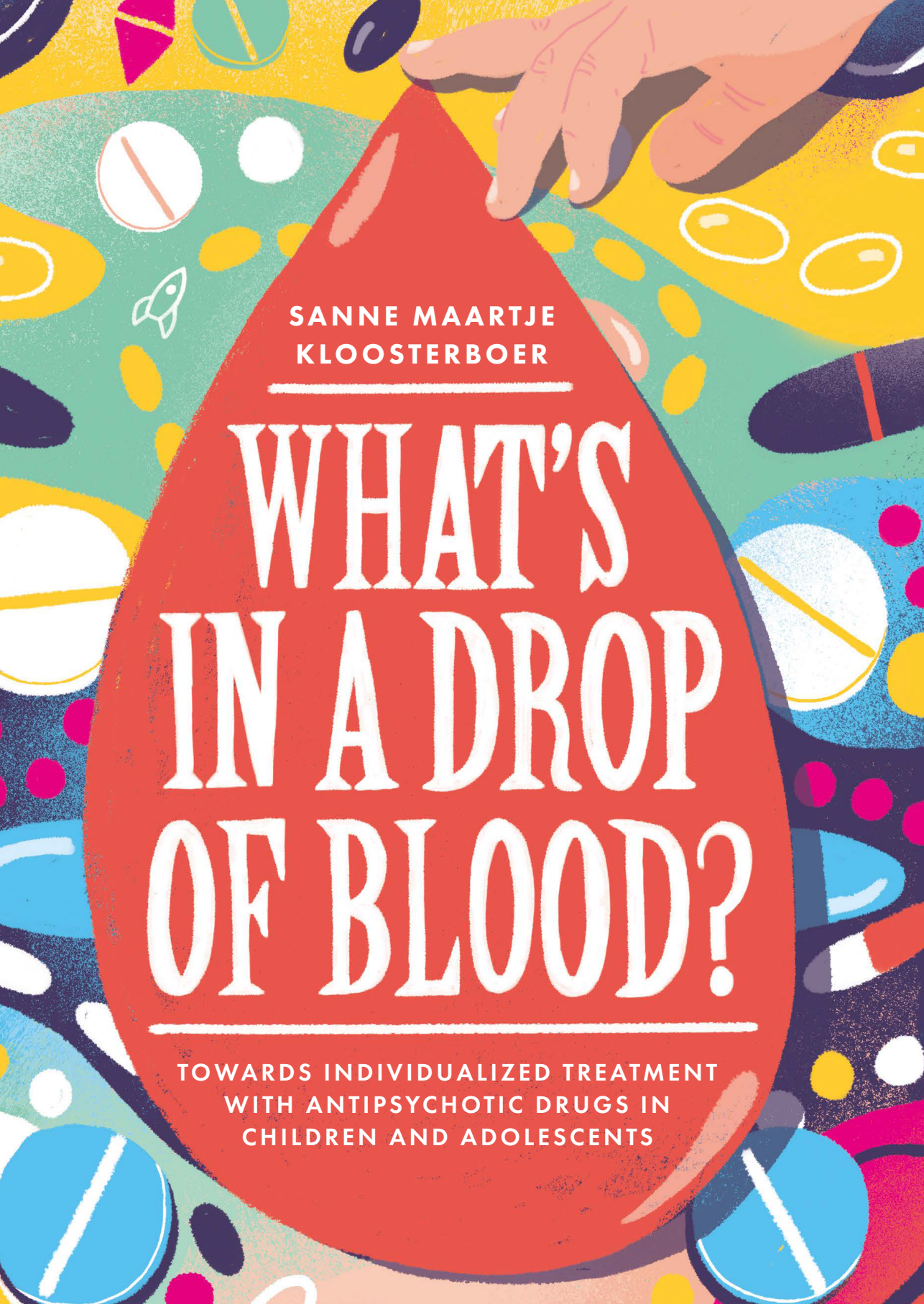
SANNE MAARTJE
KLOOSTERBOER
WHAT'S
IN A DROP
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TOWARDS INDIVIDUALIZED TREATMENT
WITH ANTIPSYCHOTIC DRUGS IN
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Sanne Maartje Kloosterboer

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WAT ZEGT EEN DRUPPEL BLOED?

OP WEG NAAR GEÏNDIVIDUALISEERDE BEHANDELING MET ANTIPSYCHOTICA BIJ KINDEREN EN ADOLESCENTEN

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. F.A. van der Duijn Schouten

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 20 januari 2021 om 13.30 uur

door

Sanne Maartje Kloosterboer
geboren te Leeuwarden.

ISBN	978-94-6423-052-9
Cover design	Iris Deppe
Layout	Ruben Lentz
Printed by	Proefschriftmaken.nl

The studies described in this thesis were financially supported by the Netherlands Organization for Health Research and Development (ZonMW), grant number 836041011. A travel grant was received from the Erasmus MC Trustfonds. Printing of this thesis was financially supported by ChipSoft and Medicidesk Rabobank Rotterdam.

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Copromotoren:	dr. B.C.P. Koch
	dr. B. Dierckx

CONTENTS

PART I

BACKGROUND

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Bart is a 9 year old boy diagnosed with Autism Spectrum Disorder. He is very curious and has a strong love for trains: his own miniature copy goes with him all the time. Routine activities are very important to Bart. As long as his schedule is not abruptly changed, he is doing fine.

However, when something unexpected occurs, Bart gets heavily irritated. When, for example, he loses a game at school or the radio in the supermarket is playing too loud, he can get very frustrated. He then bangs his own head against the wall, or can kick or bite other children and his mother. This occurs several times a week, leading to serious injuries.

The family has sought help from a social worker and a psychologist, who have intensively supported Bart, his family and school. Bart has received cognitive behavioral therapy and his parents have received parental training, but this has brought insufficient relief. His school has said that the situation is untenable and they can no longer handle Bart. Also his family is under severe pressure.

As illustrated in Bart's story, emotional and behavioral problems associated with Autism Spectrum Disorder (ASD) can deeply affect a child and his environment. When supported ineffectively, these are likely to have profound and lifelong effects on psychosocial development, leading to school drop-out, social isolation, and decreased self-sustainability.[1] Additional pharmacological treatment is deemed necessary when psychological and behavioral interventions are insufficient. Antipsychotic drugs are the first choice in the pharmacological treatment of severe behavioral problems associated with ASD in children and adolescents. But also for other psychiatric disorders in youths, such as Bipolar Disorder, Conduct Disorder or Psychotic Disorder, antipsychotic drugs play an important part in multimodal treatment.

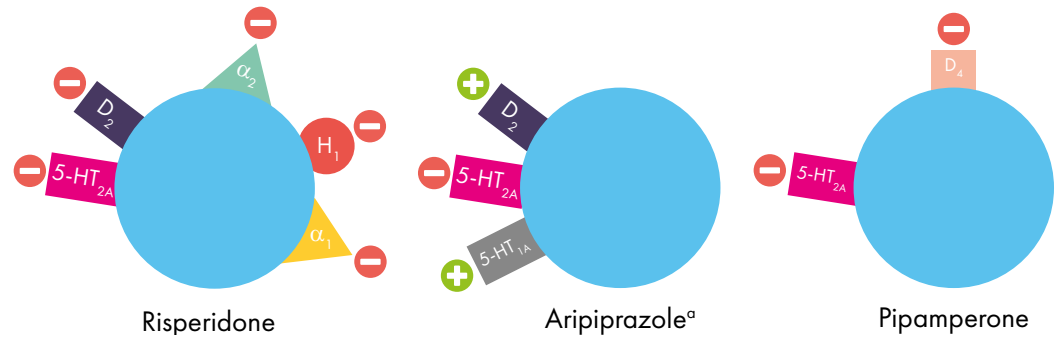
In this first chapter, the rationale and indications for antipsychotic use in children and adolescents are discussed, the pharmacokinetic and pharmacodynamic aspects are introduced, and the main aims of this thesis are presented.

ANTIPSYCHOTIC DRUGS - MECHANISM OF ACTION

Antipsychotic drugs, discovered in the 1950s, reduce dopaminergic neurotransmission in the brain. Dopamine plays an important role in several cerebral pathways, including the mesolimbic pathway which is believed to be associated with aggression, mania and psychosis.

Atypical or second-generation antipsychotic drugs were introduced in the 1980s. Typically, these drugs additionally block a wide range of other neurotransmitter receptors, including serotonin, acetylcholine, histamine and noradrenaline receptors. The difference between typical and atypical antipsychotic drugs is however arbitrary, as their pharmacological properties largely overlap.[2] The neuroreceptor affinities of three commonly used antipsychotic drugs in youths in the Netherlands (risperidone, aripiprazole and pipamperone) are shown in **figure 1**.

Figure 1 Binding affinities to neuroreceptors of risperidone, aripiprazole and pipamperone.



Only the strongest receptor affinities are shown. Risperidone and aripiprazole are regarded as second-generation antipsychotic drugs, and pipamperone as a first-generation antipsychotic drug.
[a] aripiprazole is a partial agonist for the D_2 and 5-HT_{2a} receptor. This means aripiprazole does not block, but weakly stimulates these receptors. Adapted from Correll 2008[3] and Schotte et al. 1996[2].

EFFICACY AND INDICATIONS

Numerous randomized controlled trials support the efficacy of antipsychotic drugs in children and adolescents with ASD, schizophrenia, bipolar disorder, disruptive behavior disorders and Tourette's disorder.[4] As a result, antipsychotic drugs have been taken up widely in treatment recommendations of national and international guidelines and their use in children and adolescents is widespread.[5-7]

The most common indication for antipsychotic drug prescriptions in children and adolescents in the Netherlands is behavioral problems associated with ASD, such as it is the case for Bart. ASD is characterized by impairments in social interaction, verbal and non-verbal communication, as well as by stereotypical patterns of behavior and interests.[8] The disorder is quite common, with 0.6 to 1 per 100 persons being diagnosed with ASD.[9] Strikingly, more than half of children and adolescents with ASD have serious behavioral problems.[10]

The short-term efficacy for the treatment of behavioral problems associated with ASD is relatively well studied for risperidone and aripiprazole. The general number needed to treat for this indication is two for risperidone, and three for aripiprazole[11], which reflects a very good efficacy. Pipamperone takes a special place as the evidence for its efficacy in children and adolescents is limited due to lack of research[12], but there is extensive practical experience with pipamperone, especially in children and adolescents with cognitive impairment. Mainly because of the calming effects and the availability of oral liquid dosage formulation that enables flexible dosing, pipamperone is often prescribed to children in the Netherlands, Belgium and Germany.

Despite the growing evidence for efficacy, most antipsychotic drugs are used off-label in children and adolescents, thus outside the registered indication and duration of use.[13] The registration status of risperidone, aripiprazole and pipamperone for children and adolescents in the Netherlands is shown in **table 1**.

Table 1 Registrations of the three most commonly used antipsychotic drugs in children and adolescents in the Netherlands.

Antipsychotic drug	Registration
Risperidone	Short-term treatment (up to 6 weeks) of persistent aggression in children with cognitive impairment (of at least 5 years) and young adults with a behavioral disorder.
Aripiprazole	Schizophrenia in young people aged 15 years and older. Manic episodes in bipolar I disorder in adolescents aged 13 years and older, with a maximum prescription duration of 12 weeks.
Pipamperone	Not registered for use in children and adolescents.

Retrieved from www.geneesmiddeleninformatiebank.nl

ADVERSE EFFECTS

Along with an increased popularity of antipsychotic drugs in children and adolescents, concerns have been raised in past decades about the safety in this population. Children and adolescents appeared to be prone for serious side effects, including weight gain, metabolic abnormalities, prolactin elevation, extrapyramidal symptoms, sedation and cardiac abnormalities.[3] These are the result of the broad receptor affinities of antipsychotic drugs, and involve serious short-term and long-term health risks.

Weight gain

The most important side effect concerns weight gain, with children gaining several kilograms during the first weeks of antipsychotic treatment. Antipsychotic-induced weight gain is more pronounced in youths than in adults[14], and the propensity for weight gain differs among the different antipsychotics. Olanzapine and clozapine cause most weight gain in youths, followed by risperidone, pipamperone and aripiprazole.[15] Although weight gain is heterogeneous among children and adolescents starting antipsychotic drug treatment[16], it is not well known which children and adolescents are particularly at risk.

Metabolic abnormalities

Weight gain and obesity increase the risk for metabolic abnormalities, including lipid and glucose disturbances and insulin resistance.[17] Additionally, antipsychotic drugs induce metabolic disturbances independently of weight gain.[16] Lipid and metabolic parameters increase significantly within the first weeks of antipsychotic treatment[18]. After long-term risperidone treatment, 35% of children and adolescents have at least one criterion of the metabolic syndrome phenotype.[19]

Diabetes and cardiovascular diseases

Weight gain, glucose disturbances and insulin resistance lead to a threefold increased risk of diabetes mellitus during antipsychotic drug use in children and adolescents, even within the first year of use.[20] Lipid disturbances also significantly increase the risk for cardiovascular diseases later in life, leading to significant morbidity.[21]

Extrapyramidal side effects

Antipsychotic drugs induce extrapyramidal symptoms. During long term antipsychotic treatment, up to one in three youths experience mild to moderate EPS.[22] Worldwide, extrapyramidal symptoms are the most reported adverse drug reaction of antipsychotic drugs in children and adolescents.[23]

Prolactin

More than the other antipsychotic drugs, risperidone induces substantial prolactin elevations, possibly leading to gynecomastia, galactorrhea, sexual dysfunction and irregular menses.[24] The long term risks of antipsychotic-induced prolactin elevations are not well studied, but may induce osteoporosis even in children and adolescents.

Sedation

Sedation is common in children and adolescents, especially during the first weeks of antipsychotic treatment. Children and adolescents demonstrate more antipsychotic-induced sedation than adults.[25]

Cardiac abnormalities

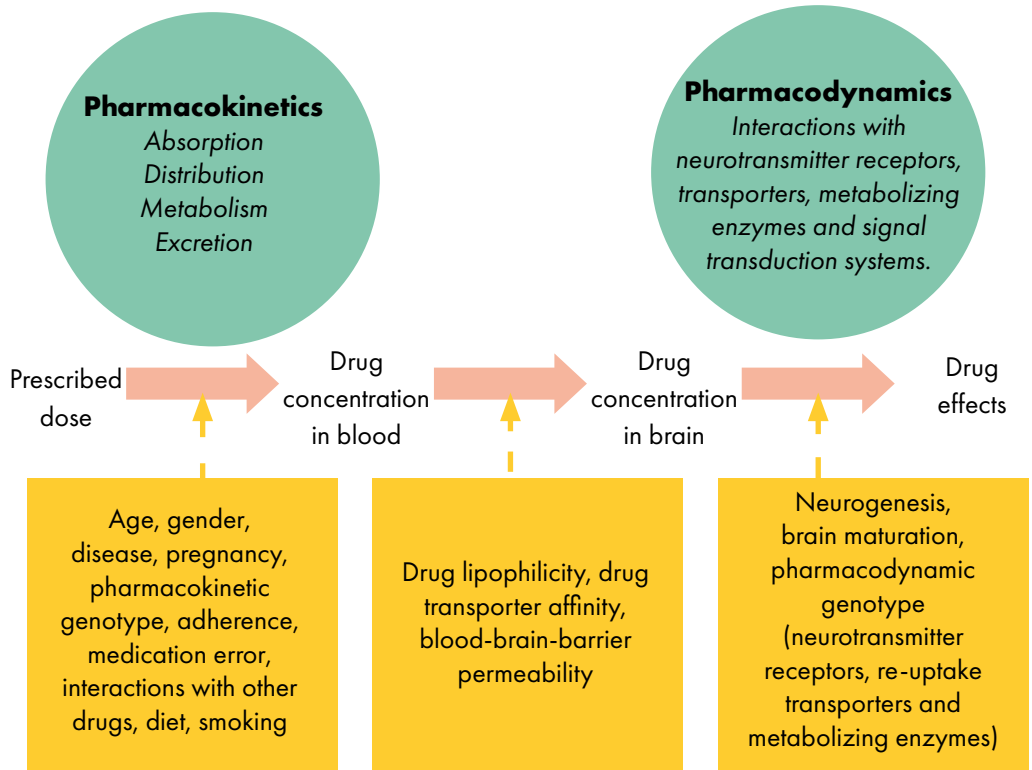
Antipsychotic drugs can increase the corrected QT (QTc) interval, risking cardiac arrhythmias.[26] In children and adolescents, the risk of pathological QTc prolongation seems rare, but should be considered when other risk factors are present.[27]

PHARMACOKINETIC AND PHARMACODYNAMIC ASPECTS

Both the therapeutic and adverse effects of antipsychotic drugs are the result of *pharmacokinetic* and *pharmacodynamic* processes in the body. Pharmacokinetics determine the drug exposure in the body by absorption, distribution, metabolism and excretion after drug administration. Pharmacodynamics describe the subsequent drug effects through the interaction with the target receptors. Many factors can influence both processes, as is shown in **figure 2**.

In childhood and adolescence, pharmacokinetics and pharmacodynamics alter rapidly. This is the result of changes in body composition, target receptor maturation and organ ripening during development[28], leading to a large variability in antipsychotic drug concentrations[29] and antipsychotic effects across the lifespan.[25] This may cause relative overdosing or underdosing of antipsychotic drugs in children and adolescents when dosages are simply extrapolated from adults.

Figure 2 Pharmacokinetic and pharmacodynamic processes and influencing factors after an administered dose.



Adapted from: AGNP guidelines for TDM 2017, Hiemke et al..[30]

THERAPEUTIC DRUG MONITORING

The relationship between pharmacokinetics, reflected in drug concentrations, and pharmacodynamics, reflected in clinical outcomes, can be used to improve the safety and efficacy of drug therapy. Therapeutic Drug Monitoring (TDM) uses the measurement of a drug concentration to titrate dosages towards the concentration range that is associated with maximal efficacy and minimal side effects, known as the therapeutic reference range.[30] TDM has proven to improve clinical outcomes of many psychotropic drugs, and is increasingly being used in adult psychiatry and other fields. The main reason for the increasing use of TDM, is that the relationship between the effects of a drug and its concentration in the blood is often better than between the dose of the drug and the observed effects.

However, the relationship between antipsychotic drug blood concentrations and clinical outcomes in children and adolescents is currently unknown. As a result, no therapeutic reference ranges for antipsychotic drugs in this population exists, hampering the use of TDM as a tool to improve safety in this population. Several studies have shown that higher antipsychotic dosages increase the risk of side effects in children and adolescents including prolactin elevation31, weight gain32, extrapyramidal symptoms22 and diabetes mellitus20, suggesting that a higher antipsychotic exposure is associated with more side effects. However, within these studies, analyses with dosages adjusted for bodyweight are generally lacking, and therefore do not inform about the exposure-response relationship. Only a few studies have assessed the relationship between risperidone blood concentrations and outcomes in children and adolescents, but these studies mainly focus on prolactin elevation and do not cover metabolic side effects.33-35 To our knowledge, no studies have been performed assessing the concentration-effect relationship in children and adolescents for aripiprazole and pipamperone. For these latter drugs, even the pediatric population pharmacokinetics are not well known.

ALTERNATIVE SAMPLING

The pharmacokinetic and pharmacodynamic research that is needed to establish a therapeutic reference range is however challenging in children using antipsychotic drugs. Bart, the boy that was introduced earlier, is already overwhelmed by simple things, let alone by a venipuncture for a drug concentration measurement. Blood sampling is often complicated due to anxiety, restlessness or aggression in children with psychiatric disorders, and therefore, alternative, minimally invasive sampling methods are highly important. A well-established alternative sampling method concerns the Dried Blood Spot (DBS) method.[36] This method can determine drug concentrations with only a fingerprick and a single drop of blood, which can be performed in the home-setting. DBS can overcome many sampling difficulties, and has therefore been suggested as a facilitator for pharmacokinetic research in pediatric populations.[37] However, although numerous DBS methods have been developed for psychotropic drug quantification, little attention have been paid to the accuracy and feasibility of DBS in clinical practice.[38] This hampers the implementation of these alternative sampling strategies for children and adolescents with psychiatric comorbidities. A

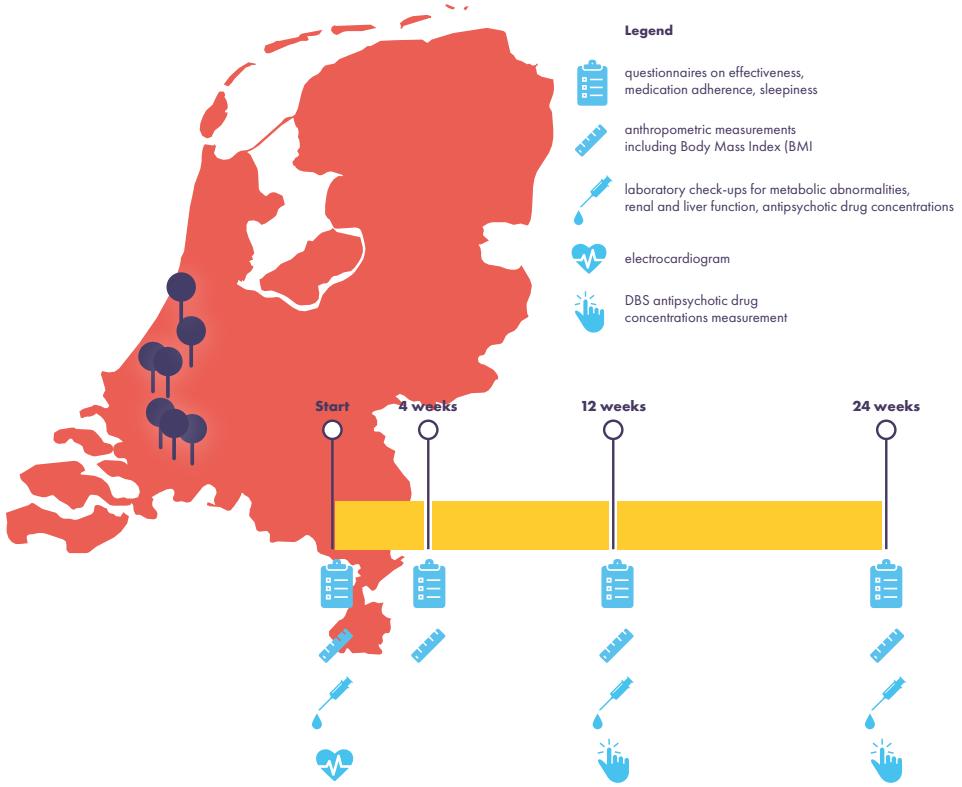
thorough clinically validated DBS method for the quantification of antipsychotic drugs in children and adolescents is therefore highly needed.

WHAT'S IN A DROP OF BLOOD?

Clarifying the relationship between pharmacokinetics and pharmacodynamics of antipsychotic drugs in children and adolescents is important, as it can provide an evidence-based basis for TDM in this population and allow us to individualize treatment to improve safety and efficacy.

To clarify this relationship, this thesis presents the results of the observational prospective cohort study SPACe, which has been performed in seven participating centers in the south-west region of the Netherlands between 2016 and 2019. Within this study, 89 children with ASD and severe behavioral problems who started with risperidone, aripiprazole or pipamperone, have been prospectively followed for 6 months. During the follow-up, both pharmacokinetic data obtained with DBS and pharmacodynamic data by measurements of side-effects and effectiveness were structurally collected (see **figure 3**).

Figure 3 Graphic illustration of study design of the SPACe study.



Participating centers were Erasmus MC, Curium-LUMC, GGZ Delfland, GGZ Breburg, Youz, Yulius and de Kroon kinderpsychiatrie.

RESEARCH QUESTIONS

The aim of this thesis is to improve the safety and effectiveness of antipsychotic drugs in children and adolescents, by individualizing antipsychotic drug dosing and monitoring. The studies presented in this thesis address the following questions:

Part II Current practice

- What is the extent of antipsychotic drug use by children and adolescents in the Netherlands?
- What are risk factors and what is the pattern of weight gain in children and adolescents starting with antipsychotic drugs?

Part III Alternative sampling

- Is the Dried Blood Spot method a suitable alternative sampling technique to measure antipsychotic drug blood concentrations in children and adolescents with severe behavioral problems?

Part IV Antipsychotic drug concentrations and clinical outcomes

- What is the relationship between antipsychotic drug blood concentrations, side effects and effectiveness in children and adolescents?

OUTLINE

Part I provides a background on the effectiveness and safety of antipsychotic drugs in children and adolescents, and introduces pharmacokinetic and pharmacodynamic aspects that are relevant to improve its safety in this population.

Part II describes the current practice of antipsychotic drug use in children and adolescents in the Netherlands. **Chapter 2** reports on the Dutch trends in antipsychotic drug prescriptions in children and adolescents between 2005 and 2015. The risk factors and pattern of weight gain in children and adolescents starting with antipsychotic drug treatment are described in **chapter 3**.

Part III investigates DBS as an alternative sampling method to measure antipsychotic drug blood concentrations by three consecutive studies. **Chapter 4** describes the development of an Ultra–High-Performance Liquid Chromatography–Mass Spectrometry method to measure risperidone, aripiprazole and pipamperone concentrations in DBS. In **chapter 5**, the accuracy of this DBS method is evaluated in a clinical setting. Subsequently, the feasibility of the DBS method in children and adolescents with behavioral problems is reported in **chapter 6**.

Part IV focuses on the relationship between antipsychotic drug concentrations and clinical outcomes in children and adolescents. **Chapter 7** presents a systematic review of the literature on this topic for all psychotropic drugs, including antipsychotic drugs. In **chapters 8** and **9**, the population pharmacokinetics in relation to clinical improvement and side effects is analyzed for pipamperone and risperidone in children and adolescents with behavioral problems.

Part V gives and overall summary of the thesis and discusses the results in a broader clinical perspective.

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PART II

CURRENT PRACTICE

CHAPTER 2

ANTIPSYCHOTICS IN DUTCH YOUTH: PREVALENCE, DOSAGES, AND DURATION OF USE FROM 2005 TO 2015

Sanne M. Kloosterboer, Catharina C.M. Schuiling-Veninga, Jens H.J. Bos, Luuk J. Kalverdijk, Birgit C.P. Koch, Gwen C. Dieleman, Manon H.J. Hillegers, and Bram Dierckx

Journal of Child and Adolescent Psychopharmacology
2018;28(3):173-9

ABSTRACT

Objectives

The use of antipsychotic drugs by youth is associated with serious side effects, especially when prescribed in higher dosages and for a longer period. Despite this, little is known about recent trends in the dosages and duration of use of antipsychotic drugs in children and adolescents. The aim of this study was to describe trends in prevalence, incidence, dosages, duration of use and preceding psychotropic medication in Dutch youth who had been prescribed antipsychotic drugs from 2005 to 2015.

Methods

We analyzed prescription data of 13.210 youths aged 0-19 years using antipsychotics between 2005-2015, derived from a large Dutch community pharmacy-based prescription database (IADB.nl).

Results

Since a peak of 9.8 users per thousand youths in 2009, prevalence rates stabilized. Dosages in milligram per kilogram declined for the most frequently prescribed antipsychotic drugs during the study period. The median duration of use was 6.0 (95% CI 5.4-6.6) months. Boys used antipsychotic drugs significantly longer than girls, with a median of 6.9 (95% CI 6.1-7.7) vs 4.6 (95% CI 3.9-5.3) months ($p<0.01$). Of the youths prescribed antipsychotics, 12.4% used them for at least 48 months. The majority of youths had used other psychotropic agents in the year prior to the start of an antipsychotic drug (62.4% in 2005 and 64.7% in 2015).

Conclusion

Despite a stabilization of usage rates and decline in dosages an duration of use, one in eight youths still used antipsychotic drugs for 4 years or longer. A substantial share of youths may therefore be at high risk for serious side-effects.

INTRODUCTION

The use of antipsychotic drugs in children and adolescents has dramatically increased worldwide since the introduction of second-generation antipsychotics in the 1980s.[1-3] Second-generation antipsychotics appeared promising for use in more vulnerable populations, such as children, as they originally had been marketed as having more favorable side effects than the first-generation agents. As a result, the number of trials supporting efficacy in youth grew, which led to numerous registered indications including schizophrenia, bipolar disorder and behavioral problems associated with autism spectrum disorder.[4]

However, in recent years concerns have been raised regarding the use of antipsychotic drugs in children. For instance, off-label prescriptions are very common, which involves particular risks as evidence for efficacy is often lacking.[5] More importantly, few studies have adequately monitored long-term antipsychotic safety profiles in children. Indeed, second-generation antipsychotics appear to have major side effects, including weight gain, metabolic abnormalities and extrapyramidal symptoms.[6] Moreover, it has been suggested that some side effects are more common in children than in adults.[7] These might have a major impact later in life, as metabolic changes and tardive dyskinesia can be irreversible even upon drug discontinuation. Children receiving antipsychotic medication indeed show an increased risk of developing type 2 diabetes and metabolic syndrome, which significantly affects future quality of life and life expectancy. [8, 9]

Given these concerns, pharmacoepidemiological research is crucial to monitor the extent of antipsychotic drug use in youth. Unfortunately, however, few studies have reported on the duration of use or the dosages of antipsychotics in children and adolescents [10-13], while especially high dosages and long duration of use might enhance the risk for serious side effects.[8, 14] Furthermore, although comedication has been widely described, to our knowledge, no study has addressed the use of psychotropic medication preceding the start of an antipsychotic drug in children. Thus, it would be valuable to evaluate if the prescription of stimulants precedes antipsychotic treatment. Although aggressive, maladaptive behavior is an important indication for antipsychotic drug use in youths [15], stimulants have emerged as an alternative to antipsychotics to treat aggression associated with Attention Deficit Disorder (ADHD)[16]. Finally, as recent research showed that prevalence rates of antipsychotic use differ remarkably between countries[17], it is of interest to know how trends on incidence and duration of therapy explain these differences. Since 2005, no such information concerning antipsychotic drug use in youth in the Netherlands is known.[10]

Therefore, the aim of this study is to examine antipsychotic drug prescription trends for children and adolescents in the Netherlands from 2005 to 2015, including the prevalence, incidence, dosages and duration of therapy. We provide, to our knowledge, the first descriptions of trends in dosages of antipsychotic drugs in youths in a Western population.

METHODS

Data source

This study was performed with pharmacy dispensing data from the population-based prescription database IADB.nl.[18] The IADB database comprises prescription drug dispensing data from community pharmacies in the northern and eastern part of the Netherlands from 1994 onwards, covering a population of approximately 600,000 people. This population largely corresponds to the composition of the general Dutch population.[18] It includes all prescriptions, irrespective of type of health insurance (also including people without insurance), prescriber and reimbursement status. Prescriptions during hospital stays are not included.

The total population estimates were based on general population statistics from the Dutch Central Bureau for Statistics. Firstly, cities completely covered by IADB pharmacies were analyzed, to determine the proportion of the population visiting the pharmacy at least once a year. This proportion was used to estimate the coverage of IADB pharmacies in all other areas.

The study database IADB.nl uses de-identified medical records which could not lead to individual patients. According to the Code of Conduct for Health Research by the Foundation Federation of Dutch Medical Scientific Societies (approved by Dutch data protection authority in 2004)[19], no ethics committee approval is needed for research using anonymous medical records. This study was conducted in accordance with the Declaration of Helsinki.

Study sample and variables

Patients aged 0 through 19 years that used at least one antipsychotic drug between January 1, 2005 and December 31, 2015 were selected. Antipsychotic drugs were defined as class N05A according to the World Health Organization's Anatomical Therapeutic Chemical/Defined Daily Dose Classification System, except from N05AN (lithium). Clozapine, olanzapine, quetiapine, sulipiride, risperidone, aripiprazole, paliperidone, serindole and lurasidone were considered second-generation antipsychotics. The remaining drugs of class N05A were considered first-generation antipsychotics.

All other psychotropic drugs were defined as anxiolytics (class N05B), hypnotics and sedatives (class N05C), antidepressants (class N06A), psychostimulants (class N06B), lithium, clonidine, carbamazepine and valproic acid.

Data Analyses

First, prevalence and incidence rates of antipsychotic drug use per year per 1000 youths in the population were calculated. A new (incidental) user was defined as a youth being present in the database for at least 90 days and receiving an antipsychotic drug prescription for the first time. Prescription data from 2004 were used to identify starters in 2005. We stratified prevalence and incidence by gender, age group (0-6 years, 7-12 years, 13-19 years) and type of drug. Incidence rates were also stratified by type of prescriber (GP or specialist). Confidence intervals (CIs) were calculated using the Score method with continuity correction .[20] Proportions were compared using the Chi square test.

Then we performed a dose analysis. Weight of the children was estimated using the Denekamp scale in order to provide milligram per kilogram dosages. [21] The Denekamp scale provides the median weight for boys and girls at different ages, based on anthropometric references of Dutch youth. Prescriptions of the four most prescribed antipsychotics were used for the dose analysis. Only prescriptions issued for at least seven days were selected in order to exclude rescue medication. Pipamperone was excluded from the dose analysis as this is often prescribed as a 40 mg/ml liquid formulation and daily dose was not consequently noted in the database as milliliters (ml) or milligrams (mg). The age on the first of January of the year of prescription was used. Means are presented as value ± the standard deviation (SD).

We calculated duration of AP drug use by median and mean survival times using Kaplan Meier analyses. The start of an episode of AP drug use was defined as described above. An episode ceased if at least the amount of days for which medication was prescribed plus 90 days had passed and the youth could still be followed in the database. All other cases were considered censored. Duration of use was stratified by gender, age groups (at time of start of the antipsychotic drug), start year and type of drug. Subgroups were compared using the Logrank test. Starters from the year 2015 were excluded from the survival analysis, as the cohort could only be followed until the end of 2015 and in this year high rates of censoring would take place. Duration of AP use was presented in months, with 30 days being considered one month.

An analysis on preceding psychotropic medication was also performed. Psychotropic prescriptions issued one year before the first antipsychotic drug prescription were considered as preceding psychotropic medication. Concurrent psychotropic treatment was defined as a psychotropic prescription being issued within the start- and stop date of an antipsychotic drug prescription. Prescriptions were stratified by year and gender.

Differences were considered significant at $p<0.05$. Statistical analyses were performed with SPSS for windows, version 21 and Microsoft Excel 2010.

Table 1 Prevalence (per thousand) of antipsychotic prescriptions among children up to age 19 years.

	2005 (n= 131,980)[a]		2010 (n=138,251)[b]		2015 (n=126,666)[c]	
	per 1000	95% CI	per 1000	95% CI	per 1000	95% CI
Total						
0-6	2.7	(2.3-3.2)	2.5	(2.1-3.0)	1.5*	(1.2-1.9)
7-12	10.1	(9.1-11.2)	13.5*	(12.4-14.7)	10.8	(9.8-11.9)
13-19	9.5	(8.7-10.4)	12.5*	(11.6-13.5)	13.8*	(12.8-14.9)
Boys						
0-6	4.1	(3.4-5.0)	3.8	(3.1-4.7)	2.0*	(1.5-2.7)
7-12	16.6	(14.8-18.5)	21.2*	(19.3-23.3)	15.5	(13.8-17.4)
13-19	13.3	(11.9-14.9)	17.0*	(15.5-18.7)	17.8*	(16.2-19.5)
Girls						
0-6	1.2	(0.8-1.8)	0.9	(0.6-1.4)	1.0	(0.6-1.6)
7-12	3.3	(2.5-4.3)	5.3*	(4.4-6.4)	5.7*	(4.7-7.0)
13-19	5.7	(4.8-6.7)	8.1*	(7.1-9.3)	9.9*	(8.7-11.2)

[a]*n=66,430 boys, n=65,550 girls*
[b]*n=70,255 boys, n=67,996 girls*
[c]*n=64,777 boys, n=61,889 girls*
**p<0.05, significantly different compared to baseline (2005)*

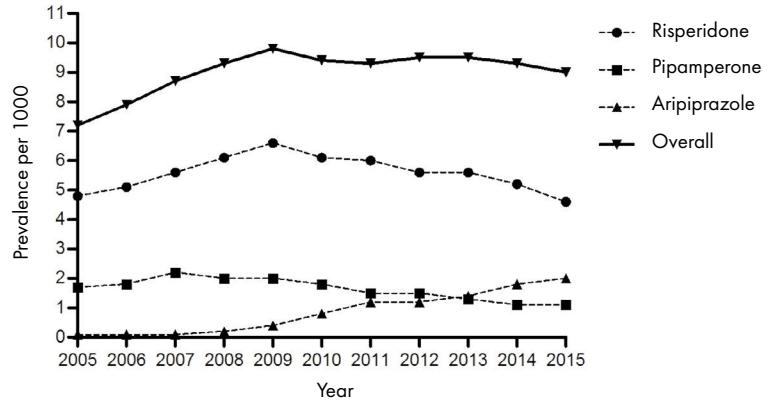
RESULTS

The total population aged 0 to 19 years ranged from 131,980 persons in 2005 to 126,666 persons in 2015.

Prevalence

The overall prevalence of antipsychotic drug use ranged from 7.2 (95% CI 6.8-7.7) in 2005 to 9.0 (95% CI 8.5-9.5) per thousand youths in 2015. The prevalence rates stratified for age, gender and year are presented in **Table 1**. The prevalence was highest in 2009 with 9.8 (95% CI 9.3-10.3) users per thousand youths (**Figure 1**). Boys were more likely to use antipsychotic drugs in all years and in all age groups. The most frequently prescribed antipsychotic drugs were risperidone (61.9% of all antipsychotic drug users), pipamperone (18.2%), aripiprazole (9.5%), quetiapine (9.3%) and olanzapine (3.7%). Trends of the three mostly prescribed antipsychotics are visualized in **Figure 1**. The prevalence of first-generation APs ranged from 2.2 (95% CI 2.0-2.5) in 2005 to 2.3 (95% CI 2.1-2.6) in 2010 and 1.6 (95% CI 1.4-1.8) in 2015 per thousand youths. The prevalence of second-generation APs increased from 5.4 (95% CI 5.0-5.8) in 2005 to 7.8 (95% CI 7.3-8.3) in 2015 per thousand youths.

Figure 1 Prevalence of antipsychotic prescriptions in youth aged 0-19 years.



Prevalence per 1000 youths. Prevalences of the unique agents add up to more than the total prevalence, as one patient can contribute to more than one line.

Incidence

The overall incidence rate was 2.0 (95% CI 1.8-2.3) per thousand minors both in 2005 and 2015. Incidence peaked in 2007 with 2.6 (95% CI 2.3-2.9) new users per thousand aged 0-19. The incidence rates, stratified for age, gender and year, are presented in **Table 2**. Risperidone was the preferred antipsychotic drug to start with for all age categories in all years. Antipsychotic drugs were mainly started by specialists (2005: 75.5%, 2015: 67.8%).

Table 2 Incidence per thousand of antipsychotic prescriptions among children up to age 19 years

	2005 (n= 131,980)[a]		2010 (n=138,251)[b]		2015 (n=126,666)[c]	
	per 1000	95% CI	per 1000	95% CI	per 1000	95% CI
Total						
0-6	1.2	(0.9-1.6)	1.1	(0.8-1.5)	0.5*	(0.3-0.8)
7-12	2.0	(1.6-2.5)	3.4*	(2.9-4.0)	2.2	(1.8-2.8)
13-19	2.8	(2.4-3.3)	3.0	(2.6-3.5)	3.1	(2.6-3.6)
Boys						
0-6	1.9	(1.4-2.6)	1.6	(1.2-2.2)	0.7*	(0.4-1.2)
7-12	3.3	(2.6-4.3)	5.1*	(4.2-6.2)	2.8*	(2.1-3.7)
13-19	2.7	(2.1-3.5)	3.7	(3.0-4.6)	2.8	(2.2-3.6)
Girls						
0-6	0.5	(0.3-0.9)	0.5	(0.3-0.9)	0.4	(0.2-0.8)
7-12	0.8	(0.5-1.4)	1.6*	(1.1-2.3)	1.6*	(1.1-2.3)
13-19	2.8	(2.2-3.6)	2.3	(1.8-3.0)	3.3	(2.6-4.1)

[a]*n=66,430 boys, n=65,550 girls*
[b]*n=70,255 boys, n=67,996 girls*
[c]*n=64,777 boys, n=61,889 girls*
**p<0.05, significantly different compared to baseline (2005)*

Dosages

For 13,006 prescriptions analysis of the dosing (in milligram per kilogram) could be performed. For the five mostly used antipsychotics, the mean dosage decreased from 2005 to 2015, with the largest decrease for aripiprazole and quetiapine. The mean dosage (± standard deviation) for aripiprazole was 0.29 mg/kg (± 0.16) in 2005 and 0.11 mg/kg (±0.09) in 2015. The mean dosage for quetiapine decreased from 3.53 mg/kg (±3.00) in 2005 to 0.93 mg/kg (±1.09) in 2015. For risperidone the mean dosage was 0.03 mg/kg (±0.03) in 2005 and 0.02 mg/kg (±0.03) in 2015, for olanzapine 0.12 mg/kg (±0.09) in 2005 and 0.09 mg/kg (±0.06) in 2015.

Duration of use

The overall median and mean duration of use of antipsychotic drugs were 6.0 months (95% CI 5.4-6.6) and 19.1 months (95% CI 17.8-20.4) respectively, with considerable differences between subgroups (**Table 3**).

The duration of use was longest for children aged 7-12 years with a median of 9.8 months (95% CI 8.1-11.4) and a mean of 25.5 months (95% CI 23.2-27.8) (Figure 2). Boys had a significantly longer duration of therapy than girls which was consistent over the years (p<0.01). Overall, risperidone was prescribed for the longest period.

A large difference between the median and mean durations of therapy can be seen. This means that a small share of patients used antipsychotic drugs for a relatively long period. Overall 12.4% of the youths used antipsychotic drugs for at least 48 months. As is visualized in **Figure 2**, 19.4% of the children aged 7-12 years used an antipsychotic drug for 48 months or longer.

Other psychotropic medication

The majority of the population used other psychotropic medication in the year prior to the first start of an antipsychotic drug. This is consistent through the years; in 2005, 62.4% and in 2015 64.7% of children starting with antipsychotic treatment used other psychotropic medication in the preceding year. Centrally acting sympaticomimetics, benzodiazepines and selective serotonin reuptake inhibitors were prescribed most often in every year, both as preceding and concomitant medication. In 2005, 32.5% of youths were prescribed a stimulant in the year preceding the first prescription of an antipsychotic drug, in 2015 this was 36.4%. Approximately half of the patients used psychotropic comedication during antipsychotic treatment from 2005-2015 (51.3%), peaking in 2009 with 55.6%. Boys used more comedication than girls (49.4% vs 47.1%), which was not statistically significant (p=0.10).

DISCUSSION

From 2005 to 2015 in the Netherlands, prevalence rates stabilized since a peak of 9.8 users per thousand youths in 2009. The duration of use decreased to a median of 6.8 months and a mean of 10.9 months in 2014. Also, dosages per kilogram for the mostly prescribed antipsychotic drugs in youth declined. Furthermore, first time antipsychotic prescriptions were preceded by the use of other psychotropic medication in the majority of cases.

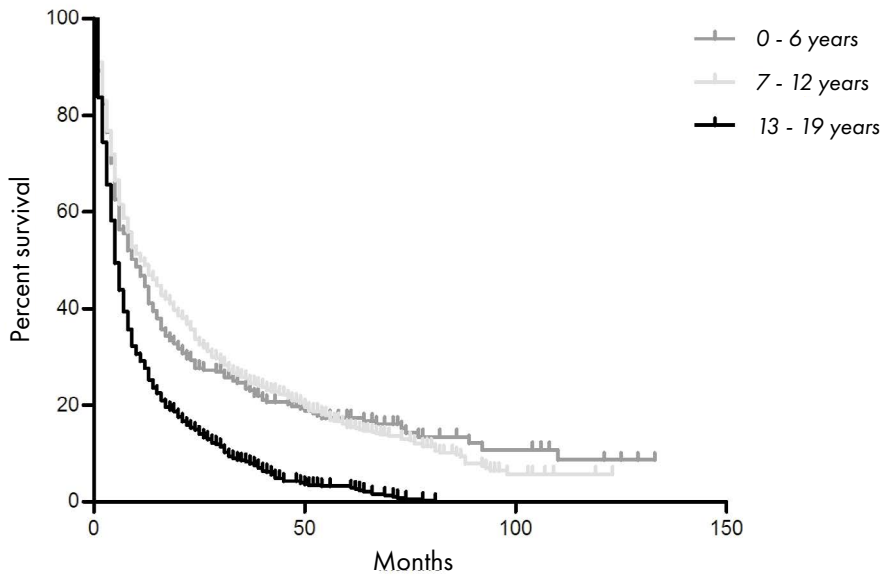
The major increase in antipsychotic drug use in youth that had been described in the Netherlands from 1997 to 2005[10], continued until 2009. The recent stabilization in usage is similar in other countries like the United States and Denmark.[22-24] We found a decline of usage rates in the youngest age group aged 6 years or younger, which mirrors findings from other studies.[15, 25] However, the prevalence of usage in adolescents increased. The overall plateauing of antipsychotic drug use in children might be attributed to more awareness of serious side effects in youth among health professionals following growing evidence from literature.[6, 26]

Table 3 Duration of use (in months) of antipsychotic drugs among children up to age 19 years.

	Median (months)	95% CI	Mean (months)	95% CI	p-value
All users*	6.0	(5.4-6.6)	19.1	(17.8-20.4)	
Age*					<0.01
0-6 years	6.2	(4.5-7.9)	24.3	(20.0-28.6)	
7-12 years	9.8	(8.1-11.4)	25.5	(23.2-27.8)	
13-19 years	4.3	(3.7-4.8)	10.7	(9.8-11.7)	
Gender*					<0.01
Boys	6.9	(6.1-7.7)	21.6	(19.9-23.3)	
Girls	4.6	(3.9-5.3)	13.9	(12.0-15.7)	
Start year					0.12
2005	7.3	(5.1-9.5)	20.7	(16.5-24.9)	
2010	4.1	(2.8-5.4)	15.4	(12.9-17.8)	
2014	6.8	(4.7-8.9)	10.9	(9.6-12.2)	
Agent**					<0.01
Risperidone	11.7	(9.5-13.9)	21.1	(19.0-23.3)	
Aripiprazole	6.5	(5.3-7.8)	14.3	(10.3-18.3)	
Pipamperone	1.0	(0.6-1.4)	7.9	(4.5-11.3)	

* *Analysis was performed for the years 2005-2014*
** *Analysis for the years 2011-2013, as in these years aripiprazole was prescribed to a considerable share of the total population*

Figure 2 Duration of antipsychotic drug use in youth aged 0-19 years.



Kaplan-Meier survival curve; plus signs indicate censored data. Analysis was performed for the years 2005-2014

Nevertheless, the usage rates of antipsychotic drugs in Dutch youth remain fairly high compared to other European countries.[17] For example, a German study carried out between 2004 and 2011 found a prevalence between 2.0 and 2.6 per thousand minors and a French study conducted between 2006 and 2013 reported usage rates between 4.6 and 4.9 per thousand youths.[11, 15] Several factors might contribute to these differences. Firstly, prevalence of psychiatric disorders may vary between countries, which can be partly due to differences in diagnosing patterns.[27] The most important registered psychiatric disorders that may warrant antipsychotic drug use by children and adolescents in the Netherlands include behavioral problems associated with autism spectrum disorder, bipolar disorder and schizophrenia. However, for example for pervasive developmental disorders like autism, differences in prevalence by geographical region are not supported by current literature.[28] Secondly, access to healthcare, child- and adolescent psychiatrists and prescription medicine might influence usage rates of antipsychotic drugs as well . In a densely populated country like the Netherlands, the access to healthcare is more easily guaranteed than in a country with great regional differences like France.[29] Furthermore, the number of psychiatrists per persons in the Netherlands is one of the highest in Europe [30], which is reflected by the high rate of first prescriptions being issued by specialists in this study. Also, access to prescription medicine for children in the Netherlands is guaranteed, as health insurance is compulsory and antipsychotics are fully reimbursed. Thirdly, prescription behavior of child- and adolescent psychiatrists might be influenced by cultural factors or differences in training. For example French psychiatrists are known to have a more psychoanalytical basis and might thus be more reluctant to prescribe psychotropic drugs.[31]

Despite the relatively high prevalence, the duration of use decreased from a median of 1.9 years in 1997-2005 [10], to a median of 6.0 months in 2005-2015. Although, a sizable share of children and adolescents used antipsychotic drugs for a long time. For example 19.4% of children treated with an antipsychotic drug aged 7 to 12 years used antipsychotics for 4 years or longer. These children might benefit substantially from such treatment. However, simultaneously, a longer duration of use might incur a greater risk to develop side effects, like dyskinetic movements, metabolic changes and the development of diabetes.[8, 14] Remarkably, few studies describe duration of antipsychotic drug use in youth. Verdoux et al reported a median duration of only 1 month in a French community based study which suggests this concerns mainly rescue medication for managing acute behavioral problems. [11] Burcu et al described a median duration of use of 180 days in a population of Medicaid-insured youth, similar to our findings.[13]

Consistent with earlier findings[10, 13], boys showed a significantly longer duration of use than girls. As mentioned, other studies have shown that antipsychotics in children are mainly prescribed to treat aggressive, impulsive and hyperkinetic behavior associated with ADHD, autism and mental retardation.[3, 15, 32] We might speculate that this explains the longer duration of use in boys, as physical aggressive behavior is more prevalent among boys than girls [33], and might exist for a longer time. However, as boys differ in a lot of ways from girls, also other factors might contribute to the difference in duration of use.

For both stimulants and antipsychotic drugs there is evidence for efficacy in aggressive and impulsive behavior associated with ADHD[16]. These agents are often found to be used concomitantly [3, 34, 35], which is also confirmed in this study. Interestingly in our study we found that most first time antipsychotic prescriptions in youth were not preceded by a stimulant prescription, although the latter agents are known to have a more favorable side effect profile. However, overall, most antipsychotic prescriptions were preceded by any type of other psychotropic medication. This is different from an earlier study conducted in adolescents with new onset psychotic symptoms, where most antipsychotics were not preceded by another psychotropic like antidepressants or benzodiazepines.[36] This finding might be explained by the sudden onset of psychotic symptoms and limited pharmacological treatment options other than antipsychotic drugs. This also might suggest that in our cohort, antipsychotics were mainly used to treat aggressive behavior rather than psychosis.

In our study, we found a trend towards lower dosages of antipsychotic drugs per kilogram from 2005 to 2015. A decrease in dosing in children has also been observed in a Japanese study.[12] This trend might be the result of the growing number of trials investigating antipsychotic drug use in children, generating new information on specific dosing schemes. For example in aripiprazole, after its introduction on the market, adult dosing schemes were advised as no specific information for children was available. For both aripiprazole and risperidone, formal dose recommendations were only available from 2009 in the Netherlands. Besides these guideline changes, also other factors might have influenced the dose changes over time. These include gender and age of the patients in the cohort, being moderators of weight and therefore influencing dosing. In this study,

we corrected for these factors using estimates of weight normalized for age and gender. Also, patients' ethnicities and socio-economic statuses might impact dosing over time. Although these latter variables were not known in our cohort, we expect these factors to be fairly constant during the study period, as the area covered by the pharmacies that delivered the prescription data did not significantly change.

Implications for future research include further investigation of international trends in duration of therapy and dosages of antipsychotic treatment in youths. In this way, country-specific exposure of antipsychotic medication to children and adolescents can be better quantified. Furthermore, by identifying specific populations at risk, tailored interventions to prevent serious side effects can be made.

This study has several limitations. Firstly, prescription rates do not represent actual usage rates, as medication might be taken in other dosages or not be taken at all. Secondly, the IADB database only includes pharmacies in the Northern and Eastern part of the Netherlands, which might not be representative for the whole country as these regions consist of more rural areas compared to the western part of the country. Some literature suggests antipsychotic drug prescribing might increase with density of population for young children [11], which might have led to an underestimation of usage rates. Nevertheless, other studies do not show such an association.[34] However, the IADB database has previously proven to be representative for the whole Dutch population.[18] Thirdly, by our definition of a new incidental users, also patients on ongoing treatment with a discontinuation of at least 3 months are defined as a new user. Also, as no inpatient prescriptions are included in the database, children that were hospitalized for a longer period of time might have been defined as a new incidental user upon discharge as well. This might have been resulted in an overestimation of incidence rates and an underestimation of duration of use. However, hospitalization in child- and adolescence psychiatry is rather rare. Moreover, a more strict definition of new users might have led to more children falsely being not identified as new users, for example when they moved into an area being covered by the database in the past months. Fourthly, no information on diagnoses was available. Lastly, the analysis of dosages per kilogram was based on median weight reference values of Dutch youth. As antipsychotics are known to induce weight gain, the weight of the children in our cohort might be above average. Therefore our analysis of dosages per kilogram might be an overestimation.

Strengths of this study are the use of a large dataset that was not limited to type of health insurance or health provider, in a country that is representative for the Western developed world. We provide an unique analysis of trends in dosages, duration of therapy and used preceding psychotropic medication which is of great importance to address the health risks of use of antipsychotics in youths.

CONCLUSIONS

In the Netherlands, overall antipsychotic drug prescription rates among children and adolescents stabilized and dosages per kilogram declined from 2005 to 2015. Although overall duration of use decreased, one in eight youths used antipsychotic drugs for at least four years.

CLINICAL SIGNIFICANCE

Youths with severe behavioral problems might substantially benefit from long term antipsychotic treatment and high dosages. However, longer duration of use and higher dosages may also increase the risk for side-effects of antipsychotics. Our study shows that a substantial share of Dutch youths who use antipsychotics use them for a considerable long time. Therefore we recommend adequate screening for side-effects in chronic users. Furthermore, health care providers should assess whether an attempt to discontinue antipsychotic drugs in chronic users is indicated.

FUNDING

S. M. K. received grant research support from The Netherlands Organisation for Health Research and Development (ZonMW), grant 836041011. ZonMW had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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CHAPTER 3

RISK FACTORS AND PATTERN OF WEIGHT GAIN IN YOUTHS USING ANTIPSYCHOTIC DRUGS

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European Journal of Child and Adolescent Psychiatry. 2020 Aug 24. doi: 10.1007/s00787-020-01614-4. Online ahead of print.

ABSTRACT

Introduction
Antipsychotic-induced weight gain is a major health concern in children and adolescents. The aim of this study was to identify risk factors for weight gain during short-, middle- and long-term treatment with antipsychotic drugs in this young population.

Methods
We analyzed a combined prospective and a retrospective observational cohort of Dutch children and adolescents, starting with risperidone, aripiprazole or pipamperone treatment. Linear mixed models were used to test whether sex, age, baseline body-mass-index (BMI) z-score, type of antipsychotic, dose equivalent/kg, duration of use, previous antipsychotic use, ethnicity, physical exercise, IQ, concomitant medication, and psychiatric classification predicted the BMI z-score for a follow-up of <15 weeks, 15-52 weeks or >52 weeks.

Results
A total of 144 patients were included with a median (interquartile range [IQR]) age of 9 (4) years and median follow-up of 30 (73) weeks. During the complete follow-up, the median (IQR) weight gain was 0.37 (0.95) BMI z-score points. Antipsychotic-induced weight gain was found to be most pronounced during the first 15 weeks of use (BMI z-score increase per week β=0.02, 95% C.I.0.01-0.03, p=0.002). A higher baseline BMI z-score and the absence of stimulant use was associated with a higher BMI z-score during the entire follow-up and after 15 weeks respectively. Previous treatment with an antipsychotic drug was associated with less weight gain during the first 15 weeks of treatment.

Conclusion
Our findings underscore the importance of close patient monitoring during the first weeks of antipsychotic treatment with a focus on patients with a high baseline BMI z-score.

INTRODUCTION

Antipsychotic-induced weight gain has been recognized as a major health concern in children and adolescents.[1] Although the magnitude of weight gain differs across type of antipsychotics and individuals, on average 1 in 7 minors gains 7% or more weight within the first 6-8 weeks of treatment.[2] This is not only highly stigmatizing, but also involves serious long-term health risks. Weight gain is associated with glucose and lipid abnormalities, thereby increasing the risk for diabetes and cardiovascular morbidity in children and adolescents using antipsychotics.[3, 4] It has been suggested that this leads to higher rates of unexpected death in this population, even at young age.[5]

The observed weight gain in youths starting antipsychotic treatment is highly heterogeneous, with some youths gaining a lot of weight, while others don't.This heterogeneity suggests that certain patient-related factors underlie the risk for antipsychotic-induced weight gain. The identification of these risk factors is an important target in the prevention of obesity, as it can facilitate early recognition and interventions for children and adolescents at risk. Such interventions can include both behavioral and pharmacological interventions, which are proven to be effective at least to some extent in the management of antipsychotic-related weight gain in this population.[6, 7]

To date, literature is inconclusive about which children and adolescents are particularly at risk for weight gain during antipsychotic treatment. While it has been suggested that girls are at higher risk than boys [4], other studies have found the opposite [8], or did not find an influence of gender at all.[9] Likewise, some studies showed that a young age is associated with more weight gain [10, 11], while others found older age increases the risk for obesity [4]. Data on clinical predictors is generally limited, although several studies found that a low baseline body mass index (BMI) is associated with more weight gain [8, 12], and the concomitant use of stimulants would not be of significant influence. [12, 13]

It can be hypothesized that, amongst other factors, different follow-up durations might have contributed to these mixed findings. Weight gain in children and adolescents has been reported to be most pronounced during the first weeks of antipsychotic use and to stabilize during continued treatment, although long-term data are limited.[14, 15] In these different phases of weight acceleration, several mechanisms that are involved in antipsychotic-related weight gain are likely to contribute differently. Although these mechanisms are only poorly understood, several neurotransmitter and endocrine systems, such as serotonergic, dopaminergic and histaminergic receptors and leptin have been implicated. [16, 17] As different patient-related factors, such as sex or comedication, might influence these mechanisms individually, the influence of risk factors might be time-dependent as well.

Therefore, the aim of this study is to describe risk factors for weight gain in children and adolescents using antipsychotic drugs with a short-, middle- and long-term duration of use.

METHODS

Population

The study population consisted of children and adolescents treated with risperidone, aripiprazole, or pipamperone in the south-west region of the Netherlands. These three antipsychotics are the most frequently prescribed antipsychotics in this population in the Netherlands. [18]

The study population consisted of a prospective and a retrospective observational cohort. The prospective cohort included children and adolescents that were enrolled in an observational Dutch multicenter trial (NTR 6050) with a follow-up of 6 months. These patients were treated in an inpatient or outpatient setting in the Erasmus Medical Center, or one of 6 other participating centers in the south-west region of the Netherlands (1 other academic tertiary care center and 5 psychiatric secondary care centers) between August 2016 and November 2018. All patients and/or their legal representatives gave written informed consent before entering the study. The study was approved by the medical ethical committee of the Erasmus Medical Centre, the Netherlands (number MEC-2016-124). The retrospective cohort consisted of children and adolescents being treated in the Erasmus medical center between 1 January 2012 and 31 December 2017. The medical ethical committee of the Erasmus Medical Centre waived informed consent for this study (MEC-2018-1613).

Patients were included based on the following criteria: 1. Treatment with risperidone, aripiprazole or pipamperone, 2. No simultaneous use of other antipsychotics, 3. Bodyweight known at least 2 weeks before or after the start of antipsychotic, 4. Minimally 1 other bodyweight known during use of antipsychotic, 5. Age up to 18 years, 6. No concomitant condition with direct influence on bodyweight (e.g. eating disorder, Prader-Willi syndrome). Patients who had used more than one antipsychotic during the study periods, were only included with the first antipsychotic that met the inclusion criteria.

Outcome

The outcome was age- and gender specific BMI z-score. To calculate the BMI z-score, BMI values were transformed into BMI z-scores based on the World Health Organization (WHO) BMI-for age reference values (5–19 years).[19] According to the WHO, a BMI z-score >1 is considered overweight, and a BMI z-score >2 is considered obesity. In the prospective cohort, weight and height were measured at baseline and at 6 months, and for a subset of patients that initiated antipsychotic treatment at start of the study, also at 1 month and at 3 months. In the retrospective cohort, weight and height were measured at variable time points during visits as part of routine clinical care. When a height measurement was missing but weight was known, a height measurement of the same patient that was performed within 5 weeks from that time point could be used. An average child or adolescent grows 6 cm per year, so 5 weeks would amount to maximal ±0,6cm difference in height which was considered insignificant.[20]

Predictors

Date of birth, sex, IQ and psychiatric classification according to *the Diagnostic and Statistical Manual of Mental Disorders* (DSM) IV or V were retrieved from the medical files. Physical exercise, country of birth of mother, father and child were collected based on questionnaires that were part of routine clinical care. For the antipsychotic drugs the following data was collected: type, dose, start date, stop date and previous use of antipsychotics. Dose equivalent/kg was calculated based on defined daily doses from the WHO which were multiplied by 100.[21] The dose equivalents were 5 mg for risperidone, 15 mg for aripiprazole and 200 mg for pipamperone. IQ was treated as a binary variable, defined as intellectual disability (IQ <70) or not (IQ >70). If IQ-scores were not available, medical records were screened for the diagnosis of intellectual disability. Ethnicity was considered a binary variable, and scored as either both parents were born in the Netherlands, or not. Concomitant stimulant use was defined as the use of methylphenidate or amphetamines at time of bodyweight and height measurement.

Statistical analysis

First, the course of weight gain within the sample was visually inspected. As the slope of the BMI z-score gain differed during the first 15 weeks of treatment (short-term), after 15 weeks (medium term) and after 52 weeks (long term), three separate analyses were performed for these time frames as described below. These timeframes were considered clinically relevant, as randomized controlled trials have shown that weight gain is significant during the first weeks of use[2] and many children use antipsychotic drugs only for a short period of time, but at the same time, a considerable proportion of children use antipsychotic drugs for more than a year.[18]

The data was analyzed per timeframe using linear mixed models with visits clustered within patients. The BMI z-score values at different time points during follow-up were used as dependent variable. Multiple imputation was used to replace missing values for IQ (4 % missing), physical exercise (15% missing) and ethnicity (10% missing). Imputation was based on the baseline data, as these variables did not change over time, and data were imputed on the individual level only. The fully conditional specification procedure in SPSS was used to impute five datasets for the analyses[22]. The imputed datasets were augmented with the repeated measurements to conduct the linear mixed models. The validity of the imputed variables was checked by computing the observed and imputed frequencies of the scores; there were no large differences between these frequencies. Subsequently, the assumptions of the linear mixed model were checked with visual inspection for the three timeframes separately, and were all met [23]. First, all predictors were analyzed in a univariate model as fixed effect with random intercept together with baseline BMI z-score (model 1). For the predictors with $p<0.15$ in the univariate model, interaction terms with time were added (model 2) to test whether they also predicted the BMI z-score increase per week (rather than for the whole timeframe). The predictors and interaction terms that were statistically significant ($p<0.05$) in one or more timeframes were combined in a multivariate model per timeframe (model 3). Variables with a p-value <0.05 in one or more timeframes were selected for the final model. SPSS Version 25 (SPSS Inc.,Chicago, IL, USA) was used for the analyses.

RESULTS

Sample

A total of 289 unique patients was screened for inclusion in the retrospective cohort, of which 90 patients were included. The main reasons for exclusion were: no use of risperidone, aripiprazole or pipamperone (n=57), no bodyweight known at baseline (n=66), simultaneous use of other antipsychotics (n=15), and no bodyweight measurements at follow-up (n=15). An additional number of 54 patients was included in the prospective cohort, resulting in a total study sample of 144 unique patients. Within this sample, 18.8% of patients were overweight and 11.8% of patients were obese at start of antipsychotic treatment. The patient characteristics can be found in **table 1**.

Table 1 Patient characteristics

Baseline BMI z-score	0.23	(1.91)
Age, years	9	(4)
Male, n (%)	109	(76)
Dutch nationality*, n (%)	90	(70)
Follow-up, weeks	30	(72.5)
No. of visits	4	(4)
Antipsychotic, n (%)		
Risperidone	93	(65)
Aripiprazole	22	(15)
Pipamperone	29	(20)
Previous antipsychotic use, n (%)	60	(42)
Psychiatric diagnosis, n (%)		
ASS	124	(86)
ADHD	68	(47)
Other	43	(30)
Comorbidities, n (%)		
Epilepsy	11	(8)
Other	78	(54)
IQ <70*, n (%)	45	(33)
Physical exercise*, n (%)	57	(47)

*Legend: Total n=144 children and adolescents. All variables are presented as medians (interquartile range, IQR) unless otherwise stated. *available data: nationality n=129 (90% complete), IQ n=138 (96% complete), physical exercise n=122 (85% complete)*

BMI z-score

During the complete follow-up, the median (interquartile range, IQR) weight gain was 0.37 (0.95) BMI z-score points. The increase in BMI z-score was most pronounced during the first 15 weeks of treatment, followed by a slower increase up to 52 weeks and a slight decrease after 52 weeks, which is shown in **figure 1**. Analyses were separately done for the timeframes <15 weeks (short-term, n=144 patients), 15-52 weeks (middle-term, n=111 patients) and >52 weeks (long-term, n=58 patients) duration of use.

When predictors were individually tested together with only baseline BMI z-score as a covariate, dose equivalent/kg, duration of use, sex, no previous antipsychotic use, and stimulant use were significantly correlated with BMI z-score during one or more follow-up time frames (see **table 2**, model 1). With addition of interaction terms with time to these models, only a higher dose-equivalent/kg (β=0.02, 95% C.I. 0.01-0.04, p=0.011), a lower baseline BMI z-score (β=-0.01, 95% C.I. -0.02- -0.01, p<0.001) and no previous antipsychotic treatment (β=0.02, 95% C.I> 0.00-0.03, p=0.01) were found to significantly predict the BMI z-score increase per week, during the first 15 weeks of use (**table 2**, model 2). The absence of stimulant use was significantly associated with a higher mean BMI z-score during the first 15 weeks, as was a higher baseline BMI z-score during all time frames.

These variables were combined in the final multivariate model per timeframe. The mean BMI z-score gain per week was higher in the first 15 weeks (duration of use β=0.02, 95% C.I. 0.01-0.03, p=0.002) than between 15 and 52 weeks (β=0.01, 95% C.I. -0.01-0.02, p=0.378) and after 52 weeks (β=0.00, 95% C.I. 0.00-0.00, p=0.01). During the first 15 weeks of follow-up, previous antipsychotic treatment was associated with less weight gain per week. A higher baseline BMI z-score was found to be strongly predictive for a higher BMI z-score during all times frames of follow-up, as was the absence of stimulant use after 15 weeks. Removal of dose equivalent and its interaction term did not remarkably change the findings of the final model. The results of the final model are shown in **table 3**.

Figure 1 Pattern of weight gain

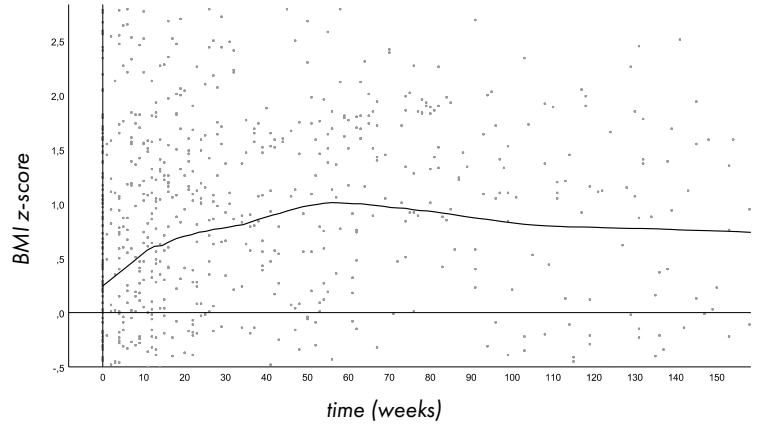

Table 2 Predictors of BMI z-score in children and adolescents during antipsychotic treatment

Model 1	<15 weeks follow-up$^{\alpha}$				15-52 weeks follow-up$^{\alpha}$				> 52 weeks follow-up$^{\alpha}$			
	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.
Dose equivalent/kg	**0.27**	**<0.001**	**0.06**	**0.16-0.38**	0.55	0.316	0.05	-0.05-0.16	-0.05	0.307	0.05	-0.14-0.04
Duration of use (weeks)	**0.03**	**<0.001**	**0.00**	**0.03-0.04**	**0.01**	**0.088**	**0.00**	**0.00-0.02**	**0.00**	**<0.001**	**0.00**	**0.00-0.00**
Baseline BMI z-score	**0.92**	**<0.001**	**0.02**	**0.90-0.96**	**0.80**	**<0.001**	**0.05**	**0.7-0.9**	**0.69**	**<0.001**	**0.07**	**0.56-0.83**
Age (years)	0.00	0.661	0.00	0.00 -0.00	0.00	0.507	0.00	0.00-0.00	0.00	0.457	0.00	0.00-0.00
Sex (male)	**0.10**	**0.076**	**0.06**	**-0.01 - 0.22**	**0.27**	**0.096**	**0.16**	**-0.05-0.60**	**0.22**	**0.328**	**0.22**	**-0.22 - 0.66**
Antipsychotic												
Risperidone	0.07	0.197	0.05	-0.04 - 0.18	0.16	0.309	0.15	-0.14-0.45	-0.07	0.756	0.22	-0.50-0.36
Aripiprazole	-0.03	0.401	0.07	-0.17-0.12	0.05	0.814	0.20	-0.35-0.45	0.16	0.950	0.26	-0.49-0.52
Pipamperone	-0.07	0.255	0.06	-0.19-0.05	-0.26	0.155	0.18	-0.61-0.10	0.08	0.762	0.26	-0.43-0.59
No previous antipsych. treatment	**0.11**	**0.026**	**0.05**	**0.01 - 0.21**	0.16	0.273	0.14	-0.12-0.43	0.13	0.524	0.20	-0.27 -0.53
Psychiatric classification												
ASS	-0.01	0.913	0.07	-0.15-0.14	0.06	0.804	0.23	-0.39-0.50	0.27	0.328	0.27	-0.27-0.80
ADHD	-0.01	0.786	0.05	-0.12-0.09	0.02	0.905	0.15	-0.27-0.31	0.23	0.249	0.20	-0.16-0.62
Other psychiatric class.	0.03	0.596	0.05	-0.08-0.14	-0.15	0.339	0.15	-0.45-0.15	-0.10	0.630	0.20	-0.49-0.29
Somatic diagnosis												
Epilepsy	-0.10	0.318	0.10	-0.29-0.09	-0.16	0.534	0.26	-0.66-0.34	-0.48	0.115	0.31	-1.08-0.12
Other	-0.05	0.325	0.05	-0.15-0.05	-0.01	0.924	0.14	-0.28-0.26	0.16	0.412	0.19	-0.21-0.53
Concomitant medication												
No stimulant	**0.13**	**0.040**	**0.06**	**0.00-0.25**	**0.38**	**0.007**	**0.14**	**-0.11-0.66**	**0.43**	**<0.001**	**0.10**	**0.24-0.62**
No other medication	0.09	0.267	0.08	-0.07 -0.25	0.12	0.543	0.22	-0.31-0.55	0.01	0.948	0.16	-0.30-0.32
Ethnicity (Dutch)	-0.04	0.709	0.06	-0.16-0.08	0.03	0.842	0.16	-0.29-0.35	0.27	0.285	0.25	-0.24-0.79
Sport	-0.05	0.310	0.05	-0.15-0.05	0.01	0.949	0.15	-0.28-0.30	-0.10	0.616	0.20	-0.48-0.29
IQ < 70	0.01	0.829	0.06	-0.10-0.12	0.04	0.779	0.14	-0.24-0.32-	-0.22	0.261	0.20	-0.61-0.16

Model 2	<15 weeks follow-up$^{\alpha}$				15-52 weeks follow-up$^{\alpha}$				>52 weeks follow-up$^{\alpha}$			
	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.
Dose equivalent/kg	-0.05	0.518	0.08	-0.21 -0.11	0.01	0.947	0.17	-0.31-0.34	-0.08	0.079	0.05	-0.17-0.01
* duration of use (weeks)	**0.02**	**0.011**	**0.01**	**0.01 -0.04**	0.00	0.792	0.00	-0.01-0.01	0.00	0.880	0.00	0.00-0.00
Baseline BMI z-score	**1.00**	**<0.001**	**0.02**	**0.96 -1.04**	**0.85**	**<0.001**	**0.11**	**0.63-1.06**	**0.68**	**<0.001**	**0.07**	**0.53-0.82**
* duration of use (weeks)	**-0.01**	**<0.001**	**0.00**	**-0.02 - -0.01**	0.00	0.667	0.00	-0.01-0.01	0.00	0.695	0.00	0.00-0.00
Sex (male)	0.07	0.284	0.07	-0.06 -0.2	0.16	0.695	0.40	-0.63-0.94	0.29	0.221	0.24	-0.17-0.75
* duration of use (weeks)	0.01	0.264	0.01	-0.01-0.02	0.00	0.04	0.01	-0.02-0.03	0.00	0.623	0.00	0.00-0.00
No previous antipsych. Drug	0.02	0.688	0.06	-0.09-1.14	0.10	0.31	0.746	-0.5-0.7	0.23	0.262	0.21	-0.17-0.64
* duration of use (weeks)	**0.02**	**0.018**	**0.01**	**0.00-0.03**	0.00	0.859	0.01	-0.02-0.02	0.00	0.141	0 .00	0.00-0.00
No stimulant	**0.16**	**0.033**	**0.07**	**0.01 -0.30**	0.49	0.114	0.31	-0.12-1.10	0.17	0.259	0.15	-0.13-0.48
* duration of use (weeks)	0.00	0.915	0.00	-0.02 -0.02	0.00	0.722	0.01	-0.02-0.02	0.00	0.201	0.00	0.00-0.00

Legend: α *<15 weeks: n= 144 patients, 15-52 weeks 111 patients, >52 weeks: 58 patients. Antipsych = antipsychotic. Class=classification. S.E.= standard error. 95% C.I. = 95% confidence interval*

Table 3 Predictors of BMI z-score in children and adolescents during antipsychotic treatment – final model

Model 3	<15 weeks follow-up$^{\alpha}$				15-52 weeks follow-up$^{\alpha}$				>52 weeks follow-up$^{\alpha}$			
	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.	β	p-value	S.E.	95% C.I.
Dose equivalent/kg	-0.02	0.812	0.08	-0.17-0.13	0.01	0.941	0.17	-0.31-0.34	-0.05	0.295	0.05	-0.14-0.04
* duration of use (weeks)	0.01	0.127	0.01	0.00-0.03	0.00	0.839	0.01	-0.01-0.01	0.00	0.826	0.00	0.00-0.00
Baseline BMI z-score	**0.99**	**<0.001**	**0.02**	**0.95-1.03**	**0.83**	**<0.001**	**0.11**	**0.61-1.04**	**0.65**	**<0.001**	**0.08**	**0.50-0.80**
* duration of use (weeks)	**-0.01**	**<0.001**	**0.00**	**-0.02-0.01**	0.00	0.565	0.00	-0.01-0.00	0.00	0.322	0.00	0.00-0.00
Previous antipsych. Drug (no)	0.00	0.975	0.06	-0.11-0.11	0.09	0.782	0.31	-0.52-0.69	0.22	0.284	0.20	-0.18-0.62
* duration of use (weeks)	**0.02**	**0.003**	**0.01**	**0.01-0.03**	0.00	0.994	0.01	-0.02-0.02	0.00	0.301	0.00	0.00-0.00
No stimulant	0.10	0.074	0.06	-0.01-0.21	**0.38**	**0.009**	**0.15**	**0.09-0.67**	**0.35**	**<0.001**	**0.10**	**0.16-0.55**
Duration of use (weeks)	**0.02**	**0.002**	**0.01**	**0.01-0.03**	0.01	0.378	0.01	-0.01-0.02	**0.00**	**0.010**	**0.00**	**0.00-0.00**

Legend: α *<15 weeks: n= 144 patients, 15-52 weeks 111 patients, >52 weeks: 58 patients*

DISCUSSION

We found that antipsychotic-induced weight gain in children and adolescents was most pronounced during the first 15 weeks of use. A higher baseline BMI z-score predicted a higher BMI z-score during follow up, while the use of stimulants was associated with lower BMI z-scores after 15 weeks. Previous antipsychotic treatment was associated with less weight gain during the first 15 weeks of treatment.

Although significant weight gain in children and adolescents on antipsychotic treatment has been widely recognized, the time course of this weight gain is only poorly documented.[2] A previous study has found that BMI z-score gain is most pronounced during the first month of treatment, while in our cohort a longer period of accelerated weight gain of almost 4 months was found.[14] Furthermore, although long-term data is generally lacking, several studies have described a slight decrease in BMI z-score after 6 months.[9, 24] This plateauing was also observed in our study, but considerably later. Despite this, the BMI z-score generally does not return to the baseline value during long-term antipsychotic use. Limited data however has shown that after antipsychotic discontinuation, baseline BMI z-score values might recover.[25]

The mechanisms behind this course of antipsychotic-induced weight gain in youths are complex and only partly understood. Both increased appetite, increased food intake and an altered metabolism contribute to an imbalance between energy intake and energy expenditure, regulated by both neurotransmitter systems and hormonal changes. Within the neurotransmitter systems, the interaction of antipsychotic drugs with both dopamine, histamine and serotonin receptors has been suggested to moderate weight gain. [17] These neurotransmitter receptor interactions are expected to happen directly, as neurotransmitter-induced side-effects like extrapyramidal symptoms and sedation can arise in several hours after initiation of antipsychotic treatment. As such, the weight-moderating effect too might start immediately. Conversely, the effects of dysregulation of the hormonal system is likely to take longer. Antipsychotic drugs affects leptin, also known as the satiety hormone. [16, 17] As this hormone is secreted by adipose tissue, the effects on appetite might only become apparent after an increase in adipocytes, and thus might need more time. Although both mechanisms are likely to reach a new homeostasis after a while, the different time courses of the induction of these two mechanisms might partly explain the non-linear development of weight gain in children and adolescents using antipsychotics. Nevertheless, numerous other mechanisms, including gene-environment interactions and neuropeptides, play a role and have not yet been clarified, especially in children and adolescents.

This study found that patients with higher BMI z-score at baseline also had higher BMI z-scores during antipsychotic treatment. However, the interaction with time suggests a faster increase in bodyweight during the first 15 weeks of treatment in children with lower bodyweight. This resembles the findings from previous, mostly short-term studies, identifying lower baseline BMI as risk factor for weight gain in children and adolescents using antipsychotics.[8, 11, 12] Nevertheless, this effect can be overestimated by the phenomenon of 'regression to the mean', indicating that extreme BMI values are naturally expected to grow closer to the population mean during follow-up. [26] Also, a low baseline BMI as predictor is easily confounded by stage of illness, as younger and thus lighter children are likely to have less prior antipsychotic exposure.[16] These children may gain more weight, as was found in our study.

Regardless of the change in weight, the finding that overweight children and adolescents are likely to remain overweight during antipsychotic treatment has important implications. Apart from the evident risk of lipid and glucose disturbances with obesity, an additional, distinct mechanism inducing metabolic abnormalities independently of weight gain is suggested for antipsychotic drugs.[17] This results in an increased prevalence of metabolic abnormalities in overweight children using antipsychotics [12], which calls for a close monitoring of overweight children at start of antipsychotic treatment. However, not all monitoring guidelines for children and adolescents on antipsychotic treatment provide such a standardized intensified monitoring schedule for overweight children.[1]

Most antipsychotic-induced side-effects monitoring guidelines, developed as a result of the growing awareness of cardiometabolic adverse effects of antipsychotic drugs in children and adolescents, recommend a first visit at 3 months after start.[1, 27] Given our findings that the most accelerated weight gain occurs in the first 15 weeks, it should be considered to bring this visit forward to 1 month after start of treatment. It has been shown previously that the weight gain at 1 month is predictive of problematic weight gain after 3 months in adolescents using antipsychotics, further confirming the added value of an early monitoring visit. [28] In addition, bodyweight controlling strategies are expected to be most beneficial when offered at an early stage, as childhood obesity is likely to persist in adulthood. [29]

Stimulant use was associated with lower BMI z-scores from 15 weeks of follow-up. It is well-known that decreased appetite is a common-side effect of stimulants,[30] thereby often leading to weight loss in children using this type of drugs. It seems obvious that concomitant stimulant use can attenuate antipsychotic-induced weight gain, although this has not been consistently demonstrated in previous studies.[12, 13] Possibly, children in our study received higher dosages of stimulant drugs, but this could not be analyzed. However, although concomitant stimulant use can possibly lower weight gain in children and adolescents using antipsychotic drugs, the risk for adverse cardiovascular adverse events remains unclear. While stimulants have been associated with an increased blood pressure and heart rate, and antipsychotics with cardiac arrhythmias, little is known about the combined cardiac risks in children and adolescents.[31 32]

Children receiving a higher dose equivalent experienced more weight gain during the first weeks of use, although this finding did not remain significant in combination with other variables. A higher dosage has been described previously as a risk factor for short-term weight gain in children and adolescents using risperidone, although findings in adults have been conflicting. [10, 33, 34] Furthermore, in this study, no difference in weight-inducing potency between different types of antipsychotic drugs was found. In randomized controlled settings however, these differences have

been clearly shown, with risperidone inducing more weight gain than aripiprazole.[35] Although the relative weight-inducing properties of pipamperone have only been limitedly described, it is likely that this antipsychotic also contributes significantly to weight gain due to its strong anti-serotonergic properties. The differences between antipsychotics can diminish in an observational settings with limited sample sized like this study, as heavier children are more likely to start treatment with antipsychotics that are associated with less weight gain, such as aripiprazole. Similarly, another relatively small retrospective study performed in Dutch children and adolescents did not show a difference in weight gain between children using risperidone or aripiprazole, while based on the literature this could have been expected.[9]

The results of this study should be interpreted in the light of its limitations. Firstly, due to the merely retrospective design, no causal relationship between patient-related factors and BMI z-score gain could be established. Also, data was collected in a real-life, clinical setting, thereby allowing changes in antipsychotic regimens upon the physician's discretion or patient preferences. This might have influenced the results, as dosages could be lowered or the antipsychotic drug could be stopped when a child gained too much weight or had other side-effects. Moreover, drug adherence was not analyzed. Lastly, the sample size of this study was relatively small and only a limited proportion of patients could be followed for several years, thereby possibly missing risk factors with a low effect size.

However, this is the first study that describes risk factors for antipsychotic-induced weight gain in children and adolescents by distinguishing between short-, middle- and long-term use. Also, the follow-up duration in our cohort was considerably longer than in previous studies. By using advanced mixed-modelling techniques, we could use all weight assessments instead of only studying endpoint differences, which is especially important as weight gain was non-linear. The findings of this study can guide further, targeted monitoring of children at risk for antipsychotic-induced weight gain, thereby increasing safety of antipsychotic use in the young.

ETHICAL STANDARDS

This study has been approved by the ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons and/or their legal representatives gave their informed consent prior to their inclusion in the prospective study; in the retrospective study, informed consent was waived by the ethics committee.

FUNDING

SMK, BD and BCK received grant research support from The Netherlands Organization for Health Research and Development (ZonMW), number 836041011.

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PART III

ALTERNATIVE
SAMPLING

CHAPTER 4

DRIED BLOOD SPOTS COMBINED WITH ULTRA–HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE QUANTIFICATION OF THE ANTIPSYCHOTICS RISPERIDONE, ARIPIPRAZOLE, PIPAMPERONE, AND THEIR MAJOR METABOLITES

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Therapeutic Drug Monitoring. 2017;39(4):429-40.

ABSTRACT

Background

Risperidone, aripiprazole and pipamperone are antipsychotic drugs frequently prescribed for the treatment of comorbid behavioral problems in children with autism spectrum disorders (ASD). Therapeutic drug monitoring (TDM) could be useful to decrease side effects and to improve patient outcome. Dried blood spot (DBS) sample collection appears to be an attractive technique to develop TDM of these drugs in a pediatric population. The aim of this work was to develop and validate a DBS assay suitable for TDM and home sampling.

Methods

Risperidone, 9-OH risperidone, aripiprazole, dehydroaripiprazole and pipamperone were extracted from DBS and analyzed by UHPLC-MS/MS using a C18 reversed phase column with a mobile phase consisting of ammonium acetate/formic acid in water or methanol. The suitability of DBS for TDM was assessed by studying the influence of specific parameters: extraction solution, EDTA, carryover, hematocrit, punching location, spot volume, hemolysis. The assay was validated with respect to conventional guidelines for bioanalytical methods.

Results

The method was linear, specific without any critical matrix effect and with a mean recovery around 90%. Accuracy and precision were within the acceptance criteria in samples with hematocrit values from 30% to 45%. EDTA or hemolysis did not skew the results and no punching carryover was observed. No significant influence of the spot volume neither the punch location was observed. The antipsychotics were all stable in DBS stored 10 days at room temperature and 1 month at 4°C or -80°C. The method was successfully applied to quantify the three antipsychotics and their metabolites in patient samples.

Conclusions

An UHPLC-MS/MS method has been successfully validated for the simultaneous quantification of risperidone, 9-OH risperidone, aripiprazole, dehydroaripiprazole and pipamperone in DBS. The assay provided good analytical performances for therapeutic drug monitoring and clinical research applications.

INTRODUCTION

Antipsychotic drugs play an important role in the treatment of comorbid behavioral problems in children with autism spectrum disorders (ASD). Risperidone, aripiprazole and pipamperone are antipsychotic drugs frequently prescribed in these patients.[1-4] Unfortunately, metabolic abnormalities, diabetes, extrapyramidal symptoms and cardiovascular disorders, as well as irreversible extrapyramidal symptoms, are major side effects of these drugs.[5-8]It is recognized that therapeutic drug monitoring (TDM) might be useful to improve treatment efficacy, to control compliance and to avoid adverse effects in adults using antipsychotics.[9-16] Some data suggest that in children as well TDM might enhance therapeutic results while lowering risk of toxicity.[17,18]

In the last few years, dried blood spot (DBS) sampling has experienced renewed interest in bioanalysis. DBS collection requires only a simple finger prick and less blood than a venipuncture. Consequently, this is less painful and stressful for the patient than conventional blood sampling. Additionally, patient samples can be collected in a home environment.[19-21] Thus, this type of sampling is particularly interesting to develop a less invasive sampling method for TDM in a pediatric population.

Several analytical methods using DBS to monitor drugs levels have been reported in the literature.22 Although quantitative methods measuring antipsychotics levels in plasma are widespread[23-29], analytical procedures able to quantify antipsychotics in DBS are sparse. To the best of our knowledge, only Patteet et al.[30] have described a DBS method to quantify risperidone, pipamperone, aripiprazole and their major metabolites. However, their assay requires to collect an accurate volume of blood in order to analyze the whole DBS. Such a strategy cannot be implemented if home sampling is intended. Besides, the extraction procedure chosen by Patteet et al. requires a time consuming step to evaporate extraction solvent because the authors aimed at quantifying simultaneously more than ten antipsychotics in a high throughput method. The approach of the present work is different. The scope of the assay is defined for an application in a specific population. Hence, less analytes are included in the method that allows a simplified analytical workflow.

When developing a quantitative DBS method, the impact of critical analytical parameters should be investigated. Although specific guidelines for DBS method validation are not yet available from regulatory agencies, recommendations are proposed by several authors[31-34] who highlight the importance to perform a thorough analytical validation before considering replacement of plasma sampling by DBS sampling.

The aim of this study was to validate a DBS assay by means of ultra-high-performance liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS), allowing an easy finger prick sample at patient's home, and suitable applications on TDM of risperidone, aripiprazole, pipamperone and their major active metabolites 9-OH-risperidone and dehydroaripiprazole.

Material and methods

Chemicals and material

Risperidone, Aripiprazole, Dehydroaripiprazole and Haloperidol-d4 were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). 9-OH-Risperidone was purchased from Bioconnect life sciences (Huissen, The Netherlands) and Pipamperone was purchased from Santa Cruz Biotechnology (Huissen, the Netherlands). Methanol and acetonitrile were purchased from Biosolve BV (Valkenswaard, the Netherlands). Ammonium acetate and formic acid were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). All reagents were of UHPLC-MS grade. Water was purified using a Milli-Q® Ultrapure Water System (Merck Millipore, Darmstadt, Germany). Whatman® 903 proteinsaver DBS cards were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). A regular puncher (Fiskars, Helsinki, Finland) was used for punching the DBS discs out of the spotting cards. Drug free human whole blood was obtained from the Dutch blood donating centre (Sanquin, the Nederlands). Blood samples were gently mixed using the HulaMixer™ sample mixer (Thermo Scientific, Waltham, USA).

UHPLC-MS-MS equipment and conditions

Instrumental

Analysis was carried out on a Thermo TSQ Vantage UHPLC-MS/MS system consisted of a Dionex Ultimate UHPLC system, connected to a triple Quadrupole mass spectrometer Thermo TSQ Vantage (Thermo Scientific, Waltham, USA). The UHPLC system consisted of an Ultimate 3000 RS UHPLC-pump, an autosampler and a column oven. XcaliburTM (2.1 SP1 w/foundation 1.0.2. SP2, Thermo Scientific), and ChromeleonTM (Dionex, Thermo Scientific) were used to acquire the data. LCquanTM (version 2.6.1.32, Thermo Scientific), was the software used for the analytes quantification process.

Chromatographic conditions

Chromatographic separation was achieved using an Acquity UPLC BEH C18 reversed phase column (2.1 x 50 mm, 1.7 µm) (Waters, Milford, USA). Gradient elution was performed with a mobile phase composed of a mixture of 0.1% formic acid and 2mM ammonium acetate in water (eluent A) or methanol (eluent B). A multistep gradient was used at a flow rate of 0.5 ml/min and was programmed as follows: equilibration at initial conditions with 15% of B, increase to 50% of B from 0 min to 0.6 min, increase to 60% of B from 0.6 min to 1.5 min, increase to 100% of B from 1.5 min to 1.8 min, stabilization at 100% of B from 1.8 min to 3.0 min, reversion to the starting conditions at 15% of B from 3.0 min to 3.3 min and re-equilibration with the initial composition from 3.3 min to 4.4. The total run time was 5.4 min. The autosampler temperature was set at 10°C and the column oven was set at 50°C.

Mass spectrometry conditions

The MS settings and conditions were as follows: sheath and auxiliary nitrogen nebulizer gas pressure were set to 50 and 20 (arbitrary units) respectively. The collision gas pressure (argon) was 1.5 mTorr. Electrospray ionization in the positive mode was applied for all compounds. The MS run time was 3.5 min performed in the selective reaction monitoring (SRM) scanning mode. Capillary temperature was 250oC, vaporizer temperature was 375oC and spray voltage was 3000 Volt. The optimized settings of each analyte are summed up in **table 1**. The most abundant SRM transition was used as quantifier ion for all analytes except for 9-OH-Risperidone. For this analyte, an interferent matrix product appeared at the same transition as the quantifier ion so the second most abundant transition ion (qualifier ion) was used.

Table 1 Mass spectrometry settings used in the DBS assay.

Analyte	Parent ion (m/z)	Daughter ions (m/z)	Rt (min)	CE (V)	S-lens (V)
Pipamperone	376.2	165.1	1.63	26	113
		123.0		43	
		291.1		17	
9-OH-Risperidone	427.2	207.1	1.79	26	126
		110.0		39	
		69.0		46	
Risperidone	411.2	191.1	1.86	27	126
		110.0		46	
		82.0		49	
Aripiprazole	448.2	285.1	2.39	25	138
		176.0		30	
		98.0		34	
Dehydroaripiprazole	446.1	285.1	2.34	22	114
		98.0		36	
		84.0		46	
Haloperidol-d4	380.2	127.0	2.15	37	114

SRM transitions, retention time (Rt), collision energy (CE) and S-lens amplitude voltage for all analytes. The bolt daughter ions are used as quantifiers for the quantitative analysis, the other ions are qualifier used for analytes identification and detection selectivity. SRM: Single reaction monitoring

Preparation of standards (Std) and quality control (QC) samples

Individual stock solutions of each analyte were prepared in methanol at a concentration of 100 mg/L. These stock solutions were combined and diluted with methanol to obtain an intermediate mixture solution containing the five analytes together at 20 mg/L for aripiprazole, dehydroaripiprazole, pipamperone and 4 mg/L for risperidone and 9-OH risperidone. The mixture solution was stored at -20°C in a brown bottle. Working solutions of each calibration standard and each QC level were daily prepared by dilution of the mixture solution in methanol.

Two batches of stock solutions per analyte were made to independently prepare two batches of mixture solutions (one for QC and one for Standard) in order to detect mistake in solutions preparation. The stock solution of Haloperidol-d4 (Internal Standard) was prepared at a concentration of 1 mg/L using methanol.

Aliquots of 950 µL of drug free human blood were spiked with 50 µL of the corresponding working solution. Thus, the percentage of organic solvent in blood was only 5%. Concentrations obtained in calibration standards and QC samples are presented in **table 2**. Tubes were gently mixed for 10 min and 15 min of stabilization time were waited before spotting blood on a DBS card.

Table 2 DBS concentrations of calibration standards and quality control

Analyte	Calibrations standards (µg/l)								Internal quality control samples (µg/l)			
	S1	S2	S3	S4	S5	S6	S7	S8	LLOQ	QC low	QC medium	QC high
9-OH-Risperidone	2	4	10	20	50	100	150	200	2	6	80	140
Risperidone	2	4	10	20	50	100	150	200	2	6	80	140
Aripiprazole	10	20	50	100	250	500	750	1000	10	30	400	700
Dehydroaripiprazole	10	20	50	100	250	500	750	1000	10	30	400	700
Pipamperone	10	20	50	100	250	500	750	1000	10	30	400	700

Blood hematocrit adjustment

For the method validation, adjustment of drug-free whole blood hematocrit was needed. The way to proceed was chosen in the light of Koster et al. findings.[35] The hematocrit value used to prepare blood samples of calibration standards and QCs, was adjusted and normalized to 35%. This is assumed to be the median normal hematocrit value of the studied population (children with ASD).[36] For the assessment of the hematocrit effect, blood samples of increasing hematocrits had to be prepared. The first step of the procedure consisted in measuring the hematocrit of the collected drug-free blood with a flux cytometer (Sysmex, Kobe, Japan). Blood was then centrifuged for 6 min at 3000 g and the appropriate amount of plasma was removed or added to obtain the target hematocrit. The proportion of red blood cells was checked again after adjustment by the automated analyzer.

Sample preparation

Quality control and calibration standard samples were prepared by pipetting 50µL of spiked blood onto a Whatman 903 paper and leaving them to dry at room temperature for 4 to 24 hours. DBS discs were punched out using a 6 mm mechanical puncher and the discs were pushed into a polypropylene microtube (*Sarstedt, Germany*). To prevent carryover, the puncher was cleaned with ethanol between a high and a low concentration sample and spots were punched out in increasing concentration order. 200 µL of the extraction solution was added (ACN/MeOH/ eluent A 1.5:1.5:1 v/v/v) with IS (haloperidol-d4 5 µg/L in sample preparation solution) or without IS to make a blank sample. Tubes were vortexed for 1 min and sonicated in an ultrasonic bath at 40°C for 20 min. After 1 min vortexing, samples were centrifuged (5min, 16000 g) and 50 µL of the supernatant were transferred to 200 µL of eluent A in an auto sampler vial. Solvents proportions in the vial matched the starting conditions of the mobile phase in the LC as good as possible to avoid chromatographic changes for early eluting analytes. Only 10 µL of the final extract was injected into the UHPLC-MS/MS apparatus.

Method development and validation

Method development and optimization

The process to quantify simultaneously analytes of interest in DBS was adapted from an in house method previously developed in plasma.[37] In order to transfer the method to whole blood and DBS, some elements were investigated.

The developed method was carried out with blood from the Dutch blood donating center (*Sanquin*). In order to prevent blood coagulation, the blood was dispensed into tubes coated with ethylene –diamine- tetra -acetic acid (EDTA). Whether EDTA has any influence on the analytical results was investigated. Fresh blood (directly obtained from the blood center) or blood kept for three days in an EDTA coated tube, were spiked with analytes at low and high concentrations. Samples (n=2 for each kind of blood and concentration level) were applied on DBS paper and analyzed. The response obtained in blood containing EDTA was compared to the one measured in fresh blood.

In order to find the best procedure to extract the antipsychotics and their metabolites from DBS, several processes based on protein precipitation were investigated. Various mixtures of precipitant agents such as methanol, acetonitrile, zinc sulphate, and trichloro-acetic acid were prepared. A liquid extraction with ethyl acetate was also tested. The optimal procedure was selected based on visual criteria and signal response. In addition, optical density at 280 nm was measured by UV-spectrophotometry in the final extract to estimate and compare efficacy of the extraction solutions regarding protein components removal.[38] Optical densities of neat solutions were controlled beforehand to avoid any bias due to the solvent intrinsic absorbance.

Method validation

Validation was performed according to international guidelines for bio-analytical method validation.[39,40] These guidelines contain no specific items for the validations of DBS. Nevertheless, based on common practice reported in the literature, the influence of specific critical parameters was also investigated. Validation was performed using whole blood with a standardized hematocrit value at 35% to be representative of the population of interest.

Selectivity, sensitivity

Selectivity was investigated using six independent sources of human blood. DBS samples were prepared from drug free blood and from blood spiked with the antipsychotic drugs and metabolites of interest at the expected lower limit of quantification (LLOQ). The lack of endogenous interferences was checked by carefully examining the chromatograms of blank samples in the retention window containing the peaks of the analyte(s) or the IS. Signal in blank should be less than 20% of the LLOQ area. The LLOQ was defined as the lowest amount of an analyte in a sample determined with precision and accuracy within ± 20%. It was identical with the concentration of the lowest calibration standard.

Linearity
The linearity of the methods was assessed by analyzing calibration samples spiked with the analytes at 8 concentration levels. Calibration standards analysis was performed over three different days. Calibration curves were established by plotting peak area ratio of the target compounds to the internal standard (IS) versus the nominal concentrations. The homoscedasticity assumption for each linear regression analysis was tested using the F-test. A minimum of 75% of the standard calibration samples had to be in the ±15% range (LLOQ:±20%) of the nominal value.

Accuracy and precision
Accuracy and precision were determined by replicate analysis of samples containing known amounts of the analytes. Quality control DBS samples were daily prepared at each concentration level (LLOQ, Low, Medium, High). Within day accuracy and precision were determined by analyzing QCs in six replicates in a single validation batch. At the LLOQ, between-day accuracy and precision were determined by repeating analysis of six replicates over three different days. For the highest QC levels, between-day accuracy and precision were determined by quantifying analytes at each concentration in duplicate over six different days. Precision was expressed by the coefficient of variation (CV). Between-day precision data were calculated using a one-way ANOVA analysis. Accuracy was reported as the relative error (RE) or bias to the theoretical value. The acceptance criteria for precision and accuracy were ±15 % except for LLOQ which should not deviate by more than 20%.

Carry over
Carry-over was evaluated by sequentially injecting an upper limit of quantification (ULOQ) sample immediately followed by two extracted blank DBS samples. The response in the first blank matrix injection at the retention time region of the analytes should be less than 20% of their mean response at the LLOQ. To avoid any additional interfering carry over from the puncher, two spots of paper soaked with methanol were punched and discarded after the highest calibrator and QC.

Extraction recovery and matrix effect
Recoveries and matrix effects were assessed with the post extraction addition technique adapted from Matuszewski[41] and using blood from six different donors. QC samples at low and high concentrations were created with 10 µL of blood spiked with analytes and precisely spotted on a DBS card in duplicate for each source. The whole DBS containing a known amount of each analyte were punched and analyzed according to the sample preparation procedure (samples A). Blank DBS were created in duplicate for each source with 10 µL of drug free blood. They were extracted and post fortified with the analytes at the same concentrations than in the QC extracts (samples B). Neat solutions (samples C) were prepared in five replicates with the extraction solution spiked with the analytes at the same concentrations than in the extracts A and B. Each extract was diluted in eluent A before injection as describe above. Recoveries were calculated as follow: recovery (%)= peak area of sample A/ peak area of sample B x100. Absolute matrix effects of each source were estimated for all analytes and the IS by matrix factors (MF) determined as MF= peak area of sample B/ peak area of sample C x100. IS corrected MF was calculated by dividing the MF of the analyte by the MF of the IS. The CV of the IS-normalized MF calculated from the 6 lots of matrix should not be greater than 15 %.

Hematocrit effect
To investigate the influence of the hematocrit on the method performances, drug-free blood samples were prepared with adjusted hematocrit values of 25, 30, 35, 40, 45, 50, 55 %. At each hematocrit level, DBS QC samples were prepared in triplicate at low, medium and high drug concentration. Samples were analyzed against a calibration curve prepared in blood with an hematocrit value normalized at 35%. The hematocrit effect was assessed by calculating analytical bias of the measurements in samples of different hematocrits versus samples made in blood with a normalized hematocrit at 35% at the corresponding concentration. A difference within ±15% was considered acceptable.

Impact of the spot volume
The influence of the spot volume on the assay must be assessed to allow self-sampling by the patient without any tool to deliver an accurate volume of blood onto the paper. DBS samples were prepared using increasing volumes of blood spiked with the analyte at three QC concentrations levels. Each QC sample made with a defined volume was analyzed in triplicate. Responses measured in QC samples made with different spot volumes were normalized to the responses obtained in DBS made with a standard volume of 50µL. A bias within ±15% was considered acceptable.

Impact of the punching location
In order to evaluate the influence of the punching position or chromatographic effect, QCs DBS samples at low and high concentrations were prepared in five-fold and punched out at the center and at the edge of the spot. Homogeneity of the spot was estimated through the bias of the response measured in spots from the edge with respect to the signal measured in spots punched at the center.

Influence of hemolysis
The influence of hemolysis on analyte determination was investigated because a small amount of hemolysis can occur during drug-free blood storage, and when it is spiked with working solution containing the analytes in methanol during preparation of QCs and calibration standards.

Blood samples spiked at the concentration of QC low, medium and high were stored 4 h at -80°C in order to fully hemolyze the blood. DBS samples were created in four replicates for each QC and were analyzed as described above. DBS QCs prepared with fresh non-hemolyzed blood were analyzed as well. The bias of QCs prepared in hemolyzed blood with respect to QCs prepared in fresh blood was calculated to assess the impact of hemolysis on the assay.

Stability

In order to study the stability of the analytes in DBS samples, QCs DBS samples at low and high concentrations were prepared and stored under several conditions. All QC's were analyzed in triplicate with fresh QC and along a freshly prepared calibration curve. Concentrations measured for each analyte were compared to the nominal value. Bias within ±15% of the nominal values was acceptable to conclude that the analyte was stable in DBS samples under the studied condition. Samples were stored with a desiccant in a sealed plastic bag. Stability of the analytes was investigated in the following conditions of storage: room temperature for 24h, 72h, 1 week, 10 days and 1 month; refrigerator (+2 to +8°C) for 72h, 1 week, 10 days, 1 month; -80°C for 20 days and 1 month. In order to mimic high temperature or humid conditions which can occur during the sample shipment by regular mail, some DBS samples were kept 36h in an oven at 60°C and, while some other samples were placed in a Dutch mail box outdoor during an April rainy week for 24h and 6 days. Stability of the extracts was also evaluated by re-injecting QC low and high kept 24 h at 10°C in the auto-sampler.

Clinical application

Before any implementation of DBS in clinical practice, a comparison of patient's paired DBS and plasma samples has to be performed. It allows establishing the correlation relationship between plasma and DBS concentrations in order to interpret results according to the appropriate therapeutic ranges. Therefore, a thorough clinical validation comparing DBS and plasma concentrations is currently started in our center. Nevertheless, as a proof of concept of the ability of the analytical method to be applied to real patients samples, the results of the first nine paired patient's samples (three per compound) are reported. Paired DBS and plasma samples were collected from patients treated by one of the antipsychotics under study, and being hospitalized in the Erasmus Medical Center, a tertiary center, or living in Middin, an institution for disabled persons in the Netherlands. Sampling occurred at a random time at steady state. Before DBS sampling, the finger was cleaned using water (no ethanol was used as this might influence the results). A single-use automatic lancet was used to prick the finger. One blood spot was collected on the DBS paper without pressuring the finger. Plasma samples were obtained by a venipuncture. Each patient's hematocrit was measured using the venous blood sample. DBS samples were analyzed according to the method described above and plasma samples were analyzed by UHPLC-MS/MS with our already developed and validated method.[37] The DBS to plasma ratio was calculated for each compound. The Medical Ethical Committee of the Erasmus MC approved the protocol of the clinical validation study.

RESULTS

Method development

There was no significant difference between response of the analytes quantified in fresh blood or in blood containing EDTA. Bias due to EDTA calculated for all analytes and concentrations were lower than 10% and ranged from -4.7% to 8.9% with an overall mean value of 1.3%. The effect of EDTA on the determination of the antipsychotics is negligible and batches of drug-free blood used to prepare QCs and calibration standards can be stored in EDTA coated tubes. Extraction with precipitant solvents mixture was preferred for its simplicity. Regarding the signal response, the highest areas were observed using an equal ratio of methanol and acetonitrile. The addition of a strong precipitant as zinc sulphate (10%) did not increase the signal response. Addition

Table 3 Within day and between day accuracy and precision of the DBS assay

Analyte	Concentration (µg/l)	Within day precision (CV%)	Between day precision (CV%)	Within day accuracy (bias %)	Between day accuracy (bias %)
9-OH-Risperidone	LLOQ	15.1	12.2	13.1	15.1
	QC low	11.6	9.3	4.4	4.6
	QC medium	4.9	4.4	2.8	1.9
	QC high	3.8	4.2	8.9	2.9
Risperidone	LLOQ	10.1	16.0	14.7	19.8
	QC low	4.1	8.4	6.4	2.7
	QC medium	4.1	4.1	4.6	4.1
	QC high	5.1	5.0	2.0	-0.7
Aripiprazole	LLOQ	12.5	10.2	-11.4	-8.5
	QC low	8.1	6.5	-2.0	-3.0
	QC medium	4.3	5.6	1.2	-0.2
	QC high	4.2	3.5	9.8	2.1
Dehydroaripiprazole	LLOQ	11.1	18.1	-11.4	-6.5
	QC low	4.2	8.9	0.3	-1.4
	QC medium	4.9	6.7	2.5	1.0
	QC high	4.2	3.8	9.4	1.7
Pipamperone	LLOQ	6.7	18.1	-1.8	1.2
	QC low	5.8	9.8	-3.8	-6.5
	QC medium	2.9	3.2	-0.7	1.6
	QC high	5.5	6.0	3.6	2.3

LLOQ: Lower limit of quantification; QC: quality control; CV: coefficient of variation

Table 4 Matrix effect and Recovery of analytes in DBS.

Analyte	Matrix effect				IS corrected matrix effect				Recovery			
	QC low		QC high		QC low		QC high		QC low		QC high	
	mean (%)	CV (%)	mean (%)	CV (%)	mean (%)	CV (%)	mean (%)	CV (%)	mean (%)	CV (%)	mean (%)	CV (%)
9-OH risperidone	94.3	9.9	110.1	6.1	102.7	3.6	99.4	1.7	90.2	3.9	90.3	6.0
Risperidone	140.3	12.1	123.4	8.7	154.7	6.0	125.7	5.2	92.7	6.6	86.3	5.7
Aripiprazole	89.1	12.2	95.8	6.0	96.5	3.9	100.0	1.7	89.6	7.1	91.1	4.8
Dehydroaripiprazole	90.8	9.7	94.7	6.1	98.9	2.4	98.9	2.3	88.2	5.3	90.7	5.8
Pipamperone	100.7	10.5	100.6	5.5	109.5	2.2	105.1	2.3	90.2	7.5	89.4	4.4

LLOQ: Lower limit of quantification; QC: quality control; CV: coefficient of variation.
Matrix samples from 6 different sources were investigated. Results express means and CV of matrix factors and recoveries determined in the different sources.

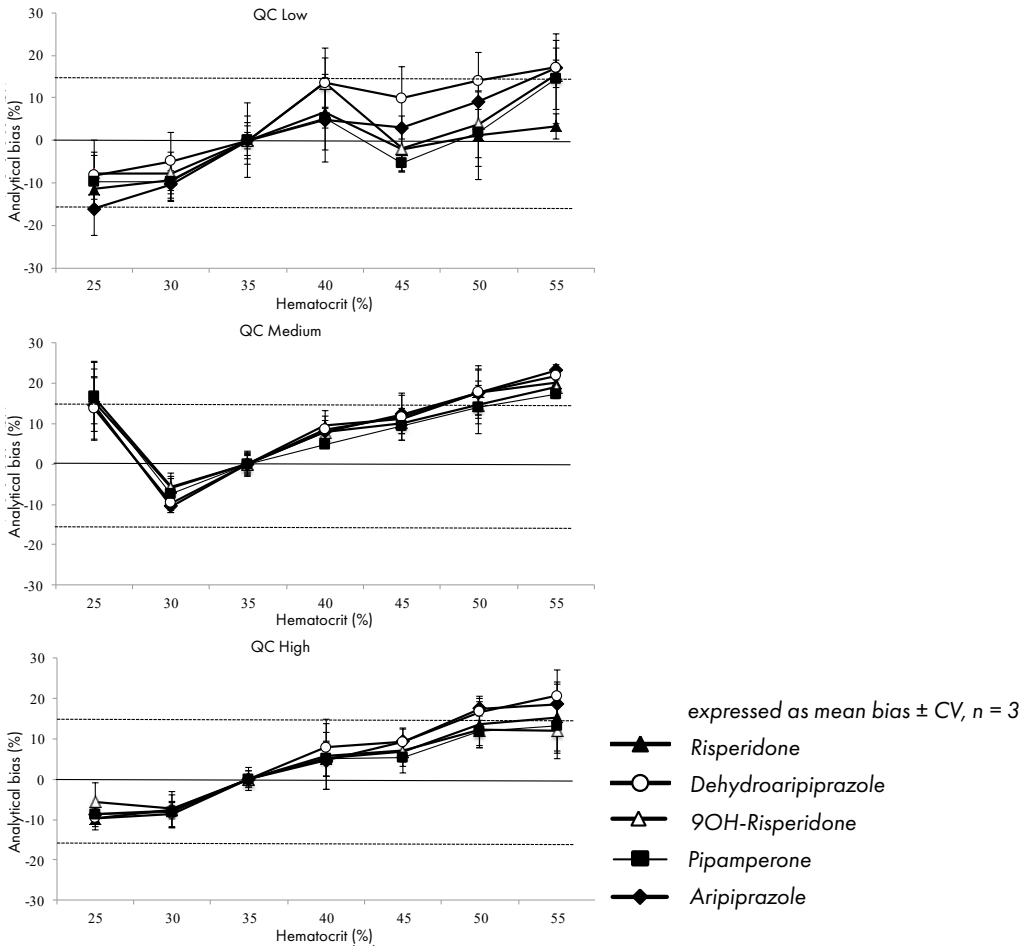
of trichloroacetic acid resulted in the paper dissolution. Addition of eluent A (acidic aqueous solution) in the mixture was needed to avoid new precipitation of proteins and opacity in the vial to inject after final dilution of the extract in the mobile phase. The efficacy of this ternary mixture was confirmed by a low value of optical density of the final extract at the absorption length of proteins (0.08 versus 0.23 for the binary mixture ACN/MeOH). Liquid extraction with ethyl acetate was very time consuming due to the solvent evaporation step and was unable to extract 9-OH risperidone. As usually described in literature, sonication was used to increase recovery of the analytes.

Validation

Selectivity, sensitivity

There were no interferences at the retention times of the analytes and IS in the six human bloods. Areas in blank samples were less than 20% of the areas in spiked samples at the LLOQ concentration. Thus, the method was found sufficiently selective and sensitive.

Figure 1 Influence of the hematocrit on analytical bias at each QC level.



Linearity

The linearity of the method was obtained for all analytes over the calibration range detailed in **table 2**. Heteroscedasticity was found for all compounds. The best fit was obtained with a weighing factor of 1/x. The back calculated concentrations of the calibration standards were within the acceptance criteria (± 15%) and the coefficient of correlation was > 0.99 for every validation analysis.

Accuracy and precision

Results of within day and between day precision and accuracy determined at four concentrations are detailed in **table 3**. They fulfilled the acceptance criteria since bias and CV did not exceed 20% for LLOQ and 15% for other QC concentrations.

Carry over

No carry-over was observed in the validation experiments for all analytes as the responses of the blank sample after injecting an ULOQ sample were less than 20% of the response of the LLOQ samples. A maximum percentage of 10% was observed for aripiprazole with a trend to increase over the time. To prevent accumulation and release of the compound in the system, two blank samples were injected after a ULOQ in analytical sequence and column was rinsed with eluent B after each run.

Extraction recovery and matrix effect

Recoveries and matrix effects determined from 6 different sources are presented in **table 4**. Recoveries were high and around 90% for all analytes at low and high concentrations. We did not observe any absolute matrix effect beyond 15% except for risperidone. Indeed, for this analyte matrix interference caused a significant positive bias since mean matrix factor was higher than 20% for both concentrations. The enhancement remained stable without variability among samples from different sources since coefficients of variation of matrix factors were less than 15%. The lack of relative matrix effect was also observed for 9-OH risperidone, aripiprazole, dehydroaripiprazole and pipamperone.

Hematocrit effect

The effect of hematocrit was investigated at low, medium and high concentration for hematocrits ranging from 25% to 55%. Results are presented in **figure 1**. It appears that hematocrit influences the accuracy of the measurement. The same pattern is observed for all analytes. Except for QC medium with a hematocrit of 25%, the bias from the standard hematocrit level (35%) tends to be negative for samples with low hematocrit and positive for those with a high hematocrit. However, deviations from the standardized QCs with a hematocrit of 35% were within +/-15% for all analytes when the hematocrit ranged between 30% and 45%. Considering analytes individually, the acceptable hematocrit range was found wider for 9-OH risperidone and pipamperone since bias was lower than 15% in samples with hematocrit ranging from 30% to 50%.

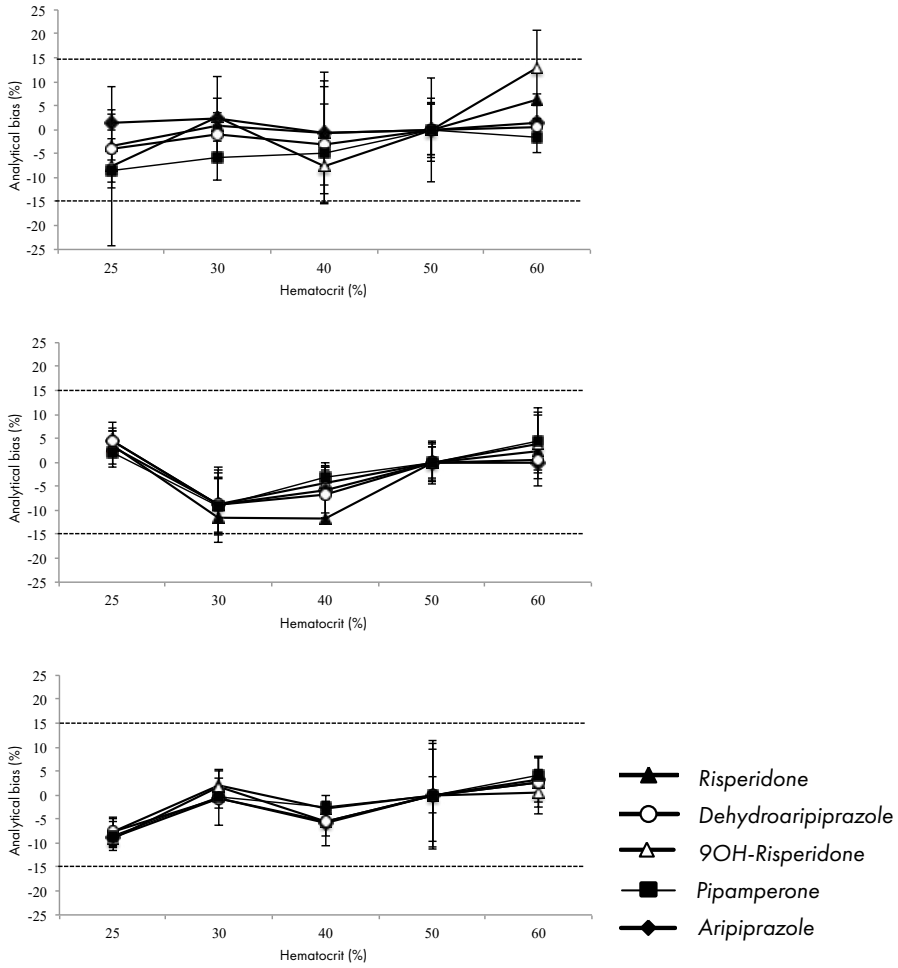
Impact of the spot volume

The spot size had a minor impact on the quantification of the antipsychotics and their metabolites. Results are presented in **figure 2**. A maximum bias of 12.8% was observed for 9-OH risperidone at low concentration for a spot of 60µL. The lack of influence of the spot volume suggests a homogeneous distribution of the blood through the paper.

Impact of the punching location

Concentrations measured in DBS punched at the edge of the spot were not substantially different from those measured in DBS punched at the center, as presented in **table 5**. Bias was less than 10% at low and high concentration. This indicates that diffusion of the blood is uniform through the paper.

Figure 2 Influence of spotting volume on analytical bias (n=3).



Expressed as mean bias ± CV, n = 3 at low (A), medium (B) and high (C) concentration levels. Nominal concentrations are respectively 3, 15 and 100 µg/l for risperidone and 9-OH risperidone and 70, 350, 500 µg/l for aripiprazole, dehydroaripiprazole and pipamperone.

Table 5 Impact of the punching location.

Analyte	Bias edge/centre (%)	
	QC Low	QC High
Risperidone	-4.1	-2.9
9-OH-Risperidone	-8.1	-1.3
Aripiprazole	6.6	1.3
Dehydroaripiprazole	3.7	0.4
Pipamperone	-0.2	-0.2

Results are reported as bias between the mean response of DBS punched at the peripheral part (of a 100µL blood drop) versus at the centre (n=5 per location) QC: quality control

Influence of hemolysis

The deviation of the response between non-hemolyzed and hemolyzed was less than 5% for all analytes at the three concentration levels (**Table 6**). It means that the slight hemolyzation which can occur in the blood used to prepare QC and calibration standards does not affect the analysis.

Table 6 Influence of hemolysis

Analyte	Bias hemolyzed blood/fresh blood (%)		
	QC Low	QC Medium	QC High
Risperidone	-3.4	-0.8	-5.0
9-OH-Risperidone	3.0	-0.8	-3.8
Aripiprazole	-0.7	3.4	-1.2
Dehydroaripiprazole	-4.4	2.4	-3.3
Pipamperone	-4.3	1.5	-2.6

Results are reported as bias between the mean response of DBS samples made with hemolyzed blood (stored 12h at -80°C before spot) versus non hemolyzed blood (n=5 per type of blood)
QC: quality control

Stability

Analytes were found to be stable in an autosampler for 24h. As shown in **table 7**, at room temperature they were stable over 10 days of storage. After 1 month, a significant degradation was observed for aripiprazole at low concentration and dehydroaripiprazole at high concentration (-17.2 % and -15.3% respectively). Samples were stable after storage at 4°C and -80°C for 1 month. Degradation of the analytes in samples stored outside in a moist atmosphere for 24h did not result in a loss higher than 15%. Except for Aripiprazole and dehydroaripiprazole, the antipsychotics could handle these conditions of storage for 1 week. Only risperidone was found stable in DBS kept 36h at 60°C.

Table 7 Stability in DBS. Results are expressed as percentage of bias compared to nominal concentration (n=3).

		9 OH-risperi-done		Aripiprazole		Dehydroaripip-razole		Pipamperone		Risperidone	
		QC low	QC high	QC low	QC high	QC low	QC high	QC low	QC high	QC low	QC high
20°C	24h	-4.8	11.4	-7.0	7.7	-6.1	9.1	-9.8	3.7	-4.1	5.6
	72h	15.2	-3.3	2.0	0.5	-2.5	-5.5	4.7	3.3	12.9	1.7
	1 week	-2.2	-6.1	-3.3	-2.9	-1.6	-4.1	-7.8	-2.3	-10.5	-7.9
	10 days	-7.5	2.8	-14.3	-5.9	-10.6	-4.0	-9.9	-5.8	3.1	-11.1
	1 month	-12	-7.2	-17.2	-10.8	-13.7	-15.3	-13.4	-8.9	-6.1	2.7
4°C	72h	18.2	-6.1	6.8	-2.4	0.8	-6.4	8.2	-1.7	12.3	0.8
	1 week	-4.8	-5.3	-6.3	-1.9	-4.4	-1.3	-7.5	-4.6	-12.2	-12.3
	10 days	4.5	10.1	-11.9	3.0	-9.0	5.6	-9.0	4.2	4.0	0.3
	1 month	-2.8	-2.7	-11.2	-4.1	-6.8	-10.3	-11.3	-9.9	-5.2	1.7
-80°C	20 days	6.8	6.3	-6.6	8.6	-2.2	2.9	-4.2	6.3	-17.5	5.5
	1 month	7.3	-13.5	-14.6	-11.9	-16.5	-17.6	-8.1	-13.3	1.4	-2.0
Humidity*	24h	5.9	-12.8	-12.1	-13.4	-10.4	-12.3	-4.9	-14.3	-2.6	-14.7
	1 week	-10.9	-10.5	-14.1	-17.1	-15.5	-13.2	-13.0	-9.4	-3.4	-13.0
60°C	36h	-8.6	-17.4	-17.6	-18.5	-17.1	-19.7	-18.4	-21.9	-7.2	-11.9

** humidity conditions: to mimic real conditions, DBS card were placed in a zip bag with desiccant bag in a plastic box (non hermetic) outdoor during a rainy week QC: quality control*

Clinical application

The method was successfully applied to the analysis of DBS samples obtained from three subjects per drug (4 females and 5 males) between June and December 2016. Delay between last drug intake and sampling ranged from 10 hours to 21 hours. Samples median hematocrit was 41%. Antipsychotics and their corresponding metabolites were detected in DBS and plasma for every patients treated. Concentrations measured in DBS and plasma and mean blood to plasma ratios are reported in **table 8**.

Table 8 DBS and plasma concentrations of the antipsychotics and their metabolites determined in nine patient samples and mean (+/- standard deviation) concentration ratios

Antipsychotics	DBS concentration (ng/mL)	Plasma concentration (ng/mL)	Mean DBS to plasma ratio ± standard deviation
Aripiprazole	56.6	133.1	0.62 ± 0.18
	18.9	23.9	
	287.4	449.0	
Dehydroaripiprazole	21.6	54.6	0.60 ± 0.18
	13.0	17.5	
	85.7	132.7	
Pipamperone	6.4	33.1	0.21 ± 0.03
	6.9	27.6	
	27.5	140.1	
Risperidone	0.1	0.1	0.74 ± 0.18
	0.5	0.8	
	0.7	0.9	
9-OH risperidone	13.2	24.3	0.57 ± 0.10
	7.1	14.4	
	14.5	21.0	

DISCUSSION

The analytical procedure for simultaneous determination of three antipsychotics and their major metabolites in DBS was extensively validated according to EMA and FDA requirements. In order to take into account specific issues due to the DBS sampling strategy, additional parameters were thoroughly investigated according to literature recommendations.[31-34]

The developed method is based on an effective and simple extraction by a precipitant mixture as demonstrated by the high recoveries and the low matrix effects observed. Interestingly, haloperidol-d4 was chosen as single IS based on the development of another method measuring the same analytes in plasma.[37] Indeed, it was found that performances of the method was good enough using only one deuterated IS instead of the deuterated form corresponding to each analyte. In DBS as well, the use of haloperidol-d4 appeared suitable to quantify every compounds with accuracy and precision. In addition, haloperidol-d4 could correct for a relative matrix effect since inter-individual CV of matrix factors from blood of six different sources were lower when IS was taken into account. Although a signal enhancement was observed for risperidone, it remained constant between matrix sources. Moreover the use of this single IS for a multiplex quantification presented obvious practical advantages.

Repeatability and accuracy were within the acceptance criteria for all analytes. However between days accuracy of risperidone at the LLOQ revealed a positive bias close to the acceptable limit (19.8%). The bias observed at low concentration might be explained by the ion enhancement suggested for risperidone in the experiments determining matrix factors. Considering the therapeutic range of the drug is largely higher than the LLOQ value (risperidone+9-OH-risperidone: 20-60 µg/L), clinical consequences of this variability may be limited and should not be an issue for dose adjustment. The European Bioanalysis Forum DBS microsampling consortium conclusions33 support this idea by considering that acceptance criteria of a DBS method could be widened if patient safety is not impacted.

Interestingly, the current assay is the only one able to provide a reliable measurement of pipamperone in DBS since in the study by Patteet et al., accuracy requirements were not met for pipamperone at low concentrations.[30]

The hematocrit effect is known to be the most important hurdle to the implementation of a DBS method since variation in hematocrit level leads to variations in the viscosity of the blood spot and consequently alters diffusion of the blood on paper. In the current method based on partial punch of the DBS, variations of hematocrit within 30% to 45 % did not produce any concerning bias. Thus, for TDM purposes it seems appropriate to analyse patients samples against calibration curves prepared using blood with hematocrit of 35%, since hematocrit values outside the validated range (30-45%) are not expected in children with autism.[36] However, it would be optimal to know the hematocrit of each sample prior to analysis. This appears challenging when home sampling is intended. The blood parameter cannot be directly determined from DBS sample since liquid blood is required. An alternative strategy has been proposed by Capiau et al.[42] The authors estimated hematocrit through potassium measurement in DBS since both parameters were found well correlated. In the case of patients treated for autism, somatic comorbidities are not likely to affect the hematocrit which should remain stable over the time.[43] Thus, a single determination of the patient hematocrit in a venous blood sample could be performed before a TDM follow up with DBS in order to identify patients whom antipsychotics concentrations would need to be corrected for the hematocrit.

Along method development common strategies reported in the literature to cope with the hematocrit effect aimed at suppressing it, were investigated. To soak the punched DBS with water before extraction tended to increase bias observed with extreme hematocrit levels. The increase of positive bias in sample with high hematocrit suggests that this approach is beneficial to improve analytes recoveries only when they are in blood of high viscosity. On the contrary, Li et *al.*[44] succeeded in decreasing hematocrit effect in lansoprazole determination using pre-soaking, however they used this strategy combined with the analysis of the whole spot of a pre-perforated DBS. This approach was also assessed to decrease bias in the antipsychotics determination due to varying hematocrits. As explained by Fan et *al.*[45], whole spot analysis avoids bias due to inhomogeneity of the blood spreading on the filter paper. The major drawback of this strategy is the need for an accurate volume of blood which is difficult to consider in the case of patient self-sampling. Results of the experiment showed a lower bias in samples with low hematocrit when analyzing the entire spot but an important negative bias was observed with increasing hematocrit values and high concentrations (data not shown). This highlights that hematocrit does not only impact spot size and spreading but also analytes recoveries from the paper. Nevertheless, reduction of the hematocrit effect by analyzing the whole spot was achieved by several authors when they associated specific systems or devices.[46-50]

During the stability study, all analytes were found stable in DBS for at least 10 days at room temperature or 4°C. This is relevant for TDM application. In addition, the impacts of high temperature (60°C) and wet atmosphere were investigated to mimic extreme conditions which can occur during sample shipment by regular mail. The effect of a moist atmosphere was not found critical up to 1 week for all the analytes but one. Except for risperidone, high temperature exposition leads to analyte degradation after 36h, therefore analysis of DBS samples received by regular mail in such hot climatic condition must be discussed.

The relation between plasma or venous blood and capillary DBS concentrations is another crucial point to take into account to implement a DBS method in clinical practice.[22,34] Current therapeutic ranges of antipsychotics were determined in plasma. Therefore, threshold concentrations might differ if the measurement comes from a whole blood sample like DBS. The results obtained from the analysis of incurred patient's DBS and plasma samples confirm these expectations. Indeed, whole blood concentrations determined in DBS were lower than plasma concentrations. This might be explained by the high level of plasma protein binding of antipsychotics drugs and thus less partition into blood cells.[51] In this way, blood cells act like a diluent, leading to a lower concentration in

whole blood samples. Moreover, a phenomenon of dilution of the capillary blood by interstitial fluid that comes with the fingerprick could also occur. Interestingly, despite the small number of subject analyzed, our results are comparable with those reported by Patteet et *al.*[52] in a clinical validation study of capillary DBS. Indeed, these authors found whole blood to serum ratios of 0.6, 0.7 and 0.7 for risperidone, 9-OH risperidone and aripiprazole respectively. We observed that DBS to plasma concentrations ratio were considerably different between drugs. This might be attributed to each drug specific distribution or binding to the blood components. For instance, for pipamperone we found a DBS to plasma ratio significantly lower than the other antipsychotics. The reason of this difference is unclear. Nevertheless, one can make the hypothesis that this is due to differences in the physicochemical properties of this first generation antipsychotic which is maybe less likely distributed in red blood cells. Unfortunately, although pipamperone was launched several decades ago, data about drug distribution in blood components are sparse.

Obviously, these results need to be further confirmed in a complete clinical validation, including a larger number of subjects. Such a study has just been started in our center and should provide additional information about the correlation relationship between DBS and plasma concentrations in vivo and the conversion from the standard analytical method using plasma to the alternative analytical method using DBS.

CONCLUSION

An UHPLC-MS/MS method has been successfully validated for the simultaneous quantification of risperidone, 9-OH risperidone, aripiprazole, dehydroaripiprazole and pipamperone in DBS.

Based on a quick and simple sample preparation procedure, the assay demonstrated good analytical performances and was applied on patient samples analysis. Because no volumetric device is required to collect blood, home sampling can be considered. An extensive clinical validation, establishing the correlation relationship between plasma and DBS concentrations, is now required to rely on DBS as an alternative to collect samples for TDM of pediatric patients with ASD in an easy, stressless and painless way.

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CHAPTER 5

DRIED BLOOD SPOT ANALYSIS FOR THERAPEUTIC DRUG MONITORING OF ANTIPSYCHOTICS: DRAWBACKS OF ITS CLINICAL APPLICATION

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Therapeutic Drug Monitoring
2018;40(3):344-50.

ABSTRACT

Background

Dried Blood Spot (DBS) sampling offers a minimally invasive sampling method for therapeutic drug monitoring of antipsychotics. To facilitate implementation in clinical practice, the aim of this study was to perform a clinical validation study of a DBS method for quantification of risperidone, aripiprazole, pipamperone and its major metabolites 9-OH risperidone and dehydro-aripiprazole in a real-life, clinical setting.

Methods

Paired DBS and venous plasma samples were analyzed (n=35 for risperidone, n=21 for aripiprazole, n=21 for pipamperone). Estimated plasma concentrations were calculated from DBS concentrations based on hematocrit and/or Deming regression formulas. Deming regression and Bland Altman analyses were used to determine the agreement between the calculated and measured plasma concentrations. For Bland Altman analysis, the following acceptance limit was used: for a minimum of 67% of the samples, the difference of the two measurements should be within 20% of their mean.

Results

The median venous plasma levels were 0.9 µg/l for risperidone, 14.8 µg/l for 9-OH risperidone, 135.4 µg/l for aripiprazole, 54.9 µg/l for dehydro-aripiprazole, and 56.4 µg/l for pipamperone. All antipsychotics required different correction formulas of DBS concentrations for best agreement. Subsequently, no constant or proportional bias was observed using Deming regression analysis. With Bland Altman analyses, for risperidone, 48% of the samples were within the 20% limits; for 9-OH risperidone, 36%; for aripiprazole, 40%; for dehydro-aripiprazole, 40%; and for pipamperone, 33%.

Conclusions

The DBS method to quantify risperidone, aripiprazole, pipamperone, and their major metabolites did not meet the acceptance criteria in the Bland Altman analyses. Therefore, this DBS method was not clinically valid. This study shows the importance of a clinical validation study with use of Bland Altman plots prior to clinical implementation.

INTRODUCTION

Antipsychotic (AP) drugs are widely used. Second-generation APs have become increasingly popular due to the belief of having more favorable side effects than the first-generation APs. This has led to an increased use of second-generation APs worldwide.[1, 2] Indications for use of APs include, among others, schizophrenia, bipolar disorder, psychosis, and behavioral problems associated with autism and/or mental retardation.

Unfortunately, both second- and first-generation APs are associated with major adverse effects. First-generation APs like haloperidol may cause extrapyramidal symptoms, while second-generation APs mainly induce metabolic changes including weight gain and the metabolic syndrome.[3] Furthermore, these side effects might have serious long-term consequences. Extrapyramidal symptoms might continue even after drug discontinuation, while the metabolic syndrome significantly increases the risk of developing diabetes and cardiovascular problems in the long term. In this way, APs might have a major negative impact on morbidity and mortality.[4]

There is evidence that therapeutic drug monitoring (TDM) can help in maximizing clinical efficacy while minimizing the risk of side effects of APs.[5] For example, for the second-generation APs risperidone and aripiprazole, a relationship is shown between plasma levels and both efficacy and safety.[6-8] TDM might thus help to find adequate dosages to reduce the incidence of the serious long-term side effects.

However, blood sampling may be challenging in populations that use antipsychotics. These include psychotic patients, mentally disabled persons, and children with autism, who may be more anxious or difficult to reach. Therefore, new sampling techniques have been explored, including the Dried Blood Spot (DBS) method.[9] DBS uses a simple fingerprick to collect a small amount of blood on filter paper. The minimally invasive technique, sample stability, and allowance for home-sampling appear to be advantageous compared to the conventional phlebotomy, especially for more vulnerable populations.

For this reason, several analytical methods for the determination of APs with DBS have been developed and reported in the literature. Patteet et al. published a clinical study of a DBS method to determine 16 APs and 8 metabolites.[10] However, blood was obtained by a capillary. Others published DBS assays to quantify clozapine and ziprasidone.[11] Recently, Tron et al. published a bioanalytical validation of a DBS method to determine the three most used APs in children in the Netherlands in this journal.[12] An advantage of the latter method is that no capillary is needed for sampling, which facilitates self-sampling in a home environment.

It is generally accepted that a thorough clinical validation is needed before implementation of a DBS method in routine care.[13, 14] Therefore, in the continuity of the work of Tron et al., we

performed a clinical validation study of the DBS method to determine risperidone, aripiprazole, and pipamperone, and their major metabolites 9-OH risperidone and dehydro-aripiprazole in a real-life clinical setting.

MATERIALS AND METHODS

Patients

81 patients aged 18 years or older who used either risperidone, paliperidone (the active metabolite of risperidone), aripiprazole, or pipamperone were included. Patients were recruited in the Erasmus Medical Center, an academic tertiary center, and Middin, a residence for mentally and/or physically disabled persons, both located in the Netherlands. All patients and/or their legal representatives gave written informed consent before entering the study. Patients were recruited between June 2016 and May 2017. The study was approved by the medical ethical committee of the Erasmus Medical Center and is registered in the Dutch Trial Register with number 6655.

Sampling

DBS samples were collected by SMK and LAH. Before sampling, the fingers of the patients were cleaned with water and regular soap or, if that was not possible, by wiping with a wet gauze. No alcohol was used as this might bias the biochemical analysis. Both a fingerprick and a venous blood sample were obtained simultaneously with a mean sampling time difference of 3 minutes. During DBS sampling, 1 to 4 falling drops were separately collected (including the first drop). After drying for several minutes, samples were stored at room temperature in an envelope containing silica desiccant. Upon arrival at the laboratory, DBS samples were stored in a desiccator at room temperature until analysis. Venous samples were centrifuged, and plasma was subsequently stored at −80 °C until analysis. Hematocrit was determined in the venous sample within 24 hours.

Analysis

DBS samples were prepared for bioanalytical analysis as described previously.[12] Concentrations of risperidone, 9-OH risperidone, aripiprazole, dehydro-aripiprazole, and pipamperone in both DBS and plasma samples were determined using ultra-high-pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), which was fully validated according to EMA and FDA guidelines.[12, 15] The Lower Limit of Quantification (LLOQ) was established as follows: for risperidone, 1.0 µg/l; 9-OH risperidone, 0.7 µg/l; aripiprazole, 10.0 µg/l; dehydro-aripiprazole, 10.0 µg/l; and pipamperone, 1.5 µg/l.

Calculation of estimated plasma concentrations

Estimated antipsychotic plasma concentrations (EPC) were calculated from DBS concentrations using two converting formulas.

Formula 1: $Plasma_{estimated\ concentration} = DBS_{concentration} / (1\text{-}hematocrit)$

This previously described formula corrects for the influence of hematocrit.[16] The fraction bound to red blood cells could be ignored in **formula 1**, as the blood:serum ratio of the investigated antipsychotics is around 0.6, which means only a small fraction partitions into blood cells.[10] Furthermore, the antipsychotics are characterized by high protein binding. The unbound fraction, which is the fraction that can partition into blood cells, can thus be concerned negligible.

For the second converting formula, the plasma and DBS concentrations were plotted with plasma levels on the x-axis and DBS levels on the y-axis. Then, a Deming regression analysis was performed. The slope and the intercept of the regression formula were used to convert DBS to estimated plasma concentrations as described in **formula 2**.

Formula 2: $Plasma_{estimated\ concentration} = (DBS_{concentration} - intercept_{Deming\ regression}) / slope_{Deming\ regression}$

Additionally, the agreement was checked with **formula 3**:

Formula 3: $Plasma_{estimated\ concentration} = DBS_{concentration} / slope_{Deming\ regression}$

All converting formulas were used separately and combined to determine the formula with the best agreement between the two methods.

Statistics

Agreement between the DBS and plasma samples was determined using Deming regression analysis and Bland Altman plots. Deming regression was used to test for a proportional and constant bias. When the 95% CI of the slope of the regression formula includes 1, no proportional bias is observed. When the 95% CI of the y-intercept includes 0, no constant bias is observed. Bland Altman plots were used to test the agreement between the two methods. According to the guideline *Bioanalytical Method Validation* of the European Medicines Agency,[17] the difference between DBS and plasma concentrations should be within 20% of the mean of the concentrations for at least 67% of the samples.

Stratification based on patients' department type was made to see if agreement between the two methods differed for somatically and non-somatically ill patients. Non-somatic departments included the psychiatric departments of the Erasmus MC and the residence for mentally and physically disabled persons. Somatic departments included all other departments, concerning all non-psychiatric departments of the Erasmus MC.

If an analyte did not meet the Bland Altman acceptance criteria, we calculated the percentage of variance (>20%), which shows an agreement for 67% of the samples.

Analyses were performed with Excel 2010 (Microsoft Corp., Redmond, WA, United States), IBM SPSS Statistics version 21 for windows (IBM Corp.,Armonk, NY, Untited States) and Graphpad Prism 5 (GraphPad Software, La Jolla California, United States).

RESULTS

A total of 82 paired samples were taken from 81 unique patients. For 4 patients, DBS sampling yielded not enough blood volume for analysis. This was due to cold extremities in bedridden patients with impaired peripheral circulation, or motoric restlessness in patients with severe mental retardation. One patient was excluded as the samples were taken directly after intake of the antipsychotic (within the distribution phase), which caused a large difference in concentrations between capillary and venous taken blood. A total of 35 samples of patients using risperidone, 21 using aripiprazole, and 21 using pipamperone could be analyzed. The baseline characteristics of the patients are presented in **table 1.**

Table 1 Baseline characteristics of patients included in the study

	Risperidone (n=32)Δ	Aripiprazole (n=19)Δ	Pipamperone (n=21)
Age (y)	49 (29-59)	45 (25-61)	54 (37-66)
Female (n)	14 (44%)	13 (68%)	10 (48%)
Length (cm)	175 (169-182)	170 (165-185)	170 (160-176)
Weight (kg)	76 (65-89)	83 (78-99)	78 (58-95)
Daily dosage (mg)	2.0 (1.6-4.0)	10 (5-20)	40 (20-70)
HtO (L/L)	0.39 (0.34-0.43)	0.43 (0.35-0.45)	0.41 (0.36-0.44)
Department* (n)			
Somatic	15 (47%)	10 (53%)	6 (29%)
Non-somatic	17 (53%)	9 (47%)	15 (71%)
Indication (n)			
Psychosis	14 (44%)	6 (32%)	1 (5%)
Behavioral problems	5 (16%)	1 (5%)	12 (57%)
Schizophrenia	4 (13%)	4 (21%)	0 (0%)
Delirium	3 (9%)	3 (16%)	0 (0%)
Bipolar disorder	1 (3%)	3 (16%)	1 (5%)
Other	5 (16%)	2 (11%)	7 (33%)

Values are presented as median (25th-75th percentile) unless stated otherwise
Δ 3 patients using risperidone and 2 patients using aripiprazole gave consent for 2 times DBS-venous sampling on 2 different days.
O Hematocrit missing for n=2 for risperidone, n=1 for aripiprazole, n=1 for pipamperone
** Non-somatic departments include the psychiatric departments of the Erasmus MC and all departments of the residence for mentally and physically disabled persons, somatic departments include all non-psychiatric departments of the Erasmus MC.*

The median plasma levels (25th – 75th percentile) measured in the venous samples were 0.9 (0.4-7.2) µg/l for risperidone, 14.8 (8.7-32.3) µg/l for 9-OH risperidone, 135.4 (74.2-226.9) µg/l for aripiprazole, 54.9 (28.7-73.0) µg/l for dehydro-aripiprazole, and 56.4 (30.3-119.7) µg/l for pipamperone. For risperidone, 18 (51%) of the samples were below the LLOQ, as well for DBS as venous samples. For 9-OH risperidone, none of the DBS or venous samples were below the LLOQ. For aripiprazole, 1 (5%) of the DBS samples was below the LLOQ, and for dehydro-aripiprazole, 2 (10%) samples of both DBS and venous samples were below the LLOQ. For pipamperone, 2 (10%) of the DBS samples were below the LLOQ.

Deming regression

Deming regression analysis showed a constant bias for all analytes. Therefore, corrections of DBS values according to formulas 1,2 and 3 were applied to derive estimated plasma concentrations (EPCs). For risperidone, aripiprazole, and their metabolites, correction based on the hematocrit using formula 1 improved agreement, while for pipamperone, hematocrit correction did not improve results. For aripiprazole, dehydro-aripiprazole, and pipamperone, (subsequent) correction based on Deming regression (formula 3) showed better agreement. The final EPC correction formulas are shown in table 2. The Deming regression analysis with these EPCs showed no proportional or constant bias for all analytes. The results of Deming regression analyses are shown in **table 2**.

Table 2 Results of Deming regression analyses for the comparison of DBS and Estimated Plasma Concentrations

Analyte	n*		Conversion formula*	Slope	95% CI	Intercept	95% CI
Risperidone	33	1,3	EPC = (DBS/(1-ht))/1.120	0.999	0.935-1.062	-0.339	-1.468-0.791
9-OH risperidone	33	1,3	EPC = (DBS/(1-ht))/0.996	1.001	0.813-1.188	0.069	-7.545-7.682
Aripiprazole	20	1,3	EPC = (DBS/(1-ht))/1.263	0.977	0.754-1.200	-22.71	-65.52-20.11
Dehydro-aripiprazole	20	1,3	EPC = (DBS/(1-ht))/1.242	0.958	0.642-1.273	-7.291	-27.58-12.99
Pipamperone	21	3	EPC = DBS/0.158	1.216	0.839-1.593	-0.682	-49.34-47.97

*Ht, hematocrit; EPC, estimated plasma concentration. *For formula 1, only cases with known hematocrit were used. Hematocrit missing for n=2 for risperidone, n=1 for aripiprazole*

Bland Altman analysis

Bland Altman analysis was applied to all analytes using the plasma concentrations and EPCs. Results are presented in **table 3**. The plots of risperidone, aripiprazole, and pipamperone are shown in **figures 1**, **2**, and **3**, respectively. None of the analytes meet the acceptance limits.

When neglecting the acceptance limits, for 67% of the risperidone samples, the difference between plasma and EPC levels lay within 30% of the mean of the samples. For 9-OH risperidone this was 35%. For aripiprazole and its metabolite, this was 47% and 52%, respectively. For pipamperone, for 67% of the samples, the difference lay within 40% of the mean.

The results of the Bland Altman analysis did not improve when only patients from non-somatic departments were analyzed. Therefore, somatic illness did not influence the agreement between DBS and venous sampling.

Table 3 Results of Bland Altman analysis of DBS versus Estimated Plasma Concentrations

Analyte	n*	Mean bias	95% limits of agreement	Δ within 20% of average (%)	Δ within 25% of average (%)
Risperidone	33	-0.349	-5.889-5.192	48	55
9-OH risperidone	33	0.085	-27.685-27.855	36	52
Aripiprazole	20	-26.308	-118.713-66.098	40	45
Dehydro-aripiprazole	20	-9.639	-47.359-28.081	40	45
Pipamperone	21	18.204	-103.599-140.006	33	52

For formulas of estimated plasma concentrations, see table 2.
**For risperidone, aripiprazole, and their metabolites, only cases with known hematocrit were analyzed.*

Hematocrit missing for n=2 for risperidone, n=1 for aripiprazole.

Figure 1 Bland Altman plot for Risperidone

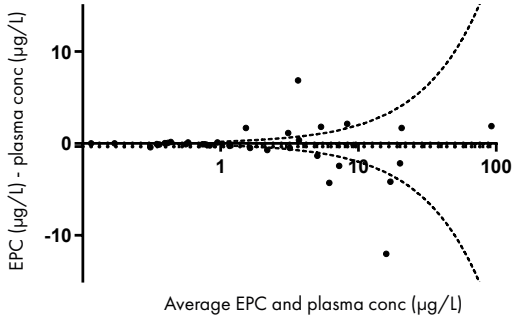


Figure 2 Bland Altman plot for Aripiprazole

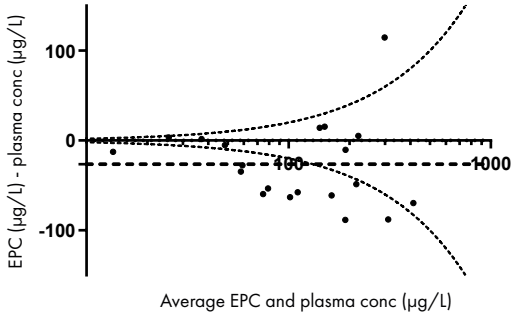
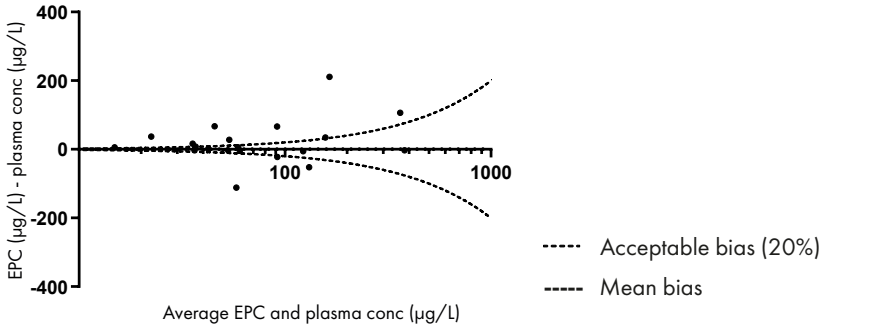


Figure 3 Bland Altman plot for Pipamperone



EPC: Estimated Plasma Concentration

DISCUSSION

The present clinical validation study shows that DBS and plasma concentrations of risperidone, aripiprazole, pipamperone, and their major metabolites have good agreement based on Deming regression analysis, but do not fulfill Bland Altman acceptance limits. Therefore, not all criteria for a completely successful clinical validation have been met.

The Bland Altman analysis is a simple way to evaluate the bias between two quantitative methods of drug measurement. Bland Altman analysis, in addition to weighted Deming or Passing Bablok regression analysis, is generally recommended for clinical validation of DBS assays.[13, 14] Despite this, Bland Altman analysis is often absent in clinical validation studies.[14] For instance, Patteet et al. performed a clinical study to determine [16] APs in DBS, only using Passing Bablok regression and a comparison of clinical interpretation. Despite the lack of Bland Altman analysis, these authors concluded that DBS can be used in routine clinical practice. However, our study demonstrates that Bland Altman might yield a large bias despite good correlation based on regression analysis. In general, this underlines the importance of Bland Altman analysis in clinical validation studies and warrants interpretation of studies without such analysis with great caution.

In this study, we used a hematocrit correction formula to assess best agreement between DBS and venous samples. For pipamperone, results did not improve with hematocrit correction, in contrast to the other compounds. This might be explained by a lower affinity of pipamperone to red blood cells, thus indicating that the DBS concentrations of pipamperone could be less influenced by hematocrit. Unfortunately, very little is known about pharmacokinetic properties of pipamperone, despite being on the market for several decades. To our knowledge, only two other clinical validation studies of DBS analysis for APs have been published, and neither of them reported using hematocrit correction.[11] A clinical validation study on Ziprasidone used a blood-to-plasma concentration ratio correction factor to derive estimated plasma concentrations,[18] while Patteet et al. did not report any conversion of DBS concentrations.[10]

The findings of this study are in contrast with the successful analytical validation of this DBS method that has been performed previously.[12] In this previous analytical validation, venous blood spotted on DBS paper showed good agreement with plasma samples. The discrepancy between the analytical and clinical validation might be explained by several factors. Firstly, with real-time DBS sampling, contamination with interstitial fluid and dermal flora takes place. These factors might have contributed significantly in our cohort. Inpatients using antipsychotics often express motoric restlessness and anxiety, which sometimes impairs adequate cleaning of the finger, or resampling in case of a limited blood flow from the fingerprick. The latter might require pressuring the finger, which might enhance hemolysis and contamination with more interstitial fluid. Secondly, the distribution phase of antipsychotics might be quite long, which means large differences between venous and capillary concentrations may occur. These factors might have complicated our clinical validation of DBS for antipsychotics.

Although not all clinical validation criteria have been met, the DBS assay for determining these antipsychotics could still be used in clinical practice, for example, as a medication adherence tool. In our study, antipsychotic drug concentrations could be determined in all analyzed DBS and plasma samples. Given this result, we can conclude that the sensitivity of DBS for qualitatively determining antipsychotic drugs is 100%. At the same time, antipsychotic drugs could not be quantified in the blanco samples that were measured during DBS analysis. Therefore, this shows that the DBS method can accurately measure if antipsychotic drugs have been taken by the patient. This might be of great value in daily clinical practice, as medication adherence for antipsychotic therapy has shown to be rather low.[19] Especially for patients that are difficult to reach, for example, because of isolated living circumstances by certain patients with schizophrenia, DBS might offer an easily applied measure to determine adherence at home.

Besides application as an adherence tool, our DBS method can also be useful for pharmacokinetic research purposes in vulnerable populations. For example, for children using antipsychotics, venous sampling can be very traumatic. A fingerprick is less invasive and of shorter sampling duration, which might increase willingness to participate in trials where repeated sampling is required. Furthermore, pharmacokinetic modeling programs, such as NONMEM, enable correction for a certain bias by considering extra variance of the DBS measurements. Different error models can be used to describe the effect of plasma samples versus DBS samples, in which the latter has a larger uncertainty.

This study has several limitations. Firstly, fewer than 40 samples were obtained per drug, despite the recommendation to include this number of samples in certain guidelines.[20] The main reason for lower number of samples was that eligible patients were often anxious, suspicious, or incompetent to give consent in case of a psychosis or delirium, which made them unsuitable for inclusion. However, other clinical validation studies have shown that it is possible to draw conclusions from smaller sample size.[21, 22] Furthermore, agreement between the two methods did not improve with larger patient numbers compared to earlier interim analyses with smaller sample sizes. This suggests that further enlargement of our sample sizes would not have improved the results. Secondly, another limitation is that somatically ill patients were also included, while the target population for at-home sampling consists of outpatient psychiatric patients who are, in general, not physically ill. Nevertheless, a stratification based on department type was made, which did not, however, show better agreement in the non-somatically ill patients. Thirdly, antipsychotic concentrations were in a relatively low range compared to the therapeutic reference range. Especially for risperidone, this led to a significant share of the samples being below the LLOQ. This might be explained by the fact that the therapeutic ranges are mainly based on treatment of patients with schizophrenia, in which higher dosages and other outcome measures are used, than for patients with, for example, behavioral problems. In our sample, only 11% of the patients used antipsychotics for schizophrenia. However, the samples with values below the LLOQ did show a similar agreement as the samples in the validated quantification range. Therefore, our method should be valid for populations with reduced dosing schedules, such as patients with delirium or in children. Lastly, in our study, we measured hematocrit in the venous sample, while this value possibly differs from the hematocrit measured in capillary blood. However, simultaneous measurement of hematocrit by DBS was not possible in our laboratory at the time of analysis.

This study also has several strengths. First of all, this is the first study that performed a complete clinical validation of a DBS method for risperidone, aripiprazole, and pipamperone using Bland Altman plots. For pipamperone, this was the first study to report the clinical validation of DBS sampling. Secondly, we performed a solid clinical validation according to current international guidelines, using typical patients in a real-life clinical setting. In this way, we have been able to simulate implementation in real clinical practice. This study can therefore be seen as an exemplary clinical validation study of a DBS method. Thirdly, to our knowledge, this is the first study that shows a negative performance of DBS sampling during the clinical validation phase. As earlier studies might not have been published due to publication bias, this study is of great importance to show the reality and drawback of DBS.

CONCLUSIONS

Our Dried Blood Spot (DBS) method to determine risperidone, aripiprazole, pipamperone, and their major metabolites was not fully clinically valid. This study shows the importance of a clinical validation study using Bland Altman plots before clinical implementation. Nevertheless, this DBS method could still be useful in clinical practice as a qualitative adherence tool.

Source of Funding
Sanne Kloosterboer received a grant from The Netherlands Organisation for Health Research and Development, grant 836041011.

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CHAPTER 6

THE FEASIBILITY OF DRIED BLOOD SPOTS IN CHILDREN WITH BEHAVIORAL PROBLEMS

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Therapeutic Drug Monitoring
2020;42(4):648-51

ABSTRACT

Background
Minimally invasive sampling methods are important to facilitate therapeutic drug monitoring (TDM) and pharmacokinetic research in children with behavioral problems. This study assessed the feasibility and pain of dried blood spot (DBS) sampling in this population.

Methods
Repeated DBS sampling was performed in children with autism spectrum disorder (ASD) and severe behavioral problems using antipsychotic drugs, aged between 6 and 18 years. The child, guardian, and DBS performer assessed pain using the numeric rating scale (NRS-11) or 5-face Faces Pain Scale. The influence of age, sex, and the fingerprick performer on the child's pain intensity was analyzed using linear mixed models.

Results
Overall, 247 fingerpricks were performed in 70 children. Seven children refused all DBS sampling. The median (IQR) NRS-11 pain scores were 2 (3) rated by children, 3 (2.5) by guardians, and 2 (2) by fingerprick performers. The child's age and sex, and fingerprick performer had no significant influence on pain intensity.

Conclusions
DBS sampling could be performed in most children with ASD and severe behavioral problems. However, 1 in 5 children refused one or more DBS fingerpricks owing to distress. The majority expressed minimal pain (NRS <4). Repeated sampling with DBS is feasible in children with ASD and severe behavioral problems.

INTRODUCTION

As an alternative to invasive venipuncture, dried blood spot (DBS) analysis is well-established for drug quantification.[1] DBS involves only one fingerprick and is especially suited for pediatric populations, in clinical and research settings. DBS can increase sampling tolerability particularly in children with behavioral problems. In these children, blood sampling by venipuncture is often challenging due to restlessness or aggression; the minimally invasive DBS procedure could overcome these challenges and can be performed at home.

Notably, among children with behavioral problems, DBS sampling is of particular interest in children prescribed antipsychotic drugs. These drugs are effective in a wide range of psychiatric disorders in childhood[2], including behavioral problems in autism spectrum disorder (ASD); however, these drugs are associated with serious side-effects. The most important side-effect concerns weight-gain[3], leading to metabolic abnormalities[3], diabetes mellitus[4], cardiovascular diseases[5], and possibly even unexpected death[6] in children using antipsychotic drugs. Therapeutic drug monitoring (TDM) could be an important tool to increase the safety of these drugs in children.[7, 8]

To facilitate TDM research and applicability in children using antipsychotic drugs, a DBS assay for the quantification of risperidone, aripiprazole, and pipamperone has been developed.[9, 10] However, the feasibility and burden of DBS in children with severe behavioral problems remains unexplored. In the current study, we evaluated the feasibility and pain levels of DBS sampling in children with ASD and severe behavioral problems and investigated the influence of sex, age, and fingerprick performer on the child's pain intensity.

MATERIALS AND METHODS

Study population
Children, aged 6-18 years old, with ASD according to the DSM IV[11] or 5[12] and severe behavioral problems were included in an observational study investigating the relationship between antipsychotic drug concentrations and effects (Netherlands National Trial Register NTR6050). All included children were prescribed risperidone, aripiprazole, or pipamperone, the three most commonly used antipsychotic drugs in the Netherlands.[13] This study, including the pain assessments, was approved by the medical ethics committee of the Erasmus MC (MEC 2016-124). All patients and/or their legal representatives provided written informed consent before entering the study.

DBS sampling
Each child received two to three fingerpricks on two separate days, with a minimum of 1 h between two fingerpricks to allow random sampling. The fingerpricks were performed with a single-use contact-activated lancet (BD Microtainer® 2.0 mm × 1.5 mm), with the first performed by the research staff at the clinic. Subsequent fingerpricks were performed by research staff, the guardian, or the child itself, at the clinic or home depending upon the patient's preference. Most children

underwent a venipuncture the same day. Antipsychotic drug concentrations were determined using a previously validated ultra-high performance liquid chromatography-mass spectrometry (LC-MS/MS) method for DBS.[9, 10]

Pain assessment

Before performing the fingerprick, the child's likely response to procedures, coping strategies, and possible interventions were discussed with parents and the child. The DBS procedure was explained and demonstrated before the performance. Immediately after the fingerprick, the child, guardian, and fingerprick performer assessed pain. Children aged 6 years used a 5-face Faces Pain Scale (FPS); older children, guardians, and fingerprick performers used the 11-point numeric rating scale (NRS-11).[14] The FPS demonstrates 5 faces from happy to extremely sad. For children with cognitive impairment, the Checklist Pain Behavior (CPG)15, including the NRS-11, was scored by the research staff, whereas the guardians only scored the NRS-11. An NRS score of 4 or higher and a CPG score of 5 or higher is regarded as an indication for pain-relieving interventions.[15, 16] After each fingerprick, the researcher evaluated the burden for each child based on pain scores, assessing whether a subsequent fingerprick could be performed together with the child and the guardian.

Statistical analyses

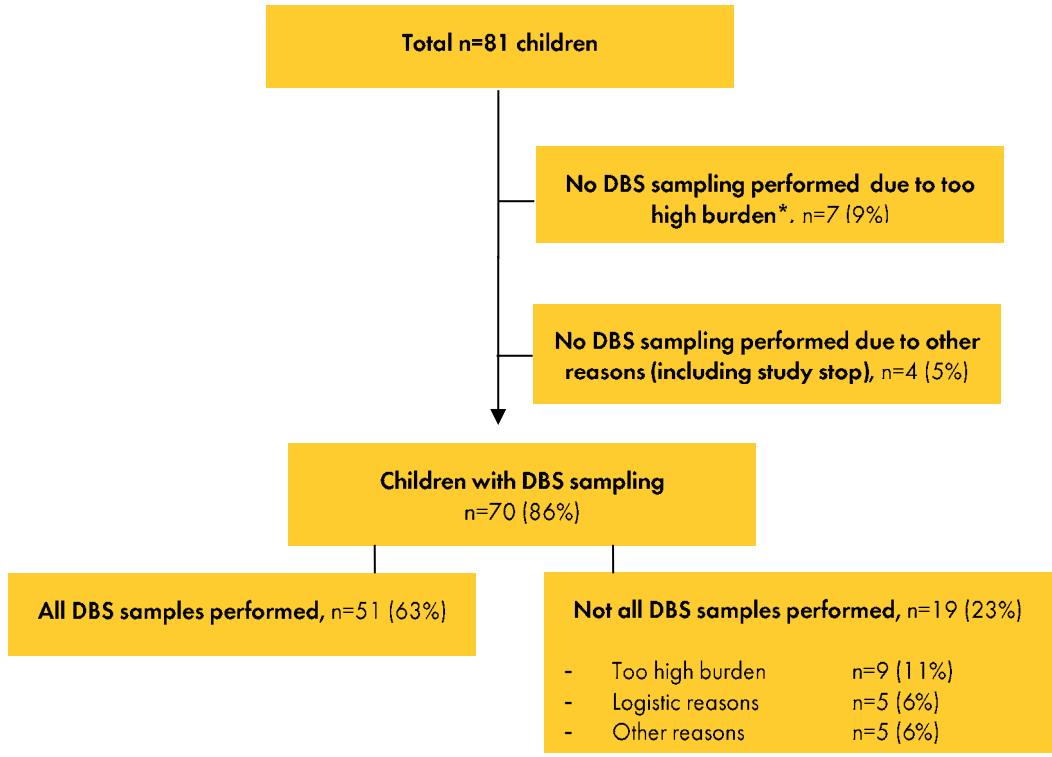
Groups were compared using the Mann-Whitney U test for continuous data (non-normal data distribution) and Fisher's exact test for categorical data. The interrater reliability of pain scores rated by the child, guardian, and research staff were analyzed with the intraclass correlation coefficient (ICC) using a two-way mixed model with absolute agreement type. To evaluate the influence of the child's age and sex, as well as fingerprick performer, on the child's pain, a linear mixed model analysis was used with a random intercept. Sampling days were clustered within patients. All variables were tested using a univariate model. Variables with $p < 0.10$ were added to the multivariate model; variables with $p < 0.05$ in this model were selected for the final model. All analyses were performed using SPSS Version 25 (SPSS Inc., Chicago, IL, USA).

RESULTS

Overall, 81 children were eligible for inclusion. Seven children refused all DBS sampling (8.6%), and in nine children (11.1%) not all DBS sampling could be performed as they expected DBS to be extremely painful or stressful (**figure 1**). Children who refused one or more DBS samples were significantly younger than those who performed all DBS samples (median age 9.3 versus 10.8 years, p=0.43). The groups did not significantly differ in terms of sex or mental retardation.

Totally, 253 DBS samples were collected from 70 children, with 21 were female (30%), and a median (IQR) age of 10.7 (5.0) years. Twelve children were diagnosed with cognitive impairment. Most DBS fingerpricks were performed by research staff (63.0%), followed by guardians (36.6%), and the child (0.4%). DBS samples obtained by guardians were performed at home. Notably, 39 (15.4%) DBS samples were not of sufficient quality for analysis.

Figure 1 DBS sampling fulfilment



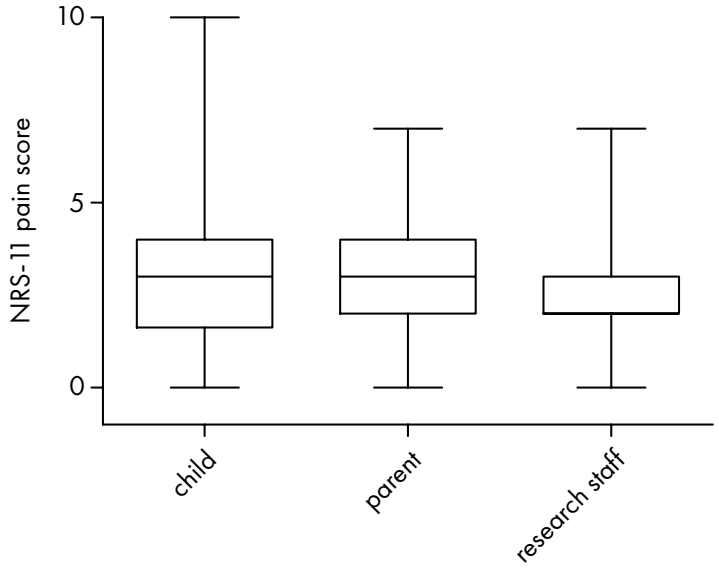
** These children expected DBS to be too painful or stressful*

For 247 DBS samples, 1 or more NRS-11 pain scores were available, including 168 pain scores reported by the child, 187 by guardians, and 126 by research staff. The median (IQR) NRS-11 pain scores were 2 (3) rated by children, 3 (2.5) by guardians, and 2 (2) by research staff. The medians and ranges are shown in **Figure 2**. Furthermore, 61 fingerpricks (36.3%) were rated ≥4 by the child. Twenty-six CPG scores were available; the median (IQR) score was 3 (1), and 11.5% were 5 or higher.

For 147 fingerpricks, both child and guardian NRS-11 pain scores were available. The ICC for guardians and children was 0.86 (95% CI 0.81-0.90) and for research staff and children was 0.79 (95% CI 0.67-0.86), considered excellent and good, respectively.

For 167 NRS-11 scores issued by the child, the age and sex of the child, as well as the nature of fingerprick performer, were known. The univariate mixed model analysis showed that none of these variables significantly influenced the child's pain intensity: age of the child (β=-0.087, p=0.134), sex of the child (β=0.476, p=0.225) and fingerpick performer (guardian β=0.266, p=0.211, self β=1.178, p=0.548, with research staff as reference category).

Figure 2 Child's pain associated with DBS sampling rated by child, guardian and research staff



whiskers indicate min-max. NRS-11: 11 point Numeric Rating Scale

DISCUSSION

Repeated DBS sampling was successfully performed in the majority of children with ASD and severe behavioral problems. Nevertheless, 1 in 5 children refused one or more DBS fingerpricks due to distress. However, most children expressed minimal pain (NRS <4) during DBS sampling.

Relatively little is known regarding pain in autistic children, although hyper- or hyporeactivity to sensory input is a feature of ASD.[12] Comparable, reduced, and increased pain thresholds have been reported in the literature.17 This is consistent with our study, where both extremely low and extremely high pain scores were observed, although most pain scores were lower than the generally accepted threshold of 4. However, pain assessment in children with ASD is challenging, as they may express pain differently due to the lack of social responsiveness and language impairment.[18] Unfortunately, pain assessment tools specifically for individuals with ASD have remained largely unexplored.

The tolerability of blood sampling is not only determined by pain, but also by distress and anxiety. Children with ASD express higher levels of distress during venipuncture than their non-impaired peers.[19, 20] DBS sampling may reduce many of these stressors as the sampling preparation phase is considerably less complex, allowing sampling by a guardian in the home setting. Hence, most children with high levels of distress are expected to prefer DBS sampling over venipuncture, as do their normally-developing peers[21, 22], but further research is necessary for validation.

In children with behavioral problems, the performance of repeated sampling studies is highly challenging. As previously suggested, sparse sampling designs in combination with DBS sampling can facilitate pediatric pharmacokinetic research.[23, 24] Both the lower burden and acceptance of home-sampling improve study recruitment, which is particularly difficult in child- and adolescent psychiatry. However, clinical validation remains challenging, e.g. our study showed a larger variability in antipsychotic drug concentrations measured with DBS than with venipuncture. As advanced pharmacokinetic modeling techniques allow the correction of this variability, the advantages of DBS can still outweigh the disadvantages. Currently, this method remains primarily useful in research settings; however, further development and study of DBS and TDM could yield a clinical, minimally invasive tool that improves the safety of antipsychotic drugs.

The current study has some limitations. As mentioned earlier, self-reporting of pain might be unreliable in children with ASD, leading to an over- or underestimation of pain. However, no pain measurement tools are currently available for pain assessment specifically in children with ASD. To optimize pain assessment, it was ensured that the DBS sampling performers had prior experience in communicating with children with ASD and that the child's guardians were present. Previous research has shown that parent involvement is essential for the interpretation and expression of pain in children with ASD.[18] A comparison with the pain associated with conventional venipuncture was not conducted, limiting the evaluation of the most optimal sampling technique in this population.

CONCLUSION

This is the first study that evaluated the feasibility of repeated DBS sampling in children with ASD and severe behavioral problems, indicating that DBS is a feasible sampling technique in this population. Although further research is needed to compare the burden of DBS and venipuncture in children with ASD and behavioral problems, DBS can facilitate pharmacokinetic research and TDM in these and other pediatric populations where sampling remains challenging.

Funding information
SMK, BD, and BCK received grant research support from The Netherlands Organization for Health Research and Development (ZonMW), number 836041011.

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PART IV

ANTIPSYCHOTIC DRUG CONCENTRATIONS AND CLINICAL OUTCOMES

CHAPTER 7

PSYCHOTROPIC DRUG CONCENTRATIONS AND CLINICAL OUTCOMES IN CHILDREN AND ADOLESCENTS: A SYSTEMATIC REVIEW

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Expert Opinion on Drug Safety 2020;19(7):873-90

ABSTRACT

Introduction
The use of psychotropic drugs in children and adolescents is widespread but associated with suboptimal treatment effects. Therapeutic drug monitoring (TDM) can improve safety of psychotropic drugs in children and adolescents, but is not routinely performed. A major reason is that the relationship between drug concentrations and effects is not well known.

Areas covered
This systematic review evaluated studies assessing the relationship between psychotropic drug concentrations and clinical outcomes in children and adolescents, including antipsychotics, psychostimulants, alpha-agonists, antidepressants and mood-stabilizers. PRISMA guidelines were used and a quality assessment of the retrieved studies was performed. 67 eligible studies involving 24 psychotropic drugs were identified from 9,298 records. The findings were generally heterogeneous and the majority of all retrieved studies was not of sufficient quality. For 11 psychotropic drugs a relationship between drug concentrations and side-effects and/or effectiveness was evidenced in reasonably reported and executed studies, but these findings were barely replicated.

Expert opinion
In order to better support routine TDM in child- and adolescent psychiatry, future work must improve in aspects of study design, execution and reporting to demonstrate drug concentration-effect relationships. The quality criteria proposed in this work can guide future TDM research.

Systematic review protocol and registration
PROSPERO CRD42018084159

ARTICLE HIGHLIGHTS

- The concentration-effect relationships of psychotropic drugs in children and adolescents are largely unknown, which hampers the routine application of Therapeutic Drug Monitoring (TDM) in this population.
- Our systematic literature search favors a concentration-effect relationship for 11 psychotropic drugs in children and adolescents with different indications, but evidence is sparse and therapeutic reference ranges are generally not evaluated or reported.
- Most retrieved studies did not accurately report or execute key aspects of TDM.
- Even when therapeutic reference ranges are not well-established, TDM can improve psychopharmacotherpay when non-compliance, drug-drug interactions or pharmacogenetic polymorphisms are suspected in children and adolescents.

INTRODUCTION

Psychotropic drugs have been proven effective for the treatment of a wide range of psychiatric disorders in children and adolescents. As a result, the use of stimulants, antipsychotics, antidepressants and mood-stabilizers in youths is widespread. [1-3]

However, the use of psychotropic drugs in youth faces several challenges. Some side effects of these drugs appear more prevalent in young patients, like metabolic and endocrine abnormalities associated with antipsychotic drug use. [4] This also applies to selective serotonin reuptake inhibitors (SSRIs), where children seem more vulnerable for restlessness and vomiting.[5] At the same time, efficacy of some psychotropic drugs may be lower in children than in adults, as demonstrated for antidepressants.[6]

Although the mechanisms behind suboptimal treatment effects in youths are not fully understood, both *pharmacokinetic* and *pharmacodynamic* changes during childhood might contribute. Pharmacokinetic changes that occur during childhood[7] may result in over- or underdosing in young patients, leading to unanticipated failures of randomized controlled drug trials in child- and adolescent psychiatry.[8] Also pharmacodynamics might influence suboptimal psychotropic treatment effects in children and adolescents, as brain development and target receptor maturation are suggested to be related to the failure of many antidepressants in youths.[9] However, age-specific pharmacokinetic and –dynamic aspects relevant for psychotropic drugs in children and adolescents are largely unknown.

Therapeutic Drug Monitoring (TDM), which comprises the quantification of drug concentrations in blood or other matrices to optimize individual drug dosing[10], incorporates individual pharmacokinetic and pharmacodynamic processes. TDM has proven to enhance efficacy and safety of many psychotropic drugs in adults and has become routine practice for mood stabilizers like lithium, tricyclic antidepressants like amitriptyline, and antipsychotics like clozapine in adult psychiatry[10]. TDM is especially indicated for patient populations with altering pharmacokinetics and pharmacodynamics, such as elderly, pregnant women, children and adolescents, where both efficacy and side-effects might be unpredictable.[10, 11] As such, TDM may also provide a measure for proactive pharmacovigilance in children and adolescents. [12]

However, TDM within child- and adolescent psychiatry is generally not routinely performed. A major reason is that the relationship between drug concentrations and effects in children and adolescents is not well known, and age- or developmental specific therapeutic reference ranges are lacking. [13, 14] The objective of this systematic review is to provide an overview of the literature investigating the relationship between blood concentrations of psychotropic drugs and clinical outcomes in children and adolescents, including stimulants, antipsychotics, antidepressants and mood-stabilizers and alpha-agonists, to further investigate the rationale for TDM in this population. Based on the findings, the current position of TDM within child- and adolescent psychiatry and future research directives are discussed.

METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline for systematic reviews.[15] This systematic review is registered under PROSPERO number CRD42018084159.

Information sources

Studies were identified by searching electronic databases and screening reference lists of relevant articles. Three databases were systematically searched without restriction of language or publication date (Embase.com, Medline Ovid and Cochrane CENTRAL). The last search was performed in November 2018. The search strategy can be found in **supplementary table 1**.

Eligibility criteria and study selection

Studies reporting the relationship between psychotropic drug concentrations and clinical outcomes (i.e. efficacy or safety) in children or adolescents aged up to 18 years were eligible for inclusion. The included psychotropic drugs were antipsychotics, psychostimulants, alpha-agonists, antidepressants and mood-stabilizers including anti-epileptics used for psychiatric indications. The eligibility criteria are presented in **table 1**.

Title abstract and full-text screen was independently performed by two reviewers (SK and DV); disagreements were resolved by consensus. References of identified studies were checked for relevant articles. Also, previous reviews and the international consensus guideline about TDM in psychiatry were checked for relevant studies.[10, 13, 14]

Table 1 In- and exclusion criteria for selection of relevant articles

Inclusion criteria	Exclusion criteria
The study concerns antipsychotics, psychostimulants, antidepressants or mood-stabilizers, alpha-agonists	No analysis on relationship between drug levels and clinically relevant outcome measures is reported
Study is performed in children or adolescents aged up to 18 years	Drug under study is used for non-psychiatric indications (f.e. epilepsy or enuresis)
Drug plasma levels are measured and reported	Maternal use during pregnancy or lactation
Direct clinical outcome measures are reported, i.e. safety or efficacy*	Non-human subjects
	Studies focusing on toxicology / overdoses
	Case reports
	Conference papers and abstracts
	Post-mortem studies

** biomarkers are not regarded a direct clinical outcome measure*

Data collection process

One reviewer (SK) extracted the following data from included studies in a data extraction form: (1) characteristics of study participants (including sex, age and diagnoses), (2) study design (including duration and dosing strategy), (3) outcome measures, (4) blood sample collection (sampling time, relation to steady state) and (5) the results as presented in the study. A second reviewer (KP) checked doubtful items identified by the first reviewer. Disagreements were resolved by discussion between reviewers.

Quality assessment of therapeutic drug monitoring

To ascertain the internal validity of the selected studies, one reviewer (SK) performed a quality assessment of the therapeutic drug monitoring component of the selected studies. A second reviewer (KP) checked doubtful quality criteria that were identified by the first reviewer during the quality assessment. Disagreements were resolved by discussion between reviewers.

Currently available quality assessment tools do not specifically address drug concentration-effect studies 16, thus criteria for quality assessment were adapted from a previously published meta-analysis of Ulrich et al. concerning the concentration-therapeutic effect relationship of haloperidol in adults.[17] As the current systematic review covers different types of psychotropic drugs with a broad range of indications, not all criteria of the total score as used by Ulrich et al. were applicable. We therefore used only the hard items of the total score. These items are indicated "sufficient" or "insufficient" and presented in **table 2**. Studies that did not report or did not realize an item were rated insufficient. Premedication was registered as study characteristic and not scored. Furthermore, "completely insufficient description of study design" was not included as score item, as individual items were already rated insufficient when the information could not be found.

Analytical method for the assay of drug concentration in serum or plasma

The analytical assay for drug quantification should be selective, able to discriminate the measured drug from other similar drugs and metabolites, and sensitive, accurately quantifying drug concentration.[10] Accurate analytical methods have become available relatively recently.[18] Examples of selective and sensitive methods include chromatographic methods, including High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS). Older analytical methods like (radio) immunoassay often present high variability in drug quantification. Analytical methods for drug quantification must be validated to demonstrate reliability and reproducibility. The quality assessment of the analytical method was checked per study by a laboratory based hospital pharmacist (BK).

Table 2 Criteria for quality assessment of the selected studies

Quality criteria		Sufficient score		Comments
1.	Analytical method for the assay of drug concentration in serum or plasma	•	Validated analytical method	
2.	Blood sample collection	•	Steady state plasma or serum concentrations	
		•	Sampling time and drug intake described	
3.	Patient selection	•	Representative sample for study outcome	With a heterogeneous sample, a sub analysis per relevant category should be provided
		•	Psychiatric classifications and associated classification system is reported	
4.	Measurement of illness severity and registration of therapeutic improvement or worsening	•	Adequate quantification of outcome measure (rating with a structured scale)	Retrospectively scored change is rated insufficient
		•	A baseline assessment of the outcome measure is provided	
		•	Adequate calculation of change in outcome measure	
		•	Sufficient time to rate effect	
5.	Comedication	•	No drug that influences pharmacokinetics or pharmacodynamics of the drug under study is taken simultaneously, or:	
		•	A sub analysis/correction is provided	
6.	Number of patients	•	At least 10 patients are included and used for analysis	

Blood sample collection

Steady state is achieved when a drug is given in a constant dose and schedule for at least 4-6 half-lives.[10] During steady state, overall bioavailability is in equilibrium with elimination, such that the drug concentration reflects the dosage given. Sampling should therefore be performed during steady state of the drug and its metabolites. An exception is when population pharmacokinetic-pharmacodynamic modelling is performed, which can correct for non-steady state concentrations. Furthermore, the concentration of a drug rises quickly after drug intake and declines afterwards as a function of time. An accurate assessment of the time interval between sampling relative to the drug intake is crucial for correct interpretation of the drug concentration. In clinical practice, sampling of the trough concentration is often the standard procedure. The trough concentration is the concentration at the end of a dosing interval, taken immediately before the subsequent dose. The concentration time curve in the final period of the dose interval is relatively flat, and therefore the exact sampling time is less critical. For normal release methylphenidate formulations, steady state sampling is not relevant due to its short half-life, and thus this item was not weighed in scoring.

Patient selection

A representative sample is important for the generalizability of results (external validity). If a heterogeneous patient group is selected, and there is concern that different relationships exist between drug plasma concentrations and (side-) effects, sub-group analysis should be performed, bearing in mind that adequate power is achieved. Furthermore, psychiatric classifications within the sample and the associated classification system should be reported, as concentration reference ranges are disorder-specific.

Measurement of illness severity and registration of therapeutic improvement or worsening

For the analysis of the relationship between drug concentrations and effect, it is important to assess the effect that is likely to be attributable to the drug. Therefore, a baseline assessment of the severity of the outcome measure, prior to drug treatment, is essential. The change from baseline should be used for analyses rather than a point measurement during treatment. Preferably a validated rating scale should be used to determine outcome measures. Lastly, a sufficient time to rate effect should be considered. For example with antipsychotics, a delay of at least one week after start of treatment is expected to observe a clinical effect.[17]

Comedication

Comedication can influence the effect of a drug trough pharmacokinetic and pharmacodynamic interactions. In particular, pharmacodynamic interactions might confound the observed clinical effects. Co-medication should be taken into account and corrected for when necessary, where possible trough strategies such as stratification or multivariate methods.

Number of patients

Power calculations are challenging in observational studies, and in the setting of observational studies in TDM. Ulrich et al [17], suggest a minimum of 10 patients, which was rated a sufficient number within our quality assessment.

RESULTS

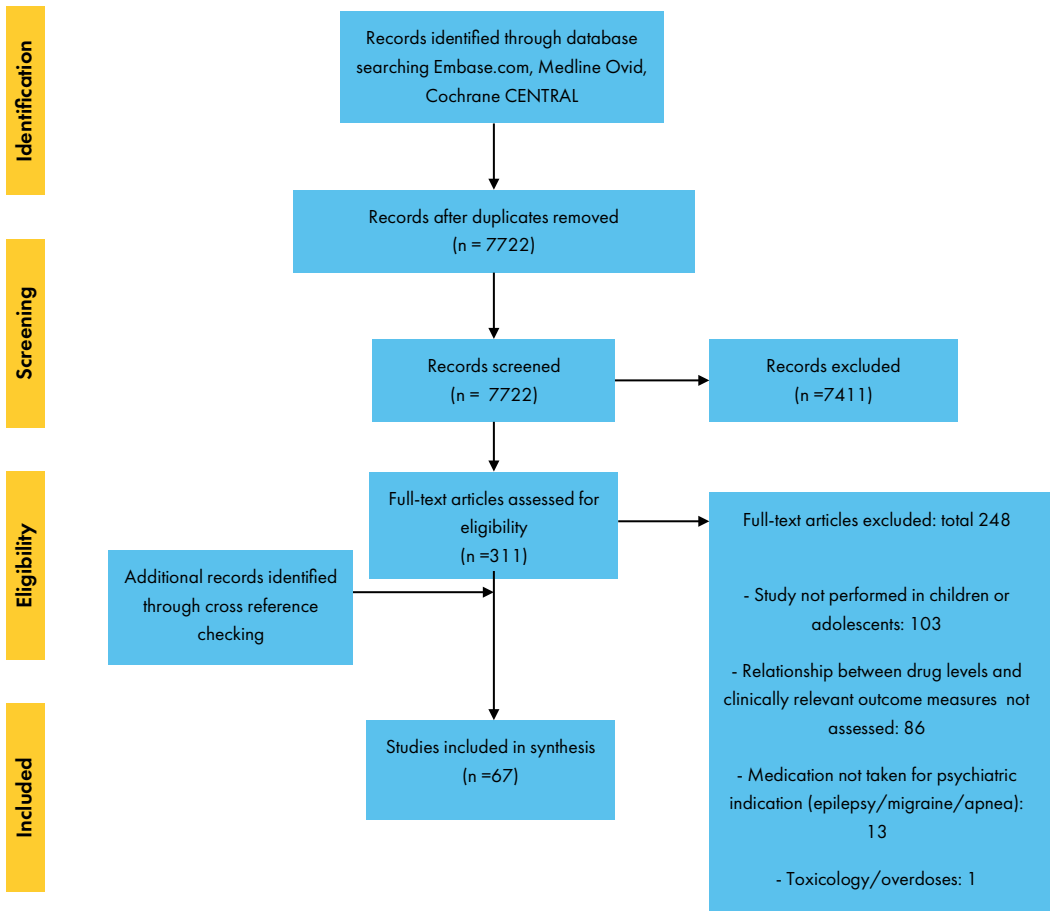
Study selection

Figure 1 shows the process by which articles were identified. Screening of title and abstract identified 311 primary studies. Full-text was not available for 43 of these.

Sixty-seven studies were included after full-text screen, representing 24 psychotropic drugs: two stimulants, one alpha-agonist, six SSRI's, five tricyclic antidepressants, one other antidepressant, seven antipsychotics and two mood stabilizers.

Of the selected studies, 35.8% evaluated efficacy measures, 32.8% evaluated side-effect measures and 31.3% evaluated both. A substantial proportion of studies was performed prior to 1995 (n=23, 34.3%). Most studies were performed in the United States (n=42, 62.7%), and 25.3% of studies was performed in Europe.

Figure 1 Flowchart



Quality assessment

Twenty-one studies met all 6 quality criteria (31.3%), while 47 studies did not meet quality criteria in full (one study described two trials, and fulfilled all criteria for one trial[19]). Five criteria were met in 25 studies (37.3%).

The most frequently missed criterion was *comedication* and *blood sample collection*. Comedication was rated as insufficient in 25 studies (37.3%); it was unreported in 9 studies (13.4%) and was not addressed in analyses in 16 studies (22.4%). Blood sample collection was insufficient in 24 studies (35.8%), where either sampling in steady state was not performed, or time point of sampling relative to the drug intake was not described. *Measurement of illness severity and registration of therapeutic improvement or worsening* was rated as insufficient in 20 studies (29.9%), principally as baseline measurement was not performed. The *analytical method* was scored insufficient in 10 studies (14.9%), the method was judged non-selective or non-sensitive in 3 studies, and the analytical method was not reported in 7 studies.

The characteristics, results and quality assessment of the studies are presented in **table 3**.

The studies meeting all quality criteria involved 15 psychotropic drugs. A concentration-efficacy relationship was found for six drugs (citalopram[20], fluoxetine[20], nortriptyline[21], buproprion[22], quetiapine[23], lithium[24]), a concentration-side-effects relationship was found for three (venlafaxine[20], desipramine[25], ziprasidone[26]), and a relationship with both efficacy and side-effects for two (methylphenidate [19, 27-29], imipramine [30-32]). The indications for use of these drugs included major depression, conduct disorder, bipolar disorder, attention-deficit disorder with or without hyperactivity and Tourette syndrome or chronic tic disorder (table 3). In 7 of the studies meeting all quality criteria, therapeutic reference ranges or concentrations for optimal treatment were reported [21-24, 29-31].

The 47 studies that did not meet all quality criteria involved 20 psychotropic drugs, for which concentration-effect relationships were reported for 13. These concerned 8 additional drugs compared to the studies judged high-quality studies: one with a concentration-efficacy relationship (atomoxetine [33]), six with a concentration-side-effect relationship (fluvoxamine [34], sertraline[35], clomipramine[36], haloperido[37], olanzapine[38, 39], risperidone[39-43]) and one with both (clozapine[44-46]). In 9 of the studies with lower quality, suggested therapeutic reference ranges or optimal concentrations were reported[36, 37, 46-52].

For 5 of the 24 psychotropic drugs that were retrieved with our search, no relationship between concentration and clinical outcomes was found in either high-quality studies or lower-quality studies (dexamphetamine, paroxetine, imipramine, loxapine, valproic acid).

Overall, findings were highly heterogeneous. Most studies were not primarily designed to assess the relationship between drug concentrations and effects. Furthermore, most of the studies were not replicated and for most drugs and outcomes, only one study was available.

CONCLUSIONS

This systematic review presents published evidence for the relationship between drug concentration and clinical outcomes of psychotropic drugs in children and adolescents. We found a minority of therapeutic drug monitoring studies were reasonably reported and executed. Among these, concentration-effect relationships were evidenced for methylphenidate, citalopram, fluoxetine, venlafaxine, desipramine, imipramine, nortriptyline, bupropion, quetiapine, ziprasidone and lithium, for various indications in children and adolescents. However, findings were often heterogeneous, barely replicated and therapeutic reference ranges were not often provided. Moreover, interpretation of data from retrieved studies was primarily complicated by inappropriately conducted or inadequately reported sampling.

EXPERT OPINION

Considering the relevance of a drug concentration-effect relationship in the clinical context

Although for a wide range of psychotropic drugs some evidence was found for a concentration-effect relationship, its relevance in clinical practice depends on several drug-related and clinical factors[53], including the time-course of the observed effects.

For instance, the relationship between systemic methylphenidate concentrations and efficacy in children and adolescents with Attention Deficit Hyperactivity Disorder was frequently reported, but might be of questionable relevance. Among 160 children and adolescents across three reasonably reported and executed trials, a higher methylphenidate concentration was associated with improved performance, though different instruments were used across the studies [29, 54, 55]. Most studies judged of lower quality reported similar findings, with one exception [56]; however, the length of the follow-up period was unclear in this work, thus the relationship might have been underestimated. These findings suggest that this stimulant might be a candidate for TDM in children and adolescents, although routine application should be carefully considered [53]. As improvement in attention is readily assessable by parents and teachers [57], it is questionable that concentration measurement would further inform clinical decision-making.

TDM may be more informative for psychotropic drugs with delayed therapeutic or side-effects, such as antidepressants or antipsychotic drugs. TDM would ideally provide important information on adequacy of therapy in an early phase, thereby preventing sub-therapeutic treatment and long-term side-effects. Given growing concerns about antipsychotic-induced metabolic abnormalities in children and adolescents [58], these drugs could be an important target for TDM. Unfortunately, no relationship was found in two studies evaluating the relationship between antipsychotic concentrations and metabolic outcomes such as weight, glucose and fatty acids in children and adolescents.[59, 60] However, these studies did not perform baseline measurements and the relationship may be underestimated. Others report associations between higher dose and weight gain [61, 62], thus a relationship with systemic concentrations is suspected.

Another aspect that should be considered when assessing the relevance of a drug concentration-effect relationship, is the margin between effective and toxic drug concentrations. If this margin is very narrow, as for example for lithium, it is important to closely monitor drug concentrations to prevent intoxications. For this reason, routine TDM is recommended for lithium in children and adolescents.[63] At the same time, for drugs with a very wide window, as generally applies to SSRIs, TDM is expected to be less useful in routine care, but may be useful to objectify non adherence. Furthermore, the drug concentrations after a given dosage should be difficult to predict. This is referred to as a high *inter-individual* pharmacokinetic variation, and means that drug concentrations differ largely between patients after administration of equal dosages. Another aspect that should be considered for the clinical application of TDM, is that a rapid and reliable method for analysis of the drug should be available. Lastly, before TDM is routinely applied, it should be demonstrated that TDM improves patient outcomes and is cost-effective in clinical practice.

Research recommendations to support TDM in child- and adolescent psychiatry

A proven drug concentration-effect relationship is the first step to provide a rationale for TDM, but this was only sparsely evidenced for most psychotropic drugs in children and adolescents. In order to better demonstrate drug concentration-effect relationships in this field, future work must improve in aspects of study design, execution and reporting. Many studies failed to perform standardized sampling, including sampling with respect to steady state and administration time. Therefore there is a need for an accepted tool for the appraisal of drug concentration-effect studies. [10, 16] The assessment criteria proposed by this report could serve as a starting point, hopefully reducing the heterogeneity observed to date, and permitting meta-analyses.

Besides the need for more adequate sampling protocols, also study designs should be considered for their feasibility and appropriateness to demonstrate a drug concentration-effect relationship. Pharmacokinetic and –dynamic research in children with psychiatric morbidities is challenging and is liable to ethical constrains. As such, observational study designs may provide initial estimates towards defining reference ranges for this patient group. However, results should be interpreted in the light of their limitations. Flexible dosing schemes might lead to an underestimation of the concentration-effect relationship due to the placebo effect that is common for psychotropic drugs. Lower dosages, and thus lower drug concentrations, are likely used in placebo-responders, weakening association estimates.[64] Furthermore, observational studies often permit dosage changes and comedication, thereby altering concentrations of the index drug and complicating analyses with respect to outcomes. In particular within child- and adolescent psychiatry, psychotropic comedication is very common and should be considered.[65] Also, non-pharmacological interventions such as behavioral interventions are commonly part of multimodal treatment, possibly influencing therapeutic outcomes. Results of observational work can nevertheless be very valuable, especially when aspects of therapeutic drug monitoring are well-reported and well-executed. It may be argued that if dose effect relationships are apparent in the setting of observational study designs, the effect would be more pronounced in a randomized controlled trial that involves titration to concentrations associated with efficacy.

However, before TDM is routinely applied in clinical practice, preferably its effect on patient outcomes is evaluated. Ideally, to demonstrate that TDM can improve clinical outcomes, randomized controlled trials would be used to evaluate TDM as an intervention, comparing clinician directed dosing with dose adjustment based on drug monitoring, or comparing different target concentrations. [10] Relevant outcomes would include response, side-effects and cost-effectiveness. An excellent example of such a trial investigating effects and side-effects is the randomization to one of three target concentrations for clozapine in adults [66], however no such RCTs have been performed for psychotropic drugs in children and adolescents. This is partly due to difficulties in performing such trials within child- and adolescent psychiatry. [67] However, in general, such RCTs are very rare in the field of TDM and are therefore not always required before its implementation in clinical practice.

Current position of TDM within child- and adolescent psychiatry

Almost no studies reported therapeutic reference ranges for psychotropic drugs in children and adolescents. Unfortunately, ranges cannot be simply extrapolated from adults, as both pharmacokinetic and pharmacodynamic processes differ considerably. This is the result of developmental changes in body composition, target receptor maturation and organ ripening[7], generally leading to lower psychotropic drug concentrations in children and adolescents than recommended therapeutic reference ranges in adults. [12] Also, psychotropic drugs may be used for other indications in children and adolescents than in adults, and in other dosages, such as antipsychotic drugs (behavioral problems versus psychosis).

The absence of established reference ranges prevents routine application of TDM on a population level. An exception applies to lithium, for which routine TDM is recommended based on a known narrow therapeutic range in adults, which is also applied in children and adolescents.[63] Within our systematic review, one well-documented and executed study found a drug concentration-effect relationship in pediatric patients with bipolar I disorder[24], but studies that systematically investigate the added value and optimal concentrations of lithium in clinical practice are lacking. For other psychotropic drugs, despite the unavailability of clear-cut concentration effect-relationships, TDM can be of added value on an individual level when non-compliance is suspected or, drug-drug interactions or pharmacogenetic polymorphisms, for example in cytochrome 2D6, are foreseen in children and adolescents using psychotropic drugs. A drug concentration measurement can identify unexpected concentrations, as for many antipsychotic drugs expected concentrations based on a given dosage in steady state are known. [68] These are called *pharmacokinetic reference ranges* and can optimize antipsychotic pharmacotherapy by guiding dose- or comedication adjustments. In this way, TDM can prevent over- or underdosing, and improve psychotropic pharmacological treatment in children and adolescents. As long-term safety data of psychotropic drugs in this population are generally lacking and these drugs are frequently prescribed off-label, TDM can provide an important tool to improve psychopharmacotherapy in children and adolescents.

LIMITATIONS

The results of this systematic review should be interpreted in the light of its limitations. Firstly, among quality assessment criteria, unreported elements were judged insufficient. However, older work might be have reported data on methodological aspects more concisely, thus might have been assessed too strictly. As such, five studies were rated insufficient for the item *comedication*. Secondly, a substantial number of articles was not available full text, primarily reflecting older work. However, based on title and abstract screen, these are not expected to have influenced our conclusions. Thirdly, the older publications also concerned drugs that are currently not widely used in children and adolescents anymore, such as tricyclic antidepressants. Fourthly,, publication bias is a possibility that due to heterogeneity we were unable to evaluate, and this may have bias our findings toward positive results. Lastly, the scope of the current review was very broad and aimed at providing an overview of the current literature, which limits a more profound discussion of the individual drugs.

Table 3 Results

Study	Drug	No of subjects (males)*	Indications	Study design Efficacy	Concentration-effect relationship	No concentration effect-relationship			Therapeutic range 1	Quality assessment					
					Side-effects	Efficacy	Side-effects			2	3	4	5	6	
ADHD drugs															
Hazell et al., 2009 [69]	Atomoxetine	156 (123)	AHDH and ODD	Cohort nested in randomized controlled trial (with non-controlled extension phase)			SNAP-IV ODD and SNAP-IV ADHD, CGI-I			X	X	X	X	X	X
Quinn et al., 2004 [27]	Methylphenidate (NR)	31 (31)	ADHD	Cohort nested in randomized controlled trial (single dose)	Math test					X	X	X	X	X	X
Shaywitz et al., 1982 [19] (acute study)	Methylphenidate (NR)	14 (14)	ADD	Prospective cohort (single dose)		GH, PRL		Glucose		X	X	X	X	X	X
Teicher et al., 2006 [28]	Methylphenidate (NR)	48 (48)	ADHD	Cohorts nested in randomized controlled trial (double-blind, parallel group of five dosing paradigms and placebo)	Computer driven vigilance task (M-MAT)					X	X	X	X	X	X
Teuscher et al., 2018 [29]	Methylphenidate (NR)	81 (53)	ADHD	Pooled analysis of 3 prospective cohort studies (single dose) and 1 cohort nested in randomized controlled trial	SKAMP scale				EC50 14.24 ng/ml	X	X	X	X	X	X
Michelson et al., 2007 [33]	Atomoxetine	338 (?)	ADHD	Pooled analysis of 14 prospective cohort studies and cohorts nested in randomized controlled trials.	ADHD Rating Scale-IV-Parent					X			X		X
Brown et al., 1979 [70]	Dexamphetamine	16 (16)	Hyperactivity	Cohort nested in randomized controlled trial (single dose)				Behavioral and and motor activity			X	X	X		X
Greenhill et al., 2001 [47]	Methylphenidate	8 (8)	ADHD	Prospective cohort	Motor performance errors		Self-rating form		EC50 10 ng/ml	X		X	X		
Gualtieri et al., 1981 [71]	Methylphenidate (NR)	27 (?)	Hyperactivity	Cohort nested in randomized controlled trial (single dose)		GH		PRL		X	X				X
Gualtieri et al., 1984 [56] study I	Methylphenidate (NR)	55 (39)	ADHD	Cohort nested in randomized controlled trial			Global response rating scale, CTRS, laboratory measures of attention and activity, behavioral observations	SERS, pulse, BP, GH, PRL		X	X	X		X	X
Gualtieri et al., 1984 [56] study II	Methylphenidate (NR)	26 (22)	ADHD	Prospective cohort (single dose)			CTRS			X	X	X		X	X
Gualtieri et al., 1984 [56] study III	Methylphenidate (NR)	11 (?)	ADHD	Prospective cohort (single dose)				GH, PRL		X	X			X	X
Gualtieri et al., 1984 [56] study VI	Methylphenidate (NR)	4 (?)	ADHD	Prospective cohort			Performance task, behavior observations, CTRS			X				X	X
Jonkman et al., 1998 [72]	Methylphenidate (NR)	12 (11)	ADHD	Prospective cohort (single dose)	Event-related brain potentials					X	X	X	X		X
Kimko et al., 2012 [73]	Methylphenidate (ER)	Unknown	ADHD	Meta-analysis	SKAMP scale, PERMP				EC50 7.55/7.69 ng/ml		X		X		X
Sebrechts et al., 1986 [74]	Methylphenidate (NR)	12 (12)	ADHD	Cohort nested in randomized controlled trial	MFFT test					X	X	X	X		X
Shaywitz et al., 1982 [19] (chronic study)	Methylphenidate (NR)	11 (?)	ADD	Prospective cohort (single dose)	CAPTRS					X	X	X	X		X
Srinivas et al., 1992 [75]	Methylphenidate (NR)	9 (9)	ADHD	Cohort nested in randomized controlled trial			SRT scores (d-enantiomer)			X	X		X	X	
Winsberg et al., 1982 [76]	Methylphenidate (NR)	25 (25)	ADHD	Cohort nested in randomized controlled trial	CTRS, WWPAS		Short term memory tasks				X	X			X
Selective Serotonin Reuptake Inhibitors															
Sakolsky et al., 2011 [20]	Citalopram	27 (?), n with plasma samples 244 (?)	MDD	Cohort nested in randomized non-controlled trial	CDRS-R, CGI-I			SEFCA		X	X	X	X	X	X
Sakolsky et al., 2011 [20]	Fluoxetine	64 (?), n with plasma samples 244 (?)	MDD	Cohort nested in randomized non-controlled trial	CDRS-R, CGI-I			SEFCA		X	X	X	X	X	X
Sakolsky et al., 2011 [20]	Venlafaxine	119 (?) ,n with plasma samples 244 (?)	MDD	Cohort nested in randomized non-controlled trial		Dizziness, cardiovascular and dermatologic adverse events (SEFCA)	CDRS-R, CGI-I			X	X	X	X	X	X
Sakolsky et al., 2011 [20]	Paroxetine	34 (?), n with plasma samples 244 (?)	MDD	Cohort nested in randomized non-controlled trial			CDRS-R, CGI-I	SEFCA		X	X	X	X	X	X

Blázquez et al., 2014) [77]	Fluoxetine	73 (24)	MDD, OCD, generalized anxiety disorder	Prospective cohort	CY-BOCS (OCD patients, n=12)		CGI-I (total sample)	UKU SERS (total sample)		X		X	X		X
Koelch et al., 2012 [78]	Fluoxetine	71 (27)	Depressive disorder or depressive symptoms	Retrospective cohort			CGI-I	UKU SERS		X		X			X
Reinblatt et al., 2009 [34]	Fluvoxam-ine	fluvox-amine: 22 (12), placebo: 23 (12)	Anxiety disorders	Prospective cohort		Activation cluster adverse events				X		X	X	X	X
Alderman et al., 1998 [79]	Sertraline	61 (33)	OCD, MDD or both	Prospective cohort				Adverse events (reported by the patient of observed by the investi-gator)		X	X	X		X	X
Alderman et al., 2006 [80]	Sertraline	43 (18)	OCD or MDD	Prospective cohort			CGI-I, CGI-S			X		X	X	X	X
Taurines et al., 2013 [35]	Sertraline	90 (41)	Children and adolescents receiving sertraline	Retrospective cohort		UKU SERS (patients with depression)	CGI-I	UKU SERS		X		X		X	X
Tricyclic Antidepressants																
Moen Olig et al., 1985 [81]	Amitrip-tyline	10 (?)	MDD	Prospective cohort			Telephone monitoring of clinical response, CDI			X	X	X	X	X	X
Flament et al., 1985 [82]	Clomip-ramine	19 (14)	OCD	Cohort nested in random-ized controlled trial			LOI-CV, OCR scale, CPRS, NIMH Global Scale, BPRS, NIMH self- rating scale			X	X	X	X	X	X
Biederman et al., 1989 [83]	Desipra-mine	56 (?)	ADHD	Randomized controlled trial followed by prospective cohort (placebo non-re-sponders)			CGI	ECG param-eters		X	X	X	X	X	X
Donnelly et al., 1986 [25]	Desipra-mine	29 (29), 15 receiving desipramine	ADHD	Cohort nested in random-ized controlled trial		HR (day 14)	CABRS (teacher ratings)	BP		X	X	X	X	X	X
Spencer et al., 2002 [84]	Desipra-mine	41 (34)	com-bined-type ADHD and chronic motor tic disorder, chronic vocal tic disorder, or Tourette disorder (TD)	Cohort nested in random-ized controlled trial			CGI and specific rating scale (ADHD Rating Scale, YGTSS, CY-BOCS, CDI, RCMAS, and GAF)	Adverse events by open ended questions, ECG		X	X	X	X	X	X
Preskorn et al., 1983 [30]	Imipra-mine	22 (17)	MDD	Prospective cohort		Diastolic BP, HR and slowing of intracar-diac conduction		Subjective reports of side-effects	<225 ng/ml	X	X	X	X	X	X
Puig-An-tich et al., 1979 [31]	Imipra-mine	13 (9)	MDD	Prospective cohort	K-SADS				> 146 ng/ml.	X	X	X	X	X	X
Puig-An-tich et al., 1987 [32]	Imipra-mine	30 (18)	MDD	Prospective cohort	K-SADS-P					X	X	X	X	X	X
Moen Olig et al., 1985 [81]	Imipra-mine	10 (?)	MDD	Prospective cohort			Telephone monitoring of clinical response, CDI			X	X	X	X	X	X
Geller et al., 1985 [85]	Nortrip-tyline	21 (14)	MDD	Prospective cohort				ECG mea-surements		X	X	X	X	X	X
Geller et al., 1986 [21]	Nortrip-tyline	22 (16)	MDD	Prospective cohort	Children depression rating scale			-	>60 ng/ml	X	X	X	X	X	X
Birmaher et al., 1998 [86]	Amitripty-line	27 (8)	MDD	Cohort nested in random-ized controlled trial			K-SADS-P, HDRS, CGI, BDI, C-GAS	Side effects scale, ECG, vital signs				X	X	X	X
Dugas et al., 1980 [36]	Clomip-ramine	10 enuretic children (10), 26 depressive children (9)	Children with depressive symptomatolo-gy or enuresis	Prospective cohort		Hospital side-effects checklist	Clinical status			X	X				X
Biederman et al., 1993 [87]	Desipra-mine	71 (?)	ADHD or depression	Cross-sectional		ECG: paired premature atrial contractions		24 h Holter, ECG, Doppler echo-car-diography, cardiac exam			X	X			X
Wilens et al., 1993a [88]	Desipra-mine	89 (79)	Various diag-noses (MDD and ADHD)	Prospective cohort				ECG-chang-es, HR		X		X	X	X	X
Ryan et al., 1986 [89]	Imipramine	34 (17)	MDD	Prospective cohort			K-SADS-P			X		X	X	X	X

Ambrosini et al., 1994 [51]	Nortriptyline	25 (14)	MDD	Prospective cohort			M-SADS, BDI		Greater response for 50-150 ng/ml (adult range)			X	X		X
Wilens et al., 1993b [52]	Nortriptyline	82 (68)	Children treated with nortriptyline	Retrospective cohort		Interventricular Conduction Delay (IVCD)		Abnormal ECG	> 150 ng/ml (IVCD)	X	X	X	X		X
Other antidepressants																
Burleson Daviss et al., 2006 [22]	Bupropion	16 (8)	MDD or depressive disorder not otherwise specified	Prospective cohort	CGI-I				7.5hours after morning dose: >37 ng/ml (provide cut-off for metabolites)	X	X	X	X	X	X
Antipsychotics																
Findling et al., 2006 [23]	Quetiapine	17 (16), analyzed n=10 at week 8	Conduct disorder and comorbid ADHD	Prospective cohort	CGI-I (week 8)				1-hour post-dose >300 ng/mL	X	X	X	X	X	X
Sallee et al., 2003 [26]	Ziprasidone	24 (19)	Tourette syndrome or chronic tic disorder	Prospective cohort (single dose)		PRL				X	X	X	X	X	X
Sallee et al., 2006 [90]	Ziprasidone	24 (19)	Tourette or chronic tics	Prospective cohort (single dose)				QTc interval		X	X	X	X	X	X
Alfaro et al., 2002 [38]	Clozapine	24 (19)	Schizophrenia or psychotic disorder not otherwise specified	Randomized controlled trials and prospective cohorts				PRL		X		X	X		X
Frazier et al., 2003 [45]	Clozapine	24 (19)	Childhood onset schizophrenia	Prospective cohort and randomized controlled trial	SANS	Subjective Treatment Emergent Symptoms Scale				X	X	X	X	X	
Sporn et al., 2007 [44]	Clozapine	6 weeks follow-up: 54 (34), long term follow-up: 33 (22)	Schizophrenia	Cohorts nested in randomized controlled trials and prospective cohort studies	desmethylclozapine /clozapine ratio : BPRS, SAPS (week 6)		Desmethylclozapine /clozapine ratio: SANS (week 6), CGAS (2-6 years). desmethylclozapine and clozapine concentrations: BPRS, SAPS, SANS, CGAS	Side effects recorded by treating physician (6 weeks)				X	X		X
Wohkittel et al., 2016 [46]	Clozapine	68 (33)	Various diagnoses, 70% childhood schizophrenia	Retrospective cohort		UKU SERS	CGI-S and CGI-I		< 317 ng/ml	X	X	X			X
Alfaro et al., 2002 [38]	Haloperidol	15 (9)	Schizophrenia or psychotic disorder not otherwise specified	Cohorts nested in randomized controlled trials and prospective cohorts				PRL		X		X	X		X

Morselli et al., 1979 [37]	Haloperidol	23 (14)	Psychotic disorder not otherwise specified	Prospective cohort		Side effects (unspecified)			< 6 ng/ml. For Gilles de la Tourette positive response in range 1-4 ng/ml (n=5)	X	X				X
Slooff et al., 2018 [91]	Haloperidol	13 (5)	Pediatric delirium	Prospective cohort				Adverse events by predefined list and Naranjo score		X		X	X	X	X
Selim et al. [92]	Loxapine	30 (13)	Non-agitated children and adolescents with chronic antipsychotic use	Single fixed dose Pharmacokinetic study (inhalation)				Sedation		X	X	X	X		X
Alfaro et al., 2002 [38]	Olanzapine	12 (7)	Schizophrenia or psychotic disorder not otherwise specified	Cohorts nested in randomized controlled trials and prospective cohorts		PRL				X		X	X		X
Fekete et al., 2017 [48]	Olanzapine	115 (47)	Various diagnoses	Retrospective cohort			CGI-I and CGI-S	UKU SERS	20-80 ng/ml	X	X	X			X
Migliardi et al., 2009 [39]	Olanzapine	13 (7)	Various diagnoses	Prospective cohort		PRL (in females)				X		X	X	X	X
Albantakis et al., 2017 [93]	Quetiapine	180 (82)	Various diagnoses	Retrospective cohort		UKU SERS	CGI-I and CGI-S		<100ng/ml	X	X	X		X	X
Gerlach et al., 2007 [59]	Quetiapine	21 (12)	Schizophrenia and schizoaffective psychosis	Prospective cohort			BPRS	DOTES, weight, ECG		X	X	X			X
Dos Santos-Junior et al., 2017 [60]	Risperidone	67 (51)	Various diagnoses	Cross-sectional				BMI z-scores; BP z-scores; serum glucose and insulin, TC and fractions; TG; LFTs; leptin		X	X	X		X	X
Duval et al., 2008 [40]	Risperidone	16 (10)	Schizophreniform disorder	Prospective cohort		PRL				X	X	X		X	X
Gagliano et al., 2004 [94]	Risperidone	20 (14)	Autistic disorder. Mental retardation was present in all subjects	Prospective cohort			CPRS-14	PRL		X		X	X	X	X

Klampfl et al., 2010 [49]	Risperidone	103 (85)	Impulsive aggressive symptoms, (74% of subjects disruptive behavior disorder)	Retrospective cohort			CGI	UKU SERS	8-26 ng/ml	X	X	X		X	X
Migliardi et al., 2009 [39]	Risperidone	29 (22)	Various diagnoses	Prospective cohort		PRL (in males)				X		X	X	X	X
Ngamsamut et al., 2016 [41]	Risperidone	103 (90)	Autism spectrum disorder according to DSM	Cross-sectional		PRL				X	X	X		X	X
Roke et al., 2012 [42]	Risperidone	51 (51) and comparison group of 47 (47)	Autism spectrum disorder or disruptive behavior disorder	Cross-sectional		PRL						X			X
Troost et al., 2007 [43]	Risperidone	25 (23)	Pervasive developmental disorder according to DSM IV	Prospective cohort		PRL				X		X		X	X
Correll et al., 2011 [95]	Ziprasidone	29 (13)	Various diagnoses	Prospective cohort				QTc duration and dispersion		X		X	X	X	X
Mood stabilizers																
Landersdorfer et al., 2017 [24]	Lithium	61 (32)	Bipolar-I disorder	Prospective cohort	YMRS				EC50 0.711 mEq/L	X	X	X	X	X	X
Amitai et al., 2014 [96]	Lithium	61 (31)	Bipolar and non-bipolar disorder	Retrospective cohort				WBCs, sCR or TSH blood levels		X		X	X	X	X
Patel et al., 2006 [97]	Lithium	27 (22)	Depression associated with Bipolar I disorder	Prospective cohort	CGI-BP		CDRS-R			X		X	X		X
Siegel et al., 2014 [98]	Lithium	30 (23)	Autism Spectrum Disorder	Retrospective cohort			CGI-I					X			X
Amitai et al., 2015 [99]	Valproic acid	104 (68)	Children treated with VPA at a psychiatric ward	Retrospective cohort				WBCs, PLT counts, TSH, LFTs, amylase levels or metabolic parameters					X	X	X

Legend

** Number of subjects with analysis on drug concentrations and effect.*
Each drug assessed within a study represents a row in the table; one study can therefore be in the table more than once. If an item was not described in the study, the item was not scored. Only outcomes that were analyzed are mentioned. Relationships may concern the mother compound, metabolite, or both.

Quality of study items: 1: Analytical method for the assay of drug concentration in serum or plasma, 2 Blood sample collection, 3. Patient selection, 4. Measurement of illness severity and registration of therapeutic improvement or worsening, 5: Comedication, 6: Number of patients
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ADD = Attention Deficit Disorder; ADHD = Attention Deficit Hyperactivity Disorder; BMI = body mass index; BP = blood pressure; BPRS = Brief Psychiatric Rating Scale; CABRS = Conners Abbreviated Rating Scale; CAPTRS = Conner's Abbreviated Parent Teacher Rating Scale; CDI = Children's Depression Inventory; CDRS-R = Children's Depression Rating Scale – revised; C-GAS = Childrens Global Assessment Scale; CGI-I = Clinical Global Impression improvement Scale; CGI-S = Clinical Global Impression severity Scale; CPRS = comprehensive Psychopathological Rating Scale; CTRS = Conner's Teaching Rating Scale; CY-BOCS = Childrens' Yale-Brown Obsessive Compulsive Scale; CY-BOCS = The Children's Yale-Brown Obsessive Compulsive Scale; DOTES = Dose Record and Treatment Emergent Symptom Scale; EC50 = half maximal effective concentration, the concentration of a drug which induces a response halfway between the baseline and maximum; ECG = electrocardiogram; ER = extended release formulation; GAF = Global Assessment of Functioning; GH = growth hormone; HDRS = Hamilton Depression Rating Scale; HR = heart rate; K-SADS= Kiddie Schedule for Affective Disorders and Schizophrenia; K-SADS-P = Schedule for Affective Disorders and Schizophrenia for School Age Children (6-18) Present Episode; LFTs = liver function tests; LOI-CV = Leyton Obsessional Inventory-Child version; MDD = Majpr Depressive Disorder; MFFT = Matching Familiar Figures Test; M-MAT = McLean Motion Attention Test; M-SADS = abbreviated Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS), just covering affective symptoms; NIMH = National Institute of Mental Health; NR = normal release; OCD = obsessive-compulsive disorder; OCR Scale = Obsessive-Compulsive Rating Scale; ODD = Oppositional defiant disorder; PERMP = Permanent Product Measure of Performance; PLT = platelet, PRL = prolactin; RCMAS = Revised Children's Manifest Anxiety Scale; sCR = serum creatinine; SEFCA = Side Effects Form for Children and Adolescents; SERS = Side Effects Rating Scale; SKAMP = Swanson, Kotkin, Agler, M-Flynn and Pelham Scale; SNAP = Swanson, Nolan, and Pelham Questionnaire; SRT = Scanning Reaction Time, part of three computer tests; TC = total cholesterol; TG = triglycerides; TSH = thyroid stimulating hormone; UKU = Udvalg for Klinske Undersogelser; WBC: white blood cell counts; WWPAS = Werry-Weiss Peters Activity Scale; YGTSS = Yale Global Tic Severity Scale

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SUPPLEMENTARY DATA

Supplementary table 1 search strategy

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CHAPTER 8

PIPAMPERONE POPULATION PHARMACOKINETICS RELATED TO EFFECTIVENESS AND SIDE-EFFECTS IN CHILDREN AND ADOLESCENTS

Sanne M. Kloosterboer, Karin M. Egberts, Brenda C.M. de Winter, Teun van Gelder, Manfred Gerlach, Manon H.J. Hillegers, Gwen C. Dieleman, Soma Bahmany, Catrien G. Reichart, Emma van Daalen, Mirjam E.J. Kouijzer, Bram Dierckx, Birgit C.P. Koch

Clinical Pharmacokinetics 2020; 59(11): 1393-1405

ABSTRACT

Background
Pipamperone is a frequently prescribed antipsychotic drug in children and adolescents in the Netherlands, Belgium and Germany. However, pediatric pharmacokinetics and the relationship with side-effects and efficacy are unknown. Currently divergent pediatric dosing recommendations exist.

Objectives
To describe the population pharmacokinetics of pipamperone in children and adolescents; to correlate measured and predicted pipamperone trough concentrations and predicted 24h area under the curves with effectiveness, extrapyramidal symptoms and sedation; to propose dose recommendations based on simulations.

Methods
Pipamperone concentrations were collected from Dutch pediatric patients in a prospective naturalistic trial (n=8), and German pediatric patients in a therapeutic drug monitoring (TDM) service (n=22). A total of 70 pipamperone concentrations were used to develop a population pharmacokinetic model with non-linear mixed-effects modeling (NONMEM®). Additionally, an additional random sample of 21 German patients with 33 pipamperone concentrations from the same TDM service was used for external validation. Pharmacokinetic parameters were related to clinical improvement, sedation, and extrapyramidal symptoms. Simulations were performed to determine optimal dosages.

Results
In a one-compartment model the apparent volume of distribution was 416 L/70kg and the apparent clearance was 22.1 L/h/70 kg. Allometric scaling was used to correct for differences in bodyweight. The model was successfully externally validated. The median [25th-75th percentile] measured pipamperone trough concentrations were numerically higher in responders (98.0 µg/L [56.0-180.5]) than in non-responders (58.0 µg/L [14.9-105.5]), although non-significant (p=0.14). A twice daily 0.6 mg/kg dosage was better than a fixed dosage to attain the concentration range observed in responders.

Conclusion
Our findings suggest that pipamperone therapeutic reference ranges may be lower for children with behavioral problems than recommended for adults with psychotic symptoms (100-400 µg/L). When dosing pipamperone in children and adolescents, bodyweight should be taken into account.

INTRODUCTION

Pipamperone is one of the most frequently prescribed antipsychotic to children and adolescents in the Netherlands, Germany and Belgium.[1-3] Between 2005 and 2015, 18% of all antipsychotics prescribed to children and adolescents in the Netherlands concerned pipamperone, with similar but slightly lower prescription rates in Germany and Belgium.[1-3] These prescription rates have been fairly constant for the past decades.

Pipamperone, being introduced in 1961 as Dipiperon® by Janssen Pharmaceutica, is considered a low-potency antipsychotic due to the relatively low affinity for the D2 receptor. The antagonism of the serotonin 5-HT2 and adrenergic alpha1 receptor is more pronounced [4], which explains its sedative effect, which is partly the result of relative hypoperfusion in the brain.[5, 6] For this reason, pipamperone has not only been explored for its antipsychotic properties [7], but also as a hypnotic in patients with sleep disorders, showing good efficacy.[8]

However, in children and adolescents pipamperone is particularly prescribed for behavioral problems, like other antipsychotics in this population.[2, 9, 10] Severe behavioral problems in youths represent the main symptoms of conduct disorder, or may occur within other psychiatric disorders such as autism spectrum disorder, attention-deficit/hyperactivity disorder, or mental retardation. Pipamperone may be a preferred antipsychotic for these indications, mainly because of the calming effects and the availability of oral liquid dosage formulation, enabling flexible dosing.[11, 12] However, despite the extensive practical experience in children and adolescents, the evidence for efficacy of pipamperone is very limited. Although two small open label studies from the 1970s showed positive results for the treatment of behavioral problems in children and adolescents, randomized controlled trials are lacking.[13, 14] For this reason, prescribing pipamperone to youths is currently considered off-label or restricted to use with particular consideration of the benefit-risk ratio, depending on the country.[15, 16] Indications for use as mentioned in the summary of product characteristics within the Netherlands, Germany and Belgium include psychomotor agitation and behavioral problems, with as major clinical contra-indication depression of the central nervous system.[4, 15, 17]

Side-effects of pipamperone mainly concern sedation and extrapyramidal symptoms.[4, 18] Prolactin elevation has also been reported, and is associated with galactorrhea and amenorrhea.[4] Although weight gain and metabolic changes have become the major concern for atypical antipsychotic use in children and adolescents[19], these side-effects have not been described in literature for pipamperone. However, as antipsychotic-induced weight gain is believed to be partly attributed to the serotonin system[20], the 5-HT2 antagonism of pipamperone is likely to induce weight gain as well.

While data on efficacy and side-effects are scarce, publicly available pharmacokinetic data of pipamperone in children and adolescents are completely missing. In adults some pharmacokinetic studies have been performed, showing a maximum plasma concentration after 1-2 hours

and an elimination half-life of 12-30 hours.[4, 21, 22] However, in children and adolescents both pharmacokinetics and pharmacodynamics are expected to be considerably different, as some consensus based-dosing guidelines advise to lower the starting dose in this population by 95% compared to recommended doses in adults, resulting in 2 mg instead of 40 mg.[16]

The aim of this study was to describe the population pharmacokinetics of pipamperone in children and adolescents. Furthermore, the relationships of pharmacokinetic parameters of pipamperone with both clinical improvement and side-effects, including sedation and extrapyramidal symptoms in children and adolescents were explored. These data will provide a more solid basis for pipamperone dose recommendations in this vulnerable patient population.

METHODS

Study population

The study population consisted of two samples. The first sample included children and adolescents who were prospectively enrolled in a Dutch multicenter observational trial (SPACe, NTR6050). Inclusion criteria were: age 6 to 18 years, documented clinical diagnosis of autism spectrum disorder according to DSM 4[23] or DSM 5[24] and comorbid behavioral problems and treatment with pipamperone. Exclusion criteria were: diabetes type I or II, congenital or acquired syndrome associated with changes in appetite, body weight or lipid profile (e.g. Prader Willi), treatment with another antipsychotic within the last 6 months or known Long QT syndrome. Eligible patients were treated in an inpatient or outpatient setting in one of the 7 participating centers in the south-west region of the Netherlands (2 academic tertiary care centers and 5 psychiatric secondary care centers). Subjects were prescribed flexible pipamperone dosages once or twice daily, as part of routine clinical care and prescribed by the treating physician. Pipamperone was prescribed as tablet formulation or oral solution. Patients were recruited between August 2016 and May 2018. All patients and/or their legal representatives gave written informed consent before entering the study. The study was approved by the medical ethics committee of the Erasmus Medical Centre, the Netherlands (number MEC 2016-124).

The second sample consisted of children and adolescents of whom pipamperone concentrations had been measured as part of the routine Therapeutic Drug Monitoring (TDM) service of the department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy at the University Hospital of Wuerzburg, Germany. Subjects were treated at this clinic or at associated clinics for child and adolescent psychiatry within the competence network of TDM in child and adolescent psychiatry, which is described elsewhere.[25] Patients were prescribed flexible dosages of pipamperone as tablets or oral solution, being administered one up to five times daily. The pipamperone samples were collected between June 2008 and February 2015 in patients with various psychiatric diagnoses. The medical ethics committee of the University of Wuerzburg approved the study (study number 27/04) and waived informed consent, as drug concentrations were measured as part of routine care.

Both studies were conducted in accordance with the Declaration of Helsinki.

Pipamperone concentrations

In the Dutch trial, a total of 6 pipamperone drug concentrations per subject were collected at random time points on 2 separate days during a 6 month follow-up with 3 to 6 months in between sampling. The time between 2 samples was at least 1 hour. Samples were collected with venipuncture and the Dried Blood Spot (DBS) method. The time of sampling, time of last pipamperone intake, the pipamperone dosages and comedication during follow-up were recorded. Both samples in steady state and in non-steady state were collected. The Dutch samples were analyzed using previously validated ultra-high performance liquid chromatography-mass spectrometry (LC-MS) methods for plasma and DBS.[26-28] The lower limit of quantification was 1.5 µg/L. The accuracy of the quality-control samples was well below a limit of relative standard deviation of 15% and the intra- and inter-assay imprecision was less than 15% during the study period.

Within the German TDM service, samples were collected with venipuncture in the morning before the first pipamperone dosage of that day (trough concentrations)[21] The pipamperone dosage and administration time (morning, midday, evening or night) was reported on the request form. Only concentrations measured in steady state were included for analyses, as for non-steady state samples previous dosages were not known. The German samples were analyzed with a validated serum high-performance liquid chromatography-ultraviolet method (HPLC-UV) for plasma. The lower limit of quantification was 8 µg/L. The method was linear in a range of 2 µg/L – 1050 µg/L (r^2 = 0.99952). Concentrations below the LLOQ were excluded, as for these no quantification of the plasma concentration was provided.

Assessment of clinical outcomes

In the Dutch trial, measures of clinical effectiveness and side-effects were collected at baseline and prospectively during the 6 month follow-up (at 6 months and for a subset of patients at 1 and 3 months). Clinical effectiveness was measured by the *Clinical Global Impression Scale* (CGI).[29] This scale describes the severity of psychopathology (CGI-S) and its improvement (CGI-I) by 7 categories, rated by the treating physician. The CGI-S describes the severity of illness relative to the physicians experience with patients with the same diagnosis: 0 = not assessable; 1 = normal; 2 = borderline; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = extremely ill. The CGI-I rates the improvement in comparison to the original medication-naive state of symptoms: 0 = not assessable, 1 = very much better, 2 = much better, 3 = moderately better, 4 = unchanged, 5 = minimally worse, 6 = much worse. Extrapyramidal symptoms were measured with the Abnormal Involuntary Movement Scale (filled in by treating physician or researcher)29 and sleepiness with the Epworth Sleepiness Scale (filled in by parents).[30] Biochemical laboratory check-ups were performed at baseline, after 6 months and for a subset of children at 3 months, and included renal function, liver function, fasting glucose, HbA1c, prolactin, cholesterol, fatty acids and albumin. During follow-up, medication adherence was measured with questionnaires (Medication Adherence Rating Scale MARS-5[31,] filled in by parents, and a visual analog scale, filled in by parents and treating physician) and during the last month of follow-up with an electronical

monitoring system (MEMS©).[32] Weight and height was measured at baseline, at time of blood sampling and for a subset of children after 1 month of follow-up.

At the German TDM service, the following information was reported at the request form at time of sampling: renal dysfunction, hepatic dysfunction, smoking status, current infection, comedication, CGI-S, CGI-I and side-effects with the Udvalg for Kliniske Undersøgelser Side Effect Rating Scale (UKU-scale).[33] The UKU scale rates the severity of the side-effects with the following categories: 0 = no side effects; 1 =mild, 2 = moderate and 3 = severe side effects. The nature of the side-effects was also recorded and classified as follows: sedation, dermatological, tension, salivation (more/less), accommodation disorder, polydipsia, delirium, extrapyramidal symptoms, cardiovascular, gastrointestinal, urogenital, other.

Population pharmacokinetic modelling

Pharmacokinetic analysis was performed by non-linear mixed-effects modelling using NONMEM® version 7.4.2 (FOCE+I; ICON Development Solutions, Ellicott City, MD, USA) and PsN® Version 4.7.0. Pirana® software version 2.9.7 was used as an interface between NONMEM® and R (version 3.4.4).

Base model development

One and two compartment models were considered with first order absorption with and without lagtime. Typical values for volume of distribution (V) and clearance (CL) were estimated as ratios, as bioavailability could not be quantified (V/F and CL/F). As the absorption rate constant (ka) could not be estimated, ka was fixed at 2 h-1, based on previous literature.[21] For each pharmacokinetic parameter inter-patient variability (IPV) was evaluated and shrinkage was calculated for all parameters for which IPV was established. A shrinkage value below 25% was considered acceptable.[34] Allometric scaling was used to account for the influence of bodyweight on pharmacokinetic parameters, which was explored with a fixed exponent 0.75 for CL/F and 1 for V/F, and with exponents estimated by the model. Residual variability was modelled as a separated additive and proportional error for the analytical method (LC-MS versus HPLC-UV) and sampling method (DBS versus venepuncture). Model selection was based on minimum objective function values (OFVs), parameter precision, error estimates, shrinkage values, and visual inspection of the goodness-of-fit plots.

Covariate model development

The following covariates were considered as potential model covariates: sex, age, body mass index (BMI), weight, comedication, psychiatric disorder, somatic comorbidities, smoking, renal function, liver function, pipamperone dose and dose/kg. For the Dutch patients, also albumin, hematocrit and medication adherence were known and evaluated as covariates. The correlation between the covariates and IPV was first evaluated graphically. Subsequently, covariates with a visual relationship with IPV were individually added to the model. Continual covariates were described using an exponential function and categorical covariates using a proportional function. The forward inclusion-backward elimination method was used.[35] Covariates that significantly improved the model with the univariate analysis ($p<0.05$), were selected for multivariate analysis. During the backward elimination process, covariates that improved the model at a level of $p<0.001$ were selected.

Internal model evaluation

Two methods were used for the internal validation of the model. Firstly, a bootstrap analysis was performed.[36] One thousand bootstrap datasets were randomly resampled from the original dataset with replacement. The validity of the model was evaluated by comparing the bootstrap estimates and their 95% confidence intervals with the values generated by the original dataset. Secondly, the model was evaluated with the visual predictive check (VPC), using a set of 1000 simulated datasets to compare the observed concentrations with the distribution of the simulated concentrations.[37]

External model evaluation

An additional dataset of another group of German patients from the same TDM service was used for external validation of the final model. Goodness-of-fit plots and a normalized prediction distribution errors (NPDE) analysis (n=1000) were used to evaluate the external validity.[38]

Pharmacodynamic analyses

The medians with 25th-75th percentiles of measured trough concentrations were correlated to CGI-I, extrapyramidal symptoms (EPS) and sedation. As trough pipamperone concentrations were not available for all Dutch patients, individual trough concentrations were also predicted for all patients, next to 24 hour areas under the concentration-time curve (AUCs24h). These pharmacokinetic parameters were also correlated to CGI-I, EPS and sedation. A subject was considered a responder when the CGI-I was rated "very much better", "much better" or "moderately better", and a non-responder when another score was given (except from "not assessable"). EPS were scored positive when at least two times "mild" or one time "moderate" in the first seven items had been filled in on the AIMS (Dutch patients), or when "EPS" was filled in as a side-effect on the application form (German patients). Sedation was considered as a score ≥1 on the Epworth Sleepiness Scale (Dutch patients) or as "sedation" being filled in as side-effect on the application form (German patients). The pipamperone trough concentration, predicted trough concentration and predicted AUC24h at the time of first response or side-effect (EPS and sedation) was used for the analyses. In this analysis efficacy was accepted as an endpoint regardless of the time interval since initiation of treatment (but within the study period). If no response or side-effect was observed, the highest concentration during follow-up was used. Laboratory findings were compared with age- (and if applicable sex-) specific reference values as being used in the Erasmus MC Rotterdam in July 2019.[39] The Mann-Whitney U test was used to compare trough concentrations and AUC24h between groups. The Fisher's Exact test was used to compare proportions. A p-value <0.05 was considered significant. Graphpad Prism 5 (GraphPad Software, La Jolla California USA) was used for the analyses.

Predictions

Pipamperone concentrations using a twice daily 30 mg dosing regimen were predicted for a patient of 25, 50 and 75 kg during a 12 hour time interval. After graphical inspection, a mg/kg dosage was chosen for optimal attainment of the concentration range that was found to be associated with response based on pharmacodynamic analyses. This mg/kg dosage was evaluated with additional predictions for a patient of 25, 50 and 75 kg. The population predictions were used as mean with a 95% confidence interval based on 1000 simulations of individual predictions.

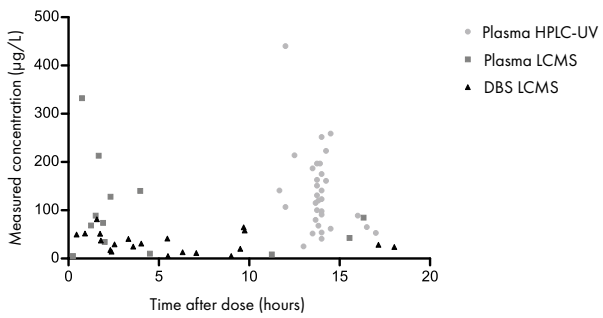
RESULTS

Thirty patients with 70 measured pipamperone concentrations were included in the model building group (Dutch patients n=8 and German patients n=22). Two German samples were below the LLOQ and were not included. The pipamperone concentrations were collected in the entire absorption and elimination phase, as can be seen in **figure 1**. Subsequently, 21 extra patients with 33 measured pipamperone concentrations were included in the external validation group (German patients). The baseline characteristics of the included patients are presented in **table 1**. Most patients were diagnosed with autism spectrum disorder, attention deficit/hyperactivity disorder or mental retardation. The median (range) measured pipamperone concentration was 66.5 µg/L (0.21-1068) in the model building group and 88 µg/L (16-337) in the model validation group.

In the model building group, n=1 sample was below the LLOQ (Dutch sample). In the model validation group, no samples below the LLOQ were included.

Indications for a pipamperone concentration measurement in the German TDM service were known for n=60 samples (90.9 % of total n=66 German samples). Most pipamperone concentrations were measured because of non-effectiveness (n=16, 26.7%) or dosage change (n=16, 26.7%). Other indications included drug-drug interaction (n=13, 21.7%), start of therapy (n=10, 16.7%) adherence (n=8, 13.3%) or side-effects (n=5, 8.3%); more than one reason could apply.

Figure 1 Measured pipamperone concentrations used for model development versus time after dose.



Pipamperone concentrations were collected during the absorption and elimination phase. The presented DBS concentrations are measured concentrations before conversion to estimated plasma concentrations. Four samples are not shown for readability of the figure (x=182, y=0.21; x=118.63, y=2.69; x=121.4, y=4.82; x=12.25, y=1068). DBS: dried blood spot; HPLC-UV: high-performance liquid chromatography-ultraviolet; LCMS: ultra-high performance liquid chromatography-mass spectrometry

Table 1 Patient characteristics

	Model building group (n=30)	Model validation group (n=21)
Male (n)	21 (70%)	13 (61.9%)
Age (years)	13.0 (5.6-17.7)	14.9 (7.2-20.6)
Body weight (kg)	50.4 (24.8-100.4)	47.4 (24.0-118.0)
Height (cm)	152 (123-180)	155 (122-186)
Body mass index (kg m-2)	20.41 (14.3-37.2)	19.0 (12.2-43.3)
Body mass index Z-score	0.98 (-2.57-3.49)	0.38 (-4.49-4.25)
Daily dosage (mg)	45 (12-400)	60 (10-180)
Psychiatric comedication		
Antipsychotic drugs	18 (60%)	8 (38.1%)
ADHD drugs*	6 (20%)	8 (38.1%)
Tricyclic antidepressant drugs	1 (3.3%)	2 (9.5%)
Selective Serotonin re-uptake inhibitors	0 (0%)	3 (14.3%)
Benzodiazepine agonists	1 (3.3%)	2 (9.5%)
Lithium	1 (3.3%)	0 (0%)
Other comedication		
Antiepileptic drugs	4 (13.3%)	5 (23.8%)
Diagnosis		
Autism spectrum disorder	22 (73.3%)	4 (19.0%)
Attention deficit/hyperactivity disorder	12 (40%)	5 (23.8%)
Schizophrenia Spectrum and Other Psychotic	2 (6.7%)	0 (0%)
Disorders	1 (3.3%)	0 (0)
Conduct disorder	9 (30%)	4 (19%)
Mental retardation		
Setting**		
Clinical	22 (73.3%)	19 (90.5%)
Outpatient	7 (23.3%)	2 (9.5%)
No of pipamperone samples per patient	1.5 (1-6)	1 (1-6)
Pipamperone concentration (µg/L) ***	66.5 (0.21-1068)	88 (16-337)
Clinical Global Impression Scale (CGI) score	5 (4-7)	5 (4-7)

Patient characteristics at time of first pipamperone concentration measurement. Presented as median and range for continuous variables.
The model building group consisted of patients from a Dutch multicenter observational trial and patients from a German TDM service. The model validation group consisted of patients from the same German TDM service.
CGI: Clinical Global Impression Scale; 1 = normal; 2 = borderline; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = extremely ill. CGI was missing for n=2 in the model building group and n=2 in model validation group.
** includes methylphenidate, amphetamine, atomoxetine*
*** unknown for n=1 patient in model building group*
**** includes DBS concentrations before conversion to estimated plasma concentrations.*

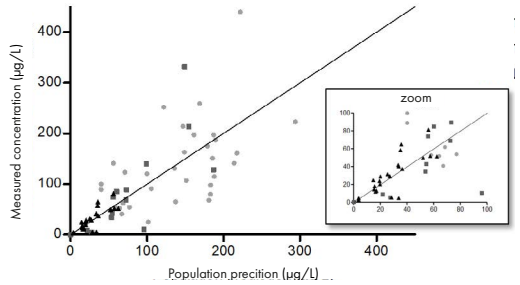
Pharmacokinetic analyses

Base model

The data were best described using a one-compartment model. This model was further improved by including an IPV on clearance. The residual error was described by a combined error model with an extra additional error for HPLC-UV concentrations. As the conversion of DBS concentrations to plasma concentrations based on the previously conducted clinical validation study[28] showed a trend towards under-estimation of the predicted concentrations by the model, a model-based conversion was calculated which showed better predictions. The GOF plots of the final model are presented in **figure 2**, and the parameter estimates of the final model are presented in **table 2**.

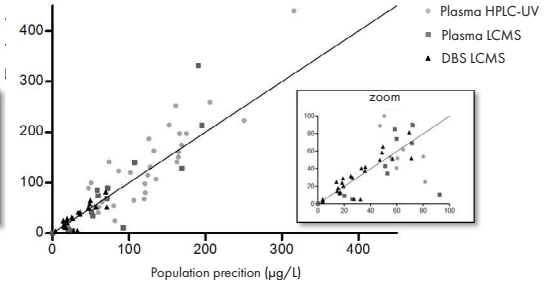
Figure 2 (2a – 2d) Goodness of Fit Plots – final model

Figure 2a Final model: measured concentrations versus population predictions



One outlier is not presented for readability of the figure (x=529.7, y=1068).

Figure 2b Final model: measured concentrations versus individual predictions.



One outlier is not presented for readability of the figure (x=755.3, y=1068).

Figure 2c Final model: the correlation of conditional weighted residuals (CWRES) with time.

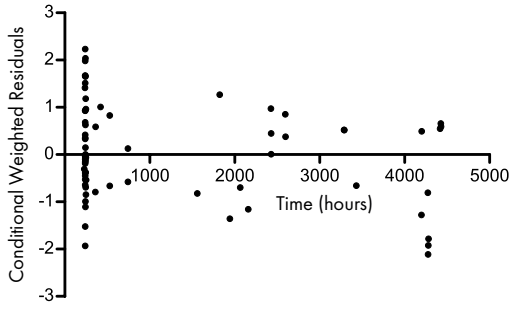
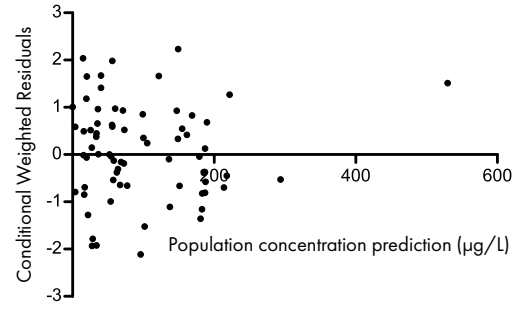


Figure 2d Final model: the correlation of conditional weighted residuals (CWRES) with population predicted concentrations.



One outlier not shown for readability of the figure (time 38232.00, CWRES 0.244350).

DBS: dried blood spot; HPLC-UV: high-performance liquid chromatography-ultraviolet; LCMS: ultra-high performance liquid chromatography-mass spectrometry

Table 2 Parameter estimates final model

Parameter	Estimate (RSE %) [shrinkage]	Bootstrap median (90th percentile)
Ka[1] (l/h)	2	2
V/F[2] (L/70 kg)	416 (32)	481 (279-2251)
CL/F[2] (L/h/70 kg)	22.1 (12) [34%][3]	22.7 (18.7-31.2)
IPV CL	20.5%	24.0 (11.6-69.7) %
Residual variability		
Additional error (µg/L)	0.21 (1)	0.21 (0.11-10.5)
Proportional error	0.39 (19) [7%]	0.33 (0.15-0.46)
Additional error HPLC-UV (µg/L)	26.6 (40) [7%]	25.6 (8.5-39.1)
DBS correction: y = ax + b		
a (µg/L)	0.33 (8)	0.31 (0.19-0.40)
b (µg/L)	3.90 (15)	3.87 (3.42-14.84)

[1] fixed; [2] Allometric scaling with exponent 1 for V and 0.75 for CL; 3 IPV: inter-patient variability. 90th percentile based on bootstrap with n=772 successful runs.
Ka: absorption rate constant; V: volume of distribution; CL: clearance; IPV: inter-patient variability; HPLC-UV: high-performance liquid chromatography-ultraviolet ; DBS: dried blood spot

Covariate analysis

After graphical analysis, the univariate analysis resulted in 3 significant covariates for the IIV on clearance (bodyweight, creatinine, BMI). These covariates were added to the base model for multivariate analysis. No covariates remained significant after backward elimination except from bodyweight, which was best described using fixed exponents with allometric scaling.

Evaluation of the final model

The model-based parameter estimates were similar to the values computed from the bootstrap analysis, indicating the stability of the model (see **table 2**). The extra additional error for HPLC-UV concentrations was 26.6 (µg/L). The VPC showed a good predictive performance (figure not shown).

The model was successfully externally validated, as is shown by the goodness-of-fit plots and NPDE. The goodness-of-fit-plots show that the model adequately describes the observed concentrations (**figure S1**), and the NPDE show a normal distribution of the normalized errors and some overestimation of variability (**figure S2**).

Pharmacodynamic analyses

Effectiveness

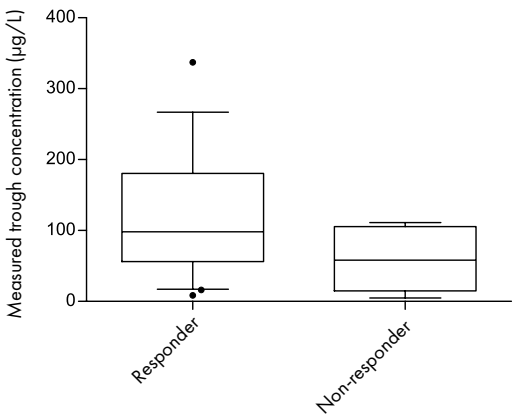
For a total of 35 patients CGI improvement scores were reported; 28 (80%) of them was rated as responder, and 7 as non-responder (20%). Psychotropic comedication was common in both responders (68%) as non-responders (57%), but non-significantly different (p=0.67).

For 29 of these patients, both CGI improvement scores and measured pipamperone trough concentrations were available. The median [25th percentile-75th percentile] pipamperone trough concentration was higher in responders (98.0 µg/L [56.0-180.5], n=24) than in non-responders (58.0 µg/L [14.91-105.5], n=5), but this difference was non-significant (p=0.14). See **figure 3a**.

For the total sample of patients with a CGI improvement score (n=35), pipamperone trough concentrations were also predicted based on the pharmacokinetic model. The median [25th percentile-75th percentile] predicted trough concentration for responders was higher than in non-responders: 80.0 µg/L [63.0-136.6] versus 51.3 µg/L [43.4-78.8], with a trend towards significance (p=0.07). **See figure 3b**. The predicted median AUC24h in responders (3448.0 µg*h/L) was also higher than in non-responders (1811.0 µg*h/L), p= 0.05.

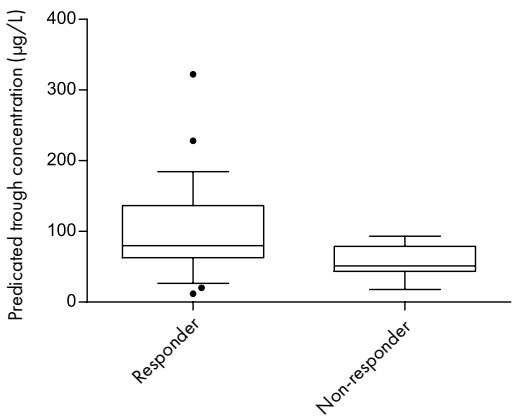
Figure 3 (3a – 3b) Pipamperone trough concentrations and clinical improvement

Figure 3a Measured trough concentrations versus response based on the Clinical Global Impression Scale.



Data available for n=29 subjects. In responders, the measured trough concentration at time of first response was used. In non-responders, the highest measured trough concentration during follow-up was used. Whiskers indicate 10th-90th percentile.

Figure 3b Predicted trough concentrations versus response based on the Clinical Global Impression Scale.



Data available for n=35 subjects. In responders, the predicted trough concentration at time of first response was used. In non-responders, the highest predicted trough concentration during follow-up was used. Whiskers indicate 10th-90th percentile.

When patients with ADHD and concurrent ADHD comedication (methylphenidate, amphetamine or atomoxetine) were excluded from the analyses, the results remained the same. The median [25th percentile-75th percentile] pipamperone trough concentration was higher in responders (113.5 µg/L [62.0-180.5], n=20) than in non-responders (58.0 µg/L [14.91-105.5], n=5), p=0.10, as was the predicted trough concentration (83.5 µg/L [66.5-136.6], n=24, versus 51.3 µg/L [43.4-78.8], n=7), p<0.05, and the AUC24h (3486 µg*h/L versus 1811 µg*h/L), p<0.05.

Extrapyramidal symptoms

In 4 patients extrapyramidal symptoms (EPS) were observed (8% of 50 patients with EPS scores). All these 4 patients came from the Dutch sample. For 2 of these patients, and 44 of the patients without EPS, measured trough concentrations were available. The median [25th percentile-75th percentile] pipamperone trough concentration was lower in patients with EPS (25.74 µg/L [8.6-42.9]) than in patients without EPS (109.5 µg/L (62.0-174.5). Predicted pipamperone trough concentrations (median [25th percentile-75th percentile]) were also lower in patients with EPS (46.4 µg/L [22.2-77.1], n=4) than in patients without (99.6 µg/L [57.7-166.8], n=46), p=0.06. The AUC24h was significantly lower in patients with EPS (1583.0 µg*h/L) than in patients without EPS (3633.0 µg*h/L), p=0.03.

Sedation

Sedation scores were available for 50 patients; 9 of them had sedation at least once. For 46 of these patients, measured trough concentrations were available. The median [25th percentile-75th percentile] pipamperone trough concentration was 77.0 µg/L [36.7-132.5] in patients with sedation (n=6) versus 114.0 µg/L [59.0-174.5] in patients without sedation (n=40), p=0.32. The median [25th percentile-75th percentile] predicted trough concentration was also non-significantly lower in patients with sedation (61.9 µg/L [38.1-88.9], n=9) than patients without sedation (103.8 µg/L [59.9-166.9], n=41), p=0.08. The AUC24h was significantly lower in patients with sedation than patients without sedation (2050 versus 3852 µg*h/L, p=0.02). All patients using benzodiazepines as comedication were not rated as having sedation.

Biochemical laboratory parameters

A total of 15 biochemical laboratory measurements was available for n=8 patients (all Dutch patients). The median duration (range) of pipamperone use of these patients was 34 months (1-54). During pipamperone treatment, the median (range) prolactin level was 0.3 Units/L (0.03-0.46); 2 patients had decreased levels (both did not have prior treatment with another antipsychotic drug), while no patients had elevated prolactin levels. The median (range) total cholesterol level was 4.2 mmol/L (3.5-5.1); no patients had elevated total cholesterol levels. The median (range) triglyceride level was 0.71 mmol/L (0.38-2.06); one patient had elevated triglyceride levels (no baseline levels known). Fasting glucose and HbA1c were normal in all patients; the median (range) glucose level was 4.7 mmol/L (4.2-5.6); the median (range) HbA1c level was 32.5 mmol/mol Hb (31-37).

Predictions

Predicted concentrations with 95% confidence intervals using a twice daily 30 mg dosing scheme in steady state for a patient of 25kg, 50kg and 75 kg are shown in **figure 4a**. The mean (95% confidence intervals) of the predicted pipamperone trough concentrations (population prediction) after a 30 mg dose were 163.2 (67.3-268.9) µg/L for a patient of 25 kg, 103.9 (49.0-167.2) µg/L for a patient of 50 kg and 79.3 (38.4-124.4) µg/L for a patient of 75 kg. The same predictions were performed with a 0.6 mg/kg dosage in a twice daily dosing scheme, showing less variability in pipamperone concentrations and better attainment of the pipamperone trough concentration range that was found to be associated with response (**figure 4b**). The mean (95% confidence intervals) of the predicted pipamperone trough concentrations (population prediction) after a 0.6 mg/kg dosage were 81.6 (33.6-134.5) µg/L for a patient of 25 kg, 103.9 (49.0-167.2) µg/L for a patient of 50 kg and 119.0 (57.6-186.7) µg/L for a patient of 75 kg.

DISCUSSION

This is the first study that describes the population pharmacokinetics of pipamperone in children and adolescents, and investigates the relationships between pipamperone concentrations, effectiveness and side-effects in this young population.

The pediatric pipamperone pharmacokinetics in this study are comparable to adult values found in a previously published study by Potgieter et al..[21] This study found a mean maximum concentration of 263-266 µg/L after a 120 mg dose for three pipamperone products in healthy volunteers (mean weight 76.8 kg), corresponding to a calculated mean volume of distribution of 451-456 L assuming a bioavailability of 100%. In our pediatric study sample, the mean apparent volume of distribution was estimated 416 L/70 kg. Both in our sample and in the study by Potgieter et al., a high variability was found. Strikingly, another study performed in adults found relatively low pipamperone concentrations after a 40 mg dose, corresponding to a more than twofold larger calculated volume of distribution of 908 L.[22] Possibly, the bodyweight of the subjects in this latter study was higher, but patient characteristics were not provided. It could also be hypothesized that non-linear pharmacokinetics underlie these differences; this however, was not seen in our sample.

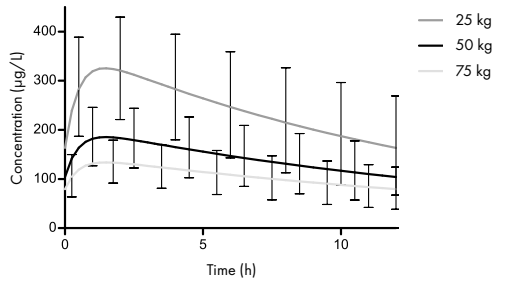
Although well-established reference ranges are lacking for pipamperone, the Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology recommends a therapeutic range between 100-400 µg/L for adults with psychotic symptoms.[40] In the absence of studies correlating pipamperone concentrations to clinical effects, this range is based on expected concentrations at an approved dose and derived from the previously mentioned pharmacokinetic study by Potgieter et al.[21]

However, ideally, reference ranges are defined by well documented concentration-effect relationships in the relevant population.[40] Recently it has been suggested that the Q1-Q3 (25th-75th percentile) range of psychotropic drug concentrations in responders would be the most optimal way to define preliminary therapeutic ranges based on observationally collected data.[41] In our study, this would result in a suggested pipamperone reference range for children and adolescents of 56.0-180.5 µg/L. Although the indications for use were mostly unknown, it is expected that the main indication concerned behavioral problems associated with autism spectrum disorders, attention-deficit/hyperactivity disorder and mental retardation, given the major share of these diagnoses in our sample. This range is substantially lower than the suggested range in adults, which might be partly explained by different indications for use, as psychotic symptoms and aggressive behavior are associated with different pathomechanisms. This however, needs further confirmation for pipamperone. Furthermore, the therapeutic reference range for children might be different from the optimal range in adolescents due to developmental pharmacodynamics changes, which should be investigated in larger prospective trials.

Based on the pipamperone concentration range in responders and the population pharmacokinetics

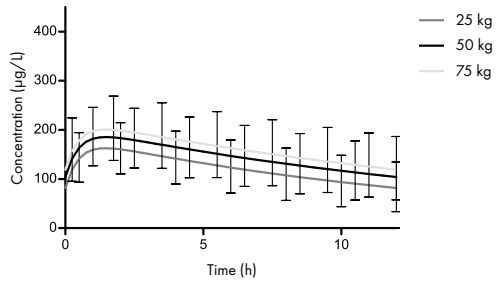
Figure 4 Simulations

Figure 4a Fixed 30 mg dose



Curves represent population predictions with 95% confidence intervals using a twice daily 30 mg pipamperone dosing scheme in steady state for a child of 25, 50 and 75 kg.

Figure 4b 0.6 mg/kg dose



Curves represent population predictions with 95% confidence intervals using a twice daily 0.6 mg/kg pipamperone dosing scheme in steady state for a child of 25 kg (15 mg), 50 kg (30 mg) and 75 kg (45 mg).

within our sample, several dose recommendations for children and adolescents could be made. Firstly, a twice daily dosing scheme should be sufficient based on the relatively long mean elimination half-life (13h), as has also been found for adults (12-30h).[4, 21] However, current guidelines are not uniform with respect to dosing intervals; while in Germany a dosing interval of 3 times per day is advised [15], the Dutch guideline states 1-2 times a day.[16] Secondly, a mg/kg dosage seems more appropriate than a fixed dosage, as weight was found to significantly influence the pharmacokinetics. Based on our simulations, a twice daily 0.6 mg/kg dosage seems appropriate to attain the concentration range that was associated with response. However, given the large variability between patients, some patients might have adequate response with lower dosages, while others need higher dosages. This calls for careful pipamperone dosage titration.

Strikingly, children and adolescents with side-effects had lower pipamperone concentrations than subjects without side-effects in our study. This is remarkable, as previous studies have found that a higher D2 receptor occupancy, as a result of higher antipsychotic drug concentrations, is associated with an increased risk for extrapyramidal symptoms 42. Certain children and adolescents might be more susceptible to side-effects based on their pharmacodynamic profile, which makes them prone to side-effects even at low pipamperone concentrations, preventing further dosage increase. It might be hypothesized that this is the case for younger children, as these have been found to be more vulnerable for other side-effects associated with antipsychotic use as well.[43] However, patients with extrapyramidal symptoms were not generally younger than patients without in our sample; neither did patients with extrapyramidal symptoms have other psychopathology than generally in the sample. Possibly, certain genetic variances underlie the elevated risk, as certain polymorphisms in the dopamine and serotonin receptor have been suggested to be a risk factor for antipsychotic-induced side-effects in adults.[44] Another explanation is that part of the side-effects may be due to the nocebo-effect, as with off-label use patients and legal representatives are informed about potential effects and side effects in a very detailed manner. More likely however, is that side-effects have been over-estimated in the Dutch patients, while underestimated in the German patients in our study. While the Dutch patients were enrolled in a clinical trial with structured screening and reporting of side-effects, it is expected that in the daily practice of the German TDM service relatively less side-effects were reported. As the pipamperone dosages and concentrations were higher in the German sample, this might have led to the finding that higher concentrations are associated with less side-effects.

In this study, the Dried Blood Spot (DBS) method was used as pharmacokinetic sampling method next to conventional venipuncture. DBS only involves one fingerprick for drug concentration measurement and can be performed in the home-setting, which makes it a promising, less invasive method for pharmacokinetic sampling in children. The development of a DBS assay requires a thorough validation process before implementation, including the assessment of agreement between DBS samples and simultaneously collected plasma samples in a real-life, clinical setting.[45] During this previously performed clinical validation study for pipamperone, the best agreement was found by dividing DBS samples by 0.158 (corresponding to a recovery of ca 16% in DBS).[28] However, in our model this conversion of the DBS samples initially led to a clear underestimation of the predicted plasma concentrations. This proportional bias was resolved by a new conversion of DBS concentrations to estimated plasma concentrations, being estimated by the model: DBS/0.33 +3.90 (see table 2). However, this finding questions the validity of the clinical validation process in our pediatric population. After consultation with the medical ethics committee the clinical validation was performed in adults due to ethical concerns. However, several factors may differentially impact DBS recovery of pipamperone in children versus adults. This could include the amount of interstitial fluid that is collected during the fingerprick, which may be more in adults. Also, sampling in children may require more pressuring on the finger to collect a full blood spot, and this may have caused hemolysis. Our findings show that results cannot simply be generalized across age groups and performing a clinical validation study in the intended target group should hence be considered. At the same time, in the clinical validation study a suboptimal agreement was already observed, which was confirmed by the findings in our population.

The results of this study must be considered in the context of its limitations. The pipamperone concentrations were collected in an observational setting with flexible dosing schemes in clinical practice. As has been suggested earlier, this study design is suboptimal to demonstrate concentration-effect relationship.[41, 46] Placebo-responders, who generally represent a substantial share of the patients in psychiatry, are likely to receive lower dosages, while non-responders might receive higher dosages. Also, dosages might be lowered when side-effects are observed. Therefore, the observed relationships between clinical effects and pipamperone concentrations could be biased. Furthermore, the analyses were performed with two different datasets. The data was collected in two countries, with different clinical and laboratory assessment methods, different inclusion criteria and prospectively versus retrospectively collected data. The data in Dutch patients was collected in a screening-based way, with questionnaires and screening-tools being applied at fixed time points, while in German patients improvement was retrospectively scored and side-effects were only reported at time of sampling. Possibly, this might have led to an overestimation of side-effects in the Dutch patients and an underestimation in the German patients. As the pharmacodynamic data on response was mainly retrospectively scored and collected in patients with various indications for use, this data did not allow for a more extensive exposure-response analysis. Despite combining the two datasets, the total number of patients was relatively low, and the relatively sparse amount of non-trough pipamperone concentrations might have limited the pharmacokinetic model development. As pipamperone is relatively lipophilic, it is expected to distribute to peripheral tissues. However, our data was possibly too sparse to support a two-compartment model, although a one-compartment model reflected the observed data best. Furthermore, different matrices and analytical methods were used to determine pipamperone concentrations, further increasing variability despite the corrections within the error-model for these influences. Lastly, the influence of metabolizing enzymes such as the cytochrome P450 could not be tested. Although the metabolism of pipamperone is assumed to take place in the liver, it is unknown which cytochrome P450 enzyme(s) are involved.[40]

CONCLUSION

This study presents the pharmacokinetics and pharmacodynamics of pipamperone in children and adolescents, based on concentrations measured in a real-life, clinical setting. Based on our findings, we recommend a twice daily dosing scheme for pipamperone in this population. Furthermore, bodyweight should be taken into account when dosing pipamperone in children and adolescents. Although more research is needed for the routine application of therapeutic drug monitoring in children and adolescents, we suggest considerably lower reference ranges than suggested for adults.

Funding
S.M.K., B.D. and B.C.K. received grant research support from The Netherlands Organization for Health Research and Development (ZonMW), number 836041011. S.M.K. received grant research support from the Erasmus Trustfonds. K.M. and M.G. received grant research support from the German Federal Institute for Drugs and Medical Devices (BfArM- reference number: 73.05/3832-397285/12). The patient registry of the 'Competence Network on Therapeutic Drug Monitoring in Child and Adolescent Psychiatry' was additionally supported by the German Federal Ministry of Education and Research (BMBF-FKZ: 01EZ0937) and the 'Verein zur Durchführung Neurowissenschaftlicher Tagungen e.V.', Berlin.

Acknowledgements
We thank the team of the TDM-laboratory of the Center of Mental Health of the University Hospital Würzburg under direction of professor Juergen Deckert for the quantification of the pipamperone concentrations in the German samples.

KEY POINTS

- Pipamperone pharmacokinetic parameters in children and adolescents are comparable to adult values from the literature.
- Children and adolescents with responses during pipamperone treatment have higher pipamperone trough concentrations and 24-h area under the curves than nonresponders.
- Bodyweight-adjusted pipamperone dosages are better than fixed dosages to attain the concentrations observed in responders. A twice-daily dosing scheme is recommended based on a relatively long elimination half-life

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SUPPLEMENTARY DATA

Figure S1 Goodness Of Fit plots – external validation

Figure S1a External validation: measured concentrations versus population predictions.

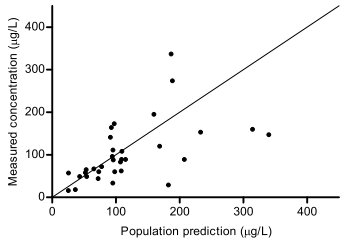


Figure S1b External validation: measured concentrations versus individual predictions.

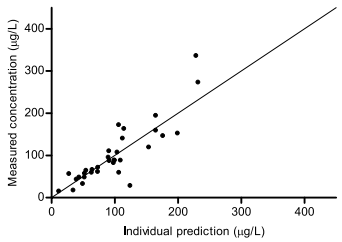


Figure S1c External validation: the correlation of conditional weighted residuals (CWRES) with time

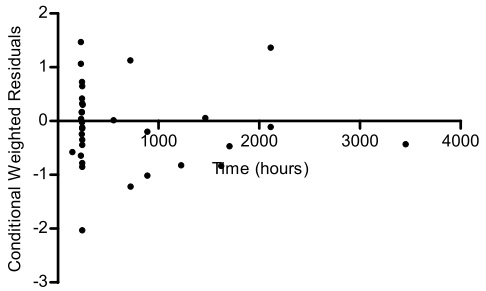
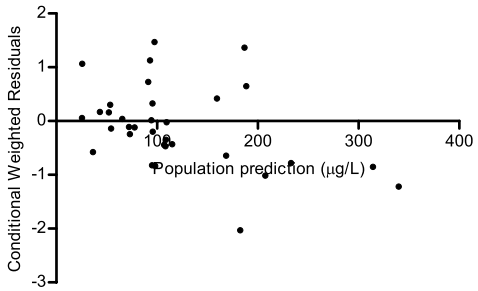
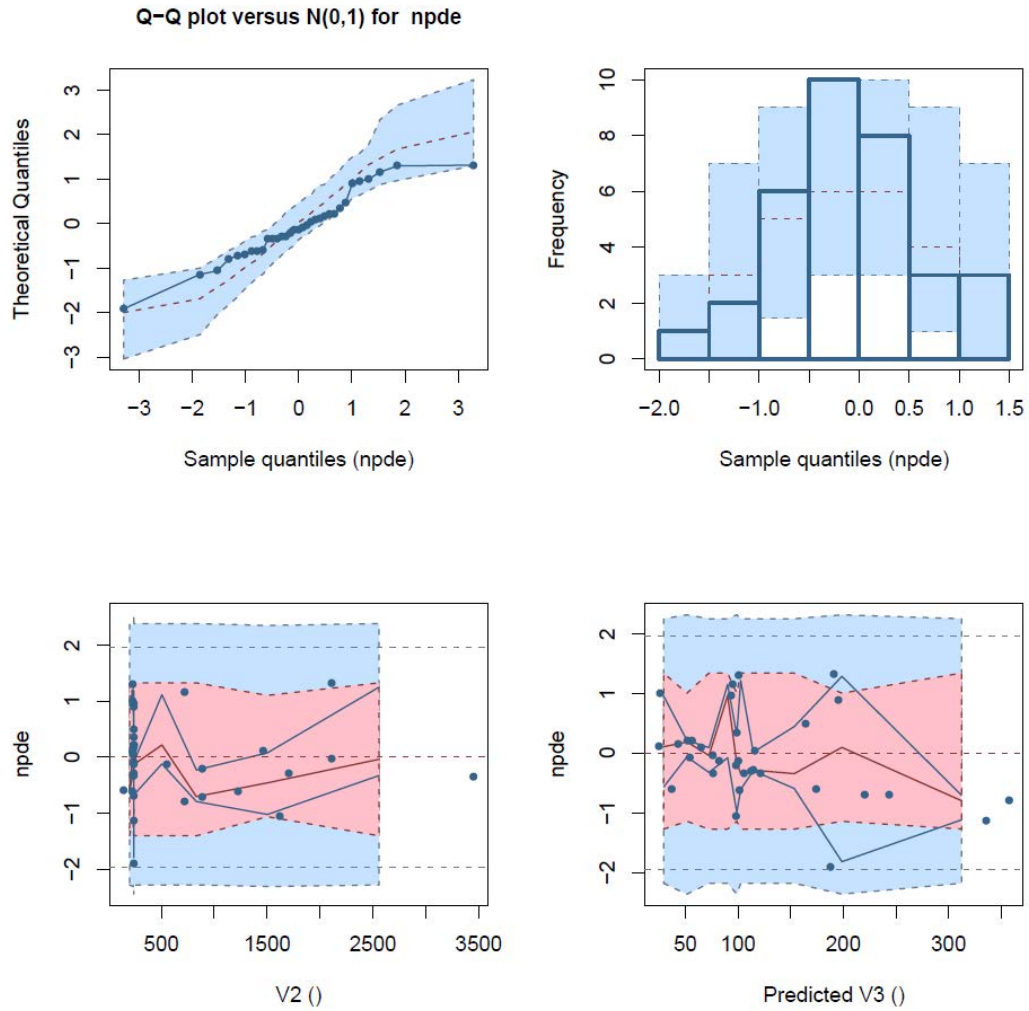


Figure S1d External validation: the correlation of conditional weighted residuals with population predicted concentrations



DBS: dried blood spot; HPLC-UV: high-performance liquid chromatography-ultraviolet; LCMS: ultra-high performance liquid chromatography-mass spectrometry

Figure S2 Normalized prediction distribution errors (npde) of external validation



CHAPTER 9

RISPERIDONE PLASMA CONCENTRATIONS ARE ASSOCIATED WITH SIDE-EFFECTS AND EFFECTIVENESS IN CHILDREN AND ADOLESCENTS WITH AUTISM SPECTRUM DISORDER

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British Journal of Clinical Pharmacology 2020. doi: 10.1111/bcp.14465. Online ahead of print.

ABSTRACT

Aim

Risperidone is the most commonly prescribed antipsychotic drug to children and adolescents worldwide, but is associated with serious side-effects, including weight gain. This study assessed the relationship of risperidone and 9-hydroxyrisperidone trough concentrations, maximum concentrations and 24-h area-under-the curves with BMI z-scores in children and adolescents with autism spectrum disorder (ASD) and behavioral problems. Secondary outcomes were metabolic, endocrine, extrapyramidal and cardiac side-effects and effectiveness.

Methods

Forty-two children and adolescents (32 males) aged 6-18 years were included in a 24 week prospective observational trial. Drug plasma concentrations, side-effects and effectiveness were measured at several time points during follow-up. Relevant pharmacokinetic covariates, including medication adherence and CYP2D6, CYP3A4, CYP3A5 and P-glycoprotein (ABCB1) genotypes, were measured. Non-linear mixed-effects modeling (NONMEM®) was used for a population pharmacokinetic analysis with 205 risperidone and 205 9-hydroxyrisperidone concentrations. Subsequently, model-based trough concentrations, maximum concentrations and 24-h area-under-the curves were analyzed to predict outcomes using generalized and linear mixed-effects models.

Results

A risperidone two-compartment model combined with a 9-hydroxyrisperidone one-compartment model best described the measured concentrations. Off all pharmacokinetic parameters, higher risperidone sum trough concentrations best predicted higher BMI z-scores during follow-up ($p<0.001$). Higher sum trough concentrations also predicted more sedation ($p<0.05$), higher prolactin levels (<0.001), and more effectiveness measured with ABC-irritability score ($p<0.01$).

Conclusion

Our results indicate a therapeutic window exists, which suggests that therapeutic drug monitoring of risperidone might increase safety and effectiveness in children and adolescents with ASD and behavioral problems.

INTRODUCTION

Risperidone is the most frequently prescribed antipsychotic drug to children and adolescents worldwide, with a prevalence ranging from 1.1 to 6.6 per 1000 youths across different countries.[1, 2] In this population risperidone is used for a broad range of mental health disorders, including disruptive behavioral disorders, schizophrenia and bipolar disorder. An important and increasing indication concerns irritability associated with autism spectrum disorder (ASD), with almost one in 9 youth with ASD using risperidone.[3] For these and other indications, the short-term efficacy of risperidone is well-established and supported by numerous randomized controlled trials.[4, 5]

However, there are growing concerns about the side-effects of risperidone in children and adolescents. Weight gain is the most important adverse effect, which is more pronounced in youths than in adults.[6] Children and adolescents gain several kilograms during the first weeks of risperidone treatment.[7] This results in serious long term health risks, including metabolic abnormalities and diabetes mellitus.[8, 9]

Other common side-effects of risperidone include extrapyramidal symptoms (EPS), prolactin elevation and sedation.[10] During long term risperidone treatment, up to one in three youths experience mild to moderate EPS, and more than half demonstrate prolactin elevations, possibly leading to gynecomastia, galactorrhea and sexual dysfunction.[11, 12] Risperidone-induced sedation is significantly more prevalent in young patients than in adults.[13] Lastly, risperidone can increase the corrected QT (QTc) interval, although clinically relevant QTc prolongation is rare.[14]

Several studies have shown that the risk of most of these side-effects, including weight gain, increases with higher risperidone dosages in children and adolescents.[9, 11, 15, 16] However, the relationship between individual exposure, reflected in risperidone plasma concentrations, and side effects remains unclear. A few studies have shown a correlation between prolactin elevation and plasma concentrations of risperidone or its active metabolite 9-hydroxyrisperidone in youths [16-20], but the relationship with weight gain and other side-effects is unknown. This hampers the use of therapeutic drug monitoring to improve safety in this population.

Also the concentration-effectiveness relationship of risperidone in children and adolescents has yet to be determined. One study showed no correlation between total plasma risperidone and 9-OH-risperidone concentrations and clinical response in a prospective cohort of children with ASD.[21] However, in that study, both trough and non-trough risperidone concentrations were analyzed together, which prevents correct interpretation. Another study neither found a concentration-effectiveness relationship in a sample of children and adolescents with different indications for risperidone use, but this study had limitations due to the naturalistic and retrospective study design.[22]

Here we study for the first time the relationship between risperidone and 9-hydroxyrisperidone plasma concentrations, weight gain, other side-effects, and effectiveness in a prospective cohort of children and adolescents with ASD. The primary aim is to investigate the relationship between

model-based individual pharmacokinetic parameters and body mass index (BMI) z-scores. For this purpose, trough concentrations, maximum concentrations and 24-h area-under-the curves are analyzed. Secondary, the relationships between the most relevant pharmacokinetic parameter and EPS, sedation, metabolic abnormalities, prolactin elevation, QTc-prolongation and effectiveness are investigated. The influence of a large number of demographic and biochemical characteristics including the cytochrome P450 enzymes *CYP2D6*, *CYP3A4*, *CYP3A5* and P-glycoprotein (*ABCB1*) genotypes is taken into account.

The findings of this study can indicate whether there is a therapeutic window and rationale for therapeutic drug monitoring of risperidone to improve safety and effectiveness in children and adolescents.

METHODS

Study population

Children aged 6-18 years with the diagnosis of ASD according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) IV [23] or 5 [24] and using or starting risperidone for irritability were eligible for inclusion in this 24-week observational prospective multicenter cohort study (Netherlands Trial Register 6050). Exclusion criteria were diabetes type I or II, congenital or acquired syndromes associated with changes in appetite, body weight or lipid profile (e.g. Prader Willi), treatment with another antipsychotic drug within the last 6 months or known Long QT syndrome. Patients were treated in one of the 7 participating centers in the south-west region of the Netherlands (2 academic tertiary care centers and 5 psychiatric secondary care centers). They were prescribed risperidone as tablet formulation or oral solution in flexible dosing schemes by their treating physician according to standard clinical care. Patients were recruited between August 2016 and October 2018. All patients and/or their legal representatives gave written informed consent before entering the study. The study was approved by the medical ethics committee of the Erasmus Medical Center, the Netherlands (number MEC 2016-124). The study has been carried out in accordance with the Declaration of Helsinki and the regulations on medical research with human subjects the Netherlands.

Drug concentration measurement

Three blood samples were repeatedly collected for risperidone and 9-hydroxyrisperidone quantification on two separate days. For patients who initiated risperidone treatment at the start of the study, blood samples were withdrawn at 12 and 24 weeks follow-up. For patients who already used risperidone at the start of the study, blood samples were collected at the start of the study and at 24 weeks follow-up. Blood samples were collected using venipuncture or Dried Blood Spot (DBS) method at random time points, with at least one hour between two samples. DBS sampling has experienced renewed interest in bioanalysis, as it requires only a simple finger prick and less blood than a venipuncture. DBS is regarded less painful and stressful for the patient than conventional blood sampling, and can be collected in a home environment, which increases the feasibility of repeated sampling in children.[25] Time of sampling, time of risperidone intake in the prior 24 hours, risperidone dose and comedication were reported during sampling. Risperidone and 9-hydroxyrisperidone plasma concentrations were measured with previously validated ultra-high performance liquid chromatography-mass spectrometry (LC-MS/MS) methods for plasma and DBS.[26-28] The lower limit of quantification (LLOQ) for risperidone was 1 µg/L and for 9-hydroxyrisperidone 0.7 µg/L for plasma and DBS samples. The lower limit of detection (LOD) for plasma risperidone samples was 0.02 µg/L and for plasma 9-hydroxyrisperidone 0.22 µg/L; for the DBS risperidone samples 0.9 µg/L and for the DBS 9-hydroxyrisperidone samples 0.5 µg/L.

DBS concentrations were converted to estimated plasma concentrations (EPC) using the following formulas with correction for hematocrit (ht), based on a previously performed clinical validation study [28]:

In this study hematocrit was standardly measured. When the hematocrit value was unknown and could not be extrapolated from a previous measurement, the median population value was used.

Assessment of outcomes

Side-effects and effectiveness were prospectively recorded at start of study and at 24 weeks for all patients using and initiating risperidone treatment.

Patients who initiated risperidone treatment when starting the study had additional assessments of side-effects and effectiveness at 4 and 12 weeks. For patients who already used risperidone at the start of the study, bodyweight, height, laboratory measurements and comedication since initiation of risperidone were retrospectively collected from the patient file.

Side-effects

Bodyweight and height were measured at each visit. EPS were measured with the Abnormal Involuntary Movement Scale (AIMS) [29], filled in by treating physician, nurse or researcher. Sedation was assessed with the Epworth Sleepiness Scale [30] and filled in by parents. Laboratory indices that were measured were triglycerides, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoproteins (LDL)-cholesterol, glucose, hemoglobin A1C (HbA1C) and prolactin. Laboratory indices were measured at the start of the study and at 24-weeks follow-up, with an additional measurement at 12 weeks for patients who had initiated risperidone treatment at the start of the study.

QT intervals were measured in triplicate from a twelve-lead ECG as described previously[31] at the start of the study and after 24 weeks of follow-up. The QT interval was measured at steady state heart rates, using preferably lead II, from the beginning of the onset of the QRS complex to the end of the T-wave. The measured QT intervals were corrected for heart rate using the Bazett's formula:

The QT times and RR intervals of the first 10 ECG's were measured by both a researcher and experienced pediatric cardiologist who were blinded for study time point. As these measurements showed good agreement (<10% difference for all measurements), the researcher individually performed the remaining QTc measurements. If there was any doubt, the ECG was also reviewed by the pediatric cardiologist.

Effectiveness

Effectiveness of risperidone was assessed by parents and the treating physician. Parents filled in the Aberrant Behavior Checklist (ABC) [32], a 58-item questionnaire that is sensitive to treatment effects in children with ASD. The ABC-irritability subscale (ABC-I) was used as measure for effectiveness; this scale reflects irritability symptoms with a maximum of 45 points. Treating physicians filled in the Clinical Global Impression Scale (CGI).[29] This scale describes the severity of psychopathology (CGI-S) and its improvement (CGI-I) by 7 categories, rated by the treating physician. The CGI-S describes the severity of illness relative to patients with the same diagnosis in ascending order, with 1 = normal and 7 = extremely ill. The CGI-I rates the improvement in comparison to the original medication-naive state of symptoms: 0 = not assessable, 1 = very much better, 2 = much better, 3 = moderately better, 4 = unchanged, 5 = minimally worse, 6 = much worse.

Assessment of covariates

Medication adherence was assessed with questionnaires (Medication Adherence Rating Scale MARS-5[33], filled in by parents, and a 100 point visual analog scale (VAS), filled in by parents and treating physician) and during the last month of follow-up with an electronical monitoring system (MEMS©).[34] A VAS score of 100, a MARS score of 25 or a MEMS adherence percentage of 100 represents optimal adherence on each scale. Comedication was retrieved from medical records and from pharmacy records. Comedication for ADHD was recorded for the following drugs which are known to influence weight: methylphenidate, amphetamine or atomoxetine.

Parents filled in a study questionnaire at every visit including questions about the child's diet, physical activity, grapefruit-juice use and over the counter self-medication use (including Saint John's Wort). Diet questions involved whether the child had visited a dietician and whether nutritional advice was followed. Physical activity questions involved the quantification of the child's high intensity (sports) and low intensity (walking or cycling) activity in hours per week. Familiar cardiometabolic risk was assessed after taking the family history at the start of the study as previously defined.[35] If the family history was unknown, it was considered positive for cardiometabolic risk.

Laboratory measurements

Renal function (ureum, creatinine), liver function (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma glutamyl transpeptidase (GGT), alkalic fosfatase (AF), albumin) and hematocrit were assessed at baseline and during follow-up.

All patients were genotyped for the following cytochrome P450 (CYP) enzymes that could influence pharmacokinetics: *CYP3A4*, *CYP3A5*, *CYP2D6* and P-glycoprotein *PGP* (*ABCB1*). The following single nucleotide polymorphisms were tested: for *CYP3A4 *22*, for *CYP3A5 *3* and **6*, for *CYP2D6 *3*, **4*, **5*, **41* and for ABCB1 (*PgP*) *3435C>T*, using genomic DNA isolated from EDTA blood, and analyzed using Taqman 5' nuclease DME assays (ThemoFisher Scientific). All pharmacogenetic testing was performed in the laboratory of the Erasmus MC, Rotterdam, the Netherlands.

Population pharmacokinetic analyses

Population pharmacokinetic analysis was performed by non-linear mixed-effects modelling using NONMEM® version 7.4.2 (FOCE+I; ICON Development Solutions, Ellicott City, MD, USA) and PsN® Version 4.7.0. Pirana® software version 2.9.7 was used as an interface between NONMEM® and R (version 3.4.4). Concentrations of 9-hydroxyrisperidone were corrected for molecular weight of risperidone.

Base model development

One, two and three compartment models were considered to describe the concentration-time data based on visual inspection of the goodness-of-fit plots and a review of the literature. Typical values for lag-time, first-order absorption rate constant (ka), volume of distribution (V), clearance (CL), and inter-compartmental clearance (Q) were estimated. As bioavailability (F) could not be quantified, certain parameters were estimated as ratios: CL/F, Q/F, and V/F. Firstly, risperidone data was described and subsequently 9-hydroxyrisperidone data was added. For each pharmacokinetic parameter inter-patient variability (IPV) was evaluated and shrinkage was calculated for all parameters for which IPV was established. A shrinkage value below 25% was considered acceptable.[36] Allometric scaling was used to account for the influence of bodyweight on pharmacokinetic parameters, which was explored with a fixed exponent (0.75 for CL and Q, and 1 for V), and with exponents estimated by the model. Residual variability was described with a combined (additive and proportional) error model, with extra errors for sampling method (DBS versus venipuncture) and concentrations below LLOQ. Concentrations measured below the LOD were set on half the value of the LOD in combination with an extra additional error of half the value of the LOD. Components of the error model estimated to approach zero were removed. Model selection criteria were a decrease in the NONMEM objective function value (OFV), goodness-of-fit plots and visual predictive checks (VPC). A decrease in the OFV of 3.84 points was considered statistically significant ($p<0.05$).

Covariate model development

The following covariates were considered as potential model covariates: sex, age, dose, dose per kilogram, bodyweight, height, BMI, CYP3A4 genotype, CYP3A5 genotype, CYP2D6 genotype, PGP genotype, comedication, somatic comorbidities, hematocrit, renal function (ureum, creatinine), liver function (ASAT, ALAT, GGT, AF), albumin, medication adherence, grapefruit juice use, Saint-John's Wort use, and smoking. First, the correlation between the covariates and IPV was evaluated graphically. Subsequently, covariates with a visual relationship with IPV were individually added to the model. Continuous covariates were described using an exponential function and categorical covariates using a proportional function. Covariates that significantly

improved the model with the univariate analysis (p<0.05), were selected for multivariate analysis. The forward inclusion-backward elimination method was used.[37] During the backward elimination process, covariates that improved the model at a level of p<0.001 were selected. A shark plot was generated for each covariate for case-deletion diagnostics.

Internal model evaluation

Firstly, a bootstrap analysis was performed with 1000 simulations.[38] The validity of the model was evaluated by comparing the bootstrap estimates and their 90 percentile range with the values generated using the original dataset. Secondly, the model was evaluated with a visual predictive check (VPC) stratified by compartment, using a set of 1000 simulated datasets to compare the observed concentrations with the distribution of the simulated concentrations.[39]

Pharmacokinetic predictions

Model-based individual pharmacokinetic predictions were used as trough concentration (Ctrough), maximum concentration (Cmax) and 24-hour AUC (AUC24h) were not available for each patient because of sparse random sampling within the study. These pharmacokinetic parameters were predicted for risperidone and 9-hydroxyrisperidone per subject for the days a BMI z-score was known. The Ctrough prior to the first risperidone administration of the day was used. The Cmax was calculated for risperidone and was defined as the concentration at 40 minutes after risperidone administration, based on visual inspection of concentrations simulated by the final model. In case of multiple dosages per day, the highest Cmax on that day was used for the analyses.

Pharmacodynamic analyses

Primary outcome

The primary outcome was BMI adjusted for age and weight, the BMI z-score. A BMI z-score of ≥1 is considered overweight and a BMI z-score ≥2 is considered obesity according to the World Health Organization (WHO).[40] BMI values were transformed into BMI z-scores based on the WHO BMI-for age reference values (5–19 years).[41]

Secondary outcomes

The following secondary outcomes were analyzed: EPS, sedation, triglycerides level, total cholesterol level, HDL-cholesterol level, LDL-cholesterol level, glucose level, HbA1C level, prolactin level, QTc-time, CGI-improvement score and ABC-irritability (ABC-I) score. EPS, sedation and CGI-improvement score were considered categorical outcomes. EPS was defined positive if at least two times mild or one time moderate was scored with the AIMS. Sedation was defined as an ESS total score of 1 or higher. Only children for whom a baseline measurement (before start of risperidone treatment) of the concerning outcome was available were included in the analyses.

Statistical analyses

Generalized and linear mixed-effects models were used to analyze our longitudinal data. Random-effects were employed to capture the heterogeneity between the patients. The BMI z-score was considered the primary outcome. Each pharmacokinetic parameter of risperidone was separately analyzed as predictor versus BMI z-score. Potential relevant covariates (duration of risperidone use, sex, age, somatic comorbidities, comedication, IQ, prematurity, physical activity, diet) were tested in each model and the best model was selected using backward variable selection. Then, the final model among all best models with different pharmacokinetic parameters of risperidone was chosen by Akaike information criterion (AIC).[42]

The secondary analyses were performed with the pharmacokinetic parameter that was selected for the final model of the primary outcome. This pharmacokinetic parameter was entered as predictor in univariable models, and if significant, relevant covariates were added in a stepwise manner. On top of the covariates for the primary analyses, psychiatric comorbidities, psychotropic treatment before start of risperidone, non-pharmacological treatment parents and/or child, and familiar cardiometabolic risk were checked when relevant.

The correlation between model-based pharmacokinetic parameters was analyzed with Pearson's correlation. Changes in BMI z-scores between baseline and the last visit was assessed with the Wilcoxon signed-rank test. The explained variance by the final model was calculated with the conditional pseudo-R2. In all analyses a p-value<0.05 was considered significant and all analyses were performed in R[43] (version 3.4.4).

RESULTS

Study sample

Forty-two patients were included, of whom 31 initiated risperidone treatment at the time they were included in the study and 11 already used risperidone before inclusion in the study. The baseline characteristics of the study sample are presented in **table 1**. The median (interquartile range, IQR) follow-up time since start of risperidone therapy was 5.7 (4.8) months. The median (IQR) risperidone daily dose at the end of follow up was 1.0 (0.5) mg and 0.02 (0.02) mg/kg. The majority of children had one or more comorbid psychiatric disorders besides ASD (64.3%), being attention deficit/hyperactivity disorder (ADHD, 52.4%), oppositional defiant disorder (11.9%), mood disorder (7.1%), post traumatic stress disorder (4.8%) or anxiety disorder (2.4%).

One-hundred and fifty-four DBS samples and 72 plasma samples were collected. Twenty-one DBS samples (13.6%) were of insufficient quality for drug quantification, resulting in a total of 205 risperidone and 205 9-hydroxyrisperidone concentrations. The median (IQR) measured risperidone concentration was 1.72 (6.19) µg/L in plasma and 3.57 (3.55) µg/L in DBS. The median (IQR) measured 9-hydroxyrisperidone concentration was 7.02 (6.13) µg/L in plasma and 6.39 (6.72) µg/L in DBS. Hematocrit was known for 85 DBS measurements in 32 patients, with a median (IQR) hematocrit of 0.40 (0.08) L/L.

The medication adherence was generally high: the median (IQR) MEMS adherence percentage was 96% (62%), VAS score filled in by treating physician 100 (4), VAS score filled in by parents 100 (2) and MARS score filled in by parents 24 (1).

Table 1 Baseline characteristics

Characteristic	
Male, n (%)	32 (76.2)
Age [a] (years)	9.7 (5.3)
Bodyweight [a] (kg)	32.4 (18.3)
Height [a] (m)	1.42 (0.34)
Body Mass Index a (kg m-2)	16.18 (4.06)
Body Mass Index z-score [a]	-0.32 (1.69)
Ethnicity, n (%)	
Both parents Dutch origin	33 (78.6)
Other [b]	8 (19.0)
Unknown	1 (2.3)
Laboratory measurements [a]	
Triglycerides (mmol/L)	0.57 (0.42)
Total cholesterol (mmol/L)	4.00 (1.00)
HDL-cholesterol (mmol/L)	1.50 (0.49)
LDL-cholesterol (mmol/L)	2.33 (0.79)
Glucose (mmol/L)	4.90 (0.40)
HbA1C (mmol/mol)	33 (4)
Prolactin (U/L)	0.12 (0.10)
Genotype, n (%) [c]	
CYP2D6	
Poor metabolizer	0 (0)
Intermediate metabolizer	27 (64.3)
Normal metabolizer	14 (33.3)
CYP3A4	
Poor metabolizer	1 (2.4)
Intermediate metabolizer	6 (14.3)
Normal metabolizer	34 (81.0)
CYP3A5	
Expressor	8 (19.0)
Non-expressor	33 (78.6)
ABCB1	
Poor metabolizer	14 (33.3)
Normal metabolizer	27 (64.3)
Unknown genotype	1 (2.4)
QTc time (ms) [a]	387 (31)
Clinical Global Impression Scale (CGI-s) score [a]	5 (2)
Comorbid psychiatric disorders other than ASD, n (%)	27 (64.3)
Comedication ADHD drugs, n (%) [d]	10 (23.8)
IQ a	100 (40)
Treatment setting, n (%)	
Outpatient	37 (88.1)
Inpatient	5 (11.9)
Prior psychotropic treatment, n (%)	25 (59.5)
Physical activity [a]	
High intensity (hours/week)	2.5 (3)
Low intensity (hours/week)	2 (2)
Increased familiar cardiometabolic risk, n (%) [e]	18 (42.9)
Formulation of risperidone administration, n (%)	
Tablet	31 (73.8)
Oral solution	11 (26.2)

All patients were diagnosed with autism spectrum disorder. The values represent start of risperidone treatment unless otherwise specified. Values represent total sample of n=42 patients, except for triglycerides (n=29), total cholesterol (n=28), HDL-cholesterol (n=28), LDL-cholesterol (n=28), glucose (n=30), HbA1C (n=23), prolactin (U/L), CGI (n=29), IQ (n=40), physical activity (n=11), QTc (n=25).
ADHD: attention deficit/hyperactivity disorder; CYP: cytochrome P450; HbA1C: hemoglobin A1C; HDL: high-density lipoprotein; LDL: low-density lipoproteins; PGP: P-glycoprotein; QTc: corrected QT
CGI-s: Clinical Global Impression Severity Scale; 1 = normal; 2 = borderline; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = extremely ill.
a Presented as median and interquartile range (IQR) for continuous variables
b Seven children had one or two parents of non-European descent. Of these, 5 children had one or two parents with African descent.
*c Metabolizing status within the sample was defined as follows: CYP2D6: poor = 2 inactive alleles (e.g. *4/*4); intermediate = 1 active and 1 inactive allele (*1/*3, *1/*4, *1/*5) OR 1 inactive and 1 decreased activity allele (*4/*41); normal = 2 active alleles (*1/*1) OR 1 active and 1 decreased activity allele (e.g. *1/*41); CP3A4: poor = *22/*22, intermediate = *1/*22, normal = *1*1; CYP3A5: expressor = at least 1 active (*1) allele (*1/*3, *1/*6); non-expressor = 2 inactive alleles (*3/*3, *3/*6); ABCB1: poor=3435TT, normal=3435CT, 3435CC.*
d Includes methylphenidate, amphetamine, atomoxetine
e As defined by American Academy of Pediatrics [35]

Population pharmacokinetic analyses

Base model

The data was best described using a two-compartment model for risperidone combined with a one-compartment model for 9-hydroxyrisperidone. IPV on CL of risperidone and CL of 9-hydroxyrisperidone significantly improved the model. The residual error was described with additional and proportional errors for risperidone and 9-hydroxyrisperidone, concentrations below the LOD, concentrations >LOD and <LLOQ, and DBS samples. Pharmacokinetic parameters are presented in **table 2** and estimates of residual variability are presented in **table S1**.

Table 2 Pharmacokinetic parameter estimates of the final model and bootstrap analysis

Parameter	Estimate [shrinkage]	Bootstrap median (90th percentile)[2]
tlag (h)	0.42	0.42 (0.40–0.50)
Ka (L/h)	18.6	34.2 (4.0–2587.1)
Vc/F1 (L/70 kg)	107	107.2 (85.5–142.1)
Vp/F1 (L/70 kg)	46.2	426.3 (39.1–9671.2)
Q/F1 (L/h/70 kg)	3.31	4.4 (1.6–14.7)
CL/F1 (L/h/70 kg)	23.9	24.2 (19.5–32.1)
IPV CL	80% [6%]	82% (66–103)
VC M /F[1] (L/70 kg)	111	101.4 (75.2–181.9)
CLM/F[1] (L/h/70 kg)	5.19	5.2 (4.6–5.7)
IPV CLM	28% [12%]	25% (17–34)

St Johns wort and grapefruit juice were not tested as covariates, as no children used these during follow-up.
1Allometric scaling with exponent 1 for V, and 0.75 for CL and Q
2 90th percentile based on bootstrap with n=279 successful runs.
tlag lag time; Ka: absorption rate constant; V: volume of distribution; CL: clearance; IPV: inter-patient variability; M: metabolite, 9-hydroxyrisperidone; P: peripheral; C: Central; RSE: Relative standard error

Covariate analysis

No covariates remained significant in multivariate analyses after backward elimination except for bodyweight, which was best described using fixed exponents with allometric scaling.

Evaluation of the final model

The bootstrap analysis yielded similar values as model-based parameter estimates, except from a larger estimate for the peripheral V (Vp). The model-based estimate of Vp however was within the bootstrap 90th percentile range, indicating the stability of the model. The goodness-of-fit-plots showed that the model adequately described the observed concentrations (**figure S1**). The VPC showed a good predictive performance, although the lower 90th percentile of the simulated concentrations was slightly lower than the measured concentrations (**figure S2**).

Pharmacokinetic predictions

For the time points a BMI z-score was known, 270 model-based individual pharmacokinetic predictions for Ctrough, Cmax, and AUC24h of risperidone and 9-hydroxyrisperidone were calculated. The correlation between the predicted pharmacokinetic parameters was moderate to strong (r>0.5, data not shown), except for risperidone Ctrough and 9-hydroxyrisperidone AUC24h, which had a weak correlation (r=0.39).

Pharmacodynamic analyses

Primary outcome; BMI z-scores

Two hundred seventy BMI z-scores were available in 42 patients; for 3 patients no baseline BMI z-score was available. The mean BMI z-score increased significantly during follow-up from -0.28 ±1.34 at baseline to 0.26 ±1.24 at end of follow-up (p<0.001).

A higher risperidone and 9-hydroxyrisperidone exposure significantly predicted higher BMI z-scores during follow up for all pharmacokinetic parameters (Ctrough, Cmax, and AUC24h p<0.001). Significant covariates in multivariate analyses were risperidone duration of use (months) and comedication for ADHD (p<0.05).

The sum Ctrough most strongly predicted BMI z-scores during follow-up, together with the significant covariates: sum Ctrough (β=0.042, p<0.001), duration of use (β=-0.009, p<0.001) and ADHD comedication (β=-0.340, p=0.004) with an intercept of -0.040 (p=0.836). The relationship between sum Ctrough and BMI z-scores is shown in **figure 1** and **table 3**.

The explained variance in BMI z-scores of the final model with sum Ctrough was 90.4%, which was higher than with risperidone dose at time of measurement (89.3%).

Secondary outcomes

Sum Ctrough significantly predicted sedation, prolactin levels, and ABC-irritability score with correction for relevant covariates. No association was found between Sum Ctrough and EPS, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, glucose, HBA1C, prolactin or QTc time, and response based on CGI-improvement score. The results of the effectiveness analyses are presented in **table 3**, the results of the secondary side-effects analyses are presented in **table S2**.

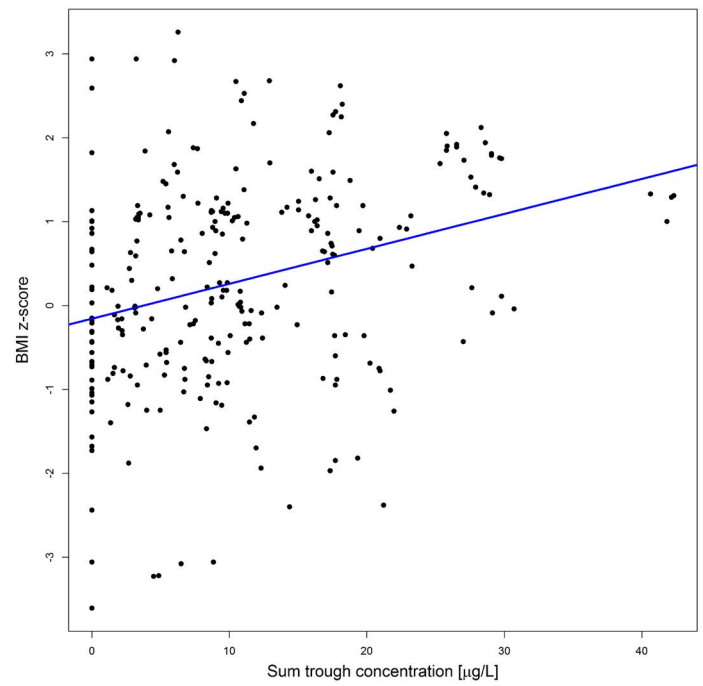
Table 3 Association between trough concentrations of risperidone + 9-hydroxyrisperidone, BMI z-score, and secondary effectiveness outcomes

Variable	N (obs)	Estimate	Standard error	p-value
Primary outcome				
BMI z-score	42 (270)			
Sum Ctrough		0.042	0.005	<0.001
Duration of use		-0.009	0.002	<0.001
Comedication ADHD		-0.340	0.116	0.004
Secondary outcomes – effectiveness				
CGI – response	29 (107)			
Sum Ctrough		0.300	0.158	0.057
Duration of use		0.445	0.183	0.015
ABC – irritability	42 (121)			
Sum Ctrough		-0.281	0.093	0.003
Age at start		-1.390	0.398	0.001

The median (IQR) predicted sum Ctrough was 10.07 (11.54) µg/L. The influence of diet and physical activity could not be analyzed due to too many missing values.

ABC-I: Aberrant Behavior Checklist – irritability score; BMI: Body Mass Index; CGI: Clinical Global Impression Scale; obs: number of observations

Figure 1 Sum trough concentration versus BMI z-scores



Legend: BMI: Body Mass Index; sum: risperidone and 9-hydroxyrisperidone

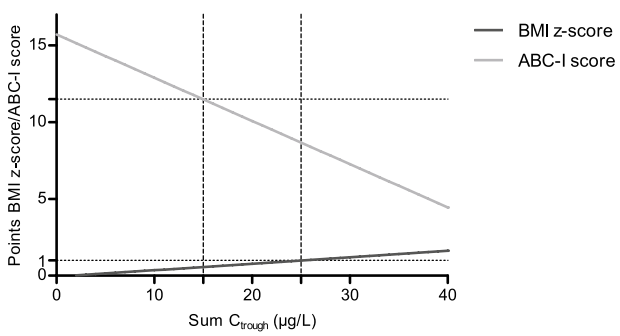
Therapeutic window
Based on the estimated coefficients for BMI z-scores, ABC-I score, and the relevant covariates, a plausible therapeutic window for a child 10 years of age, with 3 months of risperidone treatment and without ADHD comedication is visualized in **figure 2**. Assuming a BMI z-score <1 and an ABC-I score <11 as relevant cut-offs for treatment success with acceptable weight gain, the theoretical therapeutic window of the sum Ctrough would be between 15 and 25 µg/L.

DISCUSSION

This study demonstrates that higher risperidone and 9-hydroxyrisperidone plasma concentrations are associated with more weight gain, more sedation, higher prolactin levels and increased effectiveness in children and adolescents with ASD and behavioral problems. The sum trough concentration of risperidone and 9-hydroxyrisperidone was the most predictive pharmacokinetic parameter.

The risperidone and 9-hydroxyrisperidone population pharmacokinetic parameters found in this study are comparable to previously described values in children and adolescents.[44, 45] After adjusting for bodyweight, these pharmacokinetic parameters are similar to adults.[45] The effects of demographic and biochemical characteristics on risperidone plasma concentrations in youths have not been studied extensively. While several studies did not find an influence of sex [44-46], some found higher total plasma concentrations in girls than boys [47], and others the opposite.[48] Within our sample, no influence of sex was found, but our sample mainly consisted of boys. The activity of the *CYP2D6* enzyme has repeatedly been shown to strongly affect risperidone clearance in children and adolescents [44, 45, 49], but was not found as significant covariate due to the absence of poor and extensive metabolizers in our study population. The influence of polymorphisms in other cytochrome P450 enzymes *CYP3A4*, *CYP3A5* and the transport protein *PGP* on risperidone concentrations has not been previously studied in children and adolescents, despite evidence for this influence in adults.[50, 51] Our study is the first to examine these polymorphisms for risperidone in youths, but did not find any significant effect of polymorphisms in these genes on risperidone pharmacokinetics.

Figure 2 Example of theoretical therapeutic window



Legend: The relationship between BMI z-scores, ABC-I scores and sum Ctrough for a child being 10 years old, with 3 months of risperidone treatment and without ADHD comedication. The grey are indicates a theoretical therapeutic window (15-25 µg/L), for a BMI z-score < 1 and a ABC-I score <11.
BMI: Body Mass Index, ABC-I: Aberrant Behavior Checklist – irritability scale; Sum Ctrough: sum of trough concentrations of risperidone and 9-hydroxyrisperidone

Higher risperidone and 9-hydroxyrisperidone concentrations predicted higher BMI z-scores during risperidone treatment in children and adolescents. The sum trough concentration of risperidone and 9-hydroxyrisperidone, also referred to as the "active moiety", was found to be the most predictive pharmacokinetic parameter. This confirms the recommendation in current therapeutic drug monitoring guidelines[52], but was not self-evident, as for other drugs, including antibiotic drugs, it is known that maximum concentrations or area-under-the curves can predict outcomes better than trough concentrations.[53] Two other studies have investigated the relationship between both risperidone and 9-hydroxyrisperidone concentrations and BMI z-scores in children and adolescents. One of these two studies did find higher risperidone metabolite and sum concentrations in children and adolescents with higher BMI z-scores [48], the other did not find any association.[54] However, both studies had a cross-sectional design without baseline measurement, and were not able to take weight gain into account. In adults, to date no studies have investigated the relationship between risperidone plasma concentrations and weight gain.[55]

The analysis of the relationship between risperidone plasma concentrations and weight gain is complicated, as this relationship is bidirectional. Risperidone exposure may influence bodyweight in a pharmacodynamic way, but bodyweight also moderates risperidone exposure by pharmacokinetic processes. Higher body weight is expected to result in lower risperidone concentrations with an unaltered dose, as volume of distribution and clearance increase with bodyweight as a result of allometric scaling. Therefore, the pharmacodynamic explanation of the relationship is favored, as in our study higher concentrations were found with higher bodyweights. Still, it is unknown how well the established allometric scaling exponents fit pediatric populations with overweight.[56] For risperidone, a relatively lipophilic drug, the volume of distribution is likely to increase with increasing fat mass, but for 9-hydroxyrisperidone, the more hydrophilic metabolite, this is more unlikely. Further research is needed to clarify the relationship between measured risperidone concentrations, rather than predicted concentrations, and overweight in pediatric patients.

Higher risperidone sum trough concentrations were also related to more sedation, prolactin elevation and effectiveness in our sample. In adults, the risperidone exposure-response relationship has been studied almost exclusively in schizophrenia patients, yielding conflicting results. While some studies found a higher risperidone plasma concentrations predicting better response, others found the opposite.[55] The positive correlation with extrapyramidal symptoms is better established, although different plasma thresholds are reported, including >40 µg/L [57], >180 µg/L [58] or > 74 µg/L [55]. As a result, a therapeutic reference range of risperidone in adults has not yet been clearly established, but is proposed as 20-60 µg/L for schizophrenia by the AGNP consensus guideline.[52]

For children and adolescents, no therapeutic reference range is known, although it is suggested that optimal risperidone concentrations in children with impulsive-aggressive symptoms are lower than in adults with schizophrenia.22 Our findings confirm this, and show that optimal concentrations might depend on the child's age at start of risperidone therapy, duration of treatment and comedication with ADHD drugs. When accounting for these variables, a therapeutic window of risperidone in children and adolescents with autism and behavioral problems seems to exist. For example for a child of 10 years old, after 3 months of risperidone treatment without ADHD comedication, the theoretical therapeutic range would be 15-25 µg/L, when a BMI z-score <1 and ABC irritability score <11 are considered as optimal treatment outcomes. This response corresponds to a 25% reduction in ABC irritability score, which has been previously defined as clinically relevant.[59]

Although this study demonstrates a rationale to explore the added value of therapeutic drug monitoring in this population, more research is needed to better define the therapeutic reference range of risperidone in these youths. Future studies should assess measured rather than predicted sum trough concentrations, and focus on their predictive value in an early treatment phase for weight gain during later follow-up. Eventually, a randomized controlled trial should evaluate the added value of risperidone titration towards optimal concentrations versus treatment as usual. These efforts should be made before therapeutic drug monitoring of risperidone in youths can be routinely used as standard care.

The findings of this study should be interpreted in the light of its limitations. First, the study had a sparse sampling design with different sampling methods, including venous sampling and dried blood spot sampling. This design was chosen to minimalize the patient's burden and increase the study feasibility, as has been previously advised for pediatric pharmacokinetic trials.[25] This however resulted in a higher variability in measured and predicted pharmacokinetic concentrations, further enhanced by the relatively large share of the measured risperidone concentrations below the LOD. We have reduced these variabilities in the analyses with the development of an extensive pharmacokinetic residual error model, although this resulted in less accuracy in the estimation of Vp/F. Second, the analysis of the relationship between risperidone exposure and effects was done with model-based concentrations rather than the measured concentrations themselves. Although this is suboptimal compared to really measured exposure, the model-based concentrations showed a good fit with measured concentrations. Moreover, this allowed for an analysis of not only trough concentrations, but also peak concentrations and AUC24h. Third, due to a limited sample size, relatively few patients had high risperidone sum trough concentrations. These patients had a quite large impact on the regression analyses. However, the mg/kg doses and characteristics of these patients were within the range of the total sample, thus reflecting average patients. Fourth, the sample size was not powered for the secondary outcomes, thus possibly leading to non-significant results. Fifth, this study has the typical limitations of naturalistic study designs in analyzing the exposure-response relationship[60], as placebo-responders and patients with side-effects are likely to receive lower dosages, while non-responders are likely to receive higher dosages. This might have led to an over- or underestimation of the exposure-response relationship. Lastly, although a large panel of pharmacogenetic polymorphisms was tested for its pharmacokinetic influence, candidate genes that might be relevant for pharmacodynamic outcomes were currently not tested; these however are of interest for future research.[61]

This is the first study that prospectively investigated the relationship between risperidone pharmacokinetic parameters, side-effects and effectiveness in children and adolescents with autism spectrum disorder and severe behavioral problems. The finding that the risperidone sum trough concentration predicts both weight gain, other side effects and response, indicates that therapeutic drug monitoring might improve safety and efficacy of risperidone treatment in this vulnerable population.

Acknowledgements
We thank Soma Bahmany of the Erasmus MC Rotterdam for the quantification of the risperidone and 9-hydroxyrisperidone concentrations.

Funding information
SK, BD and BK received grant research support from The Netherlands Organization for Health Research and Development (ZonMW), number 836041011.

What is already known about this subject:

- Risperidone treatment in children and adolescents is associated with serious side-effects, most importantly weight gain.
- The risk of side-effects increases with higher dosages, but the relationship of concentrations of risperidone and its active metabolite 9-hydroxyrisperidone with side-effects is unknown.

What this study adds:

- In children and adolescents with Autism Spectrum Disorder (ASD), weight gain can be predicted by model-based risperidone sum trough concentrations. The sum trough concentration is a better predictor than the maximum concentration or the 24h area under the curve.
- Higher risperidone sum trough concentrations predict higher BMI z-scores, but also more sedation, higher prolactin levels, and, interestingly, more effectiveness.
- Our findings indicate that a therapeutic window for effectiveness with acceptable weight gain in children and adolescents with ASD seems to exist, but more research is needed to establish the therapeutic reference range in this vulnerable population.

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SUPPLEMENTARY DATA

Table S1 Residual variability of the final model and bootstrap analysis

Estimate	Estimate	Bootstrap median (90th percentile)
Risperidone		
ε_{add} (µg/L) =		
< LOD risperidone plasma[2]	0.01	0.01
< LOD risperidone DBS[2]	0.73	0.73
<LLOQ >LOD risperidone	0.21	0.20 (0.08-0.30)
ε_{prop} =		
Risperidone	0.27	0.26 (0.19–0.32)
Risperidone DBS	0.27	0.26 (0.12–0.38)
<LLOQ >LOD risperidone	0.37	0.37 (0.18–0.51)
9-hydroxyrisperidone		
ε_{add} (µg/L) =		
9-hydroxyrisperidone	0.94	0.80 (0.33-1.08)
9-hydroxyrisperidone DBS	0.26	0.30 (0.05–0.54)
< LOD 9-hydroxyrisperidone plasma[2]	0.11	0.11
< LOD 9-hydroxyrisperidone DBS[2]	0.42	0.42
ε_{prop} =		
9-hydroxyrisperidone	0.31 (58%)	0.32 (0.29–0.35)

Residual variability was described according to the following formula: Cobs=Cpred&prop + &add*
Shrinkage was 11% for the combined additional error and 12% for the combined proportional error.
For risperidone, 5 plasma concentrations and 74 DBS concentrations were below the LOD, and 26 plasma and 1 DBS sample below the LLOQ but above the LOD. For 9-hydroxyrisperidone, 1 DBS sample was below the LOD.
[2] *fixed value*
Obs: observed, pred: predicted, prop: proportional, add: additional, LLOQ: lower limit of quantification, LOD: lower limit of detection; RSE: Relative standard error
Bootstrap with n=279 successful runs.

Table S2 Association between trough concentrations of risperidone + 9-hydroxyrisperidone and secondary side-effect outcomes

Variable	N (obs)	Estimate	Standard error	p-value
EPS	42 (136)			
Sum Ctrough		-0.385	0.203	0.058
Sedation	41 (117)			
Sum Ctrough		0.296	0.127	0.019
Triglycerides (mmol/L)	41 (103)			
Sum Ctrough		0.002	0.005	0.681
Total cholesterol (mmol/L)	41 (99)			
Sum Ctrough		-0.010	0.007	0.158
HDL cholesterol (mmol/L)	41 (103)			
Sum Ctrough		-0.003	0.003	0.399
LDL cholesterol (mmol/L)	40 (102)			
Sum Ctrough		-0.003	0.006	0.567
Glucose (mmol/L)	42 (103)			
Sum Ctrough		0.003	0.005	0.562
HbA1C (mmol/mol)	40 (87)			
Sum Ctrough		-0.015	0.026	0.571
Prolactin (U/L)	41 (93)			
Sum Ctrough		0.027	0.007	<0.001
Age at start		0.054	0.018	0.004
QTc time (ms)	25 (52)			
Sum Ctrough		0.464	0.345	0.187

EPS: extrapyramidal symptoms; HbA1C: hemoglobin A1C; HDL: high-density lipoprotein; LDL: low-density lipoproteins; obs: observations; QTc: corrected QT

Figure S1 Goodness of fit plots of final model

Figure S1a measured concentrations versus individual predictions risperidone

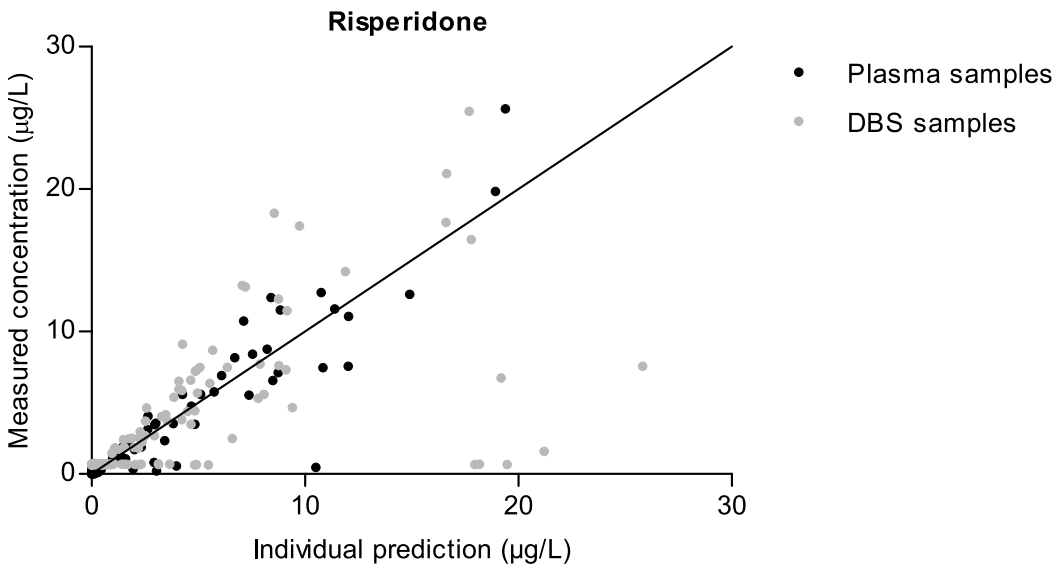


Figure S1b measured concentrations versus individual predictions 9-hydroxy-risperidone

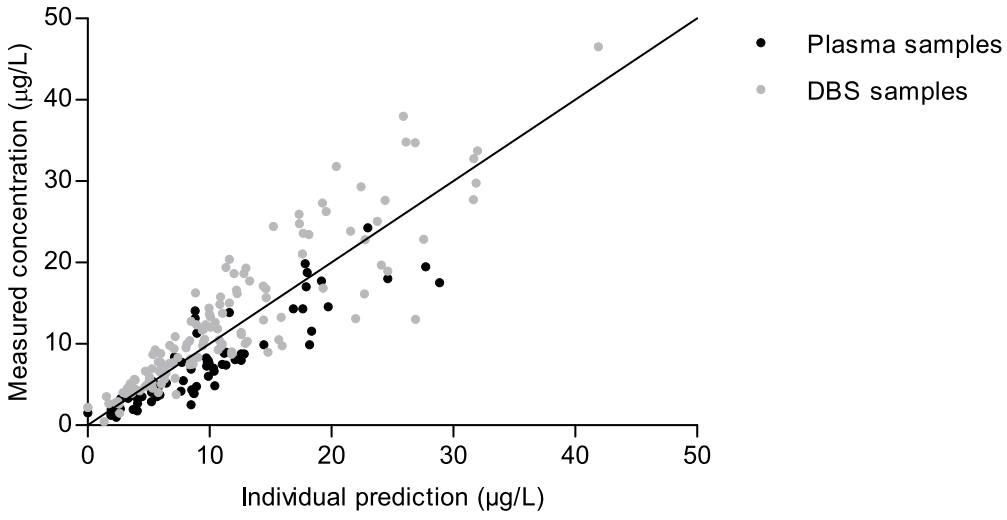


Figure S2 Visual Predictive Check (VPC)

Visual Predictive Checks (VPC). a. VPC of risperidone b. VPC of 9-hydroxyrisperidone

Legend: left figure shows VPC of risperidone, right figure shows VPC of 9-hydroxyrisperidone

PART V

GENERAL DISCUSSION, SUMMARY, AND APPENDICES

CHAPTER 10

GENERAL DISCUSSION

Autism spectrum disorder (ASD) is characterized by the impairment in two symptom domains: social communication and restricted and repetitive behaviours.[1] In addition, secondary characteristics such as emotional and behavioral problems are extremely common in children and adolescents with ASD.[2] Antipsychotic drugs are proven effective to treat these comorbid problems, but antipsychotic drugs also entail severe side effects, especially in the young.

This thesis aims to improve the safety of antipsychotic drugs in children and adolescents by using an individualized approach, focusing on antipsychotic drug concentration measurement. Within this last chapter, the findings are discussed in a broader perspective and recommendations for both clinical practice and future research are provided.

MAIN FINDINGS

- **The large extent of antipsychotic drug use**

Since the introduction of atypical antipsychotic drugs, their use in children and adolescents has increased significantly.[3] Our study in **chapter 2** showed that this trend has continued to rise, with a prevalence of 9.8 per 1000 youths in 2009, before stabilizing.[4] Dutch practitioners, including general practitioners, seem to prescribe antipsychotic drugs more commonly to youths than practitioners in other countries.[5] Among other reasons, this relatively high prescription rate in the Netherlands is explained by a considerably long duration of antipsychotic drug use, with one in eight children in whom antipsychotic drugs are initiated using it for at least 4 years (**chapter 2**). This is particularly worrying as the long-term efficacy of antipsychotic drugs in children and adolescents is not sufficiently established[6], and the risk for side-effects increases with a longer duration of use.[7-9]

- **Weight gain is most pronounced in the first weeks of antipsychotic treatment**

Side effects of antipsychotic drugs in children and adolescents are common, and especially the metabolic side effects can lead to serious long term health risks. Our study in chapter 3 has shown that the antipsychotic-induced weight gain is most pronounced in the first 15 weeks, with a less steep increase afterwards. Several monitoring guidelines for antipsychotic-related side effects in children and adolescents have been developed in response to the growing concerns about side-effects in the young population.[10-12] The most frequently used *Accare*-guideline presents an intensified monitoring schedule for children with an increased familial cardiovascular risk, non-Caucasian descent and a higher BMI at start, but the evidence on which this guideline is based is sparse and highly heterogeneous. Our study in **chapter 3** aimed to provide more evidence on predictors of excessive weight gain. In this study we found that a higher baseline BMI, adjusted for sex and age and depicted as the z-score, was associated with a higher BMI z-score during antipsychotic treatment. Moreover, we found that concomitant stimulant use can reduce weight gain during follow-up, and that prior antipsychotic use is associated with less weight gain during the first 15 weeks. Ethnicity was associated with antipsychotic-induced weight gain in our study.

- **Psychotropic drug concentrations and clinical outcomes in children and adolescents**

Although the monitoring of side-effects is important to increase the safety of antipsychotic drugs in children and adolescents, the prevention of side-effects is even more crucial. For many drugs, Therapeutic Drug Monitoring (TDM), which comprises the measurements of drug concentrations to optimize pharmacotherapy, has proven to be an excellent tool to prevent side-effects and suboptimal effectiveness.[13] By titrating dosages towards concentration ranges that are associated with minimal side-effects and maximal effectiveness, patient outcomes can be improved significantly. As such, TDM is widely applied within adult psychiatry and beyond.[13]

However, at this moment, TDM is rarely performed within child- and adolescent psychiatry. An important requirement for TDM is that a relationship between drug concentrations and clinical outcomes exists, but our systematic review in **chapter 7** reveals that this relationship is largely unexplored for psychotropic drugs in children and adolescents, including for antipsychotic drugs. The evidence that exists is sparse and highly heterogeneous. Most studies fail to report on the key aspects of well- performed TDM research, such as sampling under steady state circumstances, which complicates the interpretation of the reported concentrations. Moreover, therapeutic reference ranges for all psychotropic drugs are barely provided. This hampers the large scale application of TDM as a tool to prevent side-effects of psychotropic drugs, including antipsychotic drugs, in the young.

- **Dried Blood Spot sampling as a feasible alternative to venous sampling**

Besides established therapeutic reference ranges, also minimally invasive sampling methods are important in order to apply TDM routinely within child and adolescent psychiatry. Youths with psychiatric disorders and related behavioral problems generally have a lower tolerability for venous blood sampling due to distress, anxiety or aggression, which decreases their tolerability for venous sampling. In order to investigate the potential of Dried Blood Spot (DBS) sampling within child and adolescent psychiatry, **part III** of this thesis focused on the development, validation and feasibility of the DBS method for antipsychotic drug concentration measurement in children and adolescents. Despite a successful and extensive laboratory validation in **chapter 4**, the criteria for the clinical validation were not fully met in **chapter 5**. As such, the DBS method appeared to have a higher variability than venipuncture in quantifying antipsychotic drug concentrations, as was shown by a suboptimal agreement between these two methods. DBS however is a feasible repeated sampling technique for challenging research in children and adolescents with ASD and severe behavioral problems (**chapter 6**).

New insights on the concentration-effect relationship for antipsychotic drugs

The main objective of this thesis was to advance the application of TDM of antipsychotic drugs in children and adolescents, by providing new insights on the concentration-effect relationships of antipsychotic drugs.

Pipamperone
For pipamperone no studies on the concentration-effect relationship had been conducted, and the pharmacokinetics in youths were unknown. We studied the pharmacokinetics of pipamperone in children and adolescents (**chapter 8**), and found that these are comparable to adult values. A relatively long half-life of 13h was found, and optimal pipamperone concentrations in children and adolescents with behavioral problems were much lower than for adults with psychosis (56-180 µg/L versus 100-400 µg/L).

Risperidone
For risperidone previous concentration-effect studies in children and adolescents were designed suboptimally, and mainly focused on correlations with prolactin elevation. To investigate a wider range of effects, we have conducted a prospective study in risperidone treated children and adolescents with ASD and behavioral problems to analyze the relationship between predicted risperidone concentrations, side-effects and effectiveness (**chapter 9**), within the previously mentioned SPACe-trial. Our study showed that higher risperidone sum trough concentrations lead to more weight gain, sedation, prolactin elevation and more effectiveness in this population. The sum trough concentrations had better predictive value for weight gain than other pharmacokinetic parameters. An optimal therapeutic range seems to exist, but should be further confirmed.

Aripiprazole
For aripiprazole, another commonly used antipsychotic drug in children and adolescents in the Netherlands for ASD and comorbid severe behavioral problems, no studies assessing the concentration-response relationship have been performed yet and are highly needed. Unique data on this subject have also been collected in the SPACe-trial and are currently analyzed.

CLINICAL IMPLICATIONS

The findings of this thesis have several implications for clinical practice.

Timely discontinuation of antipsychotic drugs

Many children and adolescents use antipsychotic drugs for at least four years, but it is questionable whether all patients benefit from prolonged antipsychotic treatment. Therefore a better evaluation of timely discontinuation of antipsychotic drugs in this population is called for. To facilitate this, the prescription and follow-up of antipsychotic drugs in children and adolescents should be reserved to specialists like child- and adolescent psychiatrists. Furthermore, electronic deprescribing tools, such as clinical rules incorporated in prescribing systems, may help to identify long use of medication and thereby trigger the prescriber to assess the necessity for continued antipsychotic drug use. However, these tools are not yet commonly available for antipsychotic drugs in children and adolescents.[14]

The introduction of the *Child and Youth Act (Jeugdwet)* in 2015, leading to the transformation of child- and adolescents mental health care in the Netherlands[15], might further affect prescribing trends for the young. Local municipalities are now responsible for the organization of youth mental health care in their region, and aim to decrease the number of children and adolescents in specialized care by increasing preventive and early intervention support.[16] However, instead of demedicalizing, the *Child and Youth Act* could contribute to more psychotropic use in youths. As youth mental health care is now organized and provided by local first-line multidisciplinary teams (*wijkteams*) with limited expertise on severe psychopathology and psychopharmacology, negative consequences could be suboptimal triage, ineffective treatment and unnecessary crises. Future pharmacoepidemiological research should be part of thorough evaluations of the *Child and Youth Act*. At this moment, it is important that child and adolescent psychiatrists engage in the public debate on this transformation of youth mental health care. As a field, we must stand up for vulnerable youths and advocate adequate triage and timely and optimal care for children with psychopathology in every municipality of the Netherlands, thereby preventing unnecessary psychotropic drug use, but also under-treatment.

Improving side-effect monitoring guidelines

The *Accare* guideline[17], the most commonly used side-effect monitoring guideline in the Netherlands, recommends the first standardized follow-up visit only after 3 months. However, our study in **chapter 3** has shown that the antipsychotic-induced weight gain is most pronounced within the first 15 weeks, with a less steep increase thereafter. This means that the first weeks of antipsychotic use are an important determinant of the total weight gain, and should therefore be closely monitored. This is supported by other research, which demonstrated that the weight gain after one month is predictive of problematic weight gain after 3 months.[18] Based on these findings, we recommend continuous monitoring including a standardized visit at one month instead of three

months after the start of antipsychotic treatment. This enables a timely identification of individuals gaining excessive weight, with the possibility of an intervention. Interventions can include exercise counseling, dietary interventions or a switch in antipsychotic drug type. To overcome the currently divergent approaches and guidelines for the follow-up of youths starting antipsychotic treatment in the Netherlands[19], this recommendation should be incorporated in one uniform national monitoring guideline.

To further improve clinical side-effect monitoring practices, children and adolescents who are particularly at risk to develop weight gain and other side effects should be identified before the treatment with an antipsychotic drug, in order to offer an intensified follow-up. Our findings support the need for intensified monitoring and proactive diet and exercise counseling of children with a high baseline BMI, given the fact that these children are more prone to metabolic laboratory abnormalities.[20]

Optimizing dosing of pipamperone in young patients

Our pharmacokinetic research on pipamperone (**chapter 8**) has showed that a twice daily dosage scheme, adjusted for body weight, was best to reach optimal concentrations in children and adolescents. This finding supports an evidence based dosing recommendation for pipamperone in the Dutch *Kinderformularium*, which did not exist up till now.[21] Our findings will be incorporated in the Kinderformularium.

Therapeutic drug monitoring of antipsychotic drugs in clinical practice

Our findings in **chapters 8 and 9** support a concentration-effect relationship for antipsychotic drugs in children and adolescents, thereby providing the most important rationale for TDM. As a result the question arises what the current position of TDM is for antipsychotic drugs in children and adolescents in daily clinical practice.

Although some argue that TDM is generally indicated in child- and adolescent psychiatry given the large pharmacokinetic and pharmacodynamic variations within this population[22-24], its routine use should be carefully considered. TDM involves costs and patient burden, which must outweigh the benefits. The criteria for rational routine use of TDM[25, 26], which are shown in **table 1**, can help to determine the current position of TDM of antipsychotic drugs in child- and adolescent psychiatry. As a concentration-effect relationship for pipamperone and risperidone are demonstrated in this thesis and discussed before (Crit. 1), the other criteria will be briefly discussed.

Table 1 Criteria for routine application of TDM – the case for antipsychotic drugs in children and adolescents

Crit. 1.	There is a clinically significant relationship between drug concentration and pharmacological effect.	✓
Crit. 2.	A narrow margin exists between drug concentrations that cause therapeutic and adverse effects.	?
Crit. 3.	Drug concentrations are unpredictable after a given dosage.	✓
Crit. 4.	The pharmacological effects are not readily assessable.	✓
Crit. 5.	A rapid and reliable method for drug quantification is available.	✓
Crit. 6.	Evidence that clinical outcome is improved by doing TDM	?

Based on Soldin et al. 200225 and LeMeur et al 2011.[26] These criteria are assessed for risperidone and pipamperone, the antipsychotic drugs that have been studied in the SPACe trial and are presented in this thesis.
✓ = supported by evidence, ? = currently unknown, further research needed

Crit. 2 *A narrow margin exists between drug concentrations that cause therapeutic and adverse effects.*

The measurement of drug concentrations is only of added value if the balance between beneficial and harmful drug concentrations is delicate, and small variations in drug plasma concentrations can have potential harmful effects. This applies for example to lithium, a drug with a narrow therapeutic window, for which TDM is commonly performed. Although our findings indicate that a therapeutic window for risperidone and pipamperone exists, the therapeutic reference ranges of pipamperone, risperidone and aripiprazole in children and adolescents are not yet sufficiently established and need further confirmation.

Crit. 3 *Drug concentrations are unpredictable after a given dosage.*

Only when drug concentrations between individuals vary considerably after administration of an equal dosage, the correlation between drug concentrations and effects is likely to be better than between the dosage and effects. This variability, known as *inter-individual* variability, was found to be significant for both risperidone and pipamperone in our analyses (**chapters 8 and 9**), and is in line with previous research on antipsychotic drug concentrations in youths.[27] This means that antipsychotic concentrations are highly variable between patients.

Besides variability in drug concentrations between patients, also variability within patients may occur. This is known as the *intra-patient variability* and refers to variability in drug concentrations within the same patient after administration of an equal dosage, on different occasions. Ideally, for TDM within child and adolescent psychiatry, a low intra-patient variability exists, as this makes a drug concentration applicable to other time points for the same patient. Within our analyses, addition of intra-patient variability did not improve the pharmacokinetic model, implying that the intra-patient variability was low enough to extrapolate the pharmacokinetic parameters

concentration over time. This further supports the eligibility of TDM for antipsychotics drugs in children and adolescents.

Crit. 4 *The pharmacological effects are not readily assessable.*
Antipsychotic-induced metabolic adverse events are an illustrative example of delayed effects that are not directly assessable, as these effects generally take weeks or even months to develop. This means the measurement of a drug concentration can be of added value to predict drug effects. For other drugs, for example antihypertensive drugs, the antihypertensive effects are generally directly observable and measurable, making them less suitable for routine TDM. The delayed effect however also entails challenges for TDM, as the drug concentration measured at the time of weight assessment might not be causative for the observed weight at that time.

Crit. 5 *A rapid and reliable method for drug quantification is available.*
The introduction of mass spectrometers and other analytical developments has led to access to robust drug-monitoring assays with a short turn-around time. Also, for risperidone, aripiprazole and pipamperone, a validated ultra-high performance liquid chromatography-mass spectrometry (LC-MS/MS) is available28, enabling quick, selective and sensitive antipsychotic concentration measurements in plasma. This thesis demonstrates that also DBS is a rapid and feasible method for antipsychotic drug determination (**chapters 4, 5 and 6**).

Crit. 6 *Evidence that clinical outcome is improved by doing TDM*
Evidence-based medicine relies on interventions that improve patient outcomes in robust clinical trials. Also TDM of antipsychotic drugs should be proven to decrease weight gain and other side-effects significantly in clinical practice before it can be routinely implemented, but such trials have not been performed until now. An important reason is that randomized controlled trials are logistically hard to perform.

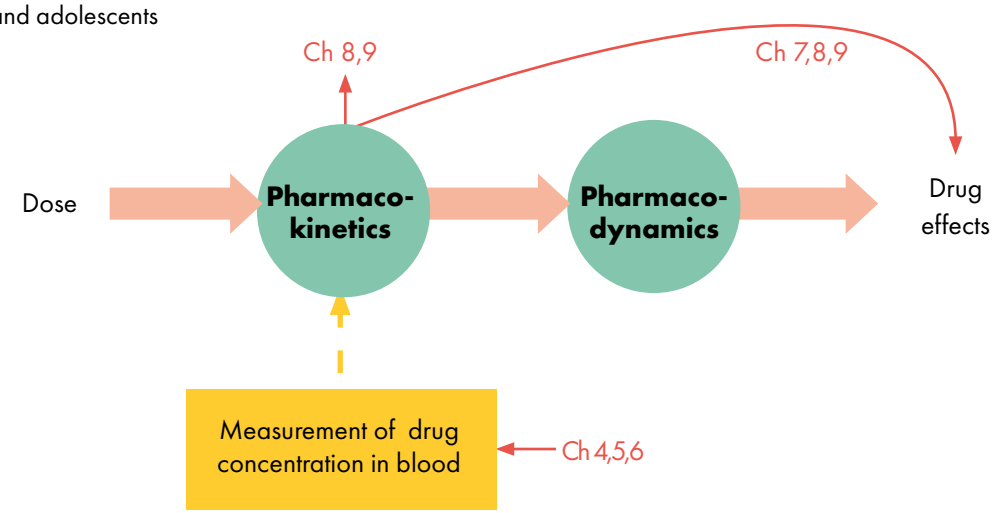
Crit. 7 *Routine TDM of antipsychotic drugs in youths – are we there yet?*
After considering the different aspects for a rational routine application of TDM, we can conclude that TDM has significant potential to enhance safety of antipsychotic drugs in children and adolescents. However, the major drawbacks for its current routine application are that no well-established therapeutic reference ranges exist yet, and that it is unknown whether TDM in this population improves the safety and efficacy outcomes in clinical practice. Therefore, there is currently not enough support for standard TDM implementation in clinical practice for all children and adolescents using antipsychotic drugs.

For the individual patient, however, TDM can certainly already improve outcomes. Indications for a antipsychotic drug concentration measurement include 1) non-response despite adequate dosages 2) excessive side-effects, including weight gain, at low dosages 3) when non-adherence is suspected 4) when drug-drug interactions are foreseen. In these cases, a drug concentration measurement can identify unexpected concentrations, for example because of Cytochrome P450 (CYP) polymorphisms, as for many antipsychotic drugs pharmacokinetic reference ranges in children and adolescents are known.[29] Especially since off-label use of antipsychotic drugs is widespread within child- and adolescent psychiatry[30], safety and effectiveness are not well established and TDM should be considered a low-threshold tool in this population.

FUTURE RESEARCH PERSPECTIVES

This thesis has contributed to knowledge on different levels of the TDM process for antipsychotic drugs in children and adolescents (see **figure 1**).

Figure 1 studies in this thesis contributing to knowledge on TDM of antipsychotic drugs in children and adolescents



Future research on improving the DBS method for antipsychotic drugs

The higher variability in measured DBS concentrations, compared to venous samples, can be successfully overcome with sophisticated statistical methods like NONMEM, as is demonstrated in **chapters 8 and 9**. However, we contend that the following efforts may decrease the variance between DBS and plasma samples, thereby improving the accuracy of the DBS assay:

- *Lowering the lower limit of quantification (LLOQ).* Within the clinical validation process, a considerable amount of samples were below the LLOQ, especially for risperidone. The measured concentrations were much lower than expected based on adult reference ranges. Concentrations below the LLOW entail more uncertainty; lowering the LLOQ

might therefore improve the agreement between DBS and plasma concentrations.

- *Performing the clinical validation study in children and adolescents.* Currently, a clinical validation specifically in children and adolescents is not recommended by the international guideline for the development and validation of DBS methods[31], as the results are expected to be comparable to adults and thus an unnecessary burden to minors should be prevented. However, it can be hypothesized that the correlation is different in children, as with DBS sampling in children less interstitial fluid contamination and more pressuring of the finger is involved due to their smaller fingers. As such, different clinical validation studies for DBS in children have been previously performed and can be considered for antipsychotic drugs as well.[32-34]

- *Minimizing the hematocrit effect.* Hematocrit influences the blood viscosity and therefore the spread of a drop, which can lead to higher or lower measured drug concentrations.35 For risperidone and aripiprazole, a correction for hematocrit was found to improve the agreement between venipuncture plasma samples and DBS in our clinical validation study, while this was not the case for pipamperone (**chapter 5**). Although the variation in hematocrit is expected to be relatively small in children and adolescents who are treated at the outpatient clinic, several approaches can be used to overcome the hematocrit effect, such as volumetric application of blood. However, as regular laboratory check-ups are indicated for metabolic follow-up in children using antipsychotic drugs, the hematocrit can easily and simultaneously be determined.

Research on therapeutic reference ranges and improvement of clinical outcomes

Following the criteria for rational, routine use of TDM of antipsychotic drugs in children and adolescents, future research should focus on establishing therapeutic reference ranges and investigating the added value on patient outcomes in clinical practice.

Therapeutic reference ranges can be based on observational and even retrospective studies, and such ranges are commonly used in TDM guidelines.[13] However, in an ideal situation, therapeutic reference ranges are determined by a prospective study design in which patients are randomized to different concentration ranges, to evaluate which group has the best clinical outcomes. Such a study has been performed for clozapine36, but is very rare in the field. Ideally, future research should confirm the concentration-effect relationship as was found in the SPACe trial in another independent sample, preferably analyzing measured instead of pharmacokinetically predicted concentrations. For risperidone, such a study should focus on the relationship between the sum trough concentration and clinical effects, as this pharmacokinetic parameter had the best correlation with weight gain in our study (**chapter 9)**. Obviously, the key aspects for TDM research, as presented in this thesis (*table 2*) and compiled by a multidisciplinary team of international experts, must be taken into account. These quality criteria are important to guide and improve future research in this field and to facilitate meta-analyses.

Table 2 Key aspects that should be considered when conducting TDM research.

Quality criteria for TDM research

1. Analytical method for the assay of drug concentration in serum or plasma
2. Blood sample collection
3. Patient selection
4. Measurement of illness severity and registration of therapeutic improvement or worsening
5. Comedication
6. Number of patients

For further explanation of these criteria, see chapter 7.

Subsequently, a randomized controlled trial to demonstrate the added value of TDM in terms of patient safety and therapeutic outcomes in clinical practice is ideal. Within this trial, antipsychotic drug concentrations should be titrated towards optimal concentrations and compared to treatment as usual in terms of efficacy and safety. Such a randomized controlled trial is planned to take place as a sequel to the SPACe trial, with as primary endpoint weight gain.

Performing pharmacokinetic-pharmacodynamic research in a challenging population

Within child and adolescent psychiatry, research on concentration-effect relationships and TDM is challenging. Patients often expose emotional and behavioral difficulties, which limits the willingness to participate in clinical trials and undergo extra sampling measurements. However, the SPACe trial has proven that challenging pharmacokinetic-pharmacodynamic research in this population can successfully be performed based on the following success factors.

- Using DBS as a minimally invasive sampling method

Blood sampling is highly challenging in children with psychiatric disorders and severe behavioral problems. Although DBS yields a higher variability in measured antipsychotic drug concentrations, in the SPACe trial the use of DBS has led to an increased willingness to participate. In this way, the advantages of DBS outweigh its disadvantage. Without DBS as alternative sampling method, the challenging SPACe recruitment would not have been as successful as it has proven, with 91% of the 81 included children accepting DBS sampling. However, some of the participants did not tolerate repeated DBS sampling as well as others. This stresses an individualized approach to sampling in children with ASD; given their heterogeneity, DBS might not be preferred over venipuncture by all.

- A flexible, patient-centered approach

During the SPACe trial, the investigator has visited the patients regularly at home for the study measurements. This has led to a personal relationship between investigator and study participants, leading to less study drop-out.

- A strong, multicenter network

Seven centers in the south-west region of the Netherlands have participated in the SPACe-trial. Such a network not only increases study-enrollment, but also facilitates sharing of new knowledge. Furthermore, international collaborations, such as the TDM-KJP network37, are important to further facilitate pharmacokinetic-pharmacodynamic research in the field of child and adolescent psychiatry.

Towards safer antipsychotic treatment in children and adolescents – that's in a drop of blood

Antipsychotic drugs can significantly improve the quality of life of children with ASD and severe behavioral problems. However, given their serious side-effects, it should be carefully identified for which children and adolescents with psychiatric conditions and comorbid behavioral problems antipsychotic treatment is truly necessary. For those children for whom antipsychotic therapy is required, it should be as brief as possible and closely monitored.

This thesis shows that a drop of blood may contribute importantly to safer and individualized antipsychotic treatment in children and adolescents. It can inform us if a child's antipsychotic concentration is adequate to achieve optimal effectiveness and safety, thereby preventing serious side-effects. This means that antipsychotic drug use in children and adolescents might become significantly safer by a single drop of blood.

KEY FINDINGS

- A large share of children and adolescents in the Netherlands who are prescribed antipsychotic drugs, use these drugs for many years, although the long term safety and efficacy is not well established
- Antipsychotic-induced weight gain in children and adolescents is most pronounced during the first 15 weeks of antipsychotic drug treatment
- DBS has a higher variability than venipuncture in quantifying antipsychotic drug concentrations, but is a feasible and suitable technique for repeated sampling in children with ASD and severe behavioral problems
- Most research on TDM in child and adolescent psychiatry does not meet the key aspects of TDM, such as sampling under steady state conditions
- The pharmacokinetics of pipamperone in children and adolescents resemble adult values, although effective concentration ranges seem to be considerably lower in children with behavioral problems than in adults with psychosis
- Risperidone sum trough concentrations can predict weight gain in children and adolescents, and are more predictive than risperidone maximum concentrations or area under the curves

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CHAPTER 11

APPENDICES

SUMMARY

The aim of this thesis is to improve the safety and effectiveness of antipsychotic drugs in children and adolescents, by individualizing antipsychotic drug dosing and monitoring.

PART I – BACKGROUND

Antipsychotic drugs are commonly prescribed to children and adolescents with severe behavioral problems associated with Autism Spectrum Disorder (ASD). For this indication, antipsychotic drugs are the cornerstone of multimodal treatment. However, despite its good efficacy, young patients frequently experience side effects of these drugs. The most important side-effect concerns weight gain, which leads to serious long-term health risks including cardiovascular diseases and diabetes mellitus. Higher antipsychotic dosages are associated with more side-effects in children and adolescents, which suggests that antipsychotic drug concentrations in the blood mediate safety outcomes. Measuring antipsychotic drug concentrations might provide a target to improve safety, as this enables the individualization of dosages towards antipsychotic drug concentrations that are associated with the most optimal treatment outcomes.

PART II – CURRENT PRACTICE

In this part, the extent of antipsychotic drug use and the associated weight gain in children and adolescents in the Netherlands are explored. The study in **Chapter 2** investigates the prescription trends of antipsychotic drugs in patients aged 0-19 years in the Netherlands between 2005 and 2015. This study finds that prescription rates have increased from 7 to almost 10 per 1000 Dutch youths in the general population from 2005 to 2009. Since then, antipsychotic drug prescriptions for children and adolescents have stabilized and slightly decreased, but they have still been substantial. Risperidone, aripiprazole and pipamperone are the most frequently prescribed antipsychotic drugs. Strikingly, one in eight youths who has been prescribed an antipsychotic drug uses it for at least four years, while good evidence for long-term efficacy is lacking in the literature. The study in **Chapter 3** examines the pattern and risk factors of antipsychotic-induced weight gain in 144 Dutch children and adolescents, and finds that weight gain is most pronounced during the first 15 weeks of treatment. A higher dose equivalent is associated with more weight gain during these weeks, but when corrected for other variables, only a higher baseline bodyweight, the absence of stimulant use and no previous antipsychotic treatment predicts more weight gain during different time frames of follow-up. These findings stress a close monitoring of side effects in the first weeks of use, especially for those children who are already overweight when starting an antipsychotic drug.

NEDERLANDSE SAMENVATTING

Het doel van dit proefschrift is het vergroten van de veiligheid en effectiviteit van antipsychotica in kinderen en adolescenten door het individualiseren van doseringen van deze geneesmiddelen.

DEEL I – ACHTERGROND

Antipsychotica worden regelmatig voorgeschreven aan kinderen en adolescenten met een autisme spectrum stoornis (ASS) en ernstige gedragsproblemen. Voor deze indicatie zijn antipsychotica de hoeksteen van de multidisciplinaire behandeling. Helaas hebben juist minderjarigen vaak bijwerkingen van antipsychotica. De belangrijkste bijwerking is gewichtstoename, wat kan leiden tot ernstige gezondheidsrisico's op de lange termijn, zoals hart- en vaatziekten en diabetes mellitus. Hogere doseringen van antipsychotica zijn geassocieerd met meer bijwerkingen bij kinderen en adolescenten, wat veronderstelt dat er een correlatie is tussen de geneesmiddelconcentraties in het bloed en de bijwerkingen die optreden. Het meten van deze geneesmiddelconcentraties kan een aangrijpingspunt zijn om de veiligheid van antipsychotica te vergroten. Immers, antipsychoticadoseringen kunnen op deze manier getitreerd worden naar concentraties met de beste behandeluitkomsten en de minste bijwerkingen.

DEEL II – DE HUIDIGE PRAKTIJK

In dit deel van het proefschrift wordt bekeken wat de huidige omvang van antipsychotica gebruik en de bijbehorende gewichtstoename in kinderen en adolescenten in Nederland is. In **hoofdstuk 2** worden de voorschrijftrends van antipsychotica aan patiënten tussen de 0 en 19 jaar in Nederland tussen 2005 en 2015 onderzocht. In deze studie wordt beschreven dat het aantal antipsychotica voorschriften fors toenam tussen 2005 en 2009, van gemiddeld 7 naar bijna 10 per 1000 minderjarigen die op enig moment een antipsychoticum gebruikte. Hoewel het aantal antipsychoticavoorschriften vanaf dat moment stabiliseerde en daarna zelfs een lichte daling liet zien, was deze nog steeds aanzienlijk. Risperidon, aripiprazol en pipamperon worden het meest voorgeschreven. Opvallend was dat één op de acht kinderen die een antipsychoticum voorgeschreven krijgt, dit middel voor minstens 4 jaar blijft doorgebruiken, terwijl de lange termijn effectiviteit niet goed onderbouwd is. In **hoofdstuk 3** worden het verloop en de risicofactoren voor antipsychotica-geïnduceerde gewichtstoename in 144 Nederlandse kinderen en adolescenten onderzocht. Er wordt geconcludeerd dat gewichtstoename het meest uitgesproken is in de eerste 15 weken van gebruik. Een hogere dosisequivalent is geassocieerd met meer gewichtstoename tijdens deze eerste weken, maar na correctie voor andere variabelen, zijn alleen een hoger uitgangsgewicht, het niet gebruiken van stimulantia en geen eerder antipsychotica gebruik voorspellend voor een gewichtstoename na verschillende gebruikersduren. Deze bevindingen onderstrepen het belang van een geïntensiveerde monitoring van bijwerkingen in de eerste weken van gebruik, vooral voor kinderen die al overgewicht hebben bij start van een antipsychoticum.

PART III – ALTERNATIVE SAMPLING

This part focuses on investigating a minimally invasive way to measure antipsychotic drug concentrations in children and adolescents with severe behavioral problems; the Dried Blood Spot (DBS) method. This method enables the quantification of drugs in a single drop of blood, collected on a filter paper by a finger prick in a home setting. Three studies are conducted to investigate the reliability and feasibility of this method in our population. First, a DBS assay is developed to measure risperidone, aripiprazole, pipamperone and their major active metabolites with Liquid Chromatography–Mass Spectrometry (LC-MS/MS). **Chapter 4** describes the successful validation of this DBS assay in the laboratory. In this study, accuracy and imprecision are well within the acceptance criteria of conventional guidelines for bioanalytical method validation. After the development, the DBS assay is tested in a real-life clinical setting and compared to simultaneously taken venous samples, which is reported in the study in **chapter 5**. Strikingly, the performance of the DBS assay in a clinical setting is worse than in the laboratory setting, and does not meet all the acceptance criteria. This results in a higher variability of antipsychotic drug concentrations when measured with DBS than with venipuncture. Lastly, the feasibility of DBS sampling in children with ASD and severe behavioral problems is studied in **chapter 6**. DBS sampling is successfully performed in most of these children, although 1 in 5 children refuses one or more DBS fingerpricks due to distress.

DEEL III – ALTERNATIEVE BLOEDAFNAME

Deel III van het proefschrift richt zich op het onderzoeken van een nieuwe manier om antipsychotica concentraties te meten in het bloed van kinderen en adolescenten met ernstige gedragsproblemen: de Dried Blood Spot (DBS) methode. Deze methode maakt het mogelijk om antipsychotica concentraties te meten in slechts één druppel bloed, verkregen met een vingerprik dat thuis kan worden uitgevoerd. Drie studies worden verricht om de betrouwbaarheid en haalbaarheid van de DBS methode te onderzoeken in deze specifieke doelgroep. Allereerst wordt een DBS methode ontwikkeld om risperidon, aripiprazol, pipamperon en de actieve metabolieten te meten met vloeistofchromatografie gecombineerd met tandem-massaspectrometrie (LC-MS/MS). **Hoofdstuk 4** beschrijft de succesvolle validatie van deze DBS methode in ons laboratorium. In deze studie vallen nauwkeurigheid en precisie ruim binnen de acceptatiecriteria van conventionele richtlijnen voor validatie van bioanalytische methoden. In de studie in **hoofdstuk 5** wordt deze DBS methode getest in een klinische setting en vergeleken met gelijktijdig afgenomen veneuze monsters. Opvallend is dat de DBS methode in deze klinische setting minder betrouwbaar is dan in de laboratoriumomgeving en niet aan alle acceptatiecriteria voldoet. De variatie in gemeten antipsychotica concentraties is groter met de DBS methode dan met de venapunctie. Ten slotte wordt de haalbaarheid van DBS-afname bij kinderen met ASS en ernstige gedragsproblemen onderzocht in **hoofdstuk 6**. DBS wordt met succes uitgevoerd bij de meeste kinderen, hoewel 1 op de 5 kinderen een of meer DBS-vingerprikken weigert vanwege angst.

PART IV – ANTIPSYCHOTIC DRUG CONCENTRATIONS AND CLINICAL OUTCOMES

Part IV investigates the relationship between antipsychotic drug concentrations and clinical outcomes in children and adolescents. Previous studies that have assessed the relationship between psychotropic drug concentrations and clinical outcomes are systematically reviewed and discussed in **chapter 7**. Based on this review, it is concluded that there is a lack of well-reported and well-executed therapeutic drug monitoring studies in this population. For pipamperone, no such studies had been conducted at all. In **chapter 8**, the pharmacokinetics of pipamperone are described and externally validated using non-linear mixed effect modelling (NONMEM®), based on measured concentrations in 51 children and adolescents from the Netherlands and Germany. It is found that pipamperone can best be dosed twice daily, with adjustment for bodyweight. Interestingly, pipamperone pharmacokinetic parameters are related to clinical improvement and side effects, showing that responders have considerably higher pipamperone concentrations than non-responders. In **chapter 9**, the relationship between risperidone plasma concentrations and side effects and efficacy is analyzed in a Dutch prospective observational study. In this study, risperidone concentrations, side effects and effectiveness are repeatedly measured in children and adolescents with ASD and severe behavioral problems. Different pharmacokinetic parameters are described using NONMEM® and correlated with clinical outcomes. We find that higher risperidone trough concentrations predict higher bodyweight, more sedation, higher prolactin levels and strikingly, also more effectiveness. The results of this study indicate that a therapeutic window for risperidone in this population might exist.

PART V – GENERAL DISCUSSION

Chapter 10 discusses the findings of the studies presented in this thesis in a broader context. The implications and advances for safer and individualized antipsychotic therapy in children and adolescents based on this thesis are discussed. In this final chapter, the findings on the relationship between antipsychotic drug concentrations and clinical outcomes are put in a larger perspective, by assessing the criteria for routine application of therapeutic drug monitoring (TDM). Also, recommendations for clinical practice and future research are made.

DEEL IV – ANTIPSYCHOTICA CONCENTRATIES EN KLINISCHE UITKOMSTEN

In deel IV van dit proefschrift wordt de relatie tussen antipsychotica concentraties en klinische uitkomsten bij kinderen en adolescenten onderzocht. Eerdere studies die deze relatie hebben onderzocht voor psychiatrische medicatie werden systematisch beoordeeld en besproken in **hoofdstuk 7**. Op basis van deze systematic review concluderen we dat er een gebrek is aan goed gerapporteerde en goed uitgevoerde therapeutic drug monitoring (TDM) studies in deze populatie. Voor pipamperon bleken dergelijke onderzoeken helemaal niet te bestaan. In **hoofdstuk 8** wordt de farmacokinetiek van pipamperon beschreven en extern gevalideerd met behulp van niet-lineaire mixed-effect modellering (NONMEM®), gebaseerd op gemeten concentraties bij 51 kinderen en adolescenten uit Nederland en Duitsland. In deze studie wordt gevonden dat pipamperon het beste twee keer per dag kan worden gedoseerd, aangepast op het lichaamsgewicht. Interessant genoeg zijn de farmacokinetische parameters van pipamperon gecorreleerd aan klinische verbetering en bijwerkingen: kinderen met goed effect van pipamperon hebben aanzienlijk hogere pipamperonconcentraties dan kinderen die geen baat hebben bij deze medicatie. In **hoofdstuk 9** wordt de relatie tussen de plasmaconcentraties van risperidon en de bijwerkingen en effectiviteit geanalyseerd in de Nederlandse prospectieve observationele studie "SPACe", onder kinderen en adolescenten met ASS en ernstige gedragsproblemen. Verschillende farmacokinetische parameters worden beschreven middels NONMEM® en gecorreleerd aan klinische uitkomsten. Er wordt gevonden dat hogere dalconcentraties van risperidon voorspellend zijn voor een grotere gewichtstoename, maar ook meer sedatie, hogere prolactinespiegels en meer effectiviteit. De resultaten van deze studie geven aan dat er mogelijk een therapeutisch venster voor optimale concentraties van risperidon in deze populatie bestaat.

DEEL V - ALGEMENE DISCUSSIE

In **hoofdstuk 10** worden de bevindingen van de studies in dit proefschrift in een bredere context geplaatst. De implicaties voor veiligere en geïndividualiseerde antipsychotische therapie bij kinderen en adolescenten op basis van dit proefschrift worden besproken. In dit laatste hoofdstuk wordt gebruik gemaakt van de criteria voor routinematige toepassing van therapeutic drug monitoring. Ook worden aanbevelingen gedaan voor de klinische praktijk en toekomstig onderzoek.

LIST OF ABBREVIATIONS

ABC	Aberrant Behavioral Checklist
ADD	Attention Deficit Disorder
ADHD	Attention-Deficit/Hyperactivity Disorder
AF	Alkalic fosfatase
AIMS	Abnormal Involuntary Movement Scale
ALAT	Alanine aminotransferase
AP	Antipsychotic
ASAT	Aspartate aminotransferase
ASD	Autism Spectrum Disorder
AUC	Area under the concentration-time curve
BP	Blood pressure
C	Central
Ctrough	Trough concentration
Cmax	Maximum concentration
CABRS	Conners Abbreviated Rating Scale
CAPTRS	Conner's Abbreviated Parent Teacher Rating Scale
CDI	Children's Depression Inventory
CDRS-R	Children's Depression Rating Scale – revised
C-GAS	Childrens Global Assessment Scale
CGI-I	Clinical Global Impression improvement Scale
CGI-S	Clinical Global Impression severity Scale
CI	Confidence Interval
CL	Clearance
CPRS	Comprehensive Psychopathological Rating Scale
CTRS	Conner's Teaching Rating Scale
CWRES	Conditional weighted residuals
CY-BOCS	Childrens' Yale-Brown Obsessive Compulsive Scale
CYP	Cytochrome P450
DOTES	Dose Record and Treatment Emergent Symptom Scale
DSM	Diagnostic and Statistical Manual of Mental Disorders
BMI	Body-mass-index
BPRS	Brief Psychiatric Rating Scale
DBS	Dried Blood Spot
EC50	Half maximal effective concentration, the concentration of a drug which induces a response halfway between the baseline and maximum
ECG	Electrocardiogram
EMA	European Medicines Agency
EPS	Extrapyramidal symptoms
ER	Extended release formulation
ESS	Epworth Sleepiness Scale
F	Bioavailability
FDA	US Food and DrugsAdministration
GAF	Global Assessment of Functioning
GH	Growth hormone
GGT	Gamma glutamyl transpeptidase
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HDRS	Hamilton Depression Rating Scale
HPLC-UV	High performance liquid chromatography-ultraviolet
HR	Heart rate
IPV	Inter-patient variability
Ka	Absorption rate constant
K-SADS	Kiddie Schedule for Affective Disorders and Schizophrenia
K-SADS-P	Kiddie Schedule for Affective Disorders and Schizophrenia - Present Episode
LDL	Low-density lipoprotein
LFTs	Liver function tests
LLOQ	Lower limit of quantification
LOD	Lower limit of detection
LOI-CV	Leyton Obsessional Inventory-Child version
NONMEM	Nonlinear mixed effects modelling
M	Metabolite
MARS	Medication Adherence Rating Scale
MDD	Major Depressive Disorder
MEMS	Medication Event Monitoring System
MFFT	Matching Familiar Figures Test
M-MAT	McLean Motion Attention Test
M-SADS	Abbreviated Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS), just covering affective symptoms
NIMH	National Institute of Mental Health
NR	Normal release
Obs	Number of observations
OCD	Obsessive-compulsive disorder
OCRS	Obsessive-Compulsive Rating Scale
ODD	Oppositional defiant disorder
P	Peripheral
PERMP	Permanent Product Measure of Performance
PGP	P-glycoprotein
PLT	Platelet
PRISMA	Preferred reporting items for systematic reviews and meta-analyses

PRL	Prolactin
Q	Intercompartmental clearance
QC	Quality control
QTc	Corrected QTc
RCMAS	Revised Children's Manifest Anxiety Scale
sCR	Serum creatinine
RSE	Relative standard error
SEFCA	Side Effects Form for Children and Adolescents
SPACe	Safety and Pharmacokinetics of Antipsychotics in Children
SD	Standard Deviation
SERS	Side Effects Rating Scale
SKAMP	Swanson, Kotkin, Agler, M-Flynn and Pelham Scale
SNAP	Swanson, Nolan, and Pelham Questionnaire
SRT	Scanning Reaction Time, part of three computer tests
tlag	Lag time
TC	Total cholesterol
TDM	Therapeutic Drug Monitoring
TG	Triglycerides
TSH	Thyroid stimulating hormone
UHPLC-MS/MS	Ultra–High-Performance Liquid Chromatography–Mass Spectrometry
UKU	Udvalg for Klinske Undersogelser
ULOQ	Upper limit of qualification
V	Volume of distribution
VAS	Visual Analogue Scale
WBC	White blood cell counts
WHO	World Health Organization
WWPAS	Werry-Weiss Peters Activity Scale
YGTSS	Yale Global Tic Severity Scale

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Wietske A. Ester	Sarr Expert Center for Autism, Youz Child and Adolescent Psychiatry, Rotterdam, The Netherlands; Department of Child and Adolescent Psychiatry, Curium-LUMC, Leiden University Medical Center, Oegstgeest, The Netherlands; Parnassia Psychiatric Institute, The Hague, The Netherlands
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Manon H.J. Hillegers	Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Center Rotterdam, the Netherlands

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Rixt A. Wijma	Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, The Netherlands
Brenda C.M. de Winter	Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, The Netherlands

LIST OF PUBLICATIONS

RELATED TO THIS THESIS

Kloosterboer SM, Schuiling-Veninga CCM, Bos JHJ , et al. Antipsychotics in Dutch Youth: Prevalence, Dosages, and Duration of Use from 2005 to 2015. *J Child Adolesc Psychopharmacol* **28**, 173-9 (2018).

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Kloosterboer SM, Egberts KM, de Winter BCM , et al. Pipamperone Population Pharmacokinetics Related to Effectiveness and Side Effects in Children and Adolescents. *Clin Pharmacokinet* **59**, 1393-1405 (2020).

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Al-Hassany L, Kloosterboer SM, Dierckx B and Koch BC. Assessing methods of measuring medication adherence in chronically ill children-a narrative review. *Patient Prefer Adherence* **13**, 1175-89 (2019).

Kloosterboer SM, Koch BCP, Hillegers MHJ and Dierckx B. [Use of antipsychotics in children; too much or too long?] Antipsychoticagebruik onder kinderen. *Ned Tijdschr Geneeskd* **163**, (2019).

Kloosterboer SM, McGuire T, Deckx L , et al. Self-medication for cough and the common cold: information needs of consumers. *Aust Fam Physician* **44**, 497-501 (2015).

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Kloosterboer SM, Koch BCP, de Winter BCM, Dierckx B. TDM van antipsychotica in de kinder- en jeugdpsychiatrie. De nieuwste inzichten. Psyfar **15**, 46-50 (2020).

ABOUT THE AUTHOR

Sanne Maartje Kloosterboer was born on October 18th in Leeuwarden, the Netherlands. She grew up in Burgum and SInt-Oedenrode. In 2007 she graduated cum laude from secondary school at Gymnasium Beekvliet and started her medical training at Utrecht University. During medical school she did an internship Obstetrics and Gynecology at the Muhimbili University of Health and Allied Sciences in Tanzania. Her enthusiasm for research started in 2014 during her research internship at the department of General Practice at the University of Queensland in Brisbane, Australia. She received the Grant for Strategic Network Development of the University Medical Center Utrecht to perform this research.

After receiving her medical degree in 2015 she started working as a resident in Geriatric Medicine at the Amphia Hospital in Breda. She started her PhD program in 2016 at the departments of Hospital Pharmacy and Child and Adolescent Psychiatry of the Erasmus MC, under supervision of prof. dr. T. van Gelder, prof. dr. F. Verhulst, dr. B.C.P. Koch and dr. B. Dierckx. After the retirement of prof. dr. F. Verhulst In 2017, prof. dr. M.H.J. Hillegers took over his role. As part of her PhD, Sanne conducted a research project at the University of Würzburg, Germany in 2018 in close collaboration with dr. K.M. Egberts and prof. dr. M. Gerlach. Alongside her PhD she fulfilled her training in clinical pharmacology. In March 2020 she started her specialist training in psychiatry at the Erasmus MC under supervision of prof. dr. W.W. van den Broek and dr. T.K. Birkenhäger.

Sanne lives together with her fiancé Ruben Lentz and their dog Wies in Rotterdam, the Netherlands.

PHD PORTFOLIO

SUMMARY OF PHD TRAINING AND TEACHING

Name PhD student: Sanne Maartje Kloosterboer Erasmus MC Department: Hospital Pharmacy, Child- and Adolescent Psychiatry Research School: Molecular Medicine	PhD period: 2016-2020 Promotor(s): prof. dr. T. van Gelder, prof. dr. M.H.J.Hillegers Supervisors: dr. B Dierckx, dr. B.C.P.Koch

1. PhD training

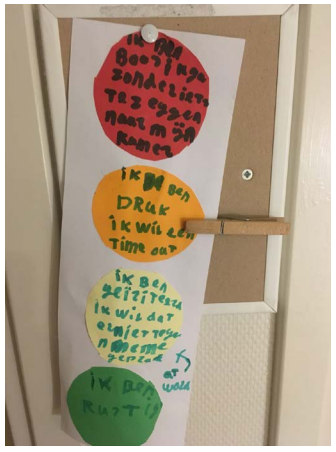
	Year	Workload (ECTS)
General courses		
Biostatistical Methods I: Basic Principles (CCO2), NIHES, Erasmus MC	2016	*5.7*
Research Management, Erasmus MC	2016	1.0
CPO mini-course, Erasmus MC	2016	0.3
BROK ('Basiscursus Regelgeving Klinisch Onderzoek'), Erasmus MC	2016	1.0
Advanced course Excel, Erasmus MC	2016	0.3
Systematic Literature Retrieval and Endnote, Medical Library, Erasmus MC	2016	1.0
Introduction in Open Clinica, Trial IT, Erasmus MC	2016	0.3
Introduction in Limesurvey/Gemstracker, trial IT, Erasmus MC	2016	0.3
Webredactie Alterian, Erasmus MC	2016	0.3
Biomedical English Writing and Communication, Erasmus MC	2017	3
Research Integrity, Erasmus MC	2018	0.3
		(13.5)
Specific courses		
Teaching the Teacher the 6step, NVKFB	2016	0.3
Principles of Pediatric Clinical Pharmacology, NIH	2016-2017	2.0
Survival Analysis Course, Erasmus MC	2017	0.6
Teach the Teacher 1, Erasmus MC	2018	0.6
Didactic workshops: e-module, tentamenvragen maken, Erasmus MC	2018	0.3
First-in-human Clinical Trials, CHDR, NVKFB	2018	0.3
Writing Succesful Grant Proposals, Erasmus MC	2019	0.3
		(4.4)
Seminars and workshops		
Golden Helix Pharmacogenomics Day, Rotterdam	2016	0.3
Lareb day, 's- Hertogenbosch	2017	0.3
Toxed symposium, Rotterdam	2017	0.3
		(0.9)
National conferences		
Research Day Clinical Pharmacology, Rotterdam (oral presentation)	2016	1.0
NVKFB Spring Symposium 2017, Nijmegen (poster presentation)	2017	1.0
Spring symposium Dutch Association for Psychiatry 2017 (oral presentation, workshop)	2017	1.0
FIGON Dutch Medicines Day 2018, Ede (poster presentation)	2018	1.0
International Society for Autism Research annual meeting 2018, Rotterdam (poster presentation)	2018	1.0
NVKFB Spring Symposium 2019, Rotterdam (poster presentation)	2019	1.0
Spring symposium Dutch Association for Psychiatry 2020 – online edition (2 oral presentations)	2020	2.0
Nominee Figon DMD PhD competition 2020	2020	1.0
		(9.0)
International conferences		
Congress of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology 2017, Kyoto, Japan (poster presentation)	2017	1.0
Flemish congress child- and adolescent psychiatry, Leuven, Belgium (oral presentation)	2019	1.0
Congress of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology 2019, Iguacu, Brazil (oral presentation, 3 poster presentations)	2019	2.0
Congress of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology 2020, Banff, Canada – online edition (oral presentation)	2020	1.0 (5.0)
Other		

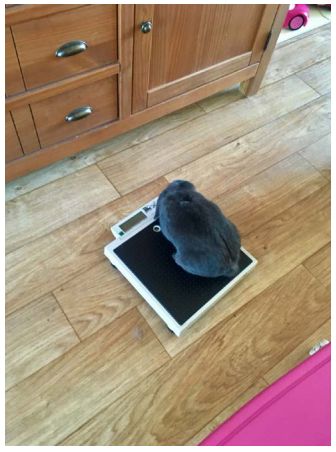
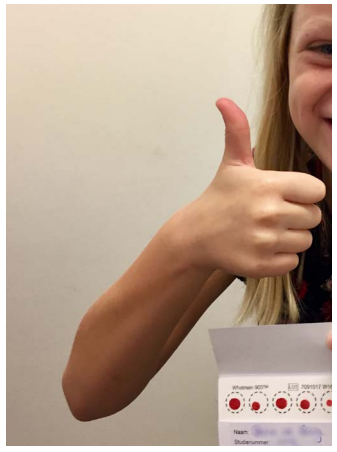
2. Teaching

	Year	Workload (Hours/ ECTS)
Lecturing		
Master Medicine Erasmus MC: Obstetrics (drugs during pregnancy and lactation) Internal medicine (polypharmacy) Psychiatry (psychopharmacology) Prescribing medication	2016-2019	2.0
Psyfar Masterclass psychopharmacology children & adolescents	2018-2020	1.0 (3.0)
Supervising Bachelor/Master's theses		
L. al-Hassany, bachelor student medicine, Erasmus University, Rotterdam	2017	1.0
E. van Eijk, bachelor student medicine, Leiden University, Rotterdam	2018-2019	1.0
C. van Esch, master student medicine, Erasmus University, Rotterdam	2019	2.0 (4.0)
Other		Tot 39.8

BEHIND THE SCENES



DANKWOORD

Onderzoek lijkt alleen over cijfers te gaan, maar niets in minder waar: het is mensenwerk van begin tot eind. Zonder de inzet van een heleboel belangrijke mensen was al dit onderzoek nooit wat geworden. Graag wil ik de kans nemen om jullie hier te bedanken.

Allereerst: **alle kinderen en hun ouders** die mee hebben gedaan aan het SPACe onderzoek. Ik vind het ontzettend bijzonder dat jullie, terwijl jullie wereld op zijn kop stond, bereid zijn geweest om uren aan vragenlijsten, talloze vingerprikken en andere extra metingen te volbrengen. Bedankt voor de openhartige gesprekken en vele koppen thee aan jullie keukentafels; het ging over zoveel meer dan de onderzoeksmetingen, en het heeft me waardevolle inzichten gegeven in het leven met autisme die ik nooit uit een boek had kunnen opdoen.

Prof. dr. van Gelder, beste Teun. Als promotor stond je deur altijd open om even stoom af te blazen, voor wijze raad, of voor gewoon een gezellig praatje. Ik heb veel geleerd van je scherpe blik als klinisch farmacoloog. Naast de onderzoekbesprekingen was eigenlijk overal voor in, of het nou de trappenloop, de Tour de Rotterdam of karaoke was. Bedankt voor je oprechte betrokkenheid.

Prof. dr. Hillegers, beste Manon. Jouw visie op de kinder- en jeugdpsychiatrie is inspirerend. Met ogenschijnlijk simpele vragen kom je meteen tot de kern. Dit heeft me vaak aan het denken gezet, niet alleen wat betreft het onderzoek, maar zeker ook wat betreft mijn eigen ambities. Veel dank daarvoor.

Prof. dr. Verhulst, beste Frank, tot je met pensioen ging was ook jij mijn promotor. In de eerste fase van het onderzoek was je doorgewinterde kennis van de deelnemende centra in de regio onmisbaar.

Dr. Koch, beste Birgit. Jouw energie en optimisme zijn echt ongekend. Je verliest de grote lijn in het onderzoek niet uit het oog en weet snel creatieve oplossingen te vinden voor hobbels op de weg. Bovendien benader je alles met een gezonde dosis humor; een verademing als ik me weer eens kon verliezen in details. Je hebt me gestimuleerd om me ook ver buiten het onderzoek te ontwikkelen; bedankt voor je onvoorwaardelijke steun en vertrouwen.

Dr. Dierckx, beste Bram. Bedankt voor je scherpe, analytische blik en eerlijkheid. Ik heb veel geleerd van onze discussies over statistische resultaten, maar ook over de Vlaamse politiek, de GGZ, en wat al niet meer. Als het op onderzoek aankomt, sta je echt voor wat je doet. Het is dankzij jou dat ik ook voor de psychiatrie gekozen heb. Ik hoop dat je wat mindert met je cola-consumptie, zodat we nog lang kunnen samenwerken.

Dr. de Winter, beste Brenda. Je bent misschien wel de slimste persoon die ik ken. Je weet werkelijk alles van NONMEM en hebt ook nog eens de gave om het heel geduldig, keer op

keer, uit te leggen. Hoewel er soms wat frustratie richting een vastlopende computer ging, stond je vooral altijd klaar om te helpen. Daarnaast is geen borrel compleet zonder jou.

Dr. Dieleman, beste Gwen, ook jij was vanaf het begin bij het project betrokken. Op de polikliniek wist je alle includerende artsen goed bij de les te houden als de inclusie inzakte. Mede dankzij jou is de SPACe studie ook in het Sophia Kinderziekenhuis een succes geworden.

Veel dank aan de leden van de leescommissie – **prof. dr. de Wildt**, **prof. dr. Hoekstra** en **prof. dr. van den Broek** voor het beoordelen van dit proefschrift. Beste Walter, als opleider vind ik het extra bijzonder dat je op deze manier ook bij mijn onderzoek betrokken bent.

Naast het Erasmus MC zijn de studies in dit proefschrift ook op vele andere plekken uitgevoerd. De hulp van een heleboel mensen was hierbij onmisbaar.

Drs. Dekker, beste Annet. Dankzij jou hadden we bij Middin binnen no time een groot aantal patiënten en hun vertegenwoordigers gemobiliseerd om mee te doen aan de DBS validatie studie. Voor de pipamperon inclusie was dat echt onze redding.

De SPACe studie had wel zeven deelnemende centra, een aardige logistieke uitdaging. De lokale hoofdonderzoekers waren op al die plekken onmisbaar om alles in goede banen te leiden. Veel dank aan **dr. van Daalen**, **dr. Reichart**, **dr. Rieken** en **drs. Ouwehand**, **dr. Kouijzer**, **drs. De Kroon** en **dr. Ester**. Daarnaast ook aan **dr. Atanasios Maras** en **prof. dr. Robert Vermeiren** voor het mogelijk maken van de SPACe studie op jullie locaties. Uiteraard veel dank aan alle **includerende artsen** die op de (poli-)klinieken de SPACe kinderen hebben vervolgd; alle genomen extra tijd en moeite (naast de overvolle poli agenda's) kan ik enorm waarderen.

Naast artsen hebben ook een aantal verpleegkundigen een belangrijke bijdrage geleverd. Op de polikliniek in het Sophia Kinderziekenhuis hebben **Roelie van Zon** en **Henriette Rijpkema** een heleboel vingerprikjes uitgevoerd, metingen gedaan, en data ingevoerd. Bij GGZ Breburg heeft **Jeske van de Velden** veel op zich genomen (en is zelfs mee gegaan op huisbezoek!). Voor de studie logistiek in het Sophia Kinderziekenhuis waren **Monique Knol** en **Q Andriessen** onmisbaar. Dank allemaal, jullie enthousiasme voor de studie gaf me veel energie.

Achter de schermen moesten een heleboel vingerprikjes geanalyseerd worden. Alle **analisten van het farmacologisch laboratorium** in het Erasmus MC hebben honderden DBS kaartjes verwerkt. Heel veel dank voor jullie geduld met al die nét niet goed ingevulde aanvraagformulieren. Één analist heeft in het bijzonder een enorme berg werk verzet: lieve **Soma Bahmany**, met jouw precisie en rust had ik dat aan geen beter persoon kunnen toevertrouwen. **Ruud Huisman**, als jij aan mijn bureau stond wist ik dat ik er ergens een rommeltje van had gemaakt, maar gelukkig hield jij altijd een oogje in het zeil.

Al vroeg in mijn promotietraject reisde ik af naar Groningen, waar we dankbaar gebruik mochten maken van de IADB.nl database. **Dr. Nynke Schuiling-Veninga**, we werden meteen met open armen ontvangen, bedankt voor het faciliteren van ons onderzoek en de fijne samenwerking. **Jens Bos**, ondanks de afstand was jij altijd bereikbaar voor ondersteuning bij data-extracties.

Op statistisch gebied heb ik dankbaar gebruik gemaakt van de hulp van **dr. Jan van der Ende** en **dr. Kazem Nasserinejad**. Ik heb ook een aantal studenten mogen begeleiden: **Linda Al-Hassany**, **Esmé van Eijk** en **Casper van Esch**. Het was erg leuk om jullie te zien groeien als onderzoekers. Daarnaast was voor jullie hulp bij de praktische uitvoer van de studie onmisbaar. Linda, het avontuur waar we bij Middin samen in terecht kwam zal ik niet snel vergeten; zonder jou had ik het niet overleefd.

Ook over de grens hebben een aantal mensen een belangrijke bijdrage geleverd aan dit proefschrift. **Herr prof. dr. Romanos**, vielen Dank dass Sie mich auf Ihrer Abteilung so herzlich Wilkommen geheissen haben. Liebe **Frau dr. Egberts**, Karin, da dein Niederländisch um ein Vielfaches besser ist als mein Deutsch, werde ich dir auf Niederländisch schreiben. Dankzij jou was mijn tijd in Würzburg onvergetelijk. Je warme onthaal en enthousiasme maakte dat ik me meteen welkom voelde. Ons onderzoek heeft veel raakvlakken, ik kom graag nog eens terug want ik hoop echt dat we zullen blijven samenwerken. Sehr geehrter **Herr prof. dr. Gerlach**, auch vielen Dank für Ihre Begleitung.

Alle **co-auteurs** die ik nog niet genoemd heb, ook aan jullie dank voor de input voor de manuscripten.

Naar goed voorbeeld wil ook ik **mijn auto** bedanken. Met 6977 gedeclareerde kilometers had ik het nooit zonder je gered.

Lieve **collega's van de apotheek**. Ik kan het iedere arts aanraden om een aantal jaar in de apotheek te werken, en dan in het bijzonder de ziekenhuisapotheek van het Erasmus MC. Ik heb veel geleerd van jullie andere perspectief en scherpe inzichten. Al vlogen de regeltjes en richtlijnen me soms om de oren, er was vooral heel veel tijd voor gezelligheid. De skivakanties waren hierbij zeker een hoogtepunt.

Lieve **mede-onderzoekers van de apotheek**, zonder jullie was mijn onderzoekstijd toch wel echt een heel stuk minder leuk geweest. Oud Na-206, **Florine, Annette, Linda, Louise, Rehana, Suzanne** en **Femke**, van werken kwam het niet perse maar dankzij jullie was ik in ieder geval op de hoogte van de Tour de France en Wie is de Mol. **Janique** (JJ), **Laura**, **Dimokrat** en **Laura**, met jullie overleefde ik de jungle van de kantoortuin, hetzij op een wat lager geluidsniveau. Tijdens de vele nutteloze rondjes lopen konden we gelukkig alsnog de laatste roddels uitwisselen, stomme grappen maken of Surinaams dan wel donuts halen. **Usha**, als "moeder" van de groep hield je altijd het hoofd koel, dankzij jou kon ik me een weg banen in het oerwoud van de AVG. Lieve **Floor**, jouw passie voor onderwijs werkt echt aanstekelijk. Je was

een geweldige partner in crime voor de Geneesmiddel van de Week Oscars, en ik ben blij dat ik je als buurvrouw nog regelmatig tegenkom. **Maya**, binnen de KJP onderzoeksgroep was jij m'n maatje, en een onovertroffen trappenloop-kampioen.

Lieve dames van de **farmafietsclub**, naast werk delen we een liefde voor fietsen. Behalve de Tour de Rotterdam en Amstel Gold Race, was de Elfstedentocht een absoluut hoogtepunt. **Annette**, als teamcaptain weet je me altijd thuis te brengen en ben ik je erg dankbaar dat ik aan jouw zijde nooit verdwaald raak. Wanneer gaan we weer?

Collega's van de afdeling psychiatrie, bedankt voor het warme welkom en de tijd die ik kon nemen om mijn proefschrift af te ronden.

Barbara, jou mag ik natuurlijk niet vergeten! Jouw ontwerp van het SPACe logo heeft vele taartjes, posters, flyers, PowerPointpresentaties, websites en chocolaatjes gesierd.

Ik heb een heleboel lieve vrienden die het leven buiten het onderzoek zo leuk maken. Een paar mensen wil ik in het bijzonder bedanken. Lieve **clubgenoten**, ook al zijn onze levens soms heel anders, ik ben blij met onze onvoorwaardelijke vriendschap. Lieve **Jen** en **Emma**, na ons Nieuw-Zeeland avontuur zoeken we ook in Nederland nog regelmatig het groene avontuur op, een fijne uitlaatklep. Lieve **Lies**, bij jou heb ik aan een half woord genoeg en je hebt me vaak beter door dan ik mezelf; Ik weet dat we elkaar niet uit het oog zullen verliezen. Lieve **Heike**, **Lara** en **Gab**, we kennen elkaar al zo lang, en ik voel me bij bijna niemand zo vertrouwd als bij jullie. Na Thailand kan ik niet wachten op alle avonturen die we nog gaan beleven, ook al is dat in Friesland, Zeeland of Noordwijk.

Mijn paranimfen. Lieve **Lotte** en **Rixt**, waar jullie begonnen als fijne collega's zijn jullie nu vooral goede vriendinnen. Lieve Lot, tijdens mijn onderzoek, maar nu nog steeds, kan ik altijd bij je terecht, of dat nou is voor een kop koffie, Disney film of een goed gesprek. Lieve Rixt, ook al zat je een paar verdiepingen hoger, je voelde nooit ver weg. Ik word eigenlijk altijd vrolijk als ik je zie. Lieve **Laura**, ik ken je vanaf de allereerste dag dat ik geneeskunde ging studeren, en gelukkig ben ik je nooit uit het oog verloren. Ook al kan je er door de coronamaatregelen waarschijnlijk niet fysiek aanwezig zijn, je bent er gevoelsmatig helemaal bij.

Lieve **schoonfamilie**, bedankt voor het warme nest. Jullie zijn echt familie voor me geworden. Tom, een bijzonder woord van dank voor jou, omdat ik nu eindelijk weet hoe ik Würzburg moet uitspreken.

Lieve broer en zus. Lieve **Marit**, ook al zijn we heel anders, ik zie steeds meer hoe erg we op elkaar lijken. Ik ben ontzettend blij met zo'n lieve, trouwe zus als jij. Lieve **Koen**, ik vind het bijzonder hoe we vaak met dezelfde thema's in ons leven bezig zijn, hoewel we zo'n ander leven leiden. Je bent een mooi mens. Lieve **Pieter** en **Anne**, ook jullie horen er voor mij meer dan bij.

Lieve **pap** en **mam**. Ik kan me nog goed herinneren dat ik jullie opzocht na mijn sollicitatiegesprek voor dit promotietraject in de stromende regen op de camping. Jullie wisten m'n gedachten te structureren en me te steunen in mijn keuze, zoals jullie zo vaak hebben gedaan. Jullie hebben me alle kansen gegeven in het leven om te groeien en mezelf te ontwikkelen tot wie ik nu ben. Jullie trots en liefde is hartverwarmend.

Liefste **Ruben**. Alle ups en downs van het promotieonderzoek heb jij van heel dichtbij mee gemaakt. Ondertussen kan je alle geneesmiddelnamen van mijn studie moeiteloos oplepelen. Als ik door de bomen even het bos niet meer zag, kan je relativeren als geen ander en zette je me met beide benen op de grond. Maar meer nog laat je me zweven en zien wat het leven zo mooi maakt: ik ben bij niemand liever dan bij jou.

Sanne

Ik ben meer

Ik ben meer dan alleen mijn naam
ik heb een hoofd waarin verlangen woont
een eigen wil heb ik, twee oren en een
mond.

Maar hoe vertel ik duidelijk: kijk, dit ben ik?
En hoe beluister ik nu wie die ander is.

Ik heb als iedereen een kostbaar hoofd
met dromerijen over hoe en wie en mij
en dat ik word erkend: ja, dat ben jij.

Hester Knibbe
stadsdichter Rotterdam 2015 - 2016

Antipsychotic drugs are effective for a wide range of disorders in children and adolescents. Unfortunately, these drugs are associated with serious side-effects in this population, including weight gain. The aim of this thesis is to improve the safety and effectiveness of antipsychotic drugs in children and adolescents, by individualizing antipsychotic drug dosing and monitoring.