of skewness of values, natural logarithm (log) values were used for statistical analysis.

For each participant the percentage difference in s-clozapine and s-norclozapine was calculated. The 12-hour time point was set as reference and compared to concentrations measured at each of the other time-points (10,11,13 and 14 hours post-dose). Likewise, the percentage changes of s-clozapine and s-norclozapine concentrations between 11 and 13-hour values and between 10 and 14-hour values (across the 12-hour time-point) were measured.
The students' *t*-test for paired data was used to analyze intra-individual differences in serum concentrations.

In order to identify potential subgroups with higher sensitivity towards deviations in sampling time, we identified both the maximum percentage difference from the 12-hour clozapine value, and the maximum percentage clozapine change across the 12-hour time-point (11 vs. 13- or 10 vs. 14-hour differences), in absolute numbers, for each subject.
Pearson's correlation was then used to test for correlations between maximum percentage clozapine differences, both compared to and across the 12-hour time-point, and the continuous variables: age, BMI, daily caffeine consumption, clozapine dosage (both daily and last dosage), reference clozapine concentration (the 12-hour or 10-hour clozapine concentration), reference norclozapine concentration, C/D ratio, combined norclozapine and clozapine C/D ratio, MR and maximum, percentage difference in norclozapine concentration.
The impact of the binary variables: gender, age ≥ 45 years <, CRP status ≥ 5 mg/l <, smoking status, co-administration of relevant medications and number of daily clozapine administrations (one vs. multiple) was also tested on maximum, percentage clozapine differences, with the student's *t*-test for un-paired data.

Results

Fifty-four subjects were screened and 48 subjects (88.9 %) were included in the study. Two subjects were subsequently excluded: One patient suffered a serious adverse reaction (SAR) i.e. agranulocytosis and sepsis, during sampling time, and one patient

had had a clozapine dose-adjustment 10 days prior to the trial and was excluded as a screening failure.

Consequently, data from 46 (85.2%) subjects was used for statistical analyses. Forty subjects had all five of their blood samples drawn on time. Five subjects had one missing value and one subject had two missing values.

Of the 46 included subjects 26 were males (56.5%). The participants had a median age of 42 years (range 20-59), a median BMI of 30.9 kg/m^2 (range 20.1 to 48.8) and they received a median daily clozapine dosage of 300.0 mg (range 50.0 to 800.0). A complete list of patient characteristics is presented in Table 1.

Clozapine

Differences across the 12-hour time-point

The median and range of 10-hour concentrations are presented in Table 1.

There was a general tendency towards a decrease in s-clozapine over time with a median decrease of 8.4% and a mean absolute difference of 14.0% (±10.2) from 10 to 14 hours post-dose (Table 2). However, 11 patients (23.9%) showed increasing levels during the four-hour period. S-clozapine time-courses are presented in the supplementary material Fig. S1.

S-clozapine values changed significantly ($P < 0.01$) across the sampling period (between 11- and 13-hour values and between 10- and 14-hour values), with individual differences up to 39.1% and 42.8%, respectively (Table 2). Seven subjects (15.9%) showed absolute differences above 25% (range 26.7 to 42.8) from 10 to 14 hours post-dose.

Differences compared to the 12-hour values

The median and range of 12-hour s-clozapine concentrations are presented in Table 1. The median and range of percentage differences in measured levels of clozapine, compared to 12-hour values, are presented in Table 2 and Fig. 1.

Measured levels before the 12-hour time-point were significantly different from the 12-hour reference concentration ($P < 0.01$) with individual differences up to 50.8% and 52.6% at – one and two hours (Table 2). Nine subjects (20.9%) showed absolute differences above 25% (range 25.1 to 52.6) between 10- and 12-hour values (Fig. 1).
Differences in s-clozapine levels from the 12-hour reference point and forth were insignificant, although individuals showed differences up to 30.5% and 44.0% at + one and two hours, respectively (Table 2). Four subjects (8.9%) showed absolute differences above 25% (range 25.2 to 44.0) between 12- and 14-hour values (Fig. 1).
Median s-clozapine differences reached a maximum of 8.0% and 8.4% at ± one and two hours, respectively (Table 2).

Norclozapine

Differences across the 12-hour time-point

The median and range of 10-hour concentrations are presented in Table 1.
There was a general tendency towards an u-shaped time-course for s-norclozapine concentrations over time with a median increase of 1.2% and a mean absolute difference of 10.6% (±10.0) from 10-14 hours post-dose (Table 2). S-norclozapine time-courses are presented in Fig. S1.
Individual changes across the 12-hour time-point,(between 11- and 13-hour values and between 10- and 14-hour values) went up to 31.3% and 38.4% respectively (Table 2), although the mean differences in serum concentrations were insignificant (Table 2). Three subjects (6.8%) showed absolute differences above 25% (range 35.3 to 38.4%) from 10 to 14 hours post-dose.

Differences compared to the 12-hour values

Median and range of 12-hour concentrations are presented in Table 1.
Individual and median percentage differences in s-norclozapine, compared to 12-hour values, are presented in Table 2 and Fig. 1.

Within the four-hour time span, individual s-norclozapine levels differed with a maximum of 31.3% to 105.0% from the reference 12-hour concentration (Table 2). Four subjects (9.3%) showed absolute differences above 25% (range 27.3 to 105.0%) between 10- and 12-hour values, and six subjects (13.3%) exceeded 25% (range 25.4 to 37.5%) in difference between 12- and 14-hour values (Fig. 1). The differences in serum concentrations were significant ($P < 0.05$), except from the difference between 11- and 12-hours post-dose values (Table 2).

Median s-norclozapine differences at ± one and two hours reached a maximum of 4.2% and 7.3%, respectively (Table 2).

Analyses for associated variables

Pearson's correlation

A positive correlation was found between maximum percentage s-clozapine difference compared to the 12-hour-value and increasing age (r = 0.30, $P = 0.045$) (Table 3).

A negative correlation was found between maximum percentage 12-hour s-clozapine difference and increasing BMI (r = -0.30, $P = 0.046$) (Table 3).

No other significant correlations were found between the magnitude of clozapine differences compared to 12-hour values and the remaining continuous variables.

No significant correlations were found between maximum percentage s-clozapine change across the 12-hour time-point (11-13 or 10-14 hours post-dose) and any of the continuous variables. The results of all tested correlations with continuous data are presented in Table 3.

T-test

When age was submitted as a binary variable, maximum percentage clozapine change across the 12-hour time-point was significantly higher for subjects aged ≥ 45 years ($P = 0.047$) (Table 4).

Subjects with chronically elevated basophiles or monocytes also showed significantly higher clozapine differences, and this was for both differences compared to and across the 12-hour time-point (Table 4).
No significant differences were observed for the remaining binary variables. The results of all *t*-tests with binary data are presented in Table 4.

Discussion

The main findings of this study were that median and mean percentage differences in measured clozapine and norclozapine concentrations, within the four-hour time span, were minor. However, some individuals experienced substantial variations in clozapine and norclozapine levels. The maximum intra-individual variation was larger for norclozapine than for clozapine.
A greater part of the change in s-clozapine occurred before the 12-hour time-point, whereas s-norclozapine changed significantly both before and after the 12-hour time-point, although in opposite directions (Table 2).
Furthermore, we found that age, BMI and the presence of chronic monocytosis or basophilia seemed to be associated with the magnitude of clozapine variation.

The mechanism behind the variation in measured plasma levels is thought to be multi-factorial: in general, some intra-individual variability in measured concentrations over time should be expected, naturally due to the elimination of drug over time, but also due to analytical laboratory variation. The coefficient of variation (CV%) for our laboratory measurements was approximately 11%, in line with other studies (28), and would have accounted for some of the varying clozapine concentrations over time. MR is an index of metabolic capacity. A high metabolic activity (a high norclozapine:clozapine ratio) would imply a greater variation in s-clozapine over time. Subjects with high clozapine clearance are likely to require a higher clozapine dosage to produce an effective level of concentration, giving a low C/D. Therefore, C/D is also a measure of clozapine clearance and would be expected to inversely correlate with the magnitude of clozapine variation (5, 30). The fact that neither MR nor C/D seemed to correlate with clozapine variation implies a noteworthy

contribution from analytical variation. However, this could also be due to lack of statistical power.

The complex individual pharmacokinetics of clozapine is also expected to have contributed: e.g. although there was a clear tendency towards a decrease in clozapine levels over time, a rather large proportion of the subjects (23.9%) showed increasing serum levels during the four-hour period. Clozapine is rather rapidly absorbed, and a delayed absorption seems unlikely. However, we cannot rule out that the absorption rate have been erratic in some patients. Although hepatic metabolism is substantial, there is indirect evidence that pre-systemic metabolism in the gastrointestinal tract may account for a large contribution of the total clozapine clearance (31). Gastro-intestinal side effects are well known to clozapine treatment (32) and could be suspected to affect absorption. Also, the co-administration of food have, in one study, been demonstrated to affect absorption rate (33), although product summary informs otherwise (11). Neither adverse effects to clozapine treatment nor food consumption in relation to trial period were registered in the present study though.

Furthermore, a reverse transformation into the parent compound has been shown for the metabolite clozapine-N-oxide and some, less important, protein reactive metabolites, which partially could explain a rise in s-clozapine long after time-to-peak (15). Therefore, it seems plausible that deviations in sampling time (as a combination of drug excretion over time, analytical- and metabolic variation) could contribute to a great deal of the reported intra-individual differences in s-clozapine concentrations.

In a clinical perspective, our findings suggest that inconsistency in sampling time could give the impression of a change in habitual clozapine levels for some patients, although no such change has occurred. Moreover, one could speculate if measured clozapine differences of this magnitude could in fact disguise or even seriously amplify an actual change in s-clozapine, subsequent to alterations in the previously described metabolism-affecting parameters, making actual changes appear either insignificant or more extreme as e.g. smoking cessation has been reported to increase s-clozapine levels with a mean of around 72% (16), and cessation of caffeine-intake may result in an approximately 50% reduction of s-clozapine levels (11). This could potentially have severe clinical implications.

Furthermore, norclozapine showed a fluctuation-pattern as well, and even though its concentrations were lower than those of clozapine, the relative differences

corresponded to those of clozapine, suggesting that one should be cautious if interpreting the combined concentrations of clozapine and norclozapine (34).

The parameters age, gender, BMI, CRP ≥ 5 mg/l, caffeine consumption, smoking status and co-administrations such as oral contraceptives, have been demonstrated to affect clozapine levels (4, 5, 11, 16, 17, 20-22) and would therefore be expected to correlate with the magnitude of clozapine variation as a result of deviations in sampling time. However, in the present study, only two of these parameters (age and BMI) showed significance in the correlation analyses. Previous studies have shown significant differences in concentration to dose ratios, when subjects aged ≥ 45 years were compared to subjects aged < 45 years (35). When we divided our participants into corresponding age groups, we also found a difference in the magnitude of clozapine fluctuation. This was, however, in the opposite direction than anticipated, i.e. older subjects showed greater variance in their clozapine concentrations, despite reports of an age dependent decrease in liver enzyme activity (4, 35). This highlights the associative, rather than causal, character of the significant correlations. Still, this indicates that patients ≥ 45 years of age, are more sensitive not only to dose, but also to sampling time. Furthermore, our findings seem to support the idea of high body fat composition as a mean to an increased clozapine half-life (4) and hence a more stable clozapine excretion. The fact that we were unable to demonstrate correlations for the other continuous variables, or found correlations only for differences either related to or across the 12-hour time-point, could be due to lack of statistical power.

Due to the high availability of WBC differential counts to clinicians, as a result of the treatment guidelines for clozapine, and the puzzling inter-individual variation in occurrence and type of clozapine induced blood dyscrasias (36), we included chronic abnormal WBC counts as a secondary endpoint variable. This was in order to test if the type of a chronic blood dyscrasia could be associated with the magnitude of s-clozapine variation in time, despite the lack of direct causality. A possible association between these parameters could help identifying risk patients, without further tests and costs, and hence be an important clinical tool in patient-specific TDM optimising. We found that patients with chronic basophilia or monocytosis had significantly higher maximum percentage differences in s-clozapine, suggesting that patients with

these particular chronic blood dyscrasias are more sensitive to deviations in sampling time. However, only 4 patients showed blood dyscrasias of this nature and one patient showed both monocytosis and basophilia. Consequently, results (especially that of basophilia) should be interpreted with caution.

Limitations of this study include the small number of subjects, which could be suspected to have influenced statistical power, e.g. as for the only few significant correlations (potential type 2 errors). Another limitation regarding statistics is the fact that an increasing number of tested variables also increase the risk of mass significance (type 1 errors) and the need of a correction factor was considered. However, our secondary endpoint analyses served only as hypothesis generating tests, showing associations (not necessarily causations) and conclusions are merely suggestive. A correction factor at this point could have eliminated potential important associations from future studying. Further examination is needed to clarify the association between clozapine pharmacokinetics and the variables in question, in order to accurately identify subgroups that are more susceptible to deviations in sampling time.

Assessment of compliance relied on reports from the patients. However, all included patients were reached by telephone at the scheduled time of clozapine administration for confirmation of their clozapine consumption. Also, all patients were re-interviewed regarding in- and exclusion criteria (including time of last clozapine administration), at the study facility, prior to final inclusion and blood sampling.

Thus, strengths of this study are the high precision of sampling-time and the documentation of individual kinetics, addressing a relevant clinical issue. This is, to our knowledge, the only study of its kind.

In conclusion, the present study argues that the general impact of deviations in clozapine-TDM-sampling-time, within the period of 10 to 14 hours post-dose, is minor. However, substantial individual variations in serum clozapine and norclozapine were observed, implying risk-patients for whom the deviations may be of clinical importance.

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Declaration of interest

Author Fink-Jensen has received an unrestricted grant form Novo Nordisk for another clinical study. Author Larsen was an investigator of the study that received the grant from Novo Nordisk and is now working at Novo Nordisk A/S.
The other authors have nothing to declare.
The protocol can be obtained by contact to the corresponding author.
Author Jakobsen performed the statistical analysis in consultancy with the Statistical Advisory Service, Department of Public Health, University of Copenhagen.
The study was registered at ClinicalTrials.gov at November 13, 2015. ID: NCT02625103.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Figure S1. Mean and individual time-courses for s-clozapine and s-norclozapine, 10-14 hours post-dose (n=46).