Clozapine OpTimal Timing for Optimal moNitoring and Side effects (COTTONS)

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PROTOCOL TITLE ‘Clozapine OpTimal Timing for Optimal moNitoring’

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AUC	**Area Under the Curve**
Cl	**Clearance**
Cmax	**Maximum Concentration**
CV	**Coefficient of Variation**
EHR	**Electronic Health Record**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IC	**Informed Consent**
ICC	**Class Correlation Coefficient**
IIV	**Interindividual Variability (synonyms are interpatient of between-patient variability)**
IOV	**Interoccasion Variability (synonyms are intrapatient of within-patient variability)**
Ke	**The elimination rate constant, is the fraction of a drug that is eliminated from the body over a set unit of time**
MIPD	**Model-Informed Precision Dosing**
NONMEM	**Nonlinear Mixed-Effects Modelling**
PANSS	**Positive and Negative Syndrome Scale**
PD	**Pharmacodynamics**
PE	**Prediction Error**
PK	**Pharmacokinetics**
PopPK	**Population pharmacokinetics**
RMSE	**Root Mean Square Error**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
Steady state	**Equilibrium where drug intake equals elimination, maintaining constant average concentration (= approx. 4-5x the half-life).**
$T_{1/2}$	**Half-life, time it takes for the concentration to be halved**
TDM	**Therapeutic Drug Monitoring**
Therapeutic window	**Range of plasma drug concentrations spanning the minimum concentration for clinical efficacy and toxicity**
Tmax	**Time to Maximum concentration**
Trough	**Lowest concentration of a drug in the bloodstream, usually just before intake of the next dose**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
Vd	**Distribution of volume**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale:
Therapeutic drug monitoring (TDM) is essential for clozapine and can enhance therapeutic outcomes and minimize side effects. As of yet, research on (nor)clozapine concentrations and their association to metabolic side effects is limited and inconclusive. Unfortunately, not enough is known about individual risk factors for developing metabolic side effects to personalize clozapine treatment. It would be desirable to have another way to predict which clozapine users are at increased risk of developing severe side effects. Current guidelines are based on limited evidence, potentially resulting in inconsistent or suboptimal monitoring and management.

Objective:
The primary objective is to evaluate the correlation between (nor)clozapine kinetics and serum level HbA1c. Secondary objectives include validating an existing population pharmacokinetic model, assessing the validity of the current 12-hour post-intake sampling practice, determining the optimal sampling window for once-daily clozapine TDM and assessing the correlation of other metabolic and multiple laboratory parameters and influence of demographic and clinical parameters on the pharmacokinetics and -dynamics of clozapine and norclozapine.

Study design:
This is a non-interventional single-center cohort study involving prospective data collection for the primary (pharmacodynamic) analysis. This will be supplemented with retrospective population pharmacokinetic analyses. Using pharmacokinetic/pharmacodynamic modelling and statistical analysis will be used to evaluate the relation to side effects as well as optimize the sampling time for clozapine. Demographic, clinical and laboratory data are collected routinely based on current standard practice.

Study population:
The study includes patients (18-70 years) diagnosed with treatment-resistant schizophrenia or schizoaffective disorder who are on a registered, stable dose of clozapine. Participants must have a known smoking status and have their times of intake and sampling registered. To ensure comparability of (nor)clozapine concentrations, pregnant women will be excluded as well as plasma levels affected by acute inflammation/infection/intoxication and/or cessation or dose change of interacting co-medication or tobacco containing products seven days prior to sampling. Clinical parameters such as baseline severity of illness, duration of illness and duration of clozapine treatment will be assessed to describe the study population.

Main study parameter/endpoint:
The main study parameter is to evaluate the correlation between (nor)clozapine kinetics and serum level HbA1c. The correlation between (nor)clozapine concentrations and ratio and HbA1c will be assessed using a PK-PD turn over model.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness.
Not applicable for this retrospective non-interventional study.

INTRODUCTION AND RATIONALE

Background clozapine

Clozapine has proven to be superior to other antipsychotics in treatment of refractory schizophrenia. Therapeutic drug monitoring (TDM) is strongly recommended for clozapine, a relatively well-studied compound among antipsychotics. As clozapine levels show large inter- and intraindividual variation, TDM helps to increase efficacy and reduce prevalence of side effects and toxicity (1-4). A systematic review and meta-analysis has shown a higher risk of non-response in the treatment of refractory schizophrenia or schizoaffective disorder when plasma levels are below the threshold of 350 to 400 µg/l. Other publications have shown that increased plasma levels (> 750 µg/L) are associated with anticholinergic side effects and the occurrence of seizures (4). Clozapine is known to cause more serious metabolic side effects than other antipsychotics (5). Up to 80% of the patients are overweight and 45 to 58% have metabolic syndrome, while hyperglycemia and hypertension are often undertreated. Cardiometabolic disorders are therefore the most common cause of premature death in schizophrenia. Clozapine-induced agranulocytosis (CIA) is much rarer, occurring in 0.4% of clozapine users and the risk of death is 10% (6). Nevertheless, fear of CIA is often a reason not to choose clozapine. Norclozapine, clozapine's main metabolite, may contribute to some extent to the therapeutic effect and side effects of clozapine. Though, its precise role is yet inconclusive (7-10). Due to its unknown clinical relevance, many laboratories do not measure norclozapine. It is likely that norclozapine, due to its stronger effect as an inverse agonist on the serotonin 5-HT2C receptor, causes weight gain and metabolic abnormalities, which are associated with a lower quality of life and somatic comorbidities such as diabetes mellitus type II, cardiovascular diseases and higher mortality (7). This is further supported by other small studies where norclozapine caused insulin resistance in animals(11), an association between norclozapine levels and elevated triglycerides, elevated cholesterol, and weight gain (12) was found or with a higher waist circumference and higher HbA1c (8). Studies with either CYP1A2 induction through smoking (8) or inhibition through fluvoxamine (13) show norclozapine concentrations to be associated with waist circumference, HbA1c, and body mass index (BMI) (8) or a lower weight and metabolic parameters (insulin, glucose and triglycerides) (8, 13, 14). Finally, two studies contradict this association as a clozapine/norclozapine ratio > 2 appears to be most beneficial regarding cardiometabolic outcomes according to Polcwiartek et al. (15), whereas Lu et al. could not confirm this at a ratio of 3.7 (14). Regarding the level of granulocytes, only one study has shown a higher norclozapine concentration to be more beneficial than a higher clozapine concentration after long-term use of clozapine (16). As of yet, research on (nor)clozapine concentrations and their association to metabolic side effects is limited and inconclusive. For both patients and healthcare providers, blood tests and fear of side effects are the main reasons to postpone or even abandon clozapine treatment (17). Unfortunately, not enough is known about individual risk factors for developing metabolic side effects to truly personalize clozapine treatment. It would be desirable to have another way to predict which clozapine users are at increased risk of developing severe side effects. Because of this, current guidelines differ in their advice regarding metabolic screening.

Therapeutic drug monitoring of clozapine

Based on the information mentioned above, the recommended window for therapeutic drug monitoring for clozapine is 350-700 µg/L for both once- and twice-daily dosing. Some patients respond well on lower or higher levels; the latter is acceptable as long as higher levels are tolerable. The therapeutic window is based on trough concentrations (i.e., approximately 12 hours after the evening dose) from studies using a divided dosing regimen(18, 19). Similar to twice-daily dosing, current practice for once-daily dosing of clozapine advises a sample to be collected at least 12 hours after intake or within a window of 10-14 hours after intake. It is however unknown if the concentration-effect relations of once- and twice-daily dosing are similar. Additionally, clinical laboratories do not have a

uniform general practice regarding sampling times, resulting in variability in preferred sampling times and windows (1, 3, 4, 20).
As of yet, while often used in daily practice, there is no conclusive evidence as to the adequacy and suitability of this sampling time for once-daily dosing. Clozapine's pharmacokinetic properties further question this suitability. It has a biphasic elimination with a mean elimination half-life of 12-16 hours (3). The range is 6-26 hours, which after a single dosage of 75 mg was reported to be 7.9 hours that increased to 14.2 hours after reaching steady state (after one week) (21). Theoretically, a mean half-life of 12 hours and sampling time of 12 hours could lead to inadequate and inconsistent samples. This might be suitable for twice-daily dosing, where a 12-hour sample would be a trough, but this might not be the case for once-daily dosing. With once-daily dosing concentrations at 12 hours after ingestion may be adequate (within the therapeutic window) but subtherapeutic after 24 hours. Furthermore, smoking influences the metabolism such that a shorter half-life is reported (21).

Pharmacokinetics of clozapine in relation to dosing

One way to understand the pharmacokinetic variability of such compounds and provide individualised treatment strategies is by using population pharmacokinetics (popPK) models. These serve as useful tools within the framework of the model-informed precision dosing (MIPD). A previous study determined the comparability of once- and twice-daily dosing based on popPK analyses as well as the effect of once-daily dosing on clozapine and norclozapine concentrations (22). Their simulations show a 30% higher 12 hr-post dose concentration with once-daily (400 mg) dosing versus twice-daily (2x 200mg) dosing and a 30% lower trough (24 hr-post dose) when dosed once-daily compared to twice-daily. A higher once-daily dosage, 700 mg, shows 12 hr-post dose concentrations to lie above the upper limit and a trough (24 hr-post dose concentration) to be within the therapeutic window but almost half the 12 hr-post dose concentration (788 µg/L vs 425 µg/L). This suggests the current sampling time of 12 hr-post dose to be inadequate for once-daily dosing. This is further supported by Procyshyn et al. as well as Zwaag et al. who noted simulated 12 hr-post dose concentrations to be 23% higher after a single evening dose versus twice-daily dosing (23, 24). Neither of these two studies included norclozapine in their assessment. This in contrast to the study by Kitagawa et al. where no significant differences were found in estimated peak and trough samples of clozapine and norclozapine dosed once- versus twice-daily (400 mg/day) (25). The Dutch Clozapine Working group recommends increasing the lower limit of clozapine's therapeutic range by 23% for 12-hr post evening dose samples in patients with once-daily dosing based on the study by Zwaag et al (4, 23).

For a valid interpretation of the clozapine/norclozapine ratio, the clozapine dosage regimen, and blood sampling interval are important. Sources of large variability in pharmacokinetics of clozapine that affect CYP1A2 metabolism are smoking behaviour (polycyclic aromatic hydrocarbons in cigarette smoke induce CYP1A2), caffeine consumption (CYP1A2 substrate), and estrogen and progesterone levels (inhibit CYP1A2). Excessive smoking is correlated with reduced plasma levels of both CLO and NCLO. Coffee consumption is correlated with increased CLO and NCLO plasma levels and negatively correlated with the CLO/NCLO plasma level ratio. In premenopausal women CLO levels are higher compared to postmenopausal women (26). Pharmacokinetic drug-drug interactions are caused by the inhibition or induction of CYPs. Fluvoxamine increases the CLO/NCLO ratio through inhibition of the CYP1A2 enzyme. The demethylation of CLO to NCLO is reduced, resulting in an increase of the CLO plasma level and decrease of the NCLO plasma level (26). Pro-inflammatory cytokines interleukin (IL)-6 and IL-1 inhibit CYP1A2, which can be detected indirectly using the biomarker C-Reactive Protein (CRP). Furthermore, CLO is highly bound to the acute phase protein alpha-1-acid glycoprotein (AGP) and less to serum albumin (95% of CLO binds to AGP). During inflammation, concentrations of AGP increase, resulting in a decreased CLO unbound fraction. Pharmacogenetics of the also affects pharmacokinetics of clozapine. For example, asian ancestry is associated with lower CYP1A2 activity compared to other ethnic groups. Additionaly, genetics may also account for variability in response tot

clozapine. It is reported that transcriptional activity of the M1 receptor gene is correlated with anticholinergic burden (26).
Finally, CLO clearance is affected by age, sex, ethnicity, and smoking status. Higher age results in a reduction in CLO clearance for both male and female smokers and non-smokers of all ethnicities. Females have decreased CLO clearance compared to males. Patients of Afro-Caribbean ethnicity have increased CLO clearance, whereas patients of Asian ethnicity have decreased CLO clearance compared to their White counterparts. Additionally, smokers have increased CLO clearance compared to non-smokers (26).
In addition to the CLO/NCLO ratio additional biomarkers to predict the CYP1A2 activity could be useful in clinical practice. Pu et al have reported that in rats the ratio of the concentrations of the amino acids phenylalanine and tyrosine in serum is highly correlated with the expression of the CYP1A2 gene and the activity of the CYP1A2 enzyme. This biomarker could be useful in prediction of the clearance of clozapine by this enzyme (27).

Pharmacokinetic modelling

Population PK models may be used to determine the sampling time or window that yields the most precise estimation of pharmacokinetic parameters through simulations and optimization techniques. Since several models have been developed for clozapine, a careful selection based on suitability is applied. In this case, a valid popPK model should be developed including Dutch patients, once-daily dosing regimen, trough samples of both clozapine and norclozapine (up until 24 hours after ingestion) and include sufficient information during the elimination phase. Geers et al. describes a popPK model for clozapine based on Dutch patients. However, this model only included samples of patients who are dosed twice-daily, fewer samples were taken between 8-24 hours after intake (and thus during the elimination phase) and their model was based on two-time point sampling strategies for AUC (28). Perera et al. studied optimal sampling points through modeling, but did so for twice-daily dosing regimen based on AUC based monitoring as well and concluded three-time point strategies. They also concluded that trough concentrations show an excellent correlation to exposure (29). Geers et al. concluded the same for twice-daily dosing regimen (AUC0-12) (28). Shang et al. used population PK/PD modeling to study the relationship between exposure of clozapine (AUC) and the Positive and Negative Syndrome Scale (PANSS) (30). They found the AUC to be a better predictor as PANSS score showed little relationship with trough concentrations of clozapine. However they mention that this is probably due to the slow onset of antischizophrenic effect after reaching steady state. Furthermore they only included Chinese patients with a twice-daily dosing regimen. In addition none of these models included norclozapine. The recent model by Beex et al. is based on Dutch patients, trough samples of both clozapine and norclozapine as well as once- and twice-daily dosing regimen (22). They mention model misspecification in the absorption phase. This might be due to the occurrence of a first-pass effect and could have been described if more samples had been taken during the absorption phase. However, this should not be relevant to this study which focuses on sampling times during the elimination phase. Therefore this model is most suitable to be used to study and find the optimal sampling time or window. Its suitability will be further examined by performance of an external validation.

Pharmacokinetics/pharmacodynamics and metabolic side effects

Metabolic side effects of clozapine comprise weight gain, dyslipidemia and hyperglycemia, which can result in a metabolic syndrome, associated with an increased risk for type 2 diabetes mellitus and cardiovascular disease (5). NCZ is hypothesised due to its stronger action as an inverse agonist on the serotonin 5-HT2C receptor to cause weight gain and metabolic changes, which are associated with a lower quality of life, somatic comorbidity, and a higher mortality (7).
In a small low-quality meta-analysis (n=120) a positive correlation was found between clozapine concentrations and elevated triglycerides, heart rate, and overall combined adverse drug reaction (12). Furthermore, a positive correlation was found between norclozapine plasma levels and triglycerides, cholesterol, and weight gain. In short, at

present there is too little evidence to identify the role of the clozapine/norclozapine plasma level ratio in the minimisation of clozapine side effects. In addition, the correlation of CLO(&CNLO) concentration with metabolic markers might be affected by cortisol and genetics of the glucocorticoid receptor (31). Finally, other genetic markers known to modulate the basic risk of metabolic syndrome might play a role in this correlation between the correlation of the CLO(&CNLO) concentration and the increase in metabolic markers (32).

Personalised clozapine treatment

Since clozapine is mostly given to patients with treatment-resistant schizophrenia, a clear defined sample time is required in order to interpret their plasma concentrations and optimize their treatment through TDM. Inadequate interpretation may lead to suboptimal treatment efficacy or increased risk of adverse effects. Furthermore the relation of adverse effects, especially metabolic and granulocyte, to (nor)clozapine concentration has yet to be researched well in order to improve screening and optimize treatment. Based on earlier studies it is suggested that current sample times may fit a twice-daily dosing schedule and not a once-daily dosing schedule. Thus in this study the difference between the (nor)clozapine concentrations at 12-hours post intake and the concentration at other time points within the 10-14 hours sampling window will be evaluated for once-daily dosed clozapine. To this end, the model by Beex et al. will be validated with independent data. Through predicted concentrations, we aim to contribute novel insights into the optimal timing of sample collection as well as insight into the development of metabolic and granulocyte side effects, addressing the existing knowledge gap and enhancing the precision of therapeutic drug monitoring in this population.

This research will not only provide more knowledge about risk factors for developing general side effects of clozapine, metabolic side effects, constipation, and chronic neutropenia, but will also improve metabolic screening in clozapine users through better implementation of metabolic screening.

OBJECTIVES

Primary Objective:

- To evaluate the correlation of (nor)clozapine kinetics and serum level HbA1c

Secondary Objectives:

- To validate an existing population model with independent data.
- To study the validity of current practice (12-hr post intake) sampling times within a window of 4 hours (10-14 hours post intake) for TDM of once daily dosed clozapine
- To identify the optimal sampling time or window for TDM of clozapine based on predicted concentration-time profiles up until 24-hours post intake.

- To investigate the influence of the following covariates on the pharmacokinetics of clozapine, the metabolite norclozapine (and CZ/NCZ ratio):
    - age
    - estradiol (indirectly through demographic data [premenopausal or postmenopausal female / male),
    - comedication with fluvoxamine (dosage) and other medication
    - ethnicity (Asian ancestry is associated with lower CYP1A2 activity compared to other ethnic groups).
    - pharmacogenetics of the metabolic enzymes *CYP2C19* and *CYP3A4*
    - smoking behaviour (yes/no and amount)
    - inflammation markers (C-reactive protein (CRP) and Erythrocyte Sedimentation (BSE))
    - phenylalanine and tyrosine (a higher ratio of phenylanlanine/tyrosine is associated with a higher CYP1A2 activity) (27).

- To evaluate the correlation of (nor)clozapine concentrations and/or ratio on:
    - Other metabolic parameters such as: fasting glucose, insulin, HOMA-IR, triglycerides, HDL, LDL, total cholesterol, BMI, abdominal circumference, blood pressure and heart rate.
    - Absolute neutrophil count (ANC) and total white cell count (TWC).
    - General side effects of clozapine using the Glasgow Antipsychotic Side-effect Scale for Clozapine (GASS-C) (33).
    - Defecation frequency per week and consistency using the Bristol Stool Form Scale (BSFS).
    - Positive and Negative Syndrome Scale (PANSS-6), regarding severity of schizophrenia

- To evaluate the effect of cortisol concentration and genetics of known relevant loci related on the correlation of (nor)clozapine concentrations and/or ratio on the metabolic markers (31,32)

STUDY DESIGN

This is a (non-interventional) single-center cohort study. (Out)patient data will be collected from the mental healthcare organization GGZ-NHN and the laboratory of NorthWest clinics. For all endpoints a baseline value is required. The inclusion period will start 01-12-2024 and will last until 01-12-2025. For each patient two years of follow-up will be collected (until a maximum of 01-12-2027). For the secondary endpoint pertaining to the sample times retrospective data will be collected from 01-02-2024 up to 01-02-2025. Remnant material will be collected in the period during the follow-up period after obtaining informed consent

STUDY POPULATION

Population (base)

The population includes adult patients (age between 18 and 70) with treatment resistant schizophrenia of schizoaffective disorder, treated with oral, once or twice-daily clozapine. Data will be collected from mental healthcare organization GGZ-NHN. All the patients have been treated with clozapine as part of standard care, as well as sampled routinely as part of standard care.

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria and provide informed consent. These criteria are partially based on covariates used the model by Beex et al., as it will be used in this study (22):

- Standard treatment of a stable, oral dose of clozapine for at least one week (at steady state).
- Age between 18-70 years.
- Registered time of intake as well as sampling time and dosage.
- Registered smoking status (yes/no).
- At least two samples in the elimination phase of clozapine with both clozapine and norclozapine measured.
- Measurement of the white cell count at least every three months or more often.
- Routine metabolic screening performed at moment of inclusion
- Subjects should be able to understand the study information and procedures and give informed consent or when incapacitated subjects are not reliably able to give informed consent their legal representatives should give informed consent under the condition that these subjects are willing to participate.

Exclusion criteria

A potential subject or specific samples that meet any of the following criteria will be excluded from this study:

- Pregnancy.
- Malignancy or treatment with immunosuppressive medication.
- Samples where cessation, start or dose change of interacting co-medication (such as valproic acid, gemfibrozil, fluvoxamine, omeprazole and cyclic oral contraceptives [21 on, 7 days off]) or changes in use of tobacco containing products occurred within seven days prior to blood sampling (21, 28, 34, 35).
- Acute inflammation, infection or samples shortly after intoxication. In case this information is unknown, it may be derived by large unexpected change in levels compared to previous or target levels.
- Not sampled at Starlet (blood collection site) or sampled by dried blood spot
- Unknown status of smoking (including vaping).
- Unknown time of intake of clozapine.
- Unknown time of blood sampling.
- If informed consent is not obtained by the patient or by a legal representative in a mentally incapacitated patient, i.e. a legally incompetent adult.

Sample size calculation

No calculation was performed since the effect of clozapine on the increase of metabolic markers is unknown. A rule of thumb with pharmacokinetic studies is an amount of 30 patients, however with the combination of pharmacokinetics and pharmacodynamics the variability in the endpoint will increase. Based on a previous PK/PD study which studied the relationship of the drug adalimumab with the PASI-score, a population of approximately 60 patients was sufficient for establishing a correlation with the pharmacokinetics of adalimumab

and the clinical response measured by the PASI (36). For the secondary endpoint investigating the effect a minimum of 30 patients with 90-120 samples is likely sufficient.

TREATMENT OF SUBJECTS

This chapter is not applicable as this is a non-interventional study.

INVESTIGATIONAL PRODUCT

This chapter is not applicable as this is a non-interventional, retrospective study that involves standard care.

NON-INVESTIGATIONAL PRODUCT

This chapter is not applicable as this is a non-interventional, retrospective study that involves standard care.

METHODS

Study parameters/endpoints

Main study parameter/endpoint

Correlation between serum level HbA1c and (nor)clozapine concentrations and ratio will be assessed using a PK-PD turn over model.

Secondary study parameters/endpoints

- The predictive performance of the model by Beex et al. (22) for our population will be assessed through calculations of the prediction error for each observation as well as the root mean square error to measure the overall prediction accuracy.

In order to study the main study point, the model by Beex et al. will have to be validated for our population first. However, since this is not the primary objective, it is noted as a secondary objective that will be performed before researching the primary endpoint. The cutoff value for the mean and median values of prediction errors is also based on clinical practice as well as the study by Veerman et al. (37) and thus set at 20%.

- Percentage difference in clozapine and norclozapine median concentrations predicted 10, 11, 13 and 14-hour post intake (based on clinical samples) compared to median concentrations at current reference sampling time of 12-hour post intake.

Based on the study of Veerman et al. an increase of 100 µg/L is regarded as clinically relevant (37). However, measurement errors may also impact the differences between concentrations and are expressed in a percentage. Therefore, clinically relevant differences will be expressed as a percentage as well. The increase of 100 µg/L is set off against the therapeutic window of 350-700 µg/L up to the alert level of 1000 µg/L. As the midpoint of this range would be 525 µg/L, 100 µg/L would correspond with a difference of 20%. This difference is defined as clinically relevant for this study, according to which concentrations would no longer be deemed similar and thus corresponding to the same time window or an adequate sampling time or window.

- Percentage difference in clozapine and norclozapine concentrations based on different sampling times across entire concentration-time profiles to identify optimal sampling time or window within current therapeutic window of 350-700 µg/L.

To identify the optimal sampling time or window, expected concentration-time profiles will be constructed on basis of individual PK parameters obtained by Bayesian analysis. In these profiles sampling times during which the concentration will be within the therapeutic window of 350-700 µg/L will be identified. These graphical ‘reference ranges’ could point to an optimal sampling time or window. In order for a sampling time window to be found, concentrations within that window would have to be deemed similar. As a percentage difference of 20% between concentrations will be regarded as clinically relevant, concentrations within the same window are allowed a difference up to 20%. As such, a new sampling time or window might be found instead of the current window of 10 to 14 hours post intake.

- Effect of covariates on the pharmacokinetics of clozapine, the metabolite norclozapine (and CZ/NCZ ratio). Covariates to be studied include: age, estradiol, comedication (e.g. fluvoxamine), ethnicity, pharmacogenetics, smoking behaviour, inflammation markers, phenylalanine and tyrosine.

- Correlation between other metabolic parameters (such as fasting glucose, insulin, HOMA-IR, triglycerides, HDL, LDL, total cholesterol, BMI, abdominal circumference, blood pressure and heart rate), ANC and TWC, GASS-C, BSFS and PANSS-6

regarding severity of schizophrenia and the (nor)clozapine concentrations and ratio will be assessed using a PK-PD turn over model.

- The effect of cortisol concentration and genetics on the correlation of (nor)clozapine concentrations and/or ratio on the metabolic markers

Other study parameters

Demographics

- Sex (male/female).
- Age (year).
- Estradiol indirectly (premenopausal/postmenopausal female / male).
- Ethnicity (Caucasian/African-Caribbean/Asian/other).

Clinical parameters

- Indication for clozapine use (diagnosis treatment resistant schizophrenia or schizoaffective disorder).
- Duration of illness.
- Duration of clozapine treatment.
- Dosage scheme.
- Baseline PANSS-6, regarding severity of schizophrenia

Other parameters

- Tobacco smoking behaviour (yes/no, including amount (prospectively)) and vaping (yes/no).
- Interacting co-medication such as strong CYP3A4-inductors (gemfibrozil, apalutamide, carbamazepine, efavirenz, enzalutamide, phenobarbital, phenytoin, St. John's wort, lumacaftor, mitotane, nevirapine, primidone, rifabutin, rifampicin) or level increasing drugs such as fluvoxamine, fluoxetine or sertraline, CYP1A2 inhibitors such as ciprofloxacin, or inductors such as omeprazole, oral contraceptives and valproic acid).
- Gemfibrozil and valproic acid use, as both inhibit renal clearance of norclozapine (38).
- Patient specific target level if this falls outside of the therapeutic window of 350-700 µg/L, in order to be able to identify these levels as expected normal target levels instead of toxic or caused by (in)adherence, interactions or infection.
- Reason for blood collection if available (routine or otherwise: possible toxicity, infection, CRP and BSE if recently measured and known, change in dosage or co-medication).

Randomisation, blinding and treatment allocation

Not applicable

Study procedures

Not applicable, patients will not undergo any (additional) procedures for research purposes. All samples will be taken as part of standard care for treatment purposes.

Withdrawal of individual subjects

Not applicable

Specific criteria for withdrawal (if applicable)

Not applicable

Replacement of individual subjects after withdrawal

Not applicable

Follow-up of subjects withdrawn from treatment

Not applicable

Premature termination of the study

Not applicable

SAFETY REPORTING

This chapter is not applicable as this is a non-interventional, retrospective study that involves standard care.

STATISTICAL ANALYSIS

Primary study parameter(s)

PK analyses will be performed using nonlinear-mixed-effects modelling (NONMEM) software (version 7.4.2, ICON Development Solution, Gaithersburg, MD, USA). Software like R (www.rproject.org), Pirana and Xpose (version 4.3.2, Niclas Jonsson and Mats Karlsson, Uppsala, Sweden) will be used for graphical display and evaluation of the models (including goodness of fit plots).

Previously Beex et al., while studying the comparability of pharmacokinetics of clozapine and norclozapine with once- and twice-daily dosing, developed a population PK model using NONMEM (13). Furthermore, their popPK model has incorporated patient-specific covariate smoking to further refine pharmacokinetic parameter estimates and improve the accuracy of dosing. Their base model describes clozapine by a two-compartment model with a transit compartment describing the delay in absorption and linear elimination consisting of combined excretion and formation of the metabolite norclozapine. This model was extended with a one-compartment model for norclozapine with first-order elimination (Figure 2, excerpt from their study).

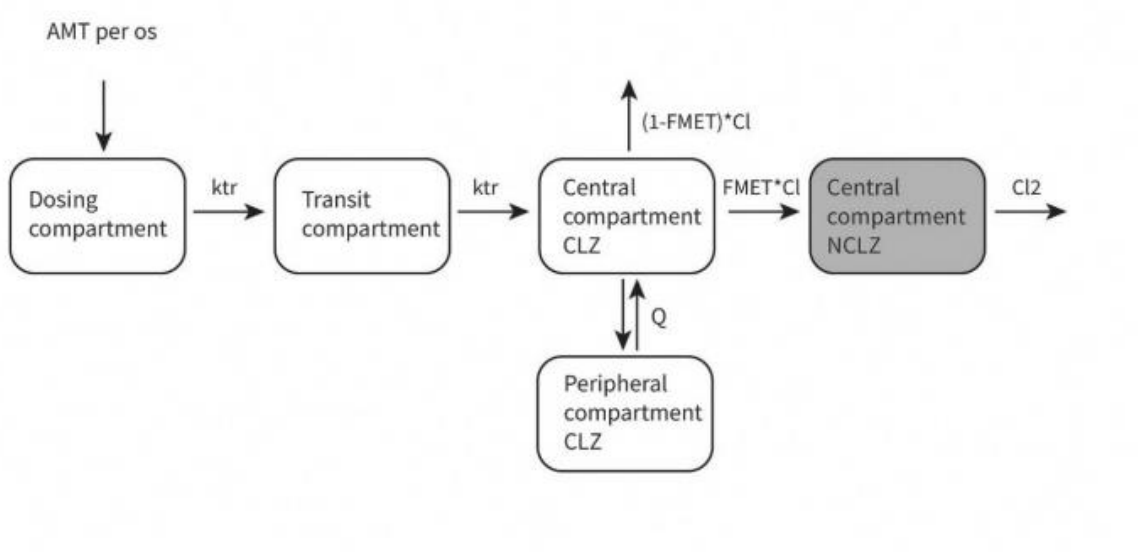

Figure 2. Schematic representation of the structural model.

AMT = amount, Cl = clearance of clozapine, Cl2 = clearance of norclozapine, CLZ = clozapine, FMET = fraction metabolised, ktr = transit compartment rate constant, NCLZ = norclozapine, Q = intercompartimental clearance

Their final model tested for several covariates: total body weight, BMI, age, sex, AGP concentrations, albumin concentrations, CRP, and smoking status. Only smoking had a significant effect on clozapine clearance (not on norclozapine clearance). Based on this model, clearance of clozapine is 55% higher in smokers than in non-smokers. Table 2 is an excerpt of the overview of parameter estimates of both base and final model.

Table 2. Overview of parameter estimates of base model, final model and SIR. SIR was run on 2000 samples and 1000 resamples. CI = confidence interval, CLZ = clozapine, Cl/F = apparent clearance of clozapine, Cl/F'= apparent clearance of norclozapine, FMET = fraction of clozapine metabolised into norclozapine, Ktr = transit rate constant, Q = intercompartimental clearance, OFV = objection function value, NCLZ = norclozapine, RSE = relative standard error, SIR = sampling importance resampling, Vcentral / F= apparent central volume of distribution of clozapine, Vperipheral / F= apparent peripheral volume of distribution of clozapine, V/ F' = apparent volume of distribution of norclozapine. FIX = fixed value

Parameter	Base model (RSE %)	Final model (RSE %)	SIR (95% CI)
Cl/F CLZ (L/h)	30.8 (7.4%)	22.2 (14.8%)	22.2 (18.0 – 28.0)
Smoking on Cl/F	-	1.55 (16.4%)	1.55 (1.20 – 1.96)
$V_{central}$/F CLZ (L)	328 (11.4%)	328 (11.3%)	328 (257 – 422)
$V_{peripheral}$/F CLZ (L)	285 (17.1%)	286 (17.0%)	286 (199 – 394)
Q (L/h)	86.0 (22.3%)	86.0 (21.9%)	86.0 (60.1 – 130.1)
Ktr (h^{-1})	1.23 (10.0%)	1.23 (10.0%)	1.23 (0.99 – 1.52)
Cl/F' NCLZ (L/h)	36.1 (8.0%)	36.1 (8.0%)	36.1 (31.3 – 41.7)
V/F' NCLZ (L)	746 (72.0%)	751 (72.2%)	751 (418 – 1796)
FMET	0.66 FIX	0.66 FIX	0.66 FIX
Interindividual variability (%)			
IIV Cl/F CLZ	49.7 (8.6%)	45.1 (10.0%)	43.0 (36.7 – 53.4)
IIV $V_{central}$ /F	63.7 (10.9%)	63.5 (12.4%)	58.2 (44.4 – 72.9)
IIV $V_{peripheral}$ / F CLZ	72.8 (28.9%)	72.7 (29.0%)	62.8 (46.8 – 84.6)
IIV Ktr	69.6 (16.6%)	69.6 (16.5%)	50.0 (42.1 – 59.4)
IIV Cl/F' NCLZ	53.3 (8.6%)	53.3 (8.9%)	65.1 (29.7 – 104.3)
Proportional error CLZ (%)	13.2 (9.1%)	13.2 (9.1%)	13.2 (12.0 - 14.7)
Proportional error NCLZ (%)	18.3 (8.3%)	18.3 (8.2%)	18.2 (16.7 – 20.0)
OFV (-2 LL)	7610.26	7602.50 (-7.76)	

Subsequently, their developed final population PK model will be used to predict concentration-time profiles for clozapine and norclozapine under various dosages and sampling schedules on basis of individual PK parameters obtained by Bayesian analysis. Collected data will be inserted into R as well as relevant patient-specific demographic, clinical and laboratory data. A new database suitable for PK analyses of clozapine will be constructed in NONMEM. In order to do this the clozapine dose, measured concentration data along with time of intake and sampling time will be taken into account. Subsequently, PK profiles will be predicted. The reference window to be targeted will be 350-700 µg/L for clozapine. Deviation from this window will be allowed for patients who, according to their psychiatrist or nurse specialist or other practitioner, have a higher or lower target level than the defined therapeutic window with good therapeutic effect. This will only be allowed based on the psychiatrist/nurse specialist/practitioner's information and help identify these levels as expected normal target levels instead of toxic or caused by inadherence, interactions or infection.

The first step before researching the primary endpoint, is the performance of an external validation (see secondary study parameter(s) below). Afterwards, the current study population will be studied in order to research the primary endpoint. To analyse this, the concentration-time data will be used to develop PK/PD turn-over models to estimate the parameters (Kin, Kout, Emax and EC50) linking the pharmacokinetics of clozapine and norclozapine to HbA1c serum levels. Once the structural PK/PD model is developed, the between subject variability and residual unexplained variability of the estimated pharmacokinetic parameters will be assessed.

A univariate covariate analysis will be performed to check for associations between the patient-related factors on parameters of the PD model using a χ2 test-statistic. Covariates with a significant association in the univariate analysis will be included in the multivariate analysis. Covariates that remain significant in the multivariate analysis will be included in the final pharmacodynamic model. This final model will be validated using visual predictive check (VPC's) and using a bootstrap procedure the robustness of the model will be tested. The latter two procedures will be performed using Piraña and Pearl speaks NONMEM (PsN) software.

Secondary study parameter(s)

For the external validation of the model, predicted profiles will be compared to the observed data of our population (which is regarded as an external data set). Prediction error (PE) for each predicted concentration (PE_i) was defined through the following equation:

$$\mathrm{PE}_i = \frac{(\mathrm{Observed}_i - \mathrm{Predicted}_i)}{\mathrm{Observed}_i} \times 100$$

where $\mathrm{Observed}_i$ and $\mathrm{Predicted}_i$ represent the *i*th observed and predicted concentrations, respectively. The root mean square error (RMSE) prediction error was also calculated using the following equation:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\mathrm{Observed}_i - \mathrm{Predicted}_i)^2}$$

where *n* represents the total number of concentrations. A population PK model was regarded as valid for our clinical settings when both the mean and median values of PE were less than 20%. As stated earlier, the external validation of the model by Beex et al. will be performed before researching our primary goal.

Based on the predicted concentration-time profile for all participants, the percentage difference in predicted clozapine and norclozapine concentrations between different sampling times will be calculated. The 12-h time point will be set as reference and compared to concentrations predicted (or measured if available) at 10, 11, 13 and 14 hours-post intake concentrations. Plasma concentrations taken at steady state should be comparable to others within an acceptable time window. As stated earlier, concentrations are regarded as comparable if the total percentage difference does not exceed 20%. This includes expected bias and imprecision in measurement and interoccassion and interindividual variability. This difference is defined as clinically relevant for this study, according to which concentrations would no longer be deemed similar and thus corresponding to the same time window or an adequate sampling time or window.
Statistical significance will be tested through generalized linear mixed models for proportions, in which the proportion of clinically relevant differences per time point versus t=12 will be compared. For descriptive statistics, continuous outcomes are presented as medians and ranges between minimum and maximum values with corresponding confidence intervals, when applicable. Categorical outcomes are reported as numbers and proportions (*n* (%)). Two tailed p-values of < 0.05 based on χ2 with one degree of freedom were considered statistically significant.

Based on the clinical patient data, predicted concentration-time profiles, averaged for each subject, will be portrayed for different oral dosages. The (new) optimal sampling window will be based on the time window during which the difference in measured clozapine and

norclozapine concentrations in the predicted profile is smaller than 20% as well as within the defined therapeutic window of 350-700 µg/L for clozapine. If needed, deviation from this window will be allowed for patients with different target levels as described before. This will be only be allowed based on the psychiatrist/nurse specialist/practitioner's information. If a window cannot be found, then sampling times will be noted during which clozapine concentrations fall within the defined therapeutic window. The student's t-test for paired data was used to analyse differences in median serum concentrations across sampling times.

Aside from the metabolic parameters mentioned in the primary endpoint, correlation with other metabolic parameters (such as fasting glucose, insulin, HOMA-IR, triglycerides, HDL, LDL, total cholesterol, BMI, abdominal circumference, blood pressure and heart rate), ANC, TWC, GASS-C and BSFS with the concentrations will be studied. The concentration-time data will be used to develop PK/PD turn-over models to estimate the parameters (Kin, Kout, Emax and EC50) linking the pharmacokinetics of clozapine and norclozapine to these parameters. Once the structural PK/PD model is developed, the between subject variability and residual unexplained variability of the estimated pharmacokinetic parameters will be assessed. A univariate covariate analysis will be performed to check for associations between the patient-related factors on parameters of the PD model using a $\chi 2$ test-statistic. Covariates with a significant association in the univariate analysis will be included in the multivariate analysis. Covariates that remain significant in the multivariate analysis will be included in the final pharmacodynamic model. This final model will be validated using visual predictive check (VPC's) and using a bootstrap procedure the robustness of the model will be tested. The latter two procedures will be performed using Piraña and Pearl speaks NONMEM (PsN) software.

Using an univariate analysis in NONMEM the association of patient-related factors on the pharmacokinetic parameters of clozapine will be tested using a $\chi 2$ test-statistic. The covariates that will be included in the univariate analysis include age, gender, premenopauzal/postmenopausal female/male, ethnicity, weight, height, CRP, BSE, smoking behaviour, comedication with fluvoxamine (dosage) and genotypes. Covariates with a significant association in the univariate analysis will be included in the multivariate analysis. Covariates that remain significant in the multivariate analysis will be included in the final pharmacokinetic model. This final model will be validated using visual predictive check (VPC's) and using a bootstrap procedure the robustness of the model will be tested. The latter two procedures will be performed using Piraña and Pearl speaks NONMEM (PsN) software. The final pharmacokinetic model will then be used to create concentration-time curves for each individual participating in the PK/PD study.

Other study parameters

Baseline characteristics will be depicted by means of summary statistics: mean ± SD for normally distributed continuous variables and median ± interquartile range for non-normally distributed continuous variables. Proportions will be calculated for categorical variables.

Interim analysis (if applicable)

Not applicable.

ETHICAL CONSIDERATIONS

This is an observational cohort study in which the data will be collected retrospectively. The subjects included in this study will not undergo any additional procedures. After gaining permission by the Advisory Committee on Local Feasibility of the GGZ-NHN, all subjects will be asked informed consent before participation. Informed consent will be asked in accordance with the General Data Protection Regulation (GDPR). Their Electronic Health Record (EHR) will be viewed by the treating psychiatrist, physician, nurse specialist, or other doctor/practitioner. Data collected by the practitioner will be used for the dataset. Additionally, information such as plasma levels is collected through Labosys rapports for which informed consent is given according to standard procedure for blood collection. Remnant material from laboratory procedures performed as part of routine clinical care will be collected and stored.

Regulation statement

This research will be conducted in accordance with Good clinical practice (GCP) and GDPR.

Recruitment and consent

This study will be submitted to the local review committee of the GGZ-NHN. Letter and informed consent are attached as separate documents.

Objection by minors or incapacitated subjects (if applicable)

Not applicable.

Benefits and risks assessment, group relatedness

Not applicable.

Compensation for injury

Not applicable.

Incentives (if applicable)

Not applicable.

ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

Handling and storage of data and documents

Data regarding sex, age, plasma levels of both clozapine and norclozapine, time of intake and sampling time will be collected through reports from Labosys (Alkmaar). This data is sourced from the blood collection site, where requesting psychiatrists, physicians, nurse specialists, or other doctors/practitioners are required to fill in this information. All specified laboratory parameters (see above) from both GGZ-NHN and other sources of requests for laboratory diagnostic procedures (i.e. general practitioner, specialist internal medicine) within the period of follow-up will be used for analysis. Time of intake will be collected from the patients before the sampling time at the collection site (Starlet) according to standard practice. Due to new ISO regulations, there are upcoming changes in standard practice for blood collection at the main site. These changes will require requesting doctors to fill in more information at every blood collection request such as: smoking (yes/no), dosage, reason for blood collection (routine or otherwise: possible toxicity, infection, change in dosage). Once these changes have followed through, this data can also be collected through Labosys. Co-medication will be retrieved through the patient's public pharmacy.

Before implementation of these changes, information on smoking, dosage and reason for blood collection will be collected through the treating psychiatrist/nurse specialist/practitioner who has access to the patient's electronic health record if needed. Other information to be gathered by the treating psychiatrist/nurse specialist/practitioner includes: weight and BMI , ethnicity, diagnosis (of schizophrenia or other psychiatric disorder), pregnancy, interacting co-medication (including newly started, stopped medication and changes in dosages within a week of sampling time). Data regarding metabolic parameters such as fasting glucose, serum level HbA1c, triglycerides, HDL, LDL, total cholesterol, abdominal circumference, BMI and blood pressure and heart rate will be collected through reports through Nexus and mConsole (Alkmaar), as well as the absolute neutrophil count (ANC) and the outcomes of the Bristol Stool Form Scale (BSS) assessment, the Glasgow Antipsychotic Side-effect Scale for Clozapine (GASS-C) questionnaire and the Positive and Negative Syndrome Scale (PANSS-6) questionnaire will be collected through Nexus and mConsole (Alkmaar). Clinical data needed from the dedicated includers (co-investigators, with an affiliation to the GGZ-NHN) will be collected through a simple case report form. This information will be collected at the moment of inclusion and at the end of the study (retrospectively) in case any changes occur during the study period. Biologic material collected as part of laboratory investigation as part of routine clinical care after obtaining informed consent will be stored at the laboratories of the North West Clinics until all laboratory procedures pertaining the investigation are completed (metabolic enzymes, the genes *CYP2C19* and *CYP3A4* and genes related to metabolic markers).

Missing data (unless described in the inclusion or exclusion criteria) will be allowed. Native speakers, if available, will be asked to deduct a patient's ethnicity based on their name in case the ethnicity is unknown before regarding this as missing data. If data, such as BMI or CRP, are not reported in the patient's medical file the event will be recorded as 'missing data' or as not occurred.

During the study period, data collected from the psychiatrist/nurse specialist/practitioner/dedicated includer will be linked to data collected from Labosys according to date of birth and name. Patient data will be encoded. The key will be safeguarded by the coordinating investigator and principal investigators. Furthermore collected data will be stored for 15 years.

The results, discussion and conclusion of the study will be reported and shared in an abstract, manuscript and registration presentation in accordance to the principles of the

ICMJE guidelines. Information concerning this study's processes and data is confidential and its rights belong to GGZ-NHN, NWZ Alkmaar and Amsterdam UMC.

Monitoring and Quality Assurance

Not applicable

Amendments

Not applicable

Annual progress report

Not applicable

Temporary halt and (prematurely) end of study report

Not applicable

Public disclosure and publication policy

Not applicable

STRUCTURED RISK ANALYSIS

Not applicable

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