Supporting successful clozapine treatment

Marieke Beex-Oosterhuis

SUPPORTING SUCCESSFUL CLOZAPINE TREATMENT

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Colofon

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The staircase on the cover is from a photograph taken by Bettina Samrén, who, as a doctor, also works at Albert Schweitzer hospital. The photo in question was taken in Kilmainham Gaol, a former prison opened in 1796 in the suburb of Kilmainham in Dublin, Ireland.

VRIJE UNIVERSITEIT

SUPPORTING SUCCESSFUL CLOZAPINE TREATMENT

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Voor Floor en Frederique

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General introduction, aims and outline of the thesis

Background

"Could there be under-treatment of patients who use the antipsychotic drug clozapine once-daily (QD) (before bedtime) and have clozapine concentrations around the upper limit of its reference range the next morning? Is it warranted to be reluctant to give these patients a higher dose, if needed, because of fear for concentration related side effects, based on concentrations halfway the concentration-time curve?" These questions came to me during a meeting of the Medication Committee of Yulius Mental Health Organisation, in which I participated on behalf of Albert Schweitzer Hospital as the responsible and advisory clinical pharmacist for Yulius' clinical departments. We, the members of the committee, consisting of psychiatrists and a clinical pharmacist at that time, were discussing the interpretation of the so-called upper threshold of clozapine's therapeutic window. We wondered how often clozapine concentrations above the upper threshold of 700 µg/L were seen and how these concentrations related to once or twice daily dosing regimens.

In the same period, on the long-stay units, we had started annual medication reviews; a recurrent, structured, and critical evaluation of pharmacotherapy by patient, physician, and pharmacist. [1] It occurred to me that these seriously ill patients were sometimes prescribed irrational combinations of antipsychotics to treat schizophrenia, often with unsatisfactory results. A trial of the antipsychotic clozapine was often justified, but for various reasons clozapine treatment had not been started before.

This observation and the discussion described above ultimately gave direction to what has now become this thesis.

Schizophrenia

Schizophrenia is a psychiatric disorder with a heterogeneous phenotype and a complex pathoetiology that affects up to 1% of the population. [2] It is characterised by a combination of positive symptoms, negative symptoms, mood dysregulation and neurocognitive impairment, including intellectual disability, and is often associated with substance abuse. Positive symptoms are abnormal mental experiences and behaviours that include hallucinations, delusions, and thought disorder. [3] Negative symptoms relate to loss of mental energy and efficiency, such as limited emotional expression, apathy, social withdrawal, and poverty of thought and speech. [3] The symptoms of schizophrenia can be tormenting to patients, greatly affecting their quality of life. In addition, lifetime suicide risk is 5% [4,5], mortality rate is 2 to 3 times higher than that of the general population [6], and life expectancy is 20% shorter [7].

Antipsychotics are the cornerstone of pharmacotherapeutic treatment of schizophrenia. However, patients with schizophrenia may manifest poor response to therapy because of intolerance to medication, poor adherence, inappropriate dosing, as well as true resistance of their illness to antipsychotic drug therapy. Moreover, antipsychotics exert their effect mainly on the positive symptoms of the disease. One fifth to one third of all patients with schizophrenia lack a full symptomatic response. [8,9] This is referred to as treatment-resistant schizophrenia (TRS). Whereas there is a lack of consistency in the definition of TRS [8], the Dutch Clozapine Collaboration Group defines TRS as non-responsive to at least two different antipsychotics including a second-generation antipsychotic, administered in adequate dosage for sufficient duration. [10] In theory, all patients with TRS should undergo a trial of clozapine, currently the only registered drug for the treatment of TRS. However, for various reasons, clozapine is highly underutilised. [11,12]

Clozapine's turbulent history

Clozapine was based on the chemical structure of the tricyclic antidepressant imipramine and first synthesized in 1958 by Wander, a Swiss pharmaceutical company that is now part of Novartis. It appeared to resemble the pharmacological properties of chlorpromazine, the first antipsychotic drug discovered in 1950, but without causing catalepsy. This latter was surprising since at the time catalepsy was considered necessary for efficacy. [13] Thus, the first 'atypical' or 'second generation' antipsychotic was born, referring to this absence of extrapyramidal side effects. Unfortunately, however, shortly after clozapine had been admitted to the European market in 1972 and clinical trials with clozapine were just beginning in the United States (US), sixteen clozapine treated patients in Finland developed agranulocytosis leading to eight deaths. Clozapine was subsequently withdrawn from the market in countries where it was already on the market and clinical trials were suspended elsewhere. [14] Yet, a 'compassionate use' programme enabled the continued use of clozapine and then, in 1988, Kane et al. conducted their landmark study that showed that clozapine could successfully treat TRS. [15] Following this study, clozapine received approval for TRS in the US and Europe, with strict guidelines regarding monitoring of white blood cell counts (WBC) (i.e., in the European Union: weekly WBC and granulocyte counts during the first eighteen weeks of treatment and every four weeks thereafter [10]). [14] (Figure 1)

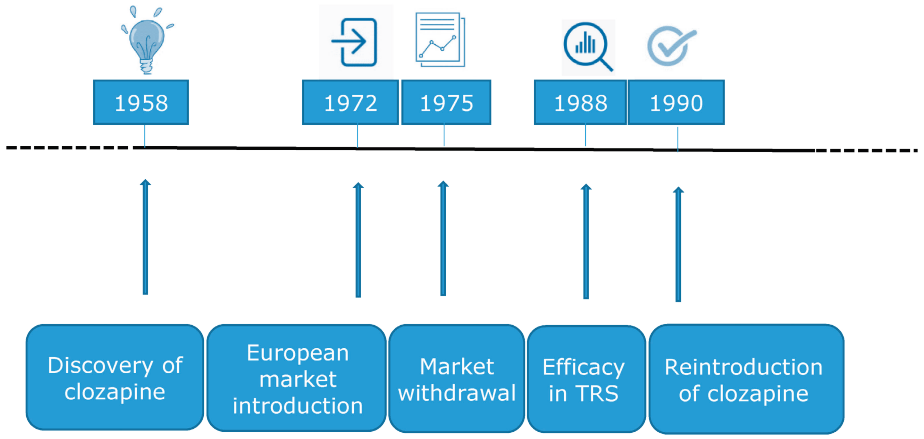

Figure 1. History of clozapine

A drug of last resort

By now, meta-analyses on efficacy of antipsychotic medications have consistently demonstrated that clozapine is superior to other antipsychotics in reducing positive psychotic symptoms in both the short and long term for people with TRS. [16,17] Also, clozapine reduces the risk of suicidal behaviour in schizophrenia patients, which appears to be selectively associated with clozapine and not with other antipsychotics. [18] Meanwhile, more knowledge regarding the incidence of clozapine-associated neutropenia has been accumulated. Clozapine-associated severe neutropenia (absolute neutrophil count < 500/ml) is now known as a relatively rare event (0.9% (95% CI: 0.7-1.1%)) with the majority occurring during the first twelve months of exposure and with a substantial decline in risk after one year of treatment. [19] Despite this, clozapine is being considered inappropriate as a drug of first choice, in view of frequently occurring, other serious side effects, such as seizures, myocarditis and bowel obstruction, and because of the frequent blood monitoring required to detect early signs of neutropenia. Over the past decades, several pharmaceutical companies have tried to develop a drug at least as effective as clozapine, but with fewer side effects, to no avail in terms of effectiveness. The pharmacological basis for the superior clinical effect of clozapine over other antipsychotics in patients with schizophrenia is still unclear. [20]

Collectively, up to 40% of all patients with schizophrenia and related disorders could benefit from clozapine. [8,21] Many candidate patients throughout the world, however, are deprived of a clozapine trial or have been treated with high doses of multiple antipsychotics, increasing the likelihood of adverse events and still only leading to limited improvement in clinical symptoms, before they are offered a trial with clozapine. [8] A mean delay of 4 years was found in a

study conducted in the United Kingdom (UK) among patients who had their first clozapine treatment between 2006-2010. [22] A recent systematic review and meta-analysis of 34 articles involving 9,386 patients with schizophrenia spectrum disorders suggested that the early use of clozapine could improve the outcome of patients with first-episode schizophrenia. [23] Another systematic review demonstrated that delayed clozapine use was related to poor treatment outcomes among patients with TRS. [24] The factors responsible for the underuse of clozapine are numerous, varied and often interconnected and related to 1) the drug itself, with its side effect profile, 2) the prescribers, with their knowledge, views, attitudes and experiences regarding clozapine treatment, 3) the patients, also with their own perceptions and often struggle with disorganised behaviour interfering with the requirements related to clozapine treatment and drug adherence, and 4) the associated infrastructure regarding the availability of clozapine in many countries and the regulatory processes related with clozapine treatment. Due to differences in the "image" of clozapine, differences in prescribing habits, availability of clozapine and in the regulations of clozapine use [25], prescription rates vary widely between countries [26-29]. For instance, in 2008, only 4.4% of patients with schizophrenia were on clozapine in the US [21], while in 2016 32% of admitted patients with schizophrenia in China used clozapine.
Thus, clozapine is still saddled with a stigma, which has relegated clozapine as being regarded a drug of last resort. However, clozapine use has increased in many countries over recent years. [27,30] In the Netherlands for example, over the past two decades, the number of outpatients being dispensed clozapine has more than doubled. [31]

Taken together, with clozapine being the only antipsychotic indicated for TRS, the graduate decrease of 'clozaphobia', and concurrent increase in the number of patients treated with clozapine, there is a critical need for more information how to successfully guide clozapine treatment.

Clozapine discontinuation

Once clozapine is prescribed, there is no guarantee that its use will be continued for an extended period. The rate of early clozapine discontinuation is disturbingly high varying from 8% to 60%, depending on how 'early discontinuation' has been defined. [32-36] Several reasons for discontinuation have been found [35,37-41], including the aforementioned side effects, although some of these do not always necessarily warrant discontinuation. [41] Considering clozapine's place in the treatment algorithm and the fact that clinical outcomes often worsen after clozapine discontinuation [42], it has important implications to know which patients are at risk of clozapine discontinuation. It would be

helpful if psychiatrists could identify patients at risk of unsuccessful treatment with clozapine early to offer them additional support. The current evidence identifying these patients is not always consistent and involves mainly inpatient populations with different follow up periods. [33-35] Also, cultural differences and differences in national policies may also cast doubt on the generalisability of international results.

Consequences of increasing numbers of women using clozapine

Pregnancy was one of the reasons for clozapine cessation in a retrospective investigation of the motives for 173 clozapine cessations. [39] Currently, about a third of Dutch clozapine users are women, including women of childbearing age. [31] As psychiatrists seem to become less reluctant to prescribe clozapine in more recent years [43], over time, more women on clozapine treatment may become pregnant and clinicians might at some point need to balance the risks of pharmacological treatment and non-treatment in the perinatal setting. [44] Pregnancy appears to worsen mental health in a subset of women with schizophrenia and untreated schizophrenia is a risk factor for adverse pregnancy outcomes for both mother and child. [45] Due to clozapine's position in the treatment algorithm, clozapine is almost always the drug of last resort, so when clozapine is initiated no alternatives with proven efficacy remain. [46] Hence, switching to another antipsychotic or discontinuation of clozapine may jeopardize the woman's mental health and even the baby's health after delivery. [45] Human data concerning the risk of perinatal clozapine use for the foetus and newborn are limited [47], however, as are the data regarding the impact on the maternal pharmacokinetics of clozapine and the pharmacological effects in the neonate.

Therapeutic Drug Monitoring and pharmacokinetics of clozapine

Up to 40% of patients with TRS demonstrate improvement in their symptoms on clozapine, but the remaining 60% of patients tend to be refractory to all antipsychotic medications. [48] One of the challenges in understanding this clozapine-resistant schizophrenia is disentangling true from pseudo resistance to clozapine. [49] A lack of response could be a legitimate reason for clozapine discontinuation, provided the patient has been treated for a sufficient period with clozapine concentrations within the 'therapeutic range'. Therapeutic drug monitoring (TDM) of clozapine concentrations is warranted to enhance efficacy, to avoid concentration-dependent toxicity (sedation, hypotension, and seizures), and to assess patient compliance. [50,51] With the side note that a 'therapeutic reference range is an orienting, population-based range which may not necessarily be applicable to all patients', Hiemke et al [50], in their consensus

guideline, recommend a therapeutic reference range of 350 – 600 µg/l for clozapine. The Guideline of the Dutch Clozapine Collaboration group (a working group of psychiatrists and other professionals such as internists, pharmacists or general practitioners who focus on treating patients with treatment-resistant psychotic disorders) adds that, if there is no response, levels to a maximum of 700 µg/l can be tried. [10]

When clozapine was marketed, it was recommended to prescribe clozapine twice daily (BID), based on clozapine's elimination half live of approximately twelve hours. [52] However, aiming for higher adherence and less sedation during the day, there seems to be a shift towards QD dosing of clozapine at the end of the day. [53] In practice, clozapine concentrations measured in samples taken twelve hours post-dose are set against the therapeutic window that has been based on clozapine trough concentrations from divided dose regimens of clozapine. This assumes the same concentration-effect relation as well as similar pharmacokinetics at QD and BID dosing, although this has not been studied before. In addition, it has yet to be determined how concentrations measured in the morning from QD dosing fit into the therapeutic window of clozapine. Concentrations measured twelve hours after clozapine intake are significantly higher than the actual trough concentrations in case of QD dosing. Specifically, this means that if a concentration of e.g., 800 µg/l is measured in the morning, the true trough concentration measured just before the next dose is estimated to be about 400 µg/l, based on the estimated halve live of twelve hours. In case of insufficient response at 800 µg/l, a psychiatrist will probably be reluctant to increase the dosage, whereas at a concentration of 400 µg/l this is more likely to be considered. The question is whether the current approach to TDM at QD dosing contributes to under-treatment of patients due to unwarranted premises about the therapeutic range at QD dosing.
In addition, TDM of clozapine and norclozapine, clozapine's main metabolite, is based on total drug concentrations, although, as for all drugs, only the unbound molecules are responsible for effect and side effects. In general, TDM of total concentrations assumes that the unbound concentration is a constant percentage of the total concentration, within the concentration range of interest. Clozapine and norclozapine are highly protein bound (95% [54] and 90% [55] respectively), with the acute phase protein alpha-1 acid glycoprotein (AGP) being the main protein in this binding. [55,56] Little is known about fluctuations in the degree of protein binding of clozapine and norclozapine, but Man et al. found that although total clozapine concentrations increased during inflammation, the unbound fraction decreased due to increasing concentrations of AGP. [57] In the light of QD dosing before bedtime, it is interesting to know if protein binding becomes saturated at these higher concentrations, potentially leading to an

increased degree of concentration-dependent side effects during the night and early morning.

Thesis objective

Thus, the population of patients who are eligible to clozapine treatment is a severely ill and vulnerable population, at high risk of non-adherence to drug treatment and suboptimal treatment due to several reasons. The overall objective of this thesis is to support successful clozapine treatment, by firstly expanding the knowledge on the predictability of early clozapine discontinuation, secondly investigating the necessity of clozapine discontinuation due to (intended) pregnancy, and thirdly expanding the knowledge on therapeutic drug monitoring (TDM) of clozapine in light of once-daily dosing and in light of possible pitfalls in the interpretation of clozapine TDM results.

Thesis outline

Chapter 2 describes the development of a prediction model to select patients who might benefit from extra support to prevent unwarranted clozapine discontinuation.

Chapter 3 focuses on the safety of clozapine in the perinatal setting to assess if clozapine discontinuation because of (intended) pregnancy is warranted. In Chapter 3.1 global pharmacovigilance data from the World Health Organisation's global individual safety report database, VigiBase, were used to compare the frequency of adverse pregnancy outcomes reported after clozapine use and after the use of other antipsychotics during pregnancy. Chapter 3.2 gives an overview of the available literature about the safety of clozapine treatment during pregnancy and lactation, focusing on both mother and child.

In Chapter 4 we studied (the pharmacokinetics of) both total and unbound clozapine and norclozapine concentrations in patients using clozapine QD and BID. Chapter 4.1 compares the pharmacokinetics of clozapine and norclozapine with once and twice daily dosing. It also simulates the impact of QD dosing on the concentration-time curves of clozapine and norclozapine. In Chapter 4.2, we studied if protein binding of clozapine and norclozapine becomes saturated at higher concentrations and how dose regimen affects the relation between total and unbound concentrations. Secondarily, we studied the degree of protein binding within a range of AGP concentrations in a naturalistic setting. To be able to analyse unbound clozapine and norclozapine concentrations, we developed a method for determination of the unbound concentrations of clozapine and norclozapine in serum, which is described in Chapter 4.3. Finally, we studied

the difference between clozapine concentrations measured in serum, lithium-heparin plasma and ethylenediamine tetraacetic acid plasma, which has been outlined in Chapter 4.4.

In the final chapter (Chapter 5) the findings of these studies are put into a broader perspective.

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2

Predicting unsuccessful clozapine treatment

2.1

Predicting unsuccessful clozapine treatment after first use in adult patients with psychotic disorders

Marieke M Beex-Oosterhuis, Eibert R Heerdink, Arthur R Van Gool, Rob J van Marum
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Abstract

Purpose/Background

Cessation of clozapine therapy and insufficient response may result in relapse of psychotic symptoms and in clinical admissions. Yet, discontinuation rates are high. Identifying patients at risk for unsuccessful clozapine use might enable clinicians to direct specific attention to them.

Methods/Procedures

Routinely collected data from a large insurance company were used to develop a simple prediction model for unsuccessful clozapine treatment in psychiatric patients 1 year after clozapine was first dispensed by a community pharmacy in the Netherlands. Multivariate logistic regression analyses were performed with the Nagelkerke R squared (R^2) statistic as a measure of the predictive value of the model.

Findings/Results

937 Patients were dispensed clozapine for the first time by their community pharmacy between January 1st 2011 and December 31st 2015 (index date). Of these, 741 patients had started their clozapine treatment in hospital before the index date (inpatient starters); the remaining 196 patients started clozapine as outpatients on the index date (outpatient starters). In 191 patients (20.4%) clozapine treatment was unsuccessful 1 year after the index date. Unsuccessful treatment was more common among outpatient starters than among inpatient starters (32.1% versus 17.3%). Using backward selection of the variables, a model consisting of 61 variables had the best predictive value overall (Nagelkerke R^2 0.301), whereas a model consisting of 52 variables had the best predictive value in outpatient starters (Nagelkerke R^2 0.676).

Implications/Conclusions

The likelihood of unsuccessful clozapine treatment after 1 year was higher among patients who started clozapine as outpatients. Despite the use of a diversity of variables and different statistical approaches, it was not possible to make a simple prediction model for unsuccessful clozapine treatment using relatively easily accessible data.

Introduction

Although clozapine is widely considered the gold standard for treatment-resistant schizophrenia and partially responsive schizophrenia (1), it is only prescribed to a proportion of eligible patients (2) and initiation is usually delayed (3, 4). This underprescribing can be attributed to a number of factors, such as its side effect profile and the need for frequent blood sampling to detect early signs of agranulocytosis. Furthermore, the rate of early clozapine discontinuation is disturbingly high, varying from 8% to 60% depending on the definition of discontinuation used, the population studied, and the duration of follow-up. (5-9) African-American ethnicity, comorbid substance abuse, social deprivation, older age at clozapine initiation, and prior history of inadequate response to traditional neuroleptics seem to be associated with clozapine discontinuation. (6-9) The most common reasons for discontinuation given by patients are an aversion to the blood tests for agranulocytosis, a negative attitude toward the drug in general, and fear of adverse effects. (8-12) Lastly, an insufficient response can lead to cessation of clozapine therapy (13). A higher number of admissions, a higher number of different antipsychotics used before treatment with clozapine (14)**,** more severely ill patients (15, 16), female gender, longer duration of illness (17), early onset of illness (17, 18), and lack of early response to clozapine (18) are predictors of a lower likelihood of a therapeutic response.

Clozapine discontinuation may lead to relapse of psychotic symptoms. Almost half of the patients treated with clozapine had an exacerbation of symptoms when the drug was withdrawn from the market in 1975. (19). Additionally, in a case series three patients were reported to have committed suicide after clozapine discontinuation (20), and excess mortality was seen in the year after clozapine discontinuation in a population-based cohort study of patients with treatment-resistant schizophrenia (21).

So far, studies of risk factors for clozapine discontinuation or insufficient response have been relatively small, involving mainly inpatient populations with different follow-up periods. Yet, identifying patients at risk of unsuccessful clozapine treatment could help psychiatrists to select patients who might need extra support to prevent early unwarranted discontinuation or relapse.

The aim of this study was to develop a database-based prediction model for unsuccessful clozapine treatment one year after the drug was first dispensed to patients with psychotic disorders by community pharmacies in the Netherlands. In addition, this study aimed to investigate whether unsuccessful treatment is influenced by whether clozapine treatment was initiated during psychiatric hospital admission or not.

Method

Design

In this predictive modelling study, outpatients were followed up from their first registered insurance claim for clozapine dispensed by a community pharmacy (index date) up to 12 months or until a patient was lost to follow-up because of termination of health insurance, termination of the database (after December 31st, 2015), or death, whichever occurred first. The study was performed in accordance with the Transparent Reporting of multivariate prediction models for Individual Prognosis Or Diagnosis (TRIPOD) statement.

Setting

Our study used data from an anonymized registry of one of the largest Dutch health insurers. The Zilveren Kruis Health Insurance Database (ZKHD) contains all reimbursements for the provision of medical care to insured patients over the last 10 years, involving over 4 million individuals throughout the Netherlands and covering approximately 25% of the total Dutch population. Health insurance is compulsory for all inhabitants in the Netherlands and the health insurance companies are legally obliged to provide inhabitants with insurance. Registrations in the ZKHD include consultations by primary care physicians, insurance claims based on diagnosis and treatment (Diagnosis Treatment Code, DTC), and prescription drugs delivered by community pharmacies. Pharmaceutical data for inpatients are not available.

The study was approved by the research committee of the ZKHD and registered with the Netherlands Trial Register number (NTR5439).

Study population

We selected all patients aged 18 years or older with a first insurance claim for clozapine as an outpatient between January 1st 2011 and December 31st 2015 (Figure S1). To include patient without prior history of clozapine use, all patients had to be insured with the health insurance company for at least 1 year before clozapine was dispensed.

To exclude patients who probably used clozapine for Parkinson's disease or dementia, we excluded patients who had used anti-Parkinson (N04BA) or anti-dementia (N06D) drugs in the year before clozapine was first dispensed and patients aged 66 years and older. Additionally, we used the DTCs to check that patients with an indication different from schizophrenia spectrum and other

psychotic disorders were excluded. The final population was then categorized into patients who probably started clozapine treatment prior to the index date while admitted to a psychiatric hospital and those who started clozapine as an outpatient.

Outcome

The main outcome was unsuccessful clozapine treatment 1 year after the index date, defined as the absence of current insurance claims for clozapine treatment 365 days after the index date. When no current insurance claim for clozapine was present after one year a patient either discontinued clozapine use or was admitted to a hospital.
To this end, we calculated the mean number of clozapine dispensing episodes for each patient by using the pharmacy dispensing data on the insurance claims. The number of days between the first and last clozapine dispensing record in the first year was counted and divided by the total number of clozapine dispensing episodes minus one. Two clozapine records for the same day, which is the case when two different strengths of clozapine tablets are used, were counted as one. In addition, we applied a maximum duration of 90 days for a mean dispensing episode, based on the maximum duration for which drugs can be dispensed on one prescription in the Netherlands.
Lastly, clozapine treatment was considered unsuccessfully if the time interval between the last clozapine-dispensing record in the first year and day 365 exceeded 1.5 times the calculated mean dispensing episode with an additional minimum of 30 days for the time interval. In this way, we allowed for some irregularity in drug compliance.
Sensitivity analyses were performed to examine the impact of reducing the maximum calculated dispensing episode to 60 days and of using a fixed dispensing episode of 60 or 90 days, with an accepted time interval of 1.5 times 60 days or 1.5 times 90 days, respectively.

Determinants

1. Demographic data:

Demographic details of age, gender, and the first number of the four-numbered, Dutch zip code were obtained from the ZKHD.

2. Clozapine initiation:

Two approaches were used to establish whether a first clozapine insurance claim in the database reflected the continuation of clozapine treatment initiated in hospital:

- It was assumed that if a patient's first clozapine record in the database were for tablets of at least 100 mg, this applied to outpatient continuation of treatment introduced earlier during hospital admission, since 100 mg exceeds the regular starting dose of clozapine (i.e., 12.5 or 25 mg).
- Patients were also categorized as having had their first clozapine prescription dispensed at their first visit of the community pharmacy or not. Based on the inclusion criteria, all patients had been insured at the insurance company for at least one year before the index date. Thus, if no insurance claims for any prescription at all were registered before the index date, the patient had probably received its medication from a hospital pharmacy during clinical admission.

3. Somatic comorbidity:

The number of somatic diagnoses, based on the number of current DTCs at the index date and in the year before the index date, was used as index of somatic comorbidity. We also recorded the number of consultations with primary care physicians in the 3 months and 1 year before the index date.

4. Medication history:

We identified the last antipsychotic delivered within 135 days of the index date, in accordance with the time interval used for the dispensing episode calculation, and whether this antipsychotic had been continued after the index date or not. Continuation was defined as there being fewer than 135 days between the last dispensing date before the index date and the first dispensing date after the index date. We also recorded whether this concerned an atypical or typical antipsychotic. In addition, we registered the number of different antipsychotics used in the year before clozapine initiation. Lastly, comedication (somatic and psychiatric) use between 6 months before and 30 days after the index date was recorded.

Statistical analysis

First, we investigated the association between the variables and the risk of unsuccessful clozapine treatment 1 year after the index date using univariate

logistic regression analysis. Patients were either a) still being successfully treated with clozapine on day 365, b) not successfully treated with clozapine on day 365, c) lost to follow-up before day 365, but still being successfully treated when lost to follow up, or d) lost to follow-up before day 365 and not successfully treated when lost to follow up. An intention-to-treat analysis was conducted with the last observation carried forward for imputation of missing data for patients who were lost to follow up during the first year after index date.

Second, we used multivariate logistic regression analysis to develop a prediction model for unsuccessful clozapine treatment 1 year after the index date with the Nagelkerke R squared (R^2) statistic as measure of the predictive value of the model. Nagelkerke R^2 is an estimation of the coefficient of determination (R^2) in the logistic regression model and summarizes the proportion of variance in the dependent variable associated with the predictor variables. The larger the R^2 value, the larger the explained variance, to a maximum of 1.

To prevent distortion of our final model by inclusion of overlapping variables in the analysis, we first defined which of the following overlapping variables should be included in the multivariate analysis: 1) the number of different antipsychotics prescribed in the year before clozapine initiation (either as a categorical or as a continuous variable); 2) the number of unique, somatic DTCs at the index date; 3) the number of unique, somatic DTCs in the year before the index date (both either as a categorical or continuous variable); 4) the number of primary care physician consultations in the 3 months before the index date; 5) the number of primary care physician consultations 1 year before the index date (also both either as a categorical or continuous variable); 6) the identity of the last antipsychotic delivered within 135 days of the index date; or 7) whether the last delivered antipsychotic was an atypical or typical antipsychotic.

Candidate predictors were entered in the multivariate logistic regression analysis, with 'absence of current clozapine treatment' as the dependent variable. By varying all possible combinations of the above-mentioned overlapping variables together with the other candidate variables, we tested which combination generated the highest Nagelkerke R^2 value. We then analysed this combination of variables again using variable selection with both backward and forward elimination. Starting with all variables in the model, variables were subsequently excluded (backward) from the model or included (forward) in the model with a covariate retention threshold of p <0.20. Relative risks were measured as odds ratios with 95% confidence intervals (95% CI). The Nagelkerke R^2 values resulting from these three analyses were compared, and the final prediction model was that with the highest Nagelkerke R^2 value.

We performed the analysis for the total population and for people who started clozapine as outpatients.

The data were analysed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 937 clozapine users were included, of whom 741 (79%) started clozapine treatment as an inpatient before the index date (Figure S1). Patient characteristics are presented in Table 1.

Table 1. Patient characteristics

	N	
Total	937	
Gender		
Male (%)	621	(66.3)
Age		
Mean age (years, (SD))	*39.5*	*(11.7)*
Mean age men (years, (SD))	37.4	(10.8)
Mean age women (years, (SD))	43.6	(12.4)
Age categories		
<30 yrs (%)	225	(24.0)
30-39 yrs (%)	271	(28.9)
40-49 yrs (%)	232	(24.8)
>=50 yrs (%)	209	(22.3)
Patient setting at clozapine initiation		
Inpatient (%)	741	(79.1)
Outpatient (%)	196	(20.9)

Most patients were men (66.3%) and the mean age at clozapine onset was 39.5 years (standard deviation (SD) 11.7), with men starting on average 6 years earlier than women (37.4 (SD 10.8) vs 43.6 (SD 12.4) years).

Unsuccessful clozapine treatment

Of the 937 patients, 583 (62.2%) did and 191 (20.4%) did not have prescriptions filled by a community pharmacy 1 year after the index date. One hundred sixty-three patients (17.4%) were lost to follow up, of whom 121 (12.9%) were still on clozapine treatment when they were lost to follow-up and 42 were not (4.5%).

Of the 196 outpatient starters and the 741 inpatient starters, 84 (42.9%) and 499 (67.3 %) had a recent clozapine prescription dispensed by the community pharmacy 1 year after the index date, respectively, whereas 63 (32.1%) and 128 (17.3%), respectively, did not. Forty-nine outpatient starters (25.0%) and 114 inpatient starters (15.3%) were lost to follow up, of whom 18 (9.2%) and 24 (3.2%), respectively, were not on clozapine treatment when lost to follow up.

Reducing the maximum number of days for a regular dispensing episode or applying a fixed number of days for the dispensing episode instead of calculating this episode, did not essentially change the proportion of patients defined as 'unsuccessful clozapine users' (Table 2). Therefore, the original definition was used in further analyses.

Table 2. Number and proportion of patients with or without current clozapine treatment on day 365 after index date (n= 937)

	Definition of 'absence of current clozapine treatment'			
	interval between 'last clozapine'# and day 365 > 1.5 * calculated mean delivery episode		interval between 'last clozapine'# and day 365 > 1.5 * fixed delivery episode	
	Max. 90 days for 'calculated delivery episode'	Max. 60 days for 'calculated delivery episode'	Fixed 90 days for 'delivery episode'	Fixed 60 days for 'delivery episode'
Current clozapine treatment on day 365 after index date (N (%))	583 (62.2)	584 (62.3)	623 (66.5)	609 (65.0)
No current clozapine treatment on day 365 after index date (N (%))	191 (20.4)	190 (20.3)	151 (16.1)	165 (17.6)
Lost to follow up before day 365 after index date – with current clozapine treatment when lost to follow up (N (%))	121 (12.9)	121 (12.9)	137 (14.6)	130 (13.9)

Table 2. *Continued*

	Definition of 'absence of current clozapine treatment'			
	interval between 'last clozapine'# and day 365 > 1.5 * calculated mean delivery episode		interval between 'last clozapine'# and day 365 > 1.5 * fixed delivery episode	
	Max. 90 days for 'calculated delivery episode'	Max. 60 days for 'calculated delivery episode'	Fixed 90 days for 'delivery episode'	Fixed 60 days for 'delivery episode'
Lost to follow up before day 365 after index date – without current clozapine treatment when lost to follow up (N (%))	42 (4.5)	42 (4.5)	26 (2.8)	33 (3.5)

#Last clozapine = the last drug-dispensing record of clozapine in the first year after index date

Prediction model

The results of the univariate logistic regression analysis are presented in Table S1. Based on data for the total group of patients, a model incorporating 1) the number of different antipsychotics in the year before clozapine initiation, as a continuous variable, 2) the number of unique, somatic DTCs in the year before the index date, as a categorical variable, 3) the number of consultations with a primary care physician 1 year before the index date, as a categorical variable, and 4) the last delivered antipsychotic within 135 days of the index date, in combination with the other candidate variables, resulted in the highest predictive value: Nagelkerke $R^2 = 0.301$. Starting with the above-mentioned variables, the same Nagelkerke R^2 (0.301) was found up until step 14 using backward selection, leaving 61 variables in the model, which was higher than the largest Nagelkerke R^2 using forward selection (0.237).

Based on data for the 196 outpatient starters, the same combination of variables, except for the number of unique, somatic DTCs at the index date instead of in the year before the index date, resulted in the highest predictive value with a Nagelkerke $R^2 = 0.676$. Starting with the above-mentioned variables, the same Nagelkerke R^2 value (0.676) was found up until step 13 using backward selection, leaving 52 variables in the model. This value was higher than the Nagelkerke R^2 found using forward selection (0.407).

The two final combinations of variables with corresponding odds ratios and 95% CI are presented in Table S1.

Discussion

In this population-based predictive modelling study, we used routinely collected data from a large insurance company to predict unsuccessful clozapine treatment 1 year after the date that clozapine was first dispensed by a community pharmacy. Our study population represents a considerable proportion of all patients starting clozapine treatment for a psychotic disorder in the Netherlands. Despite the large and diverse set of variables investigated and investigation of two populations, we were not able to successfully predict unsuccessful clozapine treatment after 1 year with a practically useful model incorporating variables recognizable and appealing to prescribing psychiatrists. Although the Nagelkerke R^2 values of the final models were respectable, the number of variables needed to be able to predict as much as 30% or 68% of the variability in unsuccessful clozapine treatment was too large for a simple prediction model to be used by psychiatrists in daily practice.

In the total patient population, the ability to predict unsuccessful clozapine treatment was more than twofold lower than that for the outpatient starter subpopulation. This is not surprising, since there was relatively less information available for the larger population. For example, the identity of the antipsychotic used before clozapine was started was known for 37% of the total population and for 75% of the outpatient starter subpopulation. In addition, the overall population was more heterogeneous in terms of duration of clozapine use, because many patients started clozapine when admitted to a psychiatric hospital, and also the duration of use before the index date was unknown and could even vary considerably between the individual patients. This heterogeneity might have hindered the prediction of unsuccessful treatment.

Although the study design does not allow us to make causal inferences, it was interesting that the clozapine failure rate was higher among the outpatient starters (41.3%) than among the inpatient starters (20.5%). One might expect that treatment adherence would be higher among patients who were considered stable enough to start their clozapine treatment as an outpatient than among patients who needed to be treated clinically first. On the other hand, the likelihood of a successful treatment might be higher among those starting treatment as an inpatient because of the extra support and daily medical supervision. Additionally, the inpatient starters would have used clozapine for longer and were likely to have benefited from clozapine by

the time they were discharged. This 'positive' experience might increase the likelihood of successful treatment continuation.

Although previously published discontinuation rates of clozapine vary widely, the rate of unsuccessful clozapine treatment of 24.9% found in our study for the total population is in line with the rate reported for both inpatient and outpatient populations by Ingimarson et al. (15.6%) and Davis et al. (30%) (5, 8). Our study used the absence of a currently dispensed clozapine prescription as a proxy for unsuccessful clozapine treatment. Since the ZKHD does not contain psychiatric admission dates or information concerning inpatient medication, patients without a current insurance claim for clozapine on day 365 could have discontinued treatment or could have been admitted to a psychiatric hospital. In addition to different definitions and different follow-up periods and settings, which might also partly explain the variation in discontinuation rates seen in previous studies, regulations concerning the prescribing and monitoring of clozapine vary widely between countries (22). For example, primary care physicians can prescribe clozapine in the Netherlands, New Zealand, the UK, and the USA, although with some restrictions in some of these countries, whereas in Ireland and China clozapine can only be prescribed by hospital physicians and dispensed through hospital pharmacies. These differences might influence the extrapolation of findings.

Although population-based healthcare databases such as the ZKHD are a valuable tool for observational studies, the data in the registry are limited to what is needed for reimbursement and the registry is not primarily designed for scientific research. For example, information about prescription episodes or the prescribed daily doses was not available, since only information about patient identity and the number of drugs delivered is required for reimbursement. Thus, a number of variables had to be derived from information provided by the database. Also, we used dispensing data as surrogate for actual drug usage, although theoretically a prescription could be filled but not used. Nevertheless, because of the financial importance of correctly paying reimbursement claims, there is intensive automated monitoring regarding the information related to research data, which guarantees the accuracy and validity of the database.
Also, the included patients might have been switched from another health insurance company before entering the ZKHD and therefore could have had an earlier clozapine treatment episode. However, we tried to overcome this limitation by inclusion of patients with at least one year of medical history at the ZKHD without having received clozapine. Hence, we believe this has no more than limited influence on our findings.
Lastly, the database does not record information about ethnicity, comorbid substance abuse, and socioeconomic status, which have been associated with

clozapine discontinuation (6, 8, 9), information about the number of admissions before treatment with clozapine (14), or the duration of illness (18), which are associated with a lower response rate. This might have influenced the ability of our model to predict unsuccessful clozapine treatment more extensively. Other researchers are encouraged to incorporate these variables into a prospective study design to further investigate the possibility to predict unsuccessful clozapine treatment 1 year after treatment initiation.

Supplemental Figure and Table

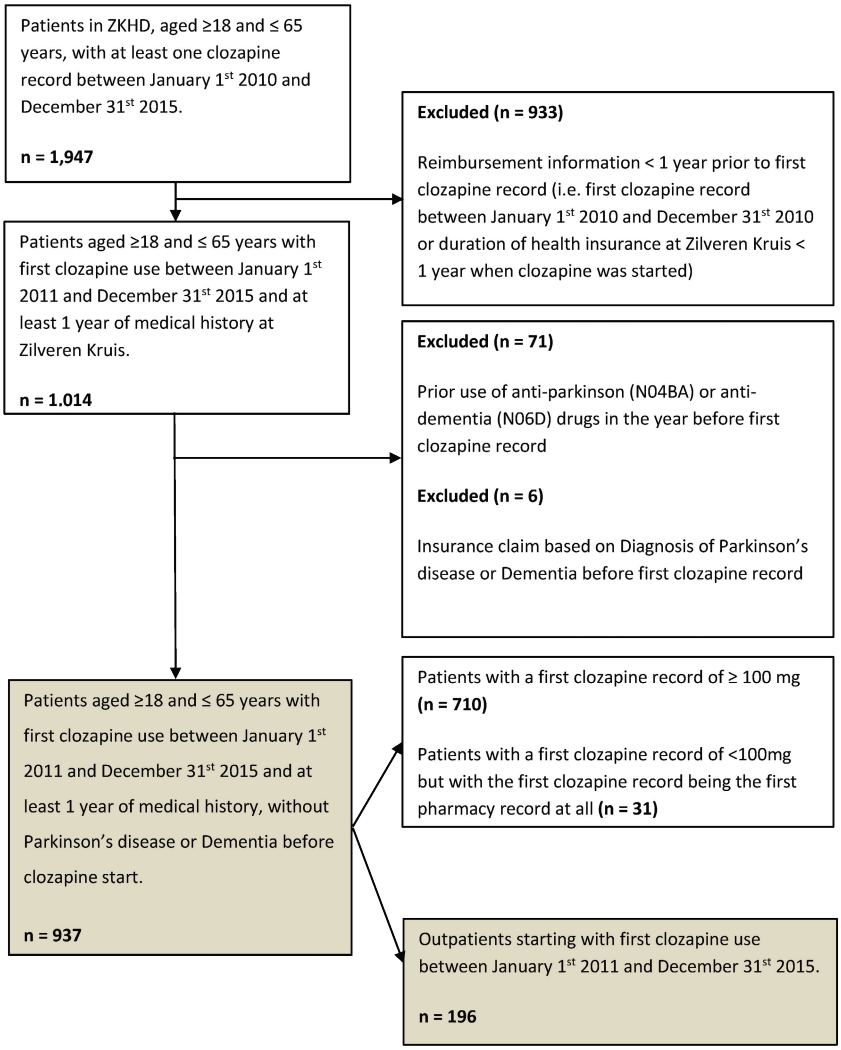

Figure S1. Process of patient inclusion

Table S1. Univariate and multivariate logistic regression analyses for unsuccessful clozapine treatment 1 year after it was first dispensed by a community pharmacy

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
Gender							‡			
Male	0.94	0.69-1.28	*621*	1.17	0.66 - 2.07	*102*			0.37	0.08 - 1.63
Age (categorized)										
<30 years	*Ref*		*225*	*Ref*		*36*	*Ref*		*Ref.*	
30-39 years	0.60	0.40 - 0.91	*271*	0.97	0.39 - 2.41	*42*	0.80	0.49 - 1.32	0.54	0.09 - 3.25
40-49 years	0.98	0.65 - 1.47	*232*	1.57	0.64 - 3.84	*44*	1.43	0.87 - 2.34	26.83	3.47 - 207.47
≥50 years	0.75	0.49 - 1.16	*209*	1.01	0.45 - 2.29	*74*	0.75	0.42 - 1.34	1.39	0.23 - 8.33
Postal code										
Missing	*		*1*			*0*	*			
1	1.58	0.81 - 3.05	*138*	0.48	0.14 – 1.66	*40*	1.24	0.57 - 2.73	0.12	0.01 - 1.14
2	2.03	1.02 - 4.07	*95*	0.71	0.20 – 2.54	*29*	1.64	0.70 - 3.80	0.57	0.05 - 6.57
3	1.60	0.87 - 2.96	*248*	0.92	0.28 – 3.03	*48*	1.47	0.71 - 3.07	0.54	0.06 - 4.83
4	2.33	0.79 - 6.88	*19*	1.33	0.21 – 8.29	*7*	2.15	0.54 - 8.54	0.50	0.01 - 33.62
5	0.80	0.21 - 3.10	*18*	*		*5*	0.37	0.07 - 1.96	*	
6	1.56	0.56 - 4.36	*25*	*		*2*	1.60	0.48 - 5.31	0.80	0.00 - 146.24
7	0.82	0.39 - 1.69	*118*	0.69	0.19 – 2.52	*27*	0.81	0.35 - 1.89	1.16	0.12 - 10.80
8	0.97	0.50 - 1.86	*195*	0.71	0.19 – 2.69	*24*	0.92	0.43 - 1.95	0.14	0.01 - 1.91

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
9	*Ref*		*80*	*Ref*		*14*	*Ref*		*Ref.*	
First clozapine record at first pharmacy visit	0.40	0.27 - 0.58	*282*	n.a.			ǂ		n.a.	
First clozapine record ≥ 100 mg	0.41	0.29 - 0.56	*710*	n.a.			0.51	0.33 - 0.78	n.a.	
Last antipsychotic - 135 days										
none / unknown	0.34	0.21 - 0.57	*586*	0.52	0.21 - 1.28	*49*	1.25	0.60 - 2.62	#	
fluphenazine (N05AB02)	*		*1*	*		*1*	*		*	
perphenazine (N05AB03)	*		*1*			*0*	*			
periciazine (N05AC01)	*		*1*	*		*1*	*		*	
pipamperone (N05AD05)	2.93	0.51 - 17.04	*6*	3.19	0.30 - 33.89	*4*	2.16	0.20 - 24.02	0.76	0.02 - 38.66
bromperidol (N05AD06)	1.47	0.20 - 10.99	*4*			*0*	2.60	0.21 - 31.63		
flupentixol (N05AF01)	1.30	0.45 - 3.76	*17*	0.85	0.19 - 3.74	*9*	1.55	0.41 - 5.89	0.52	0.01 - 21.25
chlorprothixene (N05AF03)	0.49	0.12 - 1.96	*12*	0.35	0.03 - 3.77	*4*	0.78	0.15 - 4.06	0.00	0.00 - 0.47
zuclopenthixol (N05AF05)	0.24	0.05 - 1.17	*14*	0.21	0.02 - 2.02	*6*	0.24	0.04 - 1.43	0.02	0.00 - 0.71
pimozide (N05AG02)	2.93	0.81 - 10.62	*12*	1.77	0.36 - 8.65	*8*	3.50	0.71 - 17.33	6.65	0.16 - 279.20
penfluridol (N05AG03)	1.47	0.43 - 4.98	*12*	0.71	0.10- 4.81	*5*	1.52	0.35 - 6.61	2.68	0.06 - 119.05
sulpiride (N05AL01))	4.40	0.44 - 44.34	*4*			*0*	4.46	0.23 - 88.02		

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
risperidone (N05AX08)	0.48	0.22 - 1.02	*57*	0.35	0.10- 1.20	*20*	0.58	0.24 - 1.42	0.04	0.01 - 0.43
quetiapine (N05AH04)	0.60	0.28 -1.31	*48*	1.06	0.35- 3.23	*20*	0.63	0.25 - 1.58	1.01	0.11 - 9.65
haloperidol (N05AD01)	0.51	0.22 - 1.19	*39*	0.53	0.15 - 1.90	*15*	0.53	0.20 - 1.44	0.18	0.01 - 2.29
aripiprazole (N05AX12)	0.90	0.37 - 2.17	*29*	1.49	0.39- 5.65	*12*	0.86	0.30 - 2.45	1.06	0.10 - 11.22
paliperidone (N05AX13)	0.49	0.16 - 1.49	*20*	0.43	0.07 - 2.51	*7*	0.48	0.14 - 1.64	0.34	0.02 - 7.83
olanzapine (N05AH03)	*Ref*		*74*	*Ref*		*33*	*Ref*		*Ref*	
Last antipsychotic - 135 days: (a)typical							**ǂ**		**ǂ**	
Atypical	*Ref*		*232*	*Ref*		*94*				
Typical	1.22	0.77 - 1.94	*119*	0.95	0.48 – 1.87	*53*				
Unknown	0.47	0.33 - 0.66	*586*	0.60	0.29 – 1.24	*49*				
Last antipsychotic - 135 days: continued after index date										
No	*Ref*		*157*	*Ref*		*38*	*Ref*		*Ref.*	
Yes	2.26	1.43 - 3.59	*194*	2.41	1.09 – 5.34	*109*	3.08	1.70 - 5.57	48.03	5.95 - 387.97
Unknown (due to unknown identity of last antipsychotic)	0.71	0.47 - 1.07	*586*	1.19	0.47 – 2.99	*49*	#		2.89	0.17 - 49.12
Number of different antipsychotics – 1 year	1.46	1.27 - 1.67		1.24	0.93 – 1.65		1.22	0.96 - 1.55	2.58	1.13 - 5.85

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
Number of different antipsychotics – 1 year (categorized)							ǂ		ǂ	
0 or unknown	0.31	0.18 - 0.54	*476*	0.60	0.20 – 1.78	*28*				
1	0.64	0.37 - 1.12	*245*	0.52	0.21 – 1.28	*81*				
2	0.73	0.40 - 1.32	*149*	0.76	0.30 -1.93	*62*				
≥3	*Ref*		*67*	*Ref*		*25*				
Number of somatic DTCs at index	1.29	1.01 - 1.53		1.02	0.81 – 1.29		ǂ		ǂ	
Number of somatic DTCs at index (categorized)							ǂ			
no somatic DTC	*Ref*		*751*	*Ref*		*142*			*Ref.*	
1 or 2 somatic DTCs	1.77	1.22 - 2.54	*166*	1.40	0.71 – 2.77	*44*			1.24	0.22 - 6.90
3 or more somatic DTCs	2.84	1.16 - 6.97	*20*	1.02	0.28 – 3.79	*10*			7.28	0.20 - 263.12
Number of somatic DTCs - 1 year	1.11	1.03 - 1.21		0.95	0.84 – 1.07		ǂ		ǂ	
Number of somatic DTCs - 1 year (categorized)									ǂ	

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
no somatic DTCs	*Ref*		*567*	*Ref*		*87*	*Ref*			
1 - 4 somatic DTCs	2.35	1.72 - 3.20	*325*	1.57	0.86 – 2.87	*88*	1.92	1.28 - 2.89		
≥ 5 somatic DTCs	2.20	1.14 - 4.24	*45*	0.86	0.31 – 2.35	*21*	1.88	0.76 - 4.64		
Number of primary care physician consultations - 3 months	1.17	1.10 - 1.25		1.09	1.01 – 1.18		ǂ		ǂ	
Number of primary care physician consultations - 3 months (categorized)							ǂ		ǂ	
no consultations	*Ref*		*613*	*Ref*		*72*				
1 consultation	1.38	0.93 - 2.07	*163*	1.50	0.70 – 3.23	*46*				
2 consultations	1.68	0.92 - 3.08	*56*	0.95	0.26 – 3.40	*13*				
≥3 consultations	3.13	2.03 - 4.82	*105*	2.49	1.24 – 4.98	*65*				
Number of primary care physicians consultations – 1 year	1.05	1.03 - 1.07		1.03	1.00 – 1.06		ǂ		ǂ	
Number of primary care physician consultations – 1 year (categorized)										
no consultations	*Ref*		*415*	*Ref*		*35*	*Ref*		*Ref.*	

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
1-2 consultations	1.55	1.01 - 2.39	*180*	1.26	0.45 – 3.54	*30*	1.11	0.67 - 1.83	3.25	0.50 - 21.19
3-5 consultations	2.26	1.44 - 3.53	*137*	1.34	0.52 – 3.46	*42*	1.27	0.72 - 2.25	0.42	0.07 - 2.41
6-10 consultations	2.76	1.73 - 4.42	*111*	1.64	0.64 – 4.19	*42*	1.33	0.70 - 2.54	0.39	0.05 - 2.82
≥11 consultations	4.12	2.54 - 6.68	*94*	2.48	0.99 – 6.19	*47*	3.00	1.47 - 6.15	3.37	0.48 - 23.55
Somatic co-medication										
A01 STOMATOLOGICAL PREPARATIONS	*		*1*			*0*	*	.		
A02 DRUGS FOR ACID-RELATED DISORDERS	0.95	0.67 - 1.35	*220*	0.67	0.34 – 1.32	*47*	1.10	0.71 - 1.72	1.68	0.29 - 9.65
A03 DRUGS FOR FUNCTIONAL GASTROINTESTINAL DISORDERS	0.83	0.43 - 1.61	*55*	1.45	0.41 – 5.17	*10*	0.61	0.26 - 1.42	0.13	0.00 - 6.33
A04 ANTIEMETICS AND ANTINAUSEANTS	0.60	0.07 - 5.18	*6*	*		*1*	1.42	0.15 - 13.65	ǂ	
A05 BILE AND LIVER THERAPY	*		*1*	*		*1*	*		*	
A06 DRUGS FOR CONSTIPATION	0.96	0.68 - 1.35	*227*	0.66	0.34 – 1.29	*50*	0.77	0.49 - 1.21	0.70	0.14 - 3.47

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
A07 ANTIDIARRHEALS, INTESTINAL ANTI-INFLAMMATORY/ANTI-INFECTIVE AGENTS	1.01	0.10 - 9.73	*4*	0.71	0.06 – 7.92	*3*	*		*	
A09 DIGESTIVES. INCL. ENZYMES	3.03	0.19 - 48.64	*2*	*		*1*	ǂ		ǂ	
A10 DRUGS USED IN DIABETES	0.88	0.53 - 1.47	*92*	1.48	0.59 – 3.74	*20*	0.88	0.45 - 1.74	ǂ	
A11 VITAMINS	0.57	0.27 - 1.25	*49*	0.38	0.12 – 1.18	*18*	0.22	0.08 - 0.58	0.07	0.01 - 0.71
A12 MINERAL SUPPLEMENTS	0.78	0.33 - 1.81	*34*	0.61	0.18 – 2.06	*13*	0.44	0.14 - 1.41	0.09	0.01 - 1.82
B01 ANTITHROMBOTIC AGENTS	1.07	0.56 - 2.04	*50*	1.11	0.40 – 3.13	*16*	0.69	0.28 - 1.73	0.41	0.01 - 16.38
B03 ANTIANEMIC PREPARATIONS	0.16	0.02 - 1.17	*20*	0.47	0.05 – 4.57	*4*	0.18	0.02 - 1.78	ǂ	
C01 CARDIAC THERAPY	3.05	0.61 - 15.21	*6*	*		*3*	1.74	0.15 - 20.88	*	
C02 ANTIHYPERTENSIVES	*		*1*			*0*	*			
C03 DIURETICS	1.27	0.60 - 2.70	*34*	0.39	0.08 – 1.93	*9*	1.31	0.47 - 3.63	3.79	0.09 - 165.29
C05 VASOPROTECTIVES	*		*4*	*		*2*	*		*	

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
C07 BETA-BLOCKING AGENTS	0.91	0.59 - 1.42	*128*	0.52	0.22 – 1.25	*28*	ǂ		0.02	0.00 - 0.33
C08 CALCIUM CHANNEL BLOCKERS	0.96	0.41 - 2.28	*29*	0.85	0.20 – 3.65	*8*	0.81	0.26 - 2.48	2.54	0.01 - 567.45
C09 AGENTS ACTING ON THE RENIN-ANGIOTENSIN SYSTEM	1.26	0.63 - 2.52	*41*	1.69	0.59 – 4.87	*15*	ǂ		5.23	0.15 - 185.79
C10 LIPID MODIFYING AGENTS	0.85	0.54 - 1.34	*121*	0.83	0.38 – 1.80	*32*	ǂ		0.48	0.07 - 3.46
D01 ANTIFUNGALS FOR DERMATOLOGICAL USE	1.42	0.57 - 3.53	*22*	0.95	0.15 – 5.79	*5*	ǂ		15.62	0.54 - 454.90
D02 EMOLLIENTS AND PROTECTIVES	1.35	0.65 - 2.78	*36*	1.47	0.56 – 3.89	*18*	ǂ		10.60	0.78 - 144.24
D05 ANTIPSORIATICS	1.51	0.14 - 16.76	*3*	*		*1*	1.69	0.10 - 29.06	*	
D06 ANTIBIOTICS AND CHEMOTHERAPEUTICS FOR DERMATOLOGICAL USE	3.07	0.98 - 9.63	*12*	1.44	0.28 – 7.30	*6*	2.03	0.43 - 9.50	5.79	0.11 - 309.69
D07 CORTICOSTERQDS, DERMATOLOGICAL PREPARATIONS	1.05	0.59 - 1.90	*62*	1.07	0.43 – 2.68	*21*	0.76	0.35 - 1.68	0.24	0.02 - 3.22

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
D10 ANTI-ACNE PREPARATIONS	1.01	0.20 - 5.03	*8*	*		*1*	ǂ		ǂ	
D11 OTHER DERMATOLOGICAL PREPARATIONS	2.02	0.34 - 12.18	*5*	*		*1*	ǂ		*	
G01 GYNECOLOGICAL ANTI-INFECTIVES AND ANTISEPTICS	2.02	0.34 - 12.18	*5*	1.43	0.09 – 23.12	*2*	2.92	0.32 - 26.37	*	
G02 OTHER GYNECOLOGICALS	3.03	0.19 - 48.64	*2*			*0*	12.50	0.27 - 571.09		
G03 SEX HORMONES AND MODULATORS OF THE GENITAL SYSTEM	1.14	0.58 - 2.25	*44*	1.27	0.44 – 3.64	*15*	0.63	0.25 - 1.59	0.20	0.02 - 2.71
G04 UROLOGICALS	0.62	0.23 - 1.65	*29*	0.28	0.03 – 2.40	*6*	0.42	0.13 - 1.36	0.01	0.00 - 0.40
H01 PITUITARY AND HYPOTHALAMIC HORMONES AND ANALOGUES	1.52	0.38 - 6.12	*9*	0.71	0.06 – 7.92	*3*	1.32	0.23 - 7.63	*	
H02 CORTICOSTERQDS FOR SYSTEMIC USE	2.63	1.16 - 5.96	*24*	3.53	0.89 – 14.09	*10*	2.69	0.72 - 10.04	2.18	0.08 - 61.27
H03 THYRQD THERAPY	0.87	0.41 - 1.86	*40*	0.81	0.31 – 2.16	*19*	0.35	0.12 - 1.03	0.07	0.01 - 0.69

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
H04 PANCREATIC HORMONES	*		*2*			*0*	*			
J01 ANTIBACTERIALS FOR SYSTEMIC USE	2.24	1.43 - 3.53	*89*	1.64	0.78 – 3.49	*33*	1.64	0.85 - 3.13	0.80	0.12 - 5.41
J02 ANTIMYCOTICS FOR SYSTEMIC USE	2.28	0.51 - 10.27	*7*	4.39	0.45 – 42.93	*4*	2.01	0.22 - 18.15	*	
J04 ANTIMYCOBACTERIALS	*		*1*			*0*	*			
J05 ANTIVIRALS FOR SYSTEMIC USE	*		*4*	*		*2*	*		*	
J07 VACCINES	*		*2*	*		*1*	*		*	
L01 ANTINEOPLASTIC AGENTS	1.51	0.14 - 16.76	*3*	1.43	0.09 – 23.12	*2*	*		ǂ	
L02 ENDOCRINE THERAPY	*		*2*	*		*2*	*		*	
L04 IMMUNOSUPPRESSANTS	*		*3*			*0*	*			
M01 ANTI-INFLAMMATORY AND ANTIRHEUMATIC PRODUCTS	1.39	0.78 - 2.48	*58*	2.17	0.79 – 5.97	*17*	ǂ		1.71	0.14 - 20.92

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
M03 MUSCLE RELAXANTS	0.60	0.07 - 5.18	*6*	0.47	0.05 – 4.57	*4*	0.18	0.01 - 3.79	0.17	0.00 - 75.28
M04 ANTIGOUT PREPARATIONS	*		*1*	*		*1*	*		ǂ	
M05 DRUGS FOR TREATMENT OF BONE DISEASES	*		*3*	*		*1*	*		ǂ	
N01 ANESTHETICS	*		*4*	*		*1*	*		ǂ	
N02 ANALGESICS	1.54	0.73 - 3.22	*33*	0.99	0.36 – 2.73	*17*	0.81	0.29 - 2.24	0.25	0.02 - 2.91
N07 OTHER NERVOUS SYSTEM DRUGS	1.60	0.81 - 3.19	*38*	1.27	0.44 – 3.64	*15*	5.55	0.27 - 116.05	0.26	0.02 - 3.35
P01 ANTIPROTOZOALS	3.04	0.43 - 21.70	*4*	*		*1*	0.44	0.14 - 1.46	ǂ	
R01 NASAL PREPARATIONS	1.12	0.46 - 2.69	*26*	2.20	0.61 – 8.14	*10*	0.84	0.41 - 1.71	0.69	0.05 - 8.90
R03 DRUGS FOR OBSTRUCTIVE AIRWAY DISEASES	1.42	0.84 - 2.42	*70*	1.49	0.61 – 3.61	*22*	0.54	0.08 - 3.76	ǂ	
R05 COUGH AND COLD PREPARATIONS	0.86	0.18 - 4.18	*9*	0.70	0.13 – 3.93	*6*	1.56	0.66 - 3.69	ǂ	

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
S01 OPHTHALMOLOGICALS	1.92	1.01 - 3.65	*42*	14.25	1.77 – 114.86	*10*	2.38	0.49 - 11.51	*	
S02 OTOLOGICALS	3.70	1.12 - 12.22	*11*	4.39	0.45 – 42.93	*4*	5.55	0.27 - 116.05	*	
Psychiatric comedication										
N03A ANTIEPILEPTICS	1.16	0.76 - 1.75	*132*	0.84	0.42 – 1.70	*42*	1.08	0.64 - 1.80	3.54	0.59 - 21.19
N04A ANTICHOLINERGIC AGENTS	0.97	0.65 - 1.44	*156*	074	0.37 – 1.50	*42*	0.74	0.44 - 1.25	1.98	0.28 - 13.94
N05B ANXIOLYTICS	1.08	0.80 – 1.45	*409*	0.95	0.54 – 1.67	*96*	0.81	0.49 - 1.34	1.14	0.31 - 4.24
N05C HYPNOTICS AND SEDATIVES	1.15	0.78 - 1.71	*151*	0.83	0.43 – 1.60	*50*	0.75	0.50 - 1.12	ǂ	
N06A ANTIDEPRESSANTS	0.83	0.61 - 1.15	*305*	1.00	0.57 – 1.78	*87*	1.67	0.51 - 5.52	0.29	0.09 - 1.02
N06B PSYCHOSTIMULANTS. AGENTS USED FOR ADHD AND NOOTROPICS	1.53	0.61 - 3.83	*21*	0.95	0.15 – 5.79	*5*	0.82	0.45 - 1.51	ǂ	

Table S1. *Continued*

	Univariate analysis						Multivariate analysis			
	N = 937			N = 196			N = 937		N = 196	
							Nagelkerke R^2: 0.301		Nagelkerke R^2: 0.676	
	OR	**95% C.I.**	***N***	**OR**	**95% C.I.**	***N***	**OR**	**95% C.I.**	**OR**	**95% C.I.**
R06A ANTIHISTAMINES FOR SYSTEMIC USE	1.10	0.69 - 1.74	*106*	0.83	0.38 - 1.80	*32*	1.08	0.64 - 1.80	0.76	0.13 - 4.46

*number too small to calculate an OR
not included in the multivariate analysis since the patients in the category none/unknown of variable 'Last antipsychotic - 135 days: continued after index date' are identic to those in the category none/unknown of variable 'Last antipsychotic - 135 days'
ǂ variable not included in the final prediction model
n.a.: not applicable

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3

The safety of clozapine in the perinatal setting

3.1

Safety of clozapine use during pregnancy: analysis of international pharmacovigilance data

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Abstract

Purpose

Safety data on clozapine use during pregnancy are limited. The aim of this study was to determine disproportionality in case safety reports on adverse pregnancy outcomes between clozapine and other antipsychotics (OAP) used during pregnancy.

Methods

We included all reports of suspected adverse drug reactions (ADRs) to antipsychotics registered in the World Health Organization global individual case safety report (ICSR) database (VigiBase) in children younger than 2 years and women aged 12-45 years. A case/non-case approach was used to evaluate the association between several pregnancy related ADRs and clozapine exposure during pregnancy, using 2x2 contingency tables to investigate disproportionality and Standard MedDRA Queries to select cases. Clozapine exposure was defined as all ICSR-ADR combinations with clozapine as (one of) the suspected drug(s). Non-exposure was defined as all ICSR-ADR combinations with OAP as (one of) the suspected drug(s).

Results

We identified 42,236 unique ICSR-ADR combinations related with clozapine exposure and 170,710 with OAP exposure. Of these, 494 and 4,645 ICSR-ADR combinations involved adverse pregnancy outcomes related with clozapine exposure and OAP exposure respectively. Overall, no signal of disproportionate reporting associating clozapine with the studied adverse pregnancy outcomes was found compared with OAP exposure.

Conclusions

Based on global pharmacovigilance data, we did not find any evidence that clozapine is less safe during pregnancy than OAP. Although this is not automatically equivalent to the relative safety of clozapine during pregnancy, these findings add to the convergence of proofs to allow final conclusions and decisions regarding the treatment of pregnant women with clozapine.

Introduction

The atypical antipsychotic drug clozapine is currently the only drug proven to be effective in patients with treatment-refractory schizophrenia. While it used to be under-prescribed and with a delayed onset of treatment [1,2], the importance of starting clozapine as soon as possible is gradually being recognized [3-5], and prescription rates have increased in recent years [6-8]. In the Netherlands, approximately one third of clozapine users are women of childbearing age [8]. Although fertility rates are generally lower in patients with schizophrenia than in healthy individuals of the same age [9], the fertility rate is increasing [10,11], reflected by the growing number of pregnancies in women taking atypical antipsychotics [12]. Safety data on the use of the other atypical antipsychotics quetiapine, olanzapine, and aripiprazole during pregnancy do not suggest that their use is associated with a clinically meaningful increased risk of congenital malformation [13]. However, there are few data on the risk of using clozapine during pregnancy [13]. Some anecdotal cases of congenital anomalies have been reported in association with clozapine use during pregnancy, such as a baby with gastroschisis and a horseshoe kidney [14] and a baby with a missing testicle [15], but specific patterns of anomalies have not been detected [16].

The increased prescription of clozapine and the improved fertility of women with schizophrenia make it essential to have additional pregnancy safety data. Reported adverse drug reactions (ADRs) are a valuable source of additional information about drug safety. Therefore, this study compared the frequency of reported adverse pregnancy outcomes after the use of clozapine versus other antipsychotics (OAP) during pregnancy, using the World Health Organization (WHO) global individual case safety report (ICSR) database, VigiBase [17].

Method

Data source

The Uppsala Monitoring Centre, in its role as the WHO Collaborating Centre for International Drug Monitoring, collects reports of suspected ADRs from national centres in countries participating in the WHO pharmacovigilance network. The information is stored in VigiBase, the world's largest pharmacovigilance database. The size and worldwide coverage of this database makes it particularly appropriate for exploring signals for comparatively rare events such as teratogenic events, stillbirths, and abortions. As of November 2017, VigiBase contained more than 16 million ICSRs from 130 collaborating countries and 26 associate countries [17,18]. ICSRs may be submitted by health professionals, patients, and manufacturers, depending on the reporting

strategies of the national pharmacovigilance centres and include information on patient characteristics, suspected ADRs, country of origin, and the drugs involved. An ICSR may contain information about multiple suspected drugs and multiple suspected ADRs, hence there are more drug-ADR combinations than ICSRs in the database. ADRs are coded according to the Medical Dictionary for Regulatory Authorities (MedDRA) terminology, which was developed to standardize the international medical terminology for regulatory activities [17,19]. MedDRA is a hierarchical system, starting with a very general level (the system organ classes) and ending with the more detailed preferred terms (PTs) which in turn are divided into the most specific level, namely, low-level terms (LLTs) [19].

Study population

We included all ICSRs registered in VigiBase since its establishment in 1968 until January 2018 concerning children younger than 2 years and women aged 12-45 years in which an antipsychotic drug was a suspected drug (ATC code N05A, excluding lithium (N05AN01), since lithium is not an antipsychotic and is not indicated for the treatment of schizophrenia and therefore lithium-users would represent another population). This age and gender selection were based on selection of either the affected child or the affected mother, since pregnancy outcomes can be reported for both the mother and the child in pharmacovigilance databases.

Case/non-case identification and exposure definition

In this study, a case/non-case approach was used to evaluate the association between several pregnancy-related adverse events and clozapine exposure during pregnancy. To facilitate the identification and retrieval of safety data, the International Conference of Harmonization has developed so-called Standardized MedDRA Queries (SMQs) [20]. In general, SMQs consist of preferred terms that have been grouped together, based on consistency with an overall medical condition or area of interest [20]. We used the following SMQs to select our cases: 'Pregnancy, labour and delivery complications and risk factors (excl. abortion and stillbirth' (SMQ 20000186), 'Termination of pregnancy and risk of abortion' (SMQ 20000192), 'Foetal disorders' (SMQ 20000190), 'Congenital, familial, and genetic disorders' (SMQ 20000077), and 'Neonatal disorders' (SMQ 20000191). Since the SMQ 'termination of pregnancy and risk of abortion' contains both the preferred terms 'spontaneous abortion' and 'induced abortion', we also used a modified MedDRA query, including only terms referring to spontaneous abortions.

In the included ICSRs, in which an antipsychotic was reported as a suspected drug, a case was defined as an ICSR-ADR combination with an ADR (a preferred term or low-level term) included in one of the SMQs mentioned above. Non-cases were all included ICSR-ADR combinations without ADRs of the SMQs of interest.

In addition, all ICSR-ADR combinations with an ADR included in the SMQ 'Congenital, familial, and genetic disorders' were analysed at the PT/LLT level for each ADR reported. Some ADRs included in this SMQ, such as haemoglobinopathy or dolichocolon, are not unambiguously related to a pregnancy, but are also used to report an adverse event in the actual user. Therefore, when reported for a woman aged 12-45 years, other characteristics of the individual ICSR were studied in more detail to determine final case selection.

Clozapine exposure was defined as all ICSR-ADR combinations in which the reporter had designated clozapine as (one of) the suspected drug(s). Other antipsychotic exposure was defined as all ICSR-ADR combinations with OAPs as suspected drugs. ICSRs with both clozapine and OAP as suspected drugs were defined as clozapine-exposed ICSRs.

Data analysis

Demographic data for the ICSRs were analysed using descriptive statistics.

In general, if the proportion of an ADR is greater in patients exposed to a drug or group of drugs than in patients not exposed to this drug, this suggests an association between the specific drug and the reaction and is a signal for a potential safety issue. In our study, the unit of analysis was the unique combination of the report (ICSR) and the reported suspected ADR (MedDRA code). To identify ICSR-ADR pairs with the adverse pregnancy outcomes of interest that were reported more frequently for ICSRs with clozapine as (one of) the suspected drug(s) than for ICSRs with OAP as (one of) the suspected drug(s), the reporting odds ratio (ROR) was used as a measure of disproportional reporting by assessing 2x2 contingency tables [21] (Figure 1).

	Case	Non-case
Clozapine as (one of) the suspected drug(s)	a	b
OAP as (one of) the suspected drug(s)	c	d

Figure 1. 2 x 2 contingency table for calculation of the ROR as a measure of disproportional reporting using the following formula: ROR = (a/b)/(c/d). In the analysis based on SMQs, cases are defined as ICSR-ADR pairs with an ADR included in one of the SMQs of interest. In the second analysis, cases are defined as ICSR-ADR pairs with an ADR included in the SMQ 'Congenital, familial and genetic disorders'.

This is a validated method of safety signal detection [22] and, in this study, it provided an estimate of the extent to which adverse pregnancy outcomes were reported in association with clozapine exposure compared with exposure to other antipsychotics. The ROR was defined as the ratio between proportions of reports in the "case" (reports containing the adverse pregnancy outcomes of interest) and in the "non-case" (reports containing other ADRs, without the outcomes of interest) group that are associated with clozapine exposure and with OAP exposure. A signal of disproportionate reporting was defined when the lower limit of the 95% two-sided CI for the ROR exceeded the threshold value of 1. [21] The results are presented as the RORs with the corresponding 95% confidence intervals (95%CI).

The detection of a signal of disproportionate reporting can be hampered by high frequencies of reports of events known to be strongly associated with a drug. Since clozapine was temporarily taken off the market in most European countries in 1975 after reports of life-threatening agranulocytosis in Finland shortly after it had been introduced on the Finnish market [23], it is conceivable that there is a greater alertness to adverse reactions with clozapine use in general and specifically with regard to reports of blood dyscrasia. Hence, in our study, when there is a large number of reports related to for example leukopenia, the reporting rate for other events for clozapine is mathematically reduced. To circumvent this potential masking effect, we first identified the adverse events that defined approximately 10% of the total number of ICSR-ADR combinations for clozapine. Then we removed these ICSR-ADR combinations and recalculated the RORs based on the SMQs. The same was done for the adverse events defining approximately 10% of the total number of ICSR-ADR combinations for OAP exposure.

Taking into account the complex marketing history of clozapine, a sensitivity analysis restricted to cases reported to VigiBase from 1990, the year in which clozapine was granted access to the United States' market, and onwards has also been performed.

To search for possible trends in the extent to which a specific ADR of the SMQ 'Congenital, familial, and genetic disorder' had been reported in association with clozapine exposure, the RORs of the reported ADRs were also calculated with their 95%CIs.

All statistical analyses were conducted using SPSS Statistics version 24.0.

Results

All reports

We identified a total of 18,448 unique ICSRs in which clozapine was (one of) the suspected drug(s), and 67,991 unique ICSRs in which an OAP was (one of) the suspected drug(s) (Table 1), with on average 2.3 ADRs reported per ICSR with clozapine as (one of) the suspected drug(s) and 2.5 ADRs per ICSR with OAP as (one of) the suspected drug(s).

Table 1. Characteristics of the unique individual case safety reports with clozapine or other antipsychotics as (one of the) suspected drug(s)

	Children aged < 2 years				**Women aged 12-45 years**			
	CLZ (N (%))		OAP (N (%))		CLZ (N (%))		OAP (N (%))	
Total number of unique ICSRs	125		1,426		18,323		66,565	
Total number of unique ICSR-ADR combinations	422		4,214		41,814		166,496	
Mean number of ADRs per ICSR	3.4		3.0		2.3		2.5	
Gender								
Male	59	(47%)	735	(52%)	n.a.		n.a.	
Female	53	(42%)	550	(39%)	18,323	(100%)	66,565	(100%)
Missing	13	(10%)	141	(10%)	n.a.		n.a.	
Age group								
0-28 days	60	(48%)	827	(58%)	n.a.		n.a.	
28 days – 23 months	65	(52%)	599	(42%)	n.a.		n.a.	
12-17 years	n.a.		n.a.		636	(3%)	5,631	(8%)
18-45 years	n.a.		n.a.		17,687	(97%)	60,934	(92%)
Reporting region								
European region	82	(66%)	789	(55%)	7,809	(43%)	21,538	(32%)
Region of the Americas	24	(19%)	349	(24%)	7,040	(38%)	29,152	(44%)

Table 1. *Continued*

	Children aged < 2 years				Women aged 12-45 years			
	CLZ (N (%))		OAP (N (%))		CLZ (N (%))		OAP (N (%))	
Western Pacific region	16	(13%)	260	(18%)	3,169	(17%)	10,778	(16%)
South-East Asia region	1	(1%)	17	(1%)	268	(1%)	4,235	(6%)
African region	1	(1%)	3	(0%)	23	(0%)	564	(1%)
Eastern Mediterranean region	1	(1%)	8	(1%)	14	(0%)	298	(0%)
Reporting period								
<1990	0	(0%)	70	(5%)	122	(1%)	4,789	(7%)
1990-2000	10	(8%)	81	(6%)	4,497	(25%)	5,971	(9%)
2000-2010	31	(25%)	246	(17%)	5,741	(31%)	15,299	(23%)
>2010	84	(67%)	1,029	(72%)	7,963	(43%)	40,506	(61%)

Abbreviations list:
ADR adverse drug reaction
CLZ clozapine
ICSRs individual case safety reports
n.a. not applicable
OAP other antipsychotics

Most of the reports originated from Europe, followed by the Americas. Few reports with clozapine as (one of the) suspected drug(s) originated from before 1990, the year when clozapine was introduced in the United States.

In only 6.3% (n=2,660) of the 42,236 unique ICSR-ADR combinations in which clozapine was reported as suspected drug, there was also an OAP reported as a suspected drug.

Pregnancy-related adverse events

The associations between the SMQs of interest and exposure to clozapine or an OAP are presented in Table 2.

Table 2. Number of individual case safety report-adverse drug reaction combinations for adverse pregnancy outcomes grouped by Standard MedDRA Query, with reporting odds ratios for ICSR-ADR combinations with clozapine versus other antipsychotics as suspected drug(s)

	Children aged < 2 years				Women aged 12-45 years				Combined population (children aged < 2 years and women aged 12-45 years)			
Standard MedDRA Queries (SMQ)	**CLZ (n†=422)**	**OAP (n†=4,214)**	**ROR**	**(95% CI)**	**CLZ (n†=41,814)**	**OAP (n†=166,496)**	**ROR**	**(95% CI)**	**CLZ (n†=42,236)**	**OAP (n†=170,710)**	**ROR**	**(95% CI)**
Pregnancy, labour and delivery complications and risk factors (excl. abortions and stillbirth) (20000186)	34	405	0.82	0.57 – 1.19	206	1770	0.46	0.40 – 0.53	**240**	**2175**	**0.44**	**0.39 – 0.51**
Termination of pregnancy and risk of abortion (20000192)	0	21	n.a.		64	440	0.58	0.45 – 0.75	**64**	**461**	**0.56**	**0.43 – 0.73**
Modified MedDRA query based on Termination of pregnancy and risk of abortion	0	17	n.a.		63	396	0.63	0.49 – 0.83	**63**	**413**	**0.62**	**0.47 – 0.80**

Table 2. *Continued*

	Children aged < 2 years				Women aged 12-45 years				Combined population (children aged < 2 years and women aged 12-45 years)			
Foetal disorders (20000190)	14	123	1.14	0.65 – 2.00	24	103	0.93	0.60 – 1.45	**38**	**226**	**0.68**	**0.48 – 0.96**
Congenital, familial and genetic disorders (20000077)	38	511	0.72	0.51 – 1.01	38	313	0.48	0.35 – 0.68	**76**	**824**	**0.37**	**0.29 – 0.47**
Neonatal disorders (20000191)	57	856	0.61	0.46 – 0.82	19	103	0.73	0.45 – 1.20	**76**	**959**	**0.32**	**0.25 – 0.40**

† n total number of unique ICSR-ADR combinations

Abbreviations list:

ADR adverse drug reaction
CLZ clozapine
ICSRs individual case safety reports
n.a. not applicable
OAP other antipsychotics
SMQ Standard MedDRA Queries

In total, 494 ICSR-ADR combinations were found involving adverse pregnancy outcomes with clozapine as (one of) the suspected drug(s) and 4,645 ICSR-ADR combinations with OAP as suspected drug(s). Overall, no signal of disproportionate reporting associating clozapine exposure with 'Pregnancy, labour and delivery complications and risk factors', 'Termination of pregnancy and risk of abortion', 'Foetal disorders', 'Congenital, familial, and genetic disorders', and 'Neonatal disorders' was found compared with OAP exposure. Moreover, in the combined population of children younger than 2 years and women aged 12-45 years, clozapine was statistically significantly less often associated with all the pregnancy-related adverse outcomes than OAP exposure.

To circumvent a potential masking effect resulting from high frequencies of reports of events known to be strongly associated with clozapine treatment, the adverse events that defined approximately 10% of the total number of ICSR-ADR combinations for clozapine were identified: Leukopenia (4.6%), Neutropenia (3.8%), Tachycardia (3.1%) and Granulocytopenia (2.4%). No signal of disproportionate reporting associating clozapine with one of the adverse pregnancy events was unmasked after removal of these ICSR-ADR combinations. The same was done for the adverse events defining approximately 10% of the total number of ICSR-ADR combinations for OAP exposure (i.e., Extrapyramidal disorder (1.6%), Somnolence (1.4%), Dystonia (1.3%), Tremor (1.1%), Weight increased (1.1%), Suicide attempt (1.0%), Diabetes mellitus (1.0%) and Insomnia (1.0%)). This did not influence our findings either.

The results of the sensitivity analysis restricted to ICSRs reported since 1990 are presented in Table 3. The results are almost identical to the analysis of the ICSRs reported to VigiBase since its establishment in 1968 and onwards.

Table 3. Analysis of the number of individual case safety report-adverse drug reaction combinations for adverse pregnancy outcomes grouped by Standard MedDRA Query reported in VigiBase from 1990 onwards, with reporting odds ratios for ICSR-ADR combinations with clozapine versus other antipsychotics as suspected drug(s)

	Children aged < 2 years				**Women aged 12 – 45 years**				**Combined population (children aged < 2 years and women aged 12 – 45 years)**			
Standard MedDRA Queries (SMQ)	**CLZ (n†=422)**	**OAP (n†=4,094)**	**ROR**	**(95% CI)**	**CLZ (n†=41,628)**	**OAP (n†=158,415)**	**ROR**	**(95% CI)**	**CLZ (n†=42,050)**	**OAP (n†=162,509)**	**ROR**	**(95% CI)**
Pregnancy, labour and delivery complications and risk factors (excl abortions and stillbirth) (20000186)	34	405	0.80	0.55 – 1.15	206	1769	0.44	0.38 – 0.51	**240**	**2174**	**0.42**	**0.37 – 0.48**
Termination of pregnancy and risk of abortion (20000192)	0	21	n.a.		63	427	0.56	0.43 – 0.73	**63**	**448**	**0.54**	**0.42 – 0.71**

Table 3. *Continued*

	Children aged < 2 years				Women aged 12 – 45 years				Combined population (children aged < 2 years and women aged 12 – 45 years)			
Modified MedDRA query based on Termination of pregnancy and risk of abortion	0	17	n.a.		62	383	0.62	0.47 – 0.81	**62**	**400**	**0.60**	**0.46 – 0.78**
Foetal disorders (20000190)	14	123	1.11	0.63 – 1.94	24	101	0.90	0.58 – 1.41	**38**	**224**	**0.66**	**0.47 – 0.92**
Congenital, familial and genetic disorders (20000077)	38	501	0.71	0.50 – 1.00	38	221	0.65	0.46 – 0.92	**76**	**722**	**0.41**	**0.32 – 0.51**
Neonatal disorders (20000191)	57	849	0.60	0.45 – 0.80	19	95	0.76	0.47 – 1.25	**76**	**944**	**0.31**	**0.25 – 0.39**

† n total number of unique ICSR-ADR combinations

Abbreviations list:

ADR adverse drug reaction
CLZ clozapine
ICSRs individual case safety reports
n.a. not applicable
OAP other antipsychotics
SMQ Standard MedDRA Queries

Congenital, familial, and genetic disorders

Table 4 lists the identity, number of reports, and RORs of the 76 ADRs of the SMQ 'Congenital, familial, and genetic disorders' grouped by preferred term. These ADRs were reported in 54 unique ICSRs.

Table 4. Identity, number reported, and reporting odds ratios of the adverse drug reactions of Standard MedDRA Query 'Congenital, familial, and genetic disorders' grouped by preferred term

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Atrial septal defect	8	75	0.43	0.21 – 0.89	2 clozapine-exposed ICSRs also reported a ventricular septal defect, of which 1 also reported anal atresia, cryptorchism and vitello-intestinal duct remnant and the other also reported citalopram and lithium as suspected drugs. In 1 clozapine-exposed ICSR levetiracetam and lamotrigine were also reported as suspected drugs. In 1 clozapine-exposed ICSR clomipramine was also reported as a suspected drug. 2 clozapine-exposed ICSRs also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': increased drug level, agitation, arrhythmia, cardiomegaly and confusional state, possibly referring to ADRs in a female user and not in a newborn child. In these ICSRs, topiramate was also reported as a suspected drug. Since the information in these 2 ICSRs were the same, these ICSRs were regarded as duplicate ICSRs. In 1 clozapine-exposed ICSR coarctation of the aorta, aorta hypoplasia and patent ductus arteriosus were also reported In 1 clozapine-exposed ICSR patent ductus arteriosus was also reported

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Congenital anomaly	7	39	0.73	0.32 – 1.62	In 3 of the 7 clozapine-exposed ICSRs the anomaly was also specified by one or more other MedDRA code(s): 1 ICSR also reported ear malformation 1 ICSR also reported hypospadias, congenital foot malformation and congenital hand malformation 1 ICRS also reported cleft palate
Ventricular septal defect	6	42	0.58	0.25 – 1.36	2 clozapine-exposed ICSRs also reported an atrial septal defect, of which 1 also reported anal atresia, cryptorchism and vitello-intestinal duct remnant and the other also reported citalopram and lithium as suspected drugs. 1 clozapine-exposed ICSR also reported VACTERL syndrome 1 clozapine-exposed ICSR also reported amitriptyline as a suspected drug 1 clozapine-exposed ICSR also reported aripiprazole as a suspected drug
Dysmorphism	4	18	0.90	0.30 – 2.65	3 of the 4 reported dysmorphisms referred to the same ICSR, reporting Dysmorphism as a PT, but also as the related LLTs 'Facial dysmorphism' and 'Flat philtrum'. In this ICSR valproic acid and propranolol were also reported as one of the suspected drugs and cryptorchism was also reported as an ADR. The other clozapine-exposed ICSR also reported abnormal palmar/plantar creases as an ADR and quetiapine, opipramol, simvastatin, pantoprazole and ziprasidone as suspected drugs.

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Cryptorchism	3	9	1.35	0.36 – 4.98	In 1 clozapine-exposed ICSR dysmorphism was also reported.
Patent ductus arteriosus	3	14	0.87	0.25 – 3.01	In 1 clozapine-exposed ICSR atrial septal defect was also reported. In 1 clozapine-exposed ICSR coarctation of the aorta, aorta hypoplasia atrial septal defect were also reported
Huntington's disease †	2†	2	4.04	0.57 – 28.70	
Congenital foot malformation	2	6	1.35	- 6.68	In 1 clozapine-exposed ICSRS carbamazepine was also reported as one of the suspected drugs
Microcephaly	2	8	1.01	0.21 – 4.76	In 1 clozapine-exposed ICSR valproic acid was also reported as one of the suspected drugs
Sickle cell anaemia with crisis †	2†	2	4.04	0.57 – 28.69	1 clozapine-exposed ICSR also reported the following ADR not included in the SMQ 'Congenital, familial and genetic disorders': gastrointestinal pain 1 clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': neutrophil count increased and white blood cell count increased
Cleft palate	2	24	0.34	0.08 – 1.43	1 clozapine-exposed ICSR also reported 'congenital anomaly' as an ADR
Aorta hypoplasia	2	1	8.08	0.73 – 89.16	1 clozapine-exposed ICSR also reported coarctation of the aorta, atrial septal defect and patent ductus arteriosus 1 clozapine-exposed ICSR also reported bicuspid aorta valve
Vascular malformation	2	2	4.04	0.57 – 28.70	

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Abnormal palmar/plantar creases	1	1	4.04	0.25 – 64.62	The clozapine-exposed ICSR also reported dysmorphism as an ADR and quetiapine, opipramol, simvastatin, pantoprazole and ziprasidone as suspected drugs.
Melkersson-Rosenthal syndrome †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': apathy, claustrophobia, depressed mood, drooling, increased appetite, malaise, thinking abnormal and weight abnormal.
Pulmonary hypoplasia	1	2	2.02	0.18 – 22.29	The clozapine-exposed ICSR also reported renal aplasia and renal hypoplasia
Hypospadias	1	16	0.25	- 1.90	The clozapine-exposed ICSR also reported congenital anomaly, congenital foot malformation and congenital hand malformation
Renal aplasia	1	4	1.01	0.11 – 9.04	The clozapine-exposed ICSR also reported pulmonary hypoplasia and renal hypoplasia
Renal hypoplasia	1	0	n.e.	n.e.	The clozapine-exposed ICSR also reported renal aplasia and pulmonary hypoplasia
Hepato-lenticular degeneration †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': anaemia, choreoathetosis, constipation, movement disorder, thrombocytopenia.
Congenital hydrocephalus	1	1	4.04	0.25 – 64.62	
Congenital nystagmus	1	2	2.02	0.18 – 22.29	

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Congenital hand malformation	1	10	0.40	0.05 – 3.16	
Talipes	1	42	0.10	0.01 – 0.70	The clozapine-exposed ICSR also reported flupentixol, sertraline and promethazine as suspected drugs
Scaphocephaly	1	0	n.e.	n.e.	The clozapine-exposed ICSR also reported cyamemazine and oxazepam as suspected drugs
Congenital musculoskeletal anomaly	1	15	0.27	0.04 – 2.04	
Porphyria †	1†	3	1.35	0.14 – 12.95	
Sickle cell trait †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': iron deficiency, serum ferritin decreased and viral infection
Anal atresia	1	3	1.35	0.14 – 12.95	The clozapine-exposed ICSR also reported atrial septum defect, cryptorchism, ventricular septal defect and vitello-intestinal duct remnant.
Gastroschisis	1	2	2.02	0.18 – 22.29	The clozapine-exposed ICSR also reported paroxetine as a suspected drug
Dolichocolon †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': volvulus, abdominal pain, constipation and megacolon.
Vitello-intestinal duct remnant	1	0	n.e.	n.e.	

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Gastrointestinal malformation	1	4	1.01	0.11 – 9.04	The clozapine-exposed ICSR also reported lithium as a suspected drug
Colour blindness	1	3	1.35	0.14 – 12.95	
Ear malformation	1	10	0.40	0.05 – 3.16	The clozapine-exposed ICSR also reported 'congenital anomaly' as an ADR
VACTERL syndrome	1	0	n.e.	n.e.	The clozapine-exposed ICSR also reported ventricular septum defect as an ADR
Trisomy 21	1	9	0.45	0.06 – 3.55	
Heart disease congenital	1	23	0.18	0.02 – 1.30	
Atrioventricular septal defect	1	1	4.04	0.25 – 64.62	
Hypertrophic cardiomyopathy	1	0	n.e.	n.e.	
Bicuspid aortic valve	1	0	n.e.	n.e.	The clozapine-exposed ICSR also reported aorta hypoplasia
Coarctation of the aorta	1	4	1.01	0.11 – 9.04	The clozapine-exposed ICSR also reported aorta hypoplasia, atrial septal defect and patent ductus arteriosus
Haemoglobinopathy †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': neutrophil count decreased, neutropenia and white blood cell count decreased.

Table 4. *Continued*

	Reported number				
Preferred Term	**CLZ (N)**	**OAP (N)**	**ROR**	**95%CI**	**Note**
Thalassaemia †	1†	0	n.e.	n.e.	The clozapine-exposed ICSR also reported the following ADRs not included in the SMQ 'Congenital, familial and genetic disorders': lymphocyte count increased, mean cell haemoglobin, platelet count increased, red blood cell count increased and red cell distribution width increased.

† Possible misclassification due to inclusion of an ADR or condition in a clozapine user / not applicable to perinatal use
Abbreviations list:
ADR adverse drug reaction
CI confidence interval
CLZ clozapine
ICSRs individual case safety reports
n.e. not executable (due to 0 ICSR-ADR combinations for OAP ICSRs)
OAP other antipsychotics
ROR reporting odds ratio
SMQ Standard MedDRA Queries

On closer inspection, 11 of the 76 ICSR-ADR pairs that were associated with clozapine exposure reporting ADRs of the SMQ 'Congenital, familial, and genetic disorders' were thought to be related to adverse events observed in a clozapine user or related to events for which clozapine was indicated, rather than an effect seen in the offspring of a clozapine user due to perinatal exposure. Two duplicate safety reports were identified, both describing 'atrial septal defect'. These two duplicate ICSRs were also thought to refer to ADRs in a female user instead of in an infant exposed to clozapine during pregnancy.
Atrial septal defect (ASD) (n=8), *Congenital anomaly* (n=7), and *Ventricular septal defect (VSD)* (n=6) were the most frequently reported ADRs for ICSRs with clozapine as (one of) the suspected drug(s), but these ADRs were relatively equally (congenital anomaly and VSD) or even more (ASD) often associated with OAP exposure.

Discussion

This study provides an overview of all spontaneously reported ADRs worldwide (1968-2018) that are associated with antipsychotic use during pregnancy. Our

main finding is that, based on data from this large pharmacovigilance database, we did not detect any signal of disproportionate reporting of pregnancy-related adverse events associated with clozapine exposure compared with exposure to other antipsychotics. We used SMQs, which combine multiple ADRs related to one specific topic, to select safety reports about pregnancy, labour, and delivery complications, foetal and neonatal disorders, risk of stillbirth and abortion, and congenital disorders. SMQs provide a standard and validated tool for signal detection [20]. To look for possible trends and patterns in the reporting of specific ADRs regarding congenital anomalies, we also examined these related ICSRs in more detail. Again, we did not detect an increased frequency of safety reports with clozapine rather than an OAP as (one of the) suspected drugs(s). In fact, in the combined population of children younger than 2 years and women aged 12-45 years, we even found statistically significant lower frequencies of reports with clozapine than with OAP as suspected drug(s) for all six pregnancy-related SMQs, suggesting that clozapine is less likely than other antipsychotic drugs to be related to adverse pregnancy outcomes. These lower reporting frequencies cannot be explained by a mathematical reduction in the number of reports of pregnancy-related adverse events by other adverse events with high reporting frequencies, since we have excluded this possible masking effect. Yet, it is important to emphasize that, unlike an odds ratio (OR), the ROR is not a direct risk measurement, but rather reflects imbalance in reporting frequency of a drug-associated adverse event in comparison with other events associated with the same drug. Thus, when interpreting the value of the reporting odds ratio, one should bear in mind that the true number of "exposed" and "non-exposed" patients is not available and instead the number of reports is being used as nominator and denominator, which is subject to reporting bias. In general, the number of reports associated with a drug may be influenced by the extent of its use, publicity, the nature of the reactions, and other factors. Due to clozapine's reputation as a useful but potentially harmful medicine [6], it is possible that clozapine has been used in fewer pregnancies than OAP, which could be an explanation for the significantly lower reporting frequencies of adverse pregnancy events associated with clozapine exposure. On the other hand, while we do not know if women using clozapine are more likely to have more unplanned pregnancies than women using OAP, we do know that women with psychotic disorders in general are likely to have more unplanned pregnancies than women without psychotic disorders [24,25] and thus may not consciously weigh the risks of using a potentially harmful drug. In addition, the relatively large number of ICSRs with clozapine as a suspected drug (n = 18,448) compared to the total number of ICSRs with OAP as a suspected drug (n = 67,991), does not correspond to the small proportion of actual clozapine users compared to OAP users worldwide. Owing to clozapine's stigma, there is a great awareness and willingness to report case safety data for

clozapine. Thus, although we cannot completely rule out that a smaller number of pregnancies exposed to clozapine may have influenced our findings, we believe that if this is the case, this will be, at least partly, compensated by the higher willingness to report safety data of clozapine.
Nevertheless, these lower RORs could be subject to further investigation in direct comparative studies.

Our findings are in concordance with the conclusions of Mehta and Van Lieshout, who could not detect specific patterns of anomalies [16]. They concluded that, although the evidence regarding the safety and efficacy of clozapine use during pregnancy is still very limited, the risk of congenital anomalies did not appear to exceed that of the general population.

Although disproportionality analysis of pharmacovigilance data is able to give valuable information on rare and/or nonspecific ADRs and drug safety [22], there are some important precautions. In addition to the aforementioned possibility of reporting bias, it should be borne in mind that the reports submitted to pharmacovigilance centres generally describe no more than a suspicion arisen from an observation of an unexpected or unwanted event. The reports may be incomplete and the evidence for the causality of associations is not the same in all reports and is often even lacking. So, results should be interpreted with caution, which makes it difficult to draw definite conclusions. Also, the available information about the reports is not unlimited. In our study, it would have been informative to distinguish between adult women (aged 18-45 years) and adolescent women (aged 12 – 18 years), but unfortunately nowadays VigiBase only provides the variable age as age-categories. In any case, we believe that the use of this age category, including adolescents is meaningful because adolescent women can become pregnant as well. Also, this study method did not allow for adjustment for other confounding factors. Exposure to antipsychotics during pregnancy is inevitably linked with exposure to maternal illness, and schizophrenia as such has also been associated with several adverse obstetric complications and pregnancy outcomes [26]. Other concomitant factors, such as low dietary vitamin intake, poor nutrition, reduced serum folate levels related to poor antenatal care, smoking, and alcohol and drug abuse, make it extremely difficult to separate the contribution of antipsychotics from the influence of these potentially confounding factors [27,28]. To reduce the impact of these concomitant factors as much as possible, we explicitly selected exposure to OAP as our comparator group, thereby creating a comparator group exposed to conditions that are most similar to those of patients in the clozapine-exposed ICSRs. Moreover, due to clozapine's position in the treatment algorithm, clozapine is used by the severely ill and is almost always the drug of last resort. Consequently, it is not a question of whether or

not to treat the pregnant mother, but what is the least harmful treatment in this situation for both the mother and the unborn child. Therefore, the comparator group did not consist of all other reports in VigiBase for our population, but of the theoretically available alternative of treatment with other antipsychotics. Finally, as can be seen from table 4, some ICSR-ADR combinations may have been erroneously selected as cases, owing to the inclusion of preferred terms in the SMQ 'Congenital, familial and genetic disorder' that are not unambiguously related to a pregnancy. In other words, when reported for a woman aged 12-45 years, some ADRs of this SMQ may refer to an adverse event (such as dolichocolon) associated with use of the drug rather than a congenital disorder due to in utero exposure to the drug. Since we studied the 76 ICSR-ADR combinations potentially associated with clozapine exposure in detail, but not the 824 ICSR-ADR combinations potentially associated with OAP exposure, we could not calculate an adjusted ROR. However, based on the ROR for the population of children < 2 years, it seems justified to expect that adjustment for inclusion of ICSRs related to observed adverse events in the actual users will not essentially change our results.

The therapeutic benefit of clozapine in treatment-resistant schizophrenia, is beyond doubt. However, little is known about the safety of using various neuroleptic agents during pregnancy, since this information is, understandably, lacking from randomized controlled trials. As with all pregnant women or women who are contemplating pregnancy, the risks and benefits of medical treatment have to be weighed carefully, and perhaps particularly so in women on clozapine, for whom it is likely that they have not responded adequately to other antipsychotic drugs and are unlikely to be able to do without them. Discontinuing clozapine could risk her not being able to effectively parent her child, with all the consequences that this entails.

To the best of our knowledge, this is the first study to use global pharmacovigilance data to estimate the extent to which pregnancy-related adverse events have been reported in association with clozapine as (one of the) designated suspected drug(s) compared with reports with an OAP as suspected agent. Despite its inherent limits, disproportionality analysis in pharmacovigilance databases is a valuable tool for drug safety research and surveillance, although this kind of approach should only be considered as exploratory to generate signals. Finding of a disproportionality ratio for a drug does not imply a higher risk of ADR occurrence in absolute terms and should lead to further investigation. Vice versa, in our study, the absence of a higher proportional reporting frequency is not automatically equivalent to the relative safety of clozapine during pregnancy. On the other hand, we did not find any evidence that clozapine is less safe during pregnancy than the other

antipsychotics either. This summary of pharmacovigilance data is of added value for the convergence of proofs to allow final conclusions and decisions regarding the treatment of pregnant women with clozapine.

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3.2

Clozapine treatment during pregnancy and the postpartum period: a systematic literature review

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Abstract

Objective

The objective of this systematic review was to provide a critical appraisal of the evidence related to the safety of clozapine for schizophrenia during pregnancy and lactation.

Data sources

Pubmed/Medline, Embase, and the Cochrane Library were searched from inception through December 2020. Reference lists of included studies were hand-searched. The International Clinical Trials Registry Platform and ClinicalTrials.gov were searched for unpublished trials and PROSPERO for unpublished reviews. The current marketing authorization holder of the originator brands Clozaril® and Leponex® was also contacted for pharmacovigilance data.

Study selection

Original reports published in English, German, French, or Dutch containing clinical and preclinical data were included if they provided data on maternal, fetal, and neonatal outcomes after clozapine exposure during pregnancy or lactation.

Data extraction

Two reviewers independently extracted relevant data.

Results

A total of 860 records were identified, and the full texts of 117 articles were reviewed. Forty-two studies met the inclusion criteria. Data on perinatal clozapine exposure are of limited quality and quantity. Although clozapine demonstrates partial placental passage, data thus far do not support that clozapine is teratogenic, increases the risk of stillbirth, abortion, or fetal disorders, nor that it increases the risk of delivery complications or premature birth. Information about clozapine exposure through breast milk is scarce, but based on its chemical properties, it is likely that clozapine enters the breast milk of nursing mothers taking clozapine.

Conclusions

When outweighing the risk and benefits of clozapine continuation during pregnancy and lactation versus switching to another antipsychotic, one should include severity of illness and treatment history, but also be aware of the limitations of the available safety data regarding perinatal clozapine use, including the fact that there are few studies.

https://www.crd.york.ac.uk/prospero - CRD42015032475

Introduction

Clozapine is the prototype of what has become the group of so-called atypical antipsychotics. [1] Although the number of atypical antipsychotics available has increased over the past decades, clozapine remains the only one with proven efficacy for treatment-resistant schizophrenia. [2,3] However, concerns over its side effects, which include agranulocytosis, cardiomyopathy, bowel obstruction, weight gain, sialorrhea, and sedation, have limited its use and have led to clozapine being considered the treatment of 'last resort'. To ensure that the benefits of clozapine outweigh the risk of severe neutropenia, the Food and Drug Administration has established a program called the Clozapine Risk Evaluation and Mitigation Strategy that defines the conditions for prescribing, dispensing, and using clozapine, including mandatory testing of the white blood cell count. [4]

Yet, in recent years there is a growing perception that clozapine is underused and the number of patients using clozapine is increasing. [5-8] Since schizophrenia has a peak age at onset among women in their childbearing years [9], it can be expected that, over time, the number of pregnant women treated with clozapine will increase. Although clozapine can pass the placental barrier, exposing the fetus to the drug, not treating psychosis during pregnancy and postpartum may prove harmful to both mother and child. [10-12]

While safety data suggest that antipsychotics in general are safe in pregnancy, [10,13,14] there is little literature on the safety of clozapine use during pregnancy and the postpartum period. [15] Switching to another antipsychotic for which more safety data are available is rarely an option. The objective of this study was to provide a critical appraisal of the evidence related to the safety of clozapine for schizophrenia during pregnancy and lactation, focusing on maternal, foetal, and neonatal outcomes.

Method

This study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [16] following a protocol registered with PROSPERO (registration number *CRD42015032475*).

Inclusion criteria

Eligible studies were original reports containing clinical data, including case series and case reports, on clozapine treatment during pregnancy and lactation. Animal studies describing prenatal exposure to clozapine were also eligible for inclusion. Review articles were used for cross reference checks only. Articles

not written in English, German, French, or Dutch were excluded. No year limits were applied.

Search strategy

An electronic literature search was performed in Pubmed/Medline, Embase, and the Cochrane Library in February 2019 and updated in December 2020. The Pubmed/Medline search strategy has been included as an appendix (Appendix 1) and was adapted accordingly for use in the other databases.
Two authors (MB and AVG) independently reviewed all titles and abstracts identified by the electronic searches. Articles were selected for full-text review if the two authors agreed that the inclusion criteria were met or if there was uncertainty about this. Again, full-text articles not fulfilling the inclusion criteria were removed. The reference lists of the included articles were searched for any further literature not identified in the initial database search. Additionally, the International Clinical Trials Registry Platform (ICTRP) and ClinicalTrials.gov were searched for unpublished trials and PROSPERO for unpublished reviews. Lastly, the current marketing authorization holder of the originator brands Clozaril® and Leponex® (Mylan Products Ltd) was contacted for pharmacovigilance data.

Data extraction

Data on study design, eligibility criteria, number of patients (i.e., number of foetuses or neonates or mothers), demographic information of the mother, exposure information, follow-up period and reported maternal, foetal, or neonatal outcomes were extracted.

Results

The search yielded 262 articles from PubMed, 771 from Embase, 1 from the Cochrane Library, and 9 from the reference list check. After the elimination of duplicate studies, 860 articles remained. Of these, 117 articles were considered eligible for full-text screening; 75 articles were subsequently excluded (see flow diagram in Figure 1 [16]). The ICTRP search portal, ClinicalTrials.gov and PROSPERO did not reveal any ongoing or recently completed trials or reviews and the contacted manufacturer did not provide additional pharmacovigilance data. The findings are presented below in outcomes related to the mother, foetus (including delivery), and neonate.

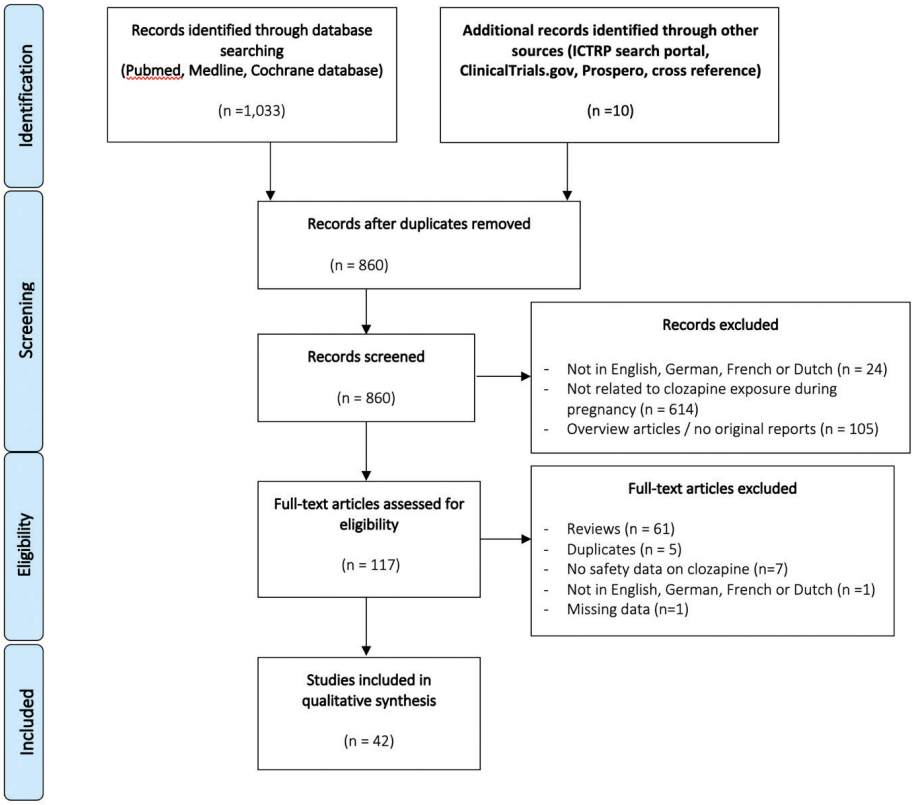

Figure 1. Flow diagram of study selection[15]

Forty-two articles were used for data extraction – 5 cohort studies, 2 case–non-case studies, 2 observational studies, 3 preclinical studies, and 30 case series, case reports, or letters to the editor (see Table 1).

Table 1 Summary of the main characteristics of the included studies of clozapine treatment during pregnancy and lactation

First author + year of publication (language if not English)	Study design	Aim	Cases
Westin – 2018 [17]	Retrospective cohort study	To elucidate to what extent pregnancy affects serum concentrations of APs in a large target population in a naturalistic setting.	103 women with 110 pregnancies of which 4 CLZ-using mothers with 4 pregnancies Number of serum CLZ concentration measurements at baseline: 114 Number of serum CLZ concentration measurements during pregnancy: 10 Number of serum CLZ concentration measurements first 12 weeks following delivery: 2
Hatters Friedman – 2016 [18]	Retrospective cohort study	To describe pregnancy outcomes for women prescribed atypical APs during pregnancy.	45 pregnancies exposed to: 21 quetiapine 19 olanzapine 7 risperidone 6 aripiprazole 1 CLZ

Exposure	Major limitations	Reported outcomes
Measured serum concentrations divided by the daily dose used by the woman at the time of sampling, providing a serum concentration/dose ratio, and then multiplied by the defined daily dose of the drug (i.e. 300mg for CLZ) Concomitant use of 'interacting drugs' was used as an exclusion criterion No information about smoking, alcohol or substance use	No data about smoking Limited generalizability of the findings due to the small number of CLZ samples No information about treatment adherence Possible varying time intervals between last dose to sampling	Maternal pharmacokinetics
No data regarding planned or unplanned pregnancies No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No individual drug data regarding co-medication ('64% being exposed to more than a single agent') Smoking during pregnancy 31% Alcohol use during pregnancy 20% Substance use during pregnancy 13% No data regarding maternal pre-pregnancy BMI	Information about drug exposure based on prescriptions Study outcomes considered for all APs as a group, possibly masking divergent frequencies among the single APs Potential determinants only provided for the group of mothers and not specifically for the single CLZ using mother No adjustments for potential confounders Exposure to more than a single psychotropic agent in 64% of the mothers Single report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Kulkarni - 2014 [19]	Observational cohort study	Not clearly specified (in the discussion paragraph: 'to identify the safest AP for use in pregnancy')	147 pregnancies exposed to APs in the first trimester; 11 of these pregnancies were exposed to CLZ (7.5%)

Exposure	Major limitations	Reported outcomes
No data regarding planned or unplanned pregnancies No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No individual drug data regarding co-medication Smoking during pregnancy 35% Alcohol use during pregnancy 26% Substance use during pregnancy 12% No individual drug data regarding maternal pre-pregnancy BMI	Study outcomes considered for all APs as a group, possibly masking divergent frequencies among the single APs Unknown distribution of possible confounders amongst the different drugs Absence of a control group No additional information regarding the two reported anomalies	Fetal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Bodén- 2012 [20]	Retrospective cohort study	To investigate the effects of maternal use of APs during pregnancy on gestational diabetes and fetal growth	Neonates exposed to (n (%): Olanzapine 159 (31.4) CLZ 11 (2.2) OAP 338 (66.7): Quetiapine 90 (17.8) Risperidone 72 (14.2) Flupentixol 58 (11.4) Haloperidol 52 (10.3 Aripiprazole 38 (7.5) Perphenazine 35 (6.9) Zuclopenthixol 30 (5.9) Ziprasidone 18 (3.6) Chlorprothixene 9 (1.8) Fluphenazine 2 (0.4) Pimozide 1 (0.2) Some of the APs were used concomitantly

Exposure	Major limitations	Reported outcomes
No data regarding planned or unplanned pregnancies Exposure defined as 'filling a prescription for an AP from last menstrual period to partition' No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No individual drug data regarding co-medication Maternal smoking in early pregnancy: 22.5% for olanzapine/CLZ exposure 31.7% for OAP exposure 6.7% for non-exposure No information about alcohol or substance use Maternal early pregnancy BMI: < 18.5 kg/m^2: 3% for olanzapine/CLZ 2.1% for OAP 2.2% for non-exposure 18.5-24.9 kg/m^2: 39.6% for olanzapine/CLZ 40.5% for OAP 55.7% for non-exposure 25.0-29.9 kg/m^2: 34.9% for olanzapine/CLZ 25.1% for OAP 22.4% for non-exposure > 30.0 kg/m^2: 14.2% for olanzapine/CLZ 23.4% for OAP 10.7% for non-exposure	Information about drug exposure based on filled prescriptions No information about drug compliance / Unknown if exposure to AP has been continued during pregnancy No information about alcohol or substance use 19.5% of the CLZ/olanzapine group also used one or more OAP throughout the pregnancy period Possible selective prescribing of olanzapine and CLZ	Maternal outcomes Fetal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Reis - 2008 [21]	Retrospective cohort study	To describe the delivery outcomes after the use of typical and atypical APs during the first trimester of pregnancy, with special stress on the risk for congenital malformations in the offspring	570 women with reported use of APs in early pregnancy of which 18 women with reported use of CLZ.
Beex-Oosterhuis – 2020 [22]	Case/non-case study	To compare the frequency of reported adverse pregnancy outcomes after the use of CLZ versus OAP during pregnancy, using data from Vigibase	494 individual case safety report-adverse drug reaction (ICSR-ADR) pairs involved adverse pregnancy outcomes related with CLZ exposure and 4645 related with OAP exposure
Montastruc - 2016 [23]	Case/non-case study	To research a signal between AP use and gastrointestinal congenital disorders by using data from Vigibase taking into account competition biases	41 safety reports of cleft palate related to in utero exposure to AP; 2 case safety reports in Vigibase of cleft palate related to in utero exposure to CLZ

Exposure	Major limitations	Reported outcomes
No data regarding planned or unplanned pregnancies No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No individual drug data regarding co-medication No individual drug data regarding smoking No information about alcohol or substance use No individual drug data regarding maternal pre-pregnancy BMI	Not possible to distinguish chronic drug users from women who used the drugs only temporarily or women who used high doses from women who used low doses No information about the contribution of CLZ to the study outcomes since this study focused on the effect of AP as a group Potential determinants only provided for the group of mothers and not specifically for the single malformation reported after CLZ exposure	Fetal outcomes
No information about planned or unplanned pregnancies No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No information about co-medication No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Risk of bias if the true number of pregnancies exposed to CLZ is relatively smaller than the number of pregnancies exposed to OAP Case safety reports only describe a suspicion and evidence for causality of associations is not the same in all reports and often even lacking No adjustment for other confounding factors	Fetal outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancies No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) Benzatropine used as co-medication in the 2 CLZ-cases No information about smoking, alcohol or substance use No pre-pregnancy BMI information	The study was designed for detection of safety signals for AP as a group and therefor only adds two casuistic reports to our data in absence of additional information about potential confounders	Fetal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Shao – 2015 [24]	Prospective observational study	To investigate the developmental effects of CLZ and other atypical APs on infants who were exposed to as fetus	CLZ (n = 33) Risperidone (n = 16) Olanzapine (n = 8) Quetiapine (n = 6)
Newham – 2008 [25]	Prospective, observational study	To determine whether atypical and typical APs differ in their effects on birth weight after maternal exposure during pregnancy	56 pregnancies exposed to typical APs and 30 to atypical APs. Exclusion of 9 infants exposed to typical (16%) and 5 exposed to atypical (17%) APs owing to premature birth Exclusion of 2 infants exposed to typical APs (4%) for postdatism.

Exposure	Major limitations	Reported outcomes
Unplanned pregnancy: CLZ 54.5% OAP 50.0% (p 0.933) Minimum CLZ dosage: 75mg Maximum CLZ dosage: 450mg Mean CLZ dosage (SD): 178.03 mg (70.37) No benzodiazepines and no mood stabilizers (all 63 women) No vitamin or folic acid taken during pregnancy: 8 CLZ using mothers (24.2%) 7 OAP using mothers (23.3%) (p 0.933) No information about alcohol or substance use Smoking during pregnancy: 1 CLZ using mother (3.0%) 1 OAP using mother (3.3%) (p 0.945) Pre-pregnancy BMI >23.9 mg/kg^2: CLZ 54.5% OAP 26.% (p 0.025)	This study used data from a previous study. Unlike in the previous study, the 13 sulpiride exposed infants are not included in the current study, without provision of any clarification No information about alcohol or substance use Unknown how the infant's sleep and mental state have been assessed Uncertain generalizability of the study results, since, according to the authors, and unlike in western countries, CLZ is popularly used for female patients with schizophrenia in China Reports of GDM without individual information about the mothers' pre-pregnancy BMI Moreover, diabetes mellitus during pregnancy was used as an exclusion criterion	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy No individual drug data regarding drug exposure (dosage, dose interval, timing of exposure, drug concentration) No information about smoking, alcohol or substance use. No pre-pregnancy BMI information	Data regarding potential confounders are not presented and thus little is known about efforts addressing potential confounders Unknown if exclusion of infants owing to premature birth or postdatism could have affected the results	Fetal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Oltulu - 2016 [26]	Preclinical study	To examine the effects of prenatal exposure to various APs on learning and memory in adult rats	4 pregnant rats receiving CLZ
Donohoe – 2008 [27]	Preclinical study	To test the hypothesis that APs affect neurodevelopment through their actions on known signaling pathways, including dopamine and serotonin receptors, and calmodulin using the model organism Caenorhabditis elegans	Unknown
Wang - 2006 [28]	Preclinical study	To evaluate the behavioral effects of chronic haloperidol and CLZ during gestation and postnatal development in mouse offspring at different ages, compared with transient treatments that stopped 1-3 weeks before the test, to know whether prenatal chronic administrations of these APs permanently or temporally influence the behavior in offspring, particularly compared with drug withdrawal	Unknown

Exposure	Major limitations	Reported outcomes
40 mg/kg CLZ as water suspension QD during the gestation period until partition compared with: 2 mg/kg haloperidol 100 mg/kg thioridazine 200 mg/kg sulpiride 20 mg/kg chlorprothixene 10 mg/kg fluphenazine 20 mg/kg chlorpromazine a control group receiving water by intragastric gavage	Unknown how the administered (relative) doses of the AP in rats relate to human doses The study seems to be designed to test the influence of the different AP chemical classes, but the conclusions refer to AP in general Preclinical study, thus the results are, at most, hypothetical, for the effects in human	Developmental outcomes
Model organisms in the fourth larval stage (prior to reproductive maturation) placed on control (solvent alone) or drug plates and allowed to mature and lay eggs, ensuring developing embryos exposed to drug Progeny developing to the third larval stage and then mounted on microscope slides	Study conducted in a non-vertebrate model organism. Unknown whether, and how, the findings of this study are likely to translate to a vertebrate nervous systems No information about the number of experimental and control groups	Developmental outcomes
Pregnant mice, 1 or 2 in each cage, housed under standard conditions with food and normal vehicle or vehicle containing 6 mg/L of haloperidol, 90 mg/L or 180 mg/L of CLZ	No information about the total number of animals used in each experiment and the number of animals in each experimental group Unknown how the administered (relative) doses of the AP in mice relate to human doses Unknown whether, and how, the findings of this study are likely to translate to human biology	Developmental outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Nguyen – 2020 [29]	Case series	To document any specific findings of obstetric, neonatal and psychiatric outcomes for pregnant women taking CLZ	n = 8 mothers, 9 pregnancies
Imaz - 2018 [30]	Case series	To provide new information on the features of CLZ pharmacokinetics that determine its placental and lactation passage, as well as the neonatal CLZ elimination half-life and neonatal and infant/child outcomes	n = 3 mothers , with 4 pregnancies

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ dose range: 100–400 mg/day Mean daily CLZ dose (SD) 258.3 mg (98.4). No change in dosing for the individual women during pregnancy Other concurrent psychotropic medications: 44.4% (such as fluvoxamine, clonazepam, aripiprazole, reboxetine and venlafaxin) Smoking 44.4% No information about alcohol or substance use Obesity at booking visit (BMI > 30 kg/m^2): 66.7% Gestational diabetes: 66.7%	The pharmacokinetic data are too limited to draw final conclusions No information regarding timing between last dose and sampling No individual information about treatment adherence, co-medication and 'smoking adherence' Little additional information about the individual cases	Maternal pharmacokinetics Maternal outcomes Fetal outcomes Delivery Neonatal outcomes
M1: planned pregnancy M2: planned pregnancy M3-1: unplanned pregnancy M3-2: unknown Clozapine exposure: M1: 550 mg/day when pregnancy was confirmed and titrated down to 350 mg/day M2: 200mg/day when pregnancy was confirmed and titrated down to 100 mg/day from the 19th week of pregnancy	Very limited number of TDM measurements Assumed linear neonatal pharmacokinetics, while from the presented individual neonatal concentrations, this linear pharmacokinetics is uncertain Unknown if the follow up information has been based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal pharmacokinetics Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases

Exposure	Major limitations	Reported outcomes
M3-1: 200 mg/day when pregnancy was confirmed, discontinued at week 16 and reintroduced (200 mg/day) at week 21. Hospitalization in week 26, until delivery with CLZ increase to 300 mg/day M3-2: 200 mg/day when pregnancy was confirmed until delivery Co-medication: M1: risperidone 50mg/month (long-acting injection) wk 0-delivery M2: no co-medication M3-1: diazepam 15 mg/day wk 30-37 M3-2: sertraline 100 mg/day wk 35-delivery Smoking / alcohol / substance use: M1: 18 cigarettes/day when pregnancy was confirmed and then reduced by 50% M2: not M3-1: alcohol, cocaine and cannabis during first 5 months of pregnancy, tobacco use daily M3-2: tobacco use daily Pre-pregnancy BMI: M1: 31.84 kg/m^2 M2: 27.78 kg/m^2 M3-1: 24.90 kg/m^2 M3-2: 28.09 kg/m^2		

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Molins – 2019 [31]	Letter to the editor / case report	Not specified	n = 1 mother, 1 pregnancy

Exposure	Major limitations	Reported outcomes
Unplanned pregnancy CLZ exposure: only started at the 24th pregnancy week along with electroconvulsive therapy dose increased up to 250 mg/day (CLZ plasma level: 495 ng/mL) > wk 33: reduction to 200 mg/day because of drowsiness and dizziness Although not clearly stated, the mother probably used aripiprazole 10 mg/day at conception At the time of admission (at 17 weeks pregnancy), she was only using tobacco Pre-pregnancy BMI of 24.7 kg/m^2	Single observation	Fetal outcomes Delivery Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Narayanaswamy – 2018 [32]	Case report	To present a case where a woman on CLZ along with folic acid supplementation gave birth to an infant with neural tube defect	n = 1 mother, 1 pregnancy

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ exposure: When the mother found to be pregnant after four months of amenorrhea: 225 mg/day (dose frequency unknown) Then CLZ stopped and haloperidol 10 mg/day CLZ restarted within a month and maintained at 150 mg/day until delivery Co-medication: haloperidol 2.5 mg/day, multivitamin tablets (containing vitamin A 2500 IU, vitamin D3 200 IU, vitamin B1 2 mg, vitamin B2 2 mg, vitamin B6 0.5 mg, niacinamide 25 mg, calcium pantothenate 10 mg, vitamin C 50 mg and 0.2 mg folic acid) Insulin for GDM from gestational week 36 No information about smoking, alcohol or substance use Pre-pregnancy BMI 23 kg/m^2	Single observation The report lacks relevant information about smoking, alcohol or substance use throughout the pregnancy Pregnancy was found out only after 4 months	Maternal outcomes Fetal outcomes Delivery Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Uygur – 2019 [33]	Case report	To present growth and neurodevelopmental outcomes of an infant exposed to CLZ during pregnancy and exposed to CLZ plus olanzapine during the lactation period	n = 1 mother, 2 pregnancies of which the most information is available of the 2nd pregnancy
Hodge - 2016 [34]	Case report	To present a case of fetal and neonatal CTG abnormalities due to CLZ use	n = 1 mother, 1 pregnancy
Köse Çinar - 2016 [35]	Case report	To present a case of two uncomplicated deliveries of healthy infants of a mother using olanzapine during her first pregnancy and CLZ during her second pregnancy	n = 1 mother, 1 pregnancy

Exposure	Major limitations	Reported outcomes
M1-1: unplanned M1-2: no information about planned or unplanned pregnancy CLZ exposure: M1-1: unknown CLZ dose M1-2: 300 mg/day when pregnancy was confirmed. Dose reduction to 100 mg/day at the third trimester No information about co-medication No information about smoking, alcohol or substance use No information about pre-pregnancy BMI, but 'no family history of diabetes'	Two, single observations with little additional information Report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy No data regarding CLZ exposure (dosage, dose interval, timing of exposure, drug concentration) The mother was on 'multi-drug therapy' (not further specified) No information about smoking, alcohol or substance use. No pre-pregnancy BMI information	Single observation with too limited data to draw conclusions	Fetal outcomes Delivery Neonatal outcomes
Unplanned and unwanted pregnancy CLZ exposure: When pregnancy was confirmed: 750 mg/day From wk 33: 350 mg/day No information about co-medication, smoking, alcohol or substance use Normal body mass index (18.5–25 kg/m^2)	Single observation, with no information about co-medication, smoking, alcohol or substance use	Maternal outcomes Fetal outcomes Delivery Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Sreeraj – 2016 [36]	Case report	To present a case of a women diagnosed with schizophrenia giving birth to triplets while on CLZ	n = 1 mother, with a triplet pregnancy
Guyon – 2015 [37]	Case report	To present a case of alteration of the fetal heart rate in a woman treated with CLZ	n = 1 mother, 1 pregnancy
Coston – 2012 [38] **(French)**	Case report	To report two cases of absence of fetal heart rate variability in fetus exposed to CLZ in utero and to show the limitations of the analysis of the fetal heart rate under CLZ by computerized CTG	n = 2 mothers, 2 pregnancies

Exposure	Major limitations	Reported outcomes
Planned pregnancy CLZ exposure: During the first trimester: reduction from 400 to 200 mg/day Co-medication prior to conception: treatment with human menopausal gonadotropin 150 mg, human chorionic gonadotropin 5000 IU, Pregnanolone 10 mg, folic acid and other vitamins supplementation in view of infertility No information about other co-medication prior to and during pregnancy No information about smoking, alcohol or substance use No information about pre-pregnancy BMI	Single observation Report of an infant with a macrocephaly, without information about other co-medication prior to and during pregnancy, as well as information about smoking, alcohol or substance use	Fetal outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy CLZ 125 mg/day for 9 years Levothyroxin 25 ug/day for mild goiter No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Single observation, with little additional data Report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy CLZ M1: 300 mg/day CLZ M2: 300mg/day M1: no other drugs M2: aripiprazole 10 mg/day No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Two, single observations with little additional information Report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Moreno-Bruna - 2012 [39] **(French)**	Case report	To present a case of neonatal delayed peristalsis and macrosomia after in-utero exposure to CLZ	n = 1 mother, 1 pregnancy
Novikova – 2009 [40]	Case report	To report a case of a young woman who poisoned herself with 10 g of CLZ late in pregnancy	n = 1 mother, 1 pregnancy
Duran – 2008 [41]	Case report	To present two cases of pregnant women receiving CLZ treatment	n = 2 mothers, three pregnancies (M1: two pregnancies; M2: one twin pregnancy)

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ: 325 mg/day with CLZ and NorCLZ drug concentrations at 2 months pregnancy of 370 mg/L and 215 mg/L respectively CLZ dose decreased to 100 mg/day at term No co-medication No information about smoking, alcohol or substance use Pre-pregnancy BMI 25 kg/m^2	Single observation Very limited number of TDM measurements Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes Neonatal pharmacokinetics
Intentional acute intoxication with approximately 10 gram CLZ prescribed for someone else at 32 weeks' gestation of an unplanned pregnancy	Single observation of an attempted suicide, without additional therapeutic drug monitoring data	Fetal outcomes Delivery Neonatal outcomes
Planned pregnancies CLZ exposure: M1-1: probably 200 mg/day M1-2: 200 mg/day M2: conception under a not clearly specified dose, but probably between 200-400mg/day. Delivery under 200 mg/day. No information about co-medication, smoking, alcohol or substance use M1-1: pre-pregnancy BMI 23.6 kg/m^2 M1-2: no pre-pregnancy BMI information M2: 24.1 kg/m^2	Three, single observations Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Klys – 2007 [42]	Case report	To describe a case of the death of a neonate after intrauterine CLZ poisoning due to ingestion by the then 9-month pregnant mother with the aim of committing suicide	n = 1 mother, 1 pregnancy
Mendhekar - 2007 [43]	Letter to the editor / case report	To report a case of a woman with schizophrenia who continued CLZ treatment throughout her 9 months of pregnancy and during lactation	n = 1 mother, 1 pregnancy
Sethi – 2006 [44]	Case report	Not specified	n = 1 mother, 1 pregnancy

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ discontinuation in the first trimester and the patient was on valproate, promethazine, risperidone and fluoxetine. According to the medical record, at 9 months pregnancy: "the patient ingested Klozapol 100 mg, in the amount of 100–200 tablets, with the aim of committing suicide" The mother did not smoke or drink alcoholic beverages No information about other substance use	Single observation of an attempted suicide, without additional therapeutic drug monitoring data	Fetal outcomes Delivery
Unplanned pregnancy CLZ 100 mg /day No co-medication No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Single observation in an unplanned pregnancy (not known when pregnancy was detected) No information about smoking, alcohol or substance use Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes
Pregnancy disclosure at the end of the first trimester, despite repeated advice to practice contraception CLZ continued at the same dose throughout the gestational period: 250 mg/day No information about co-medication, smoking, alcohol or substance use No information about pre-pregnancy BMI	Single observation, with little additional information Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Doherty – 2006 [45]	Case report	To represent the first recorded usage of CLZ in Northern Ireland during pregnancy and labor	n = 1 mother, 1 pregnancy
Walch – 2005 [46] **(German)**	Case report	Not specified	n = 1 mother, 1 pregnancy
Gupta - 2004 [47]	Letter to the editor / case report	To describe a case wherein CLZ therapy was continued successfully over 2 consecutive pregnancies	n = 1 mother, 2 pregnancies

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ treatment continued throughout pregnancy Last dose taken on the morning of admission for the C. section 'Drug levels had been monitored at regular monthly intervals and were within the therapeutic range' No information about co-medication, smoking, alcohol or substance use No pre-pregnancy BMI information, BMI at delivery 34 kg/m^2	Single observation, with little additional data	Maternal outcomes Fetal outcomes Delivery
Planned pregnancy CLZ: 6–12,5 mg CLZ/day No information about co-medication and smoking. No alcohol and substance use during pregnancy No information about pre-pregnancy BMI	Single observation No information about co-medication	Fetal outcomes Delivery Neonatal outcomes
Planned pregnancies CLZ exposure: Pregnancy 1: 200 mg/day Pregnancy 2: 100 mg/day Pregnancy 1: Folic acid started at the 10th week of pregnancy No information about other co-medication is given for both pregnancies No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Two, single observations Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Karakula – 2004 [48]	Case report	To describe the case of a neonate who had been exposed to CLZ in utero	n = 1 mother, 1 pregnancy
Mendhekar – 2003 [49]	Letter to the editor / case report	To describe a case where CLZ was continued as monotherapy during pregnancy	n = 1 mother, 1 pregnancy
Nguyen – 2003 [50] **(French)**	Case report	To survey the questions regarding perinatal CLZ use and to present a case of CLZ use during two consecutive pregnancies	n = 1 mother, 2 pregnancies

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ 200 mg/day No information about co-medication, smoking, alcohol or substance use. The father had been dependent on alcohol. No pre-pregnancy BMI information	Single observation with too limited data to draw conclusions Report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy. Pregnancy not detected until the end of the first trimester CLZ 75 mg/day, with unsuccessful attempts to reduce the dose to 50mg in the first and to 62.5 mg in second trimester No co-medication ('CLZ monotherapy') No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Single observation, lacking information about alcohol, smoking and substance use	Maternal outcomes Fetal outcomes Delivery
Planned pregnancy CLZ 350 mg/day Pregnancy 1: doxylamine used for nausea (unknown period and dose) and insulin for GDM since the 27th week of gestation. Pregnancy 2: no information about co-medication The mother continued smoking during the pregnancies (one pack of cigarettes a day) No drugs or alcohol use during the pregnancies Pregnancy 1: pre-pregnancy BMI unknown (30.4 kg/m^2 at 27th gestational week) Pregnancy 2: 23.7 kg/m^2 at the beginning of the pregnancy	Two, single observations Unknown if the follow up information is based on structured tools to assess the development of the infants or on parents' reports	Maternal outcomes Fetal outcomes Developmental outcomes Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Yogev – 2002 [51]	Case report	Not specified	n = 1 mother, 1 pregnancy
Dickson – 1998 [52]	Case report	Not clearly specified	n = 1 mother, 1 pregnancy

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy No information about CLZ dose and duration and timing of exposure No information about co-medication No information about smoking, alcohol or substance use No information about pre-pregnancy BMI	Single observation with little additional information	Fetal outcomes Delivery
Planned pregnancy CLZ: 450 mg at conception, then reduced to 200 to 250 mg daily during the second trimester and to 150 mg daily during the last two months of the pregnancy Metformin was discontinued when pregnancy was confirmed at 7 weeks of gestational age and insulin injections were initiated No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Single observation In the light of shoulder dystocia, the weight and length of the child would have been informative as well as the mothers BMI	Delivery

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Tënyi – 1998 [53]	Case series	Not specified	n = 4 mothers, 6 children

Exposure	Major limitations	Reported outcomes
M1-1: no information about planned or unplanned pregnancy M1-2: unplanned (during use of an UID) M1-3: unknown M2, M3 and M4: unknown CLZ exposure: M1-1: 100 mg/day (1-12 weeks), 50 mg/day (12-40 weeks); M1-2: 25 mg/day M1-3: 25 mg/day M2: 300 mg/day (14-19 weeks), 150 mg/day (20-34 weeks), 50 mg/day (34-37 weeks) M3: 75 mg/day (week 20-38) M4: 25 mg/day (week 16-39) No information about co-medication No information about smoking, alcohol or substance use No information about pre-pregnancy BMI	Six, single observations with little additional information Unknown if the follow up information has been based on structured tools to assess the development of the infants or on parents' reports	Fetal outcomes Delivery Developmental outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Stoner – 1997 [54]	Case report	To report the cases of two women with treatment resistant schizophrenia who received CLZ during all three trimesters and delivered at term	n = 2 mothers, two pregnancies

Exposure	Major limitations	Reported outcomes
No information about planned or unplanned pregnancy CLZ exposure: M1: conception – wk 23: 300 mg/day, but (at least) non-compliant between week 21 and 23 After week 23 CLZ was titrated up to 350mg/day M2: conception -delivery 600-625 mg/day Only partial compliance before conception Co-medication: M1: Lithium during the 1st trimester (unknown dose), during hospitalization after week 23 at least one dose of lorazepam, haloperidol, acetaminophen with and without codein, guaifenesin, magaldrate, aluminiummagnesiumhydroxide, cephalexin, metronidazole, multivitamin with folate M2: Lithium prior to learning of the pregnancy and stopped during the 1st trimester No information about smoking, alcohol or substance use No information about pre-pregnancy BMI	Two, single observations Clozapine concentrations in the neonate would have been informative in the light of the seizures Unknown if the follow up information has been based on structured tools to assess the development of the infants or on parents' reports (absent information in the second child)	Maternal outcomes Fetal outcomes Developmental outcomes Delivery Neonatal outcomes

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Di Michele – 1996 [55]	Case report	To describe a successful pregnancy in a woman undergoing treatment with CLZ and lorazepam, but whose baby developed transient Floppy Infant Syndrome	n = 1 mother, 1 pregnancy
Dev – 1995 [56]	'Review' / Case overview	Not clearly specified	102 pregnancies exposed to CLZ

Exposure	Major limitations	Reported outcomes
Planned pregnancy CLZ exposure from partition until delivery CLZ 200 mg/day, with increase up to 300 mg/day three times during the pregnancy, due to the mother's clinical condition Co-medication: lorazepam 2.5 mg three times daily, and frequently increased up to five tablets a day No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Single observation No information about several important factors, such as alcohol, smoking and substance use, CLZ concentrations during pregnancy, as well as CLZ concentrations in the neonate, which would have been informative in the light of the floppy infant syndrome, although this syndrome is mainly attributed to the use of high doses of lorazepam	Fetal outcomes Delivery Neonatal outcomes
No information about planned or unplanned pregnancy No information about CLZ dose and duration and timing of exposure No information about smoking, alcohol or substance use No pre-pregnancy BMI information	Very limited data as any additional information such as maternal age, pre-pregnancy BMI, co-medication, smoking, alcohol or substance use during pregnancy, CLZ dose and timing of exposure is absent The authors worked at Sandoz Pharma, but the source of the data is not defined	Fetal outcomes Neonatal outcomes Lactation

Table 1 *Continued*

First author + year of publication (language if not English)	Study design	Aim	Cases
Barnas - 1994 [57]	Case report	Not clearly specified	n = 1 mother, 1 pregnancy
Waldman -1993 [58]	Letter to the editor / case report	Not specified	n = 1 mother, 1 pregnancy

AP(s) Antipsychotic(s)
BMI Body Mass Index
CLZ Clozapine
GDM Gestational Diabetes Mellitus
M1 Mother 1 (and so on)
M2-1 Mother 2, pregnancy 1 (and so on)
NorCLZ Norclozapine
OAP Other antipsychotics
wk Week

Exposure	Major limitations	Reported outcomes
Planned pregnancy CLZ exposure: conception - wk 32: 100 mg/day wk 32 - delivery: 50 mg/day day 3 after delivery: 100mg/day No information about pre-pregnancy BMI, co-medication, smoking, alcohol or substance use	Single observation, with little additional information The pharmacokinetic data are too limited to draw final conclusions No information regarding timing between last dose and sampling No information about treatment adherence, co-medication and smoking Unknown if the follow up information has been based on structured tools to assess the development of the infants or on parents' reports	Maternal pharmacokinetics Fetal outcomes Developmental outcomes Deivery Lactation
No information about planned or unplanned pregnancy No information about CLZ dose and duration and timing of exposure No information about co-medication No information about smoking, alcohol or substance use No information about pre-pregnancy BMI	Single observation with little additional information Report of GDM without information about the mother's pre-pregnancy BMI	Maternal outcomes Fetal outcomes Delivery Neonatal outcomes

Mother

Pharmacokinetics during pregnancy (table 2)

Several physiological changes occur during pregnancy with the potential of altering the pharmacokinetics of clozapine in the mother. Clozapine metabolism is largely dependent on the activity of CYP1A2, a hepatic cytochrome P450 (CYP) enzyme. Studies indicate that CYP1A2 activity decreases during pregnancy, by 33% (+/- 23%) in the first trimester, 48% (+/-27%) in the second trimester, and 65% (+/- 15%) in the third trimester [59], as a result of the inhibitory effects of female sex hormones. [60,61] Decreased CYP1A2 activity will prolong clozapine clearance in pregnant women. CYP1A2 is also susceptible to changes in tobacco and caffeine use, so that if expectant mothers stop or reduce their use of tobacco- and caffeine-containing products, clozapine clearance will decrease further. However, hepatic perfusion increases during pregnancy, potentially leading to a higher hepatic clearance of clozapine.

Only one retrospective cohort study has investigated to what extent pregnancy affects serum levels of various antipsychotics.[17] This study reported no or little change in clozapine serum concentrations during pregnancy (-23% [95% CI -46 - +9 %]). However, only 10 clozapine measurements were taken, information on smoking habits was not available, and treatment adherence was unknown. One case series [29] reported clozapine levels for three of the eight women included. In one woman, concentrations were more or less stable, in the second woman there was a four-fold increase in clozapine concentration between gestational day 50 and day 200, with a nearly two-fold decrease in the last trimester, and in the third woman levels fluctuated, but particularly in the last trimester. Again, there was no information about tobacco use, treatment adherence, or the use of potentially interacting comedication.

One case report [57] described maternal plasma levels of 38–55 ng/mL in the first 32 weeks of pregnancy in a mother who took 100 mg clozapine a day. Although the dose was halved in the 32nd week, the concentrations found in the third trimester, at the day of delivery, and the day after were less than half of the concentrations found in the first 32 weeks. Unfortunately, this report also did not provide information about treatment adherence, comedication, and smoking and did not distinguish between concentrations measured in the first two pregnancy trimesters.

In summary, there is too little information about how clozapine concentrations change during pregnancy to define a net effect, and the available data are probably influenced by modifying factors.

Table 2. Studies describing neonatal and maternal clozapine concentrations as well as clozapine concentrations in breast milk

		(Mean) maternal CLZ concentrations (ng/mL)			
First author + year of publication	**Number of mothers (number of pregnancies)**	**Pre-pregnancy**	**1st trimester**	**2nd trimester**	**3rd trimester**
Nguyen – 2020 [29]	N = 3 (3)	#	Day 50: - M1: ± 200 - M2: ± 200 - M3: #	Day 100: - M1: # - M2: # - M3: ± 300 and ±200	Day 200: - M1: ±800 - M2: # - M3: ±200 Day 250: - M1: # - M2: ±300 - M3: ±500 Day 260: - M1: # - M2: # - M3: ±300
Westin - 2018 [17]	N= 4 (4)	418.6	399.7	358.8	322.1
Imaz – 2018 [30]	N= 3 (4)	#	#	#	#

	Amniotic fluid concentration (ng/mL)	Umbilical cord concentration (ng/mL)	Concentration in breast milk (ng/mL)	Neonatal concentration (ng/mL)
Since Delivery				
Day 0: - M1: ±500 - M2: ±200 - M3: ±800 Day 10: - M1: ±1400 - M2: # - M3: #	#	#	#	#
#	#	#	#	#
Day 0: M1: CLZ: 198 NorCLZ: 200 M2: CLZ: 122 NorCLZ: 79 M3-1: CLZ: 194 NorCLZ: 114 M3-2: CLZ: 148 NorCLZ: 149	 M1: CLZ: 61 NorCLZ: 39 M2: CLZ: # NorCLZ: # M3-1: CLZ: 67 NorCLZ: 52 M3-2: CLZ: # NorCLZ: #	 M1: CLZ: 77 NorCLZ: 56 M2: CLZ: 68 NorCLZ: 26 M3-1: CLZ: 113 NorCLZ: 42 M3-2: CLZ: 67 NorCLZ: 32	#	Concentrations derived from figure 1[23]: M1 – 0 hours post-delivery: CLZ: 78 NorCLZ: 52 M1 – ± 12 hours post-delivery: CLZ: 40 NorCLZ: 38 M1 – ± 80 hours post-delivery: CLZ: 12 NorCLZ: 18 M1 – ± 280 hours post-delivery: CLZ: 10 NorCLZ: 15 M1 T1/2: CLZ: 99 hours NorCLZ: 161 hours

Table 2. *Continued*

		(Mean) maternal CLZ concentrations (ng/mL)			
First author + year of publication	**Number of mothers (number of pregnancies)**	**Pre-pregnancy**	**1st trimester**	**2nd trimester**	**3rd trimester**

	Amniotic fluid concentration (ng/mL)	Umbilical cord concentration (ng/mL)	Concentration in breast milk (ng/mL)	Neonatal concentration (ng/mL)
Since Delivery				
				M2: CLZ: # NorCLZ: # M2 T1/2: CLZ: # NorCLZ: # M3-1 – 0 hours post-delivery: CLZ: 115 NorCLZ: 42 M3-1: – ± 50 hours post-delivery: CLZ: 110 NorCLZ: 38 M3-1: – ± 245 hours post-delivery: CLZ: 20 NorCLZ: 20 M3-1 T1/2: CLZ: 71 hours NorCLZ: 187 hours M3-2 – 0 hours post-delivery: CLZ: 68 NorCLZ: 32 M3-2: – ± 75 hours post-delivery: CLZ: 30 NorCLZ: 22 M3-2: – ± 245 hours post-delivery: CLZ: 18 NorCLZ: 10 M3-2 T1/2: CLZ: 107 hours NorCLZ: 131 hours

Table 2. *Continued*

		(Mean) maternal CLZ concentrations (ng/mL)			
First author + year of publication	**Number of mothers (number of pregnancies)**	**Pre-pregnancy**	**1st trimester**	**2nd trimester**	**3rd trimester**
Coston – 2012 [38]	N = 1(1)	#	#	#	#
Moreno-Bruna – 2012 [39]	N= 1 (1)	#	At 2 months pregnancy: CLZ: 370 NorCLZ: 215	#	#
Klys – 2007 [42]	N =1 (1)	#	#	#	#

	Amniotic fluid concentration (ng/mL)	Umbilical cord concentration (ng/mL)	Concentration in breast milk (ng/mL)	Neonatal concentration (ng/mL)
Since Delivery				
				M2: Day 2 (33 hours post-partum): CLZ infant/maternal plasma concentration ratio: 6.5% under mixed breastfeading.
#	#	#	#	Day 4: CLZ: 54
#	#	#	#	Day 5: CLZ: 103 NorCLZ: 47 Day 8: CLZ: 45 NorCLZ: 26
#	#	#	#	One day after admission: Post-mortem blood: CLZ: 7,300 NorCLZ: 2,600 CLZ-N-oxide: 500 Liver: CLZ: 28,000 NorCLZ: 17,100 CLZ-N-oxide 31,100 Kidney: CLZ: 10,100 NorCLZ 6,100 CLZ-N-oxide 5,800

Table 2. *Continued*

		(Mean) maternal CLZ concentrations (ng/mL)			
First author + year of publication	**Number of mothers (number of pregnancies)**	**Pre-pregnancy**	**1st trimester**	**2nd trimester**	**3rd trimester**
Barnas – 1994 [57]	N = 1 (1)	#	38 – 55		15.4

no information
Abbreviations:

CLZ	Clozapine
M1	Mother 1 (and so on)
M2-1	Mother 2, pregnancy 1 (and so on)
NorCLZ	Norclozapine

	Amniotic fluid concentration (ng/mL)	Umbilical cord concentration (ng/mL)	Concentration in breast milk (ng/mL)	Neonatal concentration (ng/mL)
Since Delivery				
Day 0: 14.1 Day 1: 14.7 Day 7: 41.4	11.6	#	Day 1: 63.5 Day 7: 115.6	Day 0: 27

Table 3. Studies describing maternal outcomes of clozapine treatment during pregnancy

First author + year of publication	**Number of mothers (number of pregnancies / infanst)**	**Maternal outcomes**
Nguyen – 2020 [29]	N = 8 (9)	No psychotic relapse during the course of the 9 pregnancies Constipation: n = 5 (55.5%), including 1 fecal impaction during labor Symptomatic orthostatic hypotension, at 34 weeks' gestation, with conservative management until delivery: n = 1 Persistent tachycardia (4 days postpartum): n = 1 (patient had been on a stable dose of 400 mg of CLZ for 1.5 years prior to conception) Pre-eclampsia: n = 0 (0%) GDM: n = 6 (66.7%) (all with pre-pregnancy BMIs in the overweight or obese range) Medication controlled: n= 2 (22.2%) Diet controlled: n= 4 (44.4%)
Narayanaswamy i– 2019 [32]	N = 1 (1)	GDM at the 36th week (normal pre-pregnancy BMI)
Uygur – 2019 [33]	N = 1 (2)	Pregnancy 1: # Pregnancy 2: GDM in the 18th week (unknown pre-pregnancy BMI, but no family history of diabetes), with successful dietary control No psychotic exacerbation

Table 3. *Continued*

First author + year of publication	Number of mothers (number of pregnancies / infanst)	Maternal outcomes
Imaz – 2018 [30]	N = 3 (4)	M1: no complications M2: GDM from week 14 Brief psychiatric hospitalization 5 days after delivery due to a relapse of manic psychotic symptoms, with rapid response to an increase in CLZ to 200 mg/day M3-1: Psychiatric hospitalization in week 26 until the end of pregnancy M3-2: Psychiatric hospitalization at 6 weeks of pregnancy after alterations in the mother's behavior, without evidence of a relapse in schizoaffective disorder
Hatters Friedman – 2016 [18]	N = 1 (1)	GDM in the single pregnancy exposed to CLZ (unknown pre-pregnancy BMI)
Köse Çinar – 2016 [35]	N = 1 (1)	Clinical admission with paranQD and persecution delusions at week 33 No metabolic diseases during pregnancy with normal pre-pregnancy BMI
Guyon – 2015 [37]	N = 1 (1)	GDM at the 26th weeks, with successful dietary management (unknown pre-pregnancy BMI)
Shao -2015 [24]	N = 33 (33)	Psychotic relapse in 3 CLZ using mothers GDM in 2 CLZ using mothers Pregnancy induced hypertension in 2 CLZ using mothers

Table 3. *Continued*

First author + year of publication	Number of mothers (number of pregnancies / infanst)	Maternal outcomes
Bodén – 2012 [20]	Group 1: N = 169 CLZ and/ or olanzapine exposed mothers (169) Group 2: N = 388 OAP exposed mothers (388)	Risk of GDM compared to non-exposed women: Group 1: OR 2.44 [95% CI, 1.14 -4.24] Group 2: OR 2.53 [95% CI, 1.48-4.34] Risk of GDM compared to non-exposed women after adjustment for early pregnancy BMI: Group 1: OR 1.46 [95% CI, 0.84-2.53] Group 2: OR 1.71 [95% CI, 0.82-3.56] Increased early pregnancy BMI seemed to be the major cause of GDM
Coston – 2012 [38]	N = 2 (2)	M1: GDM (unknown pre-pregnancy BMI) M2: GDM (unknown pre-pregnancy BMI)
Moreno-Bruna – 2012 [39]	N = 1 (1)	No known GDM, but a weight gain of 16 kg
Duran – 2008 [41]	N = 2 (3)	M1-1: no psychotic exacerbation normal fasting blood glucose levels normal lipid profile normal HbA1C levels no history of GDM in the medical records (normal pre-pregnancy BMI M1-2: no history of GDM in the medical records (no pre-pregnancy BMI information) M2: no history of GDM in the medical records (normal pre-pregnancy BMI)
Mendhekar – 2007 [43]	N = 1 (1)	Good nutritional care No exacerbation throughout the pregnancy Normal routine laboratory investigations (including blood glucose, hemoglobin, and white blood cell count)
Doherty – 2006 [45]	N = 1 (1)	BMI 34 mg/kg2 at delivery Normal blood sugar at regular antenatal visits
Sethi – 2006 [44]	N = 1 (1)	No GDM (unknown pre-pregnancy BMI)
Gupta – 2004 [47]	N = 1 (2)	Pregnancy induced hypertension in both pregnancies

Table 3. *Continued*

First author + year of publication	Number of mothers (number of pregnancies / infanst)	Maternal outcomes
Karakula -2004 [48]	N = 1 (1)	GDM (unknown pre-pregnancy BMI)
Mendhekar – 2003 [49]	N = 1 (1)	Normal routine laboratory investigations, including glucose monitoring No GDM (unknown pre-pregnancy BMI)
Nguyen – 2003 [50]	N = 1 (2)	Pregnancy 1: GDM (unknown pre-pregnancy BMI) Pregnancy 2: no GDM (normal pre-pregnancy BMI) No psychiatric exacerbations during in both pregnancies
Stoner – 1997 [54]	N = 2 (2)	M1 and M2: psychiatric symptoms intensified during pregnancy M1 and M2: uncooperative during labor
Waldman – 1993 [58]	N = 1 (1)	GDM in the second trimester (unknown pre-pregnancy BMI) Otherwise uneventful No exacerbation of psychiatric illness throughout gestation, labor, and delivery

no information
Abbreviations:

BMI	Body Mass Index
CLZ	Clozapine
NorCLZ	Norclozapine
GDM	Gestational Diabetes Mellitus
M1	Mother 1 (and so on)
M2-1	Mother 2, pregnancy 1 (and so on)
OAP	Other antipsychotics
OR	Odds ratio

Maternal outcomes (table 3)

A few cases have reported worsening of psychiatric symptoms during pregnancy in women using clozapine, but none provided information on clozapine levels. [24,30,35,54]

A retrospective cohort study found that the risk of gestational diabetes mellitus (GDM) was not increased in women who used olanzapine and/or clozapine (n=169) or other antipsychotics (n=338) compared with the risk in women who

had not been exposed to antipsychotics. [20] The actual impact of clozapine, however, was too small to draw conclusions, since only 11 of the 507 women used clozapine. While several reports mention the development of GDM in women treated with clozapine, [18,24,29,30,32,33,37,38,48,50,58] an equal number found no such development of GDM. [29,30,35,39,41,43-45,49,50] Unfortunately, many reports do not mention the mother's pre-pregnancy BMI or provide information about the presence or absence of earlier glucose intolerance. [18,24,33,37,38,48,58]
Since most data come from case reports and the number of clozapine users in the only available cohort study [20] was small, it is not possible to draw conclusions about the risk of GDM in women using clozapine. An increased BMI early in pregnancy, whether or not due to the use of antipsychotics, may be a better predictor of the development of GDM, than the use of antipsychotics as such. [20]
Beside some anecdotal reports [24,47] of pregnancy-induced hypertension in mothers on clozapine during pregnancy, we did not find any studies that investigated the risk of clozapine-associated pregnancy-induced hypertension.

Fetus

Placental passage of clozapine (table 2)

In general, the placental passage of a drug is governed by its molecular size, lipid solubility, and extent of plasma protein binding in the maternal system as well as by patient-specific factors, such as placenta transporter proteins. [62] Only the unbound fraction of a drug is able to cross membranes [63], provided the molecule is smaller than 600 Daltons.[64] Clozapine and norclozapine, the main metabolite of clozapine, are small enough to pass the placental barrier, but under 'non-pregnant' circumstances both are highly protein bound in blood, mainly to alfa-1-acid glycoprotein (AGP). [65,66] It is noteworthy that the concentration of AGP decreases by 20–30% in the third trimester of pregnancy.[67,68] Thus, toward the end of pregnancy, theoretically more drug is available to cross the placental barrier. [63] However, we did not find any literature on maternal concentrations of unbound clozapine during pregnancy, so it remains unknown whether decreasing concentrations of AGP affect the extent to which clozapine crosses the placenta.
One case series [30], describing four pregnancies in three women, reported the placental passage of clozapine and norclozapine as the ratio of umbilical cord blood to maternal plasma concentrations (UCB/MP ratio (%)). The UCB/MP ratio ranged between 39% and 58% for clozapine and between 28% and 37% for norclozapine, indicating (partial) placenta passage of clozapine toward the end of a full-term pregnancy. Since fetal CYP1A2 has negligible activity [69], norclozapine detected in the fetal system probably comes from the mother rather than arising from fetal metabolism.

Two other case reports[38,57] also showed detectable clozapine concentrations in two newborns. In one [57], the neonatal concentration at delivery was almost twice as high as the concentration in the mother on the day of delivery.
Another report [42] described an infant that died after its mother ingested 100–200 tablets (100 mg) of clozapine when she was 9 months pregnant. The high concentrations of clozapine and its metabolites in the neonate's blood, taken 1 day after admission of the mother, again reflect the placental passage of clozapine and norclozapine.

Neurodevelopment (table 4)

Since antipsychotics block dopamine and serotonin receptors and these neurotransmitters are involved in neural development, [70,71] prenatal exposure to antipsychotics may affect neural development. A preclinical study [26] examined the effects of intrauterine exposure to various antipsychotics (clozapine, haloperidol, thioridazine, sulpiride, chlorprothixene, fluphenazine, and chlorpromazine) on learning and memory in rats. The authors concluded that prenatal exposure to these antipsychotics may negatively affect problem-solving ability, rather than specifically affecting learning and memory, in adult animals.
Intrauterine exposure to clozapine during sensitive periods of brain development produced longer lasting changes in cognitive function and locomotor activity in mice pups than did similar exposure to haloperidol. [28] Clozapine induced hyperlocomotion, an effect that lasted more than 3 weeks after exposure was discontinued. In contrast to the findings of Oltulu and colleagues [26] chronic clozapine or clozapine withdrawal for 1 and 3 weeks tended to improve memory in mice pups.
Another preclinical study[27] using a non-vertebrate model organism (*Caenorhabditis elegans*), found that clozapine and fluphenazine produced greater deficits in neuronal development in *C. elegans* than did haloperidol, quetiapine, olanzapine, risperidone, or aripiprazole.
The cross-species validity, however, is one of the major limitations of the animal studies since animal models lack the underlying disease.
One prospective case–control study [24] investigated the developmental effects of foetal exposure to clozapine and other antipsychotics. More infants exposed to clozapine as foetus had delayed development of adaptive behaviour at 2 and 6 months of age than did infants exposed to other antipsychotics, and more infants exposed to clozapine had disturbed sleep and 'labile state' (depending on parents' reports) at 2 months of age. However, these differences disappeared after 6 months of age. The groups had a similar performance on cognitive, motor, social-emotional, and language scales.

A few cases [30,43,48] of neurodevelopmental delay after in utero exposure to clozapine have been reported, but it is not known whether objective instruments or parental report were used to assess the neurodevelopmental delay. One report [43] described an infant exposed to clozapine in utero as a result of an unplanned pregnancy. The child gained normal fluent speech only after 5 years. In a case series [30] one had neurodevelopmental delay at 18 months and one showed symptoms of ADHD, without fulfilling the diagnostic criteria at 6 years of age; the other two infants had normal neurodevelopment at 6 and 32 months. One case report [48] described a neonate admitted to the neonatal intensive care unit shortly after delivery for convulsions, respiratory insufficiency, and an abnormal heart shape. After 7 months, the baby could only raise its head. The mother had been using clozapine during gestation and the father had been dependent on alcohol. Several case reports describe normal neurodevelopment after different follow-up periods, [33,39,41,44,47,50,53,54,57] but these reports are generally of limited quality and, again, it is not known how the neurodevelopment of these infants had been assessed.
In summary, it remains unclear whether early developmental exposure to clozapine results in permanent changes in the brain that affect cognitive function or behaviour in both the short and long term.

Fetal disorders (table 4)

In our study [22] using global pharmacovigilance data, we compared the frequency of reported adverse pregnancy outcomes associated with the use of clozapine compared with other antipsychotics during pregnancy. We found that in utero exposure to clozapine was not associated with more 'foetal disorders' than exposure to other antipsychotics during pregnancy. However, several case reports [29,34,37,38,40,45,51] mention cardiotocography findings of reduced or absent foetal heart rate variability in the unborn infant. Unfortunately, none of the reports have provided information about clozapine concentrations. Cardiotocography is commonly used to detect changes in foetal heart rate patterns in response to hypoxia, but in the case of maternal clozapine treatment, clozapine is thought to reduce foetal heart rate variability by blocking the cholinergic and adrenergic receptors of the foetal nervous system. Misleadingly, this could mimic the symptoms of potential asphyxia.

Stillbirth and abortion (table 4)

In our pharmacovigilance study [22], we did not find evidence that clozapine exposure was associated with 'termination of pregnancy and risk of abortion' either. In fact, clozapine was even statistically significant less often associated with this endpoint than exposure to other antipsychotics.

Beside the aforementioned fatal poisoning of a neonate in the final stage of gestation after the mother had taken a clozapine overdose [42] and some anecdotal reports of stillbirth [49,50] and abortion [56] after fetal exposure to clozapine, we did not find additional studies associating the risk of stillbirth and abortion with clozapine exposure during pregnancy.

Congenital malformations (table 4)

In our pharmacovigilance study [22], we found 76 adverse drug reactions (ADRs) categorized as 'congenital, familial, and genetic disorders' with clozapine as one of the suspected drugs. 'Atrial septal defect' (ASD) (n=8) and 'ventricular septal defect' (n=6) were the most frequently reported ADRs. But relative to other antipsychotics, clozapine was equally (ventricular septal defect) or even less (atrial septal defect) often associated with these ADRs. Again, we did not find any evidence that clozapine is less safe during pregnancy than other antipsychotics in terms of 'congenital, familial, and genetic disorders'.

In a cohort study [21] comparing the pregnancy outcomes of 570 women with self-reported use of antipsychotics in the first trimester with the outcomes of unexposed pregnancies, atrial and ventricular septal defects were the most frequently reported congenital malformations after exposure to antipsychotics. Among the 18 women who used clozapine, only one malformation (ectopic anus) was reported.

Of 41 cases of cleft palate associated with the use of at least one antipsychotic drug reported in another case/non-case study [23], based on global pharmacovigilance data, two cases of cleft palate were associated with foetal exposure to clozapine.

In a cohort study [19] including eleven pregnancies of mothers exposed to clozapine in the first trimester, two major malformations after exposure to clozapine were reported: hypospadias and hypertelorism in one baby and gastroschisis and horseshoe kidney in the other baby. Unfortunately, no additional information about these two cases was available.

A number of case reports and case series [29,30,32,46,48,54,56] report various malformations but often lack information regarding possible confounders, and there does not seem to be a clear pattern of malformations. Moreover, there are at least as many reports [18,24,31,33,35,41,43,44,47,50,53,54,57] describing uncomplicated pregnancies and healthy offspring without malformations after foetal exposure to clozapine, of which one uncomplicated triplet[36] and one twin pregnancy.[41]

In summary, although the available data are limited in terms of quality and quantity, clozapine does not appear to be a teratogenic agent.

Prematurity (table 4)

In a study [20] of the risk of preterm birth after maternal use of antipsychotics, 5.1% of unexposed infants were born preterm, compared with 8.0% of the infants exposed to olanzapine and/or clozapine and 9.5% exposed to other antipsychotics. This was not a statistically significant difference. The study design did not permit a distinction to be made between clozapine and olanzapine.
In a prospective study [24] investigating 33 infants exposed to clozapine in utero compared to 30 infants exposed to other antipsychotics (risperidone, olanzapine or quetiapine), there was no significant difference in gestational age at birth between the two groups.

Delivery complications (table 4)

The abovementioned prospective study [24] did not find a significant difference in the Apgar score at 5 minutes after birth between clozapine-exposed and other antipsychotic-exposed infants. The Apgar score at 1 minute was even slightly higher in the clozapine group than in the other antipsychotic group (8.6 vs 8.3, p=0.030).
In our pharmacovigilance study [22], we also calculated reporting odds ratios for ADRs related to 'pregnancy, labour and delivery complications and risk factors', a group of diverse ADRs including breech presentation for instance, caesarean section, eclampsia, GDM, and placental problems. The relative number of reports associating clozapine with these ADRs was significantly lower than the relative number of reports associating other antipsychotics with these ADRs.
In a case series [29] of nine pregnancies, eight infants required resuscitation at birth, including suction, oxygen therapy, continuous positive airway pressure, bag and mask, intubation, external cardiac massage, or other; four of these infants required admission to special care nursery, without further specification. Six of the nine mothers were obese (BMI > 30 mg/kg^2), six developed GDM, and four used other psychotropic medications concurrently.
Shoulder dystocia has been reported in three case reports [32,52,58] in a mother with pre-pregnancy diabetes [52] and in a mother with a normal pre-pregnancy BMI who developed diabetes during pregnancy[32], unfortunately, there was no information about weight and metabolic issues in the third case report. [58]

Birth weight and height (table 4)

In a previously mentioned prospective study [24], there were no significant differences in the percentages of low birth weight (< 2.5kg), mean birth weight, and birth length between infants exposed to clozapine or other antipsychotics.

Exposure to olanzapine and/or clozapine was not associated with an increased risk of being born small or large for gestational age. [20] However, there was an increased risk of being born with a large head circumference after exposure to olanzapine and/or clozapine (adjusted OR 3.02 [95% CI, 1.60-5.71]). None of these neonates had hydrocephalus.

A possible association between foetal exposure to atypical antipsychotics and increased infant birth weight and length, particularly with use of clozapine and olanzapine, was suggested in another prospective comparison study. [25] However, only 3 of 16 infants exposed to either clozapine or olanzapine were actually exposed to clozapine, which also complicates the interpretation of clozapine's contribution to the results.

Lastly, in a preclinical study [28], the average weight of mice pre- and postnatally exposed to clozapine increased more slowly than did that of control pups at a young age but not later.

Table 4. Studies describing fetal and neonatal outcomes, including delivery outcomes, after in utero exposure to clozapine

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Beex-Oosterhuis – 2020 [22]	494 ICSR-ADR pairs with adverse pregnancy outcomes related with CLZ exposure 4,645 ICSR-ADR pairs with adverse pregnancy outcomes related with OAP exposure	Fetal disorders: ROR= 0.68 (95%CI 0.48 – 0.96) for CLZ vs OAP Termination of pregnancy and risk of abortion: ROR=0.56 (95%CI 0.43 – 0.73) for CLZ vs OAP	#	Pregnancy, labour and delivery complications and risk factors: ROR=0.44 (95%CI 0.39 – 0.51) for CLZ vs OAP	Neonatal disorders: ROR=0.32 (95%CI 0.25 – 0.40) for CLZ vs OAP Congenital, familial and genetic disorders ROR= 0.37 (95%CI 0.29 – 0.47) for CLZ vs OAP

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Nguyen – 2020 [29]	8(9)	≥ 1 Non-reactive CTG (n=7) ≥ 3 non-reactive CTGs (n=5) Mean birth weight (SD): 3,396 g (188.7)	#	Premature rupture of membranes (n=1) (11.1%) Antepartum haemorrhage (n=1) (11.1%) Unassisted vaginal delivery (n=4) (44.4%) Assisted instrumental delivery (n=1) (11.1%) Emergency caesarean (n=1) (11.1%) Elective caesarean (n=3) (33.3%) Mean gestation at birth (weeks + days, SD) 38+2 (6.58 days) Any resuscitation at birth (including suction, oxygen therapy, CPAP, bag and mask, intubation, external cardiac massage or other): n= 8 (88.9%) Special care nursery admission: n=4 (44.4%)	2 birth defects (1 pulmonary artery stenosis and atrial septal defect and 1 pyloric stenosis) 5 neonates with full blood counts taken within 7 days of birth with no evidence of agranulocytosis

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Molins – 2019 [31]	1(1)	No malformations Birth weight 3,590 gram	#	Forceps-assisted vaginal delivery at 38weeks with no perinatal complications Apgar scores of 9-10-10	Normal white blood cell count No seizures No withdrawal syndrome No other neonatal complications
Narayanaswamy – 2018 [32]	1(1)	Fetal ventriculomegaly noticed on ultrasonography in the fifth month and at term Neural tube defect in a mother who used CLZ and haloperidol and found to be pregnant after the critical period of organogenesis Birth weight 3,490 g Birth height of 46 cm Birth head circumference of 34.5 cm	#	Shoulder dystocia during labor Delivery at term Low Apgar score at 1 and 9 minutes (not further specified	Admission in the intensive care unit for further care

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Uygur – 2019 [33]	1(2)	Pregnancy 1: # Pregnancy 2: Normal ultrasound examinations	No reports of negative effects of the treatment on the infant in the first pregnancy No neurocognitive or motor delays at 2-years follow-up (¶) of the second pregnancy	Pregnancy 1 # Pregnancy 2: Delivery at 38 weeks' gestation by caesarean section Apgar scores of 7 and 9	Pregnancy 1: 'the mother and her family did not report any negative effects of the treatment on the infant'. Pregnancy 2 No perinatal complications No agranulocytosis, seizures or other neonatal complications
Imaz – 2018 [30]	3(4)	M1: physiological fetal wellbeing during pregnancy No congenital anomalies Birth weight 3,850 g	M1: symptoms, but no diagnostic criteria, of ADHD (¶) at 6 years follow up	M1: - Estimated gestational age 38+6 - Spontaneous vaginal delivery - Apgar score (1/5/10min): 9/10/10 - No perinatal complications	M1: - No agranulocytosis, seizures, or other neonatal complications,

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		M2: fetal macrosomia detected in the 29th week of gestation No congenital anomalies Birth weight 3,660 g	M2: no neurodevelopmental disorders (¶) at 32 months of age	M2: Estimated gestational age 40+5 Cesarean delivery - Apgar score (1/5/10min): 9/10/10 No perinatal complications	M2: - No agranulocytosis, seizures, or other neonatal complications,
		M3-1: Type I intrauterine growth restriction in the 28th week of gestation, breech presentation at delivery Birth weight 2,498 g Left inguinal hernia and left cryptorchidism	M3-1: generalized neurodevelopmental delay (¶) at 18 months	M3-1: Estimated gestational age 38+6 w Caesarean delivery due to breech presentation Apgar score (1/5/10min): 6/10/10 Resuscitation procedure (positive pressure ventilation) due to decreased Apgar-min 1 score	M3-1: - No agranulocytosis, seizures, or other neonatal complications - urine drug test positive for benzodiazepines

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		M3-2: no altered physiological parameters Birth weight 3,650 g No congenital anomalies	M3-2: no neurodevelopmental disorders (¶) at 6 months of age	M3-2: Estimated gestational age 39 wks (Elective) caesarean delivery - Apgar score (1/5/10min): 9/10/10 No perinatal complications	M3-2: - No agranulocytosis, seizures, or other neonatal complications
Hatters Friedman - 2016 [18]	1(1)	No malformations Birth weight of 3,095 g	#	Delivery at 38 weeks of gestation	#
Hodge – 2016 [34]	1(1)	Reduced FHR variability and absence of accelerations in a mother using multiple drugs resulting in an emergency caesarean section Normal weight	#	Emergency caesarean section Normal Apgar scores	CRP increase over the first 48 hours of life with no other concerns.

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Köse Çinar - 2016 [35]	1(1)	Birth weight 3,090 g, Birth height of 50 cm Head circumference of 34.5 cm	#	Caesarean section without any complications. Apgar score of 10-10	Healthy baby
Montastruc – 2016 [23]	2(2)	Cleft palate (2x)	#	#	#
Oltulu – 2016 [26]	n.a.	#	Deterioration of learning performance in the Morris water maze task in rats with prenatal exposure to clozapine, haloperidol, sulpiride, chlorprothixene and chlorpromazine. These rats also showed an increase in thigmotaxis	#	#
Sreeraj - 2016 [36]	1(3) (triplet pregnancy)	Macrocephaly (34.5 cm) (1x) No congenital abnormalities or major physical problems in the other two children	#	Premature rupture of membranes at term. Delivery by caesarian section. Male monozygotic triplets with Apgar-scores of 8-9-9	Transient tachypnea of newborn which settled within 12 h of delivery in the second born baby

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Guyon – 2015 [37]	1(1)	Low variability in FHR with a normal baseline and accelerations at week 32 of pregnancy which normalized from week 38 of pregnancy to term	#	Vaginal delivery at 40+5 weeks. Apgar score (1/5 min): 9/10	Normal pediatric evaluation 5 and 10 days after birth. No further cardiac investigation needed. Negative systematic screening for neonatal metabolic disorders, including hypothyrQDism
Shao – 2015 [24]	CLZ: 33 (33) Comparator group consisting of risperidone (n=16(16)), OLZ (n=8(8)) or quetiapine (n=6(6))	No significant differences between the CLZ group and the comparator group in:	Lower mean adaptive-behaviour scores of Bayley-III in CLZ-exposed infants compared with OAP-exposed infants at 2 (89.1 versus 96.3, P = 0.001) and 6 months (94.8 versus 100.5, P = 0.011) of age, but these differences disappeared at 12 months of age (98.3 versus 96.3 P = 0.712)	No significant differences between the CLZ group and the comparator group in the Apgar score at 5 minutes after birth (9.6 vs. 9.4, p = 0.176)	No significant differences between the CLZ group and the comparator group in the rates of neonatal complications between the two groups

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		Percentage of low birth weight (less than 2.5kg) (9.0% vs. 16.7%, p = 0.367)	Significantly more CLZ-exposed infants with delayed development (score <85) in the adaptive-behaviour domain compared with OAP-exposed infants at 2 (54.5% versus 16.7%, P = 0.002) and 6 months (30.3% versus 10.0%, P = 0.047) of age, but these differences disappeared at 12 months of age (21.2. % versus 6.7%, P = 0.099)	Higher Apgar score at 1 minutes in the CLZ group than in the comparator group (8.6 vs. 8.3, p = 0.030).	
		Mean birth weight (3.2 kg vs. 3.3 kg, p = 0.409) Height at birth (51.2 cm vs. 50.8 cm, p = 0.195)	More CLZ-exposed infants had disturbed sleep and labile state than OAP-exposed infants at 2 months of age (75.8% versus 26.7%, P<0.001)	No significant differences between the CLZ group and the comparator group:	

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		No malformations (not in the CLZ exposed group and not in the comparator group)	No differences in weight and height development between CLZ- and OAP exposed infants during the first year of life	in the mean gestational age at birth (39.0 vs. 38.9 weeks, p = 0.430) in complications during delivery	
Kulkarni - 2014 [19]	CLZ: 11 pregnancies N= 147 pregnancies exposed to antipsychotics in the first trimester	Two infants with major congenital anomalies: hypospadias and hypertelorism in one baby gastroschisis and horseshoe kidney in the other baby	#	Not specified for the individual drugs	#
Bodén – 2012 [20]	CLZ: 11 neonates OLZ: 159 neonates Group 1: OLZ and/or CLZ (n = 169)	OR for being born preterm: 1.58 (95% CI, 0.91-2.73) for OLZ/ CLZ exposed infants compared with non-exposed infants and 1.94 (95% CI, 1.37-2.77) for infants exposed to OAP	#	#	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
	Group 2: OAP (n=338)	No statistically significant difference regarding the risk of being SGA or LGA for weight and length for group 1 or 2 after adjusting for confounders			
	Group 3: no antipsychotics (n=357 696)	Exposure to OLZ and/ or CLZ was associated with being LGA for head circumference with an adjusted OR of 3.02 [95% CI, 1.60-5.71], but none of the neonates had a hydrocephalus diagnosis			

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Coston – 2012 [38]	2(2)	M1: physiological fetal wellbeing during pregnancy absence of FHR variability / flattening of the FHR detected at pregnancy week 34+5 M2: absence of FHR variability at pregnancy week 38 + 5. Infant weight 3,330 g	#	M1: Estimated gestational age 39+2 Eutrophic neonate 'perfect Apgar scores' Arterial pH 7.33 M2: Caesarean delivery due to insufficient cervical dilatation and moderate fetal tachycardia with late decelerations 'perfect Apgar score' Arterial pH 7.30 and venous pH 7.32	#
Moreno-Bruna – 2012 [39]	1(1)	Normal ultrasounds during pregnancy Macrosomia (4060g)	No neurodevelopmental disorders and normal growth at 2-year follow-up (¶)	Uncomplicated vaginal delivery at term (40 + 5) Apgar score (1/5 min): 10/10	Delayed peristalsis with vomiting

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Novikova – 2009 [40]	1(1)	Absence of FHR variability without acidosis at 32 weeks' gestation	#	Caesarean delivery due to the critical maternal condition and fetal distress Apgar score of 7 at 5 minutes. Placental pH 7.19	Delayed peristalsis
Donohoe - 2008 [27]	n.a.	#	Deficits in the migration of neuroblasts and axonal growth in neurons in comparison to control animals in Caenorhabditis elegans (C. elegans) organisms exposed to clozapine or fluphenazine during embryonic development.	#	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Duran – 2008 [41]	2(4) 2 mothers (M1: 2 pregnancies; M2: one twin pregnancy)	M1, pregnancy 1: Birth weight of 2,900 g Birth height of 52 cm M1, pregnancy 2: Birth weight of 3,000 g Birth height of 50 cm M2 (twins): Birth weight of 3,100 and 2,940 g Birth height of 51 and 49 cm	All four children with normal motor and mental development and haematological examinations (unknown follow-up period) (¶)	M1, pregnancy 1: a term, uncomplicated vaginal delivery Apgar-scores of 9-10 Normal white blood cell count M1, pregnancy 2: term, uncomplicated vaginal delivery. Apgar-scores of 10-10 M2 (twins): Apgar-scores of 10-10 and 9-10	M1, pregnancy 1: normal white blood cell count no neonatal seizures M1, pregnancy 2: # M2 (twins): No positive records on seizure or agranulocytosis.
Newham – 2008 [25]	3(3)	Two of the three CLZ exposed babies seemed to be LGA for weight	#	#	#
Reis – 2008 [21]	18 (18)	Ectopic anus	#	#	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Klys – 2007 [42]	1 (1)	Neonatal death shortly after delivery after 39 weeks' gestation due to an acute clozapine overdose by the mother Birth weight 4,050 g	#	Spontaneous delivery one day after the suicide attempt, following vacuum extraction Apgar score of 1, with single isolated heartbeats	
Mendhekar – 2007 [43]	1(1)	Birth weight 2,950 g	Normal development, except for speech. By the end of 5 years, the infant gained normal fluent speech (¶)	Delivery at 9 months and 2 days of gestation	No perinatal complications
Doherty – 2006 [45]	1(1)	Late fetal decelerations on the cardiotocograph Morphologically normal infant	#	Emergency caesarean section at 40 weeks Apgar scores of 9 and 10	#
Sethi – 2006 [44]	1(1)	The child showed no congenital anomaly	No developmental delay at 2-year follow-up (¶)	No complication during labour and delivery	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Wang – 2006 [28]	n.a.	#	Pups exposed to HAL/CLZ increased in body weight more slowly than control pups at the beginning of the development. After 6 weeks, neither the HAL nor CLZ treated mice differed in body weight. CLZ and CLZ-withdrawal increased locomotor activity. Chronic clozapine treatment and transient withdrawal caused no impairment in the acquisition of memory. Moreover, it tended to improve memory.	#	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Walch – 2005 [46]	1(1)	Gestational week 33: fetal retardation and oligohydramnios Birth weight 2,400 g Birth length 49 cm Head circumference 31 cm Trisomy 21	#	Caesarean delivery at week 37	Sound conduction disorder At day 4: slightly increased TSH (23 mU/l) At day 10: increased TSH (62 mU/l) At day 23 day: muscular hypotonia poor feeding increased drowsiness hypothermia enlarged thyrQD
Gupta – 2004 [47]	1(2)	No congenital malformations detected with ultrasonography at the 10th week and repeating occasions during the first pregnancy	No neurodevelopmental disorders at 20-months and 6-months follow-up (¶)	First pregnancy: Delivery with episiotomy at 39 weeks Apgar scores of 8-9 Second pregnancy: Caesarean section at 39 weeks Breech presentation. Apgar scores of 7 - 9	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Karakula -2004 [48]	1(1)	Birth weight 4,000 kg, Head circumference 36 cm, Birth length 56 cm	Major developmental delay at 7-months follow-up	Caesarean section due to fetal arrhythmia and threat of fetal asphyxiation at week 28 (?) Apgar scores (1-3-5-10 min): 7-8-8-8 Last CLZ dose 10 hours before delivery	14 hours after delivery: clonic-tonic convulsions, opistotonus, lock-jaw, apnea following tracheal intubation and administration of phenobarbital, without significant improvement, abnormal heart shape 17 hours after delivery: admission to the neonatal intensive care unit At day 3 (?): Diagnosis of 'encephalopathy as side effect of medication with convulsions and coma, respiratory insufficiency'

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
					At day 10 mandibular recess, decreased muscle tone, periodic convulsions in upper extremities, flaccid chest, dyspnea, hernia of the linea alba, left testicle not palpable At day 15: deflection of the head, hypersomnia, increased muscle flaccidity after deflection.
Mendhekar - 2003 [49]	1(1)	The mother never reported fetal movements Stillborn baby of 2,200 g with no gross congenital malformations	#	Delivery at 9 months and 9 days	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Nguyen – 2003 [50]	1(3)	Pregnancy 1 and 2: Normal ultrasounds and amniocenteses (unknown in which trimester(s)) Pregnancy 1: Birth weight 3,460 g A functional heart murmur at cardiac auscultation, without signs of any cardiomyopathy on the ultrasounds Pregnancy 2: Birth weight 3,470 g	No neurodevelopmental disorders at 5- and 3-years follow up (¶) One of the children had an average weight, height and cranial parameter. The other child was on the lower part of the curves for weight, height and cranial parameter at 5- and 3-years follow-up respectively (¶)	Pregnancy 1: At term Emergency caesarean section because of fetal distress (late desceleration) and due to decreased progression of delivery. Apgar scores of 8-9-9. Pregnancy 2: Vaginal delivery at 40 weeks using forceps because of a prolonged 2nd stage. Apgar scores of 8-9-9	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Yogev – 2002 [51]	1(1)	Unremarkable pregnancy follow-up Reduced FHR variability on all fetal surveillance tests before labor and during all stages of labor without specific time-correlation to drug administration. Birth weight of 3,420 g	#	Normal delivery at week 37 Normal fetal assessment by biophysical score Apgar score of 9-10 Normal umbilical artery pH	#
Dickson -1998 [52]	1(1)	No information regarding birth weight and length	#	Induced delivery at 38 weeks' gestation. Complicated delivery due to shoulder dystocia assisted by low mid forceps	A healthy baby was born
Tenyi – 1998 [53]	4(6)	No embryotoxic disturbances in all six pregnancies	Three children (of one mother) with normal (psychomotor) development at 6-years, 3 ½-year and 1 ½-year follow-up (¶). Follow-up of the other three infants also showed no disturbances.		#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		M1, pregnancy 1: Birth weight 4,300 g Birth length 59 cm, Head circumference 35 cm No teratogenic disturbances		M1, pregnancy 1: Cesarean section at 40 weeks due to relative spatial disproportion Apgar scores of 9 and 10	
		M1, pregnancy 2: Birth weight 3,800 g Birth length 56 cm Head circumference 35 cm No teratogenic disturbances		M1, pregnancy 2: Cesarean section at 40 weeks due to relative spatial disproportion, Apgar scores of 6 and 9	
		M1, pregnancy 3: Birth weight 3,500 g Birth length 52 cm Birth head circumference 35 cm No teratogenic disturbances		M1, pregnancy 3: Cesarean section at 40 weeks' gestation Apgar score of 7 and 9	

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
		M2, Birth weight 2,800 g Birth length 49 cm Birth head circumference 33 cm No teratogenic disturbances		M2: Delivery at 37 weeks' gestation, Apgar scores of 9 and 10	
		M3: Birth weight 3,090 g Birth length 48 cm Birth head circumference: 33 cm No teratogenic disturbances		M3: Delivery at 38 weeks' gestation Apgar scores of 9 and 9	
		M4 Birth weight 3,570 g Birth length 50 cm Head circumference: 33 cm No teratogenic disturbances		M4: Delivery at 39 weeks' gestation Apgar scores of 9 and 10,	

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Stoner – 1997 [54]	2(2)	M1: Birth weight 3,800 g M2: Birth weight 2,510 gram	M1: no physical disorders at 2-year follow-up (¶) M2: no follow-up information	M1: Delivery with vacuum extraction at 39 weeks due to lack of cooperation, Temperature of 36.9 degrees Celsius, Pulse 128 bpm 16 respirations/min Apgar scores of 8-9 Arterial cord pHs of 7.27 and 7.30	M1: abnormal findings at birth: Cephalhematoma (resolved between 2 days after delivery) Hyperpigmentation folds (resolved between 2 days after delivery) Coccygeal dimple (resolved between 2 days after delivery) One seizure at day 8 after delivery Possible mild gastroesophageal reflux No longterm sequelae from the seizure and no further seizure activity

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
				M2: Delivery at 40 weeks Apgar scores of 8 and 9	M2: No abnormalities, the baby developed low grade fever postpartum that resolved prior to discharge
Di Michele -1996 [55]	1(1)	Uneventful pregnancy under obstetric supervision Birth weight 3,300 g	#	Caesarean section at 37 weeks Apgar scores of 7 and 9, Arterial cord pH of 7.3	Benign tachypnoea Mild Floppy Infant Syndrome; with hypotonia resolving five days after delivery Normal cerebral ultrasound, electroencephalography, abdominal ultrasound and lung X-ray

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Dev – 1995 [56]	102 pregnancies The outcome of pregnancy in 22 patients is unknown	8 non-elective and 13 elective abortions 5 infants with malformations (in some instances, mothers were also taking other drugs which may have caused these malformations). Not described if these were minor or major malformations	#	#	Of the 61 babies (59 pregnancies): 51 healthy infants, 5 infants with (undefined) problems during the post-natal period
Barnas – 1994 [57]	1(1)	Uneventful pregnancy with regular ultrasonography to confirm normal fetal growth Birth weight of 3,600 g	No psychomotor disorders at 6-months follow-up (¶)	Delivery with vacuum extraction at 41 weeks of pregnancy. Apgar scores of 5 and 8 Arterial cord pH of 7.34	#

Table 4. *Continued*

First author + year of publication	Number of mothers (number of pregnancies)	Fetal outcomes	Developmental outcomes	Delivery outcomes	Neonatal outcomes
Waldman – 1993 [58]	1(1)	Birth weight of 3,700 g	#	Induced delivery with prostin gel at 38 weeks, due in part to the mother's inability to comply satisfactorily with diabetic dietary restrictions. Uncomplicated delivery except for shoulder dystocia. Apgar scores of 7 and 9	Healthy baby

no information

¶ unknown if this is based on structured tools to assess the development of the infants or on parents› reports

CI95%	95% confidence interval
CLZ	clozapine
CTG	cardiotocography
FHR	Fetal Heart Rate
LGA	large for gestational age
NorCLZ	norclozapine
n.a.	not applicable
OAP	other antipsychotics
OLZ	olanzapine
ROR	reporting odds ratio
SGA	small for gestational age

Neonate

Neonatal pharmacokinetics of clozapine (table 2)

Since some pharmacokinetic parameters, such as clearance, volume of distribution, and bioavailability, are age-related, the pharmacokinetics of clozapine in neonates may be different from those in adults. [72] In a case series [30] describing four neonates, a mean half-life value of clozapine of 92 (±18) hours was calculated for neonates, whereas it is 9–17 hours in adults [73]. Another case report [36] described a prolonged elimination half-life in neonates of approximately 2.5 days for clozapine and 3 days for norclozapine.
Available data are too limited to define an elimination half-life of clozapine and norclozapine in neonates, but the delayed clearance reported above is in line with the fact that CYP1A2 activity attains its adult level 7–8 months after birth. [69]

Neonatal outcomes (table 4)

On the basis of the above and the assumption that it takes five elimination half-lives to eliminate a drug, it would take approximately 15 days to eliminate clozapine from the neonate after delivery. Thus, it is conceivable that clozapine exerts pharmacodynamic effects in the first 2–3 weeks after birth if the neonate has been exposed to clozapine in utero.
In our previous study [22], we also calculated the reporting odds ratio for ADRs related to 'neonatal disorders'. Again, the relative number of reports associating clozapine with these ADRs was lower than the relative number of reports referring to other antipsychotics as suspected drug(s).
One case report [39] described an infant with diminished peristalsis and vomiting whose mother had been using 100 mg clozapine a day at term. The authors attributed the peristalsis and vomiting to the anticholinergic properties of clozapine.
Two cases [48,55] of floppy infant syndrome have been described. In one, the infant had also been exposed to high doses of lorazepam.[55]

Infant exposure to clozapine through breast milk (table 2)

Theoretically, infants can be exposed to clozapine via breast milk, but there have been few studies of this. Clozapine concentrations in breast milk have been reported in only one study. [57] The day after delivery, when the mother was using 50 mg clozapine a day, the clozapine level was 14.7 ng/mL in plasma and 63.5 ng/mL in the first 'portion' of breast milk. One week later, when the clozapine dose had been increased to 100 mg/day, the breast milk concentration was

115.6 ng/mL, and the maternal plasma level was 41.4 ng/mL. No additional data regarding neonatal clozapine concentrations were presented.
Dev and Krupp [56] only briefly refer to four breast-fed babies from mothers taking clozapine. One baby was extremely sleepy and another developed agranulocytosis which disappeared spontaneously when breastfeeding was discontinued. The other two babies had no apparent adverse effects.
In summary, there is very little information about clozapine in breast milk, but the lipophilicity and low molecular weight of the drug make it likely that it will enter the breast milk of nursing mothers taking clozapine.

Discussion

In this review, we summarized current knowledge on the use of clozapine during pregnancy and lactation. Although data on perinatal clozapine exposure are of limited quality and quantity, available data show that clozapine and norclozapine pass the placental barrier and that the foetus will be exposed to clozapine and norclozapine if a mother uses clozapine during pregnancy. However, clozapine appears to cross the placental barrier to a lesser extent than olanzapine but to a similar extent as risperidone. [30,62] Although it is not known at which stage of pregnancy clozapine enters the foetal system, data thus far do not support that clozapine is teratogenic or, compared to other antipsychotics, increases the risk of stillbirth, abortion, or foetal disorders, nor that it increases the risk of delivery complications or premature birth.

With exposure in the last trimester, newborns are potentially at risk of all clozapine-related adverse events post-partum, including agranulocytosis. The limited data on clozapine pharmacokinetics in neonates suggest that the elimination rate is slower, so that the neonate is exposed to clozapine for 2–3 weeks post-partum and even longer if the infant is breastfed. We did not find any reports of neonatal agranulocytosis after in utero exposure, but the incidence of agranulocytosis in adults is rare anyway. [74] Nonetheless, we recommend close antenatal monitoring of decreased white blood cell / absolute neutrophil count and other potential adverse events of clozapine, including diminished peristalsis, or possible withdrawal effects in the neonate during the first month after birth.
Yet, agranulocytosis has been reported in a breast-fed infant.[56] A lack of additional information, however, means that we do not know whether the mother also used clozapine at term and whether the symptoms developed within 2–3 weeks post-partum. Information on clozapine concentrations in breastmilk is limited to a single observation but based on the physical chemical properties of the drug, clozapine exposure through breastfeeding is highly

probable. Again, all adverse events associated with clozapine exposure can be expected if an infant is being breastfed by a mother who is using clozapine.

The physiological changes during pregnancy, the pharmacokinetic properties of clozapine, and possible changes in tobacco and caffeine use during pregnancy make it plausible that maternal clozapine concentrations change despite stable dosing. We conclude that the net result of these changes cannot be predicted, thereby emphasizing the need for close monitoring of clozapine concentrations during pregnancy in order to keep the mother's psychiatric condition stable during this period. There remains a need for well-designed, prospective studies linking maternal clozapine levels, including unbound concentrations, and dose regimens in the last trimester with the pharmacokinetics and pharmacodynamics of clozapine in neonates.

Although based on anecdotal reports, clozapine exposure might be related to an increased frequency of reduced or absent foetal heart rate variability in the unborn infant, thereby mimicking signs of foetal stress on the cardiotocography. It is unknown whether this effect is accompanied by an elevated clozapine concentration in the mother or the foetus.

The effects of maternal clozapine use during pregnancy on GDM remain unclear, as is the risk of increased infant birth weight. In the study by Bodén et al [20] the lower BMI at baseline in the group of clozapine and/or olanzapine-treated mothers compared to the group of mothers treated with other antipsychotics could be based on selective non-prescribing of clozapine and olanzapine to overweight women. We cannot exclude that this possible selection process could have masked a pharmacological effect that increased the risk of GDM. An increased BMI in early pregnancy, whether or not due to the use of antipsychotics, may be a better predictor of the development of GDM than the use of antipsychotics as such. Also, the risk and implications of the larger head circumference seen with clozapine compared with other antipsychotics remain to be elucidated. [20] Lastly, more information is needed to ascertain whether prenatal clozapine exposure results in permanent changes in the brain and how this affects the child in the short- and long-term. The results of preclinical studies suggest that clozapine might affect neurodevelopment, and the only available clinical data suggest that clozapine affects the development of adaptive behaviour to a greater extent than do other antipsychotics, at least in the short-term.

Studies of the safety of drugs in pregnancy rarely meet the gold standard of randomized controlled trial data. As a result, the best available data in this area tend to come from observational studies, and the majority of data come

from case series and case reports in which publication bias can be expected. Nevertheless, in the absence of more controlled studies, every case report on the use of clozapine in the perinatal period contributes to the accumulation of knowledge on this subject.
Another limitation of the studies included in this review is that there was often no information available about smoking and substance use during pregnancy, the use of comedication, vitamin status, maternal age, planned or unplanned pregnancy, and pre-pregnancy BMI. In addition, exposure to clozapine is a consequence of severe maternal illness, and schizophrenia as such has also been associated with a number of adverse obstetric complications and pregnancy outcomes. [75] In the absence of a control group, it is not possible to differentiate between the risks associated with drug exposure and those of the mental illness and its associated physical health problems and lifestyle factors. In addition, given the estimated baseline population rate for malformation in the general population of 1–3% [76] large numbers of exposed foetuses are needed to detect differences in the incidence of malformations and an even larger number is needed to control for confounders.

In general, when a clinician is consulted by a woman using clozapine who seeks preconception advice or who is already pregnant, one might prefer an agent with more robust pregnancy data. As a consequence, the clinician might consider a switch to another antipsychotic. However, every switch confers a risk, and a risk-benefit analysis should be made, taking into account, on the one hand, the available data and the level of evidence and, on the other hand, the timing of counselling (pre- or post-conception), lactation plans, as well as severity of illness and response to medication in the past. With regard to timing, the exposure to clozapine covers more developmental stages of the foetus in case a woman presents for preconception counselling on clozapine, compared with the situation that conception has already taken place or in the case of an advanced pregnancy.
In the first situation, there is more time to gather information, make a thorough decision and, in some cases, involve family members. Also, when the mother plans to breastfeed the child after birth, the scarce safety data regarding clozapine exposure through breastmilk in combination with probable neonatal exposure based on its chemical properties, should be considered.
On the other hand, given clozapine's place in the treatment algorithm of schizophrenia, it is likely that a woman has been diagnosed with treatment-resistant schizophrenia when she uses clozapine and thus has not responded adequately to other antipsychotics. One should know if previous psychotic episodes were associated with danger to the patient (e.g., suicidal behaviour) or to others, and if previous antipsychotic drugs were associated with serious

side effects (e.g., severe akathisia or parkinsonism) before deciding if stopping or changing clozapine would outweigh the risk of relapse in this context.

Altogether, at some point, clinicians can be faced with the challenge of planned and unplanned pregnancies in their clozapine treated patients. When carefully outweighing the risk and benefits of clozapine continuation during pregnancy versus switching to another antipsychotic, one should include severity of illness and treatment history, but also be aware of the limitations of the available safety data regarding perinatal clozapine use, including the fact that there are few studies.

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Appendix 1

Search strategy Pubmed/ Medline

((("clozapine"[MeSH Terms] OR "clozapine"[All Fields] OR "leponex"[All Fields] OR "clozaril"[All Fields])) AND (((((((Pregnancy[Mesh] OR pregnan*[tiab] OR gestation*[tiab])) OR ("Maternal Exposure"[Mesh] OR maternal[tiab] OR "in utero"[tiab] OR prenatal*[tiab])) OR (Fetus[Mesh] OR fetus*[tiab] OR fetal[tiab] OR foetus*[tiab] or foetal[tiab])) OR ("infant, newborn"[MeSH Terms] OR "infant"[All Fields] OR "newborn"[All Fields] OR "newborn infant"[All Fields] OR "neonate"[All Fields] OR neonat*[All fields] OR offspring[All fields]))) OR (("lactation"[MeSH Terms] OR "lactation"[All Fields] OR "breast feeding"[MeSH Terms] OR ("breast"[All Fields] AND "feeding"[All Fields]) OR "breast feeding"[All Fields])))

4

Total and unbound clozapine and norclozapine concentrations in patients using clozapine once and twice daily

4.1

The impact of once-daily dosing on the pharmacokinetics of clozapine and norclozapine

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Abstract

Aim

Clinicians increasingly favour a once-daily (QD) over a divided dosing regimen for clozapine. This study aimed to determine whether the pharmacokinetics of clozapine and norclozapine with QD use are similar to twice-daily (BID) use. Additionally, the effect of QD dosing on the concentration-time curves of (nor) clozapine was studied through simulations.

Methods

Multiple blood samples were collected within a dosing interval from patients using clozapine QD or BID in steady state. (Nor)clozapine concentrations were analysed with a validated LC-MS/MS method. Population pharmacokinetic (PPK) analyses were performed by means of nonlinear mixed-effects modelling. The influence of the dosing regimen was tested as a covariate.

Results

Forty-four patients (319 samples) were included. For clozapine, the best structural model was a two-compartment model with a transit compartment and linear elimination consisting of combined excretion and formation of norclozapine. For norclozapine, a one-compartment model with first-order elimination best described the data. Dosing regimen did not affect any of the pharmacokinetic parameters. The simulations showed that clozapine concentrations sampled in the morning with QD use in the evening are markedly higher than its trough concentrations and higher than if the same daily dose had been used in two divided doses. The relative amounts of clozapine to norclozapine also vary to a greater extent than with BID dosing.

Conclusion

The pharmacokinetics of clozapine and norclozapine are not influenced by the dosing regimen. Nonetheless, clozapine concentrations obtained at a given total daily dose as well as the metabolic ratio will be affected by the dosing regimen and the time of blood sampling. The implications of this on clozapine's therapeutic window at QD dosing are yet unknown.

Dutch Trial Register (NL6913 - NTR7108) – date of registration: 30/11/2017

Introduction

Considering the high frequency of non-adherence among patients with schizophrenia taking antipsychotics [1], and the considerable negative clinical and economic consequences that are associated with it [2], every effort to increase adherence should be fully explored. This certainly applies to clozapine, the only antipsychotic drug effective in treatment-resistant schizophrenia and therefore a drug of last resort in most cases.

While many factors are known to affect adherence, simplifying dosing regimens is one means to this end. [3] With a relatively short elimination half-life of 12-16h [4], and even shorter in smokers [5], clozapine was originally administered in divided doses. Yet, nowadays, clinicians increasingly seem to favour a once-daily (QD) over a divided dosing regimen aiming for better adherence [6]. In addition, the Dutch 'Guideline on the use of clozapine' states that clozapine can be given as a single dose before the night to reduce the risk of day-time sedation. [7]

Therapeutic drug monitoring (TDM) is considered useful to guide clozapine therapy. A therapeutic window of 350 - 700 µg/L is applied, based on trough concentrations from studies using a divided dosing regimen. Clozapine's effects, both beneficial and adverse, have been proposed to be partially attributable to clozapine's main metabolite, norclozapine and therefore TDM for clozapine includes measurement of norclozapine in several laboratories. However, norclozapine's precise role is yet inconclusive. [8-11]

In clinical practice, the same reference values are used for QD as for twice-daily (BID) dosing regimens, although it is unknown if the concentration-effect relations of BID and QD dosing are similar. Theoretically, higher peak concentrations, due to higher single doses, might lead to an increase in concentration-related side effects, such as sedation, hypersalivation, seizures and tachycardia. [12-14] In contrast, a recently published meta-analysis of 8 cohorts (n=2810 clozapine-treated individuals) suggested slightly better tolerability in patients receiving once- vs. multiple dosing of clozapine, although this finding did not survive in their sensitivity analysis and the results are limited by the designs of the included studies. [15]

For practical reasons, blood samples for TDM of clozapine are usually drawn in the morning, i.e., approximately 12 hours post-dose regardless of the dosing regimen. However, unlike with BID dosing, these morning samples are measured only halfway down the concentration time curve in case of QD dosing and do not reflect trough concentrations. Pharmacokinetic models can be used to estimate the influence of QD dosing on the concentration-time curves of clozapine. [12-14] But for this to be legitimate, it must be established whether the pharmacokinetics at QD and BID doses are similar.

This study therefore aims to determine whether the pharmacokinetics of clozapine and norclozapine with QD use are comparable to BID use, based on population pharmacokinetic (PPK) analyses.
In addition, the effect of QD dosing on clozapine and norclozapine concentrations are studied through simulations, based on the developed model.

Method

Study design and data collection

This multicentre, non-randomised, open label pharmacokinetic study has been conducted among male and female in- and outpatients, aged 18-70 years, diagnosed with schizophrenia or other psychotic disorders, and receiving a stable dose and dose regimen of clozapine QD or BID for at least one week as part of their regular treatment. From February 2019 until February 2020, patients were recruited from two Dutch mental health organisations: Yulius in Dordrecht and Reinier van Arkel in 's-Hertogenbosch.

Subjects were not included in case of compulsory psychiatric treatment, active suicidality, and inability to decide on participation in the present study, as assessed by the patient's attending psychiatrist. Additional exclusion criteria were pregnancy, initiation, cessation, or dose change of interacting comedication or tobacco containing products within seven days prior to blood sampling, and acute inflammation or infection (derived from having a body temperature >38.0 degrees Celsius or using an antibiotic), as verified by the research nurse or physician at the study day.

Multiple venous blood samples were drawn from each participant, using a peripheral venous catheter. The sampling times refer to different pharmacokinetic phases necessary to characterize the pharmacokinetic profile of clozapine and include the optimal sampling time points established in two previous studies [15,16:] immediately before clozapine intake (t=0), between 0.5 and 1 h post-dosing, and at 2 (± 30 min), 3 (± 30 min), 4 (± 1 h), 5 (± 30 min), 8 (± 1 h) (only BID), and 12 h (± 2 h) (only for patients using clozapine QD and BID in unequally divided doses) post-dosing. To avoid sampling during the night and early morning respectively, patients on an QD dosing regimen took their evening dose between 5pm and 6pm, and the 12 hours post-dosing samples were allowed to be taken until 9am.

Serum was separated by centrifugation and stored at -20 degrees Celsius until analysis. Plasma samples for the analyses of C-reactive protein (CRP), albumin,

creatinine and urea concentrations and serum samples for the analysis of AGP were taken at t=0.

The following clinicodemographical data were also collected: age, sex, weight, height, clozapine dosing regimen, total daily clozapine dose and individual dose at each time point (in case of BID dosing), comedication, smoking habits and average number of caffeine-containing drinks per day over the past seven days, and ethnic origin.

Power calculation

A formal power analysis could not be performed since the effect of the alternative dosing regimen (i.e., QD) on pharmacokinetic parameters was unknown.
However, Bonate et al. demonstrated that a subgroup of 30 in a total population of 60 patients would detect a covariate with a power of 90% in a study where 2 samples per patient are drawn. [17] Furthermore, Lee showed that increasing the number of samples per patient from 2 to 3 dramatically increased power. [18] The same effect was seen for allowing variability in sampling times.
In our study, inclusion of patients was expected to be more complex than collecting sufficient data within one patient once included. Therefore, the study was designed to include 50 patients: 25 on each dosing regimen, with optimized data collection in terms of number of samples (7 or 8 per patient), allowing for variable sampling times within a certain time frame.

Ethical requirements

The study has been conducted in accordance with the Declaration of Helsinki and approval was provided by the Medical research Ethics Committees United (MEC-U) (number NL63635.101.18) as well as by the local ethics committees of the participating centres. The study protocol has been registered in the Dutch Trial Register (NL6913 - NTR7108). Patients were included after providing written informed consent.

Bio-analysis

Quantification of clozapine and norclozapine concentrations in serum was performed by the clinical and pharmaceutical laboratory of the University Medical Centre of Groningen (the Netherlands) using a LC-MS/MS method validated in accordance with the FDA [19] and EMA guidelines.[20] Linearity of the calibration curve was proven on an eight-point calibration curve, with a Lower Limit of Quantification (LLQ) of 10 µg/L, a low QC sample of 20 µg/L, a medium

QC sample of 400 µg/L, a high QC sample of 800 µg/L and a four-times-diluted QC sample of 2000 µg/L.

Albumin, creatinine, urea, and CRP concentrations in plasma were quantified as routinely by the Result Laboratory of the Albert Schweitzer hospital in Dordrecht, the Netherlands on the Siemens Dimension® Vista 1500 (Siemens Healthcare Diagnostics). Assays were photometric (albumin, creatinine, urea) and nephelometric (CRP).

Serum AGP concentrations were determined at the Leiden University Medical Centre (the Netherlands) using the Tina-quant α1-Acid Glycoprotein Gen.2 (AAGP2) immunoturbidimetric assay on the Roche cobas c502 analyser (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analyses

Patients' baseline characteristics were tested for differences between the treatment groups (QD and BID regimen) using Chi-square and t-test. Statistical analyses were performed using R, version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

Population pharmacokinetic analyses

PPK analyses were performed using the software package NONMEM (non-linear mixed effects modelling, version 7.5 Icon, Hanover, MD, USA), combined with Pirana version 2.9.9, R version 4.0.5, Xpose4 version 4.7.1 and PsN version 5.0.0. Concentrations were expressed in nanomoles per litre, using the molecular weights of clozapine (326.82 g/mol) and norclozapine (312.80 g/mol).

The first order conditional estimation option with interaction (FOCE-I), was used as estimation method with subroutine ADVAN6 TOL6, as implemented in NONMEM.

Structural model development

For the structural model for clozapine and norclozapine one- and two-compartment models were tested. Different approaches were tested to describe the absorption process: first and zero order, as well as the incorporation of lag time and a transit compartment. Regarding elimination, first-order elimination was applied. Like previous reports [21-23], the structural model assumed that the metabolised fraction of clozapine to norclozapine was 0.66. No first-pass effect was considered in the model.

To evaluate whether the pharmacokinetics of clozapine and norclozapine differ between QD and BID dosing regimens, several steps were taken. Since delayed absorption or saturation of elimination might occur at higher concentrations with QD dosing, the data were visually inspected for non-linearities. Furthermore, Michaelis-Menten kinetics was tested for both absorption (formation in case of norclozapine) and elimination of both parent and metabolite. In addition, dosing regimen was tested as a covariate for the PK parameters.

Inter-individual variability (IIV) was assumed to follow a log-normal distribution and was therefore implemented into the model as:

$$P_i = Ppop * e^{\eta i}$$

where P^i depicts the individual or post-hoc value of the parameter for the i^{th} individual, P_{pop} the population mean for the parameter, and ηi the empirical Bayes estimate of interindividual variation (IIV) for the i^{th} individual, sampled from a normal distribution with a mean of zero and a variance of ω^2. Residual error was evaluated for clozapine and norclozapine separately, as a proportional or additive error, or as a combination of both:

$$P_{obs} = P_i * (1 + \varepsilon_{proportional}) + \varepsilon_{additive}$$

where P_{obs} is the observed value, $\varepsilon_{proportional}$ the proportional-, and $\varepsilon_{additive}$ the additive-error component. Residual error components are sampled from a normal distribution with mean of zero and variance σ.

Discrimination between hierarchical models was guided by the likelihood ratio test, by comparison of the objective function value (OFV) [i.e. −2 log likelihood (−2LL)] between nested models. A *P* value of <0.05, representing a change in the OFV [ΔOFV] of −3.84 for one degree of freedom, was considered statistically significant.

A visual inspection of model performance was done through evaluation of goodness-of-fit plots for both compounds (observed versus individual-predicted concentrations, observed versus population-predicted concentrations, conditional weighted residuals [CWRES] versus time after dose, and CWRES versus population-predicted concentrations). Furthermore, precision of parameter estimates, the correlation matrix, relative standard error (RSE), condition number, eta-shrinkage and improvement in the individual plots were used to evaluate the model. [24]

Covariate model development

Once the structural model for clozapine and norclozapine was developed, the possible influence of the dosing regimen was studied as described above.

In addition, other covariates tested were total body weight, body mass index (BMI), age, sex, AGP concentrations, albumin concentrations, CRP, and smoking status. Covariates were plotted independently against the eta estimates of the pharmacokinetic parameters to visualize potential relations. Continuous covariates were tested using linear and power equations:

(1) $P_i = P_p + Y*(COV-COV_{median})$

(2) $P_i = P_p * (COV/COV_{median})^X$

where P_i and P_p represent the individual and population parameter estimates, respectively, COV the covariate, and COV_{median} the median value for the covariate for the population. Y represents a correlation factor between the population pharmacokinetic parameter and the change in the covariate value for a linear function, and X represents the exponent for a power function.

Categorical covariates were examined by calculation of a fractional parameter. Potential covariates were entered into the model one at a time and statistically tested by the likelihood ratio test. In addition, if applicable, a reduction in IIV (omega) of the parameter was evaluated upon inclusion of the covariate on the parameter. Further, trends in the random effects of the parameter versus the covariate involved were explored. When more than one significant covariate was identified, the covariate-adjusted model with the largest decrease in the OFV was chosen as a basis to sequentially explore the influence of additional covariates with the use of the same criteria. Finally, after forward inclusion ($p < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($p < 0.01$).

Model evaluation

The precisions of the final model parameters were evaluated using relative standard error (RSE) generated from covariance steps and sampling-importance-resampling (SIR)[25]-based 95% confidence intervals (CIs). Furthermore, the final model was evaluated using prediction-corrected visual predictive checks [24] (VPCs) stratified for clozapine and norclozapine, using 1000 simulated datasets of individuals from the original dataset.

Simulations

To illustrate the influence of dosing regimen on the concentration-time curves and especially on trough concentrations in relation to the currently applied therapeutic window, the PPK model was used to simulate the concentration-time curves of both clozapine and norclozapine for QD and BID (both equally and unequally divided doses) dosing regimens. To make our results comparable with those of two previous simulation studies [12,13], simulations were performed for a daily dose of 400 mg of clozapine in a smoking patient. In addition, concentration-time curves for a relatively high daily dose (700 mg) in a smoking patient was also simulated.

Results

Forty-four patients were included in this study with the majority (n = 28) in the QD group. In total, 319 clozapine and norclozapine serum samples were collected, with a median of 7 per patient (range 5-8). The patient characteristics are summarised in Table 1.

Table 1. Clinicodemographics of the included patients

Variable	QD (n=28)	BID (n=16)
Male (%)	17 (61%)	9 (56%)
Smoker (%)	20 (71%)	13 (81%)
Age (years)	43.5 (12.7)	46.1 (13.9)
Body weight (kg)	97.6 (27.1)	90.3 (27.0)
BMI (kg m-2) *[18.5-25.0]*	31.1 (8.6)	29.8 (9.4)
Albumin (g L-1) *[35-50]*	39.2 (5.0)	38.2 (3.1)
Creatinine (µmol L-1) *[45-90 for women]* *[60-110 for men]*	75.6 (20.2)	76.7 (14.9)
Urea (mmol L-1) *[1.8 – 7.1]*	4.9 (1.8)	4.9 (1.3)
CRP (mg L-1) *[< 10]*	6.6 (6.5)	7.8 (8.6)
AGP (g L-1) at t=0 *[0.5-1.2]* *(median, range)*	0.92 (0.58 – 1.52)	0.97 (0.42 – 1.64)

Table 1. *Continued*

Variable	QD (n=28)	BID (n=16)
Ethnicity (n, %)	Caucasian: 23 (82%) Asian: 1 (4%) African: 3 (11%) Other: 1 (4%)	Caucasian: 13 (81%) Asian: 1 (6%) African: 2 (13%)
Daily dosage of clozapine (mg) *(median, range)*	313 (25-800)	388 (150-750)

Values are expressed as means (standard deviation) unless specified otherwise.
Reference values (if relevant) are shown between square brackets in the first column.
BMI = body mass index
CRP = C-Reactive protein
AGP = alpha-1 acid glycoprotein

There were no significant differences in the demographical or clinical data between the QD and BID group.

Figure 1 shows the concentration-time profiles of all participants, stratified on substance and dosing regimen. No concentrations fell below the LLQ of the analytical method.

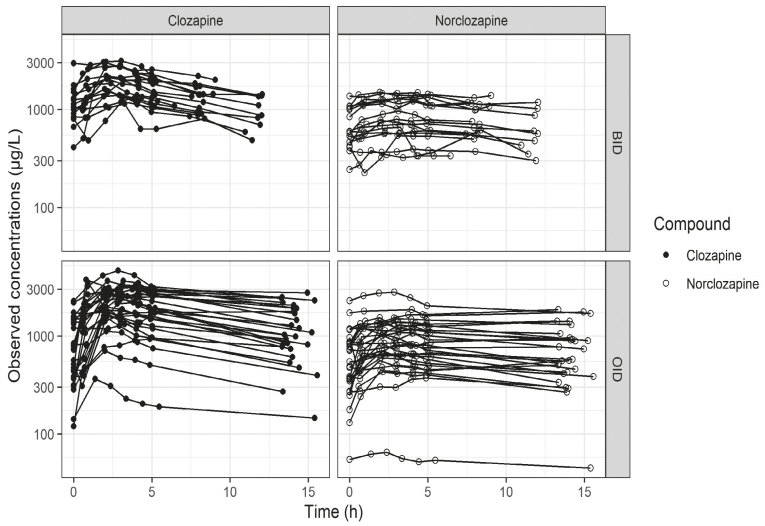

Figure 1. Observed concentrations (in µg/L) versus time plots for clozapine and norclozapine, stratified on once-daily (QD) or twice-daily (BID) at a semi-logarithmic scale.

PPK model

Base model development

For clozapine, data were best described 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).

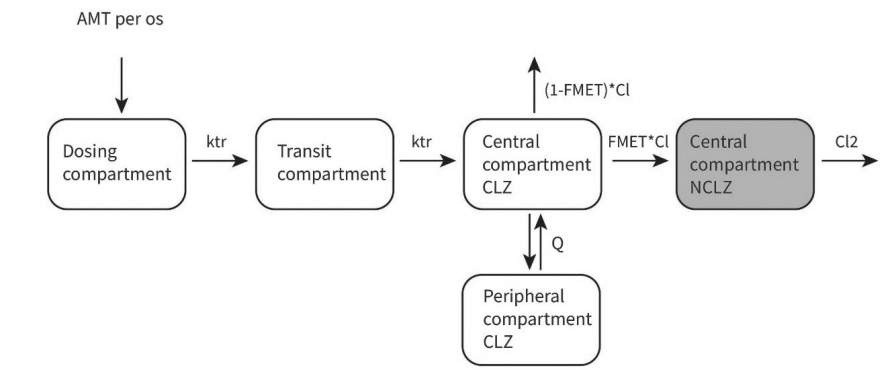

Figure 2. Schematic representation of the structural model.
AMT = amount, Cl = clearance of clozapine, Cl2 = clearance of norclozapine, CLZ = clozapine, FMET = fraction metabolised, ktr = transit compartment rate constant, NCLZ = norclozapine, Q = intercompartimental clearance

The parameter estimates of the base model without covariates are shown in Table 2. The percent relative standard error (% RSE) of the parameter estimates were acceptable (<30% for all estimated parameters) except for the apparent volume of distribution (V/F') of norclozapine (72.0%).

Final model

Visual inspection of the data did not imply non-linear PK at the higher concentrations in de QD regimen. Also, application of Michaelis-Menten kinetics in both absorption and elimination phase, was not supported by the data.

None of the pharmacokinetic parameters of clozapine and norclozapine was found to be different between the two dosing regimens when dosing regimen was applied as a covariate.

The population-predicted values for apparent clearance of clozapine and norclozapine in non-smokers were 22.2 and 36.1 L/h, respectively. The population-predicted apparent volumes of distribution for clozapine (central and peripheral) and norclozapine were 328 L, 286 L and 751 L, respectively (Table 2).

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)	

The mean-transit time was estimated at 1.23 h-1, resulting in a mean transit time for absorption of 1.6h.

Of the other covariates tested, only 'smoking' had a significant effect on clozapine clearance (delta OFV = -7.76). This effect was not seen on norclozapine clearance. Based on the developed model, clearance of clozapine is 55% higher in smokers than in non-smokers.

Model evaluation

The goodness-of-fit plots are shown in Figure 3. Due to eta shrinkage towards 0, no IIV could be reliably quantified for V/F' for norclozapine as well as on intercompartmental clearance (Q). The parameter uncertainties remained relatively similar after SIR (table 2).

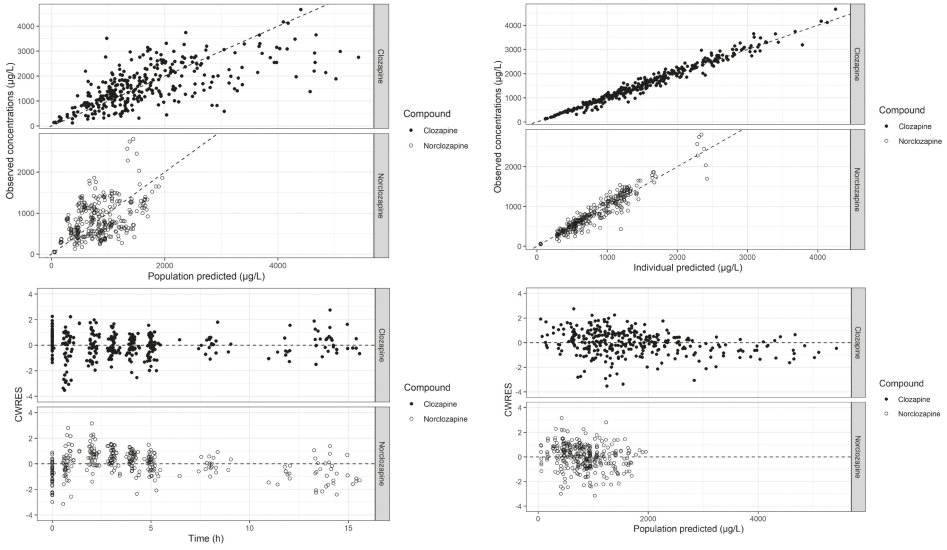

Figure 3. Goodness-of-fit plots. Observed versus population predicted concentrations (top left) and observed versus individual predicted concentrations (top right) of both clozapine and norclozapine. CWRES versus time (bottom left) and CWRES versus population predicted concentrations (bottom right) of both clozapine and norclozapine.

(The clozapine concentrations are represented by closed circles; the norclozapine concentrations by open circles.)

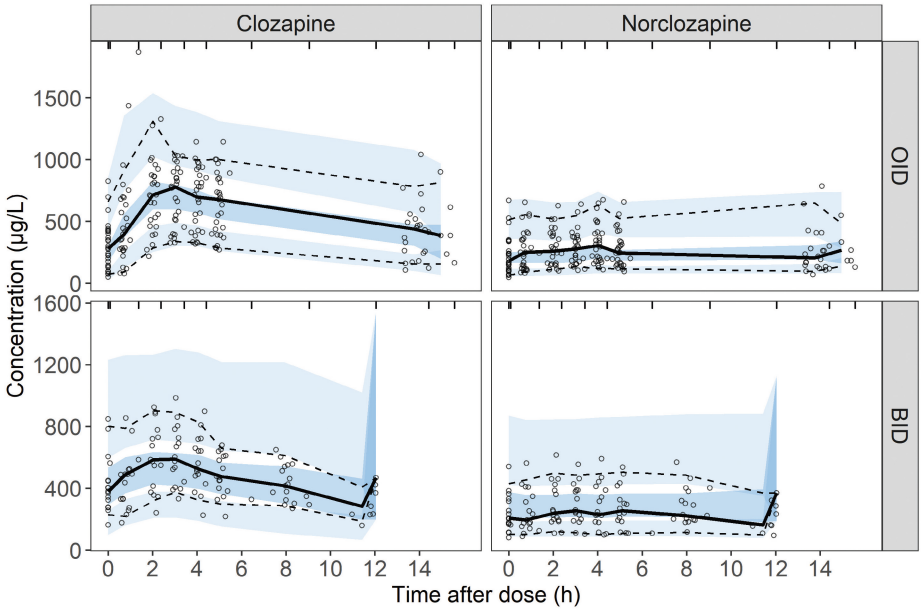

Figure 4. Visual predictive checks (VPCs) of prediction-corrected concentrations of CLZ (left) and NCLZ (right). The upper plots indicate

(*VPCs of once-daily, while the lower plots indicate VPCs of twice-daily regimen. VPCs were based on 1000 simulations. The solid line represents the median prediction-corrected observed serum concentrations with the semitransparant field representing a simulation-based 90% confidence interval for the median. The observed 5% and 95% percentiles are presented with dashed lines, and the 90% confidence intervals for the corresponding model predicted are shown as semitransparant fields. The prediction-corrected serum concentrations are represented by open circles.)*

Some model misspecification was observed in the goodness-of-fit plots (Figure 3) and visual predictive checks (Figure 4). Overprediction of clozapine and underprediction of norclozapine concentrations occurred in the absorption phase of clozapine.

Simulations

In Figure 5 and 6, the impact of the dosing regimen on clozapine and norclozapine trough concentrations is illustrated as is the impact of sampling time on the obtained concentrations when clozapine is used QD.

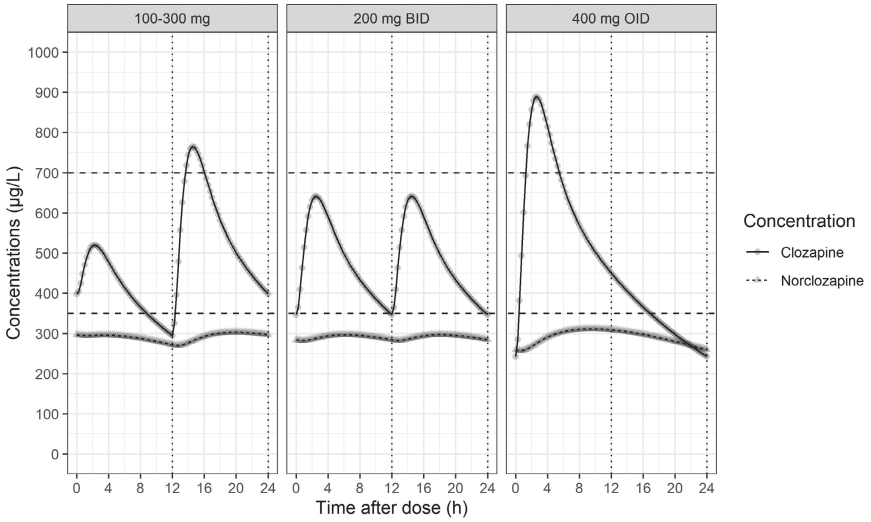

Figure 5. Simulated clozapine (black, dotted) and norclozapine (grey, triangles) concentrations over time for a dosing regimen of 400 mg per day for a smoking patient. Left: 100-300 mg, middle: 200 mg twice-daily, right: 400 mg once-daily.

(Dashed lines (horizontal): therapeutic window for clozapine (350 - 700 µg/L). Dashed lines (vertical): indication of sampling time (trough concentration or 12 h post-dose))

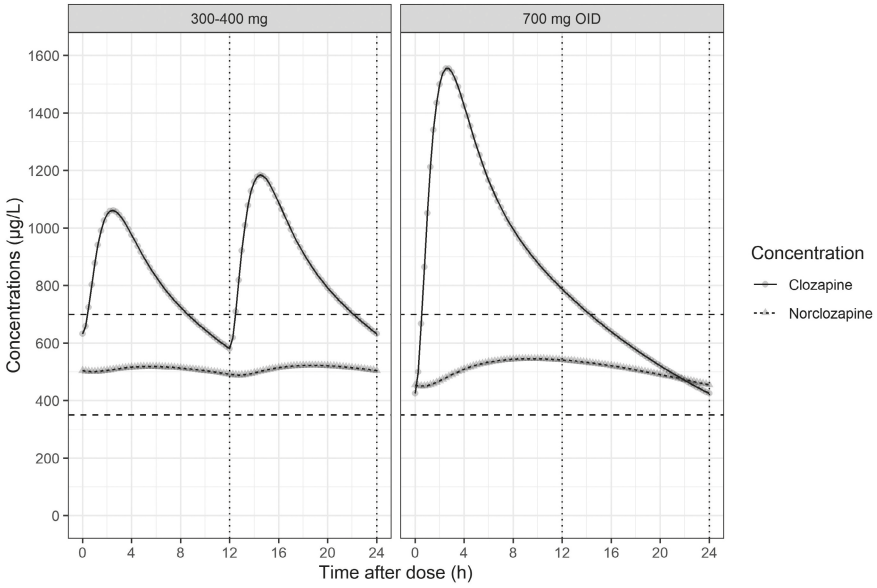

Figure 6. Simulated clozapine (black, dotted) and norclozapine (grey, triangles) concentrations over time for a dosing regimen of 700 mg per day, a typically high dose in our dataset. Left: 300-400 mg, right: 700 mg once-daily. Dashed lines (horizontal): therapeutic window (350 µg/L - 700 µg/L).

(Dashed lines (vertical): indication of sampling time (trough concentration or 12 h post-dose))

Figure 5 shows that, based on our PPK model, the clozapine trough concentration of the BID dosing regimen almost equals the lower limit of clozapine's therapeutic window (346 µg/L) when 400 mg clozapine is used in two divided doses. With QD dosing of the same daily dose, however, the 12 h post-dose concentration is 30% higher (450 µg/L) than the trough concentration at BID dosing. And, in contrast, the true trough concentration 24 hours after the single dose is approximately 30% lower (243 µg/L) than the trough concentration at BID dosing. In fact, this concentration even drops below the lower limit of the therapeutic window.

The impact of this on obtained concentrations is further illustrated in Figure 6 when a higher daily dose (700 mg) is used. In case of QD dosing in the evening, concentrations drawn in the morning lie above the currently applied upper limit, while the actual trough concentration lies within the therapeutic window. The true trough concentration is almost half the 12 h post-dose concentration measured in the morning (788 µg/L vs 425 µg/L).

For norclozapine, the impact of the dosing regimen on the concentration-time curves is much less pronounced. In the first simulation (Figure 5), the norclozapine trough concentration is 284 µg/L in the BID dosing regimen, and 260 µg/L when 400 mg is used QD. In the second simulation (Figure 6), it is seen that for the QD regimen, the pre-dose norclozapine concentration is 454 µg/L, while it is 541 µg/L twelve hours post-dose. Yet in QD dosing, the relative amount of clozapine over norclozapine (metabolic ratio) throughout the day is markedly different than in BID dosing.

Discussion

Several studies on the pharmacokinetics of clozapine have been conducted, using classical pharmacokinetic methodologies and, more recently, PPK approaches. [5] To our knowledge, our study is the first, however, to address possible differences in the pharmacokinetics of clozapine and norclozapine when clozapine is used QD instead of BID.

Theoretically, QD dosing could change the pharmacokinetics of a drug in several ways, which should be considered when changing dose regimens and applying the same reference values. First, intrusion of nonlinear elimination kinetics at higher doses has been suggested before [14], but our study found no proof of metabolic enzyme saturation at higher (QD) doses. Also, due to clozapine's anticholinergic properties, higher (QD) doses might prolong gastrointestinal transit time of clozapine, as has been seen after overdose. [26] In our dataset, a transit compartment between the dosing compartment and the central

compartment was indeed found to improve the model, but this was regardless of the dosing regimen applied.

Additionally, the simulations based on our final PPK model showed that with QD use, numerically, clozapine concentrations sampled in the morning (12 h after dosing) are markedly different from trough concentrations. This is consistent with two other studies simulating the influence of the dosing regimen and sampling time on clozapine concentrations [14,17], but contradicts the results of another study [16] that compared estimated peak and trough plasma concentrations of clozapine between QD and BID dosing regimens in a Japanese population based on a PPK model developed in a Canadian population.

Based on the simulation by VanderZwaag et al [17], the Dutch Clozapine Working group recommends increasing the lower limit of clozapine's therapeutic range by 23% for samples taken in the morning with QD use. This also follows the reasoning formerly applied for lithium concentrations at QD doses. Whether this numerical approach is appropriate or oversimplified will have to be studied using a pharmacokinetic/pharmacodynamics approach, since therapeutic and adverse effects are not only related to clozapine trough concentrations. The area under the concentration time curve (AUC) has been suggested to be a better predictor of clozapine effect [30], although for BID use trough concentrations have been found to correlate well with the AUC and moderately well for QD use.[18] Furthermore, higher peak concentrations at QD dosing, could affect the pharmacodynamic profile of clozapine. Norclozapine concentrations were not incorporated in the simulation studies by VanderZwaag et al [17] and Procyshyn et al [14] and as acknowledged in a 2019 review on the clinical utility of the metabolic ratio [31], variations of the metabolic ratio during the day have not been studied so far. Our study illustrates that the relative amounts of clozapine to norclozapine at QD are much different than at BID dosing, predominantly driven by the course of the clozapine concentrations within a dose-interval as norclozapine concentrations are relatively constant (Figure 5 and 6). Consequently, the calculated ratios depend numerically on the time of sampling: the calculated ratio at QD will be higher than at BID when peak concentrations are measured, also higher when measured in the morning, but will be lower when trough concentrations will be compared. In general, the relative amount of clozapine to norclozapine during the first 12 hours after dosing is higher with QD dosing than with BID dosing. The clinical relevance of this remains to be investigated. Surprisingly, metabolic ratio studies do not always report the dosing regimen or time of sampling. The literature is inconclusive on the clinical relevance of the metabolic ratio; heterogeneity in the calculation of the ratio may contribute to the lack of conclusiveness. Our

simulations illustrate the importance of clear reporting of dosing regimens and sampling times in studies on the relevance of the clozapine/norclozapine ratio.

Thus, our findings show that a disproportionate increase in clozapine concentrations, and hence a disproportionate increase in (adverse) effects, is not to be expected when (QD) doses are increased and vice versa when doses are reduced. Future studies are nonetheless needed to determine whether the therapeutic window of clozapine in QD use is the same as in BID use, considering both AUCs and trough and peak concentrations.

A two-compartment model for clozapine, with a single transit compartment addressing clozapine absorption, and a one-compartment model for norclozapine best described our data. A recently published review by Albitar et al [5] yields twelve studies on the PPK of clozapine. In most of the included studies 5 the structural model developed was a one-compartment model for clozapine with first-order absorption and elimination. The possibility of a two-compartment model of clozapine was only tested in six of the studies [30,32-36], of which three also found that a two-compartment model best fit the data. 30,33,34 The other three studies might have had too little data to be able to detect a two-compartment model, since the data involved either a trough or random blood sampling schedule. Norclozapine population pharmacokinetics was also only evaluated in six of the studies. [24,32,34,36-38]
In line with the literature [24,38], clozapine's clearance among smokers was found to be higher in our population, whereas norclozapine clearance was not affected by smoking. The literature is not conclusive about the latter effect, but Olmos et al also found no effect of smoking on norclozapine clearance.[38] The effects of sex on the exposure to clozapine have been consistent in the literature, with a higher clozapine clearance in men compared to women. This difference was not detected in our study, which is most likely attributable to the imbalance in the number of male and female participants.

Our study has some limitations. Model misspecification in the absorption phase of clozapine was found, which might be due to the occurrence of a first-pass effect. Previous research modelled a fixed fraction varying from 1.5%, 18.3% or 40% of clozapine undergoing first-pass effect. [32,36] In our dataset, a few patients seemed to have a higher fraction of first-pass effect. To be able to describe the first-pass effect, more dense sampling in the absorption phase is required. However, we considered neglecting the first pass effect in this model of little importance to assess potential differences in pharmacokinetic parameters between QD and BID.
Since our study population consisted of both in- and outpatients, full adherence to the prescribed dosing regimen is not guaranteed. We have tried to overcome

this as much as possible by verifying the time since last dose at the study day. Moreover, only 18 patients were outpatients living independently.
The intended inclusion of fifty patients was not met due to restrictions from March 2020 onwards as a result of the Covid-19 pandemic. With the rich number of samples per patient, however, the number of patients in the QD group was considered adequate to detect any differences in pharmacokinetic parameters between the two groups.[20]
The influence of comedication on the pharmacokinetics of clozapine and norclozapine was not investigated. However, it is likely to have contributed to the residual variability in our PPK model. Yet, we did record all comedication, including over-the-counter drugs. To assure steady state pharmacokinetics, we did not allow for any dose changes of known inhibitors or inducers of clozapine such as fluvoxamine, oral contraceptives, and valproic acid within seven days prior to blood sampling.
Finally, external validation was not feasible in this patient group, since our dataset was already a unique data set with a rich sampling strategy and both QD and BID users.

In summary, the pharmacokinetics of clozapine and norclozapine are not influenced by the applied dosing regimen. Our simulations have shown that the clozapine concentration obtained at a given total daily dose as well as the metabolic ratio will be affected by the dosing regimen as well as by the time between last dose and blood sampling. Thus, doctors wishing to switch a patient from BID to QD dosing cannot simply adjust doses based on the classic therapeutic window. Given the importance of adherence and the supposed positive contribution of once-daily dosing to it, it is necessary to further optimise treatment of patients using clozapine QD. This extended knowledge of the pharmacokinetic profile is valuable to define an evidence based therapeutic window for clozapine when used QD. Current knowledge regarding effectiveness and safety of clozapine once- versus multiple dosing regimens is hampered by the limitations of retrospective cohort studies. [15] Therefore, prospective studies targeting efficacy and tolerability are needed to determine whether current practice regarding TDM for once-daily dosing of clozapine is adequate or whether a different therapeutic window should be applied to improve the treatment of patients on clozapine on a once-daily regimen.

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4.2

The impact of once-daily dosing on protein binding of clozapine and norclozapine

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Abstract

Aim

Clozapine and norclozapine are highly protein bound. Currently, clozapine is increasingly prescribed once-daily (QD). Higher QD doses may lead to saturation of protein binding of (nor)clozapine, resulting in increased unbound fractions. We investigated whether protein binding of clozapine and norclozapine becomes saturated at higher concentrations. Secondly, we investigated the correlation between unbound (nor)clozapine fractions and alpha-1 acid glycoprotein (AGP) concentrations.

Methods

A total of 319 blood samples were collected at different time points within a dose interval from forty-four patients taking clozapine QD or twice daily. AGP-concentrations were measured in samples drawn just before clozapine intake. The relation between total and unbound concentrations and fractions, and between unbound fractions and AGP-concentrations were investigated using univariate linear regression analysis. The effect of dosing regimen on protein binding was assessed using multivariate linear regression analysis and split ANOVA analysis.

Results

(Nor)clozapine concentrations correlated well with its unbound concentrations (R2 = 0.87, p <0.001 and R2 =0.73, p <0.001 respectively) within the studied concentration range. The dosing regimen did not affect the relation between total (nor)clozapine concentrations and its unbound fractions. A moderate (clozapine) and small (norclozapine) correlation were found between AGP-concentrations and unbound fractions of clozapine (R^2=0.15, p<0.001) and norclozapine (R^2=0.055, p<0.001).

Conclusion

No disproportional increase in unbound concentrations were found with clozapine concentrations up to 1500 µg/L. Total concentrations remain suitable for therapeutic drug monitoring in QD clozapine regimens in general. However, occasional abnormal unbound fractions, even in the absence of infection, could possibly modify clozapine's concentration-effect relation.

Dutch Trial Register (NL6913 - NTR7108) – date of registration: 30/11/2017

Introduction

The antipsychotic drug clozapine is the most effective drug for patients with treatment-resistant schizophrenia. [1,2] Clozapine was originally used in divided doses, based on its relatively short elimination half-life of 12-16h [3], which is even shorter in smokers 4. Yet, nowadays, there appears to be a shift towards once-daily (QD) dosing of clozapine in the evening, aiming for better adherence and reduced day-time sedation [5]. Poor adherence to drug treatment is a well-known problem in the treatment of patients with schizophrenia. Considering clozapine's place in the treatment algorithm and the importance of treatment adherence for relapse prevention [6], every effort to increase clozapine adherence should be fully explored. Simplifying dosing regimens is one way to meet this end [7].

Therapeutic drug monitoring (TDM) of clozapine is recommended to guide clozapine therapy. Concentrations of clozapine's main metabolite, norclozapine, are also often measured, although the role of norclozapine remains a matter of debate. [8-11] It is customary to measure total clozapine (and norclozapine) concentrations, although the unbound molecules are responsible for the (side) effects. In general, the unbound fraction of a drug is constant [12] until the total concentration passes a point at which protein binding sites become saturated, leading to disproportionally increased concentrations of unbound molecules (non-linear protein binding) and hence disproportional increases in (side) effects. In that case, unbound rather than total concentrations may be more suitable for TDM, as total concentrations no longer align with unbound concentrations.

Clozapine and norclozapine are both highly protein bound (95% [13] and 90% [14] respectively), mainly to the acute-phase protein alpha-1 acid glycoprotein (AGP).[14-17] It is unknown whether at higher (nor)clozapine concentrations, either from QD dosing or when reaching peak concentrations, protein binding becomes saturated, as has been studied for rifampicin for example [18]. This knowledge would provide valuable information on how to monitor (nor) clozapine concentrations.

Furthermore, the binding to AGP may be altered in certain pathological and physiological states, due to alterations in the AGP molecules or in the concentration of AGP molecules. [15] Elevated AGP concentrations during infection or inflammation, for example, are suggested to compensate for the decreased clozapine clearance by CYP1A2, the key enzyme for clozapine metabolism, which' activity is known to decrease during inflammation. As a net result, the unbound concentrations will be relatively unchanged despite elevated total clozapine plasma concentrations. [19, 20]

We aimed to investigate whether protein binding of clozapine and norclozapine becomes saturated at higher concentrations. Secondly, we wanted to investigate the correlation between unbound clozapine and norclozapine fractions and AGP concentrations measured in our study population.

Method

Study design and data collection

This study was performed in the framework of the INPUT-study (Dutch Trial Register (NL6913 - NTR7108), in which serum samples were collected for a non-randomized, open label pharmacokinetic study to assess the influence of dosing regimen on the pharmacokinetics of clozapine and norclozapine. Between February 2019 and February 2020, in- and outpatients, aged 18-70 years, diagnosed with schizophrenia spectrum disorders, and receiving clozapine therapy orally, QD or twice daily (BID), as part of their regular treatment were recruited from two Dutch mental health organisations: Yulius in Dordrecht and Reinier van Arkel in 's-Hertogenbosch.

Study subjects were in steady state with respect to clozapine therapy. Subjects were excluded in case of enforced psychiatric treatment, pregnancy, initiation, cessation, or dose change of interacting comedication, tobacco or caffeine containing products within seven days prior to blood sampling, and acute inflammation or infection (derived from having a body temperature >38.0 degrees Celsius or using an antibiotic), as verified by the research nurse or physician at the study day.

Multiple blood samples were drawn from each participant, using a peripheral venous catheter: immediately before clozapine intake (t=0), between 0.5 and 1 h post-dosing, and at 2 (± 30 min), 3 (± 30 min), 4 (± 1 h), 5 (± 30 min), 8 (± 1 h) (only BID), and 12 h (± 2 h) (only for patients using clozapine QD and BID in unequally divided doses) post-dosing. Serum samples for the analysis of AGP were taken at t=0 h.

Plasma samples for the analyses of C-reactive protein (CRP), albumin, creatinine and urea concentrations were taken at t=0. Demographic and clinical patient characteristics were collected using a data collection form. Serum was separated by centrifugation and stored at -20 degrees Celsius until analysis. Plasma samples were stored at 2-8 degrees Celsius and analysed within 24 hours after sampling.

Power calculation

Since the effect of the QD dosing regimen on protein binding was unknown, a formal power analysis could not be performed. However, Bonate et al. demonstrated that a subgroup of 30 in a total population of 60 patients would detect a covariate with a power of 90% in a study where 2 samples per patient are drawn.[21] Furthermore, Lee showed that increasing the number of samples per patient from 2 to 3 dramatically increased power.[22] The same effect was seen for allowing variability in sampling times.
In our study, inclusion of patients was expected to be more complex than collecting sufficient data within one patient once included. Therefore, the study was designed to include 50 patients: 25 on each dosing regimen, with optimized data collection in terms of number of samples (7 or 8 per patient), allowing for variable sampling times within a certain time frame.

Statistical analyses were performed using R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical requirements

The study has been conducted in accordance with the Declaration of Helsinki and approval was provided by the Medical research Ethics Committees United (number NL63635.101.18) as well as by the local ethics committees of the participating centres.

The study protocol has been registered in the Dutch Trial Register (NL6913 - NTR7108). Patients were included after providing written informed consent.

Bio-analysis

Quantification of total and unbound (nor)clozapine concentrations in serum was performed by the clinical and pharmaceutical laboratory of the University Medical Centre of Groningen, the Netherlands, using a LC-MS/MS method validated in accordance with the FDA [23] and EMA guidelines [24]. All quality control samples (QC's) were prepared in fivefold. Linearity of the calibration curve was proven on an eight-point concentration range, with a Lower Limit of Quantification (LLQ) of 10 µg/L, a low QC sample of 20 µg/L, a medium QC sample of 400 µg/L, a high QC sample of 800 µg/L and a four-times-diluted QC sample of 2000 µg/L. Ultrafiltration with the Centrifree Ultrafiltration Device and Ultracel PL membrane 30KDa filters (Merck) was used to separate the protein bound and unbound (nor)clozapine molecules. For analysis of the unbound (nor)clozapine concentrations, the LLQ was set at 1.0 µg/L. Accuracy could

not be determined since it is not possible to relate the results to a known true unbound concentration Albumin, creatinine, urea, and C-reactive protein (CRP) concentrations in plasma were analysed as routinely by the Result Laboratory of the Albert Schweitzer hospital in Dordrecht, the Netherlands on the Siemens Dimension® Vista 1500 (Siemens Healthcare Diagnostics) and testing was performed per manufacturer's instructions. Assays were photometric (albumin, creatinine, urea) and nephelometric (CRP).

Serum AGP concentrations were determined at the Leiden University Medical Centre (Leiden, The Netherlands) using the Tina-quant α1-Acid Glycoprotein Gen.2 (AAGP2) immunoturbidimetric assay on the Roche cobas c502 analyser (Roche Diagnostics GmbH, Mannheim, Germany). In this assay, anti AGP antibodies react with antigen in the sample to form an antigen/antibody complex, which is measured turbidimetrically.

Statistical analyses

Baseline characteristics of both treatment groups (QD and BID regimen) were tested for differences using Chi-square and t-tests. Univariate linear regression analyses were performed to describe the relation between total (nor)clozapine concentrations and its corresponding unbound concentrations, as well as total (nor)clozapine concentrations and the corresponding unbound fractions. Clozapine and norclozapine unbound fractions were expressed as percentages and calculated by dividing the unbound concentrations by the total concentrations.

A multivariate linear regression analysis and split ANOVA analysis were performed to test the influence of the dosing regimen on the relation between total (nor)clozapine concentrations and their unbound fractions.

Finally, univariate linear regression analysis was performed to describe the relation between unbound fractions of (nor)clozapine and AGP-concentrations.

Results

Forty-four patients were included in this study, of which two patients participated in both treatment groups. In total, 319 blood samples were collected for quantification of total and unbound (nor)clozapine concentrations.

Demographics

The patient characteristics are summarized in Table 1. There were no significant differences in the demographic and clinical characteristics between the QD and BID groups.

Table 3. Clinicodemographics of the included patients (n=44)

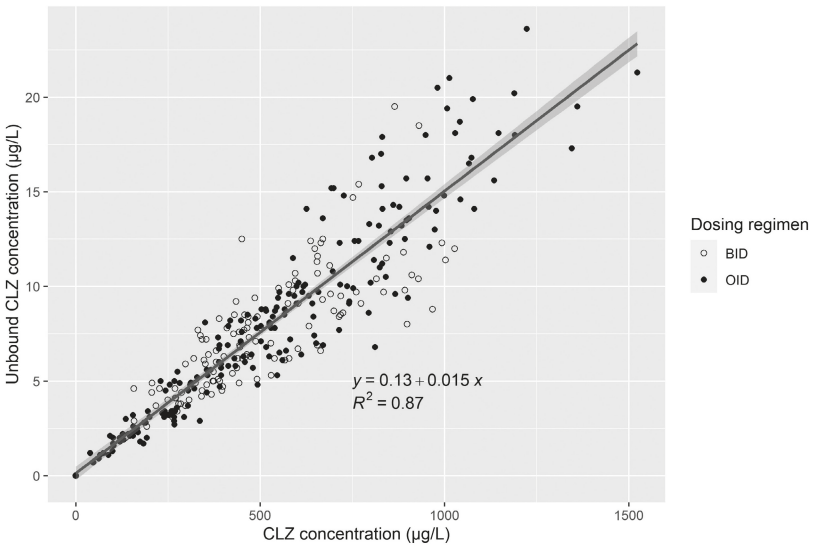
Variable	QD (n=28)	BID (n=16)
Male (%)	17 (61%)	9 (56%)
Smoker (%)	20 (71%)	13 (81%)
Age (years)	43.5 (12.7)	46.1 (13.9)
Height (cm)	177.3 (10.9)	174.3 (12.5)
Body weight (kg)	97.6 (27.1)	90.3 (27.0)
BMI (kg/m^2) *[18.5-25.0]*	31.1 (8.6)	29.8 (9.4)
Albumin (g/L) *[35-50]*	39.2 (5.0)	38.2 (3.1)
Creatinine (µmol/L) *[45-90 for women]* *[60-110 for men]*	75.6 (20.2)	76.7 (14.9)
Urea (mmol/L) *[1.8 – 7.1]*	4.9 (1.8)	4.9 (1.3)
CRP (mg/L) *[< 10]*	6.6 (6.5)	7.8 (8.6)
AGP (g/L) at t=0 *[0.5-1.2]*	0.92 (range 0.58 – 1.52)	0.97 (range 0.42-1.64)
Ethnicity (n, %)	Caucasian: 23 (82%) Asian: 1 (4%) African: 3 (11%) Other: 1 (4%)	Caucasian: 13 (81%) Asian: 1 (6%) African: 2 (12%)
Daily dosage of clozapine (mg)	313 (25-800)	388 (150-750)

Values are expressed as mean (standard deviation) unless specified otherwise.
Reference values (if relevant) are shown between square brackets in the first column.
BMI = body mass index
CRP = C-Reactive protein
AGP = alpha-1 acid glycoprotein

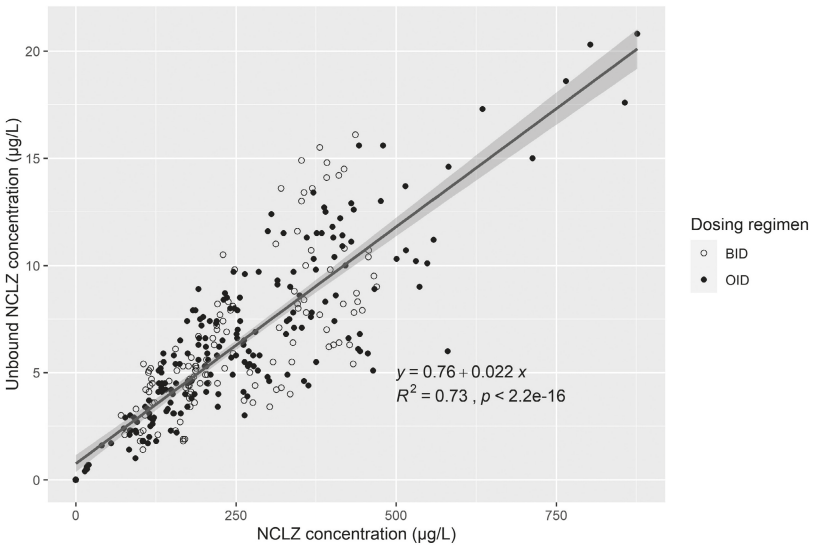
Association between total and unbound concentrations

The correlations between total and unbound concentrations of clozapine and norclozapine are shown in Figure 1a and 1b.

a)


Unbound CLZ concentration (µg/L)
CLZ concentration (µg/L)
y = 0.13 + 0.015 x
R2 = 0.87
Dosing regimen
BID
OID

b)


Unbound NCLZ concentration (µg/L)
NCLZ concentration (µg/L)
y = 0.76 + 0.022 x
R2 = 0.73 , p < 2.2e-16
Dosing regimen
BID
OID

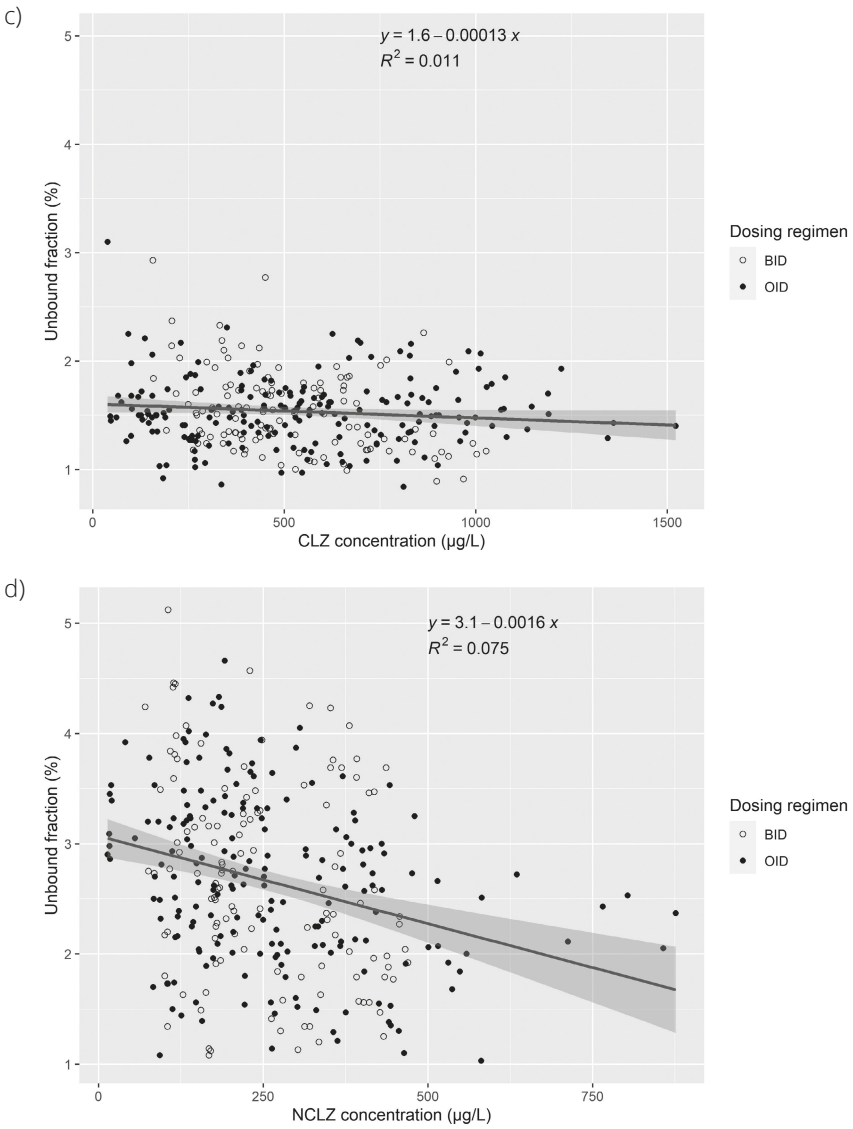


Figure 1. a. Unbound vs total clozapine concentrations, b. Unbound vs total norclozapine concentrations, c. Unbound clozapine fractions vs total clozapine concentrations, d. Unbound norclozapine fractions vs total norclozapine concentrations. Linear regression lines with standard errors with their equations and R^2 values are shown.

(CLZ = clozapine; NCLZ = norclozapine; black bullets = concentrations from once-daily dosing; grey bullets = concentrations from twice daily dosing)

Both clozapine and norclozapine concentrations correlated well with its unbound concentrations (R^2 = 0.87, p <0.001 and R^2 =0.73, p <0.001 respectively) within the studied concentration range.

Association between total concentrations and unbound fractions – impact of dosing regimen

No correlation is found between all total concentrations of clozapine and its unbound fractions (R^2 = 0.011, p=0.056), and a moderate but significant correlation between all total concentrations of norclozapine and its unbound fractions. (R^2 = 0.075, p<0.001) (Figure 1c and 1d).

Both the multivariate linear regression analysis and split ANOVA analysis did not reveal a statistically significant effect of the dosing regimens (QD or BID) on the relation between total (nor)clozapine concentrations and the unbound fractions.

Association between AGP-concentrations and unbound fractions

The correlations between AGP-concentrations and the unbound fractions of clozapine and norclozapine are visualised in Figure 2.

Linear regression analysis showed a moderate but significant correlation between AGP concentrations and unbound fractions of clozapine (R^2 = 0.15, p<0.001). The correlation between AGP concentrations and unbound fractions of norclozapine was small, but also significant (R^2 = 0.055, p< 0.001). The intra-individual variation of the unbound clozapine fractions appeared to be smaller than that of unbound norclozapine fractions.

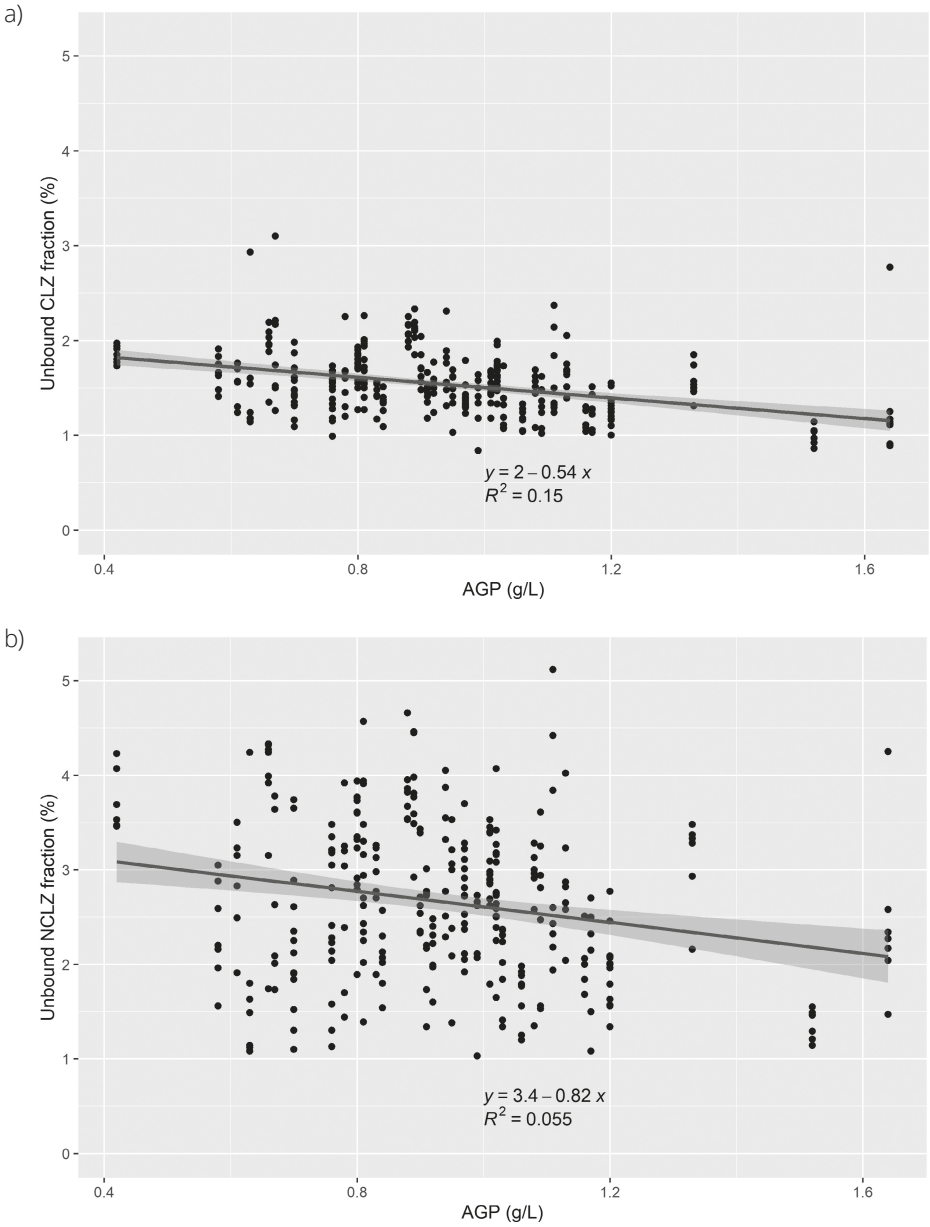


Figure 2. a. unbound CLZ fractions vs AGP at time point at which CLZ trough level was drawn, b. unbound NCLZ fractions vs AGP. Linear regression lines with standard errors with their equations and R2 values are shown.

(CLZ = clozapine; NCLZ = norclozapine)

Discussion

This study investigated whether protein binding of clozapine and norclozapine becomes saturated at higher concentrations from QD dosing or when reaching peak concentrations. Protein binding saturation of clozapine was not detected with clozapine concentrations up to approximately 1500 µg/L) (i.e., 4.6 µmol/L). This contrasts with what might have been expected knowing that saturation effects for compounds with high affinity to AGP often start at a drug concentration of approximately 2-5 µmol/L.[25] When the corresponding amount of norclozapine is also considered, the summed amount will in fact even exceed the 5 µmol/L. One explanation for the absence of saturation effect could be that clozapine and norclozapine also bind to albumin, although with considerably lower binding affinity than for AGP.[16] Also, the above calculation assumes a single drug binding site per protein molecule as well as binding by clozapine and norclozapine to the same binding site, which might be too simplified.
Secondly, we wanted to investigate the correlation between unbound clozapine and norclozapine fractions and AGP concentrations measured in our study population. In our study, variability in free fractions of clozapine is determined by the variability of AGP concentrations to a small extent (R^2 0.15). The negative correlation seems predominantly driven by the unbound fractions of the four patients with AGP values outside (one below and three above) the reference range. The lower fractions seen with AGP concentration above the reference range correspond to the findings of Man et al.[20] who found a significant association between elevated AGP concentrations during inflammation and lower clozapine unbound fractions (norclozapine concentrations were not studied). In our study, two of the three patients with an AGP concentration above the reference range turned out to have an elevated CRP concentration (22 and 35 mg/L), suggesting inflammation. The other patient, however, did not demonstrate clinical or chemical signs of infection or inflammation. As several other factors have been described with the potential of influencing AGP concentrations [15], our findings argue for looking beyond clinical manifest infection or inflammation as possible indicators of elevated AGP concentrations and, consequently, of decreased unbound fractions.

Overall, we found lower unbound fractions for both clozapine and norclozapine than was expected based on the literature. The most obvious reason for this is that the ultrafiltration technique, used to separate the protein bound molecules from the unbound molecules, introduces a variable that increases the imprecision of the measured value, affecting the accuracy of the data but not the precision
In addition, there might be a bioanalytical rather than physiological explanation for the observed larger inter- and intraindividual variation in norclozapine

unbound concentrations and fractions compared to clozapine. During the validation process of the LC-MS/MS method, we found that the overall variation coefficient (CV) of the unbound concentrations of norclozapine was higher than the CV of unbound clozapine concentrations.

Our study has some other limitations. Three of the unbound clozapine concentrations fell below the lower limit of quantification of the validated LC-MS/MS method. Although this decreases the precision and accuracy of these three values, this will not have affected the conclusions, given the total amount of samples.
Protein binding is influenced by more than just the amount of protein. Co-administration of other drugs can also significantly change the unbound fraction of drugs by displacing the drug molecules from its protein binding sites.[26] Due to the small sample size, we were not able to investigate this influence.

Nevertheless, we conclude that there is no need to account for any disproportional increase in unbound concentrations (and thus in (side) effects) at higher total clozapine concentrations from QD dosing. We have therefore found no arguments pleading against the currently applied 'once-daily' dosing regimens, based on suspected saturation of protein binding. This provides valuable information on how to monitor (nor)clozapine levels in patients using clozapine QD. Total concentrations remain suitable for therapeutic drug monitoring in general. However, while unbound fractions of clozapine and norclozapine were constant within the normal range of AGP concentrations, the unbound fractions tended to decrease in patients with increased AGP concentrations. Future research should focus on the relation between the degree of (side) effect, and unbound fractions at AGP concentrations outside the reference range or when possible displacing agents are used.

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4.3

Pitfalls in the development of a method to determine the unbound clozapine and norclozapine concentrations in serum using liquid chromatography-tandem mass spectrometry and ultrafiltration

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Abstract

Background

It is customary to measure total concentrations of clozapine and norclozapine, although only the unbound molecules are responsible for (side) effects. This study describes a strategy to develop a method for quantification of the unbound concentrations of (nor)clozapine in serum, using ultrafiltration (UF) and a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, considering several variables.

Materials and methods

First, the lower limit of quantification of an existing LC-MS/MS method to analyze (nor)clozapine in serum was lowered. To test for non-specific adsorption to the filter, the method was cross-validated to be used in water. Five different UF-filters, with and without pre-treatment of the membranes, were tested to choose the best filter and optimize recovery in ultrapure water. Finally, the total procedure was applied in serum.

Results

The calibration curves remained linear over the expanded concentration range of 1-1000 µg/L with acceptable regression coefficients, within-run, and between-run coefficients of variation (CVs) (<15%), as well as assay parameters for cross-validation and matrix effects.

The 30KDa UF-filter from Merck without pre-treatment yielded the highest recovery in ultrapure water, which was stable (13.6-15.6%) within a range of 5 – 50 µg/L before UF. The free fractions in serum remained constant over a representative concentration range for actual total clozapine and norclozapine concentrations. The within run, between run and overall CVs of the calibration series were acceptable, except for the overall CV of the free norclozapine concentrations.

Discussion

This study demonstrates that the choice of the ultrafilter is a crucial part of the development of. A method to measure unbound concentrations using UF. Adequate control of the factors influencing the NSA is essential, as well as factors influencing the stability of the protein-drug complex.

Introduction

While the number of different atypical antipsychotics is still growing, clozapine's efficacy remains unique, as it is the only antipsychotic drug with proven efficacy in the treatment of patients with treatment resistant schizophrenia (TRS).[1]
Clozapine and its main metabolite, norclozapine, are highly protein bound (95% and 90% respectively [2]) with alpha-1 acid glycoprotein (AGP) and albumin being the main proteins involved.[3-6] When performing therapeutic drug monitoring (TDM) for clozapine, it is customary to measure total clozapine and norclozapine concentrations, although, as for all drugs, only the unbound molecules are responsible for the pharmacological activity. It is unknown whether unbound concentrations of clozapine and norclozapine are better markers for its (side) effects than its total concentrations. Elevated AGP concentrations during inflammation are suggested to cause increased plasma clozapine-AGP binding, which might compensate for the elevated total clozapine plasma concentrations during infection. The net result is that unbound concentrations do not change significantly, whilst unbound fractions are mathematically decreased.[7,8] The clinical relevance of this, has yet to be investigated. Also, it is unknown whether at higher clozapine concentrations, from once-daily dosing or when reaching peak concentrations for instance, protein binding becomes saturated. This knowledge would provide valuable information on how to monitor (nor) clozapine concentrations, but an accurate and reproducible method for the determination of unbound concentrations is needed first.
In the past, limited sensitivity of analytical methods hindered the analysis of the low free concentrations of highly protein bound drugs, but the more widely availability of sensitive analytical equipment such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) has opened the way to this. The Food and Drug Administration (FDA) and European Medicines Agency (EMA) have well defined requirements for development and validation of bioanalytical assays, [9,10] but the requirements of the separation of unbound fractions are scarcely defined and not uniform.
The gold standard for determination of unbound concentrations is equilibrium dialysis. This method, however, is time consuming and not suitable for high sample throughput. Due to its simple and rapid separation technique, ultrafiltration (UF) is one of the most widely used methods.[11] Yet, one must overcome several challenges to develop an accurate and reproducible method for determination of unbound concentrations. For instance, non-specific adsorption of drugs to the filter membranes should be taken into account, as well as the concentration-dependency of this adsorption.
In this study, we describe a strategy to develop a method for determination of the unbound concentrations of clozapine and norclozapine in serum, using UF and considering several variables.

Materials and methods

Materials/chemicals

Clozapine was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA), norclozapine from Cerilliant (Round Rock, Texas, USA) and the internal standard ([13C,2H3]-clozapine) was purchased from Alsachim (Illkirch-Graffenstaden, France).

Nanosep® Centrifugal Devices with Omega™ Membrane 30KDa were purchased from Pall Corporation (New York, New York, USA), Centrifree Ultrafiltration Device with Ultracel PL membrane 30KDa from Merck (Darmstadt, Germany), and three different Vivaspin® filters from Sartorius (Göttingen, Germany): Polyethersulfone (PES) 30 KDa, Hydrosart® regenerated Cellulose 30 KDa, and Cellulosetriacetat 20 KDa.

Blank human serum was purchased from Merck (Darmstadt, Germany).

Ultrapure water was produced in the laboratory with the use of the Milli-Q® Advantage van Millipore (Molsheim, France).

Chemicals were of analytical or LC-MS/MS quality. Sodium hydroxide was purchased from Merck (Darmstadt, Germany), Methanol from Biosolve (Valkenswaard, the Netherlands), Ammoniumformate from Thermo Fisher Scientific (Waltham, Massachusetts, USA), and Formic Acid Merck (Darmstadt, Germany).

Analysis of clozapine and norclozapine concentrations in serum (10-1000 µg/L)

To analyze the concentrations of clozapine and norclozapine before and after UF, a validated LC-MS/MS assay on a Thermo Fisher Scientific triple quadrupole Quantiva MS/MS system with a Thermo Fisher Scientific Vanquish UPLC and autosampler system (Waltham, Massachusetts, USA) was used. The method has been validated for both serum and EDTA-plasma, in accordance with the Guidance for Industry Bioanalytical Method Validation of the FDA [10] and Guideline on Bioanalytical method validation of the EMA.[9]

Chromatography was achieved using a Thermo Accucore C18 column (50 mm x 2.1 mm, particle size 2.6 µm) purchased from Thermo (Waltham, Massachusetts, USA) and held at 60°C. The mobile phase consisted of 0.02 M ammonium formate pH 3.5 (A) and methanol (B). Gradient elution started with a flow of

1.0 mL/min at 30% B, at 0.1 minute 37.5% B for 0.7 minutes, at 0.85 minutes 95% B for 0.3 minutes, and at 1.2 minutes 30% B. Detection was achieved using a Thermo Quantiva triple-quadrupole mass spectrometer (Waltham, Massachusetts, USA), that operated in positive electrospray ionization (ESI) mode at a temperature of 350 °C with a voltage of 3500 V. Sheath gas is set at 50 (Arbitrary units, AU), Sweep gas at 0 (AU) and Auxiliary gas at 20 (AU). The ion-transfer-tube temperature was set at 140°C. The method was run in multiple reaction monitoring (MRM) mode, and set to detect the precursor and product ions of clozapine, its isotopic-labeled internal standard ([13C,2H3]-clozapine) and norclozapine.

For the sample preparation 500 µL precipitation reagent with internal standard (methanol containing 0.05 mg/L [13C,2H3]-clozapine) was added to 100 µL human blank serum. The calibration samples and quality control samples (QCs) were prepared by adding the required amount of a clozapine and norclozapine stock solution to obtain the desired concentrations. The sample was vortexed for 1 minute and centrifuged for 5 minutes at 9500 g. After this, 0.1 µL of the supernatant was injected into the LC-MS/MS system.

The validation was carried out over three days and QCs were prepared in fivefold. Linearity of the calibration curve was measured on an eight-point concentration range (10, 20, 50, 100, 250, 500, 750, and 1000 µg/L), with a Lower Limit of Quantification (LLQ) of 10 µg/L, a low (LOW) QC sample of 20 µg/L, a medium (MED) QC sample of 400 µg/L, a high (HIGH) QC sample of 800 µg/L and a four-times-diluted (DIL) QC sample of 2000 µg/L for both clozapine and norclozapine.

Analysis of clozapine and norclozapine in ultrafiltrate (1-1000 µg/L)

The LC-MS/MS setup and sample preparation method to analyze clozapine and norclozapine in ultrafiltrate was identical to the setup and sample preparation method described above except for the injection volume and the precipitation reagent and internal standard being added after ultrafiltration, to prevent an unknown fraction of the internal standard from adsorbing to the filter.

Lowering the LLQ in ultrafiltrate

Since clozapine and norclozapine are highly protein bound, [4] and accounting for potential reduced recovery of the free molecules after UF due to adsorption to the filter membrane, we aimed for a LLQ of 1.0 µg/L for both clozapine and norclozapine. Hereto, the injection volume of the supernatant that is injected into the LC-MS/MS system was increased from 0.1 to 1.5 µL. This procedure was

carried out over three days, linearity of the range was measured on an eight-point calibration curve (1-1000 µg/L) and all QCs were prepared in threefold (1, 10, 30, 60, 100, 300, 600, 1000 µg/L).

Cross-validation of serum to water and matrix effect of ultrafiltrate compared to water

The matrix effect is the impact of components being endogenously present in the biological samples (i.c. serum and serum ultrafiltrate) on the ionization efficiency of the LC-MS/MS assay.[12] In this study, we assumed that ultrapure water could be used as a matrix to mimic the protein-free serum matrix after UF. To assess the appropriateness of this assumption, we compared the effect of water as a matrix with the effect of the ultrafiltrate on the quantification of clozapine and norclozapine.
Hereto, we performed ion-suppression tests in twofold, by infusing methanol with clozapine and norclozapine (3 µg/L) and injecting prepared blank samples of water, serum, and ultrafiltrate. For comparison with real life measurements, we also injected two serum samples with clozapine and norclozapine at LLQ level (1.0 µg/L) and one higher concentration (3.5 µg/L).

To cross-validate the method, we prepared a calibration curve in serum and QCs in ultrapure water. The validation was performed on a single day. Since linearity was proven before, the QCs was measured on a single-point calibration curve (0-1000 µg/L), and all QCs were prepared in fivefold, with a LLQ QC sample of 1 µg/L, a low (LOW) QC sample of 5 µg/L, a medium (MED) QC sample of 50 µg/L, a high (HIGH) QC sample of 100 µg/L, for both clozapine and norclozapine. In addition, a sample of 500 µg/L was also measured.

Non-specific adsorption (NSA) of clozapine and norclozapine using different UF filters and pre-treatment techniques

Since the determination of unbound concentrations of drugs by UF may be flawed by adsorption of drugs onto the filter membranes, the conditions for optimized recovery of clozapine and norclozapine concentrations *in vitro* in ultrapure water were established testing five different UF-filters (see: materials and methods). Filters were operated as per manufacturers' instructions.

One QC with a concentration of 1000 µg/L clozapine and norclozapine was prepared in ultrapure water and measured in fivefold with the five different filters. Aliquots of 250 mL were transferred into the upper chamber of the filter device. The ultrafiltrate was obtained by centrifugation of the samples at 2000 g for 20 minutes at 20 °C using a MIKRO 220R centrifuge from Hettich

(Kirchlengern, Germany) for the two UF-filters by Pall and Merck and at 4000 g for 20 minutes at 20 °C using a ROTANTA 460R centrifuge from Hettich (Kirchlengern, Germany) for the three different UF-filters by Sartorius.

UF methodology was performed with and without pre-treatment of the filter membranes to limit possible NSA. In the case of pre-treatment, the filters were pre-treated with different solvents: 0.1 M NaOH, 0.05 M ammoniumformate pH 5.5, pH 7.0, and pH 9.0. For each solvent, aliquots of 200 µL were filtrated as described above and dried for 2 hours at room temperature. Clozapine and norclozapine concentrations before and after UF were analyzed using the LC-MS/MS assay and setup as described in the corresponding section.

Method development: NSA of clozapine and norclozapine at different concentrations in ultrapure water

Subsequently, the relationship between clozapine and norclozapine concentrations before and after UF was investigated over a wide clozapine and norclozapine concentration range (5 – 200 µg/L) in ultrapure water. To be able to detect any concentration-dependent adsorption in water due to saturation of the filter membrane, a higher concentration was used than the maximum expected unbound concentration (i.e. 50 to 100 µg/L, based on 5% and 10% free fractions for clozapine and norclozapine respectively and a maximum total serum concentration of 1000 µg/L). Hereto, the filter and pre-treatment technique resulting in the highest recovery, as determined in one of the previous steps, was applied.

The sample preparation is identical as described in the 'Analysis of clozapine and norclozapine in ultrafiltrate'-section. QC samples of increasing clozapine and norclozapine concentrations (5, 7.5, 10, 15, 25, 50, 100, 200 µg/L) in ultrapure water were prepared and analyzed before and after UF in fivefold on three different days.

For each day, the means and between-run and within-run coefficients of variation (CV) were calculated of the concentrations after UF. In general, due to the imprecision added by the UF step, the acceptance criterion for the CV of the mean concentration after UF has been agreed to be somewhat more generous than for normal bioanalysis [13] and we accepted a CV of 20%.

Method development: recovery of clozapine and norclozapine from serum after UF

Finally, we applied the established technique on a set of human serum samples spiked with clozapine and norclozapine to define the precision of the unbound concentrations in serum and to detect any concentration dependent adsorption to the filter in serum.

On three different days, calibration series with increasing clozapine and norclozapine concentrations (range 10 – 1000 µg/L), representing very low and reasonable total clozapine and norclozapine concentrations *in vivo,* were prepared in threefold and analyzed before and after UF, using the described method above.

Again, the means, within-run and between run CV were calculated before and after UF, with an accepted CV of the mean concentration before UF of 15% and of 20% for the mean concentrations after UF. Since the true unbound clozapine and norclozapine concentrations are unknown, accuracy cannot be tested.

Mean concentrations of clozapine and norclozapine before (total) and after (unbound) ultrafiltration were compared using Deming regression analysis (XLSTAT 2023.1.1 for Excel version 16.0). Linearity of the NSA of clozapine and norclozapine was visually and statistically assessed over the full calibration range.

Homogenization

To explore the potential influence of time and sample homogenization on the degree of protein binding of clozapine and norclozapine in spiked serum samples we measured the total and unbound clozapine and norclozapine concentrations in serum in fivefold at several time points, between 0 and 255 minutes, after spiking the samples with 800 µg/L of the respective substances. Then we calculated the free fractions at these time points.

Freeze-thaw

Additionally, when assessing the degree of protein binding of a drug for clinical practice, one must also consider the stability of the protein binding of the drug from freezer storage conditions to room temperature.
Hereto, we measured the total and unbound clozapine and norclozapine concentrations in serum, spiked with 800 µg/L clozapine and norclozapine, between and after five cycles of freezing (24 hours) and thawing (4 to 5 hours).

Results

Analysis of clozapine and norclozapine concentrations in serum (10-1000 µg/L)

Linearity and bias

For clozapine, the calibration curves were linear with a regression coefficient (R^2) of 0.9992 with an overall bias of -6.3% at LLQ, -7.0% at LOW, 1.4% at MED, 2.1% at HIGH and 0.0% at DIL.

For norclozapine, the calibration curves were also linear with a R^2 of 0.9931, an overall bias of -0.9% at LLQ, -3.9% at LOW, -2.4% at MED, -0.3% at HIGH and 0.0% at DIL.

Precision

The within-run CV for clozapine was 4.2% at LLQ, 1.0%, at LOW, 0.7% at MED, 0.5% at HIGH, 1.0% at DIL and the between-run showed a CV of 0.0% at LLQ, 0.0% at LOW, 0.2% at MED, 0.3% at HIGH and 3.0% at DIL.

The within-run CV for norclozapine was 4.0% at LLQ, 1.8%, at LOW, 0.9% at MED, 0.6% at HIGH, 0.8% at DIL and the between-run showed a CV of 3.2% at LLQ, 0.0% at LOW, 0.3% at MED, 0.5% at HIGH and 4.8% at DIL.

Selectivity and Specificity

The method was found to be selective and specific using six different blank serum samples (data not shown).

Stability and Freeze thaw cycles

Samples were stable for six days in the autosampler and at ambient temperature. Stability of the samples was proven for three freeze-thaw cycles (data not shown).

Analysis of clozapine and norclozapine in ultrafiltrate (1-1000 µg/L)

Lowering the LLQ in ultrafiltrate

For clozapine, the calibration curve was proven to be linear over the expanded concentration range of 1-1000 µg/L, with a R^2 of 0.9896, showing an overall bias

of -2.6% at LLQ (1.0 µg/L) and -3.0% at LOW (2.5 µg/L). The within-run CV was 6.0% at LLQ and -3.0% at LOW and the between-run showed a CV of 5.1% at LLQ and 5.7% at LOW.

For norclozapine, the calibration curves also remained linear over the expanded concentration range of 1-1000 µg/L, with a R^2 of 0.9917, showing an overall bias of -3.8% at the LLQ (1.0 ug/L) and -4.4% at LOW (2.5 µg/L). The within-run CV was 4.1% at LLQ and 3.7% at LOW and the between-run showed a CV of 4.1% at LLQ and 6.2% at LOW.

Cross-validation of serum to water and matrix effect of ultrafiltrate compared to water

The ion-suppression test did not show any influence of the blank ultrafiltrate and water matrices on the LC-MS/MS signal of clozapine and norclozapine (data not shown).

For clozapine, the QC samples spiked in water showed an overall bias of -18.1% at LLQ (1.0 µg/L), -6.6% at LOW (5 µg/L), 4.3% at MED (50 µg/L), 4.8% at HIGH (100 µg/L) and 9.0% at the Extra QC (500 µg/L). The within-run CV was 8.3% at LLQ, 3.1%, at LOW, 2.3% at MED, 2.1% at HIGH and 1.6% at the Extra QC.

For norclozapine, the QC samples spiked in water showed an overall bias of -19.6% at LLQ (1.0 µg/L), -7.1% at LOW (5 µg/L), 5.0% at MED (50 µg/L), 6.0% at HIGH (100 µg/L) and 10.0% at the Extra QC (500 µg/L). The within-run CV was 9.2% at LLQ, 2.6%, at LOW, 2.2% at MED, 2.2% at HIGH and 1.5% at the Extra QC.

Non-specific adsorption (NSA) of clozapine and norclozapine using different UF filters and pre-treatment techniques

All filters investigated showed NSA of clozapine and norclozapine to a greater or lesser extent. Use of the Centrifree Ultrafiltration Device with Ultracel PL membrane 30KDa from Merck resulted in the highest recovery of clozapine (52.6%) and norclozapine (45.9%) after filtration of QC samples with clozapine and norclozapine concentrations of 1000 ug/L in ultrapure water when compared to the other filters (Table 1).

Table 1. Recovery (%) of clozapine and norclozapine concentrations of 1000 µg/L after ultrafiltration with different filter devices in purified water.

Filter type	Recovery of clozapine (%)	Recovery of norclozapine%
Merck	52.6	45.9
Pall	0.3	0.2
Vivaspin PES	0.4	0.7
Vivaspin Hydrosart	11.5	8.5
Vivaspin CTA	2.2	1.7

Using the 30KDa UF-filter from Merck, UF methodology without pre-treatment of the filters showed the highest recovery of clozapine and norclozapine after UF, indicating the lowest NSA in water with the use of these filters (Table 1).

Method development: NSA of clozapine and norclozapine at different concentrations in ultrapure water

Table 2 shows the mean concentrations of clozapine and norclozapine measured in five-fold on three different days, before and after UF, with the corresponding recovery percentage after UF.

Table 2. Mean clozapine and norclozapine concentrations before and after ultrafiltration (UF), recovery, within run, between run and overall variation coefficients (CVs) of the calibration series of increasing clozapine and norclozapine concentrations in ultrapure water.

Clozapine					
Mean concentration before UF (µg/L)	Mean concentration after UF (µg/L)	Mean recovery (%)	Within Run CV (%)	Between Run CV (%)	Overall CV (%)
3.84	0.52*	**	17.6	0.0	17.6
6.07	0.79*	**	11.4	9.3	14.7
7.87	1.23	15.6	15.8	10.9	19.2
11.94	1.63	13.7	13.6	3.1	14.0
21.62	3.07	14.2	11.8	3.7	12.3
45.56	7.12	15.6	12.8	12.2	17.7
90.97	18.25	20.1	11.2	10.6	15.4
196.53	50.56	25.7	15.5	7.7	17.3
Norclozapine					
Mean concentration before UF (µg/L)	Mean concentration after UF (µg/L)	Mean recovery (%)	Within Run CV (%)	Between Run CV (%)	Overall CV (%)

Table 2. *Continued*

Clozapine					
Mean concentration before UF (µg/L)	Mean concentration after UF (µg/L)	Mean recovery (%)	Within Run CV (%)	Between Run CV (%)	Overall CV (%)
3.7	0.43*	**	20.3	6.0	21.2
5.9	0.68*	**	11.0	6.0	12.5
8.3	1.13	13.7	17.7	11.5	21.2
12.4	1.44	11.6	14.2	0.0	14.2
22.7	2.79	12.3	13.1	7.2	14.9
51.1	6.99	13.7	15.3	8.6	17.6
105.7	18.06	17.1	10.9	7.0	13.0
230.3	50.19	21.8	18.0	9.5	20.4

*Concentration under the LLQ; ** Free fraction not calculated due to free concentration < LLQ, UF = ultrafiltration

For clozapine, the recovery is stable within a range of 5 – 50 µg/L clozapine in ultrapure water before UF, with recoveries between 13.6 and 15.6%. However, the percentage in the ultrafiltrate increases with 50% to 100% when the concentration before UF exceeds 50-100 µg/L, possibly due to saturation of the filter. The same is seen for norclozapine.

Method development: recovery of clozapine and norclozapine from serum after UF

The mean total and free concentrations, free fractions, within-run, between run and overall CV of the measurement of clozapine and norclozapine in serum before and after UF are shown in table 3.

Table 3. Mean total and free (unbound) concentrations, free fractions, within run, between run and overall variation coefficients (CVs) of the calibration series of increasing clozapine and norclozapine concentrations in serum.

Clozapine					
Mean total concentration (before UF) (µg/L)	Mean free concentration (after UF) (µg/L)	Mean free fraction (%)	Within Run CV (%)	Between Run CV (%)	Overall CV (%)
9.85	0.25*	**	14.2	11.2	18.1
30.29	0.72*	**	8.1	13.6	15.8
60.86	1.41	2.35	7.7	14.7	16.6
102.84	2.25	2.19	6.9	14.1	15.7

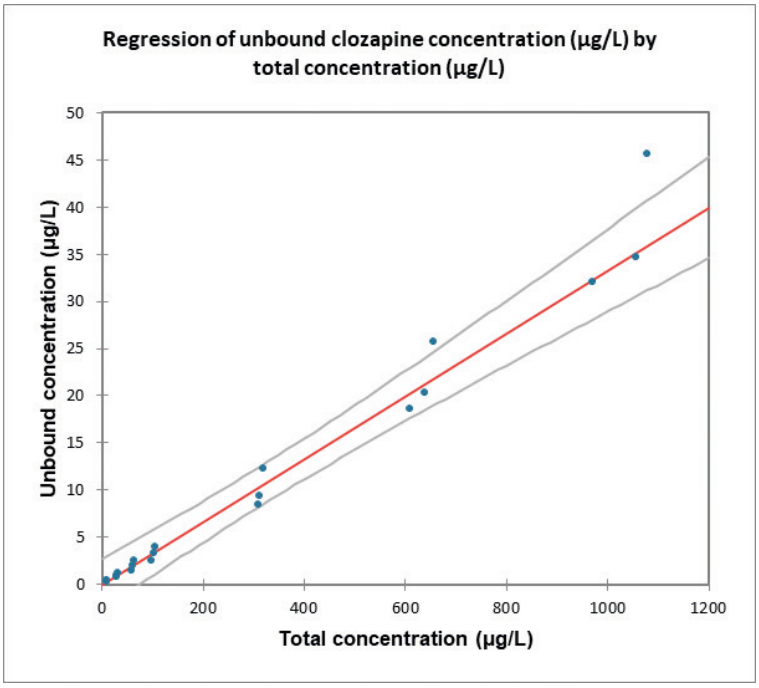
313.40	7.00	2.24	3.1	13.5	13.8
641.06	15.06	2.35	2.6	8.6	8.9
1075.01	26.26	2.45	6.1	9.5	11.3
Norclozapine					
Mean total concentration (before UF) (µg/L)	Mean free concentration (after UF) (µg/L)	Mean free fraction (%)	Within Run CV (%)	Between Run CV (%)	Overall CV (%)
9.84	0.34*	**	14.3	21.9	26.2
29.99	0.99*	**	10.2	21.2	23.5
60.79	2.00	3.28	7.3	24.3	25.4
102.56	3.28	3.18	4.4	22.8	23.2
313.10	10.06	3.21	3.3	19.3	19.5
634.47	21.58	3.39	4.3	16.5	17.0
1034.92	37.45	3.61	5.4	19.2	20.0

*Concentration under the LLQ; ** Free fraction not calculated due to free concentration < LLQ, UF = ultrafiltration

Due to high variation between the runs, the overall CV of the free concentration of norclozapine has exceeded the acceptance level.

Unlike in ultrapure water, the free fractions of clozapine and norclozapine, found in serum over a concentration range that is representative for total clozapine and norclozapine concentrations for TDM, seem to be constant. Figure 1 depicts the relationship between the mean clozapine (a) and norclozapine (b) concentrations of the various QCs before and after UF, with a R^2 of 0.987 for clozapine and a R^2 of 0.993 for norclozapine.

a)



b)

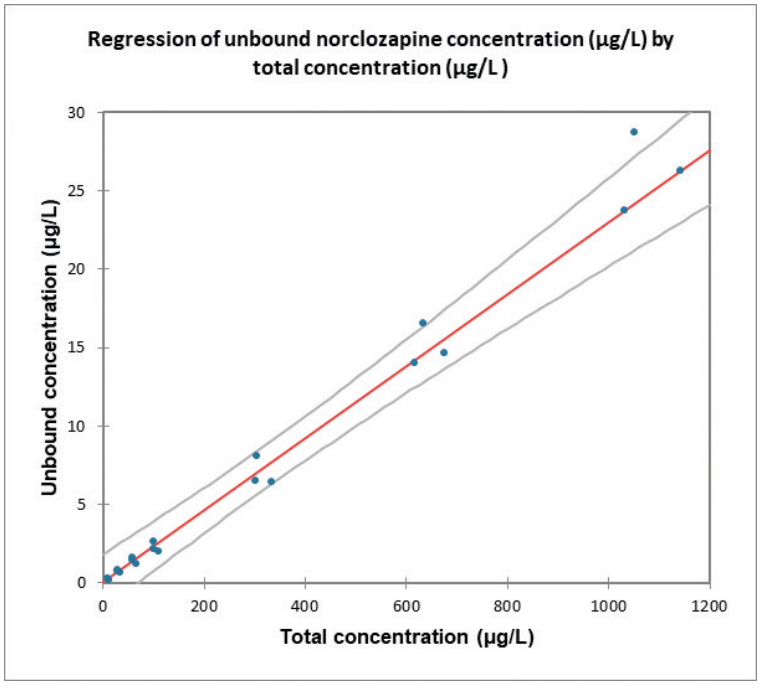


Figure 1. Deming regression analysis of the concentrations of clozapine (a) and norclozapine (b) in serum before and after ultrafiltration (UF).

Demming regression showed linearity over the full calibration range with an intercept (95% CI) of 0.030 (-0.033 – 0.093) µg/L for clozapine and 0.007 (-0.095 – 0.108) µg/L for norclozapine. The mean fee fraction in the spiked samples was 2.3% and 3.3% for clozapine and norclozapine, respectively.

Concentrations of AGP and albumin in these serum samples were within the physiological range (AGP 0.64 g/L and albumin 40 g/L).

The mean free fraction for clozapine in these spiked samples was 2.37% and 3.35% for norclozapine. Concentrations of AGP and albumin in these serum samples were within the physiological range (AGP 0.64 g/l and albumin 40 g/l). The overall CV of the free concentrations of norclozapine exceeded the acceptance level of the CV, mainly because of high between run CVs.

Homogenization

Table 4 shows the unbound fractions of both clozapine and norclozapine at different time points after spiking the serum samples with 800 µg/L clozapine and norclozapine. Since similar unbound fractions are seen after 5 and after 255 minutes, the binding process of clozapine and norclozapine to its plasma proteins reaches its equilibrium within less than 5 minutes.

Table 4. Free fractions of both clozapine and norclozapine measured at several time points after spiking the serum samples with 800 µg/L clozapine and norclozapine.

	Clozapine			Norclozapine		
Time (minutes)	Free Fraction (%)	CV before UF	CV after UF	Free Fraction (%)	CV before UF	CV after UF
5	5.72	1.50	2.30	10.96	1.43	1.82
15	5.64	0.83	0.43	10.75	0.76	0.30
30	5.47	0.59	2.63	10.47	0.88	1.73
60	5.60	0.54	1.94	10.42	0.66	1.74
105	5.68	0.97	4.68	10.59	0.90	3.24
170	5.51	0.76	2.49	10.33	0.68	1.90
255	5.73	2.40	2.30	10.76	2.15	1.44

CV = coefficient of variation; UF = ultrafiltration

Since similar unbound fractions are seen after 5 and after 255 minutes, the binding process of clozapine and norclozapine to its plasma proteins reaches its equilibrium within less than 5 minutes.

Freeze-thaw

Freezing and thawing did not influence the degree of binding of clozapine and norclozapine to its plasma proteins after five freeze-thaw cycles (data not shown).

Discussion

The standard method to separate free drug concentrations remains equilibrium dialysis, but UF is frequently used as an alternative because this method is less time consuming and easier to implement. However, when applying UF, several variables should still be considered.

To the best of our knowledge, this is the first study describing a strategy for the development of a method to determine the unbound clozapine and norclozapine concentrations considering several variables and describing the hurdles to be taken.
First, one should be aware of possible NSA of drugs to the filter membranes resulting in a lower recovery. Several UF filters and several pre-treatment techniques can be applied to reduce the binding of free drug to the surface of the filter device. In this study, we saw that only the use of the 30KDa filters of Merck resulted in measurable clozapine concentrations in purified water after UF. Pre-treatment of the filter with buffers with a different pH did not improve the recovery.
It is important to determine if the degree of NSA is influenced by the concentration of the drug(s) under investigation, since, in case of concentration-dependent binding, higher free fractions observed at higher total concentrations in clinical samples, may be misinterpreted as saturation of protein binding, while in fact saturation of the UF filter explains the higher free fractions. When we assessed the NSA of clozapine and norclozapine in ultrapure water, we found a constant degree of NSA (85-87%) within a range of reasonable free clozapine and norclozapine concentrations (5-50 µg/L), but higher recoveries with clozapine concentrations of 100 and 200 µg/L. This suggests that the adsorption of clozapine and norclozapine to the filter surface becomes saturated in ultrapure water at higher 'free' clozapine concentrations. However, we did not detect this saturation effect in our serum samples (total drug concentrations 10 – 1000 mg/L). This is thought to be due to plasma and serum components displacing clozapine and norclozapine from the filter and thereby 'protecting' these drug molecules from adsorption.[14] We have not investigated whether this also holds at higher (supratherapeutic) levels, since dilution is not desirable because it would also dilute the concentration of AGP

and other proteins, with unpredictable effects on protein binding and filter saturation. This is a limitation of the study.
In addition, the main problem with protein binding studies is that the true unbound fraction/concentration is unknown and thus accuracy cannot be determined. We found free clozapine fractions of approximately 2.5% in the spiked serum samples, which are higher than expected based on the 85% NSA in water and 95% protein binding in *vivo*. This is also thought to be due to the 'protective effects' of plasma and serum components.[14]
Compared with normal bioanalysis, there is also a bigger risk for batch- to-batch variations and the imprecision is usually approximately twice as high [13], which might explain the large between run CV in the analyses of the free concentrations of norclozapine.

In addition to the attention paid to examining NSA in this study, the study has several other strengths. In order to preclude varying degrees of protein binding due to different concentrations of AGP and albumin in the spiked serum samples, we used the same serum samples for the method development in serum. The serum samples used for the homogenization experiment, however, originated from a different serum pool. The concentrations of AGP and albumin were lower in the samples used for the homogenization experiment than in the samples used for the method development (AGP 0.55 g/L vs 0.64 g/L and albumin 34 g/L vs 40 g/L). For AGP, both concentrations are at the lower physiological range. If the different AGP concentrations explain the discrepancy in the free fractions, is yet unknown. A previous study, however, did find a significant association between increased AGP concentrations and lower clozapine unbound fractions. [8]
For practical reasons, we used ultrapure water as a matrix to mimic the matrix of the free drug concentrations for development of the UF method. To justify this, we first cross-validated the use of water over serum and excluded the presence of matrix effect.
Another variable to be considered when using UF, is protein leakage through the UF membrane, possibly affecting the amount of drug after UF and influencing interpretation of the free concentration. After adding methanol to ultrafiltered serum, no precipitate was observed and therefore we have assumed that protein leakage did not occur. Additionally, protein leakage of AGP or albumin was not expected, since the applied filter in this study has a cut-off value of 30 kDa, being smaller than the molecular size of these proteins (41-43 kDa for AGP [15] and about 66 kDa for albumin [16]).
Finally, one should consider that NSA and protein binding require a minimum period of time to reach equilibrium. Therefore, we analyzed the free fraction in serum samples at different time points after preparation of the serum samples. Protein binding seemed to have reached equilibrium within five minutes after

spiking the samples. However, we did not study the effect of time on the degree of NSA.

In addition, our study has some other limitations. *In vivo,* when the drug is in equilibrium with the binding proteins, this equilibrium is affected by changes in pH, temperature, protein concentration and the concentration of other drugs and circadian rhythm of endogenous compounds present.[13] The normal human body temperature is commonly accepted to be 37 °C. According to Nilsson the unbound fraction at room temperature is usually approximately 50% of the unbound fraction at 37 °C,[13] which has been reported for phenytoin.[17] Thus, to minimize experimental bias, experiments to measure absolute free drug concentrations ideally should be performed under physiological circumstances and stable protein concentrations. We used human serum samples spiked with clozapine and norclozapine, which have been analyzed at a constant room temperature of 21°C. In the event that ambient temperature affected the balance between bound and unbound clozapine and norclozapine concentrations in the serum samples, we expect this to introduce at most a systematic error, not affecting the precision of the results.

The pH in circulating plasma is 7.40 ± 0.05. Since we used serum samples instead of plasma, the pH in our serum samples is expected to be slightly higher. For drugs with a pKa value close to 7.4, such as clozapine [18] binding to AGP, the unbound fraction might decrease with an increase in pH, mainly due to the change in ionization states as the nonionized fraction is preferentially bound to the protein. Again, we expect that this only might have affected the accuracy of the data, rather than the precision.

Conclusion

In summary, this study demonstrates the complexity and potential pitfalls of measuring unbound drug concentrations using UF.

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4.4

Clozapine levels in serum and plasma, are they really comparable?

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Abstract

Background

Clozapine levels are widely measured in both plasma and serum. Knowledge regarding the interchangeability of these matrices is limited and conflicting.

Objective

This study aims to assess whether clozapine concentrations measured in plasma collected with tubes containing ethylenediaminetetraacetic (EDTA) and lithium-heparin (LH) as anticoagulants are comparable to clozapine concentrations measured in serum.

Design and Methods

Paired residual EDTA- and LH-derived plasma and routine serum samples were obtained for analysis of clozapine concentrations. Clozapine concentrations in serum were compared with the concentrations found in EDTA- and LH-plasma using linear regression. For clinical interpretation, clozapine concentrations measured in the three different matrices were categorized into three categories: subtherapeutic (< 300 µg/L), therapeutic (300 – 700 µg/L) and supratherapeutic (> 700 µg/L). Differences in clinical interpretation between clozapine concentrations measured in the two plasma matrices and serum were assessed.

Results

Compared to the serum samples (n=36), clozapine levels measured in LH-derived plasma samples (n=19) were on average 150 µg/L lower (95% CI [-200; -110]). Clozapine levels measured in EDTA-derived plasma samples (n=35) were on average 20 µg/L higher (95% CI [-10; 50]). In 37% of the LH samples, the clinical interpretation differed from the interpretation based on serum clozapine levels, whilst there was high agreement between serum and EDTA plasma samples (97%).

Conclusions

The use of LH-derived plasma samples for therapeutic drug monitoring of clozapine may lead to different clinical interpretations than when using serum samples. Therefore, interchangeable use of LH-derived plasma and serum samples to measure clozapine levels in clinical practice is not recommended.

Introduction

Clozapine is the only drug with proven efficacy in the treatment of patients with treatment-resistant schizophrenia [1]. To improve efficacy in patients with suboptimal response and to prevent dose-related adverse events such as sedation, hypersalivation and seizures, therapeutic drug monitoring (TDM) of clozapine concentrations is highly recommended [2-6].

In our clinic, serum without a separating gel is the preferred matrix for clozapine measurement, since previous research [7,8] showed that test tubes containing gel could decrease clozapine concentrations due to adsorption of clozapine to the gel layer. However, incidentally, serum may not be available while ethylenediaminetetraacetic acid (EDTA) and lithium heparin (LH) plasma are. In the literature clozapine levels are widely measured in both plasma and serum. Conflicting results about the interchangeability of clozapine levels in different matrices have been reported, however. Some studies [9-11] found that clozapine levels measured in EDTA-derived plasma and serum were similar. Another study [12] found that clozapine concentrations were 10% lower in serum than in LH-derived plasma and proposed that only plasma samples should be used to avoid significant underestimation of clozapine levels. Yet, in a recently published systematic review and meta-analysis [13] about the 'therapeutic window' of clozapine, all clozapine levels of 1,019 participants were pooled. These clozapine levels were taken from serum in five studies and from plasma in 14 studies. Of these 14 studies with plasma samples, eight studies did not specify the anticoagulant used to obtain plasma, three studies used plasma samples obtained from LH-tubes, two used EDTA-derived plasma samples, and one used balanced ammonium and potassium oxalate solution as anticoagulant. Additionally, a recent update of the Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology [4] states that: "The few available comparisons indicate that values obtained from serum or plasma can be used interchangeably."

The conflicting results in literature and our own clinical observations prompted us to investigate the interchangeability of clozapine measurements in plasma and serum.

Aim of the Study

The aim of this study was to assess whether the clozapine concentrations measured in plasma collected from EDTA and LH vacuum containers are equivalent to our conventional measurements in serum. Hereto, we also

compared the differences in clinical interpretation between clozapine concentrations measured in EDTA- and LH-derived plasma and in serum.

Materials and methods

This study was conducted in the Albert Schweitzer hospital, a teaching hospital in Dordrecht, the Netherlands, between January and February 2018. Under Dutch legislation, no approval was needed from the institutional review board nor from an ethics committee, as patients were not subjected to additional handling.

Patient samples

After performing required laboratory tests in plasma samples that were drawn simultaneously with the serum samples for the analysis of clozapine concentrations, these residual plasma samples were paired with these routine serum samples. These residual plasma samples were obtained from vacuum containers from BD (Plymouth, UK) with either LH or EDTA as anticoagulant and were anonymized and stored at 4°C until further analysis. Serum samples were derived from BD 6.0 ml clot activator vacuum containers (Plymouth, UK). Serum clozapine levels were routinely measured for therapeutic evaluation and anonymized afterwards according to the anonymization process of the other samples.

Analysis of clozapine

Serum and plasma samples were centrifuged for 5 min at 2880 *g*. For extraction, 1 mL serum or plasma was added to 1 mL internal standard solution containing 1 mg/L doxepin in water, 1 mL methanol, 100 µL extraction buffer (1 M sodium carbonate) and 6 mL extraction solution (n-Hexane 99%:isoamylalcohol 1%). After mixing, the solution was centrifuged at 10,000 *g* for 5 min. The hexane layer was transferred and dried at 50°C under air. The residue was dissolved in 160 µL mobile phase (38% acetonitrile in water containing 3% phosphoric acid 85%, adjusted to pH 3.35). The vacuum containers were vortexed and gently shaken for 1 h. Vials were centrifuged at 2880 *g* for 5 min. The supernatant was then analyzed with high performance liquid chromatography equipped with a Primaide 1210 autosampler, a Primaide 1110 isocratic pump and a 7450 A diode array detector from Hitachi (Tokyo, Japan). Ultraviolet detection was performed at 205 nm. Clozapine and internal standard doxepin were separated using a Luna analytical column from Phenomonex (Torrance, CA), 15.0 cm by 4.6 mm internal-diameter, 5 µm particle size, equipped with a pre-column of

Phenomonex Security Guard C8 4 cm x 3.0 mm. The flow rate was set at 2.0 ml/min.

The clozapine assay was validated for clinical use, with a lower limit of quantification of 60 µg/L. Intra-run and between-run coefficient of variation were less than 7% at all concentrations of clozapine (90 – 710 µg/L). The average accuracy of external quality control samples ranged from 95% to 107%. The mean recovery of clozapine spiked in blanc EDTA- and LH-derived plasma was 94%. This was linear over the studied concentration range, ruling out a significant matrix effect of plasma in the clozapine assay.

Clinical interpretation of differences

Clozapine concentrations were categorized into three categories: subtherapeutic (< 300 µg/L), therapeutic 300 – 700 µg/L) and supratherapeutic (> 700 µg/L), based on the reference concentrations used in our clinic. [4,5] Subsequently, categorization of the concentrations in LH- or EDTA-plasma were compared with the assigned categories of the paired serum samples and labeled as follows: incorrectly categorized (too low), correctly categorized, incorrectly categorized (too high). Two investigators (CL and MB) discussed differences in clinical interpretation between clozapine concentrations around the cut-off values of the three categories. Discrepancies were discussed with a third researcher (GT).

Statistical analysis

R version 4.0.3 (R foundation for statistical computing, Vienna, Austria) was used for statistical analysis. A power analysis was performed with G*Power version 3.1.3 (Düsseldorf, Germany). Assuming matched pairs, an effect size equal to the clinically acceptable value for deviation error in the analytical method validation (42 µg/L), a power of 80% and α of 0.05, we needed a sample size of n= 10 sample pairs. Analytical results were compared by means of a linear regression. A sensitivity analysis was performed by means of a Passing-Bablok regression.

Results

The collected samples contained 36 serum, 35 EDTA and 19 LH samples. The clozapine levels in serum ranged from 130 to 840 µg/L, and included 8 so-called subtherapeutic (<300 µg/L) (22%), 25 therapeutic (300 – 700 µg/L) (69%) and 3 supratherapeutic (> 700 µg/L) (8%) serum levels of clozapine (Figure 1).

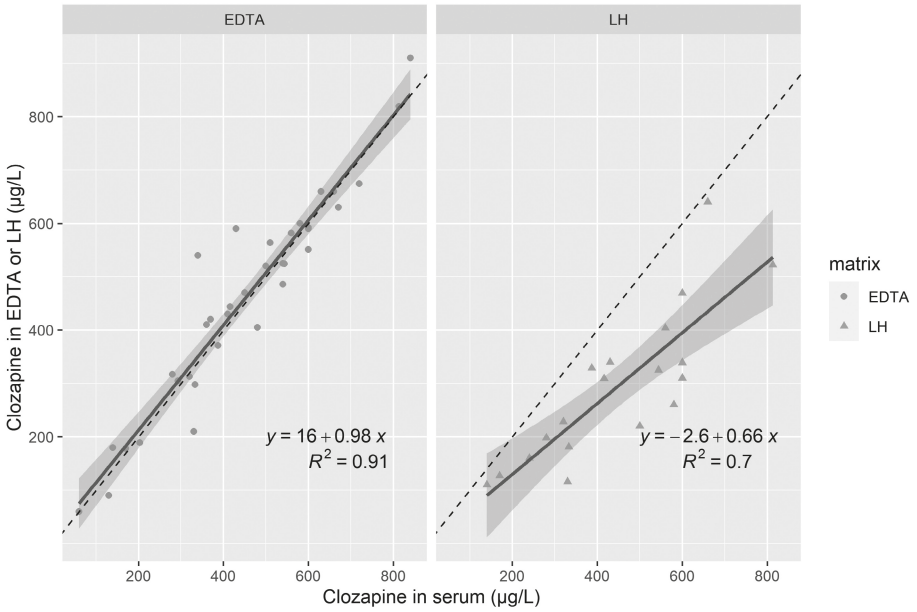

Figure 1. Plots of clozapine levels measured in EDTA-derived plasma versus serum (n=34, red, open dots) or LH-derived plasma versus serum (n=19, blue, triangles).

Linear regression lines with 95% confidence intervals are shown. All points except for one outlier were included in the calculation of the regression equations.

Based on a Z-score outside -3 and +3, one outlier in the EDTA versus serum samples was excluded in the regression analysis.

Linear regression analysis showed that clozapine levels measured in LH vacuum containers were on average 34% lower than in serum (Figure 1). This corresponds to an average difference of 150 ug/L (95% CI [-200; -110]) over the studied concentration range. In EDTA vacuum containers, clozapine levels were on average 20 µg/L higher than in serum (95% CI [-10; 50]). The Passing-Bablok regressions (graphs not shown) showed similar regression results (y= -0.5 + 1.01 x for EDTA R2 = 0.91 and y = -7.3 + 0.73 x for LH R2 = 0.70) as obtained with linear regression.

More than one-third (37%) of the LH plasma samples would have been categorized differently from the corresponding serum samples, possibly leading to a different clinical interpretation (Table 1).

Table 1. Assessment of the clinical interpretation of measurements in plasma compared to the values in serum.

	Incorrectly categorized (too low) †	Correctly categorized ‡	Incorrectly categorized (too high)
LH	7 / 19 (37%)	12 / 19 (63%)	N/A
EDTA	1 / 34 (3%)	33 / 34 (97%)	N/A

† Categorized as subtherapeutic levels in plasma, while serum levels were therapeutic, or categorized as therapeutic levels in plasma, while serum levels were supratherapeutic. ‡ Plasma and serum levels were categorized into the same category. N/A = not applicable. The outlier in EDTA versus serum was not included in this table.

The measured concentrations in these samples were either categorized as 'subtherapeutic' in LH-plasma, while serum levels were 'therapeutic', or categorized as 'therapeutic' in LH plasma, while serum levels were considered 'supratherapeutic'. In contrast, there is a high agreement between serum and EDTA plasma samples (97%).

Discussion

We performed this study to assess whether clozapine concentrations measured in EDTA-derived and LH-derived plasma are indeed comparable to clozapine concentrations in serum, as has been assumed in a recent consensus guideline [4] and a recent meta-analysis [13]. We found that EDTA-derived plasma samples and serum samples were indeed interchangeable in a validated serum clozapine assay. In contrast, the clozapine concentrations measured in LH-derived plasma were on average 34% lower over the studied concentration range than when measured in serum. In comparison, in their study on the safety of COVID-19 vaccination in patients on clozapine, Veerman et al, considered 100 ug/L to be a clinically relevant increase in clozapine blood levels.[14] This positions the average difference of 150 ug/L between concentrations measured in serum and in LH plasma. When these matrices would be used interchangeably within patients, wrong conclusions may be drawn.

According to current consensus, an analytical method validated for serum needs cross-validation when another matrix (e.g. plasma) is used. In case of plasma, cross-validation is also needed when another anticoagulant is used[15]. In this study, we found that EDTA-derived plasma can indeed be used instead of serum in our validated clozapine serum assay, whereas LH-derived plasma cannot be used. Our results for clozapine in EDTA-derived plasma are in line with research of Handley *et al.* [11], who also showed that the differences between

clozapine levels measured in serum and EDTA-derived plasma are so small as to be not relevant.
However, in our study, we also found that clozapine levels measured in LH-derived plasma were on average 150 µg/L (-34%) lower than in serum. This finding contrasts with a previous report from Kaladjian *et al.*[12] who showed that mean clozapine concentrations were 11% higher in LH-derived plasma than in serum. Our results are also in contrast to the findings of Hermida *et al.* [9], who found that clozapine levels in LH-derived plasma were similar to corresponding hematocrit-corrected EDTA-derived plasma levels.

The cause for the discrepancies between clozapine levels measured in serum and in LH-derived plasma is unclear [16]. Kaladjian *et al.* [12] hypothesized that clozapine is degraded by neutrophil myeloperoxidase released during blood coagulation, leading to lower clozapine levels in serum, but they were not able to confirm this. In addition, differences in clozapine levels could be due to differences in stability in different matrices. However, clozapine is stable for 5 days at ambient temperature and for 4 weeks at 2-8°C in EDTA-derived plasma, LH-derived hemolyzed whole blood and serum [16]. Since corresponding samples were withdrawn and stored under the same conditions, it is not likely that the differences between clozapine levels measured in LH-derived plasma and serum are due to differences in the stability of clozapine.

Since the results of our study are not likely to be attributable to matrix effect, another explanation for the difference in clozapine concentrations measured in serum and LH plasma is due to the LH tubes itselves. It is known that collection tubes can also be a major source of preanalytical error in laboratory testing.[17] Blood collection tubes can generally be comprised of rubber stoppers, tube wall materials, surfactants, anticoagulants or clot activators, separator gels, and surfactants, all of which may interfere with analytical assay. First the anticoagulans used may affect the measurable quantity of clozapine molecules. Heparin is hypothesized to increase the concentrations of non-esterified fatty acids. These non-esterified fatty acids can displace numerous drugs from plasma proteins. Consequently, a shift of the drug from the plasma into the erythrocytes can occur, resulting in lower plasma drug levels [18]. This has for example been shown *in vitro* for several psychotropic drugs such as amitriptyline, imipramine and maprotiline [19]. Perhaps, as adsorption of drugs onto the gel-layer sometimes present in collection tubes, may cause falsely low concentrations in therapeutic drug monitoring of various drugs, the found discrepancies in our study may also be attributable to adsorption of clozapine molecules to the constituents on the inside of the tubes.

Our study is based on a range of serum concentrations between 130 - 840 µg/L, which includes the therapeutic range of clozapine. We do not know whether our results can be extrapolated to higher supratherapeutic or potentially toxic levels.
Some laboratories also report concentrations of clozapine's main metabolite, norclozapine. Since this is not common practice in our laboratory, we cannot comment on the effect of different blood collection tubes on the norclozapine concentrations obtained.

In summary, it is important that medical professionals realize that results of measurements in different matrices are not simply interchangeable. Our findings show that if serum and LH plasma collection tubes are used in the same patient at different occasions for TDM of clozapine, this may lead to apparent, clinically relevant deviations in clozapine concentrations with possible false conclusions such as unjustified questions about compliance.

Based on this study, interpretation of subsequent clozapine levels measured in different matrices should be done with caution. EDTA-derived plasma levels of clozapine seem in high agreement with serum levels, while LH-derived plasma levels were not. Contrary to current consensus, we advise against the interchangeable use of LH-derived plasma and serum samples to measure clozapine in clinical practice as well as in guidelines and reviews regarding TDM of clozapine.

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5

General Discussion

This year (2022) marks exactly 50 years since clozapine was first marketed in Austria and Switzerland, under the European brand name "Leponex". Even though it has been on the market for half a century and the target population is relatively limited, an undiminished interest in this drug remains among researchers worldwide. This is evidenced by the number of scientific articles with "clozapine" in the title and collected in Pubmed/Medline over the past five years, which is more than four times the number of publications for hydrochlorothiazide, a diuretic patented a year later than clozapine. The pharmaceutical industry has made several attempts to develop a drug with at least the same efficacy as clozapine, but without the serious side effects. So far, in vain.

While clozapine is still the most effective antipsychotic, its mechanism of action remains elusive. [1] In contrast to the typical antipsychotics, showing high striatal dopamine receptor binding, clozapine has multiple sites of action such as dopamine (D), serotonine (5HT), muscarine (M), and histamine (H1-) receptors, with high affinity to D4 and 5HT2A receptors and low affinity to D1, D2 and D3 receptors. [2] Unlike the first-generation antipsychotics, clozapine does not tend to produce extrapyramidal symptoms due to its low affinity for striatal D2 receptors, here its atypical profile. However, despite clozapine's superior efficacy, other side effects (e.g., agranulocytosis, myocarditis, seizures, bowel obstruction, sedation, hypersalivation) and associated controls limit its widespread use. [3]

To date, clozapine is the only registered drug for the treatment of people with treatment-resistant schizophrenia (TRS) and has even been designated an essential drug by the World Health Organisation since 2013. [4]

Yet, it is noteworthy that clozapine treatment is one of the areas in psychiatry with the biggest mismatch between efficacy and utilisation in clinical practice. [5] Clozapine is often started reluctantly, with significant delays and preceded by antipsychotic polypharmacy, frequently prescribed in high doses, making clozapine a drug of last resort for many patients. Yet, for various reasons, subsequent discontinuation of clozapine treatment is common in clinical practice.

In short, clozapine is difficult to start with, and it is also difficult to maintain.

The overall objective of this thesis is to support successful clozapine treatment, by firstly expanding the knowledge on the predictability of early clozapine discontinuation, secondly investigating the necessity of clozapine discontinuation due to (intended) pregnancy, and thirdly expanding the

knowledge on therapeutic drug monitoring (TDM) of clozapine in light of once-daily (QD) dosing and in light of possible pitfalls in the interpretation of clozapine TDM results.

First, using data of a large, Dutch insurance company, a prediction model was developed for 'unsuccessful clozapine treatment' one year after the drug was first dispensed by a community pharmacy to patients with psychotic disorders (Chapter 2). With the available information, 30% (total population) and 68% (outpatient starters) of the variability in unsuccessful clozapine treatment could be predicted. The original research plan aimed to develop a model that could easily be used in clinical practice by psychiatrists to identify patients at increased risk of early discontinuation of clozapine treatment, so that these patients could receive additional support to prevent unwarranted discontinuation. The number of variables in our prediction model, however, was too large to be practically useful. Also, due to the Dutch insurance system, the available drug information only covered outpatient dispensations. This restricted the final study population to outpatients in whom a prescription for clozapine was first declared by a community pharmacy. However, a significant proportion of patients taking clozapine is expected to reside (long-term) in an inpatient unit of a mental health institution. Indeed, in our study population, almost 80% of patients had already started clozapine treatment as inpatients by the time their treatment continued on an outpatient basis. Although it was beyond the scope of our study to define determinants predictive for successful clozapine treatment, it is noticeable that the likelihood of successful clozapine treatment was higher among patients who had started using clozapine as an inpatient. Explanations for this remain speculative. Patients who had started clozapine and continued their treatment long-term as inpatients, thus representing the most seriously ill patients, remained outside the scope of our study. An alternative definition of successful clozapine treatment, however, could be the transition from inpatient to outpatient treatment after starting clozapine treatment clinically, which could be the subject of future research into variables predictive of successful clozapine treatment.
Finally, in the light of the studies presented in sections 4.1 and 4.2, it would be interesting to examine dosing regimen as a possible determinant of successful clozapine treatment, which was not possible with the insurance data because they do not provide insight into the doses and dosing regimens used.

Pregnancy has been cited as a motive for discontinuing clozapine [6] and there is a specific reluctance to continue clozapine in women with TRS who might become pregnant.[7] However, there is a lack of well-designed studies focusing on maternal, foetal, or neonatal outcomes of perinatal clozapine treatment. This may not be surprising since clozapine has long been underprescribed,

especially when pregnancy was concerned. To assess the validity of this reluctance, the safety of perinatal clozapine treatment was investigated in this thesis (Chapters 3.1 and 3.2). It is clear that clozapine and its main metabolite, norclozapine, enter the foetal system at some point in pregnancy and that the foetus is then exposed to clozapine and norclozapine if a mother uses clozapine during pregnancy. The data so far have not shown that clozapine is a teratogenic drug, and compared with other antipsychotics, clozapine does not appear to increase the risk of stillbirth, abortion, or foetal disorders, nor the risk of birth complications or preterm birth. Later in this chapter, the guidance of women taking clozapine during pregnancy and their affected children is discussed in more detail.

Nowadays, clozapine is increasingly prescribed once-daily (QD) at bedtime, aiming for increased drug adherence and less daytime sedation. Currently, the same reference trough values are used for once and twice daily (BID) dosing regimens. But simply assuming the reference values to be interchangeable for once and multiple dosing regimens, overlooks the possible influence of higher once-daily doses on the pharmacokinetics of clozapine and norclozapine. In our study to compare the pharmacokinetics of clozapine and norclozapine with QD and BID use, no evidence was found for increased half-lives due to saturation of metabolic enzymes at QD dosing or decreased absorption rates at (higher) QD dosing (Chapter 4.1). In addition, no evidence was found for saturation of protein binding at higher concentrations (Chapter 4.2). This implies that it is not necessary to consider differences in pharmacokinetics when conceptualizing a therapeutic window for QD dosing of clozapine. Also, a disproportionate increase in (unbound) clozapine and norclozapine concentrations, and hence a disproportionate increase in (adverse) effects, is not to be expected when (QD) doses are increased and vice versa when doses are reduced. The clinical study of the effect of QD dosing on the degree of protein binding required the development and validation of the method to determine unbound (nor) clozapine concentrations in serum using ultrafiltration (UF) to separate the bound and unbound molecules (Chapter 4.3). This study demonstrates the pitfalls and concerns regarding the accuracy and interchangeability of results from current protein binding studies with (nor)clozapine using ultrafiltration. The results of the study described in Chapter 4.4 plead for restraints in the interchangeable use of clozapine concentrations measured in serum and lithium-heparin (LH) derived plasma, both in clinical practice and in review articles.

In this general discussion, three themes are put into a broader perspective. First, there is a plea for more standardisation, clear definitions, and transparency in clinical trials reporting TDM results of clozapine. Second, the positioning of

measuring unbound clozapine (and norclozapine) concentrations is discussed in more detail. Finally, we discuss how to guide pregnant women using clozapine and their offspring.

Optimization of therapeutic drug monitoring to guide clozapine treatment: a plea for more standardisation, clear definitions, and transparency

Introduction

The currently applied therapeutic window of clozapine defines a trough level of 350 µg/l as the lower limit for efficacy, but it is generally accepted that patients with satisfactory response under lower clozapine concentrations do not require dose adjustments just to achieve this threshold value. [8] The upper target concentration of clozapine for effect remains a matter of debate [9], but is often referred to as 600-750 µg/l. However, some patients may require higher levels to show adequate response. [10,11] In addition, there is a lack of well-designed prospective studies, examining the association between clozapine concentrations and serious side effects.[9] Yet, an alert level of 1000 µg/l has been suggested [12,13], indicating "the concentration above the recommended therapeutic reference range that obliges the laboratory to feedback immediately to the prescribing physician". [12]

As already highlighted 25 years ago in a study by VanderZwaag and colleagues [14], "*We must interpret plasma or serum clozapine levels with caution. The level obtained at a given total daily dose will be substantially affected by the specific dosing schedule according to which the dose was administered, the duration of time between the last dose and blood sampling.*" Thus, as will be argued in the following section, to be able to interpret TDM results properly, it is important that the reference values are based on studies with similar dosing schedules and sampling times. And, consequently, these reference values only apply to the dosing schedules and sampling times used to establish this range, until proven to be valid for other dosing regimens as well. Also, the effect of different collection tubes on the results obtained should be assessed before pooling studies with plasma and serum concentrations, assuming that serum and plasma concentrations are interchangeable. In this light, it is noteworthy that this standardisation and transparency in the reporting of dosing schedules, sampling times, and blood collecting tubes in studies about the concentration-effect relationship of (nor)clozapine concentrations is frequently deficient. The awareness of the importance of this topic among researchers and reviewers of scientific journals needs to be improved.

Dosing regimens: the same therapeutic window for clozapine at once and twice daily dosing?

The therapeutic window of clozapine is based on studies referring to a twice- or thrice-daily schedule, with blood samples generally taken at trough concentrations (although one study even referred to samples collected at the midpoint of the multiple dosing interval [15]). To the best of our knowledge, it has not been previously investigated whether concentrations measured after 12 or 24 hours should be used to guide clozapine therapy at QD dosing and what reference range should then be used. Yet, in clinical practice the same reference values are used for QD as for divided dosing regimens, and in the Netherlands, samples are drawn in the morning approximately 12 hours post-dose.

Contrary to BID dosing, samples taken in the morning 12 hours after the last dose do not reflect trough concentrations in case of QD dosing (although sometimes even referred to as such in the literature [16]). The term 'trough concentrations' appears unambiguous, referring to the lowest concentration in the concentration-time curve, but in practice it proves open to more interpretation than expected. Clinicians should realise that a plasma or serum concentration sampled 12 hours post-dose (QD) will be higher compared to when the same daily dose was used in multiple doses (Chapter 4.1).

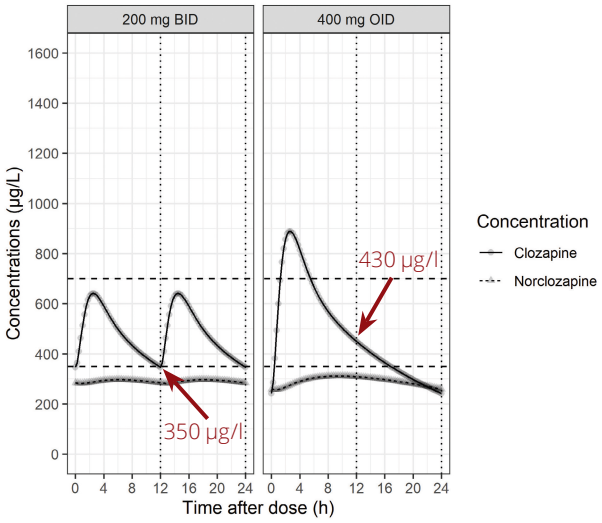

Figure 1. Simulated clozapine (black, dotted) and norclozapine (grey, triangles) concentrations over time for a dosing regimen of 400 mg per day for a smoking patient. Left: 200 mg twice-daily, right: 400 mg once-daily. Dashed lines (horizontal): therapeutic window for clozapine (350 - 700 µg/L).

(*Dashed lines (vertical): indication of sampling time (trough concentration or 12 h post-dose))*

In the Netherlands, the Dutch Clozapine Collaboration group (DCCG) (a working group of psychiatrists and other professionals such as internists, pharmacists or general practitioners who focus on treating patients with treatment-resistant psychotic disorders) therefore recommends applying 430 µg/l instead of 350 µg/l as the threshold for effect for samples taken after 12 hours in case of QD dosing. [8] This numerical approach is based on a study from VanderZwaag et al. [14] and consistent with the results of our simulations, but the validity of this approach has never been proven in clinical practice. Additionally, the DCCG does not comment on increasing the upper threshold for clozapine by the same factor, to approximately 925 µg/L, although this might be plausible based on the same reasoning.
However, there is an important caveat to this approach. It assumes similar concentration-effect relations with QD and BID use, whilst higher peak concentrations at QD dosing, for example, might affect the pharmacodynamics of clozapine. Moreover, as has been demonstrated recently for clozapine [17] trough levels provide only limited information about the exposure in time. The area under the concentration time curve (AUC) is a commonly used marker to estimate drug exposure in time and might therefore be a better predictor of clozapine response and safety. Specifically, Geers et al demonstrated that trough clozapine concentrations were not able to properly predict the AUC for QD dosing. [17] They even suggest that "the relatively wide therapeutic range may be the result of the poor predictive performance of the trough level for the AUC and thus exposure". Extrapolating this reasoning tentatively further, the question is whether current practice contributes to undertreatment of patients on a QD regimen who experience a suboptimal effect with good tolerability at clozapine concentrations, measured 12 hours post-dose, around or above the currently applied upper limit. Because of the risk of serious side effects, psychiatrists are obviously reluctant to increase clozapine doses once the upper limit of the currently applied therapeutic window has been reached. Therefore, this upper limit should be studied more thoroughly, considering the different markers for clozapine exposure.

The clozapine/norclozapine ratio

Although norclozapine itself has no antipsychotic properties, norclozapine might contribute (positively or negatively) to the manifestation of some of clozapine's side effects. [18,19]
Sedation is probably the most common side effect associated with clozapine [20], explained by clozapine's high affinity and antagonism toward H1- receptors. Norclozapine is thought to have similar affinities for H1 receptors, but norclozapine and not clozapine concentrations were correlated with total

sleeping hours [21]. Thus, norclozapine is probably important for clozapine-induced sedation.
Due to clozapine's antagonism of the muscarine-1(M1)-receptors, cognition may worsen under clozapine treatment. Norclozapine, however, possesses cholinergic activity. The balance between M1 antagonism and agonism, as expressed by the relative concentrations of clozapine to norclozapine (i.e., metabolic ratio), could underlie the better clinical outcome in terms of cognition in individuals with relatively higher concentrations of norclozapine. [22,23] The authors of two recently published reviews [18,19] agreed that the association of the metabolic ratio with cognitive outcomes is unclear, however, and additional data from longitudinal studies are needed to explore the link between the metabolic ratios and cognitive impairment.
Conversely, a positive association is suggested between the metabolic ratio and better cardiometabolic outcomes [18], which is contributed to the more potent serotonin (5-HT2c) receptor blocking capacity of norclozapine compared to clozapine. Recently, norclozapine serum concentrations were found to correlate with waist circumference, being considered a valuable predictor for metabolic syndrome. [24]

The study presented in Chapter 4.1 shows that the relative amount of clozapine over norclozapine throughout the day in QD dosing is markedly different than in BID dosing. Again, in studies on the metabolic ratio [25-27], the dose regimen is not always listed, nor is the time of sampling [26,27]. Also, the two recent reviews [18,19] have not addressed the possible impact of the dosing regimen and time of sampling on the calculated ratio. This possible heterogeneity could attribute to the conflicting results in the available literature and interferes with the requirements to define the presence or absence of an association. This demonstrates that transparent reporting of dosing regimens and sampling times is also required in studies on the relevance of the clozapine/norclozapine ratio.

Serum or plasma

Conflicting results about the interchangeability of clozapine levels in different matrices have been reported. [28-31] Yet, in a recently published systematic review and meta-analysis about the 'therapeutic window' of clozapine all clozapine levels of 1,019 study subjects were pooled. [32] These clozapine levels were taken from serum in five studies and from plasma in 14 studies. Of the 14 studies with plasma samples, eight studies did not specify the anticoagulant used to obtain plasma. In Chapter 4.4, the TDM results of clozapine measured in serum samples have been compared with the concentrations found in plasma derived from plasma collecting tubes containing lithium-heparin (LH) and

ethylenediamine tetraacetic acid as anticoagulants. The significant differences found between clozapine concentrations measured in serum samples and in plasma samples from LH-containing collecting tubes questions the validity of considering plasma and serum samples as interchangeable. This emphasizes the need for clear reporting of the matrices used to collect samples for TDM of clozapine (and norclozapine).

Future perspectives

The reliance on total plasma or serum kinetics as the main basis for dosing (regimens) of antipsychotics, has been questioned. Previous studies have shown a discrepancy between the duration of D2-receptor occupancy and peripheral half-lives of antipsychotics including clozapine, with the striatal D2 receptor occupancy lasting much longer than expected based on their peripheral half-lives. [33-35] For QD dosing this could suggest that it is not necessary to aim for trough concentrations above 350 µg/l (i.e., sustained clozapine concentrations above 350 µg/l within the 24 hour-interval after the single evening dose). Maybe, as with BID dosing, 12 hours post-dose values of 350 µg/l can be pursued for QD dosing.

In the meantime, a large prospective longitudinal study is needed comparing QD and BID dosing of clozapine in terms of efficacy and side effects (both peripheral and central) and their relationships with both trough concentrations and concentrations measured after twelve hours, as well as peak concentrations and the AUCs (which might be predicted with a recently proposed limited sampling strategy [17]). Concentrations should be measured in a single matrix type and the matrix used should be clearly indicated.

Optimization of clozapine treatment based on unbound concentrations

Introduction

As with all drugs, the unbound rather than total amount of molecules is responsible for (side) effects. And for drugs acting on the central nervous system (CNS) such as clozapine, the unbound concentration at the brain target site, rather than peripheral unbound concentrations, is the driving force for drug-receptor binding in the brain, along with the drug's affinity to the receptor. On the other hand, most peripheral side effects are related to peripheral (unbound) concentrations. [16] In clinical practice, for obvious practical reasons, total concentrations are measured in serum or plasma to guide therapy.

Clozapine and norclozapine are both highly protein bound [36], primarily to alpha-1-acid glycoprotein (AGP) and to a lesser extent to albumin. The degree of protein binding by clozapine and norclozapine, generally referred to as 95% and 90% respectively, is based on a study among 15 patients, using equilibrium dialysis. [36] Equilibrium dialysis is the gold standard to separate the unbound from the bound molecules, but it is time consuming and not suitable for high sample throughput necessary for routinely clinical practice. Ultrafiltration is often used as an alternative method, due to its simple and rapid separation technique. However, the ultrafiltration technique to separate bound and unbound molecules does not generate absolute concentrations, as part of the clozapine and norclozapine molecules adsorb to the filter as we have demonstrated in our study on the validation of the ultrafiltration technique (Chapter 4.3). Quantifying the extent of non-specific adsorption is complex and accuracy of the results cannot be determined since it is not possible to relate the retrieved concentrations after filtration to known concentrations of an internal standard. Also, the binding of the protein-drug complex is influenced by variables such as temperature and pH, so that free concentrations measured outside the body can be different from those inside the body. [37] This requires strict control of these variables and currently limits the interchangeability of the retrieved unbound concentrations between studies, as well as the interpretation of a single measurement of unbound clozapine and norclozapine concentrations in an individual patient.

Total versus unbound clozapine and norclozapine concentrations

With the above limitations in mind, the correlation between total and unbound clozapine and norclozapine concentrations in view of higher (QD) dosing and at peak concentrations was explored in this thesis (Chapter 4.2). Within this context, the focus was on relative values rather than on absolute quantification of the unbound concentrations. Our findings support the use of total clozapine and norclozapine concentrations as a proxy for the amount of unbound clozapine molecules present in plasma or serum, with some possible exceptions. First, the unbound fractions of clozapine and norclozapine tended to decrease at higher AGP-concentrations. As AGP is an acute-phase protein, AGP levels increase in various disease states such as infections and inflammation. In our study, three of the 44 included patients had AGP-concentrations above the reference range of 0.5-1.2 g/L. Indeed, one of them (AGP 1.64 g/L; unbound clozapine fraction of 0.9%) turned out to have an elevated CRP value (35 mg/L) despite a normal body temperature at the study day. But the other two did not. Increased AGP levels have also been observed in obese individuals [38]; one of the other two patients in our study (AGP 1.52 g/L, unbound clozapine fraction of 0.9%) had a normal CRP concentration, but a body mass index (BMI) of 36.0 kg/m2. Thus,

although total clozapine and norclozapine concentrations seem to correlate well with the unbound concentrations on a population level (R2 = 0.87), we observed a case with an increased AGP-concentration in the absence of a clear infection. Furthermore, possible changes in the binding affinity of AGP caused by several physiological and pathological situations, including the presence of other agents binding to AGP, should be part of the clinical reasoning in case of unexpected (side) effect.

Future perspectives

The ultimate goal would be to establish a therapeutic window for the free clozapine concentrations on which dose adjustments could be based, rather than on total concentrations in the event of infection, inflammation, pregnancy (Chapter 3.2) or the presence of drugs possibly interfering with protein binding, for example. The clinical significance of binding displacement drug interactions has been understudied anyway and should be further explored. Currently, the unbound concentrations measured with ultrafiltration as a separation method should be considered as relative rather than absolute values, which interferes with the demand for absolute reference concentrations for the therapeutic window of the unbound clozapine and norclozapine concentrations. As the EMA and FDA have well defined requirements for development and validation of bioanalytical assays [39,40], they should also develop these uniform requirements for separation techniques, so that concentrations from different studies (in different laboratories) can be compared. In the meantime, as a next step in optimizing treatment, in each patient who is stable on clozapine, consideration might be given to determining some sort of "individual or personal reference value" for free and total clozapine and norclozapine concentrations in conjunction with a simultaneous determination of AGP concentration.

Perinatal clozapine treatment

Introduction

Since clozapine does not elevate prolactin levels unlike other atypical antipsychotics, ovulation may resume when clozapine is initiated, increasing the chance of pregnancy and at some point, clinicians can be faced with planned and unplanned pregnancies in their patients. The Summary of Product Characteristics of Leponex states that animal studies do not indicate any direct or indirect adverse effects with respect to pregnancy, embryonic/foetal development, partus or postnatal development. [41] But caution should be exercised when prescribing clozapine to pregnant women. Newborns exposed

to clozapine during the third trimester of pregnancy are at risk of adverse reactions. Therefore, newborns should be closely monitored.

Exposure to antipsychotics during pregnancy is inevitably coupled with exposure to maternal illness, and schizophrenia as such has also been associated with several adverse obstetric complications and pregnancy outcomes. [42] Other concomitant factors, such as low dietary vitamin intake, poor nutrition, reduced serum folate levels related to poor antenatal care, smoking, and alcohol and drug abuse, make it extremely difficult to separate the contribution of antipsychotics from the influence of these potentially confounding factors. Nevertheless, our study using global pharmacovigilance data (Chapter 3.1) did not reveal a safety signal necessitating a switch from clozapine to a second-choice antipsychotic in the treatment algorithm of patients with TRS.

Continuation of clozapine during pregnancy; how?

When the decision is made to continue clozapine during pregnancy, efforts must be made to (maintain) effective maternal treatment and to minimize foetal exposure to clozapine and norclozapine.
Therefore, close perinatal care is required, including nutritional counselling and laboratory monitoring to ensure sufficient vitamin use and to detect early signs of gestational diabetes. As with all mothers, excessive weight gain should be avoided. An increased risk of gestational diabetes mellitus seemed to be best predicted by an increased early pregnancy BMI rather than by the treatment with clozapine itself. [43] Based on theoretical knowledge of the physiological changes during pregnancy, the pharmacokinetic properties of clozapine and possible changes in tobacco and caffeine use during pregnancy, it is likely that both total and unbound clozapine concentrations will change despite stable doses. It has been shown that the net result of these changes cannot be predicted, which emphasizes the need of TDM to assist in keeping a mother's psychiatric condition stable during this period. Considering the previously discussed degree of protein binding of clozapine and norclozapine, it would be especially relevant to know whether and if so, how the expected decrease in AGP concentrations during pregnancy affects both maternal outcomes and placental passage, due to changes in unbound concentrations. As always, but especially during pregnancy, the mother should be treated with the lowest possible dose to minimise foetal drug exposure, and stable clozapine levels should be pursued. However, changes in protein binding may lead to difficulties in the interpretation of total clozapine concentrations since the total drug concentration may no longer be a valid reference value. Therefore, as also suggested above, it might be helpful to have a 'pre-pregnancy', reference (trough or AUC) value for clozapine and norclozapine exposure at which there

was an optimal ratio between effect and the degree and severity of side effects. At least theoretically, this reference value could assist the doctor in adjusting the dose during pregnancy (and after birth), responding to any changes in the pharmacokinetics and degree of protein binding.

Perhaps, as with lithium, during pregnancy it is better to aim for more constant blood concentrations throughout the (24 h) day to avoid higher (unbound) peak concentrations leading to increased foetal exposure. However, no research has been done on this to date. Since there is no slow-release formulation of clozapine, this would imply divided dosing of clozapine. Thus, whilst QD dosing might be better for adherence and reduced sedation, it is unknown if it is also appropriate during pregnancy.

Continuation of clozapine during pregnancy; what to bear in mind during and after birth?

During birth, a cardiotocogram (CTG) is commonly used to detect changes in foetal heart rate (FHR) patterns to identify foetuses unable to initiate or maintain cardiovascular compensatory defence mechanisms in response to hypoxia, leading to acidosis. Isolated interpretation of the computerized recording of a CTG, however, can be falsely alarming on foetuses with no other signs of foetal stress. Clozapine is thought to be able to reduce this FHR variability by blocking the cholinergic and adrenergic receptors of the foetal nervous system after crossing the placental barrier which, misleadingly, could mimic the symptoms of asphyxia. Therefore, when interpreting a CTG pattern it is important to consider the use of medication such as clozapine. As mentioned above, it is recommended to keep the clozapine dose as low as possible, but especially during the days that immediately precede birth to reduce foetal exposure shortly before birth. Also, close monitoring of the neonate for potential adverse events such as delayed peristalsis and possible withdrawal effects of clozapine is recommended. Due to the lack of knowledge about neonatal risks for clozapine-induced agranulocytosis, haematological monitoring of the newborn in the first two to three weeks postpartum is warranted. This period is based on expected decreased neonatal clozapine clearance and the absence of breastfeeding. The influence of medications during breastfeeding should always be evaluated against the background of the exposure that already took place during pregnancy. However, based on clozapine's side effect profile and the expected decreased neonatal clozapine clearance, lactation should preferably be avoided since clozapine accumulates in breastmilk. [43,44] Yet, if breastfeeding is undertaken by a mother who is taking clozapine, close monitoring of the infant for excessive sedation, constipation and periodic monitoring of the infant's white blood cell count is advisable.

Finally, as with all psychotropic drugs, there are insufficient data on the long-term development of the children after intrauterine exposure to clozapine.

Future research

To further support the treatment of pregnant women taking clozapine and to be able to timely anticipate on any changes, prospective studies are needed to determine the effect of pregnancy on maternal pharmacokinetics of total and unbound clozapine and norclozapine concentrations. Studies of maternal blood, umbilical cord blood and neonatal (free) concentrations immediately post-partum should provide more insight into the extent of foetal exposure to clozapine and norclozapine shortly before birth. By comparing these concentrations in mothers receiving clozapine once or twice daily, more knowledge will be gained about the extent of foetal exposure in relation to the dosing regimen.

Prospective cohort-studies with children who have been exposed to antipsychotics during pregnancy are needed to determine long-term effects on several developmental outcomes.

Final conclusion

Given its place in the treatment algorithm, it is concerning that it is estimated that only half of people with TRS is expected to respond adequately to clozapine [45] and that discontinuation rates are high.
We found that in 20.4% of the patient clozapine treatment was unsuccessful one year after clozapine was first dispensed by a community pharmacy. Discontinuation could not easily be predicted with a limited number of variables derived from an insurance database, suggesting multifactorial causes and influence of variables that could not be inferred from the information in the database.

Pregnancy or the wish to become pregnant should not be a reason to discontinue clozapine unless after careful consideration of the costs and benefits.

Although we found that the pharmacokinetics of clozapine and norclozapine are similar at QD and BID dosing, prospective studies are needed to define the therapeutic window at QD dosing of clozapine. Also, further studies on the variation in unbound clozapine and norclozapine concentrations is needed.
Finally, this thesis demonstrates the need for improvement in the standardization and transparency in the reporting of dosing schedules, sampling times, and

blood collecting tubes in studies about the concentration-effect relations of (nor) clozapine. Researchers and reviewers of scientific journals, as well as doctors should be aware of the importance of this topic when interpreting clozapine and norclozapine concentrations for TDM. Moreover, this standardization and transparency is necessary to fully utilize the added value of TDM.

Clinical pharmacists and pharmacologists are the ambassadors to transfer this knowledge to clinical practice and translate this knowledge into individual treatment recommendations. They should have a close working alliance with clinicians initiating and monitoring long-term treatment with clozapine, and most notably should be structurally involved in the treatment of patients with suspected clozapine-resistant schizophrenia to disentangle true from pseudo resistance to clozapine.

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6

Summary & Samenvatting

Summary

Antipsychotics are the cornerstone of pharmacotherapeutic treatment of schizophrenia. However, one fifth to one third of all patients with schizophrenia lack a full symptomatic response. This is referred to as treatment-resistant schizophrenia (TRS). Clozapine, the first so-called 'atypical antipsychotic', referring to absence of extrapyramidal side effects, is currently the only registered drug for the treatment of TRS. Meta-analyses have consistently demonstrated that clozapine is superior to other antipsychotics in reducing positive psychotic symptoms in both the short and long term for people with TRS. The pharmacological basis for this superior effect of clozapine is still unclear. Nevertheless, clozapine is considered unsuitable as a drug of first choice because of its side effect profile and required laboratory controls. These side effects include but are not limited to the risk of agranulocytosis, for which the drug was withdrawn from the market shortly after its introduction 50 years ago, as well as the risk of seizures, myocarditis, metabolic syndrome, and bowel obstruction. Yet, at least in theory, all patients with TRS should undergo a trial of clozapine, but clozapine is highly underutilised for several reasons. Thus, clozapine is still saddled with a stigma, which has relegated clozapine as being regarded a drug of last resort. However, there seems to be a gradual decline in "clozaphobia", and clozapine use has increased in many countries over recent years. (**Chapter 1**) The overall objective of this thesis is to support successful clozapine treatment, by firstly expanding the knowledge on the predictability of early clozapine discontinuation (**Chapter 2**), secondly investigating the necessity of clozapine discontinuation due to (intended) pregnancy (**Chapter 3**), and thirdly expanding the knowledge on therapeutic drug monitoring (TDM) of clozapine in light of once-daily dosing and protein binding, as well as in light of possible pitfalls in the interpretation of these TDM results (**Chapter 4**).

The aim of the study described in **chapter 2.1** was to develop a database-based prediction model for unsuccessful clozapine treatment one year after the drug was first dispensed to patients with psychotic disorders by community pharmacies in the Netherlands. Identifying patients at risk for unsuccessful clozapine use might enable clinicians to direct specific attention to these patients to prevent unwarranted discontinuation. In addition, this study aimed to investigate whether unsuccessful treatment is influenced by initiation of clozapine treatment during psychiatric hospital admission or during ambulatory treatment.
Routinely collected data from a large insurance company were used to develop a simple prediction model. Unsuccessful clozapine treatment was defined as the absence of current insurance claims for clozapine treatment 365 days after the index date. When no current insurance claim for clozapine was present

after one year a patient either discontinued clozapine use or was admitted to a hospital. Multivariate logistic regression analyses were performed with the Nagelkerke R squared (R^2) statistic as a measure of the predictive value of the model.
937 Patients were dispensed clozapine for the first time by their community pharmacy between January 1st 2011 and December 31st 2015 (index date). Of these, 741 patients had started their clozapine treatment during admission before the index date (inpatient starters); the remaining 196 patients started clozapine as outpatients on the index date (outpatient starters). In 191 patients (20.4%) clozapine treatment was unsuccessful 1 year after the index date. Unsuccessful treatment was more common among outpatient starters than among inpatient starters (32.1% versus 17.3%). Using backward selection of the variables, a model consisting of 61 variables had the best predictive value overall (Nagelkerke R^2 0.301), whereas a model consisting of 52 variables had the best predictive value in outpatient starters (Nagelkerke R^2 0.676).
The likelihood of unsuccessful clozapine treatment after 1 year was higher among patients who started clozapine as outpatients. The number of variables needed to be able to predict as much as 30% or 68% of the variability in unsuccessful clozapine treatment was too large, however, for a simple prediction model to be used by psychiatrists in daily practice.

Currently, about a third of Dutch clozapine users are women, including women of childbearing age. As psychiatrists seem to become less reluctant to prescribe clozapine in more recent years, over time, more women on clozapine treatment may become pregnant. Safety data on clozapine use during pregnancy are limited, but pregnancy has been cited as a motive for discontinuing clozapine and there is a specific reluctance to continue clozapine in women with TRS who might become pregnant. In **chapter 3**, the safety of perinatal clozapine treatment was investigated. First, disproportionality in case safety reports on adverse pregnancy outcomes between clozapine and other antipsychotics (OAP) used during pregnancy was determined in **chapter 3.1**. In this study, all reports of suspected adverse drug reactions (ADRs) to antipsychotics registered in the World Health Organization global individual case safety report (ICSR) database (VigiBase) in children younger than 2 years and women aged 12-45 years were included. A case/non-case approach was used to evaluate the association between several pregnancy related ADRs and clozapine exposure during pregnancy, using 2x2 contingency tables to investigate disproportionality and Standard MedDRA Queries to select cases. Clozapine exposure was defined as all ICSR-ADR combinations with clozapine as (one of) the suspected drug(s). Non-exposure was defined as all ICSR-ADR combinations with OAP as (one of) the suspected drug(s).

In total, 42,236 unique ICSR-ADR combinations were identified related with clozapine exposure and 170,710 with OAP exposure. Of these, 494 and 4,645 ICSR-ADR combinations were related to adverse pregnancy outcomes due to exposure to clozapine and OAP, respectively. Overall, no signal of disproportionate reporting associating clozapine with the studied adverse pregnancy outcomes was found. Based on this, we did not find any evidence that clozapine is less safe during pregnancy than OAP. However, this is not automatically equivalent to the relative safety of clozapine during pregnancy.
Chapter 3.2 provides a critical appraisal of the available evidence related to the safety of clozapine for schizophrenia during pregnancy and lactation.
Pubmed/Medline, Embase, and the Cochrane Library were searched from inception through December 2020. Reference lists of included studies were hand-searched. The International Clinical Trials Registry Platform and ClinicalTrials.gov were searched for unpublished trials and PROSPERO for unpublished reviews. The current marketing authorization holder of the originator brands Clozaril® and Leponex® was also contacted for pharmacovigilance data. Original reports published in English, German, French, or Dutch containing clinical and preclinical data were included if they provided data on maternal, foetal, and neonatal outcomes after clozapine exposure during pregnancy or lactation. Two reviewers independently extracted relevant data.
A total of 860 records were identified, and the full texts of 117 articles were reviewed. Forty-two studies met the inclusion criteria. Data on perinatal clozapine exposure are of limited quality and quantity. Although clozapine demonstrates partial placental passage, data thus far do not support that clozapine is teratogenic, increases the risk of stillbirth, abortion, or foetal disorders, nor that it increases the risk of delivery complications or premature birth. Information about clozapine exposure through breast milk is scarce, but based on its chemical properties, it is likely that clozapine enters the breast milk of nursing mothers taking clozapine. When outweighing the risk and benefits of clozapine continuation during pregnancy and lactation versus switching to another antipsychotic, one should include severity of illness and treatment history, but also be aware of the limitations of the available safety data regarding perinatal clozapine use, including the fact that there are few studies.

Aiming for better adherence and reduced daytime sedation, clinicians increasingly favour a once -daily (QD) over a divided dosing regimen for clozapine. In **chapter 4.1.**, as part of the INPUT study (INfluence of dose interval on the Pharmacokinetics of both Unbound and Total fractions of clozapine and norclozapine in psychiatric patients in the Netherlands), we determined whether the pharmacokinetics of clozapine and norclozapine with QD use are

similar to twice-daily (BID) use. Additionally, the effect of QD dosing on the concentration-time curves of clozapine and norclozapine was studied through simulations.
Multiple blood samples were collected within a dosing interval from patients using clozapine QD or BID in steady state. Clozapine and norclozapine concentrations were analysed with a validated LC-MS/MS method. Population pharmacokinetic (PPK) analyses were performed by means of nonlinear mixed-effects modelling. The influence of the dosing regimen was tested as a covariate. Forty-four patients (319 samples) were included. For clozapine, the best structural model was a two-compartment model with a transit compartment and linear elimination consisting of combined excretion and formation of norclozapine. For norclozapine, a one-compartment model with first-order elimination best described the data. Dosing regimen did not affect any of the pharmacokinetic parameters. The simulations showed that clozapine concentrations sampled in the morning with QD use are markedly different than its trough concentrations and higher than if the same daily dose had been used in two divided doses. The relative amounts of clozapine to norclozapine also vary to a greater extent than with BID dosing. Thus, we concluded that the pharmacokinetics of clozapine and norclozapine are not influenced by the dosing regimen. Nonetheless, clozapine concentrations obtained at a given total daily dose as well as the metabolic ratio will be affected by the dosing regimen and the time of blood sampling. The implications of this on clozapine's therapeutic window at QD dosing is yet unknown.
In **chapter 4.2**, the impact of (higher) QD dosing on unbound clozapine and norclozapine concentrations was investigated. Clozapine and norclozapine are highly protein bound in plasma, mainly to alpha-1 acid glycoprotein (AGP), and theoretically, higher (QD) doses may lead to saturation of protein binding of clozapine and norclozapine, resulting in increased unbound fractions. We investigated whether protein binding of clozapine and norclozapine becomes saturated at higher concentrations from QD dosing or when reaching peak concentrations. Secondly, we investigated the correlation between unbound clozapine and norclozapine fractions and alpha-1 acid glycoprotein concentrations.
Unbound and total clozapine and norclozapine concentrations were measured in the 319 blood samples collected at different time points from forty-four patients taking clozapine QD or twice daily as part of the above-mentioned INPUT study. In addition, AGP-concentrations were measured in samples drawn just before clozapine intake. The relation between total and unbound concentrations and fractions, and between unbound fractions and AGP-concentrations were investigated using univariate linear regression analysis. The effect of dosing regimen on protein binding was assessed using multivariate linear regression analysis and split ANOVA analysis.

Clozapine and norclozapine concentrations correlated well with its unbound concentrations (R^2 = 0.87, p <0.001 and R^2 =0.73, p <0.001 respectively) within the studied concentration range. The dosing regimen did not affect the relation between total clozapine and norclozapine concentrations and its unbound fractions. A moderate (clozapine) and small (norclozapine) correlation were found between AGP-concentrations and unbound fractions of clozapine (R^2=0.15, p<0.001) and norclozapine (R^2=0.055, p<0.001).
Thus, no disproportional increase in unbound concentrations were found with clozapine concentrations up to 1500 µg/L. Total concentrations remain suitable for therapeutic drug monitoring in QD clozapine regimens in general. However, occasional abnormal unbound fractions, even in the absence of infection, could possibly modify clozapine's concentration-effect relation.
To be able to measure the unbound clozapine and norclozapine concentrations for the study presented in chapter 4.2, a method was developed for quantification of the unbound concentrations of clozapine and norclozapine in serum. **Chapter 4.3** describes the hurdles to be taken when developing a method for quantification of the unbound concentrations of clozapine and norclozapine in serum, using ultrafiltration (UF).
First, the lower limit of quantification of an existing LC-MS/MS method to analyse clozapine and norclozapine in serum was lowered. To test for non-specific adsorption to the filter, the method was cross-validated to be used in water. Five different UF-filters, with and without pre-treatment of the membranes, were tested to choose the best filter and optimise recovery in purified water. Finally, the total procedure was applied in serum.
The calibration curves remained linear over the expanded concentration range of 1-1000 µg/L with acceptable regression coefficients, within-run, and between-run coefficients of variation (CVs) (<15%), as well as assay parameters for cross-validation and matrix effects.
The 30KDa UF-filter from Merck without pre-treatment yielded the highest recovery for clozapine in purified water, which was stable (13.6-15.6%) within a range of 5 – 50 µg/L before UF. The free fractions in serum remained constant (2.35-2.45% and 3.28-3.61% for clozapine and norclozapine respectively) over a representative concentration range for actual total clozapine and norclozapine concentrations. The within run, between run and overall CVs of the calibration series were acceptable, except for the overall CV of the free norclozapine concentrations.
This study demonstrates that the choice of the ultrafilter is a crucial part of the development of a method to measure unbound concentrations using UF. Adequate control of the factors influencing the NSA is essential, as well as factors influencing the stability of the protein-drug complex.
The study in **chapter 4.4** assessed whether clozapine concentrations measured in plasma collected with tubes containing ethylenediaminetetraacetic

(EDTA) and lithium-heparin (LH) as anticoagulants are comparable to clozapine concentrations measured in serum. Clozapine levels are widely measured in both plasma and serum, but knowledge regarding the interchangeability of these matrices is limited and conflicting.
Paired residual EDTA- and LH-derived plasma and routine serum samples were obtained for analysis of clozapine concentrations. Clozapine concentrations in serum were compared with the concentrations found in EDTA- and LH-plasma using linear regression. For clinical interpretation, clozapine concentrations measured in the three different matrices were categorized into three categories: subtherapeutic (< 300 µg/L), therapeutic (300 – 700 µg/L) and supratherapeutic (> 700 µg/L). Differences in clinical interpretation between clozapine concentrations measured in the two plasma matrices and serum were assessed.
Compared to the serum samples (n=36), clozapine levels measured in LH-derived plasma samples (n=19) were on average 150 µg/L lower (95% CI [-200; -110]). Clozapine levels measured in EDTA-derived plasma samples (n=35) were on average 20 µg/L higher (95% CI [-10; 50]). In 37% of the LH samples, the clinical interpretation differed from the interpretation based on serum clozapine levels, whilst there was high agreement between serum and EDTA plasma samples (97%).
The use of LH-derived plasma samples for therapeutic drug monitoring of clozapine may lead to different clinical interpretations than when using serum samples. Therefore, interchangeable use of LH-derived plasma and serum samples to measure clozapine levels in clinical practice is not recommended.

In **chapter 5**, the findings of this thesis were put into a broader perspective. Given its place in the treatment algorithm, it is concerning that it is estimated that only half of people with TRS is expected to respond adequately to clozapine and that discontinuation rates are high. We found that in 20.4% of the patient clozapine treatment was unsuccessful one year after clozapine was first dispensed by a community pharmacy. Discontinuation could not easily be predicted with a limited number of variables derived from an insurance database, suggesting multifactorial causes and influence of variables that could not be inferred from the information in the database.
Pregnancy or the wish to become pregnant should not be a reason to discontinue clozapine unless after careful consideration of the costs and benefits.
Although we found that the pharmacokinetics of clozapine and norclozapine are similar at QD and BID dosing, prospective studies are needed to define the therapeutic window at QD dosing of clozapine. Also, further studies on the variation in unbound clozapine and norclozapine concentrations as well as its clinical impact is needed.

Finally, this thesis demonstrates the need for improvement in the standardisation and transparency in the reporting of dosing schedules, sampling times, and blood collecting tubes in studies about the concentration-effect relations of clozapine and norclozapine. Researchers and reviewers of scientific journals, as well as doctors should be aware of the importance of this topic when interpreting clozapine and norclozapine concentrations for TDM. Moreover, this standardisation and transparency is necessary to fully utilise the added value of TDM.

Samenvatting

Antipsychotica vormen de hoeksteen van de farmacotherapeutische behandeling van schizofrenie. Echter, een vijfde tot een derde van alle patiënten met schizofrenie heeft geen volledige symptomatische respons. Dit wordt 'treatment-resistant schizophrenia' (TRS) genoemd. Clozapine, het eerste zogenaamde "atypische antipsychoticum", verwijzend naar de afwezigheid van extrapyramidale bijwerkingen, is momenteel het enige geneesmiddel dat geregistreerd is voor de behandeling van TRS. Meta-analyses hebben aangetoond dat clozapine superieur is aan andere antipsychotica in het verminderen van positieve psychotische symptomen op zowel de korte als de lange termijn bij mensen met TRS. De farmacologische basis voor dit superieure effect van clozapine is nog onduidelijk. Toch wordt clozapine ongeschikt geacht als middel van eerste keuze vanwege het bijwerkingenprofiel en de vereiste laboratoriumcontroles. Deze bijwerkingen omvatten, maar zijn niet beperkt tot, het risico van agranulocytose, waarvoor het middel kort na de introductie 50 jaar geleden van de markt werd gehaald, alsmede het risico van aanvallen, myocarditis, metabool syndroom en darmobstructie. Toch zouden, althans theoretisch, alle patiënten met TRS de kans moeten krijgen om een proefbehandeling met clozapine te ondergaan. Echter, er is wereldwijd sprake van een hoge mate van ondergebruik, om verschillende redenen. Zo draagt clozapine nog steeds een stigma, waardoor clozapine wordt beschouwd als een middel van laatste keuze. "Clozafobie" lijkt geleidelijk wel af te nemen en het gebruik van clozapine is de laatste jaren in veel landen toegenomen. (**Hoofdstuk 1**) Het algemene doel van dit proefschrift is het ondersteunen van een succesvolle clozapinebehandeling, door ten eerste de kennis over de voorspelbaarheid van vroegtijdig stoppen met clozapine uit te breiden (**Hoofdstuk 2**), ten tweede de noodzaak van stoppen met clozapine vanwege (voorgenomen) zwangerschap te onderzoeken (**Hoofdstuk 3**), en ten derde de kennis over 'therapeutic drug monitoring' (TDM) van clozapine uit te breiden in het licht van eenmaal daags doseren en eiwitbinding, alsmede in het licht van mogelijke valkuilen bij de interpretatie van deze TDM-resultaten (**Hoofdstuk 4**).

Het doel van het in **hoofdstuk 2.1** beschreven onderzoek was het ontwikkelen van een database-gebaseerd predictiemodel voor het voorspellen van niet-succesvolle clozapinebehandeling één jaar na de eerste verstrekking van het middel aan patiënten met psychotische stoornissen door openbare apotheken in Nederland. Het identificeren van patiënten met een risico op niet-succesvol clozapinegebruik zou behandelaren in staat kunnen stellen specifieke aandacht aan deze patiënten te besteden om onterecht staken van de behandeling te voorkomen. Daarnaast wilden we in deze studie onderzoeken of niet-

succesvolle behandeling wordt beïnvloed door start van clozapinebehandeling tijdens psychiatrische ziekenhuisopname of tijdens ambulante behandeling.
Routinematig verzamelde gegevens van een grote verzekeringsmaatschappij werden gebruikt om een eenvoudig predictiemodel te ontwikkelen. Niet-succesvolle clozapinebehandeling werd gedefinieerd als de afwezigheid van lopende verzekeringsclaims voor clozapine 365 dagen na de indexdatum. Wanneer na één jaar geen lopende verzekeringsclaim voor clozapine aanwezig was, werd de patiënt geacht te zijn gestopt met clozapinegebruik of te zijn opgenomen in een ziekenhuis. Multivariate logistische regressieanalyses werden uitgevoerd met de Nagelkerke R squared (R^2) statistiek als maat voor de voorspellende waarde van het model.
937 Patiënten kregen tussen 1 januari 2011 en 31 december 2015 voor het eerst clozapine verstrekt door hun openbare apotheek (indexdatum). Hiervan werden 741 patiënten geacht vóór de indexdatum tijdens een klinische opname te zijn begonnen met hun clozapinebehandeling (intramurale starters); de overige 196 patiënten waren op de indexdatum als poliklinische patiënt begonnen met clozapine (ambulante starters). Bij 191 patiënten (20,4%) was de clozapinebehandeling 1 jaar na de indexdatum niet succesvol. Niet-succesvolle behandeling kwam vaker voor bij poliklinische starters dan bij intramurale starters (32,1% versus 17,3%). Bij 'backward' selectie van de variabelen had een model bestaande uit 61 variabelen de beste voorspellende waarde overall (Nagelkerke R^2 0,301), terwijl een model bestaande uit 52 variabelen de beste voorspellende waarde had bij ambulante starters (Nagelkerke R^2 0,676).
De kans op een niet-succesvolle clozapinebehandeling na 1 jaar was groter bij patiënten die poliklinisch met clozapine begonnen. Echter, het aantal variabelen dat nodig was om maar liefst 30% of 68% van de variabiliteit in niet-succesvolle clozapinebehandeling te kunnen voorspellen was te groot voor een eenvoudig te gebruiken predictiemodel.

Momenteel is ongeveer een derde van de Nederlandse clozapinegebruikers vrouw, waaronder vrouwen in de vruchtbare leeftijd. Aangezien psychiaters de laatste jaren minder terughoudend lijken te zijn met het voorschrijven van clozapine, is de verwachting dat er op termijn meer vrouwen die clozapine gebruiken zwanger worden. Veiligheidsgegevens over clozapinegebruik tijdens de zwangerschap zijn beperkt, maar zwangerschap is genoemd als motief voor het staken van clozapine. Bovendien bestaat er terughoudendheid om clozapine voort te zetten bij vrouwen met TRS die zwanger zouden kunnen worden. In **hoofdstuk 3** is de veiligheid van het gebruik van clozapine rondom de zwangerschap onderzocht. In **hoofdstuk 3.1** is allereerst vastgesteld of er disproportionaliteit bestaat in 'case safety reports' over ongunstige zwangerschapsuitkomsten gerelateerd aan clozapine gebruik en het gebruik van andere antipsychotica (OAP). In deze studie werden alle meldingen van

vermoedelijke bijwerkingen (ADR's) van antipsychotica geïncludeerd die zijn geregistreerd in de wereldwijde 'individuele case safety report' (ICSR) database van de Wereldgezondheidsorganisatie (VigiBase) bij kinderen jonger dan 2 jaar en vrouwen van 12-45 jaar. Een case/non-case benadering werd gebruikt om de associatie tussen verschillende zwangerschapsgerelateerde bijwerkingen en clozapineblootstelling tijdens de zwangerschap te evalueren, met behulp van 2x2 contingency-tabellen om disproportionaliteit te onderzoeken en Standard MedDRA Queries om cases te selecteren. Blootstelling aan clozapine werd gedefinieerd als alle ICSR-ADR-combinaties met clozapine als (een van) de 'verdachte' geneesmiddel(en). Niet-blootstelling werd gedefinieerd als alle ICSR-ADR-combinaties met OAP als (een van) de 'verdachte' geneesmiddel(en). In totaal werden 42.236 unieke ICSR-ADR-combinaties geïdentificeerd met blootstelling aan clozapine en 170.710 met blootstelling aan OAP. Hiervan waren 494 en 4.645 ICSR-ADR-combinaties gerelateerd aan ongunstige zwangerschapsuitkomsten door blootstelling aan respectievelijk clozapine en OAP. Er werd geen disproportionele rapportage gevonden van de onderzochte ongunstige zwangerschapsuitkomsten gerelateerd aan clozapine. Op basis hiervan hebben wij geen aanwijzingen gevonden dat clozapine gebruik minder veilig is tijdens de zwangerschap dan gebruik van OAP. Echter, dit betekent niet automatisch dat clozapine gebruik tijdens de zwangerschap veilig is.

Hoofdstuk 3.2 geeft een kritische beoordeling van de literatuur met betrekking tot de veiligheid van clozapine gebruik tijdens zwangerschap en borstvoeding. Pubmed/Medline, Embase en de Cochrane Library werden doorzocht vanaf het begin tot december 2020. Referentielijsten van geïncludeerde studies werden met de hand doorzocht. Het International Clinical Trials Registry Platform en ClinicalTrials.gov werden doorzocht op ongepubliceerde trials en PROSPERO op ongepubliceerde reviews. Er werd ook contact opgenomen met de huidige vergunninghouder van de oorspronkelijke merken, Clozaril® en Leponex®, voor geneesmiddelenbewakingsgegevens. Originele, in het Engels, Duits, Frans of Nederlands gepubliceerde onderzoeken met klinische en preklinische gegevens werden geïncludeerd indien zij gegevens bevatten over maternale, foetale of neonatale uitkomsten na blootstelling aan clozapine tijdens de zwangerschap of borstvoeding. Twee beoordelaars extraheerden onafhankelijk van elkaar de relevante gegevens.

In totaal werden 860 records geïdentificeerd en van 117 artikelen werd de volledige tekst beoordeeld. Tweeënveertig studies voldeden aan de inclusiecriteria. Gegevens over perinatale blootstelling aan clozapine zijn van beperkte kwaliteit en kwantiteit. Hoewel clozapine een gedeeltelijke placentapassage vertoont, wijzen de beschikbare gegevens er tot nu toe niet op dat clozapine teratogeen is, het risico op doodgeboorte, abortus of foetale aandoeningen verhoogt, noch dat het het risico op bevallingscomplicaties of vroeggeboorte verhoogt. Informatie over blootstelling aan clozapine via de

moedermelk is schaars, maar op grond van de chemische eigenschappen is het waarschijnlijk dat clozapine in de moedermelk terechtkomt. Bij het afwegen van de risico's en voordelen van voortzetting van clozapine tijdens de zwangerschap en borstvoeding versus overschakeling op een ander antipsychoticum dient men rekening te houden met de ernst van de ziekte en de voorgeschiedenis, maar ook met de beperkingen van de beschikbare veiligheidsgegevens over perinataal clozapinegebruik, waaronder het feit dat er weinig studies zijn.

Met het oog op een betere therapietrouw en minder sedatie overdag geven clinici steeds vaker de voorkeur aan een eenmaal daags (QD) boven een verdeeld doseringsschema voor clozapine. In **hoofdstuk 4.1.** is in het kader van de INPUT studie (INfluence of dose interval on the Pharmacokinetics of both Unbound and Total fractions of clozapine and norclozapine in psychiatric patients in the Netherlands) bepaald of de farmacokinetiek van clozapine en norclozapine bij QD gebruik vergelijkbaar is met tweemaal daags (BID) gebruik. Daarnaast werd het effect van QD-dosering op de concentratie-tijdcurven van clozapine en norclozapine bestudeerd door middel van simulaties.
Meerdere bloedmonsters werden verzameld binnen een doseringsinterval van patiënten die clozapine QD of BID in steady state gebruikten. Clozapine- en norclozapineconcentraties werden geanalyseerd met een gevalideerde LC-MS/MS-methode. Populatie farmacokinetische (PPK) analyses werden uitgevoerd door middel van niet-lineaire mixed-effects modellering. De invloed van het doseringsschema werd getest als covariaat.
Vierenveertig patiënten (319 monsters) werden geïncludeerd. Voor clozapine was het beste structurele model een twee-compartimentenmodel met een transitcompartiment en lineaire eliminatie bestaande uit gecombineerde excretie en vorming van norclozapine. Voor norclozapine beschreef een één-compartimentmodel met eerste-orde-eliminatie de gegevens het best. Het doseringsschema had geen invloed op de farmacokinetische parameters. Uit de simulaties blijkt dat de clozapineconcentraties die 's ochtends bij QD-gebruik worden bemonsterd, duidelijk verschillen van de dalconcentraties en hoger zijn dan wanneer dezelfde dagelijkse dosis in twee verdeelde doses zou zijn gebruikt. De relatieve hoeveelheden clozapine ten opzichte van norclozapine variëren ook sterker dan bij BID-dosering. Wij concluderen derhalve dat de farmacokinetiek van clozapine en norclozapine niet wordt beïnvloed door het doseringsschema. Wel worden clozapineconcentraties bij een bepaalde totale dagdosis en de metabole ratio beïnvloed door het doseringsschema en het tijdstip van bloedafname. Wat dit betekent voor het therapeutische venster van clozapine bij QD-dosering is nog niet bekend.
In **hoofdstuk 4.2** is het effect van (hogere) QD-dosering op ongebonden clozapine- en norclozapineconcentraties onderzocht. Clozapine en norclozapine zijn sterk eiwitgebonden in plasma, voornamelijk aan alfa-1 zuur glycoproteïne

(AGP), en theoretisch kunnen hogere (QD) doseringen leiden tot verzadiging van de eiwitbinding van clozapine en norclozapine, waardoor de ongebonden fracties toenemen. Wij onderzochten of de eiwitbinding van clozapine en norclozapine verzadigd raakt bij hogere concentraties bij QD doseren of bij het bereiken van piekconcentraties. Ten tweede onderzochten we de correlatie tussen ongebonden clozapine en norclozapine fracties en AGP-concentraties. Ongebonden en totale clozapine- en norclozapineconcentraties werden gemeten in de 319 bloedmonsters die op verschillende tijdstippen werden afgenomen bij vierenveertig patiënten die clozapine QD of BID gebruikten in het kader van bovengenoemde INPUT-studie. Daarnaast werden AGP-concentraties gemeten in monsters die vlak voor de inname van clozapine werden afgenomen. De relatie tussen totale en ongebonden concentraties en fracties, en tussen ongebonden fracties en AGP-concentraties werd onderzocht met behulp van univariate lineaire regressieanalyse. Het effect van het doseringsschema op de eiwitbinding werd beoordeeld met behulp van multivariate lineaire regressieanalyse en gesplitste ANOVA-analyse.
De clozapine- en norclozapineconcentraties correleerden goed met de ongebonden concentraties (respectievelijk R^2 = 0,87, p <0,001 en R^2 =0,73, p <0,001) binnen het bestudeerde concentratiebereik. Het doseringsschema had geen invloed op de relatie tussen de totale clozapine- en norclozapineconcentraties en de ongebonden fracties ervan. Er werd een matige (clozapine) en een kleine (norclozapine) correlatie gevonden tussen AGP-concentraties en ongebonden fracties van clozapine (R^2=0,15, p<0,001) en norclozapine (R^2=0,055, p<0,001).
Bij clozapineconcentraties tot 1500 µg/L werd dus geen onevenredige toename van ongebonden concentraties gevonden. De totale concentraties blijven in het algemeen geschikt voor TDM bij QD doseren van clozapine. Echter, incidentele abnormale ongebonden fracties, zelfs in afwezigheid van infectie, kunnen mogelijk de concentratie-effect relatie van clozapine wijzigen.
Om voor het in hoofdstuk 4.2 gepresenteerde onderzoek de ongebonden clozapine- en norclozapineconcentraties te kunnen meten, is een methode ontwikkeld voor kwantificering van de ongebonden concentraties van clozapine en norclozapine in serum. In **hoofdstuk 4.3** worden de hindernissen beschreven die genomen moeten worden bij het ontwikkelen van een dergelijke methode, met behulp van ultrafiltratie (UF).
Ten eerste werd de ondergrens (LOQ) van een bestaande LC-MS/MS-methode voor de analyse van clozapine en norclozapine in serum verlaagd. Om te testen op niet-specifieke adsorptie aan het filter werd de methode 'ge-cross-gevalideerd' voor gebruik in water. Vijf verschillende UF-filters, met en zonder voorbehandeling van de filtermembranen, werden getest om het beste filter te kiezen en de 'recorvery' in gezuiverd water te optimaliseren. Ten slotte werd de totale procedure toegepast in serum.

De kalibratiecurven bleven lineair over het uitgebreide concentratiebereik van 1-1000 µg/L met aanvaardbare regressiecoëfficiënten, variatiecoëfficiënten binnen en tussen reeksen (CV's) (<15%), alsmede parameters voor crossvalidatie en matrixeffect.
Het 30KDa UF-filter van Merck zonder voorbehandeling leverde de hoogste recovery op in gezuiverd water, die stabiel was (13,6-15,6%) binnen een bereik van 5 - 50 µg/L vóór UF. De vrije fracties in serum bleven constant (2,35-2,45% en 3,28-3,61% voor respectievelijk clozapine en norclozapine) over een representatief concentratiebereik voor werkelijke totale clozapine- en norclozapineconcentraties. De CV's binnen de reeks, tussen de reeksen en de totale CV's van de ijkreeksen waren acceptabel, behalve de totale CV van de vrije norclozapineconcentraties.
Deze studie toont aan dat de keuze van het ultrafiltratiefilter een cruciaal onderdeel is van de ontwikkeling van een methode om ongebonden concentraties te meten met behulp van UF. Adequate controle van de factoren die de NSA beïnvloeden is essentieel, evenals van factoren die de stabiliteit van het eiwit-geneesmiddelcomplex beïnvloeden.
In **hoofdstuk 4.4** is beoordeeld of clozapineconcentraties gemeten in plasma dat is verzameld met bloedbuizen die ethyleendiaminetetraaceticum (EDTA) en lithium-heparine (LH) als anticoagulans bevatten, vergelijkbaar zijn met clozapineconcentraties gemeten in serum. Clozapinespiegels worden op grote schaal gemeten in zowel plasma als serum, maar de kennis over de uitwisselbaarheid van deze matrices is beperkt en tegenstrijdig.
Gepaarde residuele EDTA- en LH-plasma- en routineserummonsters werden verzameld voor analyse van clozapineconcentraties. Clozapineconcentraties in serum werden met behulp van lineaire regressie vergeleken met de concentraties in EDTA- en LH-plasma. Voor de klinische interpretatie werden clozapineconcentraties gemeten in de drie verschillende matrices ingedeeld in drie categorieën: subtherapeutisch (< 300 µg/L), therapeutisch (300 - 700 µg/L) en supratherapeutisch (> 700 µg/L). Verschillen in klinische interpretatie tussen clozapineconcentraties gemeten in de twee plasmamatrices en serum werden beoordeeld.
Vergeleken met de serummonsters (n=36) waren de clozapinespiegels gemeten in LH- plasmamonsters (n=19) gemiddeld 150 µg/L lager (95% CI [-200; -110]). Clozapinespiegels gemeten in EDTA-plasmamonsters (n=35) waren gemiddeld 20 µg/L hoger (95% CI [-10; 50]). In 37% van de LH-monsters verschilde de klinische interpretatie van de interpretatie op basis van de clozapinespiegel in serum, terwijl er een hoge mate van overeenstemming was tussen serum- en EDTA-plasmaconcentraties (97%).
Het gebruik van LH- plasmamonsters voor TDM van clozapine kan leiden tot andere klinische interpretaties dan bij gebruik van serummonsters. Daarom

wordt het gebruik van LH-plasmamonsters en serummonsters voor het meten van clozapinespiegels in de klinische praktijk niet aanbevolen.

In **hoofdstuk 5** zijn de bevindingen van dit proefschrift in een breder perspectief geplaatst. Gezien de plaats van clozapine in het behandelingsalgoritme is het zorgwekkend dat naar schatting slechts de helft van de mensen met TRS naar verwachting adequaat zal reageren op clozapine en dat het stoppercentage hoog is. Wij vonden dat bij 20,4% van de patiënten clozapinebehandeling niet succesvol was één jaar nadat clozapine voor het eerst was verstrekt door een openbare apotheek. Discontinueren kon niet gemakkelijk worden voorspeld met een beperkt aantal variabelen afkomstig uit een onderzoeksdatabank met verzekeringsgegevens, wat wijst op multifactoriële oorzaken en invloed van variabelen die niet konden worden afgeleid uit de informatie in de databank.
Zwangerschap of de wens om zwanger te worden mag geen reden zijn om te stoppen met clozapine, tenzij na zorgvuldige afweging van kosten en baten.
Hoewel wij vonden dat de farmacokinetiek van clozapine en norclozapine vergelijkbaar is bij QD- en BID-dosering, is prospectief onderzoek nodig om het therapeutische venster bij QD-dosering van clozapine te bepalen. Ook is verder onderzoek nodig naar de variatie in ongebonden clozapine- en norclozapineconcentraties en de klinische impact daarvan.
Ten slotte toont dit proefschrift aan dat er behoefte is aan verbetering van de standaardisatie en transparantie in de rapportage van doseringsschema's, afnametijden en bloedafnamebuizen in studies over de concentratie-effect relaties van clozapine en norclozapine. Onderzoekers en beoordelaars van wetenschappelijke tijdschriften, alsmede artsen moeten zich bewust zijn van het belang van dit onderwerp bij de interpretatie van clozapine- en norclozapineconcentraties voor TDM. Bovendien is deze standaardisatie en transparantie noodzakelijk om de meerwaarde van TDM ten volle te benutten.

7

Appendices

7.1

Dankwoord

En dan is er 'opeens', na 8 jaar, een einde gekomen aan deze reis: een leerzaam avontuur waar ik met veel plezier aan terugdenk. Ik had verwacht een gevoel van euforie te ervaren, maar het is beter te vergelijken met het gevoel na thuiskomst van een mooie reis: moe, maar voldaan, vol ervaringen en herinneringen die langzaam een plaatsje gaan krijgen en alvast voorzichtig plannen makend voor de volgende reis.
Ik schrijf dit dankwoord terwijl m'n gedachten teruggaan naar hoe het begon. De route was bij tijd en wijle, en zeker in de eerste jaren, uitdagend. Privé liep deze reis namelijk volledig parallel met de basisschooltijd van onze oudste dochter, terwijl onze jongste dochter de eerste 2 jaar nog niet naar school ging. Ook doorkruiste de coronapandemie deze reis. Dit heeft de route beïnvloed, maar de reis niet gestopt.
Soms geldt dat de route belangrijker is dan de eindbestemming. Voor mij was de route in ieder geval net zo belangrijk als de eindbestemming, niet in het minst door alle mensen die ik op m'n route ben tegengekomen. Ik wil dan ook iedereen die hieronder niet met name wordt genoemd, maar op enigerlei wijze bij heeft gedragen aan de totstandkoming van dit proefschrift bedanken voor hun inbreng.

Een aantal mensen wil ik in het bijzonder bedanken.

Allereerst mijn promotieteam; Rob, Rob en Arthur. Ik heb me geregeld gelukkig geprezen met jullie begeleiding. We waren een goed en gevarieerd team, waarin jullie elkaar aanvulden, elk met jullie eigen kennis, expertises en karakters, en mij begeleidden vanuit gelijkwaardigheid. Onze overleggen vonden de eerste jaren plaats bij Rob (Heerdink) op het David de Wiedgebouw op de Uithof in Utrecht, maar later voornamelijk online. De laatste tijd zagen we elkaar niet vaak, maar we spraken elkaar op de juiste momenten.

Beste Rob (van Marum), als mijn promotor bewaakte jij de grote lijnen, floot je me soms terug als ik te veel wilde of als ik vond dat het niet snel genoeg ging. Ook wist je met je feedback op m'n manuscripten de inhoud aan te scherpen en leerde je me korter en bondiger te zijn (iets wat niet blijkt uit dit dankwoord …). Zo nu en dan kwam je op vrijdagochtend bij me langs voor een overleg in het Albert Schweitzer ziekenhuis in Dordrecht. Steevast was je eerste vraag: "Hoe gaat het? Vind je het nog leuk?". Een ogenschijnlijk simpele, maar belangrijke vraag. Onderzoek doen is immers niet altijd 'leuk' en vereist flexibiliteit, optimisme en geregeld ook incassatievermogen. Mede dankzij de wijze waarop jij dit promotietraject hebt begeleid, heb ik deze vraag altijd met 'ja' beantwoord. Onze samenwerking heb ik te danken aan mijn oud-opleider Ed de Vogel. Jullie kenden elkaar uit jouw opleidingstijd in het Albert Schweitzer ziekenhuis en waren elkaar tegen het lijf gelopen op de dijk in Sliedrecht, nabij jouw huis, waar

Ed 'toevallig' aan de wandel was. Ed legde zijn ideeën omtrent een nog op te zetten onderzoekslijn en daaraan te koppelen promotieonderzoek aan je voor, vervolgens hadden wij een maand later ons eerste kennismakingsgesprek en zo geschiedde. Bedankt dat je toentertijd deze onverwachte uitdaging aan wilde gaan met mij als 'promovendus op afstand'!

Beste Rob (Heerdink), onze eerste kennismaking was op de dag dat Rob (van Marum) zijn inaugurele reden als bijzonder hoogleraar uitsprak aan de VU. Een paar dagen later zat ik al met jou om de tafel om concrete plannen te maken. Jij zorgde ervoor dat ik op donderdagen welkom was bij jou op de afdeling om daar aan m'n onderzoek te werken. De onderzoeken uit hoofdstuk 2.1 en 3.1 zijn geworden tot wat ze zijn, dankzij jouw ijzersterke methodologische kennis. Bij elk overleg dat ik met je had, wist je direct inhoudelijk aan te haken bij het punt waar ik ondertussen was aanbeland en me zo nodig weer op het juiste spoor te zetten. Ook jij hebt me begeleid zoals elke promovendus, denk ik, begeleid zou willen worden; altijd constructief en met een vleugje humor. Dankjewel!

Beste Arthur, wij kenden elkaar al voordat dit avontuur begon. Jij was toentertijd directeur van de Yulius Academie en waarnemend A-opleider van Yulius en ik was net als ziekenhuisapotheker verantwoordelijk geworden voor de farmaceutische zorg aan de klinische afdelingen van Yulius. Maar ik heb je pas echt leren kennen toen ook jij mijn copromotor werd. Je hebt oog voor alle en ieders verschillende belangen en was binnen ons team de alfa onder de bèta's. In jou moet ik mijn meerdere erkennen als het om purisme gaat. Ik heb genoten van de opbouwend-kritische manier waarop je soms vraagtekens zette bij bepaalde woorden of zinnen en suggesties voor verbetering deed. Jij was altijd consciëntieus en de eerste die een stuk van commentaar wist te voorzien. Ook jou wil ik heel erg bedanken voor je betrokkenheid en begeleiding.

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Beste Ed, lieve Hetty, mijn oud-opleiders en later collega-ziekenhuisapothekers, dit promotietraject was er zonder jullie niet gekomen. In 2007 hebben jullie mij aangenomen als ziekenhuisapotheker in opleiding. Ik ben jullie hier nog steeds dankbaar voor. In de jaren daarna heb ik onbeschrijflijk veel van jullie geleerd. Meer nog dan ik me waarschijnlijk bewust van ben. Ed, jouw brein borrelde altijd al van de ideeën en hoe kan het ook anders dan dat jij met het idee kwam om onderzoek te doen naar clozapine. Hetty, jij was degene die inzag dat dit traject alleen kon slagen als ik een dag in de week niet in de apotheek zou zijn. Die vier jaar die je voor ogen had, zijn alleen wel verdubbeld ;) Inmiddels genieten jullie

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7.2

List of publications

Publications related to this thesis

Beex-Oosterhuis MM, Heerdink ERR, Van Gool AR, van Marum RJ. Predicting Unsuccessful Clozapine Treatment After First Use in Adult Patients With Psychotic Disorders. J Clin Psychopharmacol. 2018 Dec;38(6):604-608.

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7.3

About the author

Marieke Beex-Oosterhuis was born in Dongen, The Netherlands on April 27th, 1980. She attented her secondary school at the Sint Oelbert Gymnasium in Oosterhout, where she received her gymnasium diploma in 1998. Afterwards she started studying Pharmaceutical Sciences at Utrecht University.

From 2001 to 2002, she was a member of the board of the study association "Unitas Pharmaceuticorum". In 2003 she obtained her doctoral exam, followed by her Master's degree in 2005. As part of her doctoral program, she worked on a research project investigating prescription of potentially inappropriate medications to older people (supervisor prof.dr. A.C.G. Egberts) at the hospital pharmacy of the then "TweeSteden ziekenhuis" (currently " Elisabeth-TweeSteden Ziekenhuis") in Tilburg. In summer 2005, she went to Ghana for 2 months for an internship in Accra, Agroyesum (St. Martin's Hospital) and Tamale.

After graduation, she worked in the hospital pharmacy of the Zuwe Hofpoort ziekenhuis in Woerden from 2005-2006 and from 2006-2007 in the hospital pharmacy of the Leiden University Medical Centre in Leiden.

In May 2007, she started her hospital pharmacy residency at the Albert Schweitzer hospital in Dordrecht (supervisors drs. H.G. Dieleman and drs. E.M. de Vogel). As part of this residency, she performed a research project on detection and correct handling of prescribing errors in Dutch hospital pharmacies for which she received the first price for best research project of her year (supervisors prof.dr. P.M.L.A. van den Bemt, drs. E.M. de Vogel).

After finishing her residency in June 2011, she continued her career as a hospital pharmacist at the hospital pharmacy of the Albert Schweitzer hospital where she is still holding a position. By the end of 2014 she started her PhD research project under supervision of prof.dr. R.J. van Marum, dr. E.R. Heerdink and dr. A.R. Van Gool. The results of this project are described in this thesis.

Marieke Beex-Oosterhuis is married to Jeroen Beex and they have two daughters, Floor (December 2010) and Frederique (January 2013).