Aus dem Zentralinstitut für Seelische Gesundheit Mannheim
der Medizinischen Fakultät Mannheim
(Direktor: Prof. Dr. med. Andreas Meyer-Lindenberg)

How to find and validate therapeutic reference ranges for psychotropic drugs

Inauguraldissertation
zur Erlangung des Doctor scientiarum humanarum (Dr. sc. hum.)
der
Medizinischen Fakultät Mannheim
der Ruprecht-Karls-Universität
zu
Heidelberg

vorgelegt von
Xenia Marlene Hart

aus
Wiesbaden
2022

Dekan: Prof. Dr. med. Sergij Goerdt
Referent: Prof. Dr. med. Gerhard Gründer

TABLE OF CONTENTS

ABBREVIATIONS

ADR	Adverse drug reaction
ADS	Antidepressant discontinuation syndrome
AGNP	Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie
AIMS	Abnormal Involuntary Movement Scale
AM	Active moiety, sum of concentrations of parent compound and major active metabolite
ARI	Aripiprazole
BD	Bipolar Disorder
BL	Blood level; serum or plasma drug concentration in ng/ml
BPRS	Brief Psychiatric Rating Scale
C/D	Concentration-to-dose ratio (mean concentration / mean dose)
CGI	Clinical Global Impression
CGI-I	Clinical Global Impression- Improvement
CGI-S	Clinical Global Impression - Severity
CL	Total clearance
Cmin	Minimum (trough) steady-state concentration
CS	Cohort study
CSS	Cross-sectional Study
CYP	Cytochrome P450
d	Day
D-ARI	Dehydroaripiprazole
di	Dosing interval
Dm	Daily maintenance dose
Dx	Diagnosis
EPS	Extrapyramidal symptoms
ESC	Escitalopram
F	Bioavailability
FN	False negative
FP	False positive
HAMD	Hamilton rating scale for depression
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th edition
IQR	Interquartile range; 25th to 75th percentile
m	Month
MADRS	Montgomery–Åsberg Depression Rating Scale
MDD	Major depressive disorder
Mod.	Modified
MPR	Metabolite to parent compound ratio
ODV	O-desmethylvenlafaxine
OLZ	Olanzapine
PANSS	Positive and Negative Syndrome Scale
PET	Positron emission tomography
RCT	Randomized controlled clinical trial
ROC	Receiver operating characteristic
SCZ	Schizophrenia

SD	Standard deviation
SERT	Serotonin transporter occupancy
TDM	Therapeutic Drug Monitoring
TN	True negative
TP	True positive
UKU	UKU side effect rating scale
VEN	Venlafaxine
w	Week
WFSBP	World Federation of Societies of Biological Psychiatry
Δt	Time interval between intake of the last dose and blood withdrawal

1 INTRODUCTION

1.1 Background

Many psychotropic medications have been in use for over 30 years. They have been shown effective in placebo controlled clinical trials. Nevertheless, many patients fail to respond or do not tolerate the drugs due to pharmacokinetic or pharmacodynamic peculiarities. Pharmacokinetic abnormalities can be controlled by measuring drug concentrations in blood, i.e. by Therapeutic Drug Monitoring (TDM). A key principle of TDM is the comparison of individual drug concentrations in the blood of a patient to a reference system, the drug-specific therapeutic reference range, thereby optimizing individual dosage regimens. Ranges for 154 neuropsychiatric drugs, along with levels of recommendation for use of TDM in clinical practice, have been reported by the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP), an association of German-speaking neuro- and psychopharmacological researchers and psychiatrists, in their Consensus Guidelines on Therapeutic Drug Monitoring (Hiemke et al., 2018). Clinical experience has led to more or less well established therapeutic reference ranges. As indicated in the guidelines of the World Federation of Societies of Biological Psychiatry (WFSBP) on how to grade treatment evidence for clinical guideline development, a low-quality systematic review would be based on “an unstructured conglomerate of a selective choice of open and controlled original studies, regardless of their quality, previously published systematic reviews and/or meta-analyses, previous guidelines and expert opinions which are not based on empirical studies” (Hasan et al., 2019). This was a major criticism of previously published guidelines reporting therapeutic reference ranges. Methods for the estimation of ranges were reported, but there has not been a clear stratification on how the cited work was analyzed (Hiemke et al., 2018). A clinical validation was missing. As a result, a high variation of ranges reported in the literature evokes the notion of an arbitrary estimation of published ranges. Understandably, this has led to criticism among clinicians, and reported ranges are more or less considered experts’ opinions. The uncertain validity of reference ranges has led to a systematic underestimation of TDM’s clinical value in psychiatry. TDM is primarily used as a tool to identify adherence problems, improve drug safety or for problem solving, not for dose titration.

1.2 Aim and Scope

The main objective of the following doctoral thesis is to provide a stepwise methodology for the determination and validation of therapeutic reference ranges in two exemplary antidepressant and two exemplary antipsychotic substances by combining i) up-to-date and systematic search of available evidence including a quality control of these publications, and the grading of available evidence (Hart et al., 2021; Hasan et al., 2019) with ii) patients’ data from clinical studies and TDM databases. Therapeutic reference ranges for other drug classes lie beyond the scope of this work. Nonetheless, methodological principles may be applied for other drug classes for which TDM has been established.

1.3 Project description and contributions

1.3.1 Determination of a therapeutic reference range for four exemplary substances

Between October 2020 and Mai 2022, four systematic reviews including metaanalyses for the psychotropic substances aripiprazole (ARI), escitalopram (ESC), venlafaxine (VEN), and

olanzapine (OLZ) were performed and supervised by X.M. Hart. X.M. Hart designed the methodology on how to find a therapeutic reference range using literature-based and metaanalytical methodology and exemplary performed the project for the antipsychotic drug aripiprazole. A method protocol was published in the course of this work (Hart et al., 2021). The work was accepted for publication in the Journal “Psychopharmacology” (IF 4.415 (2021)) on September 1, 2022. The additional projects for escitalopram, venlafaxine, and olanzapine were part of three respective dissertation projects to obtain a medical doctorate (L. Eichentopf, X.M. Lense, and K. Wesner). The present work provides a short summary of the results of these projects.

1.3.2 Validation of a therapeutic reference range for four exemplary substances

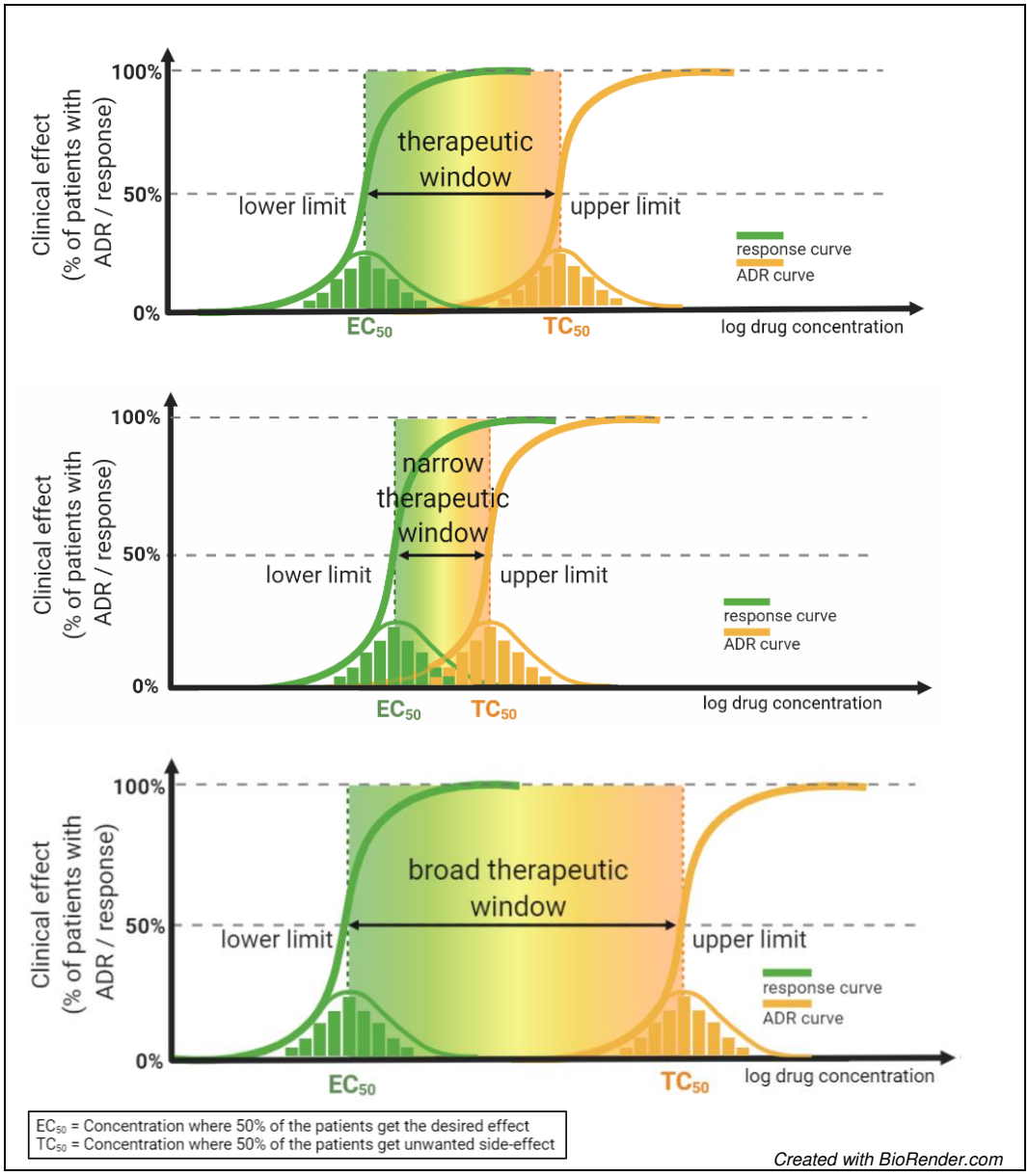
Validation studies were performed for each of the four substances using unpublished as well as previously published datasets. Permissions for the use of this data within this work was obtained beforehand. For the validation of aripiprazole’s oral therapeutic reference range, three previously published and two unpublished datasets were used. The published datasets comprised a prospective clinical trial (Lin, Chen, & Liu, 2011), a prospective cross-sectional TDM study (Kirschbaum et al., 2008) and data from a retrospective TDM database (Jukić, Smith, Molden, & Ingelman-Sundberg, 2021). In addition, unpublished anonymized concentration data was extracted from the routine archive of a TDM laboratory (MVZ Medizinisches Labor Bremen GmbH, Haferwende 12, 28357 Bremen), provided by Dr. Gabriela Zurek. Another dataset comprised retrospective patient TDM data from the Central Institute of Mental Health, which has been collected within the present doctorate project. The respective ethical vote has been appended. For the validation of escitalopram’s therapeutic reference range, one previously published and two unpublished datasets have been used. One data set was provided by Prof. U. Havemann-Reinecke (University of Göttingen) and Prof. C. Hiemke (University of Mainz) as part of a cross-sectional TDM data collection and has not been published before. X.M. Hart performed data analysis and writing of a manuscript that was accepted for publication in the Journal “European Archives of Psychiatry and Clinical Neuroscience” (IF 5.760 (2021)) on September 13, 2022. Patient TDM data concerning escitalopram was collected from the Central Institute of Mental Health by a retrospective evaluation of medical records, analysed and included in the present work. A respective manuscript prepared by X.M. Hart is currently in revision. Last, previously published data was used that derived from a cohort nested in a randomized controlled clinical trial, namely the “EMC trial” (Engelmann et al., 2021; Tadić et al., 2016). This data was also used for the validation of venlafaxine’s therapeutic reference range. For the validation of olanzapine’s therapeutic reference range, two unpublished datasets were available. Anonymized concentration data was extracted from the routine archive of a TDM laboratory (MVZ Medizinisches Labor Bremen GmbH, Haferwende 12, 28357 Bremen), provided by Dr. Gabriela Zurek. In addition, patient data from the Central Institute of Mental Health was collected and evaluated.

1.4 Definition of a therapeutic reference range

The theoretical concept of a therapeutic reference range (sometimes also called “therapeutic window”) of a drug has been described in detail in the literature such as pharmacology textbooks (Hilal-Dandan & Brunton, 2014). In order to illustrate a therapeutic reference range of a drug as the gap between two sigmoidal curves, clinical effects, either the response to the drug or an adverse drug reaction (ADR) (y-axis, linear) are plotted against the drug concentration (x-axis, logarithmic) (Figure 1). The lower limit would then be the drug concentration on a drug concentration/response-curve, in which a certain percentage of patients are responding. For

the upper limit, two possible assumptions exist: (i) The drug concentration on a drug concentration/ADR-curve, in which a certain percentage of patients show an ADR. (ii) The drug concentration on a drug concentration/response-curve, above which the number of responders does not further increase. This definition requires the evaluation of drug`s risk of harm for the purpose of finding an upper limit. Following this concept, the AGNP defined a therapeutic reference range as a drug concentration range between a “lower limit below which a drug-induced therapeutic response is relatively unlikely to occur and an upper limit above which tolerability decreases or above which it is relatively unlikely that therapeutic improvement may be still enhanced” (Hiemke et al., 2018). Therapeutic reference ranges yield pharmacodynamic information on increased likelihoods for the occurrence of desired drug effects (referred to as drug-induced therapeutic response) and ADR’s (Buclin, Gotta, Fuchs, Widmer, & Aronson, 2012). Upper and lower limits thereby refer to daily minimum (trough) blood concentrations of a drug in the steady state. Each reference range is based on a distribution of drug concentrations from a sample of reference patients. Hence, the individual drug concentrations of the majority of patients should be within this range. Nonetheless, some individuals will reach optimal therapeutic response at drug concentrations outside the range. Some will show adverse drug reactions within this range (Patsalos, Spencer, & Berry, 2018). The methodology used to estimate a therapeutic reference range determines the scope, validity and as a result the applicability in clinical practice. The characteristics of the reference population primarily defines the characteristics of the resultant range. Depending on the reference sample, the scope of a therapeutic reference range may be for instance restricted to a specific subpopulation, an indication, route of administration, dosage, age range or drug formulation.

Figure 1. Theoretical concept: Therapeutic reference ranges. The ordinate is linear; the abscissa is logarithmic

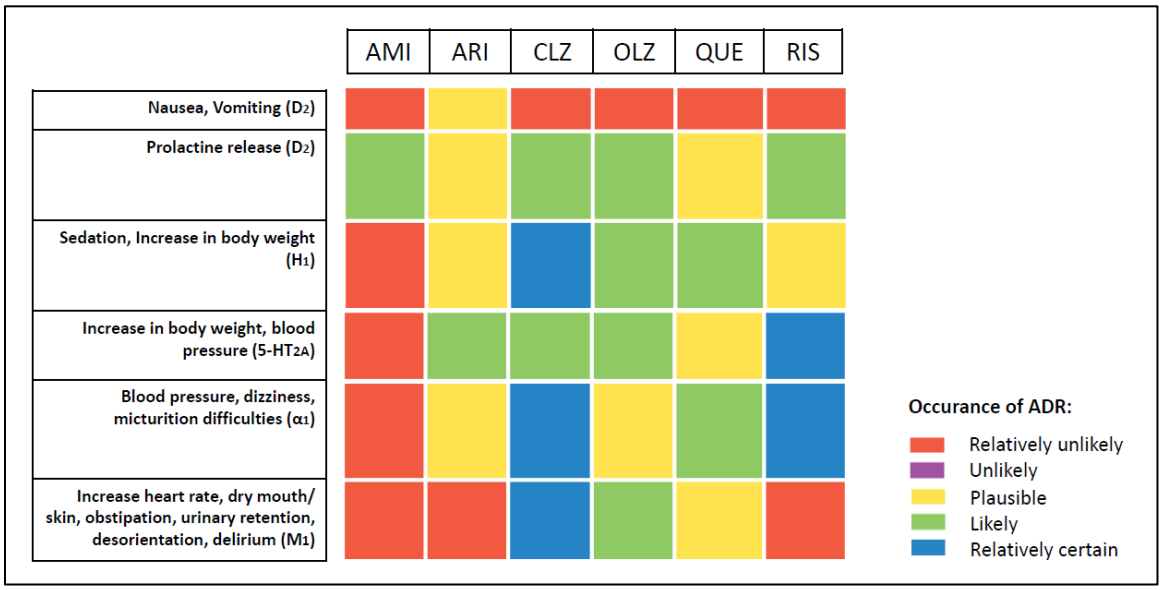


1.5 The concept of the upper limit

As described before, an upper limit of a therapeutic reference range corresponds either to a decreasing tolerability which is generally the onset of an ADR or to maximum therapeutic improvement. Psychotropic drugs can be discerned in drugs with a high risk of harm and drugs with a low risk of harm as based on their safety and tolerability profile. There is no standard definition of what constitutes a low or a high risk of harm for psychotropic drugs. Solmi et al. investigated risks of harm for 18 first- and second-generation antipsychotics in a systematic review by linking the evidence for treatment related ADRs to pharmacokinetic profiles of these drugs (Solmi et al., 2017). In that respect, safety measures such as the therapeutic index and the standard safety margin have been proven useful. Probability maps for the occurrence of ADRs that are based on receptor-binding profiles may help to narrow down clinical relevant

ADRs (Figure 2). For drugs with a low risk of harm, the upper limit will refer to the maximum therapeutic effect. Due to their relatively low toxicity, selective serotonin reuptake inhibitors (SSRIs) can be classified as drugs with low risk of harm. Their reported upper threshold reflects the concentration above which therapeutic improvement does not further increase (Tomita et al., 2014). For drugs with a high risk of harm, the upper limit should refer to the onset of the first moderate or severe dose-, better concentration-related ADR (Müller et al., 2009). Upper thresholds in the latest AGNP Consensus Guidelines for most psychotropic drugs were defined by an increased risk of distinct ADRs (Hiemke et al., 2018), such as for haloperidol (Rao, Bishop, & Coppen, 1980), for paroxetine (Hegerl et al., 1998), for citalopram (Yin et al., 2006) and for tricyclic antidepressants (Dawling, 1982; Gupta, Shah, & Hwang, 1999). With tricyclic antidepressants, anticholinergic effects will appear before (at lower drug concentrations) the onset of the desired effect. Hence, the upper limit will refer either to CNS- or to cardiovascular toxicity. This example shows, why the use of general ADR-scales (such as UKU (Lingjaerde, Ahlfors, Bech, Dencker, & Elgen, 1987)) will not provide a sound upper limit. Estimates of the likelihood of specific ADRs can be provided by positron emission tomography (PET) studies, which investigate a drug's receptor occupancy-profile (Cumming, Abi-Dargham, & Gründer, 2021). To conclude, assumptions about the risks of harm from an investigated drug is most essential for choosing an appropriate outcome and finding meaningful upper thresholds for clinical practice. Upper limits are ideally obtained from established relationships on drug concentrations and response to a drug or the occurrence of a specific ADR. However, very few prospective studies directly address drugs' risk of harm. Data is usually obtained from retrospective observational studies, cohort studies, case-control studies or case series.

Figure 2. Probability map for the occurrence of ADRs estimated at lower therapeutic drug concentrations based on receptor-binding profiles for six antipsychotic drugs, modified from Klein Haen et al. 2018 (Klein H.-G. et al., 2018)



2 MATERIAL AND METHODS

2.1 Lessons from the past

Sound concentration/response-relationships set a minimum requirement for evidence-based TDM. However, in the latest AGNP Consensus Guidelines prospective studies investigating therapeutic reference ranges were found for only 17 of 154 neuropsychiatric drugs (Hiemke et al., 2018). The majority of studies, which attempted to relate clinical response to drug concentrations, were retrospective analyses of TDM databases, which included data from flexible dose studies (Hiemke et al., 2018). Most of them failed to find significant concentration/effect-relationships. For most psychotropic drugs, a clear relationship between drug concentration and drug effect (drug-induced therapeutic response or ADRs) is not well established (Lopez & Kane, 2013). A drug concentration, which is more efficacious than placebo or which indicates maximal efficacy cannot be reported for these drugs. Therefore, therapeutic reference ranges were in the past often assigned to psychotropic drugs based on individual studies with small sample sizes, which compared drug concentrations from approved doses with clinical effects (Hiemke et al., 2018). For the antidepressant drug doxepin, a poor reference range has been used in TDM for many years. This range was not based on an adequate concentration/response-analysis, but rather on individual studies with small sample sizes and case reports. An evaluation of measured doxepin concentrations from a TDM database found only 9% of all samples (N= 217) within the reference range (Leucht et al., 2001), meaning very little clinical value for referring individual drug concentrations. A revised lower limit for the preliminary reference range was proposed after a reevaluation of the available evidence. The following section will highlight frequent pitfalls, which arise when attempting to find a relationship between drug concentration and clinical improvement for a psychotropic drug and will furthermore unravel methodological limitations in clinical studies' designs.

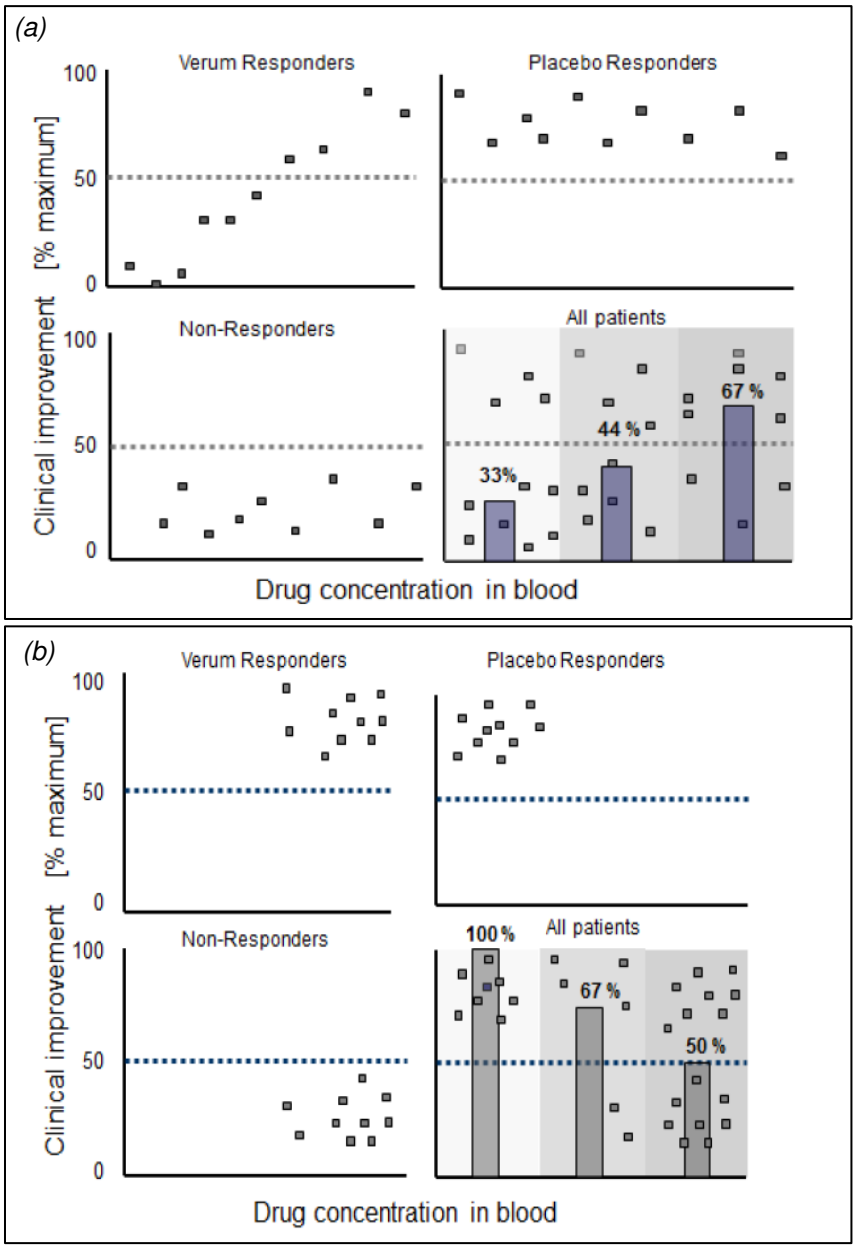
2.1.1 Drug concentration/response-relationships for psychotropic drugs: Explaining high signal-to-noise ratios

As for other drug classes, high pharmacokinetic variation in patients treated with psychotropic drugs produce high noise in dose/response- and in concentration/response-studies. Small sample sizes have been used in the past when aiming at finding a concentration/response-relationship for a psychotropic drug (Eggart, Hiemke, & Zernig, 2011). However, artificial results have been published from these studies. In a study by Zabala and colleagues, olanzapine was titrated up to effective doses in a small sample of 23 patients (Zabala et al., 2017). The authors aimed at finding a concentration threshold that relates to response, which was defined as a minimum of 30% decrease in total score of the positive and negative syndrome scale (PANSS) (Kay, Fiszbein, & Opler, 1987). Due to the high variation in measured drug concentrations from this limited sample (signal-to-noise problem), the estimated threshold concentration was not specific enough to distinguish between responders and non-responders. As in this example, clinical trial designs often allow for flexible dosing in order to optimize drug effects. In theory, flexible dose regimes are generally inappropriate for determination a positive concentration/response-relationship.

When treated with antidepressant (Preskorn, 2014) or antipsychotic drugs (Hiemke, 2019), patients are considered to fall into three groups according to their clinical improvement: verum responders, placebo responders and nonresponders. A correlation of response to increasing drug concentrations can only be expected for verum responders (Hiemke, 2019). Poor drug concentration/response-relationships are obtained from studies, which do not take the concept

of heterogeneous response of psychiatric patients into account. Following the theoretical concept of heterogeneity in patients' responses, the use of flexible dose regimens will produce a high signal-to-noise ratio in a clinical study (Figure 3b, all patients). To obtain the highest response rates, verum responders and nonresponders will be titrated towards higher concentrations. Placebo responders will benefit at low doses, usually related to low drug concentrations. Attempting to correlate clinical effects with drug concentrations will increase the noise and may even result in a negative relationship. These theoretical assumptions have been supported by findings from metaanalyses for antipsychotic (Woods, Gueorguieva, Baker, & Makuch, 2005) and antidepressant drugs (Funk et al., 2022; Khan, Khan, Walens, Kolts, & Giller, 2003). A large metaanalysis including RCTs that applied antidepressant drugs found an inverse relationship between concentration and efficacy from flexible dose studies while reporting a trend towards the expected relationship in those studies using fixed dosing strategies (Funk et al., 2022).

Figure 3: Theoretical concentration-clinical improvement relationship in psychiatric patients in fixed-dose (a) and flexible-dose (b) studies (Hiemke, 2019)



2.1.2 U-shaped concentration/effect-relationships: fact or artefact?

U-shaped or even inverse u-shaped relationships between drug concentration and clinical effects have been published in the past for antipsychotic and antidepressant drugs (Asberg, Crönholm, Sjöqvist, & Tuck, 1971; Cellini et al., 2022; Florio, Porcelli, Saria, Serretti, & Conca, 2017; Santos et al., 1989). This type of relationship implies decreasing clinical efficacy with increasing drug concentrations. It may, however, be the result of nonresponders in the study population titrated to high doses/ concentrations. Pharmacologically reasonable concentration/response models that are based on pharmacodynamic receptor occupancy assumptions (Gründer, Hiemke, Paulzen, Veselinovic, & Vernaleken, 2011) comprise ascending and descending logistic equations (Eggart et al., 2011; Ulrich & Lauter, 2002; Zernig & Hiemke, 2020). However, some psychotropic medications seem to have a descending concentration/response-relationship above a certain point, most likely because of the adverse drug effects at these high concentrations (e.g., sedation and extrapyramidal side-effects (EPS)). In this term, bisigmoidal equations comprising two logistic equations, an ascending one at the lower concentration range and a descending one at higher concentrations, can be appropriate (Palao et al., 1994; Ulrich & Lauter, 2002; Ulrich, Wurthmann, Brosz, & Meyer, 1998).

Equation 1: bisigmoidal concentration effect relationship
$$\% \text{ change in score} = \left(m_1 / (1 + e^{a1-b1 * c}) \right) - \left(m_2 / (1 + e^{a2-b2 * c}) \right)$$

A reevaluation of published data may help to unravel artificial findings (Eggart et al., 2011). After three months of flexible dosing with escitalopram, Florio and colleagues reported a positive quadratic concentration/effect-curve for the antidepressant drug (r = 0.56, Figure 4a) (Florio et al., 2017). This curve indicates a decrease in clinical effects above an escitalopram blood concentration of about 100 ng/ml with only one data point marking the descending part of the curve. Using an exponential curve-fitting model with an asymptotic part approximately starting about 60 ng/ml would be more appropriate for this data (r = 0.57, Figure 4b). Care should be taken if just a few or one drug concentration describes the descending part of the concentration/efficacy curve (De Donatis et al., 2019; Florio et al., 2017). The main benefit of this model is a corresponding semi-logistic equation that allows for the computation of an EC_{50} values, referring to 50% of total clinical efficacy, which here is a concentration of 20 ng/ml. Interestingly, this value does not only correspond to the threshold gathered from the receiver operating characteristic (ROC) analysis of this data after dichotomization by 50% HAMD score reduction from baseline (*Figure 6*). It is also in line with PET data indicating 80% serotonin transporter occupancy above approximately 17 ng/ml (Eichentopf et al., 2022). As shown in a metaanalysis of the antipsychotic drug aripiprazole, in order to maximize treatment effects, studies with fixed dosing often use higher dosages resulting in higher mean concentrations when compared to flexible dose studies (Hart et al., 2022). However, prerequisite in order to find a concentration efficacy relation is the inclusion of a concentration range with concentrations below the efficacy threshold (Funk et al., 2022; Zernig & Hiemke, 2020). In studies with high fixed doses, most patients will experience drug concentrations above the therapeutic threshold and in the asymptotic part of the concentration response curve. A relationship can then not be modelled adequately (see *Figure 5).*

Figure 4. Remodeled data reporting inverse u-shaped concentration/effect relationship for escitalopram (Florio et al., 2017). (a) published relationship using a quadratic function (r= 0.56), (b) remodeled relationship using an exponential function (r= 0.57)

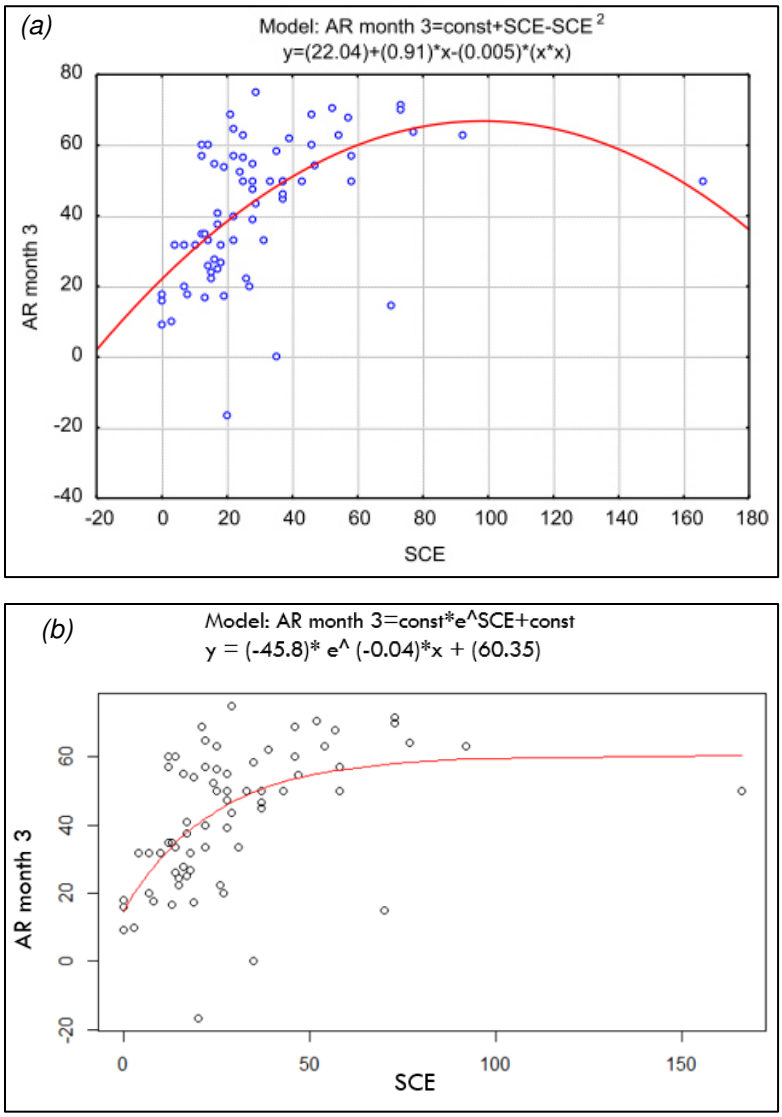


Figure 5. Aripiprazole concentrations in relation to PANSS score reduction. No good fit for a hyperbolic or linear model possible, almost all concentrations lie within current reference range (100-350 ng/ml) (Lin et al., 2011)

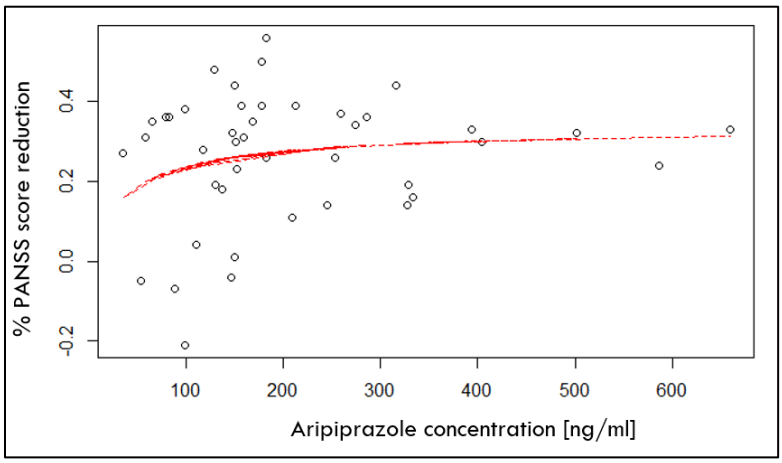
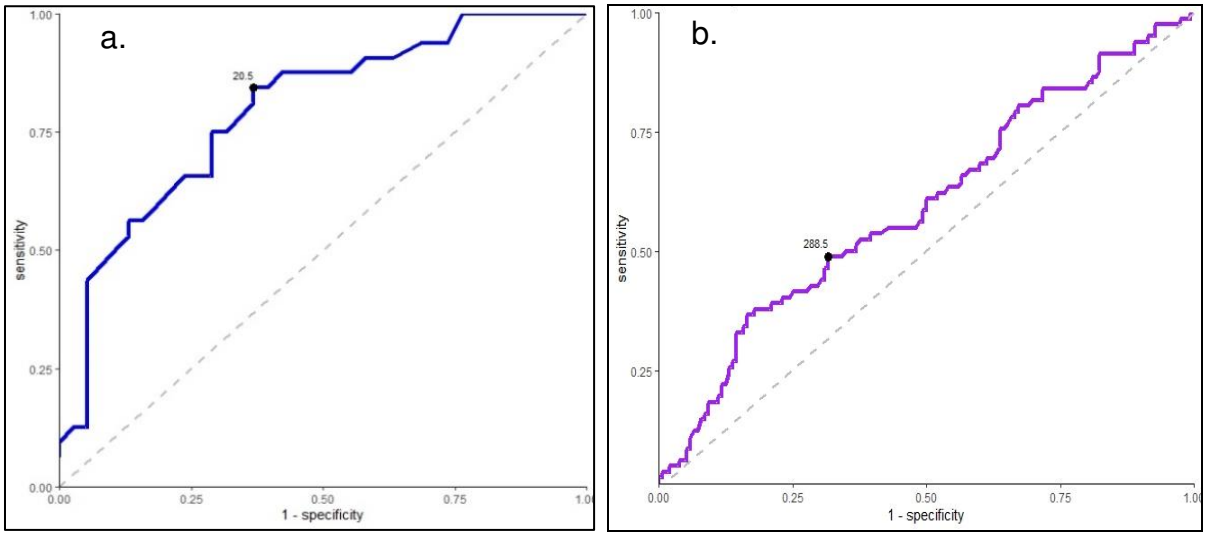


Figure 6. ROC analysis for escitalopram (a) and venlafaxine (b) after dichotomization into responders (≥ 50% HAMD score reduction) and nonresponders (< 50% HAMD score reduction) (Engelmann et al., 2021; Florio et al., 2017)



Examples of studies reporting positive and studies reporting presumably artificially negative concentration effect relations for psychotropic drugs have been summarized in Tables 1 and 2. A positive correlation between antidepressant response and drug concentration was for example shown in a fixed-dose study for duloxetine (De Donatis et al., 2019) or for nortryptiline (Asberg et al., 1971). Similar results have been published for the antipsychotic agents haloperidol (Palao et al., 1994) and for olanzapine (Perry, Sanger, & Beasley, 1997). In these designs, patients were often dichotomized according to their individual clinical improvement into responders and nonresponders. Semi-structured interviews, but also simple global scales (e.g., the Clinical Global Impression, CGI, scale (Guy, 1976)), have been used to discriminate responders and nonresponders. Perry and colleagues for example defined a drug-induced response by 20% reduction in Brief Psychiatric Rating Scale (BPRS) scores and a Clinical Global Impression (CGI) Severity scale score of < or = 3 (Perry, Lund, Sanger, & Beasley, 2001). An upper limit is then the drug concentration, which is able to distinguish between both groups (ROC-analysis). Considerably few studies, however, initially assessed placebo response by e.g. using a placebo lead-in phase as proposed (Asberg et al., 1971; Zernig & Hiemke, 2020). However, nonresponders also remain in the study population. This work will therefore provide further guidance on how to carefully interpret potential therapeutic thresholds from available studies in view of additional evidence e.g. from pharmacokinetic and neuroimaging studies in psychiatry.

Table 1. Exemplary studies reporting a positive or negative concentration/effect-relationship in terms of design for antidepressant drugs

Substance (Author, Year)	Design	Clinical efficacy measure	Dose de-sign	BLs below range	Concentration/ effect-relationship	Implication for therapeutic reference range
Amitriptylin (Ulrich & Lauter, 2002)	Metaana-lysis, N = 339	HAMD % improve-ment	Fi-xed/ fle-xible	Y	Positive continuous (bisigmoidal)	*Optimal range be-tween 80-200 ng/ml using data from 13 studies*
Duloxetine (De Donatis et al., 2019)	CS, N = 66	HAMD-21 % improve-ment	Fixed	Y	Positive continuous (u-shaped)	*Confirms range*
Escitalopram (Hodgson et al., 2014)	RCT, N = 266	MADRS % improve-ment	Fle-xible	PN	*Negative continuous (linear)*	*Levels below 10 ng/ml excluded*
Escitalopram (Florio et al., 2017)	CS, N = 70	HAMD-21 % improve-ment	Fle-xible	Y	*Positive continuous* (u-shaped)	*ROC predicts 21 ng/ml (Eichentopf et al., 2022)*
Nortriptyline (Asberg et al., 1971)	CS, N = 29	Depression rating score mod. from Cronholm/ Ottoson	Fixed	Y	*Positive continuous* (u-shaped)	*Placebo lead-in phase, improvement within range of 50-139 ng/ml*
Paroxetin (Eggart et al., 2011; Tasker, Kaye, Zussman, & Link, 1989)	Cohort from RCTs, N = 94	CGI	Fixed	Y	*Positive continuous* (curvilinear)	*Confirms SERT oc-cupancy curve with lower threshold of 20-30 ng/ml corre-sponding to 80% SERT occupancy*
Venlafaxine (Schoretsanitis et al., 2019)	CSS, N = 858	CGI-S	Fle-xible	Y	*Negative dichotomized*	
Venlafaxine (Berm, Kok, Hak, & Wilffert, 2016)	RCT, N = 40	MADRS, HAMD	Fle-xible	Y	*Negative dichotomized*	
Venlafaxine (De Donatis et al., 2021)	CS, N = 52	HAMD-21 % improve-ment	Fle-xible	PY	*Positive continuous* (u-shaped)	*Confirms current range of 100–400 ng/mL*
Venlafaxine (Scherf-Clavel et al., 2020)	CS, N = 23	HAMD-21 % improve-ment	Fle-xible	N	*Positive continuous (linear)*	*ROC predicts remis-sion (HAMD ≤ 7) above 393 ng/ml*
Venlafaxine (Charlier, Pinto, Ansseau, & Plomteux, 2002)	CS, N = 22	MADRS total score	Fle-xible	Y	*Positive continuous (linear)*	*Suggested range: 125 – 400 ng/ml*
Venlafaxine (Hoencamp, Haffmans, Dijken, & Huijbrechts, 2000)	CS, N = 37	HAMD-17, MADRS	Fixed	PY	*Positive continuous (linear)*	*VEN only, not for ODV at week 7*
Multiple Sub-stances (Cellini et al., 2022)	Combined analysis, N = 206	HAMD-21 % improve-ment	Fixed & fle-xible	Y	*Positive continuous* (u-shaped)	

Positive dichotomized = Responders had higher concentrations than non-responders; Negative dichotomized = Nonresponders had higher concentrations than responders; Positive continuous = Clinical response scale initially increased with higher concentrations; Negative continuous = Clinical response scale initially decreased with higher concentrations; Y = Yes; N = No; PY = Probably yes; PN = Probably no

Table 2. Exemplary studies reporting a positive or negative concentration effect relationship in terms of design for antipsychotic drugs

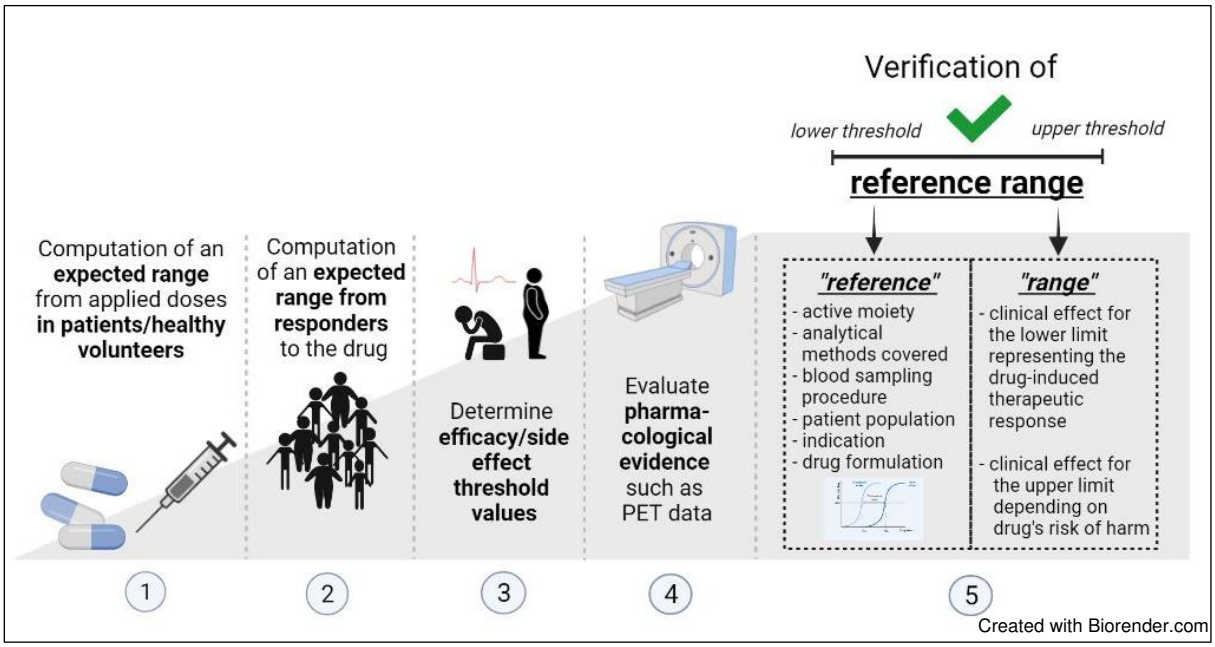
Substance (Author, Year)	Design	Clinical efficacy measure	Dose de-sign	BLs below range	Concentration/ effect-relationship	Implication for therapeutic reference range
Aripiprazole (Lin et al., 2011)	CS, N = 45	PANSS % improve-ment	Fle-xible	N	*Positive dichoto-mized* (20%)	*Only for sum, not for ARI, unexpected high threshold by ROC*
Haloperidol (Santos et al., 1989)	CS, N = 30	BPRS % improve-ment	Fixed	Y	*Negative continuous (u-shaped)*	
Haloperidol (Palao et al., 1994)	RCT, N = 22	BPRS % improve-ment	Fixed	N	*Positive continuous (sigmoidal)*	*Optimal range 1-10 ng/ml*
Haloperidol (Ulrich et al., 1998)	Metaana-lysis, N = 552	SCZ rating scores, mainly % BPRS	Fixed & fle-xible	Y	*Positive continuous (bisigmoidal)*	*Optimal range 1-10 ng/ml*
Olanzapine (Perry et al., 1997 and Perry et al., 2001)	CS, N = 79	BPRS % improve-ment	Fixed	Y	*Positive continuous (curvilinear)*	*ROC threshold of 9 ng/ml (12h di) and 23 ng/ml (24h di)*
Olanzapine (Laika et al., 2010)	CS, N = 124	CGI-I	Fle-xible	Y	*Positive continuous (linear)*	*co-medication al-lowed*
Olanzapine (Lu et al., 2016)	CSS, N = 151	PANSS total score	Fle-xible	Y	*Positive continuous (linear)*	*ROC threshold of 22.8 ng/ml*
Olanzapine (Mauri et al., 2005)	CS, N = 54	BPRS and PANSS % improve-ment	Fle-xible	Y	*Positive continuous (curvilinear)*	*IQR in responders is 19-37 ng/ml*
Olanzapine (Zabala et al., 2017)	CS, N = 23	PANSS	Fle-xible	N	*Negative continuous (curvilinear)*	*Nonresponders higher BL*
Quetiapine (Mauri, Volonteri, Fiorentini, Pirola, & Bareggi, 2007)	CS, N = 18	PANSS	Fle-xible	NI	*Positive continuous (linear)*	*Normalized BLs (doses/kg)*
Risperidone (Yasui-Furukori et al., 2010)	CS, N = 51	BPRS total score	Fixed	Y	*Positive continuous (linear)*	*Correlation only for BPRS total score, not for % improve-ment*

Positive dichotomized = Responders had higher concentrations than non-responders; Negative dichotomized = Nonresponders had higher concentrations than responders; Positive continuous = Clinical response scale initially increased with higher concentrations; Negative continuous = Clinical response scale initially decreased with higher concentrations; Y = Yes; N = No; NI = no information

2.2 Five-step approach on how to find a therapeutic reference range

As stated in the introduction, for most psychotropic drugs, evidence with a low risk of bias is scarce, resulting in a level of evidence as low (“C”) or even absent (“D”). The present work demonstrates how to overcome obstacles from unsatisfactory study designs and introduces a five-step approach on how to find and validate a sound therapeutic reference range (Figure 7) using examples from antipsychotic and antidepressant drugs. A protocol for a state-of-the-art systematic literature search including a grading of available evidence has been published in the course of this work (Hart et al., 2021).

Figure 7: Overview of the methodology to determine a therapeutic reference range for a psychotropic drug



2.2.1 Computation of expected drug concentrations in somatically healthy populations

To obtain pharmacokinetically meaningful concentration ranges based on prescribed doses, theoretically expected concentration ranges in a patient population or healthy controls should be computed in a first step. For the calculation of this range, data from a reference sample of patients, preferentially without concomitant medication or pharmacogenetic abnormalities, should be used. This data is often obtained from pharmacokinetic studies, in which blood samples of a large cohort of patients were taken after administration of approved drug doses. The minimum (trough) steady-state concentration (C_{min}) of a drug expected in a patient can be calculated when the daily maintenance dose (D_m), the dosing interval (d_i), the total clearance (CL), the bioavailability (F), the half-life ($t_{1/2}$) and time interval between intake of the last dose and blood withdrawal (Δt) are known (Equation 1 and 2). The daily maintenance dose may vary depending on factors such as the clinical indication, population and drug formulation. An example calculation for olanzapine is given in the data supplement.

Equation 2: Computation of pharmacokinetic-based minimum steady-state concentration (Cmin) at approved doses

$$C\min = \left[\left(\frac{Dm}{di}\right) \times \left(\frac{F}{CL}\right)\right] \times \left[\frac{(ke \times di)}{(1 - e^{-ke \times di})}\right] \times (e^{-ke \times \Delta t})$$

Equation 3: Computation of elimination rate (k_e)

$$ke = \ln 2 \,/ t_{1/2}$$

2.2.2 Computation of expected drug concentrations in real-world populations

The pharmacokinetically expected concentrations should then be compared to concentrations from patients under clinically effective doses (e.g. under flexible dose regimens). In this term, pharmacokinetic modelling techniques have been introduced in the last years that allow for an evaluation of pharmacokinetically influencing factors on drug concentrations (Korell, Green, Rae, Remmerie, & Vermeulen, 2018). As a result, for aripiprazole, the 80% fluctuation range for approved dosages (10-30 mg once daily), herein suggested as preliminary reference range, lies between 53-186 and 159-557 ng/ml. This range rather represents the expression a high intraindividual fluctuation of this drug than a useful model to guide therapeutic decisions. For this reason, an alternative concept has been introduced. Interquartile concentration ranges from large patient populations for whom TDM was requested in a clinical setting have been proven useful, e.g. pooled across multiple studies (Hart et al., 2022; Hiemke, 2019).

Retrospective data mining of TDM-databases

Retrospective or prospective collections of data from routine TDM (TDM-databases) can be a rich source of generating pharmacokinetically expected concentrations, especially when also comprising pharmacodynamic information such as CGI-scores. Comprehensive data from a naturalistic setting reflect the ideal reference population and cover variables such as comorbidity, co-medication, subpopulations and specific indications. Bias may occur using TDM routine data, because certain clinical circumstances (flexible dosing, ADRs, suspected non-adherence or inconsistent analytical methods) contribute to inconsistent data that may not be evaluated in retrospect. Including subpopulations such as geriatric patients or treatment-resistant patients within the reference population may cause bias in the resultant range. On the other hand, when dosing an individual patient from a subpopulation to a concentration range, which derived from an overall patient population, the result may not be reliable for this patient. If the observed drug concentrations differ in sub-populations, partitioning, which means a division of the reference population into subgroups, may be necessary to take into account influencing factors such as sex, age or ethnic group.

2.2.3 Computation of therapeutically effective ranges using efficacy data

Ranges of blood concentrations from only responders to a drug have been published for many psychotropic drugs (Hart et al., 2022; Hiemke, 2019; Kirschbaum et al., 2008; Müller, Regenbogen, Härtter, Eich, & Hiemke, 2007) and usually derive from systematic reviews or TDM-databases. Response must be defined by a change in an objective symptom rating scale (e.g. CGI, BPRS, HAMD) or retrospectively from medical records (Hart et al. 2022). There is no consistent method for calculating these ranges. The use of mean ± one or two standard deviations (SD) or interquartile ranges (IQR; 25th to 75th percentile) have been proposed

(Bengtsson, 2004). Parametric computation methods such as mean ± SD ranges as introduced by the AGNP Consensus Guidelines (Hiemke et al., 2018) presume a Gaussian distribution of drug concentrations and should not be used in case the concentration data is skewed (non-Gaussian). In addition, better correspondence of interquartile ranges to current reference range recommendations than mean ± one SD has been shown in a work comparing both ranges for six antipsychotics and seven antidepressants (Hiemke, 2019). As a result, IQRs of drug concentrations in blood of responders represent effective working ranges for psychotropic drugs. However, since these ranges by nature include data from placebo responders as well, they should be regarded as preliminary. An alternative methodology for collecting population-based therapeutic effective ranges is based on pharmacokinetic/pharmacodynamic modeling of the range (Derendorf & Meibohm, 1999). These techniques provide the unique advantage of the identification of potential moderating factors on therapeutic response or adverse effects, such as genetic polymorphisms (Ahmed et al., 2019), age, gender and pathophysiological conditions. However, in psychiatry, these are barely available.

2.2.4 Computation of concentration thresholds

ROC-analysis

In the past, ROC-analyses have frequently been used to identify a cut-off value in a drug`s blood concentration that predicts response (decrease in a clinical surrogate) or the occurrence of a specific ADR in a psychotropic drug (Fellows et al., 2003; Härtter, Wetzel, Hammes, Torkzadeh, & Hiemke, 1998; Kagawa et al., 2014; Lin et al., 2011; Müller et al., 2007; Perry et al., 2001; Perry, Zeilmann, & Arndt, 1994; Ulrich et al., 2003; Waldschmitt, Vogel, Pfuhlmann, & Hiemke, 2009). Hereby, response must be defined by a change in an objective symptom rating scale (e.g. CGI, BPRS, HAMD or retrospectively from medical records) (Huguet, Castiñeiras, & Fuentes-Arderiu, 1993). The ROC-curve predicts the ability to distinguish between two groups, i.e. responders and nonresponders at various threshold concentrations. The higher the area under the curve (AUC) of the ROC probability curve, the better the model is capable of identifying a response or an ADR. Estimated thresholds reflect a concentration that provides optimal sensitivity (*true positive rate*) with highest concurrent specificity (*true negative rate*). Preferably, data is gathered from fixed-dose studies, comprising therapeutic, subtherapeutic and supratherapeutic drug levels. A noteworthy example estimation of an upper limit by ROC-analysis is published for paroxetine (Yasui-Furukori et al., 2011). In a prospective, fixed dose study, patients without moderate to severe side effects at paroxetine doses of 20 mg/day were treated with fixed-doses of 40 mg/day. After six weeks of treatment, drug concentrations in blood were compared with drug-induced therapeutic response. A cut-off concentration of 64 ng/ml paroxetine was determined from the ROC-analysis. Similar ROC-analyses to determine a response concentration threshold have been conducted e.g. for fluvoxamine (Härtter et al., 1998), tricyclic antidepressant drugs (Perry et al., 1994), duloxetine (Waldschmitt et al., 2009), lamotrigine augmentation therapy (Kagawa et al., 2014) and clozapine (Ulrich et al., 2003). An efficacy threshold derived from a ROC-analysis marks an expectancy limit of a certain therapeutic effect relating to a certain % of clinical improvement after a certain time of continuous drug treatment. For the lower limit, we are interested in patients that respond above an efficacy threshold (true positives; TP). True negatives are patients that have drug levels below the threshold and do not respond. Sensitivity characterizes the amount of patients with levels above a threshold that responded (TP)/(the amount of patients with a level above threshold that responded (TP) + the amount of patients with a level below threshold that

responded (FN)). Specificity characterizes the amount of patients with a level below a threshold that not responded (TN)/(the amount of patients with a level below threshold that not responded (TN) + the amount of patients with a level above threshold who not responded (FP)). Optimal lower limits for a therapeutic reference range are characterized by a high sensitivity and a high specificity; expressed by the sum-score of both.

Equation 4: Sensitivity of a lower limit

Sensitivity = true positive/(true positive + false negative)

Equation 5: Specificity of a lower limit

Specificity = true negative/(true negative + false positive)

For the upper limit, we are interested in characterizing patients that do not respond above a certain threshold or alternatively show a certain side effect (TP). Patients that have a level below this threshold and responded/without side effect are classified as true negatives (TN). Patients with a concentration above the threshold that responded/without side effect (formerly marked as TP) are in this scenario false positives (FP). These patients show concentrations above the nonresponse/side effect threshold but do respond to the treatment/ do not show the side effect. Likely, patients with a concentration below the threshold that not responded/ with side effects (formerly marked as TN) are in this scenario false negatives (FN). These patients show concentrations below the nonresponse/ side effect threshold but are nonresponders to the treatment/ have the certain side effect. In the further analyses, sensitivity characterizes the amount of patients with a level above a threshold that not responded (TP; former FP)/amount of patients with a level above a threshold that not responded (TP; former FP) + amount of patients with a level below a threshold that not responded (FN; former TN). For the upper limit, a “normal” ROC analysis can be performed and the sensitivity can be computed by 1- specificity. Specificity characterizes the amount of patients with a level below a certain threshold that responded (TN; former FN)/(the amount of patients with a level below a threshold that responded (TN; former FN) + amount of patients with a level above a threshold who responded (FP; former TP)). The specificity can be computed from a normal ROC-analysis by 1- sensitivity. Optimal upper limits for a therapeutic reference range are characterized by a high value for 1- specificity and a high value for 1- sensitivity; expressed by the sum-score of both.

Equation 6: Sensitivity of an upper limit

Sensitivity = 1-specificity lower limit

Equation 7: Specificity of an upper limit

Specificity = 1-sensitivity lower limit

Concentration/efficacy-curves

Despite several potential caveats when approaching concentration/efficacy-curves (see section 2.1), they are of essential value for the determination of a therapeutic reference range. A practical approach on how to compute a threshold from these curves has however barely described in the literature. 50% effective concentration (EC_{50}) values have been introduced as clinical efficacy markers indicating 50% of therapeutic response in an average patient. Ulrich

and Lauter have suggested a concentration threshold of 60% improvement in HAMD total score for the lower limit of amitriptyline's reference range (Ulrich & Lauter, 2002). Data from 13 studies were pooled in order to approximate a bisigmoidal concentration efficacy model. Pooling of data from individual studies is highly recommended since the definition of the limits is very sensitive to few data points above and below the suspected range (Ulrich & Lauter, 2002). Furthermore, study population specific parameters such as geriatric age and concurrent medication may shift the limits of the therapeutic reference range. A formal test of homogeneity between studies is a crucial requirement.

2.2.5 Evaluation of pharmacological evidence such as PET data

PET studies can strongly support the definition of a therapeutic reference range (Gründer et al., 2011; Hart, Schmitz, & Gründer, 2022). For antipsychotics, a characterization of receptor occupancy by a drug combined with drug concentration measurements allows for a calculation of EC_{60} and EC_{80} values, the drug concentration predicted to provide 60% and 80% of the maximum attainable receptor occupancy. 60-80% receptor occupancy has been related to optimal efficacy for D_2 antagonistic antipsychotic drugs (Gründer et al., 2011). Above 80%, the risk for EPS increases significantly. For partial agonists at dopamine D_2 receptors, presumably a minimum target engagement of 90% is required for antipsychotic drug action (Hart et al., 2022). PET studies on occupancy of the primary molecular target by the respective drug may also help to estimate the significance of contradictory studies. Especially for antipsychotics, but also for some antidepressants, PET studies have provided essential information on the relationship between plasma concentrations of psychotropic drugs on one hand and clinical effects and side effects on the other hand. For the well-studied paroxetine, PET studies detected systematic mistakes in conducted metaanalysis as the reason for contradictory data. Serum concentrations used for metaanalyses lay within the asymptotic part of the curve and thus suggested that there is no linear concentration/effect relationship for SSRIs (Adli, Baethge, Heinz, Langlitz, & Bauer, 2005; Rasmussen & Brosen, 2000). A re-analysis of these data found a clear-cut correlation, which was almost identical with the in vivo occupancy of serotonin transporters (Eggart et al., 2011). Pharmacological evidence also comprises pharmacokinetic assumptions e.g. gained from TDM data under naturalistic settings, which also include information on subpopulations.

2.3 Validation of a therapeutic reference range

2.3.1 In a prospective clinical trial

At best, a therapeutic reference range that is systematically derived from available evidence is then verified by a prospective randomized-controlled trial using objective symptom rating scales (e.g. PANSS, BPRS, MADRS) as efficacy measures. An adequate sample size should be included in such a study and dosing should allow for sub-/ or supratherapeutic and therapeutic drug levels. A study may for example compare outcomes, usually continuous outcome measures, between two or more concentration ranges, below, or above a certain threshold for the same drug (Cooney et al., 2017). Van der Zwaag developed such a confirmatory concentration-based study design for clozapine (VanderZwaag et al., 1996). In this study, patients were randomly assigned to a 12-week double-blind treatment at one of three serum concentration ranges. Individual doses were adjusted weekly to the midpoint of their assigned drug concentration range. To detect the clozapine concentration in blood for maximum therapeutic improvement, therapeutic response was measured as the change in a symptom severity scale from baseline, along with tolerability measures. This study design requires prior investigation

of appropriate ranges or threshold values for comparisons. If evidence for a likely position of a range is lacking, studies risk comparing inadequate concentration ranges, thereby generating artificial results (Volavka, Cooper, Czobor, & Meisner, 1996). Another example is given by Ostad Haji et al. (2011) for citalopram. Based on findings from PET studies, Ostad Haji et al. for example investigated citalopram concentrations in blood below and above a threshold of 50 ng/ml in 55 patients. After seven days of treatment, citalopram concentrations above 50 ng/ml were associated with a more favorable treatment outcome than concentrations below this threshold (Ostad Haji et al., 2011). For the four exemplary substances investigated in this work such studies were not available.

2.3.2 Using data from previous trials and TDM databases

Instead of using data from one individual study, pooling of raw data from multiple studies conducted under comparable conditions has been proven useful in different research contexts (Mathew & Nordström, 1999; Sung et al., 2014). Various methods have been proposed that use combined individual concentration efficacy data from previous trials in order to find and validate a certain reference range (Ulrich & Lauter, 2002; Ulrich et al., 1998). Methods include (i) comparison of efficacy scores in patients within and outside the therapeutic reference range (ii) estimation of effect sizes for treatment within and outside the therapeutic reference range (log odds ratios) (iii) sensitivity/specificity analyses of specific thresholds. For amitriptyline, Ulrich and Lauter (Ulrich & Lauter, 2002) showed that all methods mentioned before provided comparable results. Of note, these assumptions have only been shown in efficacy data that are based on psychiatric rating scales such as the HAMD, PANSS or the CGI rating scale. Data from previously published studies further used in the present work has been either provided by the authors of respective publications or data was extracted from original manuscripts using a web-based software (WebPlotDigitizer, https://automeris.io/WebPlotDigitizer, last access 29.08.2022).

TDM database containing patient data from the CIMH: Assessment of treatment failure

Additionally, patient data was retrospectively obtained from routine therapeutic drug monitoring data at the Central Institute of Mental Health in Mannheim in patients treated between Jan 21 2014 and Dec 18 2018. Patients treated with an oral dose of escitalopram, aripiprazole, or olanzapine for a psychiatric indication were included. Patients were excluded if concentration was not at steady state, treatment compliance was not achieved, medical records were not available, depot medication was applied (for aripiprazole and olanzapine) or death occurred during ongoing treatment. Data from medication records such as patient's demographics and medication profile at date of discharge were collected from patients for whom TDM was requested to guide drug therapy. Steady state conditions were confirmed from medical records. Only one level per patient was selected, the last sample for which the daily dose was given. The use of anonymised patients' data for the purpose of this study was approved by the ethics committee of the university medical center Mannheim. Written informed consent was not required for this study. Treatment failure was estimated by the switch of the respective drug to another antidepressant/antipsychotic or by discontinuation of this drug at date of discharge. We hypothesized that a switch or the onset to/of another drug within the same residence most likely represents a treatment failure within the current episode of depression/exacerbation in schizophrenia. Treatment responders were defined as patients being discharged with the respective drug. Secondly, information on adverse effects were extracted from medical records. Medication effects were investigated (i) in a sample of patients with depressive/psychotic disorder, and (ii) in the total sample. The analytical assays were validated and certified for routine

TDM (Limbach, 2022). Calibration curves were linear (r^2 >0·99) in validated ranges: 0-800 ng/ml (escitalopram), 5-1000 (aripiprazole), 2-100 ng/ml (olanzapine). Imprecision and inaccuracy parameters of the assays were lower than 11%. For escitalopram, results reported as < 10 ng/ml (N= 27) were set to 5 ng/ml. For descriptive analyses, mean values and standard deviations (SD) were calculated. For primary analyses, correlation analyses were applied to test for an association between treatment failures, serum concentration and dose. Pearson correlation was used for data that were normally distributed as measured by the Shapiro–Wilks test for normality. Spearmen correlation was used for data that were not normally distributed. For all analyses $p \leq 0.05$ was defined as statistically significant. A Kruskal–Wallis test was applied to compare concentrations in different patient groups (patient with/without treatment failures; patients with/ without antidepressant/ antipsychotic comedication). Receiver operating characteristic (ROC) analysis was used to define a threshold in concentration in order to predict therapeutic failure. For the analysis of therapeutic thresholds, only patients were included whose dose remained stable from time of measurement to discharge. All statistical analyses were performed with IBM SPSS Statistics for Windows, version 26.0 (IBM, Armonk, N.Y.).

Concentration data from a routine TDM laboratory

We retrospectively obtained concentration data from a routine TDM laboratory (MVZ Medizinisches Labor Bremen GmbH, Haferwende 12, 28357 Bremen) without regard to dosing, sampling conditions such as steady state, or trough sampling. Multiple concentrations from one patient could be included as data was provided anonymized. Concentration data was used to fit distributions that deviate from Gaussian distribution. Allocation of individual data was compared between previously published (“old”) and hereby, according to our methodology, proposed (“new”) reference ranges. Distributions that were fitted for comparison included “Normal”, “Lognormal”, “Exponential”, “Weibull”, “Gamma”, “Logistic”, and “Loglogistic”. Data was analyzed by R version 4.0.3 (2020-10-10) and Minitab Statistical Software.

3 RESULTS

3.1 Therapeutic Reference Range for the Antipsychotic Drug Aripiprazole

A systematic literature search and grading of available studies that describe relationships between concentration and clinical or side effects has been lately conducted for the antipsychotic drug aripiprazole (Hart et al., 2022). Prescribing information recommends a once daily dose regimen of 10 - 30 mg aripiprazole for the treatment of schizophrenia. Evidence for a concentration/efficacy-relationship is scarce (Level C1; low). Only one prospective study without relevant psychiatric add-on therapy describes in part a positive relationship with a continuous scale (20% PANSS score reduction; only for the active moiety (sum of aripiprazole and dehydroaripiprazole), not for aripiprazole alone) (Lin et al., 2011). No study established a concentration/efficacy curve nor described a meaningful relationship with side effects (Level D; absent). The dose/concentration relationship has been shown to be linear for aripiprazole ($r^2 = 0.72$, $p < .0001$) and the active moiety ($r^2 = 0.62$, $p = .007$).

3.1.1 Computation of an expected range from approved doses

The expected concentrations of aripiprazole and the active moiety in healthy volunteers after the administration of 10 - 30 mg daily are *117 - 352 ng/ml* and *165 - 494 ng/ml*, respectively (Hiemke et al., 2018). In patients, these ranges must be adjusted towards higher values of *138 - 415 ng/ml* and *182 - 545 ng/ml* (Table 3).

Table 3. Population-based expected reference range for approved dose range of aripiprazole

Administered Dose [mg/day]	Expected ARI BL [ng/ml] based on C/D ratio 13.82 (Hart et al., 2022)	Dose-related range based on TDM Guidelines 11.72 (Hiemke et al., 2018)	Expected AM BL [ng/ml] based on C/D ratio 18.18 (Hart et al., 2022)	AM dose-related range based on TDM Guidelines 16.45 (Hiemke et al., 2018)
10	138 [124, 153]	117 [82, 153]	182 [166, 197]	165 [112, 219]
20	276 [248, 305]	234 [163, 306]	364 [333, 395]	329 [224, 438]
30	415 [372, 458]	352 [245, 459]	545 [499, 592]	494 [336, 657]

3.1.2 Expected concentration range in real world patients

The IQR of patients with schizophrenia and other schizophrenia spectrum disorders that were treated with aripiprazole under flexible dosing among eight studies ($N = 3{,}373$, $p < .0001$, $I^2 = 93.24$) was *120 - 273 ng/ml*.

3.1.3 Computation of therapeutically effective ranges using efficacy data

Two studies reported interquartile concentrations from responders after flexible dosing i) *127 - 278 ng/ml* for aripiprazole and *196 - 385 ng/ml* for the active moiety, based on 20% reduction in PANSS scores in patients with schizophrenia or schizoaffective disorder (Lin et al., 2011) and *ii) 124 - 286 ng/ml* for aripiprazole based on CGI-improvement of “much improved” and “very much improved” in patients with schizophrenia (Kirschbaum et al., 2008).

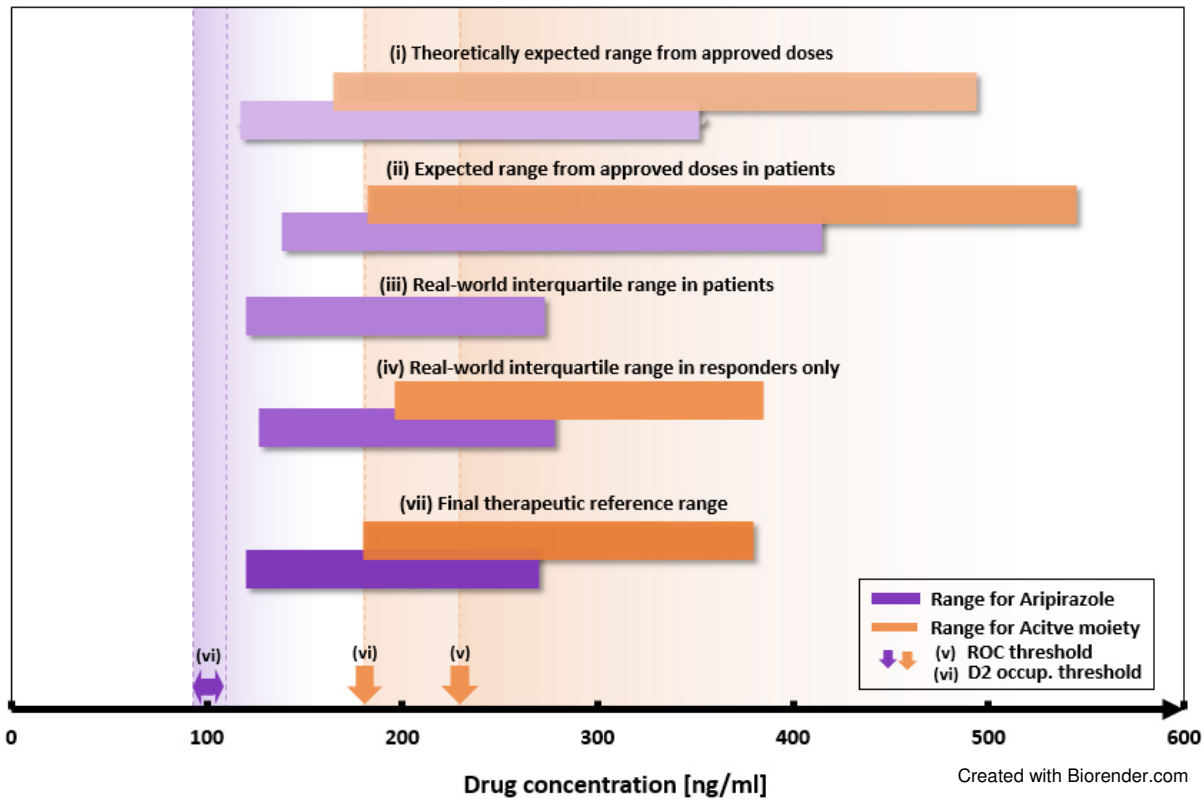
3.1.4 Estimation of concentration thresholds for the upper and lower limit

ROC analysis revealed a threshold of *170 ng/ml* and *224 ng/ml* for aripiprazole (not significant) and the active moiety (significant) (Lin et al., 2011).

3.1.5 Molecular imaging to measure target receptor occupancy

Three PET studies report findings that can be used in order to support a reference range for the partial D_2 agonist. A target engagement of >90% D_2 receptor occupancy can be reached with blood concentrations above *90 - 110 ng/ml* for aripiprazole and approximately *180 ng/ml* for the active moiety (Hart et al., 2022).

Figure 8. Summary of findings for aripiprazole's reference range



*We suggest a therapeutic reference range for aripiprazole of **120 - 270 ng/ml** and for the active moiety of **180 - 380 ng/ml** (Figure 8). Above the lower threshold, a higher response is expected. The upper limit reflects a therapeutic optimum derived from concentrations in a representative population.*

3.1.6 Validation of the proposed therapeutic reference range

The validation sample used for the computation comprises 167 patients from two studies in which aripiprazole and active moiety blood levels were measured together with clinical effects after flexible dosing (Table 4) (Kirschbaum et al., 2008; Lin et al., 2011). Aripiprazole concentrations ranged from 32 - 869 ng/ml (mean 218 ± 138). Active moiety concentrations ranged from 47 – 1,031 ng/ml (mean 301 ± 177, median 264). IQR in responders was *136 - 273 (50 -*

869) and *194 - 366 (74 - 366)* ng/ml. The concentration between responders and nonresponders did not differ significantly. For aripiprazole and the active moiety local sensitivity/specificity maxima were observed at 115 ng/ml and at 98 ng/ml and 194 ng/ml, respectively. Tables 5 and 6 show sensitivity and specificity scores at different cut-off points.

Table 4: Demographic data of patient population of the validation sample for aripiprazole

	Lin et al., 2011	Kirschbaum et al., 2008	Validation sample
Country	Taiwan	Germany	
Design	Prospective cohort study	Cross-sectional TDM study	
Subjects	N = 45, patients with SCZ or schizoaffective disorder	N = 159, efficacy sample of patients with SCZ: N = 122	N = 167
Clinical effects	PANSS after 6 weeks	CGI improvement score	
- **mean age (years)** - **% males** - **mean ARI dose (mg/day)**	40 ± 11 (19 - 59) 42 14.2 ± 6.3	33 ± 11 (19 - 66) 66 20.2 ± 8.1	35 ± 11* 59 18.6 ± 8.1*
Mean ARI BL (range) in ng/ml	208 ± 136	221.5 ± 138.9 (32 - 869)	218 ± 138 (32 - 869)
Mean AM BL (range) in ng/ml	296 ± 188	303.7 ± 172.9 (47 -1031)	301 ± 177 (47 - 1031) (N = 150)
Comment	Higher ARI BL in responders (20% decrease in PANSS score, p = 0.05).	No differences in ARI or AM BLs among responders and nonresponders	No differences in ARI or AM BLs in responders and nonresponders

*computed using Cochrane's Formula

Table 5: Sensitivity/specificity-scores at selected thresholds for aripiprazole using combined data from two studies (N = 167) (Kirschbaum et al., 2008; Lin et al., 2011)

	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	100	87	15	48	17	0.8529412	0.2615385	1.1144796
2	115	84	18	45	20	0.8235294	0.3076923	1.1312217
3	120	82	20	44	21	0.8039216	0.3230769	1.1269985
4	150	70	32	38	27	0.6862745	0.4153846	1.1016591
	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
5	270	26	76	19	46	0.25490196	0.7076923	0.9625943
6	280	25	77	19	46	0.24509804	0.7076923	0.9527903
7	300	20	82	16	49	0.19607843	0.7538462	0.9499246
8	407	8	94	5	60	0.07843137	0.9230769	1.0015083

TP = true positive, FN = false negative, FP = false positive, TN = true negative

Table 6: Sensitivity/specificity-scores at selected thresholds for active moiety of aripiprazole using combined data from two studies (N = 150) (Kirschbaum et al., 2008; Lin et al., 2011)

	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	150	79	15	45	11	0.8404255	0.1964286	1.0368541
2	200	68	26	37	19	0.7234043	0.3392857	1.0626900
3	180	71	23	38	18	0.7553191	0.3214286	1.0767477
	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
4	380	20	74	16	40	0.2127660	0.7142857	0.9270517
5	400	19	75	14	42	0.2021277	0.7500000	0.9521277
6	500	15	79	7	49	0.1595745	0.8750000	1.0345745

TP = true positive, FN = false negative, FP = false positive, TN = true negative

3.1.7 Evaluation of TDM data from the Central Institute of Mental Health

1,219 aripiprazole serum levels have been measured between Jan 2014 and Dec 2018 at the Central Institute of Mental Health. Of these, 234 patients were included in the final analysis with mean aripiprazole and active moiety steady state levels of 225.1 ± 155.5 ng/ml (N = 234) and 329.3 ± 195.4 ng/ml (N = 41), respectively. 49% of patients were males. Mean age was 39.1 ± 13.9 years. Mean aripiprazole dose was 16.5 ± 7.0 (5 - 40) mg/day. Most applied doses were 15 mg (30% of patients) and 20 mg (25% of patients). Applied daily doses showed a good linear correlation with i) aripiprazole concentration (*p < 0.001, F = 73.2, r = 0.49, beta = 10.96)* and with the active moiety concentration (*p < 0.001, F = 21.98, r = 0.60, beta = 15.86*). 57% were treated as inpatients at time of measurement, 22% were semi-in patients, and 21% were outpatients. Trough sampling could be confirmed from records in 58% of cases. Of all 234, 125 of patients were diagnosed with schizophrenia (ICD 10, F20.X). 88% of patients with schizophrenia received aripiprazole treatment at the date of discharge. Their mean concentration did not differ from patients being discontinued on aripiprazole within current episode. Interquartile concentrations of patients was *119 - 305 ng/ml* and *189 - 390 ng/ml* for aripiprazole and the active moiety.

3.1.8 Distribution of aripiprazole and active moiety concentrations within old and new range

Two datasets have been evaluated in terms of data distribution (Figure 9). Distributions followed a lognormal curve for aripiprazole (i) location 5.23306, scale 0.58413, threshold -33.3866, N = 1,269, (ii) location 5.41699, scale 0.55962, threshold -55.5334, N = 3,169, Figure 9) and for the active moiety (location 5.55142, scale 0.54791, threshold -48.358, N = 1,262).
The 25-75% quantile range of the dataset comprising 3,169 aripiprazole concentration levels from German patients (“Bremen Data”) was 99 - 273 ng/ml. 61.3% of values lied within, 24.1% below and 14.6% above the therapeutic reference range of 100 - 350 ng/ml (Figure 10).
The second study comprised data from a Norwegian TDM database (N = 1,269). 25-75% quantiles were 93 - 245 ng/ml and 130 - 324 ng/ml for aripiprazole and the active moiety. For aripiprazole, 61.9% of values lied within, 27.0% below and 11.1% above the therapeutic reference range in current guidelines of 100 - 350 ng/ml. 36.3% and 21.2% of values lied below 120 ng/ml and above 270 ng/ml. For the active moiety, 60.5% of values lied within, 31.5% below and 8.0% above the therapeutic reference range in current guidelines of 150 - 500 ng/ml. 41.9% and 18.7% of all levels lie below and above the suggested range of 180 - 380 ng/ml.

Figure 9: Histogram with lognormal density curve for aripiprazole concentrations from Bremen (N = 3,169)

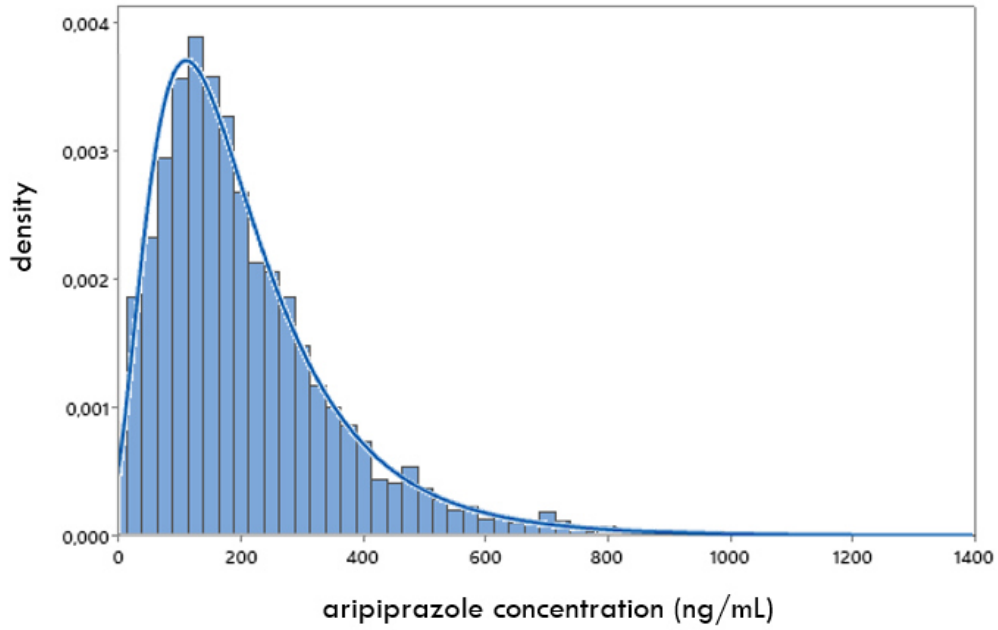
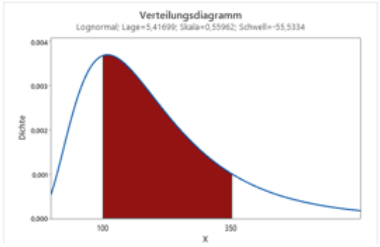


Figure 10: Distribution of aripiprazole concentrations within old (top) vs. new (bottom) reference range using Bremen Data (N = 3,169)

763/3169 values <100 = **24.08%**

462/3169 values >350 = **14.58%**

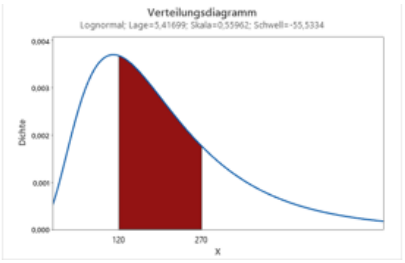
Sum 38.66%



992/3169 values <120 = **31.30%**

810/3169 values >270 = **25.56%**

Sum 56.86%



3.2 Therapeutic Reference Range for the Antipsychotic Drug Olanzapine

For olanzapine, a standard dose range would consider a dose between 5 – 20 mg once daily in the evening. In practice, blood samples are usually taken in the morning, approx. 12h after the last intake, not at c_{min} after 24h (Wesner et al., 2022). Olanzapine has linear kinetics and dose proportionality can be assumed within the approved dose range (Callaghan, Bergstrom, Ptak, & Beasley, 1999; Wesner et al., 2022). Ambivalent findings exists from concentration/efficacy studies with an overall low level of evidence. EPS have been found infrequently and no concentration-dependency could be confirmed (Wesner et al., 2022).

3.2.1 Computation of an expected range from approved doses

The expected concentration range (c_{min}) for sampling after 12h and 24h is *9 - 37 ng/mL* and *7 - 29 ng/ml* (see S1 for example calculation) after the administration of 5 – 20 mg/day.

3.2.2 Expected concentration range patients under real world conditions

After a once daily dose of 5 mg and 20 mg, a 9 - 14h concentration between *7 - 21 ng/ml* and *28-86 ng/ml* is expected (Korell et al., 2018).

3.2.3 Computation of therapeutically effective ranges using efficacy data

An interquartile concentration range in patients who responded to olanzapine drug treatment were available from solely one study that did not report an artificial finding. OLZ IQR was *19 - 37 ng/ml* in 20 responders (Mauri et al., 2005).

3.2.4 Estimation of concentration thresholds for the upper and lower limit

Lower limit: Three studies have consistently reported a threshold of *23 ng/ml* from ROC analysis (Fellows et al., 2003; Lu et al., 2016; Perry et al., 2001) (20% decrease in PANSS/ BPRS score; PANSS score > or ≤ 58) 12h post dosing. 24h post dosing a threshold of 9 ng/ml was suggested (Perry et al., 1997).

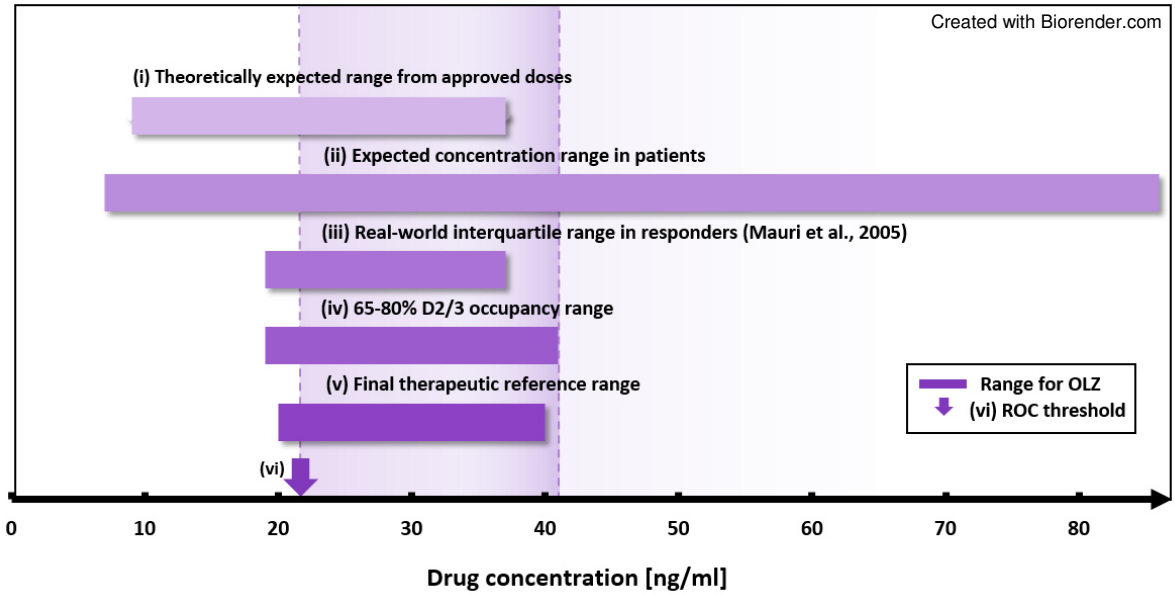
Upper limit: Concentration efficacy curves suggest a maximum treatment effect at olanzapine concentrations of around *50 ng/ml* (Mauri et al., 2005) to *78 ng/ml* (Zabala et al., 2017). However, the latter represents an artificial finding and will not be used to support olanzapine reference range (see section 2.1.).

3.2.5 Molecular imaging to measure target receptor occupancy

For oral olanzapine, an ED_{50} of 10.3 ng/ml in terms of plasma levels (N = 15, *r = 0.83*) was published (Kapur et al., 1998). A therapeutic range that refers to 65 - 80% receptor occupancy would be associated to olanzapine plasma levels between *19 - 41 ng/ml.* This range was confirmed in an olanzapine pamoate long-acting injectable formulation (range 20 - 44 ng/ml (Mamo et al., 2008).

*We propose a therapeutic reference range for olanzapine of **20 - 40 ng/ml** (Figure 11) when sampling 12-15h post dose. Above the lower threshold, a higher response is expected. The upper limit reflects a therapeutic optimum derived from concentration/efficacy curves.*

Figure 11. Summary of findings for olanzapine's reference range



3.2.6 Validation of the proposed therapeutic reference range

The validation sample comprises 57 patients with schizophrenia from two studies in which olanzapine blood levels were measured together with clinical effects (Table 8) (Carrillo et al., 2003; Mauri et al., 2005). Patients were classified as responders when showing a minimum improvement of 20% in BPRS (Carrillo et al., 2003) or PANSS score (Mauri et al., 2005) after 15 or 14 days of continuous treatment. Data was extracted from original manuscripts using a web-based software. Olanzapine concentrations ranged from 4 - 121 ng/ml (mean 33 ± 26, median 30). The concentration between responders (N = 31) and nonresponders (N = 26) differed significantly (median 34 ng/ml vs. 22 ng/ml; p = .027). IQR in responders was 22 - 50 ng/ml (6 - 121). Table 7 shows sensitivity and specificity scores at different cut-off points. Local sensitivity/specificity maxima were observed at 25.5 and 27.5 ng/ml.

Table 7. Sensitivity/specificity-scores at selected thresholds for olanzapine using combined data from two studies (N = 57) (Carrillo et al., 2003; Mauri et al., 2005)

Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	10	26	5	20	0.83870968	0.2307692	1.069479
2	20	24	7	15	0.77419355	0.4230769	1.197270
3	23	23	8	13	0.74193548	0.5000000	1.241935
4	27	21	10	10	0.67741935	0.6153846	1.292804
5	40	12	19	3	0.38709677	0.8846154	1.271712
6	60	7	24	0	0.22580645	1.0000000	1.225806
7	80	4	27	0	0.12903226	1.0000000	1.129032
8	100	2	29	0	0.06451613	1.0000000	1.064516

Table 8. Demographic data of patient population of the validation sample for olanzapine

	Carillo et al., 2003	Mauri et al., 2005	Validation sample
Country	Spain	Italy	
Design	Prospective cohort study	Prospective cohort study	
Subjects	N = 17, 10 patients with SCZ, 5 with schizoaffective disorder, and 2 with delusional disorder	N = 54, inpatients with acute SCZ, efficacy sample N = 40	N = 57
Clinical effects	BPRS after 15 days	PANSS after 14 days	
- **mean age (years)** - **% males** - **mean OLZ dose (mg/day)**	37 ± 16 (18-70) 53 8.4 ± 2.3	35.6 ± 12.4 (18-75) (N=54) 70 15.3 ± 5.5 (N = 54)	35.9 ± 13.2* 66 13.7 ± 5.7 (N = 71)*
Mean OLZ BL (range) in ng/ml	35 ± 22 (4 - 69.5)	33 ± 28 (6 - 121) (N = 40)	33 ± 26
Comment	% decrease in BPRS was correlated with BL	Curvilinear correlation between BLs and clinical improvement (PANSS and BPRS)	Higher concentrations in responders compared to non-responders (p = .027)

*computed using Cochrane's Formula

3.2.7 Evaluation of TDM data from the Central Institute of Mental Health

Our database comprised 1,588 olanzapine blood levels that have been measured at the Central Institute of Mental Health between Jan 2, 2014 and Dec 27, 2018. Of these, 231 patients with oral olanzapine dosing in the steady state were eligible for analysis. Since 12 patients received additional electroconvulsive therapy during the time of blood level assessments, the efficacy sample comprised 219 patients aged from 14 to 83 years (41.0 ± 16.6 years; 57.5% males). The majority of patients were inpatients (72%) and day-care patients (15.1%) (outpatients 11%). Most patients were diagnosed with schizophrenia (F20.X, N = 113). In the total sample, mean olanzapine dose was 19.5 ± 9.2 mg/day (range 5 - 50 mg/day). The most common doses were 20 mg (25.6%), 30 mg (18.3%), 10 mg (17.8%), and 40 mg (4.6%) daily. 19 patient received a lower dose than 10 mg and one patient was treated with a dose of 50 mg per day. Mean olanzapine concentration was 45.7 ± 38.8 ng/ml (range 2.5 - 378 ng/ml, *IQR 22.7-58.1 ng/ml*). Linear regression analysis revealed a good correlation between olanzapine concentration and dose (r = 0.395, p < 0.001, beta = 1.67 [1.15, 2.19]). For the majority of patients (70.8%, N = 155), olanzapine serum levels lied within the current therapeutic reference range of 20 - 80 ng/ml. 19.6% (N = 43) and 9.6% (N = 21) of patients had levels below and above this range, respectively. For patients with schizophrenia a similar picture was observed (70.8% within, 17.7% below and 11.5% above the range). When assessing longitudinal effects, no differences were found in patients that were discharged with or without olanzapine.

3.2.8 Distribution of olanzapine concentrations within old and new range

5,657 OLZ blood levels were available for inclusion. Distribution followed a lognormal curve (location 3.52925, scale 0.68502, threshold -1.89933, Figure 13) with an interquantile range between *19.6 - 52.3 ng/ml*. 64.1% of values lied within, 25.8% below and 10.1% above the therapeutic reference range in current guidelines of 20 - 80 ng/ml (Figure 13). 39.0% of all concentrations lied above the proposed threshold of 40 ng/ml.

Figure 12. Histogram with lognormal density curve for olanzapine concentrations from Bremen (N = 5,657)

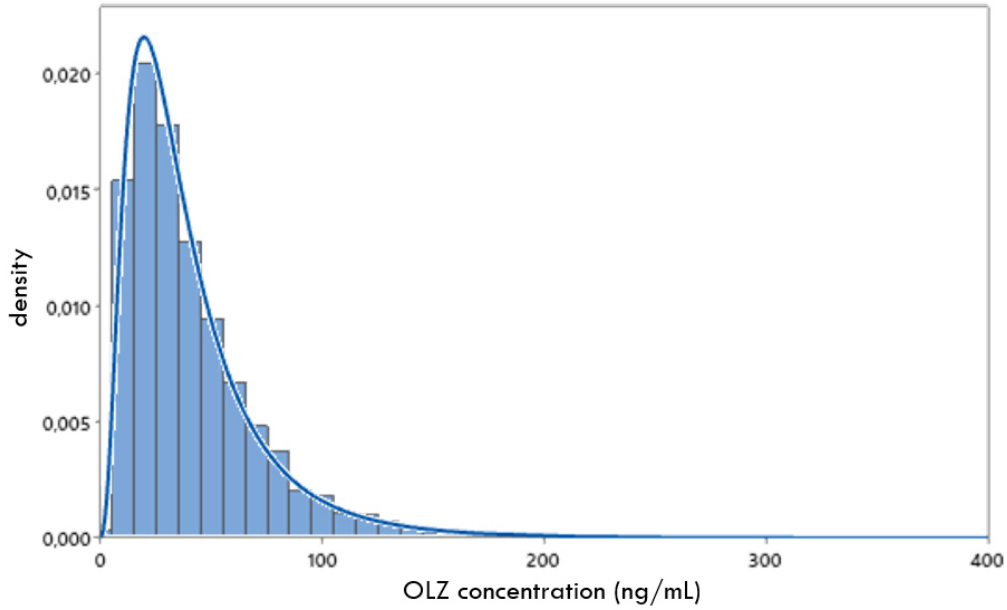
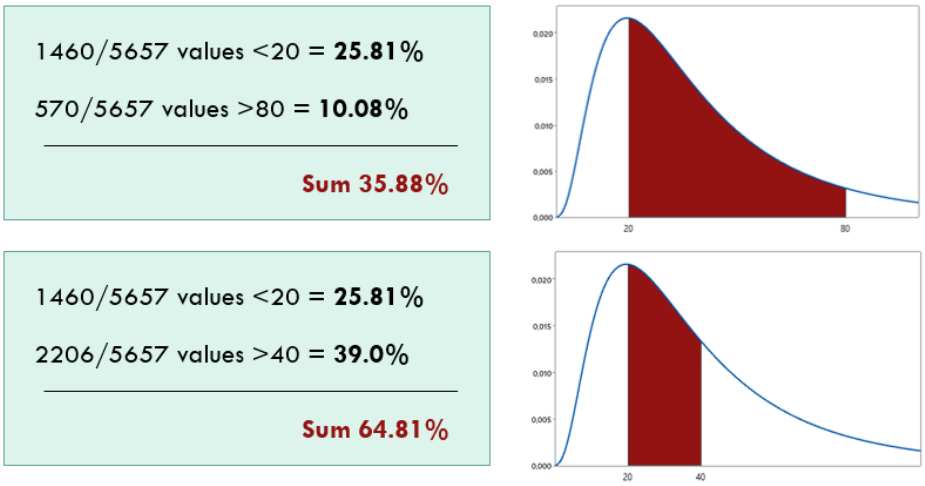


Figure 13. Distribution of olanzapine concentrations within old (top) vs. new (bottom) reference range (N = 5,657)



3.3 Therapeutic Reference Range for the Antidepressant Drug Escitalopram

A systematic literature search and grading of available studies that describe relationships between concentration and clinical or side effects has been conducted by Eichentopf et al. for the antipsychotic drug escitalopram (Eichentopf et al., 2022). Prescribing information recommend an once daily dose regimen of 10 - 20 mg/day escitalopram for the treatment of depression (MDD). Evidence for a concentration/efficacy relationship is scarce (Level C1; low). Only one prospective flexible dose study describes a positive relationship between blood levels and HAMD-21 scores (Florio et al., 2017). After eight weeks of treatment, one study reported a lower MADRS improvement in patients with higher blood levels (inverse correlation) (Hodgson et al., 2014). No study established a concentration efficacy curve nor described a meaningful relationship with side effects (Level D; absent).

3.3.1 Computation of an expected range from approved doses

The expected concentrations of escitalopram after a dose of 10 - 20 mg/day is *11 - 21 ng/ml*. This range lies around the lower limit of the current reference range of 15 - 80 ng/ml (Hiemke et al., 2018). Despite barely reported C/D ratios, some studies suggest that this range must most likely be adjusted towards higher values in patients.

3.3.2 Expected concentration range in real world patients

The interquartile range of patients with depression that were treated with escitalopram (mostly under flexible dosing) in seven studies (N = 4,295, $p < .0001$, $I^2 = 96.59$) was *15 - 39 ng/ml*.

3.3.3 Computation of therapeutically effective ranges using efficacy data

Two studies report interquartile concentrations from patients with depression who responded (50% reduction in HAMD-21 scores) two drug treatment after flexible dosing i) *24 - 54 ng/ml* after 3 months (N = 32) (Florio et al., 2017), ii) *20 - 41 ng/ml* after 4 weeks (N = 360) (Tadić et al., 2016). Combined responders had an interquartile range between *20 - 40 ng/ml* (N = 394).

3.3.4 Estimation of concentration thresholds for the lower limit

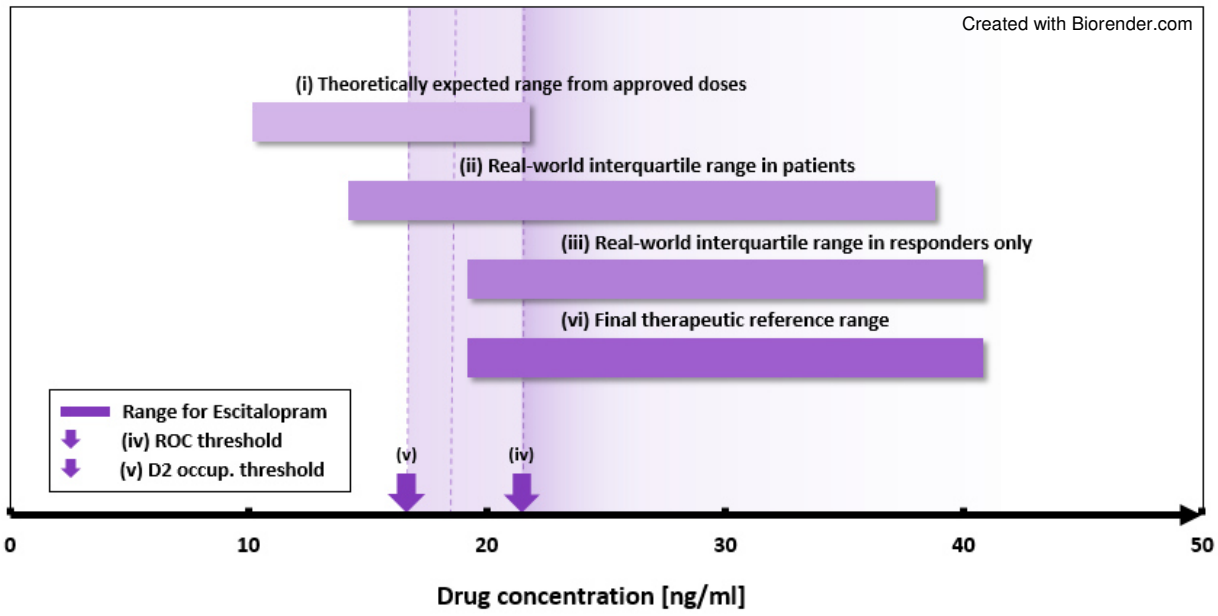
A ROC analysis was performed using data from Florio and colleagues that identified a threshold concentration of *20.5 ng/ml* separating responders from nonresponders (Figure 5a) (Florio et al., 2017).

3.3.5 Molecular imaging to measure target receptor occupancy

PET studies suggest that there is a significant relationship between SERT occupancy and escitalopram blood levels. EC_{80} values consistently lie between *16 - 18 ng/ml* (thalamus/putamen) (Arakawa et al., 2016; Kim et al., 2017; Lanzenberger et al., 2012).

*We propose a therapeutic reference range for escitalopram of **20 - 40 ng/ml** (Figure 14). Above the lower threshold, a higher response is expected. The upper limit reflects a therapeutic optimum derived from concentrations in a representative population.*

Figure 14. Summary of findings for escitalopram's reference range



3.3.6 Validation of the proposed therapeutic reference range

The validation sample used for the computation comprises 750 patients with depression from two studies in whom escitalopram was measured together with clinical effects after flexible dosing (Florio et al., 2017; Tadić et al., 2016). Escitalopram blood levels ranged from 0 - 166 ng/ml (mean 34.4 ± 20.5, median 29). The concentration between responders and nonresponders did not differ. Table 9 shows sensitivity and specificity scores at different cut-off points. Local sensitivity/specificity maximum was observed at 18.5 ng/ml.

Table 9. Sensitivity/specificity-Scores at selected thresholds for escitalopram using combined data from two clinical trials (Florio et al., 2017; Tadić et al., 2016) (N = 749)

	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	15	349	45	319	36	0.88578680	0.1014085	0.9871953
2	17	333	61	306	49	0.84517766	0.1380282	0.9832058
3	20	297	97	273	82	0.75380711	0.2309859	0.9847930
	Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
4	38	117	277	137	218	0.29695431	0.6140845	0.9110388
5	40	105	289	122	233	0.26649746	0.6563380	0.9228355
6	45	85	309	97	258	0.21573604	0.7267606	0.9424966
7	80	13	381	10	345	0.03299492	0.9718310	1.0048259

Table 10. Demographic data of patient population of the validation sample for escitalopram

	Florio et al, 2017	Tadic et al., 2016	Validation sample
Country	Italy	Germany	
Design	Prospective cohort study	RCT	
Subjects	N = 70 with major depression	N = 679 with major depression	N = 749 with major depression
Clinical effects	50% HAM-D 21 improvement after 3 months	50% MADRS improvement after 4 weeks	
- **mean age (years)** - **% males** - **mean ESC dose (mg/day)**	46.2 ± 16.63 40 15.2 ± 5.1	40.6 ± 11.8 42 19.5 ± 2.1	41.1 ± 12.4 42 19.1 ± 2.8
Mean ESC BL (range) in ng/ml	30.2 ± 25.6	34.9 ± 19.8	34.4 ± 20.5
Comment	Higher BLs predicting higher treatment response.	Early ESC improvers were excluded	No higher BLs in responders compared to nonresponders

*computed using Cochrane's Formula

3.3.7 Evaluation of TDM data from the Central Institute of Mental Health

535 escitalopram blood levels have been measured between Jan 21 2014 and Dec 18 2018 at the Central Institute of Mental Health. Of these, 134 patients were included in the final analysis aged from 14 to 89 years (47 ± 19 years; 41.8% males). The majority of patients were inpatients (65.7%) and day-care patients (33.6%). Most patients were diagnosed with a depression (ICD 10, F32.X or F33.X, N = 103) with five, 42, and 56 patients, respectively experiencing a minor (ICD 10, F32.1 or F33.1), moderate (ICD 10, F32.2 or F33.2), or severe depressive episode (ICD 10, F32.3 or F33.3) at time of escitalopram Drug Monitoring. Other antidepressant drugs were given in 54 (40.3%) of all patients, most preferred was mirtazapine (N= 27). Additional interventions with antidepressive effects were noted in six patients with five of them receiving periodic electroconvulsive therapy and one patient being treated with transcranial magnetic stimulation therapy. In all patients, mean (± SD) escitalopram dose was 17 ± 6 mg/day (range 5 - 40 mg/day). The most common doses were 20 mg (43.3%), 10 mg (28.4%) and 15 mg (22.4%) daily. One patient received a lower dose of 5 mg and seven patients were treated with doses above 20 mg per day. Mean escitalopram concentration was 24 ± 17 ng/ml (range 5-76 ng/ml, IQR 11 - 34 ng/ml). While six patients were excluded because of additional antidepressant interventions, the efficacy sample comprised 128 patients. Of those, 97 patients were treated with escitalopram for depressive disorders (ICD 10, F32.X or F33.X). For the majority of patients with depression (62%), escitalopram serum levels lied within the current therapeutic reference range of 15 - 80 ng/ml. 38% of patients had levels below this range. Overall, higher escitalopram concentrations (mean: 21 vs. 11 ng/ml, p = .006, Figure 2) and higher dose-corrected escitalopram concentrations (mean C/D ratio: 1.4 vs. 0.63 (ng/ml)/(mg/day), p = .03) were found in patients that were discharged with escitalopram (N = 95) compared to patients not discharged with escitalopram (N = 33), whereas doses did not differ between both groups. This holds also true when selecting patients experiencing a depressive episode (ICD 10, F32.X or F33.X) at this point in time (BL: p = 0.01; C/D ratio: p = .046, N = 97, Figure 15) and when excluding patients, whose dose has been increased or decreased within sampling and discharge time (BL: p = 0.002; C/D ratio: p = .04; dose: p = .02). For the sample of patients whose doses remained stable, as well as for the subsample

of depressive patients with stable doses, ROC curve identified a cut-off point of 18.5 ng/ml (AUC = 0.686 [CI 0.566; 0.807], p = .002, N = 111) and 15 ng/ml (AUC = 0.695 [CI 0.562; 0.827], p = .003, N = 85) that discriminates responders from nonresponders (Figure 16). Of depressive patients, 81% of patients with a drug level above 15 ng/ml were discharged with escitalopram (N = 58 of 72; responders). The “response rate” below this threshold was 51.3% (N = 20/39). Interquartile concentration range of “responders” with depression to escitalopram treatment was *16 - 36 ng/ml* (N = 57).

Figure 15. Escitalopram as discharge medication in patients with a depressive episode (N = 96, p = .011, median BLs: 7.8 ng/ml vs. 21.5 ng/ml)

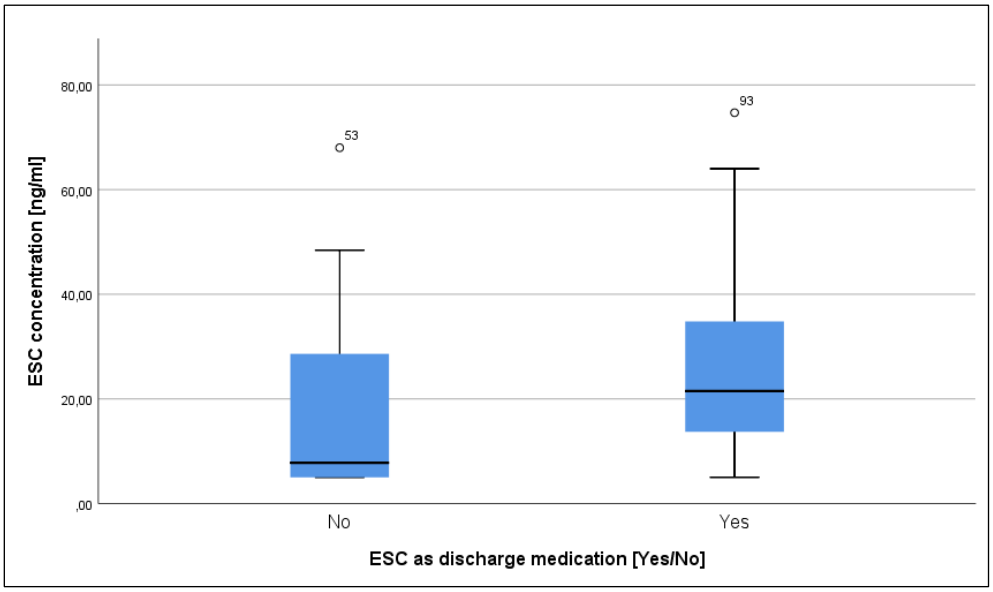
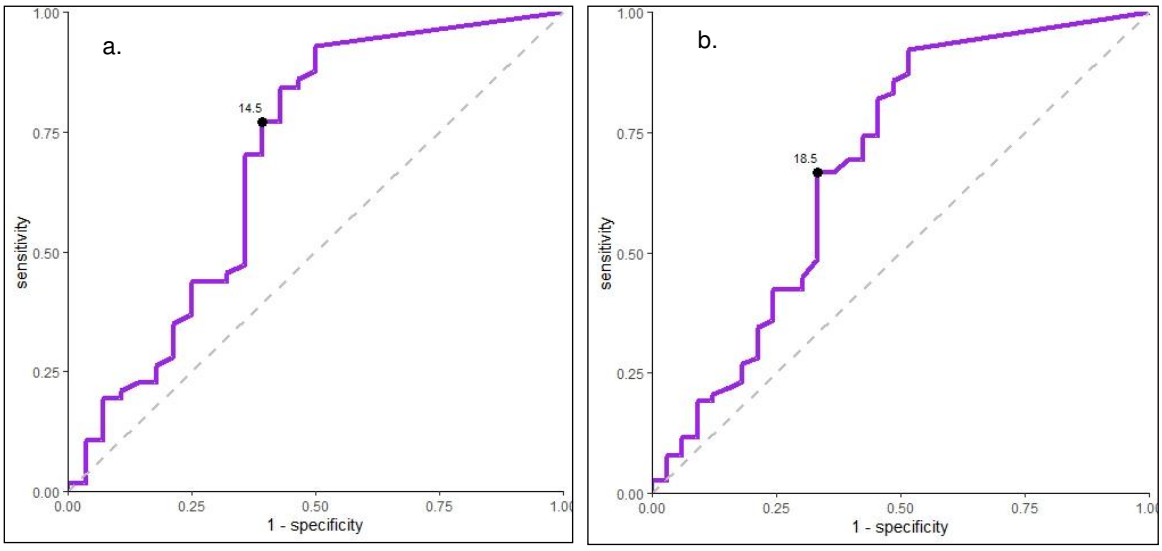


Figure 16. ROC curve escitalopram at discharge. Y/N for patients with stable dose from sampling time point to discharge in a. patients with depression (AUC = 0.695 [CI 0.562; 0.827], p = .003, closest top left 14.5 ng/ml, N = 85) b. complete sample (AUC 0.686 [CI 0.566; 0.807], p = .002, closest top left 18.5 ng/ml, N = 111)



3.3.8 Distribution of escitalopram concentrations within old and new range

Concentration data of a patient cohort from a large randomized controlled clinical trial was tested to find the optimal distribution. It followed a lognormal curve (location 3.40589, scale 0.54262, threshold 0, N = 679, Figure 17) (Tadić et al., 2016). IQR was 21 - 44 ng/ml. 87.8% of values lied within, 9.4% below and 2.8% above the therapeutic reference range in current Guidelines of 15 - 80 ng/ml. 46.8% of values lie within, 22.2% below and 30.9% above the range of 20 - 40 ng/ml (Figure 18).

Figure 17. Histogram with lognormal density curve for escitalopram concentrations (N = 679) (Tadić et al., 2016)

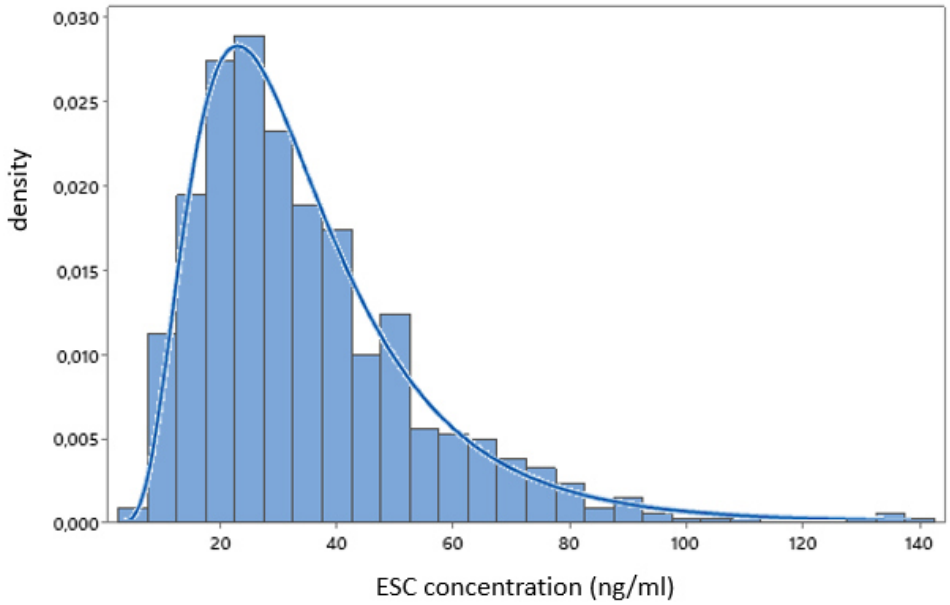
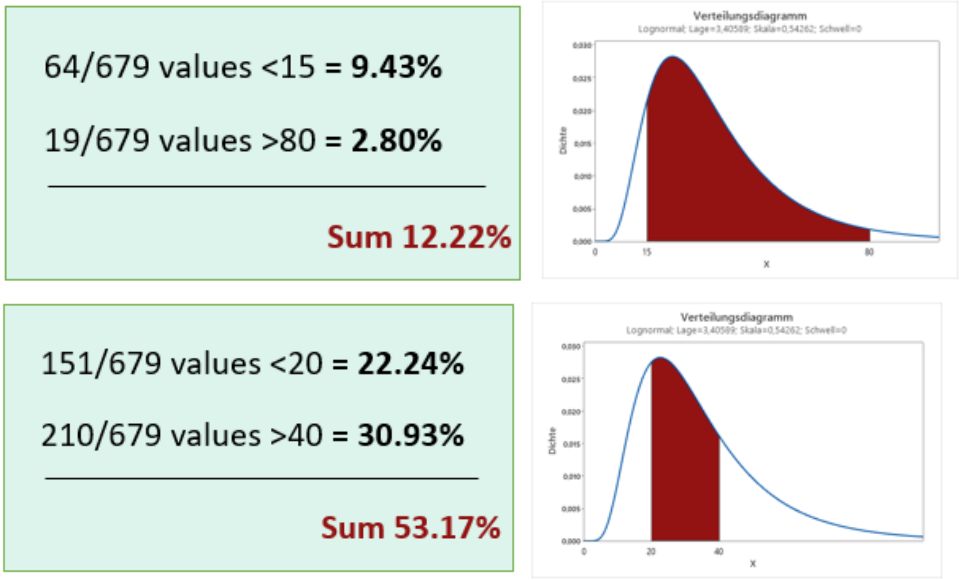


Figure 18. Distribution of escitalopram concentrations within old (top) vs. new (bottom) reference range (Tadić et al., 2016) (N = 679)



3.4 Therapeutic Reference Range for the Antidepressant Drug Venlafaxine

A systematic literature search and grading of available studies that describe relationships between concentration and clinical or side effects has been conducted for venlafaxine (VEN) and its active moiety (AM; venlafaxine + O-desmethylvenlafaxine) (Lense et al., 2022). Five cohort-studies reported a positive correlation between VEN, ODV, or AM blood levels and antidepressant effects (Level C; low) (Charlier et al., 2002; De Donatis et al., 2021; Hoencamp et al., 2000; Scherf-Clavel et al., 2020; Stamm et al., 2014). Two studies reported a negative correlation respectively (Berm et al., 2016; Schoretsanitis et al., 2019). Overall, the metaanalysis across four studies in adult patients found higher concentrations in responders compared to nonresponders (N = 360; EE = 0.35 [0.10, 0.59], $p \leq 0.05$). One study found concentration-dependent tremor in patients treated with venlafaxine for depression (Level C1; low) (Engelmann et al., 2021). The relationship between dose and active moiety concentration has been shown linear within approved doses (75 - 225 mg/day).

3.4.1 Computation of an expected range from approved doses

The expected concentrations of the active moiety and O-desmethylvenlafaxine (XR release) after a daily dose of 75 - 225 mg are *96 - 288 ng/ml and 78 - 234 ng/ml* (Hiemke et al., 2018). In study patients, higher C/D values have been reported resulting in a range between *140 - 421 ng/ml* and *85 - 254 ng/ml* for the active moiety and for O-desmethylvenlafaxine, respectively (Table 11).

Table 11. Population-based expected reference range for venlafaxine XR maintenance doses

Administered dose [mg/day]	Expected ODV BLs [ng/ml] based on C/D ratio 1.13 (Lense et al., 2022)	ODV dose-related range based on TDM Guidelines 1.04 (Hiemke et al., 2019)	Expected AM BLs [ng/ml] based on C/D ratio 1.87 (Lense et al., 2022)	AM dose-related range based on TDM Guidelines 1.28 (Hiemke et al., 2019)
75	85 [79, 91]	78 [59, 98]	140 [131, 149]	96 [68, 125]
150	170 [158, 182]	156 [117, 195]	281 [261, 299]	192 [135, 251]
225	254 [236, 497]	234 [176, 293]	421 [392,448]	288 [203, 376]

3.4.2 Expected concentration range in real world patients

The interquartile active moiety and O-desmethylvenlafaxine concentration ranges of patients under flexible dosing that were treated with venlafaxine in 11 studies (N = 3,200) were *225 - 450 ng/mL* (mean BL 358 ng/ml, $p < .0001$, $I^2 = 85.8\%$) *and 144 - 302 ng/mL* (mean BL 223 ng/ml, $p < .0001$, $I^2 = 92.9\%$).

3.4.3 Computation of therapeutically effective ranges using efficacy data

IQR of responders (N = 82) from a patient cohort treated with venlafaxine for depression was 213 - 382 ng/mL for O-desmethylvenlafaxine and 305 - 534 ng/ml for the active moiety (Engelmann et al., 2021). Antidepressant effects were assessed after eight weeks of treatment. Response was defined as 50% reduction in HAMD scores.

3.4.4 Estimation of concentration thresholds for the upper and lower limit

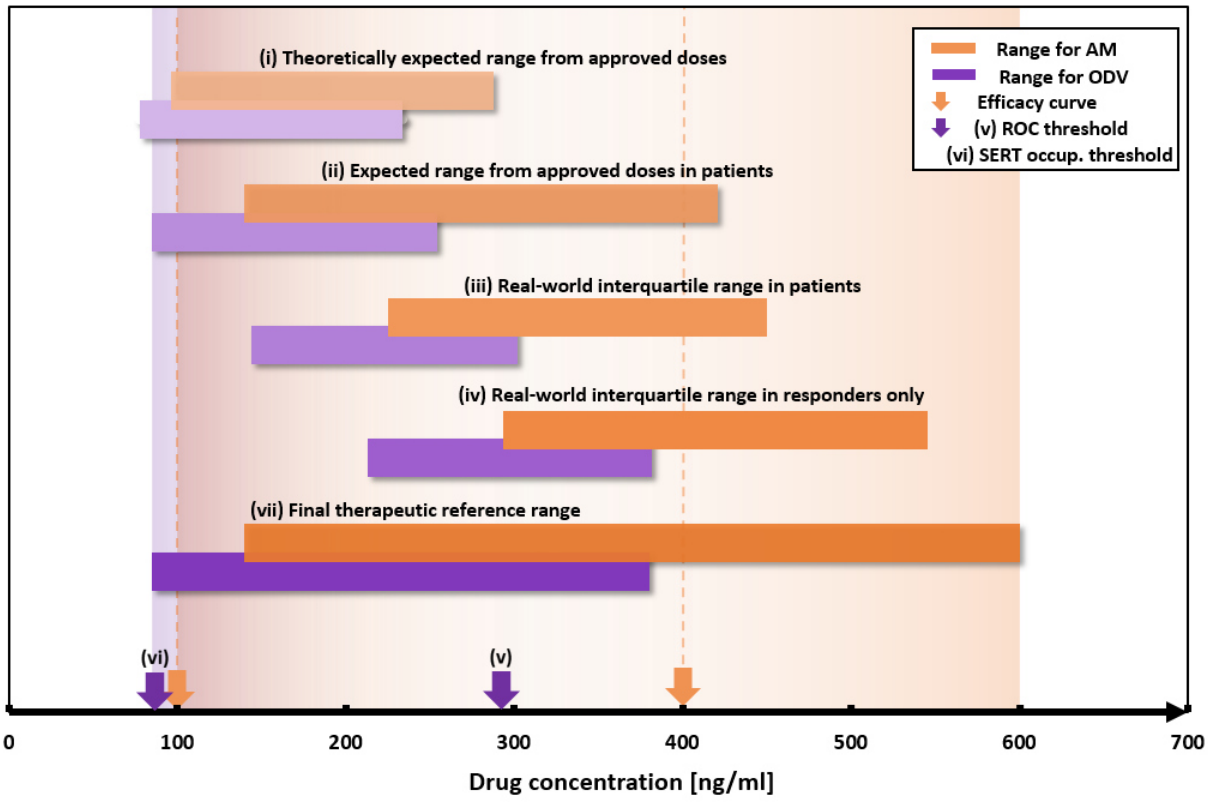
A ROC analysis was performed using data from Engelmann et al. that identified an O-desmethylvenlafaxine BL threshold for antidepressant response of 289 ng/mL (Figure 6b) (Engelmann et al., 2021). De Donatis and colleagues reported an u-shaped active moiety concentration/effect-relationship with optimal efficacy within *100 - 400 ng/ml*, referring to a range between the onset (30%) and maximum (42%) reduction in HAMD-21 score after three months of treatment (De Donatis et al., 2021). Patients with active moiety concentrations *above 400 ng/ml* were more often found to develop a tremor compared to patients within the current reference range of 100 - 400 ng/ml (Engelmann et al., 2021).

3.4.5 Molecular imaging to measure target receptor occupancy

One PET study reports SERT occupancy in relation to O-desmethylvenlafaxine BLs (Frankle et al., 2018). 80% SERT occupancy is reached above 85 ng/ml (EC_{80}).

*We propose a therapeutic reference range for venlafaxine active moiety and O-desmethylvenlafaxine of **140 - 600 ng/ml** and **85 - 380 ng/ml** (Figure 19). Above the lower threshold, a higher response is expected. The upper limit reflects a therapeutic optimum. Increased occurrence of side effects, in particular tremor is expected at higher drug concentrations.*

Figure 19. Summary of findings for venlafaxine's reference range



3.4.6 Validation of the proposed therapeutic reference range

The validation sample comprises 234 patients with depression from one study in which venlafaxine was measured together with clinical effects after flexible dosing. O-desmethylvenlafaxine and active moiety blood levels ranged from 28 - 874 ng/ml (mean 272 ± 123, median 263) and 96 - 997 ng/ml (mean 424 ± 170, median 402). O-desmethylvenlafaxine, but not active moiety concentration between responders and nonresponders differed significantly. Tables 12 and 13 show sensitivity and specificity scores at different cut-off points. For the active moiety, a local sensitivity/specificity maximum was observed at 419 ng/ml. For O-desmethylvenlafaxine, a local sensitivity/specificity maxima were observed at 289 and 344 ng/ml.

Table 12: Sensitivity/specificity-scores at selected thresholds for the venlafaxine active moiety *using combined data from Engelmann and colleagues (Engelmann et al., 2021) (N = 234)*

Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	100	82	0	150	1.0000000	0.01315789	1.013158
2	140	82	0	147	1.0000000	0.03289474	1.032895
3	220	77	5	140	0.9390244	0.07894737	1.017972
4	290	66	16	121	0.8048780	0.20394737	1.008825
Cut-off	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Sum-Score**
5	400	47	35	72	0.5731707	0.52631579	1.099487
6	450	39	43	49	0.4756098	0.67763158	1.153241
7	600	14	68	19	0.1707317	0.87500000	1.045732

Table 13: Sensitivity/specificity-scores at selected thresholds for O-desmethylvenlafaxine *using combined data from Engelmann and colleagues (Engelmann et al., 2021) (N = 234)*

Cut-off	TP	FN	FP	TN	Sensitivity	Specificity	Sum-Score
1	85	80	2	144	0.9756098	0.05263158	1.028241
2	100	78	4	141	0.9512195	0.07236842	1.023588
3	215	61	21	97	0.7439024	0.36184211	1.105745
4	290	40	42	48	0.4878049	0.68421053	1.172015
Cut-off	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Sum-Score**
5	380	21	61	21	0.2560976	0.86184211	1.117940
6	400	15	67	17	0.1829268	0.88815789	1.071085
7	450	9	73	9	0.1097561	0.94078947	1.050546

3.4.7 Distribution of O-desmethylvenlafaxine and active moiety concentrations within old and new range

6,332 O-desmethylvenlafaxine and 3,505 active moiety blood levels were eligible for inclusion. O-desmethylvenlafaxine blood level distribution followed a lognormal curve (location 5.6758, scale 0.39842, threshold -107,886, Figure 20) with an interquartile range between 115 - 274 ng/ml. 73.0% of values lied within, 19.7% below and 7.3% above the therapeutic reference range in current guidelines of 100 - 400 ng/ml (Figure 22).
Active moiety BL also followed a lognormal curve (location 5.913, scale 0.4652, threshold -94.676, Figure 21) with an interquartile range between 176 - 411 ng/ml. 65.7% of values lied within, 8.3% below and 26.0% above the therapeutic reference range in current guidelines (Figure 23).

Figure 20. Histogram with lognormal density curve for O-desmethylvenlafaxine concentrations from Bremen (N = 6,332)

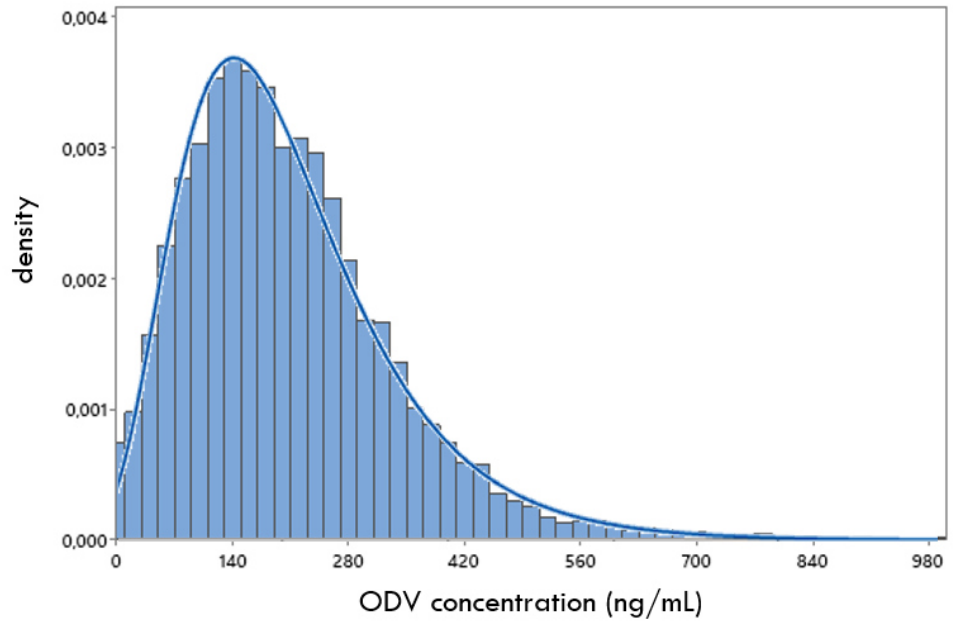


Figure 21. Histogram with lognormal density curve for venlafaxine active moiety concentrations from Bremen (N = 3,505)

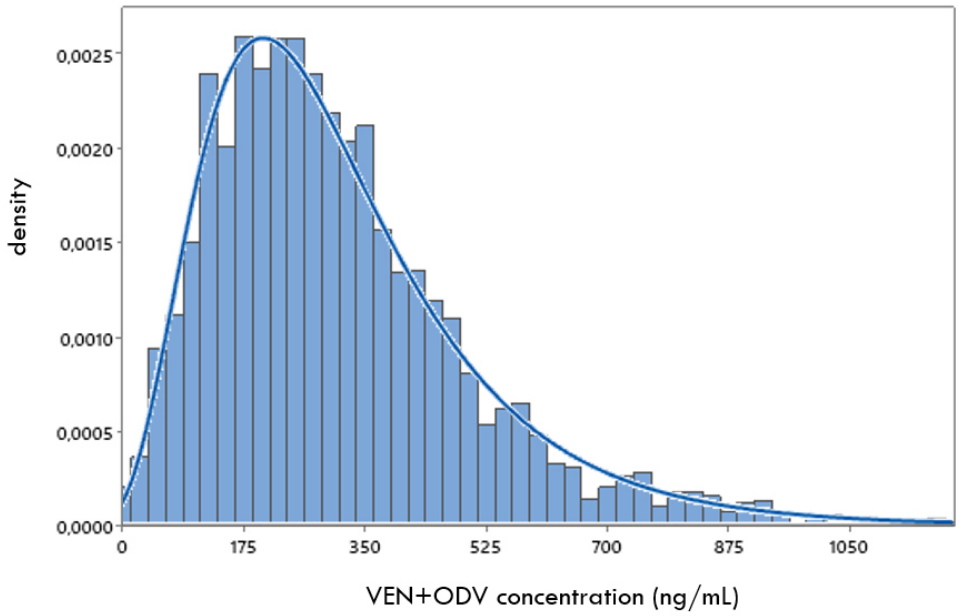


Figure 22. Distribution of active moiety *concentrations within old (top) vs. new (bottom) reference range (N = 3,505)*

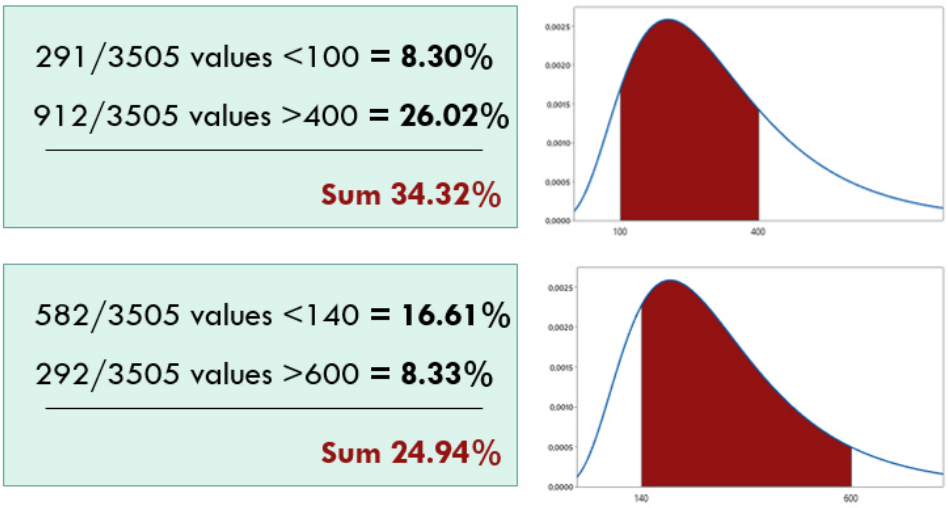
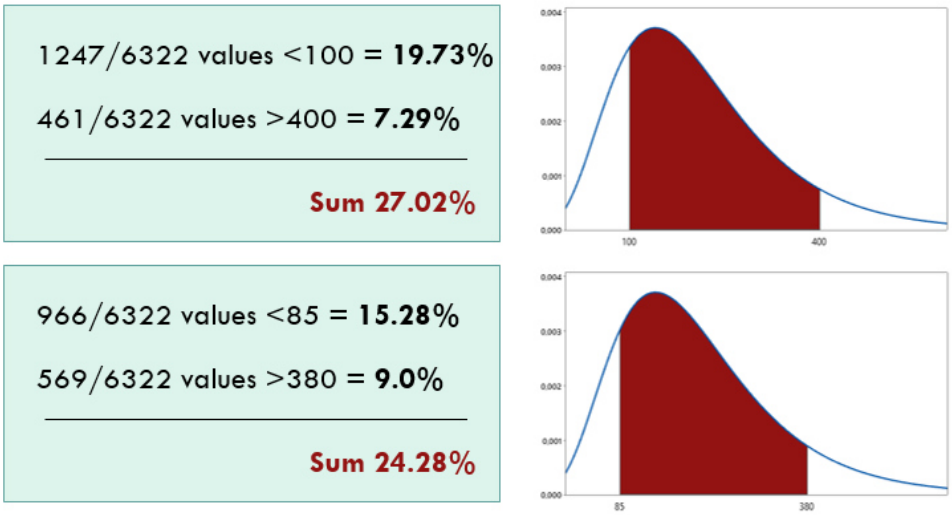


Figure 23. Distribution of O-desmethylvenlafaxine *concentrations within old (top) vs. new (bottom) reference range (N = 6,322)*



4 DISCUSSION

4.1 Therapeutic reference ranges: Scope and clinical implications

The scope of a reference range may be restricted to certain categorical items, for instance the measured analyte, analytical methodology, a dosing scheme, a drug formulation, specific patient populations or the state of a disease. The documentation of these elements gives a minimum requirement when publishing a therapeutic reference range. Optimal drug concentration ranges may depend on reference patients' characteristics such as age and gender or the state of the disease. Although age- and gender-related differences in blood levels have been demonstrated for various psychoactive substances, partitioning of ranges has not been established yet. An example substance for which gender-dependent TDM has been discussed is olanzapine. Weight corrected olanzapine levels were found to be increased by an average of 34% in women compared to men (Weiss, Marksteiner, Kemmler, Saria, & Aichhorn, 2005). For lithium, it is well known that acute treatment requires higher concentrations than the maintenance therapy (Amdisen, 1977; Wilting et al., 2009). For clozapine, despite patients showing up to 40% lower serum concentrations in maintenance as compared to acute treatment (Gaertner, Gaertner, Vonthein, & Dietz, 2001), the published reference range has not been subdivided (Hiemke et al., 2018). An efficacy of lower doses in maintenance treatment compared to acute therapy has been discussed by dose/efficacy metaanalysis for antipsychotic drugs (Leucht et al., 2021; Uchida, Suzuki, Takeuchi, Arenovich, & Mamo, 2011) . In the present work, studies have been included irrespective of former treatment duration. It remains unclear, if this may affect the clinical transferability of the suggested reference ranges.
Naturalistic TDM-studies can provide valuable information about varying drug levels in subgroups, as they usually include a widespread group of patients. By way of conclusion, narrowing the scope of a range may be useful to decrease interindividual variability within partitioned groups and thereby increase the clinical utility of a reported range. A key consideration for the need to partition is the likely effect of TDM in clinical practice.
The validity of a reference range crucially depends on whether a relationship between drug concentration and clinical improvement has been established or not, in particular in regard to the lower limit. When reporting a therapeutic reference range with a lower threshold based on a ROC-analysis, a risk of poor response can be expected at subtherapeutic drug concentrations. ROC-analysis represents so far the ideal method marking a lower therapeutic threshold of a reference range. Yet, it has to be shown whether the drug concentration from a ROC-analysis conforms to the concentration threshold, which indicates the onset of response compared to placebo. An estimate of the number needed to treat was calculated for a discussion of the clinical application of TDM for amitriptyline.
For many psychotropic drugs, a relationship between drug concentration and therapeutic response is not well established. A range for referring individual drug concentrations for these drugs has to be computed from drug concentration data without a clear relation to clinical effects. A preliminary therapeutic reference range refers to a range of drug concentrations in blood that specify a cluster of individual drug concentrations in the blood of patients. Further studies must verify or correct this range (Hiemke et al., 2018).
Another useful tool of TDM is the laboratory alert level. It indicates "drug concentrations above the recommended therapeutic reference range that oblige the laboratory to feedback immediately to the prescribing physician" (Hiemke et al., 2018). It is important to differentiate between upper level of a therapeutic reference range and the laboratory alert level that indicates a safety threshold and is especially important in drugs with a high risk of harm. Reflecting the toxicity

threshold for this drug, the laboratory alert level ideally derives from reports of severe ADRs or intoxications. For most psychotropic drugs, this evidence is still not available. In these cases, the AGNP Consensus Guidelines have calculated laboratory alert levels as twice the upper limit drug concentration (Hiemke et al., 2018). Using low quality evidence or non-evidence based computation, the resultant thresholds have, however small informative value.

4.1.1 Aripiprazole

Aripiprazole parent compound

Data from flexible dose studies showed that about 50% of patients with schizophrenia and related disorders treated under effective doses present aripiprazole trough concentrations (16-24h after last dose) between 120 and 270 ng/ml (Hart et al., 2022). This finding is quite consistent with previously reported interquartile ranges of patients with schizophrenia or schizoaffective disorders in single studies who responded to aripiprazole treatment ("responders"). Response was defined as at least 20% reduction in PANSS scores compared to baseline and was assessed after six weeks of continuous treatment (Lin et al., 2011). Supported by PET studies that report a 90% dopamine D_2 receptor occupancy above 90 - 110 ng/ml, a lower and upper level of 120 ng/ml and 270 ng/ml seems plausible for aripiprazole's therapeutic reference range. Concentrations above the lower limit will increase the probability of response in nonresponders. Concentrations above the upper limit are unlikely to further improve treatment response, but the incidence of adverse events seems equally unlikely to increase.
As stated before, the optimal lower limit for a therapeutic reference range is characterized by a high sensitivity and a high specificity; expressed by the sum-score of both. The validation sample showed that above 120 ng/ml, 80.4% of patients are correctly classified as responders (true positive rate or sensitivity) (Table 5). 19.6% of patients responded below this threshold. A local sensitivity/specify maximum, that is close to the suggested threshold, was found at 115 ng/ml. The 115 and 120 ng/ml threshold both provided a better sum-score than the lower limit of the current reference range of 100 ng/ml.
The optimal upper limit for a therapeutic reference range is characterized by a high value for 1-specificity and a high value for 1-sensitivity. At 270 ng/ml, 29.2% of patients are correctly identified as nonresponders (sensitivity); these patients had concentrations above the nonresponse threshold and did not respond. Accordingly, 70.8% of nonresponders had concentrations below 270 ng/ml. In here, a high specificity is more important indicating patients who respond below this threshold. 74.5% of patients with concentrations below 270 ng/ml responded to drug treatment. Only 25.5% of patients responded above this threshold. Higher thresholds above 270 ng/ml lead to a further decrease in sensitivity (1-specficity) but an increase in specificity.
Interquartile concentration range of patients from the CIMH was *119 - 305 ng/ml* and higher compared to both larger TDM databases from Oslo and Bremen that reported IQRs of 95 - 251 and 102 - 274 ng/ml (Table 6). 43.1% of values (Bremen Data) lie within the new range of 120 - 370 ng/ml whereas 61.3% lie within the old broader range of 100-350 ng/ml (Figure 24). The 75th interquartile concentrations confirmed the applicability of a suggested lower upper threshold of 270 ng/ml in clinical practice when compared to the old range.

Aripiprazole active moiety

The lower limit of the suggested range of the active moiety (180 ng/ml) represents a concentration at which 90% of receptor occupancy is expected, but it also showed a higher sensitivity/specificity score than the other suggested thresholds with 75.5% sensitivity and 32.1%

specificity. From simple pharmacokinetic assumptions, a lower threshold of 170 ng/ml (computed from MPR 0.4 and threshold 120 ng/ml) for the active moiety seems plausible. It is unclear, whether the metabolite dehydroaripiprazole has the same clinical efficacy as aripiprazole alone would have, but it is pharmacodynamically implausible that clinical effects simply add up in case of two substances with differing inhibitory constants at the dopamine D_2 receptor. The upper limit of 380 ng/ml is computed from the metabolite-to-parent compound ratio (MPR 0.4) and represents a pharmacokinetically expected concentration. Lin et al., 2011 reported a 75th interquartile concentration in aripiprazole responders with schizophrenia or schizoaffective disorder that confirms this threshold. In contrast, Jukic et al. report a quite low interquartile range of 129 - 332 ng/ml for the active moiety (N = 1,262, MPR 0.33). As a result, 41.9% and 18.7% of all levels in this sample lie below and above the suggested range of 180 - 380 ng/ml. 31.5% and 8.0% of values lied above the therapeutic reference range of 150 - 500 ng/ml in suggested by former guidelines (Hiemke et al., 2018). A correction of the active moiety reference range towards a lower upper threshold seems plausible. The validation sample showed that above 380 ng/ml, 78.7% of responders showed concentrations below this threshold (sensitivity). 28.6% of patients did not respond with concentrations above this nonresponse threshold meaning 71.4% of nonresponders had concentrations within or below the suggested range of 180 - 380 ng/ml (Table 6).
Population specific differences in pharmacokinetics, expressed by differing MPRs and aripiprazole/active moiety ratios, complicate the clear definition of an upper threshold for the active moiety. Problems may occur in patients that are comedicated with CYP2D6 inhibitors or that are CYP2D6 poor metabolizers. Aripiprazole levels will increase while dehydroaripiprazole levels remain constant. In clinical practice, both levels, aripiprazole and the active moiety drug level, have to be taken into account. Hence, optimal therapeutic efficacy is expected in patients with trough concentrations that lie within the proposed ranges. Some patients might require concentrations above this ranges. As aripiprazole is well tolerated with blood levels exceeding 270 ng/ml (aripiprazole)/370 ng/ml (active moiety), levels above the upper threshold do not require dose reduction in case of good clinical response and tolerance. Starting doses that will in most patients result in drug concentrations within the proposed ranges can be computed from dose/concentration-relationships (Hart et al., 2022). A starting dose of 10 mg will result in effective concentrations in blood and brain of most patients; 5 mg might be sufficient in known CYP2D6 poor metabolizers. Further studies must differentiate patients according to diagnoses i.e. bipolar disorders, schizophrenia, schizoaffective disorders and should also report relevant CYP interfering comedication.

4.1.2 Olanzapine

For olanzapine, the evidence of single studies is most eminent since multiple studies report consistent efficacy thresholds. The highest response rate (defined by a minimum decrease of 20% of PANSS score and constant dosing for one to six weeks, Table 2) is expected above a threshold of 20 ng/ml. Concentrations above this limit will increase the probability of response in nonresponders. PET studies confirm this threshold (19 ng/ml) and at the same time report an upper limit of 40 ng/ml that refers to 80% dopamine D_2 receptor occupancy. This threshold is also confirmed by the 75th interquartile concentration in responders with schizophrenia (Mauri et al., 2005). The validation sample showed that above 20 ng/ml, 77.4% of patients are correctly classified as responders (true positive rate or sensitivity) (Table 7). Local sensitivity/specify maximum was reached at 27 ng/ml. However, sensitivity was below 70% at this threshold. Above 40 ng/ml, 11.5% of patients are correctly identified as nonresponders (sensitivity). 61.3% of patients with concentrations below 40 ng/ml responded to drug treatment

(specificity). Specificity (=1-sensitivity) further decreased with higher thresholds but sensitivity (=1-specificity) decreased to 0% above 52 ng/ml. The 40 ng/ml thresholds provides the best sensitivity/specificity-score when compared with higher thresholds. The interquartile ranges among two real-world datasets were 23 - 58 ng/ml (N = 219) and 20 - 52 ng/ml (N = 5,657). 35.2% of concentrations (Bremen Data) would lie within a smaller range of 20 – 40 ng/ml compared to 64.1% that lie within the current range of 20 - 80 ng/ml. Almost 40% of all drug levels lie above 40 ng/ml (Figure 24). As shown before, dose escalation will most likely not increase the probability of response in those patients with drug concentrations above the 20 ng/ml threshold; but as our data suggest, is still commonly practiced in clinical as well as in study settings to maximize treatment effects. On the other side, olanzapine is well tolerated with blood levels exceeding 40 ng/ml and a serum level above the upper threshold does not necessarily require dose reduction in case of good clinical response and tolerance. Of note, the therapeutic reference range discussed refers to a 12 - 15h sampling time point after once daily dosing and does not reflect trough level conditions. 1.6- fold lower concentrations are expected when sampling 24h post dose (Wesner et al., 2022).

4.1.3 Escitalopram

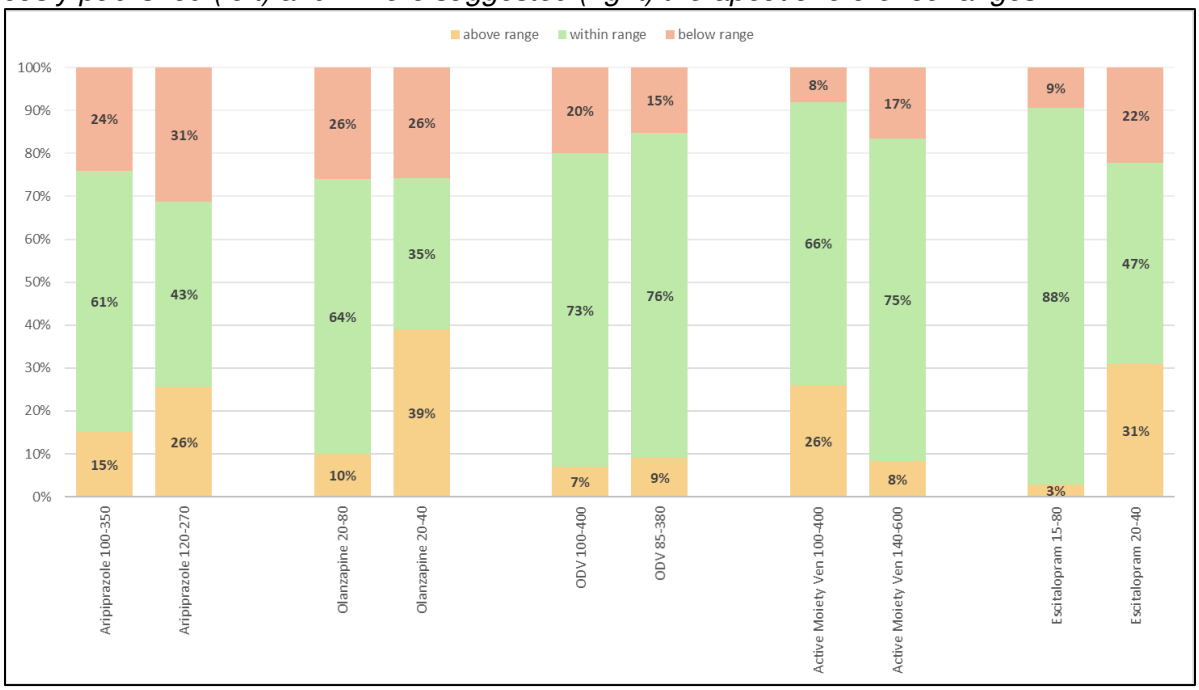
Combined responders to escitalopram treatment, all treated for depression, had an interquartile range between 20 - 40 ng/ml (N = 394). The lower threshold was also confirmed by a ROC analysis (20.5 ng/ml refers to 50% reduction in HAMD-21 after three months of treatment) and by findings from neuroimaging studies (EC_{80} 16 - 18 ng/ml). The validation sample showed that above 20 ng/ml, 75.4% of patients are correctly classified as responders (true positive rate or sensitivity) (Table 9). Local sensitivity/specify maximum was reached at 19 ng/ml. 73.4% of patients with concentrations below 40 ng/ml responded to drug treatment (specificity). Providing a very poor sensitivity of only 2.8%, the 80 ng/ml threshold should be rejected.
TDM data from the Central Institute of Mental Health confirms a threshold of 19 ng/ml in patients (across diagnoses) that were discharged with escitalopram compared to patients that were switched to another or no antidepressant during the hospital stay.
46.8% of concentrations (Bremen Data) would lie within the smaller range of 20 - 40 ng/ml compared to 87.8% that lie within the current range of 15 - 80 ng/ml (Figure 24). In support of the 20 ng/ml threshold, the validation sample showed a higher specificity (23.1%) at 20 ng/ml compared to the low specificity at 15 ng/ml of only 10.1%. As a result, the highest response rate (defined by a minimum decrease of 50% in HAM-D 21 score after three months) is expected above a threshold of 20 ng/ml. The upper threshold most likely reflects a therapeutic optimum of escitalopram that can be easily reached with approved maximum dosage of 20 mg/day. However, some patients might require doses above 20 mg/day to reach the efficacy range and TDM should here be used to guide (off-label) dosing.

4.1.4 Venlafaxine

Our metaanalysis proved a concentration/antidepressant effect-relationship for the active moiety of venlafaxine but not for venlafaxine or O-desmethylvenlafaxine alone. At low doses, venlafaxine predominantly expresses serotonin reuptake inhibiting effects whereas at high doses (≥ 150 mg/day, corresponding to ≥ 170 ng/ml O-desmethlyvenlafaxine and ≥ 282 ng/ml active moiety (Lense et al., 2022), it also acts as a noradrenaline reuptake inhibitor. Based on our results, we suggest a target range of 85 - 380 ng/mL for ODVs' antidepressant efficacy. The lower level hereby indicates an expected concentration from the lowest dose (75 mg/day) recommended for maintenance therapy in real world patients and is furthermore supported by

SERT occupancy findings (EC_{80}) from a neuroimaging study (Frankle et al., 2018). For venlafaxine, 25th interquartile concentrations of patients (144 ng/ml) and of responders (213 ng/ml) to the drug treatment are quite high compared to the SERT occupancy threshold. However, some patients might benefit already from low concentrations and some might require the additional NET actions at higher drug concentrations to reach optimal antidepressant efficacy. A dose titration within the proposed reference range is indicated for venlafaxine in case of insufficient response within the lower part of the range. Even at high doses, the incidence of adverse drug reactions in venlafaxine-treated patients was in general low and the upper level of the reference range is most likely best described by a maximum in clinical response. The suggested upper level of O-desmethylvenlafaxine's efficacy range of 380 ng/ml is based on the 75th interquartile concentration in responders only. The target range of 140 - 600 ng/ml for the active moiety represents a pharmacokinetically expected concentration range (MPR 0.6, N = 2,751). As expected, the validation sample confirmed the lower limit for O-desmethylvenlafaxine's and the active moiety target range being not sensitive in terms of treatment response (Tables 11 and 12). Local sensitivity/specify maxima lie right within the proposed ranges. 81.7% of patients with concentrations below 400 ng/ml (O-desmethylvenlafaxine's) responded to drug treatment (specificity). Above 400 ng/ml, 11.2% of patients are correctly identified as nonresponders (sensitivity). The 600 ng/ml threshold for the active moiety provided similar results with a specificity of 82.9% (sensitivity 12.5%). Interquartile range of real world patient data also lied right within the suggested target ranges for both, O-desmethylvenlafaxine and the active moiety. Around 75 - 76% of all values lie within the newly suggested target ranges (Figure 24). Sex, age and CYP2D6 metabolizer status were identified as clinically relevant factors on venlafaxine, O-desmethylvenlafaxine and active moiety concentrations. Dose related concentrations strongly varied in different trials. As for aripiprazole, patients that are comedicated with CYP2D6 inhibitors, but also CYP2C19 inhibitors or that are CYP2D6 or CYP2C19 poor metabolizers will show increased venlafaxine levels with constant O-desmethylvenlafaxine levels. Polymorphisms in CYP2D6 have furthermore been shown ethnicity related. In clinical practice, both, the O-desmethylvenlafaxine and the active moiety blood levels should be measured and evaluated.

Figure 24. Comparison of percentage of concentration data within, below and above previously published (left) and in here suggested (right) therapeutic reference ranges



4.2 Limitations

For all four exemplary substances, real-world concentration data presented in this work were best described by lognormal distributions. The use of interquartile ranges provides a good estimate for concentration ranges, in which about 50% of patients will lie after flexible dosing (see comparison with curve-fitted quantiles, Table 14). Interquartile ranges from TDM databases usually present patients that are titrated towards an optimal effective concentration by the use of recommended dose regimes. As a result, these ranges comprise indirect information on clinical efficacy but they are also strongly influenced by common clinical practices. Influences in this context are e.g. i) a systematic over- or underdosing in specific patient groups, ii) the inclusion of patients with multiple in-label and off-label diagnoses, iii) multiple samplings per patient, iv) the lack of confirmation of steady-state and trough sampling, and v) the concomitant use of psychiatric comedication and interventions. For olanzapine, a titration towards higher doses in clinical practice resulted in 39% of patients' concentrations being above the upper efficacy threshold that is related to 80% dopamine receptor occupancy. In these patients, the probability of side effects, i.e. extrapyramidal symptoms (EPS) will increase. Problems may also occur when evaluating reference ranges for sum concentrations of parent compounds plus active metabolites as required for venlafaxine and aripiprazole. The present work showed that population-specific differences might have an influence on resulting ranges in dependence of the underlying dataset used to determine a population-based range. For aripiprazole, a metabolite-to-parent compound ratio of 0.45 was reported in the literature. Our metaanalysis among nine studies (N = 3,332) found a ratio of 0.40. Patients included in a large Scandinavian genotyping database had a considerable lower mean ratio of 0.33 (Jukic et al., 2021). A much lower interquartile range derives from this data for the active moiety with 42% of all levels in this sample lying below the suggested range of 180 - 380 ng/ml.

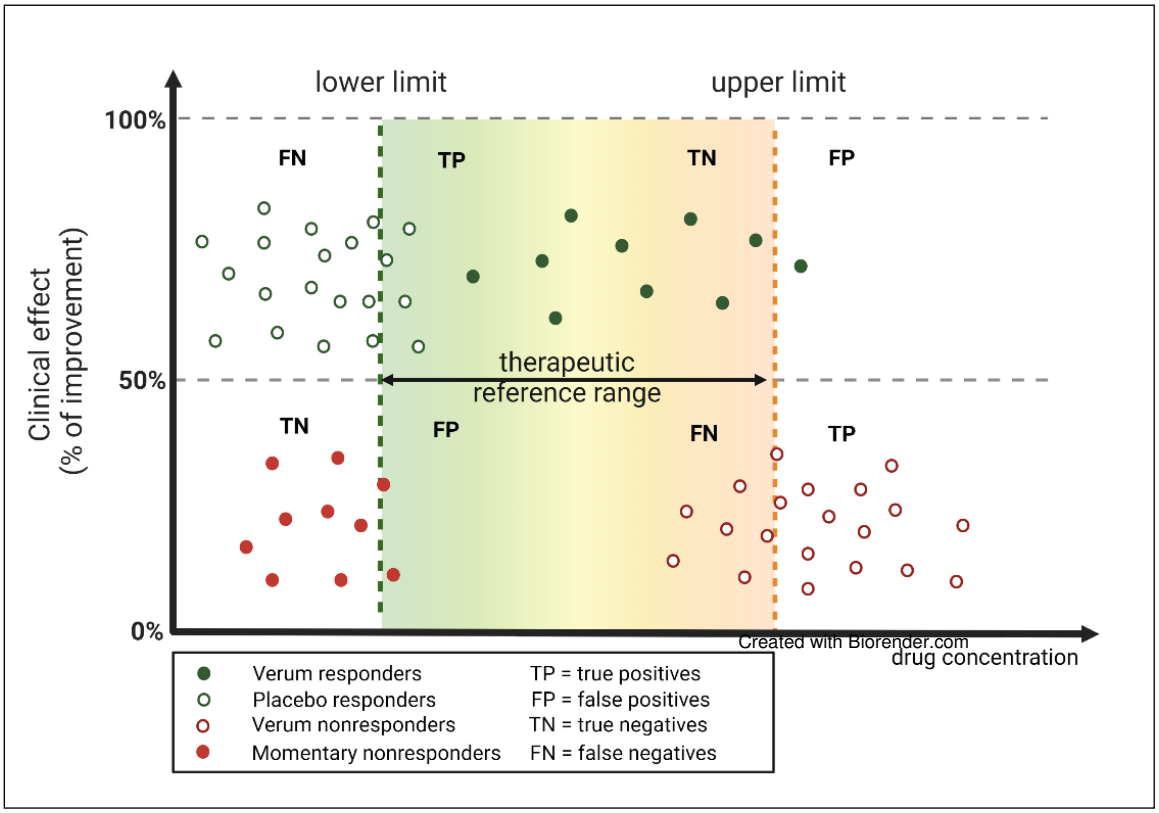
Table 14. Population-based distributions in patients treated in naturalistic settings. Median and interquartile ranges computed from data in differences to median and interquartile ranges from fitted density curve quantiles.

Substance	Dataset	N	Mean, SD (Min-Max)	Median	Diff. Median (%)	Diff. 25/75 IQ (%)	25-75 IQ	TRR old	TRR new	Specifiicity/ sensitivity lower limit (%)	Specifiicity/ sensitivity upper limit (%)
Aripiprazole	Bremen Data, 2022	3,169	208 ± 56 (1-1,410)	170	0	-3/-1	102-274	100-350	**120-270**	32.3/ 80.4	74.5/ 29.2
Aripiprazole	Jukic et al., 2021	1,269	189 ± 139 (1-1,185)	152	+2	-2/-6	95-251	100-350	**120-270**	s.o.	s.o.
ARI active moiety	Jukic et al., 2021	1,262	251 ± 175 (2-1,578)	207	+3	0/-8	129-332	150-500	**180-380**	32.1/ 75.5	78.7/ 28.6
Olanzapine	Bremen Data, 2022	5,657	41 ± 32 (1-427)	32	-13	0/-1	19-53	20-80	**20-40**	42.3/ 77.4	61.3/ 11.5
Escitalopram	Tadić et al., 2016	679	35 ± 20 (5-140)	30	0	0/-1	21-44	15-80	**20-40**	23.1/ 75.4	73.4/ 34.4
VEN active moiety	Bremen Data, 2022	3,505	317 ± 205 (2-1825)	277	-2	-1/ 2	176-409	100-400	**140-600**	3.3/ 100	82.9/ 12.5
O-desmethyl-VEN	Bremen Data, 2022	6,332	208 ± 132 (1-1760)	187	-3	-2/1	117-273	100-400	**85-380**	5.3/ 97.6	81.7/ 11.2

Clinical efficacy data from prospective trials are in general of higher value than retrospectively mined data, however also prone to error as emphasized in chapter 2.1. In his equation, Preskorn identified three main moderators for clinical response that have to be taken into account when performing drug monitoring: i) drug affinity for and activity at the site of action ii) drug concentration at the site of action, and iii) the underlying patient biology (genetics, age, disease) (Preskorn, 2010). As shown in this work, the activity required at the site of action that relates to an optimal therapeutic target range can be precisely estimated from EC values reported in neuroimaging studies (i). To complicate the picture even more in drugs with prominent active metabolites, the interpretation of sum drug concentrations and clinical effects remains obscure. According to common pharmacological assumptions, different affinities to drug targets will result in similar but not in equal intensities in clinical i.e. antidepressant or antipsychotic effects. Linear increase in sum concentrations may not result in a comparable collateral increase in drug effects, as pointed out for the antidepressant drug venlafaxine. A concentration/antidepressant efficacy-relationship was proven for the active moiety, but this finding could neither be replicated for the parent compound nor for the metabolite alone despite using exactly the same patient samples. Studies report a relationship for venlafaxine alone, for venlafaxine plus the active metabolite, or for the metabolite alone. No study found a relation for all of three drug levels. Most studies however have used flexible dosing regimens, which might, as pointed out in chapter 2.1, also have blurred treatment effects. As described, nonresponse is common among psychotropic drug trials and has to be specifically addressed by the methodology used to evaluate data. Two forms of nonresponse exist when evaluating concentration/efficacy-data: i) verum nonresponders: patients that will not respond at subtherapeutic or therapeutic drug concentrations (see Hiemke et al. 2019). ii) momentary nonresponders: patients that will respond at higher drug concentrations but do currently have too low concentrations at the site of action. Verum nonresponders and placebo responders (patients that will respond at subtherapeutic and therapeutic drug concentrations) were purported to capture 1/3 of the patient sample each, meaning that verum responders (patients that respond with sufficient drug concentrations) and momentary nonresponders share the other one third. In flexible dose trials, verum nonresponders (1/3) will be titrated to high concentrations whereas momentary nonresponders (1/6) will in general show low drug concentrations. Verum responders (1/6) will most likely have concentrations within the efficacy range and placebo responders (1/3) will show low drug concentrations (i.e. below the range). When dichotomizing data, only momentary nonresponders compared to verum responders will result in a positive correlation. Taken this into account, the results from sensitivity/specificity analysis should be interpreted with care. ROC curves show a trade-off between true and false positive rates at different thresholds where the sensitivity is high and 1-specificity is low i.e. misclassifications are low. As shown in Figure 25, the false negative (FN) group for a ROC curve finding a lower limit will mainly comprise placebo responders. Sensitivity (true response rate = TP/(TP + FN)) will be affected towards lower values. In addition, the false positive (FP) group will be affected by verum nonresponders with high drug concentrations. 1-specificity (false response rate = FP/(TN + FP) will be biased towards higher values. The resulting ROC thresholds will be biased towards higher drug concentrations. In concordance, reported specificity (true nonresponse) rates are biased towards lower values. These assumptions firstly explain the findings of about 12-24% higher thresholds from ROC analyses when compared to efficacy thresholds from neuroimaging studies in olanzapine, escitalopram and aripiprazole. They also debase negative implications or interpretations from low specificity and sensitivity rates in sensitivity/specificity studies (see Table 14). Interpretation of sensitivity/specificity results for the upper limit is even more challenging since it represents a cut-off dividing verum nonresponders from verum responders (see Figure 25). The true negative (TN) group is hereby influenced by the presence of placebo

responders and the false negative group (FN) is biased by (momentary) nonresponders with corresponding low drug concentrations. As a result, lower values will be computed for the sensitivity and higher values will derive for specificity. Table 14 confirms these assumptions.

Figure 25. Theoretical assumptions on risk for bias when using sensitivity/specificity studies when using clinical trial data



5 CONCLUSION

Unsystematic summaries of existing evidence on the one hand and wrong methodological assumptions, i.e. the use of Gaussian-based descriptive statistics to compute preliminary target ranges, and the disregard of population-specific pharmacokinetics on the other hand, have in the past led to poor reference ranges for psychotropic drugs. The proposed methodology presented in this work sets a new standard on how to find a therapeutic reference range. A short critical view on the reported state of the art of reference ranges is given, including an outlook and discussion of suggested ranges in four highly heterogeneous examples. A therapeutic reference range can be used to titrate a drug`s dose, when it is based upon an established concentration/response-relationship. If a concentration/response-relationship is not well established for a drug, the resultant range needs to be regarded as preliminary and should rather be used as an orienting range than for dose titration. Hence, the methodology, which is used to compute a therapeutic reference range, specifies its scope, validity and clinical utility. For all example drugs, the proposed reference range indicate therapeutic maxima. Serum concentrations above the upper threshold do not require dose reduction in case of good clinical response and tolerance.

6 SUMMARY

A key principle of Therapeutic Drug Monitoring is the comparison of individual drug concentrations in the blood of a patient to a reference system, the drug-specific therapeutic reference range. Inconsistent methodologies concerning the way that reference ranges were determined has led to a high variation of ranges reported in the literature. Reported ranges from previous guidelines are more or less considered experts' opinions. Therapeutic reference ranges yield pharmacodynamic information from a reference population on increased likelihoods for the occurrence of desired drug effects and adverse drug reactions. The present work addresses methodological difficulties, which arise when following this concept. Based on examples from the literature, a methodology for finding a therapeutic reference range is introduced. The most robust method to find a therapeutic reference range is a well-conducted systematic literature review including a meta-analysis of prospective data. However, prospective studies, showing concentration/response-relationships, are scarce. For most psychotropic drugs, a relationship between drug concentration and therapeutic response is not well established. For these drugs, a preliminary range for referring individual drug concentrations can be, for instance, computed using population-based concentration ranges. In this context, retrospective data, ideally comprising pharmacodynamic information, can be helpful. The methodology used to estimate the limits of a reference range determines the validity of this range. Valid ranges are not based solely on a single (concentration efficacy) study. Recommendations should also consider insights from e.g., pharmacokinetic findings and neuroimaging studies. Ranges for four exemplary drugs have been determined and discussed in the present work. Furthermore, datasets from clinical studies and from TDM databases have been used to verify these ranges.

7 SUPPLEMENTARY MATERIAL

1. Supplementary Data Material
   S1. Example calculation for olanzapine using equation 1
   S2. Plot aripiprazole sensitivity/specificity dataset
   S3. Data aripiprazole sensitivity/specificity dataset
   S4. Plot aripiprazole active moiety sensitivity/specificity dataset
   S5 Data aripiprazole active moiety sensitivity/specificity dataset
   S6. Plot olanzapine sensitivity/specificity datasets
   S7. Data olanzapine sensitivity/specificity dataset
   S8. Plot escitalopram sensitivity/specificity dataset
   S9 Data escitalopram sensitivity/specificity dataset
   S10. Plot venlafaxine active moiety sensitivity/specificity dataset
   S11. Data venlafaxine active moiety sensitivity/specificity dataset
   S12. Plot o-desmethylvenlafaxine sensitivity/specificity dataset
   S13. Data o-desmethylvenlafaxine sensitivity/specificity dataset

2. Ethical vote for patient data collection at the CIMH

3. Publication “Therapeutic Reference Ranges for Psychotropic Drugs: A Protocol for Systematic Reviews”

4. Accepted manuscript “Therapeutic Reference Range for Aripiprazole in Schizophrenia Revised: a Systematic: Review and Metaanalysis”

5. Accepted manuscript “Concentrations of escitalopram in blood of patients treated in a naturalistic setting: Focus on patients with alcohol and benzodiazepine use disorder”

6. Publication “Molecular Imaging of Dopamine Partial Agonists in Humans: Implications for Clinical Practice”

8 REFERENCES

Adli, M., Baethge, C., Heinz, A., Langlitz, N., & Bauer, M. (2005). Is dose escalation of antidepressants a rational strategy after a medium-dose treatment has failed? A systematic review. *Eur Arch Psychiatry Clin Neurosci, 255*(6), 387-400. doi:10.1007/s00406-005-0579-5

Ahmed, A. T., Biernacka, J. M., Jenkins, G., Rush, A. J., Shinozaki, G., Veldic, M., . . . Frye, M. A. (2019). Pharmacokinetic-Pharmacodynamic interaction associated with venlafaxine-XR remission in patients with major depressive disorder with history of citalopram / escitalopram treatment failure. *J Affect Disord, 246*, 62-68. doi:10.1016/j.jad.2018.12.021

Amdisen, A. (1977). Serum level monitoring and clinical pharmacokinetics of lithium. *Clin Pharmacokinet, 2*(2), 73-92. doi:10.2165/00003088-197702020-00001

Arakawa, R., Tateno, A., Kim, W., Sakayori, T., Ogawa, K., & Okubo, Y. (2016). Time-course of serotonin transporter occupancy by single dose of three SSRIs in human brain: A positron emission tomography study with [(11)C]DASB. *Psychiatry Res Neuroimaging, 251*, 1-6. doi:10.1016/j.pscychresns.2016.03.006

Asberg, M., Crönholm, B., Sjöqvist, F., & Tuck, D. (1971). Relationship between plasma level and therapeutic effect of nortriptyline. *Br Med J, 3*(5770), 331-334. doi:10.1136/bmj.3.5770.331

Bengtsson, F. (2004). Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit, 26*(2), 145-151.

Berm, E., Kok, R., Hak, E., & Wilffert, B. (2016). Relation between CYP2D6 Genotype, Phenotype and Therapeutic Drug Concentrations among Nortriptyline and Venlafaxine Users in Old Age Psychiatry. *Pharmacopsychiatry, 49*(5), 186-190. doi:10.1055/s-0042-105443

Buclin, T., Gotta, V., Fuchs, A., Widmer, N., & Aronson, J. (2012). An agenda for UK clinical pharmacology: Monitoring drug therapy. *British Journal of Clinical Pharmacology, 73*(6), 917-923. doi:https://doi.org/10.1111/j.1365-2125.2012.04237.x

Callaghan, J. T., Bergstrom, R. F., Ptak, L. R., & Beasley, C. M. (1999). Olanzapine. Pharmacokinetic and pharmacodynamic profile. *Clin Pharmacokinet, 37*(3), 177-193. doi:10.2165/00003088-199937030-00001

Carrillo, J. A., Herráiz, A. G., Ramos, S. I., Gervasini, G., Vizcaíno, S., & Benítez, J. (2003). Role of the smoking-induced cytochrome P450 (CYP)1A2 and polymorphic CYP2D6 in steady-state concentration of olanzapine. *J Clin Psychopharmacol, 23*(2), 119-127. doi:10.1097/00004714-200304000-00003

Cellini, L., De Donatis, D., Zernig, G., De Ronchi, D., Giupponi, G., Serretti, A., . . . Florio, V. (2022). Antidepressant efficacy is correlated with plasma levels: mega-analysis and further evidence. *Int Clin Psychopharmacol, 37*(2), 29-37. doi:10.1097/yic.0000000000000386

Charlier, C., Pinto, E., Ansseau, M., & Plomteux, G. (2002). Venlafaxine: the relationship between dose, plasma concentration and clinical response in depressive patients. *J Psychopharmacol, 16*(4), 369-372. doi:10.1177/026988110201600413

Cooney, L., Loke, Y. K., Golder, S., Kirkham, J., Jorgensen, A., Sinha, I., & Hawcutt, D. (2017). Overview of systematic reviews of therapeutic ranges: methodologies and recommendations for practice. *BMC Med Res Methodol, 17*(1), 84. doi:10.1186/s12874-017-0363-z

Cumming, P., Abi-Dargham, A., & Gründer, G. (2021). Molecular imaging of schizophrenia: Neurochemical findings in a heterogeneous and evolving disorder. *Behav Brain Res, 398*, 113004. doi:10.1016/j.bbr.2020.113004

Dawling, S. (1982). Monitoring of tricyclic antidepressant therapy. *Clin Biochem, 15*(1), 56-61. doi:10.1016/s0009-9120(82)90511-2

De Donatis, D., Florio, V., Porcelli, S., Saria, A., Mercolini, L., Serretti, A., & Conca, A. (2019). Duloxetine plasma level and antidepressant response. *Prog Neuropsychopharmacol Biol Psychiatry, 92*, 127-132. doi:10.1016/j.pnpbp.2019.01.001

De Donatis, D., Porcelli, S., Zernig, G., Mercolini, L., Giupponi, G., Serretti, A., . . . Florio, V. (2021). Venlafaxine and O-desmethylvenlafaxine serum levels are positively associated with antidepressant response in elder depressed out-patients. *World J Biol Psychiatry*, 1-8. doi:10.1080/15622975.2021.1938668

Derendorf, H., & Meibohm, B. (1999). Modeling of Pharmacokinetic/Pharmacodynamic (PK/PD) Relationships: Concepts and Perspectives. *Pharmaceutical Research, 16*(2), 176-185. doi:10.1023/A:1011907920641

Eggart, V., Hiemke, C., & Zernig, G. (2011). "There is no dose-response relationship in psychopharmacotherapy" vs "pharmacotherapy in psychiatry is based on ligand-receptor interaction": a unifying hypothesis and the need for plasma concentration based clinical trials. *Psychopharmacology (Berl), 217*(2), 297-300. doi:10.1007/s00213-011-2319-z

Eichentopf, L., Hiemke, C., Conca, A., Engelmann, J., Gerlach, M., Havemann-Reinecke, U., . . . Hart, X. M. (2022). Systematic review on the therapeutic reference range for escitalopram: blood concentrations, effects and SERT occupancy [accepted for publication at Frontiers in Psychiatry].

Engelmann, J., Wagner, S., Solheid, A., Herzog, D. P., Dreimüller, N., Müller, M. B., . . . Lieb, K. (2021). Tolerability of High-Dose Venlafaxine After Switch From Escitalopram in Nonresponding Patients With Major Depressive Disorder. *J Clin Psychopharmacol, 41*(1), 62-66. doi:10.1097/jcp.0000000000001312

Fellows, L., Ahmad, F., Castle, D. J., Dusci, L. J., Bulsara, M. K., & Ilett, K. F. (2003). Investigation of target plasma concentration-effect relationships for olanzapine in schizophrenia. *Ther Drug Monit, 25*(6), 682-689. doi:10.1097/00007691-200312000-00006

Florio, V., Porcelli, S., Saria, A., Serretti, A., & Conca, A. (2017). Escitalopram plasma levels and antidepressant response. *Eur Neuropsychopharmacol, 27*(9), 940-944. doi:10.1016/j.euroneuro.2017.06.009

Frankle, W. G., Robertson, B., Maier, G., Paris, J., Asmonga, D., May, M., . . . Narendran, R. (2018). An open-label positron emission tomography study to evaluate serotonin transporter occupancy following escalating dosing regimens of (R)-(-)-O-desmethylvenlafaxine and racemic O-desmethylvenlafaxine. *Synapse, 72*(3). doi:10.1002/syn.22021

Funk, C. S. M., Hart, X. M., Gründer, G., Hiemke, C., Elsner, B., Kreutz, R., & Riemer, T. G. (2022). Is Therapeutic Drug Monitoring Relevant for Antidepressant Drug Therapy? Implications From a Systematic Review and Meta-Analysis With Focus on Moderating Factors. *Front Psychiatry, 13*, 826138. doi:10.3389/fpsyt.2022.826138

Gärtner, I., Gärtner, H. J., Vonthein, R., & Dietz, K. (2001). Therapeutic drug monitoring of clozapine in relapse prevention: a five-year prospective study. *J Clin Psychopharmacol, 21*(3), 305-310.

Gründer, G., Hiemke, C., Paulzen, M., Veselinovic, T., & Vernaleken, I. (2011). Therapeutic plasma concentrations of antidepressants and antipsychotics: lessons from PET imaging. *Pharmacopsychiatry, 44*(6), 236-248. doi:10.1055/s-0031-1286282

Gupta, S. K., Shah, J. C., & Hwang, S. S. (1999). Pharmacokinetic and pharmacodynamic characterization of OROS and immediate-release amitriptyline. *Br J Clin Pharmacol, 48*(1), 71-78. doi:10.1046/j.1365-2125.1999.00973.x

Guy, W. N. I. o. M. H. P. R. B. E. C. D. E. P. (1976). *ECDEU assessment manual for psychopharmacology*. Rockville, Md.: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs.

Hart, X. M., Brückner, A., Dörfler, S., Wedekind, D., Heesen, S., Schmitz, C., . . . Havemann-Reinecke, U. (2022). Concentrations of escitalopram in blood of patients treated in a naturalistic setting: Focus on patients with alcohol and benzodiazepine use disorder [accepted for publication at European Archives of Psychiatry and Clinical Neuroscience].

Hart, X. M., Eichentopf, L., Lense, X., Riemer, T., Wesner, K., Hiemke, C., & Gründer, G. (2021). Therapeutic Reference Ranges for Psychotropic Drugs: A Protocol for Systematic Reviews. *Front Psychiatry, 12*, 787043. doi:10.3389/fpsyt.2021.787043

Hart, X. M., Hiemke, C., Eichentopf, L., Lense, X. M., Clement, H. W., Conca, A., . . . Gründer, G. (2022). Therapeutic Reference Range for Aripiprazole Revised: a Systematic Review and Metaanalysis [accepted for publication at Psychopharmacology].

Hart, X. M., Schmitz, C. N., & Gründer, G. (2022). Molecular Imaging of Dopamine Partial Agonists in Humans: Implications for Clinical Practice. *Frontiers in Psychiatry, 13*. doi:10.3389/fpsyt.2022.832209

Härtter, S., Wetzel, H., Hammes, E., Torkzadeh, M., & Hiemke, C. (1998). Serum concentrations of fluvoxamine and clinical effects. A prospective open clinical trial. *Pharmacopsychiatry, 31*(5), 199-200. doi:10.1055/s-2007-979327

Hasan, A., Bandelow, B., Yatham, L. N., Berk, M., Falkai, P., Moller, H. J., & Kasper, S. (2019). WFSBP guidelines on how to grade treatment evidence for clinical guideline development. *World J Biol Psychiatry, 20*(1), 2-16. doi:10.1080/15622975.2018.1557346

Hegerl, U., Bottlender, R., Gallinat, J., Kuss, H. J., Ackenheil, M., & Moller, H. J. (1998). The serotonin syndrome scale: first results on validity. *Eur Arch Psychiatry Clin Neurosci, 248*(2), 96-103.

Hiemke, C. (2019). Concentration-Effect Relationships of Psychoactive Drugs and the Problem to Calculate Therapeutic Reference Ranges. *Ther Drug Monit, 41*(2), 174-179. doi:10.1097/ftd.0000000000000582

Hiemke, C., Bergemann, N., Clement, H. W., Conca, A., Deckert, J., Domschke, K., . . . Baumann, P. (2018). Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry, 51*(1-02), 9-62. doi:10.1055/s-0043-116492

Hilal-Dandan, R., & Brunton, L. (2014). *Goodman and Gilman's Manual of Pharmacology and Therapeutics, 2e.*: McGraw Hill.

Hodgson, K., Tansey, K., Dernovsek, M. Z., Hauser, J., Henigsberg, N., Maier, W., . . . McGuffin, P. (2014). Genetic differences in cytochrome P450 enzymes and antidepressant treatment response. *J Psychopharmacol, 28*(2), 133-141. doi:10.1177/0269881113512041

Hoencamp, E., Haffmans, J., Dijken, W. A., & Huijbrechts, I. P. (2000). Lithium augmentation of venlafaxine: an open-label trial. *J Clin Psychopharmacol, 20*(5), 538-543. doi:10.1097/00004714-200010000-00008

Huguet, J., Castiñeiras, M. J., & Fuentes-Arderiu, X. (1993). Diagnostic accuracy evaluation using ROC curve analysis. *Scand J Clin Lab Invest, 53*(7), 693-699. doi:10.3109/00365519309092573

Jukić, M. M., Smith, R. L., Molden, E., & Ingelman-Sundberg, M. (2021). Evaluation of the CYP2D6 Haplotype Activity Scores Based on Metabolic Ratios of 4,700 Patients Treated With Three Different CYP2D6 Substrates. *Clin Pharmacol Ther, 110*(3), 750-758. doi:10.1002/cpt.2246

Kagawa, S., Mihara, K., Nakamura, A., Nemoto, K., Suzuki, T., Nagai, G., & Kondo, T. (2014). Relationship between plasma concentrations of lamotrigine and its early therapeutic effect of lamotrigine augmentation therapy in treatment-resistant depressive disorder. *Ther Drug Monit, 36*(6), 730-733. doi:10.1097/ftd.0000000000000088

Kapur, S., Zipursky, R. B., Remington, G., Jones, C., DaSilva, J., Wilson, A. A., & Houle, S. (1998). 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiatry, 155*(7), 921-928. doi:10.1176/ajp.155.7.921

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull, 13*(2), 261-276. doi:10.1093/schbul/13.2.261

Khan, A., Khan, S. R., Walens, G., Kolts, R., & Giller, E. L. (2003). Frequency of positive studies among fixed and flexible dose antidepressant clinical trials: an analysis of the food and drug administration summary basis of approval reports. *Neuropsychopharmacology, 28*(3), 552-557. doi:10.1038/sj.npp.1300059

Kim, E., Howes, O. D., Kim, B. H., Chon, M. W., Seo, S., Turkheimer, F. E., . . . Kwon, J. S. (2017). Regional Differences in Serotonin Transporter Occupancy by Escitalopram: An [(11)C]DASB PK-PD Study. *Clin Pharmacokinet, 56*(4), 371-381. doi:10.1007/s40262-016-0444-x

Kirschbaum, K. M., Müller, M. J., Malevani, J., Mobascher, A., Burchardt, C., Piel, M., & Hiemke, C. (2008). Serum levels of aripiprazole and dehydroaripiprazole, clinical response and side effects. *World J Biol Psychiatry, 9*(3), 212-218. doi:10.1080/15622970701361255

Klein H.-G., H. E. (2018). *Pharmakogenetik und Therapeutisches Drug Monitoring* (W. d. G. GmbH Ed.). Berlin.

Korell, J., Green, B., Rae, A., Remmerie, B., & Vermeulen, A. (2018). Determination of plasma concentration reference ranges for oral aripiprazole, olanzapine, and quetiapine. *Eur J Clin Pharmacol, 74*(5), 593-599. doi:10.1007/s00228-018-2419-3

Lanzenberger, R., Kranz, G. S., Haeusler, D., Akimova, E., Savli, M., Hahn, A., . . . Kasper, S. (2012). Prediction of SSRI treatment response in major depression based on serotonin transporter interplay between median raphe nucleus and projection areas. *Neuroimage, 63*(2), 874-881. doi:10.1016/j.neuroimage.2012.07.023

Lense, X. M., Gründer, G., Riemer, T., Funk, C., Hefner, G., Hiemke, C., & Hart, X. M. (2022). Concentration/ antidepressant effect relationship for Venlafaxine [in preparation].

Leucht, S., Bauer, S., Siafis, S., Hamza, T., Wu, H., Schneider-Thoma, J., . . . Davis, J. M. (2021). Examination of Dosing of Antipsychotic Drugs for Relapse Prevention in Patients With Stable Schizophrenia: A Meta-analysis. *JAMA Psychiatry, 78*(11), 1238-1248. doi:10.1001/jamapsychiatry.2021.2130

Leucht, S., Steimer, W., Kreuz, S., Abraham, D., Orsulak, P. J., & Kissling, W. (2001). Doxepin plasma concentrations: is there really a therapeutic range? *J Clin Psychopharmacol, 21*(4), 432-439. doi:10.1097/00004714-200108000-00011

Limbach, L. (2022). Service specification [accessed on April 22, 2022]. Retrieved from https://lv.limbachgruppe.com/onlinelvz/11_de#/detailView/6238

Lin, S. K., Chen, C. K., & Liu, Y. L. (2011). Aripiprazole and dehydroaripiprazole plasma concentrations and clinical responses in patients with schizophrenia. *J Clin Psychopharmacol, 31*(6), 758-762. doi:10.1097/JCP.0b013e3182356255

Lingjaerde, O., Ahlfors, U. G., Bech, P., Dencker, S. J., & Elgen, K. (1987). The UKU side effect rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. *Acta Psychiatr Scand Suppl, 334*, 1-100. doi:10.1111/j.1600-0447.1987.tb10566.x

Lopez, L. V., & Kane, J. M. (2013). Plasma levels of second-generation antipsychotics and clinical response in acute psychosis: a review of the literature. *Schizophr Res, 147*(2-3), 368-374. doi:10.1016/j.schres.2013.04.002

Lu, M. L., Wu, Y. X., Chen, C. H., Kuo, P. T., Chen, Y. H., Lin, C. H., & Wu, T. H. (2016). Application of Plasma Levels of Olanzapine and N-Desmethyl-Olanzapine to Monitor Clinical Efficacy in Patients with Schizophrenia. *PLoS One, 11*(2), e0148539. doi:10.1371/journal.pone.0148539

Mamo, D., Kapur, S., Keshavan, M., Laruelle, M., Taylor, C. C., Kothare, P. A., . . . McDonnell, D. (2008). D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. *Neuropsychopharmacology, 33*(2), 298-304. doi:10.1038/sj.npp.1301409

Mathew, T., & Nordström, K. (1999). On the equivalence of meta-analysis using literature and using individual patient data. *Biometrics, 55*(4), 1221-1223. doi:10.1111/j.0006-341x.1999.01221.x

Mauri, M. C., Steinhilber, C. P., Marino, R., Invernizzi, E., Fiorentini, A., Cerveri, G., . . . Barale, F. (2005). Clinical outcome and olanzapine plasma levels in acute schizophrenia. *Eur Psychiatry, 20*(1), 55-60. doi:10.1016/j.eurpsy.2004.09.009

Mauri, M. C., Volonteri, L. S., Fiorentini, A., Pirola, R., & Bareggi, S. R. (2007). Two weeks' quetiapine treatment for schizophrenia, drug-induced psychosis and borderline personality disorder: a naturalistic study with drug plasma levels. *Expert Opin Pharmacother, 8*(14), 2207-2213. doi:10.1517/14656566.8.14.2207

Müller, M. J., Eich, F. X., Regenbogen, B., Sachse, J., Härtter, S., & Hiemke, C. (2009). Amisulpride doses and plasma levels in different age groups of patients with schizophrenia or schizoaffective disorder. *J Psychopharmacol, 23*(3), 278-286. doi:10.1177/0269881108089806

Müller, M. J., Regenbogen, B., Härtter, S., Eich, F. X., & Hiemke, C. (2007). Therapeutic drug monitoring for optimizing amisulpride therapy in patients with schizophrenia. *J Psychiatr Res, 41*(8), 673-679. doi:10.1016/j.jpsychires.2005.10.003

Ostad Haji, E., Tadic, A., Wagner, S., Dragicevic, A., Müller, M. J., Boland, K., . . . Hiemke, C. (2011). Association between citalopram serum levels and clinical improvement of patients with major depression. *J Clin Psychopharmacol, 31*(3), 281-286. doi:10.1097/JCP.0b013e318218f503

Palao, D. J., Arauxo, A., Brunet, M., Bernardo, M., Haro, J. M., Ferrer, J., & Gonzalez-Monclus, E. (1994). Haloperidol: therapeutic window in schizophrenia. *J Clin Psychopharmacol, 14*(5), 303-310.

Patsalos, P. N., Spencer, E. P., & Berry, D. J. (2018). Therapeutic Drug Monitoring of Antiepileptic Drugs in Epilepsy: A 2018 Update. *Ther Drug Monit, 40*(5), 526-548. doi:10.1097/ftd.0000000000000546

Perry, P. J., Lund, B. C., Sanger, T., & Beasley, C. (2001). Olanzapine plasma concentrations and clinical response: acute phase results of the North American Olanzapine Trial. *J Clin Psychopharmacol, 21*(1), 14-20. doi:10.1097/00004714-200102000-00004

Perry, P. J., Sanger, T., & Beasley, C. (1997). Olanzapine plasma concentrations and clinical response in acutely ill schizophrenic patients. *J Clin Psychopharmacol, 17*(6), 472-477. doi:10.1097/00004714-199712000-00006

Perry, P. J., Zeilmann, C., & Arndt, S. (1994). Tricyclic antidepressant concentrations in plasma: an estimate of their sensitivity and specificity as a predictor of response. *J Clin Psychopharmacol, 14*(4), 230-240.

Preskorn, S. H. (2010). Outliers on the dose-response curve: how to minimize this problem using therapeutic drug monitoring, an underutilized tool in psychiatry. *J Psychiatr Pract, 16*(3), 177-182. doi:10.1097/01.pra.0000375714.93078.a8

Preskorn, S. H. (2014). Therapeutic Drug Monitoring (TDM) in psychiatry (part I): why studies attempting to correlate drug concentration and antidepressant response don't work. *J Psychiatr Pract, 20*(2), 133-137. doi:10.1097/01.pra.0000445247.54048.68

Rao, V. A., Bishop, M., & Coppen, A. (1980). Clinical state, plasma levels of haloperidol and prolactin: a correlation study in chronic schizophrenia. *Br J Psychiatry, 137*, 518-521. doi:10.1192/bjp.137.6.518

Rasmussen, B. B., & Brosen, K. (2000). Is therapeutic drug monitoring a case for optimizing clinical outcome and avoiding interactions of the selective serotonin reuptake inhibitors? *Ther Drug Monit, 22*(2), 143-154.

Santos, J. L., Cabranes, J. A., Vazquez, C., Fuentenebro, F., Almoguera, I., & Ramos, J. A. (1989). Clinical response and plasma haloperidol levels in chronic and subchronic schizophrenia. *Biol Psychiatry, 26*(4), 381-388. doi:10.1016/0006-3223(89)90054-1

Scherf-Clavel, M., Hommers, L., Wurst, C., Stonawski, S., Deckert, J., Domschke, K., . . . Menke, A. (2020). Higher venlafaxine serum concentrations necessary for clinical improvement? Time to re-evaluate the therapeutic reference range of venlafaxine. *J Psychopharmacol, 34*(10), 1105-1111. doi:10.1177/0269881120936509

Schoretsanitis, G., Haen, E., Gründer, G., Hiemke, C., Endres, K., Ridders, F., . . . Paulzen, M. (2019). Pharmacokinetics of venlafaxine in treatment responders and non-responders: a retrospective analysis of a large naturalistic database. *Eur J Clin Pharmacol, 75*(8), 1109-1116. doi:10.1007/s00228-019-02675-4

Solmi, M., Murru, A., Pacchiarotti, I., Undurraga, J., Veronese, N., Fornaro, M., . . . Carvalho, A. F. (2017). Safety, tolerability, and risks associated with first- and second-generation antipsychotics: a state-of-the-art clinical review. *Ther Clin Risk Manag, 13*, 757-777. doi:10.2147/tcrm.S117321

Stamm, T. J., Becker, D., Sondergeld, L. M., Wiethoff, K., Hiemke, C., O'Malley, G., . . . Adli, M. (2014). Prediction of antidepressant response to venlafaxine by a combination of

early response assessment and therapeutic drug monitoring. *Pharmacopsychiatry, 47*(4-5), 174-179. doi:10.1055/s-0034-1383565

Sung, Y. J., Schwander, K., Arnett, D. K., Kardia, S. L., Rankinen, T., Bouchard, C., . . . Rao, D. C. (2014). An empirical comparison of meta-analysis and mega-analysis of individual participant data for identifying gene-environment interactions. *Genet Epidemiol, 38*(4), 369-378. doi:10.1002/gepi.21800

Tadić, A., Wachtlin, D., Berger, M., Braus, D. F., van Calker, D., Dahmen, N., . . . Lieb, K. (2016). Randomized controlled study of early medication change for non-improvers to antidepressant therapy in major depression--The EMC trial. *Eur Neuropsychopharmacol, 26*(4), 705-716. doi:10.1016/j.euroneuro.2016.02.003

Tasker, T. C., Kaye, C. M., Zussman, B. D., & Link, C. G. (1989). Paroxetine plasma levels: lack of correlation with efficacy or adverse events. *Acta Psychiatr Scand Suppl, 350*, 152-155. doi:10.1111/j.1600-0447.1989.tb07201.x

Tomita, T., Yasui-Furukori, N., Nakagami, T., Tsuchimine, S., Ishioka, M., Kaneda, A., . . . Kaneko, S. (2014). Therapeutic reference range for plasma concentrations of paroxetine in patients with major depressive disorders. *Ther Drug Monit, 36*(4), 480-485. doi:10.1097/ftd.0000000000000036

Uchida, H., Suzuki, T., Takeuchi, H., Arenovich, T., & Mamo, D. C. (2011). Low dose vs standard dose of antipsychotics for relapse prevention in schizophrenia: meta-analysis. *Schizophr Bull, 37*(4), 788-799. doi:10.1093/schbul/sbp149

Ulrich, S., Baumann, B., Wolf, R., Lehmann, D., Peters, B., Bogerts, B., & Meyer, F. P. (2003). Therapeutic drug monitoring of clozapine and relapse--a retrospective study of routine clinical data. *Int J Clin Pharmacol Ther, 41*(1), 3-13. doi:10.5414/cpp41003

Ulrich, S., & Lauter, J. (2002). Comprehensive survey of the relationship between serum concentration and therapeutic effect of amitriptyline in depression. *Clin Pharmacokinet, 41*(11), 853-876. doi:10.2165/00003088-200241110-00004

Ulrich, S., Wurthmann, C., Brosz, M., & Meyer, F. P. (1998). The relationship between serum concentration and therapeutic effect of haloperidol in patients with acute schizophrenia. *Clin Pharmacokinet, 34*(3), 227-263. doi:10.2165/00003088-199834030-00005

VanderZwaag, C., McGee, M., McEvoy, J. P., Freudenreich, O., Wilson, W. H., & Cooper, T. B. (1996). Response of patients with treatment-refractory schizophrenia to clozapine within three serum level ranges. *Am J Psychiatry, 153*(12), 1579-1584. doi:10.1176/ajp.153.12.1579

Volavka, J., Cooper, T. B., Czobor, P., & Meisner, M. (1996). Effect of varying haloperidol plasma levels on negative symptoms in schizophrenia and schizoaffective disorder. *Psychopharmacol Bull, 32*(1), 75-79.

Waldschmitt, C., Vogel, F., Pfuhlmann, B., & Hiemke, C. (2009). Duloxetine serum concentrations and clinical effects. Data from a therapeutic drug monitoring (TDM) survey. *Pharmacopsychiatry, 42*(5), 189-193. doi:10.1055/s-0029-1220890

Weiss, U., Marksteiner, J., Kemmler, G., Saria, A., & Aichhorn, W. (2005). Effects of age and sex on olanzapine plasma concentrations. *J Clin Psychopharmacol, 25*(6), 570-574.

Wesner, K., Hiemke, C., Bergemann, N., Fekete, S., Gerlach, M., Havemann-Reinecke, U., . . . Hart, X. M. (2022). New therapeutic reference range for olanzapine - A systematic review and metaanalysis [under review].

Wilting, I., Heerdink, E. R., Mersch, P. P., den Boer, J. A., Egberts, A. C., & Nolen, W. A. (2009). Association between lithium serum level, mood state, and patient-reported adverse drug reactions during long-term lithium treatment: a naturalistic follow-up study. *Bipolar Disord, 11*(4), 434-440. doi:10.1111/j.1399-5618.2009.00699.x

Woods, S. W., Gueorguieva, R. V., Baker, C. B., & Makuch, R. W. (2005). Control group bias in randomized atypical antipsychotic medication trials for schizophrenia. *Arch Gen Psychiatry, 62*(9), 961-970. doi:10.1001/archpsyc.62.9.961

Yasui-Furukori, N., Nakagami, T., Kaneda, A., Inoue, Y., Suzuki, A., Otani, K., & Kaneko, S. (2011). Inverse correlation between clinical response to paroxetine and plasma drug concentration in patients with major depressive disorders. *Hum Psychopharmacol, 26*(8), 602-608. doi:10.1002/hup.1252

Yasui-Furukori, N., Saito, M., Nakagami, T., Furukori, H., Suzuki, A., Kondo, T., & Kaneko, S. (2010). Clinical response to risperidone in relation to plasma drug concentrations in acutely exacerbated schizophrenic patients. *J Psychopharmacol, 24*(7), 987-994. doi:10.1177/0269881109104849

Yin, O. Q., Wing, Y. K., Cheung, Y., Wang, Z. J., Lam, S. L., Chiu, H. F., & Chow, M. S. (2006). Phenotype-genotype relationship and clinical effects of citalopram in Chinese patients. *J Clin Psychopharmacol, 26*(4), 367-372. doi:10.1097/01.jcp.0000227355.54074.14

Zabala, A., Bustillo, M., Querejeta, I., Alonso, M., Mentxaka, O., Gonzalez-Pinto, A., . . . Segarra, R. (2017). A Pilot Study of the Usefulness of a Single Olanzapine Plasma Concentration as an Indicator of Early Drug Effect in a Small Sample of First-Episode Psychosis Patients. *J Clin Psychopharmacol, 37*(5), 569-577. doi:10.1097/jcp.0000000000000770

Zernig, G., & Hiemke, C. (2020). Pharmacokinetic and Pharmacodynamic Principles. In P. Riederer, G. Laux, T. Nagatsu, W. Le, & C. Riederer (Eds.), *NeuroPsychopharmacotherapy* (pp. 1-19). Cham: Springer International Publishing.

9 CURRICULUM VITAE

Name:	Xenia Marlene Hart
Date of birth:	1 February 1994 in Wiesbaden
Address:	Department of Molecular Neuroimaging Central Institute of Mental Health University of Heidelberg J 5, 68159 Mannheim Phone: +49-621-1703-6061 E-mail: xenia@xeniahart.de

Current position:	Research Assistant
Since 05/2018	Research Assistant and PhD student in the Department of Molecular Neuroimaging at the Central Institute of Mental Health
12/2017	Johannes Gutenberg University Mainz Degree as registered medical pharmacist
05/2017 – 11/2017	6 months pre-registration training in pharmaceutical industry, Pfizer Manufacturing GmbH, Freiburg
11/2016 – 05/2017	6 months pre-registration training in public pharmacy Neue Apotheke, Wiesbaden
10/2014 – 10/2016	Johannes Gutenberg University Mainz Main study period, pharmacy degree
10/2012 – 09/2014	University of Würzburg Basic study period, pharmacy degree

10 ACKNOWLEDGEMENT

I would like to thank Prof. Dr. Gerhard Gründer for providing me with the topic of this thesis and the excellent opportunities to work on it, for important factual guidance and suggestions on the way to this dissertation.

I would also like to thank the Central Institute of Mental Health in Mannheim (directed by Prof. Dr. Andreas Meyer-Lindenberg) for the excellent opportunities for clinical research.

For the support and provision of laboratory results I would like to thank the Limbach Laboratory in Heidelberg, especially Prof. Dr. Peter Findeisen and Jonas Brand.

I also owe special thanks to Professor Dr. Christoph Hiemke from Mainz, who often helped me to understand correlations and tirelessly gave me important feedback over the years.

I thank my family and friends, but especially my parents, for constant support and encouragement to always strive for higher goals.

SUPPLEMENTARY MATERIAL

HOW TO FIND AND VALIDATE THERAPEUTIC REFERENCE RANGES FOR PSYCHOTROPIC DRUGS

1. SUPPLEMENTARY DATA MATERIAL

S1. Example calculation for olanzapine using equation 1.

Pharmacokinetic data suggest a mean plasma CL/F of 372 ml/min (Hiemke et al., 2018), considering the before mentioned, 5 mg/ once daily would be expected to yield 9 ng/ml (Cmin 7 ng/mL). For the 20 mg dose the expected concentration is 37 ng/mL (Cmin 29 ng/mL).

Cl/F = 372 ml/min = 22,32 l/h $t_{1/2}$ = 33 h t = 12 h $k_e = \frac{\ln(2)}{t_{1/2}} = \frac{\ln(2)}{33h} = 0{,}021\ h^{-1}$

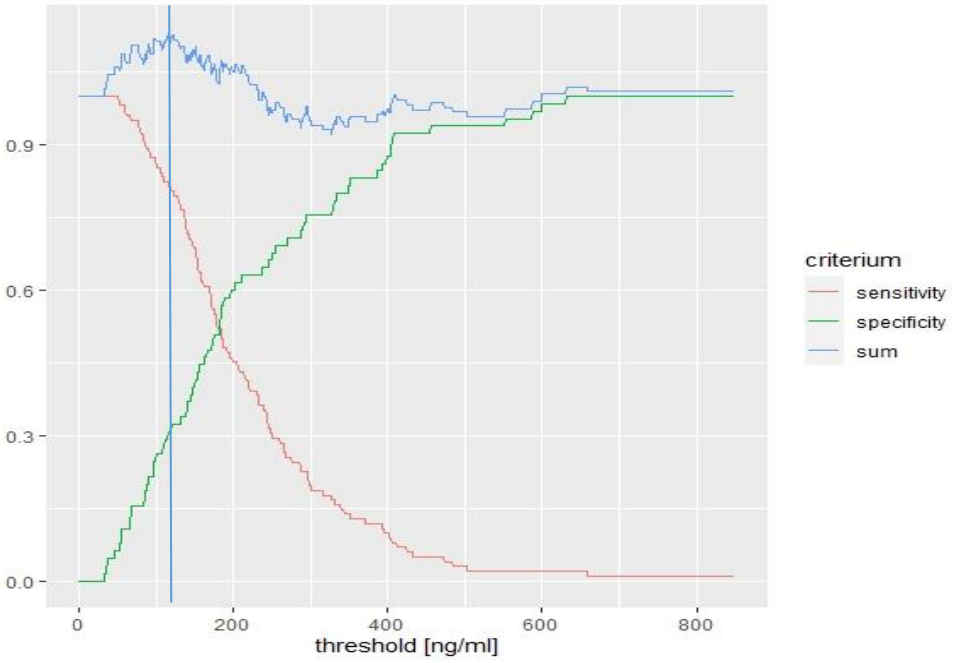
For a 5 mg dose, once in the evening (20:00), blood sampling in the morning at 08:00:

$$\text{C min} = \left[\left(\frac{5mg}{24h}\right) \times \left(\frac{1}{22{,}32\frac{l}{h}}\right)\right] \times \left[\frac{(0{,}021),\times 24\ h)}{(1-e^{-0{,}021\times 24\ h})}\right] \times (e^{-0{,}021\times 12h}) = 0{,}00923\frac{mg}{l} = \mathbf{9{,}23\ ng/ml}$$

For a 20 mg dose, once in the evening (20:00), blood sampling in the morning at 08:00:

$$\text{C min} = \left[\left(\frac{20mg}{24h}\right) \times \left(\frac{1}{22{,}32\frac{l}{h}}\right)\right] \times \left[\frac{(0{,}021),\times 24\ h)}{(1-e^{-0{,}021\times 24\ h})}\right] \times (e^{-0{,}021\times 12h}) = 0{,}0369\frac{mg}{l} = \mathbf{36{,}94\frac{ng}{ml}}$$

S2. Plot aripiprazole Sensitivity Specificity dataset (local maximum: 115)



S3. Data aripiprazole Sensitivity Specificity dataset (local maximum: 115)

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
31	102	0	65	0	1,0000	0,0000	1,0000
32	102	0	65	0	1,0000	0,0000	1,0000
33	102	0	64	1	1,0000	0,0154	1,0154
34	102	0	64	1	1,0000	0,0154	1,0154
35	102	0	64	1	1,0000	0,0154	1,0154
36	102	0	62	3	1,0000	0,0462	1,0462
37	102	0	62	3	1,0000	0,0462	1,0462
38	102	0	62	3	1,0000	0,0462	1,0462
39	102	0	62	3	1,0000	0,0462	1,0462
40	102	0	62	3	1,0000	0,0462	1,0462
41	102	0	62	3	1,0000	0,0462	1,0462
42	102	0	62	3	1,0000	0,0462	1,0462
43	102	0	62	3	1,0000	0,0462	1,0462
44	102	0	62	3	1,0000	0,0462	1,0462
45	102	0	62	3	1,0000	0,0462	1,0462
46	102	0	61	4	1,0000	0,0615	1,0615
47	102	0	61	4	1,0000	0,0615	1,0615
48	102	0	61	4	1,0000	0,0615	1,0615
49	102	0	61	4	1,0000	0,0615	1,0615
50	102	0	61	4	1,0000	0,0615	1,0615
51	101	1	61	4	0,9902	0,0615	1,0517
52	100	2	60	5	0,9804	0,0769	1,0573
53	100	2	60	5	0,9804	0,0769	1,0573
54	100	2	60	5	0,9804	0,0769	1,0573
55	100	2	58	7	0,9804	0,1077	1,0881
56	100	2	58	7	0,9804	0,1077	1,0881
57	100	2	58	7	0,9804	0,1077	1,0881
58	100	2	58	7	0,9804	0,1077	1,0881
59	99	3	58	7	0,9706	0,1077	1,0783
60	98	4	58	7	0,9608	0,1077	1,0685
61	98	4	58	7	0,9608	0,1077	1,0685
62	98	4	58	7	0,9608	0,1077	1,0685
63	98	4	58	7	0,9608	0,1077	1,0685
64	98	4	58	7	0,9608	0,1077	1,0685
65	98	4	57	8	0,9608	0,1231	1,0839
66	98	4	57	8	0,9608	0,1231	1,0839
67	97	5	56	9	0,9510	0,1385	1,0894
68	97	5	55	10	0,9510	0,1538	1,1048
69	97	5	55	10	0,9510	0,1538	1,1048
70	97	5	55	10	0,9510	0,1538	1,1048
71	97	5	55	10	0,9510	0,1538	1,1048
72	97	5	55	10	0,9510	0,1538	1,1048
73	97	5	55	10	0,9510	0,1538	1,1048
74	97	5	55	10	0,9510	0,1538	1,1048
75	97	5	55	10	0,9510	0,1538	1,1048
76	96	6	55	10	0,9412	0,1538	1,0950
77	96	6	55	10	0,9412	0,1538	1,0950
78	95	7	55	10	0,9314	0,1538	1,0852
79	95	7	55	10	0,9314	0,1538	1,0852
80	94	8	55	10	0,9216	0,1538	1,0754
81	94	8	55	10	0,9216	0,1538	1,0754
82	94	8	55	10	0,9216	0,1538	1,0754
83	93	9	55	10	0,9118	0,1538	1,0656
84	92	10	54	11	0,9020	0,1692	1,0712
85	92	10	53	12	0,9020	0,1846	1,0866
86	91	11	53	12	0,8922	0,1846	1,0768
87	91	11	52	13	0,8922	0,2000	1,0922
88	91	11	52	13	0,8922	0,2000	1,0922
89	91	11	52	13	0,8922	0,2000	1,0922
90	91	11	51	14	0,8922	0,2154	1,1075
91	90	12	51	14	0,8824	0,2154	1,0977
92	89	13	51	14	0,8725	0,2154	1,0879
93	89	13	51	14	0,8725	0,2154	1,0879
94	89	13	51	14	0,8725	0,2154	1,0879
95	89	13	51	14	0,8725	0,2154	1,0879
96	89	13	49	16	0,8725	0,2462	1,1187
97	89	13	49	16	0,8725	0,2462	1,1187
98	89	13	49	16	0,8725	0,2462	1,1187
99	89	13	49	16	0,8725	0,2462	1,1187
100	87	15	48	17	0,8529	0,2615	1,1145
101	87	15	48	17	0,8529	0,2615	1,1145
102	87	15	48	17	0,8529	0,2615	1,1145
103	87	15	48	17	0,8529	0,2615	1,1145
104	87	15	48	17	0,8529	0,2615	1,1145
105	86	16	48	17	0,8431	0,2615	1,1047
106	86	16	48	17	0,8431	0,2615	1,1047
107	85	17	48	17	0,8333	0,2615	1,0949
108	85	17	47	18	0,8333	0,2769	1,1103
109	85	17	47	18	0,8333	0,2769	1,1103
110	84	18	47	18	0,8235	0,2769	1,1005
111	84	18	46	19	0,8235	0,2923	1,1158
112	84	18	46	19	0,8235	0,2923	1,1158
113	84	18	46	19	0,8235	0,2923	1,1158
114	84	18	46	19	0,8235	0,2923	1,1158
115	84	18	45	20	0,8235	0,3077	1,1312
116	83	19	45	20	0,8137	0,3077	1,1214
117	83	19	45	20	0,8137	0,3077	1,1214
118	82	20	45	20	0,8039	0,3077	1,1116
119	82	20	44	21	0,8039	0,3231	1,1270
120	82	20	44	21	0,8039	0,3231	1,1270
121	82	20	44	21	0,8039	0,3231	1,1270
122	82	20	44	21	0,8039	0,3231	1,1270
123	81	21	44	21	0,7941	0,3231	1,1172
124	81	21	44	21	0,7941	0,3231	1,1172
125	81	21	44	21	0,7941	0,3231	1,1172
126	81	21	44	21	0,7941	0,3231	1,1172
127	81	21	44	21	0,7941	0,3231	1,1172
128	80	22	44	21	0,7843	0,3231	1,1074
129	80	22	44	21	0,7843	0,3231	1,1074
130	79	23	44	21	0,7745	0,3231	1,0976
131	79	23	44	21	0,7745	0,3231	1,0976
132	78	24	43	22	0,7647	0,3385	1,1032
133	78	24	43	22	0,7647	0,3385	1,1032
134	78	24	43	22	0,7647	0,3385	1,1032
135	78	24	43	22	0,7647	0,3385	1,1032
136	77	25	43	22	0,7549	0,3385	1,0934
137	76	26	43	22	0,7451	0,3385	1,0836
138	75	27	43	22	0,7353	0,3385	1,0738
139	73	29	42	23	0,7157	0,3538	1,0695
140	73	29	41	24	0,7157	0,3692	1,0849
141	73	29	41	24	0,7157	0,3692	1,0849
142	73	29	41	24	0,7157	0,3692	1,0849
143	72	30	41	24	0,7059	0,3692	1,0751
144	72	30	41	24	0,7059	0,3692	1,0751
145	72	30	40	25	0,7059	0,3846	1,0905
146	71	31	40	25	0,6961	0,3846	1,0807
147	71	31	39	26	0,6961	0,4000	1,0961
148	70	32	39	26	0,6863	0,4000	1,0863
149	70	32	39	26	0,6863	0,4000	1,0863
150	70	32	38	27	0,6863	0,4154	1,1017
151	69	33	38	27	0,6765	0,4154	1,0919
152	67	35	38	27	0,6569	0,4154	1,0722
153	66	36	37	28	0,6471	0,4308	1,0778
154	66	36	37	28	0,6471	0,4308	1,0778
155	65	37	37	28	0,6373	0,4308	1,0680
156	65	37	36	29	0,6373	0,4462	1,0834
157	65	37	36	29	0,6373	0,4462	1,0834
158	63	39	36	29	0,6176	0,4462	1,0638
159	63	39	36	29	0,6176	0,4462	1,0638
160	63	39	36	29	0,6176	0,4462	1,0638
161	62	40	36	29	0,6078	0,4462	1,0540
162	62	40	35	30	0,6078	0,4615	1,0694
163	62	40	35	30	0,6078	0,4615	1,0694
164	62	40	35	30	0,6078	0,4615	1,0694
165	62	40	35	30	0,6078	0,4615	1,0694
166	62	40	34	31	0,6078	0,4769	1,0848
167	62	40	34	31	0,6078	0,4769	1,0848
168	62	40	34	31	0,6078	0,4769	1,0848
169	61	41	34	31	0,5980	0,4769	1,0750
170	60	42	34	31	0,5882	0,4769	1,0652

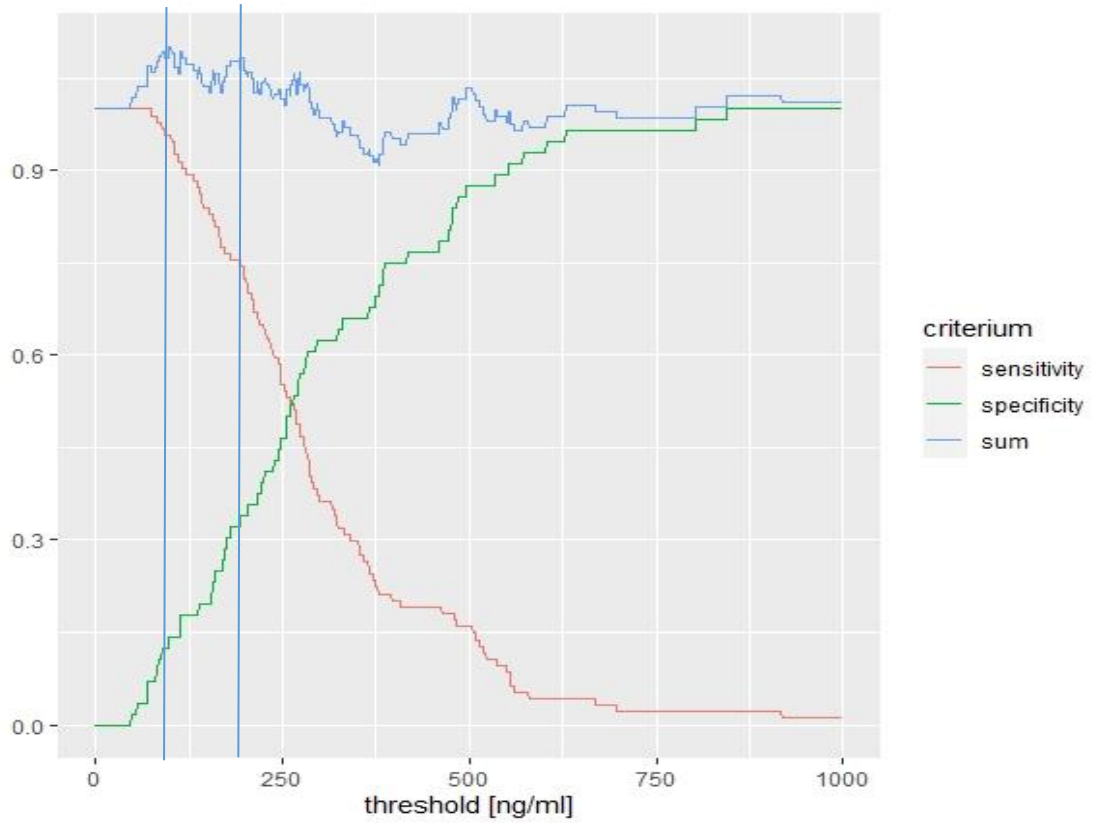
171	58	44	34	31	0,5686	0,4769	1,0456
172	57	45	33	32	0,5588	0,4923	1,0511
173	57	45	33	32	0,5588	0,4923	1,0511
174	57	45	32	33	0,5588	0,5077	1,0665
175	57	45	32	33	0,5588	0,5077	1,0665
176	56	46	32	33	0,5490	0,5077	1,0567
177	56	46	32	33	0,5490	0,5077	1,0567
178	54	48	32	33	0,5294	0,5077	1,0371
179	53	49	32	33	0,5196	0,5077	1,0273
180	53	49	32	33	0,5196	0,5077	1,0273
181	53	49	32	33	0,5196	0,5077	1,0273
182	53	49	31	34	0,5196	0,5231	1,0427
183	53	49	29	36	0,5196	0,5538	1,0735
184	51	51	28	37	0,5000	0,5692	1,0692
185	51	51	28	37	0,5000	0,5692	1,0692
186	49	53	28	37	0,4804	0,5692	1,0496
187	49	53	28	37	0,4804	0,5692	1,0496
188	49	53	27	38	0,4804	0,5846	1,0650
189	49	53	27	38	0,4804	0,5846	1,0650
190	49	53	27	38	0,4804	0,5846	1,0650
191	49	53	27	38	0,4804	0,5846	1,0650
192	48	54	27	38	0,4706	0,5846	1,0552
193	48	54	27	38	0,4706	0,5846	1,0552
194	48	54	27	38	0,4706	0,5846	1,0552
195	47	55	27	38	0,4608	0,5846	1,0454
196	47	55	26	39	0,4608	0,6000	1,0608
197	47	55	26	39	0,4608	0,6000	1,0608
198	46	56	26	39	0,4510	0,6000	1,0510
199	46	56	26	39	0,4510	0,6000	1,0510
200	46	56	26	39	0,4510	0,6000	1,0510
201	46	56	26	39	0,4510	0,6000	1,0510
202	46	56	25	40	0,4510	0,6154	1,0664
203	46	56	25	40	0,4510	0,6154	1,0664
204	46	56	25	40	0,4510	0,6154	1,0664
205	45	57	25	40	0,4412	0,6154	1,0566
206	45	57	25	40	0,4412	0,6154	1,0566
207	44	58	25	40	0,4314	0,6154	1,0468
208	44	58	25	40	0,4314	0,6154	1,0468
209	44	58	25	40	0,4314	0,6154	1,0468
210	44	58	24	41	0,4314	0,6308	1,0621
211	44	58	24	41	0,4314	0,6308	1,0621
212	44	58	24	41	0,4314	0,6308	1,0621
213	44	58	24	41	0,4314	0,6308	1,0621
214	43	59	24	41	0,4216	0,6308	1,0523
215	43	59	24	41	0,4216	0,6308	1,0523
216	42	60	24	41	0,4118	0,6308	1,0425
217	42	60	24	41	0,4118	0,6308	1,0425
218	42	60	24	41	0,4118	0,6308	1,0425
219	41	61	24	41	0,4020	0,6308	1,0327
220	40	62	24	41	0,3922	0,6308	1,0229
221	40	62	24	41	0,3922	0,6308	1,0229
222	40	62	24	41	0,3922	0,6308	1,0229
223	40	62	24	41	0,3922	0,6308	1,0229
224	40	62	24	41	0,3922	0,6308	1,0229
225	40	62	24	41	0,3922	0,6308	1,0229
226	40	62	24	41	0,3922	0,6308	1,0229
227	40	62	24	41	0,3922	0,6308	1,0229
228	40	62	24	41	0,3922	0,6308	1,0229
229	40	62	24	41	0,3922	0,6308	1,0229
230	39	63	24	41	0,3824	0,6308	1,0131
231	39	63	24	41	0,3824	0,6308	1,0131
232	38	64	24	41	0,3725	0,6308	1,0033
233	37	65	24	41	0,3627	0,6308	0,9935
234	37	65	24	41	0,3627	0,6308	0,9935
235	37	65	24	41	0,3627	0,6308	0,9935
236	37	65	24	41	0,3627	0,6308	0,9935
237	37	65	23	42	0,3627	0,6462	1,0089
238	37	65	23	42	0,3627	0,6462	1,0089
239	36	66	23	42	0,3529	0,6462	0,9991
240	36	66	23	42	0,3529	0,6462	0,9991
241	36	66	23	42	0,3529	0,6462	0,9991
242	35	67	23	42	0,3431	0,6462	0,9893
243	35	67	23	42	0,3431	0,6462	0,9893
244	34	68	23	42	0,3333	0,6462	0,9795
245	32	70	23	42	0,3137	0,6462	0,9599
246	32	70	22	43	0,3137	0,6615	0,9753
247	32	70	22	43	0,3137	0,6615	0,9753
248	31	71	22	43	0,3039	0,6615	0,9655
249	31	71	22	43	0,3039	0,6615	0,9655
250	30	72	21	44	0,2941	0,6769	0,9710
251	30	72	21	44	0,2941	0,6769	0,9710
252	30	72	21	44	0,2941	0,6769	0,9710
253	30	72	21	44	0,2941	0,6769	0,9710
254	30	72	20	45	0,2941	0,6923	0,9864
255	30	72	20	45	0,2941	0,6923	0,9864
256	30	72	20	45	0,2941	0,6923	0,9864
257	30	72	20	45	0,2941	0,6923	0,9864
258	30	72	20	45	0,2941	0,6923	0,9864
259	30	72	20	45	0,2941	0,6923	0,9864
260	29	73	20	45	0,2843	0,6923	0,9766
261	29	73	20	45	0,2843	0,6923	0,9766
262	29	73	20	45	0,2843	0,6923	0,9766
263	29	73	20	45	0,2843	0,6923	0,9766
264	29	73	20	45	0,2843	0,6923	0,9766
265	28	74	20	45	0,2745	0,6923	0,9668
266	27	75	20	45	0,2647	0,6923	0,9570
267	27	75	20	45	0,2647	0,6923	0,9570
268	26	76	20	45	0,2549	0,6923	0,9472
269	26	76	20	45	0,2549	0,6923	0,9472
270	26	76	19	46	0,2549	0,7077	0,9626
271	26	76	19	46	0,2549	0,7077	0,9626
272	26	76	19	46	0,2549	0,7077	0,9626
273	26	76	19	46	0,2549	0,7077	0,9626
274	26	76	19	46	0,2549	0,7077	0,9626
275	25	77	19	46	0,2451	0,7077	0,9528
276	25	77	19	46	0,2451	0,7077	0,9528
277	25	77	19	46	0,2451	0,7077	0,9528
278	25	77	19	46	0,2451	0,7077	0,9528
279	25	77	19	46	0,2451	0,7077	0,9528
280	25	77	19	46	0,2451	0,7077	0,9528
281	25	77	19	46	0,2451	0,7077	0,9528
282	25	77	19	46	0,2451	0,7077	0,9528
283	25	77	19	46	0,2451	0,7077	0,9528
284	25	77	19	46	0,2451	0,7077	0,9528
285	25	77	19	46	0,2451	0,7077	0,9528
286	24	78	19	46	0,2353	0,7077	0,9430
287	23	79	19	46	0,2255	0,7077	0,9332
288	23	79	18	47	0,2255	0,7231	0,9486
289	23	79	18	47	0,2255	0,7231	0,9486
290	23	79	18	47	0,2255	0,7231	0,9486
291	23	79	17	48	0,2255	0,7385	0,9640
292	23	79	17	48	0,2255	0,7385	0,9640
293	23	79	17	48	0,2255	0,7385	0,9640
294	23	79	16	49	0,2255	0,7538	0,9793
295	23	79	16	49	0,2255	0,7538	0,9793
296	22	80	16	49	0,2157	0,7538	0,9695
297	20	82	16	49	0,1961	0,7538	0,9499
298	20	82	16	49	0,1961	0,7538	0,9499
299	20	82	16	49	0,1961	0,7538	0,9499
300	20	82	16	49	0,1961	0,7538	0,9499
301	19	83	16	49	0,1863	0,7538	0,9401
302	19	83	16	49	0,1863	0,7538	0,9401
303	19	83	16	49	0,1863	0,7538	0,9401
304	19	83	16	49	0,1863	0,7538	0,9401
305	19	83	16	49	0,1863	0,7538	0,9401
306	19	83	16	49	0,1863	0,7538	0,9401
307	19	83	16	49	0,1863	0,7538	0,9401
308	19	83	16	49	0,1863	0,7538	0,9401
309	19	83	16	49	0,1863	0,7538	0,9401
310	19	83	16	49	0,1863	0,7538	0,9401
311	19	83	16	49	0,1863	0,7538	0,9401
312	19	83	16	49	0,1863	0,7538	0,9401
313	19	83	16	49	0,1863	0,7538	0,9401
314	19	83	16	49	0,1863	0,7538	0,9401
315	19	83	16	49	0,1863	0,7538	0,9401
316	18	84	16	49	0,1765	0,7538	0,9303

317	18	84	16	49	0,1765	0,7538	0,9303
318	18	84	16	49	0,1765	0,7538	0,9303
319	18	84	16	49	0,1765	0,7538	0,9303
320	18	84	16	49	0,1765	0,7538	0,9303
321	18	84	16	49	0,1765	0,7538	0,9303
322	18	84	16	49	0,1765	0,7538	0,9303
323	18	84	16	49	0,1765	0,7538	0,9303
324	18	84	16	49	0,1765	0,7538	0,9303
325	18	84	16	49	0,1765	0,7538	0,9303
326	18	84	16	49	0,1765	0,7538	0,9303
327	17	85	16	49	0,1667	0,7538	0,9205
328	17	85	15	50	0,1667	0,7692	0,9359
329	17	85	15	50	0,1667	0,7692	0,9359
330	17	85	14	51	0,1667	0,7846	0,9513
331	16	86	14	51	0,1569	0,7846	0,9415
332	16	86	14	51	0,1569	0,7846	0,9415
333	16	86	14	51	0,1569	0,7846	0,9415
334	16	86	13	52	0,1569	0,8000	0,9569
335	16	86	13	52	0,1569	0,8000	0,9569
336	16	86	13	52	0,1569	0,8000	0,9569
337	16	86	13	52	0,1569	0,8000	0,9569
338	16	86	13	52	0,1569	0,8000	0,9569
339	15	87	13	52	0,1471	0,8000	0,9471
340	15	87	13	52	0,1471	0,8000	0,9471
341	15	87	13	52	0,1471	0,8000	0,9471
342	15	87	13	52	0,1471	0,8000	0,9471
343	14	88	13	52	0,1373	0,8000	0,9373
344	14	88	13	52	0,1373	0,8000	0,9373
345	14	88	13	52	0,1373	0,8000	0,9373
346	14	88	13	52	0,1373	0,8000	0,9373
347	14	88	13	52	0,1373	0,8000	0,9373
348	14	88	13	52	0,1373	0,8000	0,9373
349	14	88	12	53	0,1373	0,8154	0,9526
350	14	88	12	53	0,1373	0,8154	0,9526
351	13	89	11	54	0,1275	0,8308	0,9582
352	13	89	11	54	0,1275	0,8308	0,9582
353	13	89	11	54	0,1275	0,8308	0,9582
354	13	89	11	54	0,1275	0,8308	0,9582
355	13	89	11	54	0,1275	0,8308	0,9582
356	13	89	11	54	0,1275	0,8308	0,9582
357	13	89	11	54	0,1275	0,8308	0,9582
358	13	89	11	54	0,1275	0,8308	0,9582
359	13	89	11	54	0,1275	0,8308	0,9582
360	13	89	11	54	0,1275	0,8308	0,9582
361	13	89	11	54	0,1275	0,8308	0,9582
362	13	89	11	54	0,1275	0,8308	0,9582
363	13	89	11	54	0,1275	0,8308	0,9582
364	13	89	11	54	0,1275	0,8308	0,9582
365	13	89	11	54	0,1275	0,8308	0,9582
366	13	89	11	54	0,1275	0,8308	0,9582
367	13	89	11	54	0,1275	0,8308	0,9582
368	13	89	11	54	0,1275	0,8308	0,9582
369	13	89	11	54	0,1275	0,8308	0,9582
370	13	89	11	54	0,1275	0,8308	0,9582
371	12	90	11	54	0,1176	0,8308	0,9484
372	12	90	11	54	0,1176	0,8308	0,9484
373	12	90	11	54	0,1176	0,8308	0,9484
374	12	90	11	54	0,1176	0,8308	0,9484
375	12	90	11	54	0,1176	0,8308	0,9484
376	12	90	11	54	0,1176	0,8308	0,9484
377	12	90	11	54	0,1176	0,8308	0,9484
378	12	90	11	54	0,1176	0,8308	0,9484
379	12	90	11	54	0,1176	0,8308	0,9484
380	12	90	11	54	0,1176	0,8308	0,9484
381	12	90	11	54	0,1176	0,8308	0,9484
382	12	90	11	54	0,1176	0,8308	0,9484
383	12	90	11	54	0,1176	0,8308	0,9484
384	12	90	11	54	0,1176	0,8308	0,9484
385	12	90	11	54	0,1176	0,8308	0,9484
386	12	90	10	55	0,1176	0,8462	0,9638
387	12	90	10	55	0,1176	0,8462	0,9638
388	12	90	10	55	0,1176	0,8462	0,9638
389	12	90	10	55	0,1176	0,8462	0,9638
390	12	90	10	55	0,1176	0,8462	0,9638
391	12	90	10	55	0,1176	0,8462	0,9638
392	12	90	10	55	0,1176	0,8462	0,9638
393	11	91	9	56	0,1078	0,8615	0,9694
394	11	91	9	56	0,1078	0,8615	0,9694
395	10	92	9	56	0,0980	0,8615	0,9596
396	10	92	9	56	0,0980	0,8615	0,9596
397	10	92	9	56	0,0980	0,8615	0,9596
398	10	92	8	57	0,0980	0,8769	0,9750
399	10	92	8	57	0,0980	0,8769	0,9750
400	10	92	8	57	0,0980	0,8769	0,9750
401	10	92	8	57	0,0980	0,8769	0,9750
402	9	93	8	57	0,0882	0,8769	0,9652
403	9	93	8	57	0,0882	0,8769	0,9652
404	9	93	7	58	0,0882	0,8923	0,9805
405	8	94	6	59	0,0784	0,9077	0,9861
406	8	94	6	59	0,0784	0,9077	0,9861
407	8	94	5	60	0,0784	0,9231	1,0015
408	8	94	5	60	0,0784	0,9231	1,0015
409	8	94	5	60	0,0784	0,9231	1,0015
410	8	94	5	60	0,0784	0,9231	1,0015
411	8	94	5	60	0,0784	0,9231	1,0015
412	7	95	5	60	0,0686	0,9231	0,9917
413	7	95	5	60	0,0686	0,9231	0,9917
414	7	95	5	60	0,0686	0,9231	0,9917
415	7	95	5	60	0,0686	0,9231	0,9917
416	7	95	5	60	0,0686	0,9231	0,9917
417	7	95	5	60	0,0686	0,9231	0,9917
418	7	95	5	60	0,0686	0,9231	0,9917
419	7	95	5	60	0,0686	0,9231	0,9917
420	7	95	5	60	0,0686	0,9231	0,9917
421	7	95	5	60	0,0686	0,9231	0,9917
422	7	95	5	60	0,0686	0,9231	0,9917
423	7	95	5	60	0,0686	0,9231	0,9917
424	7	95	5	60	0,0686	0,9231	0,9917
425	6	96	5	60	0,0588	0,9231	0,9819
426	6	96	5	60	0,0588	0,9231	0,9819
427	6	96	5	60	0,0588	0,9231	0,9819
428	6	96	5	60	0,0588	0,9231	0,9819
429	6	96	5	60	0,0588	0,9231	0,9819
430	6	96	5	60	0,0588	0,9231	0,9819
431	6	96	5	60	0,0588	0,9231	0,9819
432	5	97	5	60	0,0490	0,9231	0,9721
433	5	97	5	60	0,0490	0,9231	0,9721
434	5	97	5	60	0,0490	0,9231	0,9721
435	5	97	5	60	0,0490	0,9231	0,9721
436	5	97	5	60	0,0490	0,9231	0,9721
437	5	97	5	60	0,0490	0,9231	0,9721
438	5	97	5	60	0,0490	0,9231	0,9721
439	5	97	5	60	0,0490	0,9231	0,9721
440	5	97	5	60	0,0490	0,9231	0,9721
441	5	97	5	60	0,0490	0,9231	0,9721
442	5	97	5	60	0,0490	0,9231	0,9721
443	5	97	5	60	0,0490	0,9231	0,9721
444	5	97	5	60	0,0490	0,9231	0,9721
445	5	97	5	60	0,0490	0,9231	0,9721
446	5	97	5	60	0,0490	0,9231	0,9721
447	5	97	5	60	0,0490	0,9231	0,9721
448	5	97	5	60	0,0490	0,9231	0,9721
449	5	97	5	60	0,0490	0,9231	0,9721
450	5	97	5	60	0,0490	0,9231	0,9721
451	5	97	5	60	0,0490	0,9231	0,9721
452	5	97	5	60	0,0490	0,9231	0,9721
453	5	97	5	60	0,0490	0,9231	0,9721
454	5	97	5	60	0,0490	0,9231	0,9721
455	5	97	5	60	0,0490	0,9231	0,9721
456	5	97	4	61	0,0490	0,9385	0,9875
457	5	97	4	61	0,0490	0,9385	0,9875
458	5	97	4	61	0,0490	0,9385	0,9875
459	5	97	4	61	0,0490	0,9385	0,9875
460	5	97	4	61	0,0490	0,9385	0,9875
461	5	97	4	61	0,0490	0,9385	0,9875
462	5	97	4	61	0,0490	0,9385	0,9875

463	5	97	4	61	0,0490	0,9385	0,9875
464	5	97	4	61	0,0490	0,9385	0,9875
465	5	97	4	61	0,0490	0,9385	0,9875
466	5	97	4	61	0,0490	0,9385	0,9875
467	5	97	4	61	0,0490	0,9385	0,9875
468	5	97	4	61	0,0490	0,9385	0,9875
469	5	97	4	61	0,0490	0,9385	0,9875
470	5	97	4	61	0,0490	0,9385	0,9875
471	5	97	4	61	0,0490	0,9385	0,9875
472	5	97	4	61	0,0490	0,9385	0,9875
473	4	98	4	61	0,0392	0,9385	0,9777
474	4	98	4	61	0,0392	0,9385	0,9777
475	4	98	4	61	0,0392	0,9385	0,9777
476	4	98	4	61	0,0392	0,9385	0,9777
477	4	98	4	61	0,0392	0,9385	0,9777
478	4	98	4	61	0,0392	0,9385	0,9777
479	4	98	4	61	0,0392	0,9385	0,9777
480	4	98	4	61	0,0392	0,9385	0,9777
481	4	98	4	61	0,0392	0,9385	0,9777
482	4	98	4	61	0,0392	0,9385	0,9777
483	4	98	4	61	0,0392	0,9385	0,9777
484	3	99	4	61	0,0294	0,9385	0,9679
485	3	99	4	61	0,0294	0,9385	0,9679
486	3	99	4	61	0,0294	0,9385	0,9679
487	3	99	4	61	0,0294	0,9385	0,9679
488	3	99	4	61	0,0294	0,9385	0,9679
489	3	99	4	61	0,0294	0,9385	0,9679
490	3	99	4	61	0,0294	0,9385	0,9679
491	3	99	4	61	0,0294	0,9385	0,9679
492	3	99	4	61	0,0294	0,9385	0,9679
493	3	99	4	61	0,0294	0,9385	0,9679
494	3	99	4	61	0,0294	0,9385	0,9679
495	3	99	4	61	0,0294	0,9385	0,9679
496	3	99	4	61	0,0294	0,9385	0,9679
497	3	99	4	61	0,0294	0,9385	0,9679
498	3	99	4	61	0,0294	0,9385	0,9679
499	3	99	4	61	0,0294	0,9385	0,9679
500	3	99	4	61	0,0294	0,9385	0,9679
501	3	99	4	61	0,0294	0,9385	0,9679
502	3	99	4	61	0,0294	0,9385	0,9679
503	2	100	4	61	0,0196	0,9385	0,9581
504	2	100	4	61	0,0196	0,9385	0,9581
505	2	100	4	61	0,0196	0,9385	0,9581
506	2	100	4	61	0,0196	0,9385	0,9581
507	2	100	4	61	0,0196	0,9385	0,9581
508	2	100	4	61	0,0196	0,9385	0,9581
509	2	100	4	61	0,0196	0,9385	0,9581
510	2	100	4	61	0,0196	0,9385	0,9581
511	2	100	4	61	0,0196	0,9385	0,9581
512	2	100	4	61	0,0196	0,9385	0,9581
513	2	100	4	61	0,0196	0,9385	0,9581
514	2	100	4	61	0,0196	0,9385	0,9581
515	2	100	4	61	0,0196	0,9385	0,9581
516	2	100	4	61	0,0196	0,9385	0,9581
517	2	100	4	61	0,0196	0,9385	0,9581
518	2	100	4	61	0,0196	0,9385	0,9581
519	2	100	4	61	0,0196	0,9385	0,9581
520	2	100	4	61	0,0196	0,9385	0,9581
521	2	100	4	61	0,0196	0,9385	0,9581
522	2	100	4	61	0,0196	0,9385	0,9581
523	2	100	4	61	0,0196	0,9385	0,9581
524	2	100	4	61	0,0196	0,9385	0,9581
525	2	100	4	61	0,0196	0,9385	0,9581
526	2	100	4	61	0,0196	0,9385	0,9581
527	2	100	4	61	0,0196	0,9385	0,9581
528	2	100	4	61	0,0196	0,9385	0,9581
529	2	100	4	61	0,0196	0,9385	0,9581
530	2	100	4	61	0,0196	0,9385	0,9581
531	2	100	4	61	0,0196	0,9385	0,9581
532	2	100	4	61	0,0196	0,9385	0,9581
533	2	100	4	61	0,0196	0,9385	0,9581
534	2	100	4	61	0,0196	0,9385	0,9581
535	2	100	4	61	0,0196	0,9385	0,9581
536	2	100	4	61	0,0196	0,9385	0,9581
537	2	100	4	61	0,0196	0,9385	0,9581
538	2	100	4	61	0,0196	0,9385	0,9581
539	2	100	4	61	0,0196	0,9385	0,9581
540	2	100	4	61	0,0196	0,9385	0,9581
541	2	100	4	61	0,0196	0,9385	0,9581
542	2	100	4	61	0,0196	0,9385	0,9581
543	2	100	4	61	0,0196	0,9385	0,9581
544	2	100	4	61	0,0196	0,9385	0,9581
545	2	100	4	61	0,0196	0,9385	0,9581
546	2	100	4	61	0,0196	0,9385	0,9581
547	2	100	4	61	0,0196	0,9385	0,9581
548	2	100	4	61	0,0196	0,9385	0,9581
549	2	100	4	61	0,0196	0,9385	0,9581
550	2	100	4	61	0,0196	0,9385	0,9581
551	2	100	4	61	0,0196	0,9385	0,9581
552	2	100	3	62	0,0196	0,9538	0,9735
553	2	100	3	62	0,0196	0,9538	0,9735
554	2	100	3	62	0,0196	0,9538	0,9735
555	2	100	3	62	0,0196	0,9538	0,9735
556	2	100	3	62	0,0196	0,9538	0,9735
557	2	100	3	62	0,0196	0,9538	0,9735
558	2	100	3	62	0,0196	0,9538	0,9735
559	2	100	3	62	0,0196	0,9538	0,9735
560	2	100	3	62	0,0196	0,9538	0,9735
561	2	100	3	62	0,0196	0,9538	0,9735
562	2	100	3	62	0,0196	0,9538	0,9735
563	2	100	3	62	0,0196	0,9538	0,9735
564	2	100	3	62	0,0196	0,9538	0,9735
565	2	100	3	62	0,0196	0,9538	0,9735
566	2	100	3	62	0,0196	0,9538	0,9735
567	2	100	3	62	0,0196	0,9538	0,9735
568	2	100	3	62	0,0196	0,9538	0,9735
569	2	100	3	62	0,0196	0,9538	0,9735
570	2	100	3	62	0,0196	0,9538	0,9735
571	2	100	3	62	0,0196	0,9538	0,9735
572	2	100	3	62	0,0196	0,9538	0,9735
573	2	100	3	62	0,0196	0,9538	0,9735
574	2	100	3	62	0,0196	0,9538	0,9735
575	2	100	3	62	0,0196	0,9538	0,9735
576	2	100	3	62	0,0196	0,9538	0,9735
577	2	100	3	62	0,0196	0,9538	0,9735
578	2	100	3	62	0,0196	0,9538	0,9735
579	2	100	3	62	0,0196	0,9538	0,9735
580	2	100	3	62	0,0196	0,9538	0,9735
581	2	100	3	62	0,0196	0,9538	0,9735
582	2	100	3	62	0,0196	0,9538	0,9735
583	2	100	3	62	0,0196	0,9538	0,9735
584	2	100	3	62	0,0196	0,9538	0,9735
585	2	100	3	62	0,0196	0,9538	0,9735
586	2	100	3	62	0,0196	0,9538	0,9735
587	2	100	2	63	0,0196	0,9692	0,9888
588	2	100	2	63	0,0196	0,9692	0,9888
589	2	100	2	63	0,0196	0,9692	0,9888
590	2	100	2	63	0,0196	0,9692	0,9888
591	2	100	2	63	0,0196	0,9692	0,9888
592	2	100	2	63	0,0196	0,9692	0,9888
593	2	100	2	63	0,0196	0,9692	0,9888
594	2	100	2	63	0,0196	0,9692	0,9888
595	2	100	2	63	0,0196	0,9692	0,9888
596	2	100	2	63	0,0196	0,9692	0,9888
597	2	100	2	63	0,0196	0,9692	0,9888
598	2	100	2	63	0,0196	0,9692	0,9888
599	2	100	1	64	0,0196	0,9846	1,0042
600	2	100	1	64	0,0196	0,9846	1,0042
601	2	100	1	64	0,0196	0,9846	1,0042
602	2	100	1	64	0,0196	0,9846	1,0042
603	2	100	1	64	0,0196	0,9846	1,0042
604	2	100	1	64	0,0196	0,9846	1,0042
605	2	100	1	64	0,0196	0,9846	1,0042
606	2	100	1	64	0,0196	0,9846	1,0042
607	2	100	1	64	0,0196	0,9846	1,0042
608	2	100	1	64	0,0196	0,9846	1,0042

609	2	100	1	64	0,0196	0,9846	1,0042
610	2	100	1	64	0,0196	0,9846	1,0042
611	2	100	1	64	0,0196	0,9846	1,0042
612	2	100	1	64	0,0196	0,9846	1,0042
613	2	100	1	64	0,0196	0,9846	1,0042
614	2	100	1	64	0,0196	0,9846	1,0042
615	2	100	1	64	0,0196	0,9846	1,0042
616	2	100	1	64	0,0196	0,9846	1,0042
617	2	100	1	64	0,0196	0,9846	1,0042
618	2	100	1	64	0,0196	0,9846	1,0042
619	2	100	1	64	0,0196	0,9846	1,0042
620	2	100	1	64	0,0196	0,9846	1,0042
621	2	100	1	64	0,0196	0,9846	1,0042
622	2	100	1	64	0,0196	0,9846	1,0042
623	2	100	1	64	0,0196	0,9846	1,0042
624	2	100	1	64	0,0196	0,9846	1,0042
625	2	100	1	64	0,0196	0,9846	1,0042
626	2	100	1	64	0,0196	0,9846	1,0042
627	2	100	1	64	0,0196	0,9846	1,0042
628	2	100	1	64	0,0196	0,9846	1,0042
629	2	100	1	64	0,0196	0,9846	1,0042
630	2	100	1	64	0,0196	0,9846	1,0042
631	2	100	0	65	0,0196	1,0000	1,0196
632	2	100	0	65	0,0196	1,0000	1,0196
633	2	100	0	65	0,0196	1,0000	1,0196
634	2	100	0	65	0,0196	1,0000	1,0196
635	2	100	0	65	0,0196	1,0000	1,0196

S4. Plot aripiprazole Active Moiety Sensitivity Specificity dataset (local maxima: 98, 194)



S5 Data aripiprazole Active Moiety Sensitivity Specificity dataset

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
45	94	0	56	0	1,0000	0,0000	1,0000
46	94	0	56	0	1,0000	0,0000	1,0000
47	94	0	55	1	1,0000	0,0179	1,0179
48	94	0	55	1	1,0000	0,0179	1,0179
49	94	0	55	1	1,0000	0,0179	1,0179
50	94	0	55	1	1,0000	0,0179	1,0179
51	94	0	55	1	1,0000	0,0179	1,0179
52	94	0	55	1	1,0000	0,0179	1,0179
53	94	0	55	1	1,0000	0,0179	1,0179
54	94	0	55	1	1,0000	0,0179	1,0179
55	94	0	54	2	1,0000	0,0357	1,0357
56	94	0	54	2	1,0000	0,0357	1,0357
57	94	0	54	2	1,0000	0,0357	1,0357
58	94	0	54	2	1,0000	0,0357	1,0357
59	94	0	54	2	1,0000	0,0357	1,0357
60	94	0	54	2	1,0000	0,0357	1,0357
61	94	0	54	2	1,0000	0,0357	1,0357
62	94	0	54	2	1,0000	0,0357	1,0357
63	94	0	54	2	1,0000	0,0357	1,0357
64	94	0	54	2	1,0000	0,0357	1,0357
65	94	0	54	2	1,0000	0,0357	1,0357
66	94	0	54	2	1,0000	0,0357	1,0357
67	94	0	54	2	1,0000	0,0357	1,0357
68	94	0	54	2	1,0000	0,0357	1,0357
69	94	0	52	4	1,0000	0,0714	1,0714
70	94	0	52	4	1,0000	0,0714	1,0714
71	94	0	52	4	1,0000	0,0714	1,0714
72	94	0	52	4	1,0000	0,0714	1,0714
73	94	0	52	4	1,0000	0,0714	1,0714
74	93	1	52	4	0,9894	0,0714	1,0608
75	93	1	52	4	0,9894	0,0714	1,0608
76	93	1	52	4	0,9894	0,0714	1,0608
77	93	1	52	4	0,9894	0,0714	1,0608
78	93	1	52	4	0,9894	0,0714	1,0608
79	93	1	52	4	0,9894	0,0714	1,0608
80	93	1	52	4	0,9894	0,0714	1,0608
81	93	1	51	5	0,9894	0,0893	1,0786
82	92	2	51	5	0,9787	0,0893	1,0680
83	92	2	51	5	0,9787	0,0893	1,0680
84	92	2	50	6	0,9787	0,1071	1,0859
85	92	2	50	6	0,9787	0,1071	1,0859
86	92	2	50	6	0,9787	0,1071	1,0859
87	92	2	50	6	0,9787	0,1071	1,0859
88	92	2	50	6	0,9787	0,1071	1,0859
89	91	3	49	7	0,9681	0,1250	1,0931
90	91	3	49	7	0,9681	0,1250	1,0931
91	91	3	49	7	0,9681	0,1250	1,0931
92	91	3	49	7	0,9681	0,1250	1,0931
93	90	4	49	7	0,9574	0,1250	1,0824
94	90	4	49	7	0,9574	0,1250	1,0824
95	90	4	49	7	0,9574	0,1250	1,0824
96	90	4	49	7	0,9574	0,1250	1,0824
97	90	4	49	7	0,9574	0,1250	1,0824
98	90	4	48	8	0,9574	0,1429	1,1003
99	90	4	48	8	0,9574	0,1429	1,1003
100	90	4	48	8	0,9574	0,1429	1,1003
101	90	4	48	8	0,9574	0,1429	1,1003
102	89	5	48	8	0,9468	0,1429	1,0897
103	89	5	48	8	0,9468	0,1429	1,0897
104	89	5	48	8	0,9468	0,1429	1,0897
105	87	7	48	8	0,9255	0,1429	1,0684
106	87	7	48	8	0,9255	0,1429	1,0684
107	87	7	48	8	0,9255	0,1429	1,0684
108	87	7	48	8	0,9255	0,1429	1,0684
109	87	7	48	8	0,9255	0,1429	1,0684
110	86	8	48	8	0,9149	0,1429	1,0578
111	86	8	48	8	0,9149	0,1429	1,0578
112	86	8	48	8	0,9149	0,1429	1,0578
113	86	8	46	10	0,9149	0,1786	1,0935
114	86	8	46	10	0,9149	0,1786	1,0935
115	86	8	46	10	0,9149	0,1786	1,0935
116	85	9	46	10	0,9043	0,1786	1,0828
117	85	9	46	10	0,9043	0,1786	1,0828
118	85	9	46	10	0,9043	0,1786	1,0828
119	85	9	46	10	0,9043	0,1786	1,0828
120	85	9	46	10	0,9043	0,1786	1,0828
121	84	10	46	10	0,8936	0,1786	1,0722
122	84	10	46	10	0,8936	0,1786	1,0722
123	84	10	46	10	0,8936	0,1786	1,0722
124	84	10	46	10	0,8936	0,1786	1,0722
125	84	10	46	10	0,8936	0,1786	1,0722
126	84	10	46	10	0,8936	0,1786	1,0722
127	84	10	46	10	0,8936	0,1786	1,0722
128	84	10	46	10	0,8936	0,1786	1,0722
129	84	10	46	10	0,8936	0,1786	1,0722
130	84	10	46	10	0,8936	0,1786	1,0722
131	84	10	46	10	0,8936	0,1786	1,0722
132	83	11	46	10	0,8830	0,1786	1,0616
133	83	11	46	10	0,8830	0,1786	1,0616
134	83	11	46	10	0,8830	0,1786	1,0616
135	83	11	46	10	0,8830	0,1786	1,0616
136	82	12	46	10	0,8723	0,1786	1,0509
137	82	12	46	10	0,8723	0,1786	1,0509
138	82	12	45	11	0,8723	0,1964	1,0688
139	81	13	45	11	0,8617	0,1964	1,0581
140	81	13	45	11	0,8617	0,1964	1,0581
141	81	13	45	11	0,8617	0,1964	1,0581
142	80	14	45	11	0,8511	0,1964	1,0475
143	79	15	45	11	0,8404	0,1964	1,0369
144	79	15	45	11	0,8404	0,1964	1,0369
145	79	15	45	11	0,8404	0,1964	1,0369
146	79	15	45	11	0,8404	0,1964	1,0369
147	79	15	45	11	0,8404	0,1964	1,0369
148	79	15	45	11	0,8404	0,1964	1,0369
149	79	15	45	11	0,8404	0,1964	1,0369
150	79	15	45	11	0,8404	0,1964	1,0369
151	79	15	45	11	0,8404	0,1964	1,0369
152	78	16	45	11	0,8298	0,1964	1,0262
153	78	16	45	11	0,8298	0,1964	1,0262
154	78	16	45	11	0,8298	0,1964	1,0262
155	78	16	45	11	0,8298	0,1964	1,0262
156	78	16	43	13	0,8298	0,2321	1,0619
157	77	17	43	13	0,8191	0,2321	1,0513
158	77	17	43	13	0,8191	0,2321	1,0513
159	76	18	43	13	0,8085	0,2321	1,0407
160	76	18	42	14	0,8085	0,2500	1,0585
161	76	18	42	14	0,8085	0,2500	1,0585
162	76	18	42	14	0,8085	0,2500	1,0585
163	76	18	42	14	0,8085	0,2500	1,0585
164	76	18	42	14	0,8085	0,2500	1,0585
165	75	19	42	14	0,7979	0,2500	1,0479
166	74	20	42	14	0,7872	0,2500	1,0372
167	73	21	42	14	0,7766	0,2500	1,0266
168	73	21	42	14	0,7766	0,2500	1,0266
169	73	21	42	14	0,7766	0,2500	1,0266
170	73	21	41	15	0,7766	0,2679	1,0445
171	73	21	41	15	0,7766	0,2679	1,0445
172	73	21	41	15	0,7766	0,2679	1,0445
173	72	22	40	16	0,7660	0,2857	1,0517
174	72	22	40	16	0,7660	0,2857	1,0517
175	72	22	39	17	0,7660	0,3036	1,0695
176	72	22	39	17	0,7660	0,3036	1,0695
177	72	22	39	17	0,7660	0,3036	1,0695
178	72	22	39	17	0,7660	0,3036	1,0695
179	72	22	39	17	0,7660	0,3036	1,0695
180	71	23	38	18	0,7553	0,3214	1,0767
181	71	23	38	18	0,7553	0,3214	1,0767
182	71	23	38	18	0,7553	0,3214	1,0767
183	71	23	38	18	0,7553	0,3214	1,0767
184	71	23	38	18	0,7553	0,3214	1,0767

185	71	23	38	18	0,7553	0,3214	1,0767
186	71	23	38	18	0,7553	0,3214	1,0767
187	71	23	38	18	0,7553	0,3214	1,0767
188	71	23	38	18	0,7553	0,3214	1,0767
189	71	23	38	18	0,7553	0,3214	1,0767
190	71	23	38	18	0,7553	0,3214	1,0767
191	71	23	38	18	0,7553	0,3214	1,0767
192	71	23	38	18	0,7553	0,3214	1,0767
193	70	24	38	18	0,7447	0,3214	1,0661
194	70	24	37	19	0,7447	0,3393	1,0840
195	70	24	37	19	0,7447	0,3393	1,0840
196	70	24	37	19	0,7447	0,3393	1,0840
197	70	24	37	19	0,7447	0,3393	1,0840
198	69	25	37	19	0,7340	0,3393	1,0733
199	68	26	37	19	0,7234	0,3393	1,0627
200	68	26	37	19	0,7234	0,3393	1,0627
201	68	26	37	19	0,7234	0,3393	1,0627
202	67	27	37	19	0,7128	0,3393	1,0521
203	67	27	37	19	0,7128	0,3393	1,0521
204	66	28	36	20	0,7021	0,3571	1,0593
205	66	28	36	20	0,7021	0,3571	1,0593
206	66	28	36	20	0,7021	0,3571	1,0593
207	66	28	36	20	0,7021	0,3571	1,0593
208	66	28	36	20	0,7021	0,3571	1,0593
209	65	29	36	20	0,6915	0,3571	1,0486
210	65	29	36	20	0,6915	0,3571	1,0486
211	64	30	36	20	0,6809	0,3571	1,0380
212	63	31	36	20	0,6702	0,3571	1,0274
213	63	31	36	20	0,6702	0,3571	1,0274
214	63	31	36	20	0,6702	0,3571	1,0274
215	63	31	36	20	0,6702	0,3571	1,0274
216	63	31	36	20	0,6702	0,3571	1,0274
217	63	31	35	21	0,6702	0,3750	1,0452
218	61	33	35	21	0,6489	0,3750	1,0239
219	61	33	35	21	0,6489	0,3750	1,0239
220	61	33	35	21	0,6489	0,3750	1,0239
221	61	33	35	21	0,6489	0,3750	1,0239
222	61	33	34	22	0,6489	0,3929	1,0418
223	61	33	34	22	0,6489	0,3929	1,0418
224	61	33	34	22	0,6489	0,3929	1,0418
225	61	33	34	22	0,6489	0,3929	1,0418
226	60	34	33	23	0,6383	0,4107	1,0490
227	60	34	33	23	0,6383	0,4107	1,0490
228	59	35	33	23	0,6277	0,4107	1,0384
229	59	35	33	23	0,6277	0,4107	1,0384
230	59	35	33	23	0,6277	0,4107	1,0384
231	59	35	33	23	0,6277	0,4107	1,0384
232	59	35	33	23	0,6277	0,4107	1,0384
233	58	36	33	23	0,6170	0,4107	1,0277
234	58	36	33	23	0,6170	0,4107	1,0277
235	58	36	33	23	0,6170	0,4107	1,0277
236	57	37	33	23	0,6064	0,4107	1,0171
237	57	37	33	23	0,6064	0,4107	1,0171
238	57	37	33	23	0,6064	0,4107	1,0171
239	56	38	32	24	0,5957	0,4286	1,0243
240	56	38	32	24	0,5957	0,4286	1,0243
241	56	38	32	24	0,5957	0,4286	1,0243
242	56	38	32	24	0,5957	0,4286	1,0243
243	56	38	32	24	0,5957	0,4286	1,0243
244	56	38	32	24	0,5957	0,4286	1,0243
245	55	39	31	25	0,5851	0,4464	1,0315
246	55	39	31	25	0,5851	0,4464	1,0315
247	54	40	31	25	0,5745	0,4464	1,0209
248	52	42	30	26	0,5532	0,4643	1,0175
249	52	42	30	26	0,5532	0,4643	1,0175
250	52	42	30	26	0,5532	0,4643	1,0175
251	52	42	30	26	0,5532	0,4643	1,0175
252	52	42	30	26	0,5532	0,4643	1,0175
253	51	43	30	26	0,5426	0,4643	1,0068
254	51	43	30	26	0,5426	0,4643	1,0068
255	50	44	29	27	0,5319	0,4821	1,0141
256	50	44	28	28	0,5319	0,5000	1,0319
257	50	44	28	28	0,5319	0,5000	1,0319
258	50	44	28	28	0,5319	0,5000	1,0319
259	50	44	28	28	0,5319	0,5000	1,0319
260	49	45	28	28	0,5213	0,5000	1,0213
261	49	45	27	29	0,5213	0,5179	1,0391
262	49	45	27	29	0,5213	0,5179	1,0391
263	49	45	27	29	0,5213	0,5179	1,0391
264	49	45	26	30	0,5213	0,5357	1,0570
265	48	46	26	30	0,5106	0,5357	1,0464
266	48	46	26	30	0,5106	0,5357	1,0464
267	48	46	26	30	0,5106	0,5357	1,0464
268	47	47	26	30	0,5000	0,5357	1,0357
269	46	48	26	30	0,4894	0,5357	1,0251
270	46	48	26	30	0,4894	0,5357	1,0251
271	46	48	25	31	0,4894	0,5536	1,0429
272	46	48	24	32	0,4894	0,5714	1,0608
273	46	48	24	32	0,4894	0,5714	1,0608
274	44	50	24	32	0,4681	0,5714	1,0395
275	44	50	24	32	0,4681	0,5714	1,0395
276	44	50	24	32	0,4681	0,5714	1,0395
277	44	50	24	32	0,4681	0,5714	1,0395
278	44	50	24	32	0,4681	0,5714	1,0395
279	43	51	24	32	0,4574	0,5714	1,0289
280	42	52	23	33	0,4468	0,5893	1,0361
281	42	52	23	33	0,4468	0,5893	1,0361
282	42	52	23	33	0,4468	0,5893	1,0361
283	41	53	22	34	0,4362	0,6071	1,0433
284	41	53	22	34	0,4362	0,6071	1,0433
285	40	54	22	34	0,4255	0,6071	1,0327
286	39	55	22	34	0,4149	0,6071	1,0220
287	38	56	22	34	0,4043	0,6071	1,0114
288	38	56	22	34	0,4043	0,6071	1,0114
289	37	57	22	34	0,3936	0,6071	1,0008
290	37	57	22	34	0,3936	0,6071	1,0008
291	36	58	22	34	0,3830	0,6071	0,9901
292	36	58	22	34	0,3830	0,6071	0,9901
293	36	58	22	34	0,3830	0,6071	0,9901
294	36	58	22	34	0,3830	0,6071	0,9901
295	36	58	21	35	0,3830	0,6250	1,0080
296	36	58	21	35	0,3830	0,6250	1,0080
297	35	59	21	35	0,3723	0,6250	0,9973
298	35	59	21	35	0,3723	0,6250	0,9973
299	34	60	21	35	0,3617	0,6250	0,9867
300	34	60	21	35	0,3617	0,6250	0,9867
301	34	60	21	35	0,3617	0,6250	0,9867
302	34	60	21	35	0,3617	0,6250	0,9867
303	34	60	21	35	0,3617	0,6250	0,9867
304	34	60	21	35	0,3617	0,6250	0,9867
305	34	60	21	35	0,3617	0,6250	0,9867
306	34	60	21	35	0,3617	0,6250	0,9867
307	34	60	21	35	0,3617	0,6250	0,9867
308	34	60	21	35	0,3617	0,6250	0,9867
309	34	60	21	35	0,3617	0,6250	0,9867
310	34	60	21	35	0,3617	0,6250	0,9867
311	34	60	21	35	0,3617	0,6250	0,9867
312	34	60	21	35	0,3617	0,6250	0,9867
313	34	60	21	35	0,3617	0,6250	0,9867
314	34	60	21	35	0,3617	0,6250	0,9867
315	34	60	21	35	0,3617	0,6250	0,9867
316	33	61	21	35	0,3511	0,6250	0,9761
317	33	61	21	35	0,3511	0,6250	0,9761
318	33	61	21	35	0,3511	0,6250	0,9761
319	33	61	21	35	0,3511	0,6250	0,9761
320	32	62	21	35	0,3404	0,6250	0,9654
321	32	62	21	35	0,3404	0,6250	0,9654
322	31	63	21	35	0,3298	0,6250	0,9548
323	31	63	21	35	0,3298	0,6250	0,9548
324	30	64	20	36	0,3191	0,6429	0,9620
325	30	64	20	36	0,3191	0,6429	0,9620
326	30	64	20	36	0,3191	0,6429	0,9620
327	30	64	20	36	0,3191	0,6429	0,9620
328	30	64	20	36	0,3191	0,6429	0,9620
329	30	64	20	36	0,3191	0,6429	0,9620
330	30	64	19	37	0,3191	0,6607	0,9799

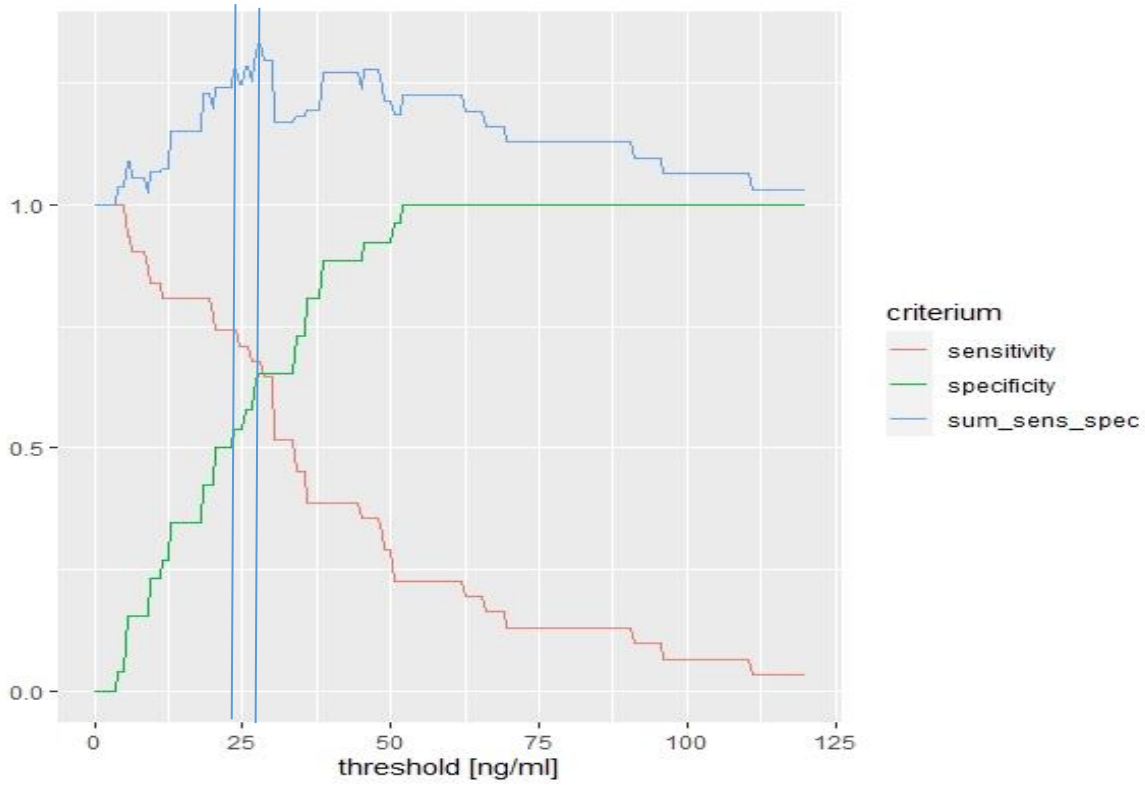
331	30	64	19	37	0,3191	0,6607	0,9799
332	30	64	19	37	0,3191	0,6607	0,9799
333	29	65	19	37	0,3085	0,6607	0,9692
334	29	65	19	37	0,3085	0,6607	0,9692
335	29	65	19	37	0,3085	0,6607	0,9692
336	29	65	19	37	0,3085	0,6607	0,9692
337	29	65	19	37	0,3085	0,6607	0,9692
338	29	65	19	37	0,3085	0,6607	0,9692
339	29	65	19	37	0,3085	0,6607	0,9692
340	29	65	19	37	0,3085	0,6607	0,9692
341	28	66	19	37	0,2979	0,6607	0,9586
342	28	66	19	37	0,2979	0,6607	0,9586
343	28	66	19	37	0,2979	0,6607	0,9586
344	28	66	19	37	0,2979	0,6607	0,9586
345	28	66	19	37	0,2979	0,6607	0,9586
346	28	66	19	37	0,2979	0,6607	0,9586
347	28	66	19	37	0,2979	0,6607	0,9586
348	28	66	19	37	0,2979	0,6607	0,9586
349	28	66	19	37	0,2979	0,6607	0,9586
350	28	66	19	37	0,2979	0,6607	0,9586
351	28	66	19	37	0,2979	0,6607	0,9586
352	27	67	19	37	0,2872	0,6607	0,9479
353	26	68	19	37	0,2766	0,6607	0,9373
354	26	68	19	37	0,2766	0,6607	0,9373
355	26	68	19	37	0,2766	0,6607	0,9373
356	26	68	19	37	0,2766	0,6607	0,9373
357	26	68	19	37	0,2766	0,6607	0,9373
358	26	68	19	37	0,2766	0,6607	0,9373
359	25	69	19	37	0,2660	0,6607	0,9267
360	25	69	19	37	0,2660	0,6607	0,9267
361	25	69	19	37	0,2660	0,6607	0,9267
362	25	69	19	37	0,2660	0,6607	0,9267
363	25	69	19	37	0,2660	0,6607	0,9267
364	25	69	19	37	0,2660	0,6607	0,9267
365	24	70	18	38	0,2553	0,6786	0,9339
366	24	70	18	38	0,2553	0,6786	0,9339
367	23	71	18	38	0,2447	0,6786	0,9233
368	23	71	18	38	0,2447	0,6786	0,9233
369	23	71	18	38	0,2447	0,6786	0,9233
370	23	71	18	38	0,2447	0,6786	0,9233
371	22	72	18	38	0,2340	0,6786	0,9126
372	22	72	18	38	0,2340	0,6786	0,9126
373	22	72	18	38	0,2340	0,6786	0,9126
374	22	72	18	38	0,2340	0,6786	0,9126
375	21	73	17	39	0,2234	0,6964	0,9198
376	21	73	17	39	0,2234	0,6964	0,9198
377	21	73	17	39	0,2234	0,6964	0,9198
378	20	74	17	39	0,2128	0,6964	0,9092
379	20	74	16	40	0,2128	0,7143	0,9271
380	20	74	16	40	0,2128	0,7143	0,9271
381	20	74	16	40	0,2128	0,7143	0,9271
382	20	74	16	40	0,2128	0,7143	0,9271
383	20	74	16	40	0,2128	0,7143	0,9271
384	20	74	16	40	0,2128	0,7143	0,9271
385	20	74	15	41	0,2128	0,7321	0,9449
386	20	74	14	42	0,2128	0,7500	0,9628
387	20	74	14	42	0,2128	0,7500	0,9628
388	20	74	14	42	0,2128	0,7500	0,9628
389	20	74	14	42	0,2128	0,7500	0,9628
390	20	74	14	42	0,2128	0,7500	0,9628
391	20	74	14	42	0,2128	0,7500	0,9628
392	20	74	14	42	0,2128	0,7500	0,9628
393	20	74	14	42	0,2128	0,7500	0,9628
394	20	74	14	42	0,2128	0,7500	0,9628
395	20	74	14	42	0,2128	0,7500	0,9628
396	19	75	14	42	0,2021	0,7500	0,9521
397	19	75	14	42	0,2021	0,7500	0,9521
398	19	75	14	42	0,2021	0,7500	0,9521
399	19	75	14	42	0,2021	0,7500	0,9521
400	19	75	14	42	0,2021	0,7500	0,9521
401	19	75	14	42	0,2021	0,7500	0,9521
402	19	75	14	42	0,2021	0,7500	0,9521
403	19	75	14	42	0,2021	0,7500	0,9521
404	19	75	14	42	0,2021	0,7500	0,9521
405	19	75	14	42	0,2021	0,7500	0,9521
406	19	75	14	42	0,2021	0,7500	0,9521
407	19	75	14	42	0,2021	0,7500	0,9521
408	18	76	14	42	0,1915	0,7500	0,9415
409	18	76	14	42	0,1915	0,7500	0,9415
410	18	76	14	42	0,1915	0,7500	0,9415
411	18	76	14	42	0,1915	0,7500	0,9415
412	18	76	14	42	0,1915	0,7500	0,9415
413	18	76	14	42	0,1915	0,7500	0,9415
414	18	76	14	42	0,1915	0,7500	0,9415
415	18	76	14	42	0,1915	0,7500	0,9415
416	18	76	14	42	0,1915	0,7500	0,9415
417	18	76	13	43	0,1915	0,7679	0,9593
418	18	76	13	43	0,1915	0,7679	0,9593
419	18	76	13	43	0,1915	0,7679	0,9593
420	18	76	13	43	0,1915	0,7679	0,9593
421	18	76	13	43	0,1915	0,7679	0,9593
422	18	76	13	43	0,1915	0,7679	0,9593
423	18	76	13	43	0,1915	0,7679	0,9593
424	18	76	13	43	0,1915	0,7679	0,9593
425	18	76	13	43	0,1915	0,7679	0,9593
426	18	76	13	43	0,1915	0,7679	0,9593
427	18	76	13	43	0,1915	0,7679	0,9593
428	18	76	13	43	0,1915	0,7679	0,9593
429	18	76	13	43	0,1915	0,7679	0,9593
430	18	76	13	43	0,1915	0,7679	0,9593
431	18	76	13	43	0,1915	0,7679	0,9593
432	18	76	13	43	0,1915	0,7679	0,9593
433	18	76	13	43	0,1915	0,7679	0,9593
434	18	76	13	43	0,1915	0,7679	0,9593
435	18	76	13	43	0,1915	0,7679	0,9593
436	18	76	13	43	0,1915	0,7679	0,9593
437	18	76	13	43	0,1915	0,7679	0,9593
438	18	76	13	43	0,1915	0,7679	0,9593
439	18	76	13	43	0,1915	0,7679	0,9593
440	18	76	13	43	0,1915	0,7679	0,9593
441	18	76	13	43	0,1915	0,7679	0,9593
442	18	76	13	43	0,1915	0,7679	0,9593
443	18	76	13	43	0,1915	0,7679	0,9593
444	18	76	13	43	0,1915	0,7679	0,9593
445	18	76	13	43	0,1915	0,7679	0,9593
446	18	76	13	43	0,1915	0,7679	0,9593
447	18	76	13	43	0,1915	0,7679	0,9593
448	18	76	13	43	0,1915	0,7679	0,9593
449	18	76	13	43	0,1915	0,7679	0,9593
450	18	76	13	43	0,1915	0,7679	0,9593
451	18	76	13	43	0,1915	0,7679	0,9593
452	18	76	13	43	0,1915	0,7679	0,9593
453	18	76	13	43	0,1915	0,7679	0,9593
454	18	76	13	43	0,1915	0,7679	0,9593
455	18	76	13	43	0,1915	0,7679	0,9593
456	18	76	13	43	0,1915	0,7679	0,9593
457	18	76	13	43	0,1915	0,7679	0,9593
458	18	76	13	43	0,1915	0,7679	0,9593
459	18	76	12	44	0,1915	0,7857	0,9772
460	18	76	12	44	0,1915	0,7857	0,9772
461	18	76	12	44	0,1915	0,7857	0,9772
462	18	76	12	44	0,1915	0,7857	0,9772
463	17	77	12	44	0,1809	0,7857	0,9666
464	17	77	12	44	0,1809	0,7857	0,9666
465	17	77	12	44	0,1809	0,7857	0,9666
466	17	77	12	44	0,1809	0,7857	0,9666
467	17	77	12	44	0,1809	0,7857	0,9666
468	17	77	12	44	0,1809	0,7857	0,9666
469	17	77	12	44	0,1809	0,7857	0,9666
470	17	77	12	44	0,1809	0,7857	0,9666
471	17	77	12	44	0,1809	0,7857	0,9666
472	17	77	11	45	0,1809	0,8036	0,9844
473	17	77	11	45	0,1809	0,8036	0,9844
474	17	77	11	45	0,1809	0,8036	0,9844
475	17	77	11	45	0,1809	0,8036	0,9844
476	17	77	10	46	0,1809	0,8214	1,0023

477	17	77	10	46	0,1809	0,8214	1,0023
478	17	77	9	47	0,1809	0,8393	1,0201
479	17	77	9	47	0,1809	0,8393	1,0201
480	17	77	9	47	0,1809	0,8393	1,0201
481	16	78	9	47	0,1702	0,8393	1,0095
482	16	78	9	47	0,1702	0,8393	1,0095
483	15	79	9	47	0,1596	0,8393	0,9989
484	15	79	8	48	0,1596	0,8571	1,0167
485	15	79	8	48	0,1596	0,8571	1,0167
486	15	79	8	48	0,1596	0,8571	1,0167
487	15	79	8	48	0,1596	0,8571	1,0167
488	15	79	8	48	0,1596	0,8571	1,0167
489	15	79	8	48	0,1596	0,8571	1,0167
490	15	79	8	48	0,1596	0,8571	1,0167
491	15	79	8	48	0,1596	0,8571	1,0167
492	15	79	8	48	0,1596	0,8571	1,0167
493	15	79	8	48	0,1596	0,8571	1,0167
494	15	79	8	48	0,1596	0,8571	1,0167
495	15	79	7	49	0,1596	0,8750	1,0346
496	15	79	7	49	0,1596	0,8750	1,0346
497	15	79	7	49	0,1596	0,8750	1,0346
498	15	79	7	49	0,1596	0,8750	1,0346
499	15	79	7	49	0,1596	0,8750	1,0346
500	15	79	7	49	0,1596	0,8750	1,0346
501	15	79	7	49	0,1596	0,8750	1,0346
502	15	79	7	49	0,1596	0,8750	1,0346
503	15	79	7	49	0,1596	0,8750	1,0346
504	15	79	7	49	0,1596	0,8750	1,0346
505	14	80	7	49	0,1489	0,8750	1,0239
506	14	80	7	49	0,1489	0,8750	1,0239
507	14	80	7	49	0,1489	0,8750	1,0239
508	13	81	7	49	0,1383	0,8750	1,0133
509	13	81	7	49	0,1383	0,8750	1,0133
510	13	81	7	49	0,1383	0,8750	1,0133
511	13	81	7	49	0,1383	0,8750	1,0133
512	13	81	7	49	0,1383	0,8750	1,0133
513	13	81	7	49	0,1383	0,8750	1,0133
514	12	82	7	49	0,1277	0,8750	1,0027
515	12	82	7	49	0,1277	0,8750	1,0027
516	12	82	7	49	0,1277	0,8750	1,0027
517	12	82	7	49	0,1277	0,8750	1,0027
518	12	82	7	49	0,1277	0,8750	1,0027
519	11	83	7	49	0,1170	0,8750	0,9920
520	11	83	7	49	0,1170	0,8750	0,9920
521	11	83	7	49	0,1170	0,8750	0,9920
522	11	83	7	49	0,1170	0,8750	0,9920
523	10	84	7	49	0,1064	0,8750	0,9814
524	10	84	7	49	0,1064	0,8750	0,9814
525	10	84	7	49	0,1064	0,8750	0,9814
526	10	84	7	49	0,1064	0,8750	0,9814
527	10	84	7	49	0,1064	0,8750	0,9814
528	10	84	7	49	0,1064	0,8750	0,9814
529	10	84	7	49	0,1064	0,8750	0,9814
530	10	84	7	49	0,1064	0,8750	0,9814
531	10	84	7	49	0,1064	0,8750	0,9814
532	10	84	7	49	0,1064	0,8750	0,9814
533	10	84	7	49	0,1064	0,8750	0,9814
534	10	84	7	49	0,1064	0,8750	0,9814
535	10	84	6	50	0,1064	0,8929	0,9992
536	10	84	6	50	0,1064	0,8929	0,9992
537	9	85	6	50	0,0957	0,8929	0,9886
538	9	85	6	50	0,0957	0,8929	0,9886
539	9	85	6	50	0,0957	0,8929	0,9886
540	9	85	6	50	0,0957	0,8929	0,9886
541	9	85	6	50	0,0957	0,8929	0,9886
542	9	85	6	50	0,0957	0,8929	0,9886
543	9	85	6	50	0,0957	0,8929	0,9886
544	9	85	6	50	0,0957	0,8929	0,9886
545	9	85	6	50	0,0957	0,8929	0,9886
546	9	85	6	50	0,0957	0,8929	0,9886
547	9	85	6	50	0,0957	0,8929	0,9886
548	9	85	6	50	0,0957	0,8929	0,9886
549	9	85	6	50	0,0957	0,8929	0,9886
550	8	86	6	50	0,0851	0,8929	0,9780
551	8	86	6	50	0,0851	0,8929	0,9780
552	8	86	5	51	0,0851	0,9107	0,9958
553	8	86	5	51	0,0851	0,9107	0,9958
554	8	86	5	51	0,0851	0,9107	0,9958
555	7	87	5	51	0,0745	0,9107	0,9852
556	6	88	5	51	0,0638	0,9107	0,9745
557	6	88	5	51	0,0638	0,9107	0,9745
558	6	88	5	51	0,0638	0,9107	0,9745
559	6	88	5	51	0,0638	0,9107	0,9745
560	5	89	5	51	0,0532	0,9107	0,9639
561	5	89	5	51	0,0532	0,9107	0,9639
562	5	89	5	51	0,0532	0,9107	0,9639
563	5	89	5	51	0,0532	0,9107	0,9639
564	5	89	5	51	0,0532	0,9107	0,9639
565	5	89	5	51	0,0532	0,9107	0,9639
566	5	89	5	51	0,0532	0,9107	0,9639
567	5	89	5	51	0,0532	0,9107	0,9639
568	5	89	5	51	0,0532	0,9107	0,9639
569	5	89	5	51	0,0532	0,9107	0,9639
570	5	89	5	51	0,0532	0,9107	0,9639
571	5	89	5	51	0,0532	0,9107	0,9639
572	5	89	4	52	0,0532	0,9286	0,9818
573	5	89	4	52	0,0532	0,9286	0,9818
574	5	89	4	52	0,0532	0,9286	0,9818
575	5	89	4	52	0,0532	0,9286	0,9818
576	5	89	4	52	0,0532	0,9286	0,9818
577	5	89	4	52	0,0532	0,9286	0,9818
578	5	89	4	52	0,0532	0,9286	0,9818
579	5	89	4	52	0,0532	0,9286	0,9818
580	4	90	4	52	0,0426	0,9286	0,9711
581	4	90	4	52	0,0426	0,9286	0,9711
582	4	90	4	52	0,0426	0,9286	0,9711
583	4	90	4	52	0,0426	0,9286	0,9711
584	4	90	4	52	0,0426	0,9286	0,9711
585	4	90	4	52	0,0426	0,9286	0,9711
586	4	90	4	52	0,0426	0,9286	0,9711
587	4	90	4	52	0,0426	0,9286	0,9711
588	4	90	4	52	0,0426	0,9286	0,9711
589	4	90	4	52	0,0426	0,9286	0,9711
590	4	90	4	52	0,0426	0,9286	0,9711
591	4	90	4	52	0,0426	0,9286	0,9711
592	4	90	4	52	0,0426	0,9286	0,9711
593	4	90	4	52	0,0426	0,9286	0,9711
594	4	90	4	52	0,0426	0,9286	0,9711
595	4	90	4	52	0,0426	0,9286	0,9711
596	4	90	4	52	0,0426	0,9286	0,9711
597	4	90	4	52	0,0426	0,9286	0,9711
598	4	90	4	52	0,0426	0,9286	0,9711
599	4	90	4	52	0,0426	0,9286	0,9711
600	4	90	4	52	0,0426	0,9286	0,9711
601	4	90	4	52	0,0426	0,9286	0,9711
602	4	90	4	52	0,0426	0,9286	0,9711
603	4	90	3	53	0,0426	0,9464	0,9890
604	4	90	3	53	0,0426	0,9464	0,9890
605	4	90	3	53	0,0426	0,9464	0,9890
606	4	90	3	53	0,0426	0,9464	0,9890
607	4	90	3	53	0,0426	0,9464	0,9890
608	4	90	3	53	0,0426	0,9464	0,9890
609	4	90	3	53	0,0426	0,9464	0,9890
610	4	90	3	53	0,0426	0,9464	0,9890
611	4	90	3	53	0,0426	0,9464	0,9890
612	4	90	3	53	0,0426	0,9464	0,9890
613	4	90	3	53	0,0426	0,9464	0,9890
614	4	90	3	53	0,0426	0,9464	0,9890
615	4	90	3	53	0,0426	0,9464	0,9890
616	4	90	3	53	0,0426	0,9464	0,9890
617	4	90	3	53	0,0426	0,9464	0,9890
618	4	90	3	53	0,0426	0,9464	0,9890
619	4	90	3	53	0,0426	0,9464	0,9890
620	4	90	3	53	0,0426	0,9464	0,9890
621	4	90	3	53	0,0426	0,9464	0,9890
622	4	90	3	53	0,0426	0,9464	0,9890

623	4	90	3	53	0,0426	0,9464	0,9890
624	4	90	3	53	0,0426	0,9464	0,9890
625	4	90	3	53	0,0426	0,9464	0,9890
626	4	90	3	53	0,0426	0,9464	0,9890
627	4	90	3	53	0,0426	0,9464	0,9890
628	4	90	3	53	0,0426	0,9464	0,9890
629	4	90	2	54	0,0426	0,9643	1,0068
630	4	90	2	54	0,0426	0,9643	1,0068
631	4	90	2	54	0,0426	0,9643	1,0068
632	4	90	2	54	0,0426	0,9643	1,0068
633	4	90	2	54	0,0426	0,9643	1,0068
634	4	90	2	54	0,0426	0,9643	1,0068
635	4	90	2	54	0,0426	0,9643	1,0068
636	4	90	2	54	0,0426	0,9643	1,0068
637	4	90	2	54	0,0426	0,9643	1,0068
638	4	90	2	54	0,0426	0,9643	1,0068
639	4	90	2	54	0,0426	0,9643	1,0068
640	4	90	2	54	0,0426	0,9643	1,0068
641	4	90	2	54	0,0426	0,9643	1,0068
642	4	90	2	54	0,0426	0,9643	1,0068
643	4	90	2	54	0,0426	0,9643	1,0068
644	4	90	2	54	0,0426	0,9643	1,0068
645	4	90	2	54	0,0426	0,9643	1,0068
646	4	90	2	54	0,0426	0,9643	1,0068
647	4	90	2	54	0,0426	0,9643	1,0068
648	4	90	2	54	0,0426	0,9643	1,0068
649	4	90	2	54	0,0426	0,9643	1,0068
650	4	90	2	54	0,0426	0,9643	1,0068
651	4	90	2	54	0,0426	0,9643	1,0068
652	4	90	2	54	0,0426	0,9643	1,0068
653	4	90	2	54	0,0426	0,9643	1,0068
654	4	90	2	54	0,0426	0,9643	1,0068
655	4	90	2	54	0,0426	0,9643	1,0068
656	4	90	2	54	0,0426	0,9643	1,0068
657	4	90	2	54	0,0426	0,9643	1,0068
658	4	90	2	54	0,0426	0,9643	1,0068
659	4	90	2	54	0,0426	0,9643	1,0068
660	4	90	2	54	0,0426	0,9643	1,0068
661	4	90	2	54	0,0426	0,9643	1,0068
662	4	90	2	54	0,0426	0,9643	1,0068
663	4	90	2	54	0,0426	0,9643	1,0068
664	4	90	2	54	0,0426	0,9643	1,0068
665	4	90	2	54	0,0426	0,9643	1,0068
666	4	90	2	54	0,0426	0,9643	1,0068
667	4	90	2	54	0,0426	0,9643	1,0068
668	4	90	2	54	0,0426	0,9643	1,0068
669	3	91	2	54	0,0319	0,9643	0,9962
670	3	91	2	54	0,0319	0,9643	0,9962
671	3	91	2	54	0,0319	0,9643	0,9962
672	3	91	2	54	0,0319	0,9643	0,9962
673	3	91	2	54	0,0319	0,9643	0,9962
674	3	91	2	54	0,0319	0,9643	0,9962
675	3	91	2	54	0,0319	0,9643	0,9962
676	3	91	2	54	0,0319	0,9643	0,9962
677	3	91	2	54	0,0319	0,9643	0,9962
678	3	91	2	54	0,0319	0,9643	0,9962
679	3	91	2	54	0,0319	0,9643	0,9962
680	3	91	2	54	0,0319	0,9643	0,9962
681	3	91	2	54	0,0319	0,9643	0,9962
682	3	91	2	54	0,0319	0,9643	0,9962
683	3	91	2	54	0,0319	0,9643	0,9962
684	3	91	2	54	0,0319	0,9643	0,9962
685	3	91	2	54	0,0319	0,9643	0,9962
686	3	91	2	54	0,0319	0,9643	0,9962
687	3	91	2	54	0,0319	0,9643	0,9962
688	3	91	2	54	0,0319	0,9643	0,9962
689	3	91	2	54	0,0319	0,9643	0,9962
690	3	91	2	54	0,0319	0,9643	0,9962
691	3	91	2	54	0,0319	0,9643	0,9962
692	3	91	2	54	0,0319	0,9643	0,9962
693	3	91	2	54	0,0319	0,9643	0,9962
694	3	91	2	54	0,0319	0,9643	0,9962
695	3	91	2	54	0,0319	0,9643	0,9962
696	3	91	2	54	0,0319	0,9643	0,9962
697	2	92	2	54	0,0213	0,9643	0,9856
698	2	92	2	54	0,0213	0,9643	0,9856
699	2	92	2	54	0,0213	0,9643	0,9856
700	2	92	2	54	0,0213	0,9643	0,9856
701	2	92	2	54	0,0213	0,9643	0,9856
702	2	92	2	54	0,0213	0,9643	0,9856
703	2	92	2	54	0,0213	0,9643	0,9856
704	2	92	2	54	0,0213	0,9643	0,9856
705	2	92	2	54	0,0213	0,9643	0,9856
706	2	92	2	54	0,0213	0,9643	0,9856
707	2	92	2	54	0,0213	0,9643	0,9856
708	2	92	2	54	0,0213	0,9643	0,9856
709	2	92	2	54	0,0213	0,9643	0,9856
710	2	92	2	54	0,0213	0,9643	0,9856
711	2	92	2	54	0,0213	0,9643	0,9856
712	2	92	2	54	0,0213	0,9643	0,9856
713	2	92	2	54	0,0213	0,9643	0,9856
714	2	92	2	54	0,0213	0,9643	0,9856
715	2	92	2	54	0,0213	0,9643	0,9856
716	2	92	2	54	0,0213	0,9643	0,9856
717	2	92	2	54	0,0213	0,9643	0,9856
718	2	92	2	54	0,0213	0,9643	0,9856
719	2	92	2	54	0,0213	0,9643	0,9856
720	2	92	2	54	0,0213	0,9643	0,9856
721	2	92	2	54	0,0213	0,9643	0,9856
722	2	92	2	54	0,0213	0,9643	0,9856
723	2	92	2	54	0,0213	0,9643	0,9856
724	2	92	2	54	0,0213	0,9643	0,9856
725	2	92	2	54	0,0213	0,9643	0,9856
726	2	92	2	54	0,0213	0,9643	0,9856
727	2	92	2	54	0,0213	0,9643	0,9856
728	2	92	2	54	0,0213	0,9643	0,9856
729	2	92	2	54	0,0213	0,9643	0,9856
730	2	92	2	54	0,0213	0,9643	0,9856
731	2	92	2	54	0,0213	0,9643	0,9856
732	2	92	2	54	0,0213	0,9643	0,9856
733	2	92	2	54	0,0213	0,9643	0,9856
734	2	92	2	54	0,0213	0,9643	0,9856
735	2	92	2	54	0,0213	0,9643	0,9856
736	2	92	2	54	0,0213	0,9643	0,9856
737	2	92	2	54	0,0213	0,9643	0,9856
738	2	92	2	54	0,0213	0,9643	0,9856
739	2	92	2	54	0,0213	0,9643	0,9856
740	2	92	2	54	0,0213	0,9643	0,9856
741	2	92	2	54	0,0213	0,9643	0,9856
742	2	92	2	54	0,0213	0,9643	0,9856
743	2	92	2	54	0,0213	0,9643	0,9856
744	2	92	2	54	0,0213	0,9643	0,9856
745	2	92	2	54	0,0213	0,9643	0,9856
746	2	92	2	54	0,0213	0,9643	0,9856
747	2	92	2	54	0,0213	0,9643	0,9856
748	2	92	2	54	0,0213	0,9643	0,9856
749	2	92	2	54	0,0213	0,9643	0,9856
750	2	92	2	54	0,0213	0,9643	0,9856
751	2	92	2	54	0,0213	0,9643	0,9856
752	2	92	2	54	0,0213	0,9643	0,9856
753	2	92	2	54	0,0213	0,9643	0,9856
754	2	92	2	54	0,0213	0,9643	0,9856
755	2	92	2	54	0,0213	0,9643	0,9856
756	2	92	2	54	0,0213	0,9643	0,9856
757	2	92	2	54	0,0213	0,9643	0,9856
758	2	92	2	54	0,0213	0,9643	0,9856
759	2	92	2	54	0,0213	0,9643	0,9856
760	2	92	2	54	0,0213	0,9643	0,9856
761	2	92	2	54	0,0213	0,9643	0,9856
762	2	92	2	54	0,0213	0,9643	0,9856
763	2	92	2	54	0,0213	0,9643	0,9856
764	2	92	2	54	0,0213	0,9643	0,9856
765	2	92	2	54	0,0213	0,9643	0,9856
766	2	92	2	54	0,0213	0,9643	0,9856
767	2	92	2	54	0,0213	0,9643	0,9856
768	2	92	2	54	0,0213	0,9643	0,9856

769	2	92	2	54	0,0213	0,9643	0,9856
770	2	92	2	54	0,0213	0,9643	0,9856
771	2	92	2	54	0,0213	0,9643	0,9856
772	2	92	2	54	0,0213	0,9643	0,9856
773	2	92	2	54	0,0213	0,9643	0,9856
774	2	92	2	54	0,0213	0,9643	0,9856
775	2	92	2	54	0,0213	0,9643	0,9856
776	2	92	2	54	0,0213	0,9643	0,9856
777	2	92	2	54	0,0213	0,9643	0,9856
778	2	92	2	54	0,0213	0,9643	0,9856
779	2	92	2	54	0,0213	0,9643	0,9856
780	2	92	2	54	0,0213	0,9643	0,9856
781	2	92	2	54	0,0213	0,9643	0,9856
782	2	92	2	54	0,0213	0,9643	0,9856
783	2	92	2	54	0,0213	0,9643	0,9856
784	2	92	2	54	0,0213	0,9643	0,9856
785	2	92	2	54	0,0213	0,9643	0,9856
786	2	92	2	54	0,0213	0,9643	0,9856
787	2	92	2	54	0,0213	0,9643	0,9856
788	2	92	2	54	0,0213	0,9643	0,9856
789	2	92	2	54	0,0213	0,9643	0,9856
790	2	92	2	54	0,0213	0,9643	0,9856
791	2	92	2	54	0,0213	0,9643	0,9856
792	2	92	2	54	0,0213	0,9643	0,9856
793	2	92	2	54	0,0213	0,9643	0,9856
794	2	92	2	54	0,0213	0,9643	0,9856
795	2	92	2	54	0,0213	0,9643	0,9856
796	2	92	2	54	0,0213	0,9643	0,9856
797	2	92	2	54	0,0213	0,9643	0,9856
798	2	92	2	54	0,0213	0,9643	0,9856
799	2	92	2	54	0,0213	0,9643	0,9856
800	2	92	2	54	0,0213	0,9643	0,9856

S6. Plot olanzapine Sensitivity Specificity datasets (local maxima: 23.5, 28.5)

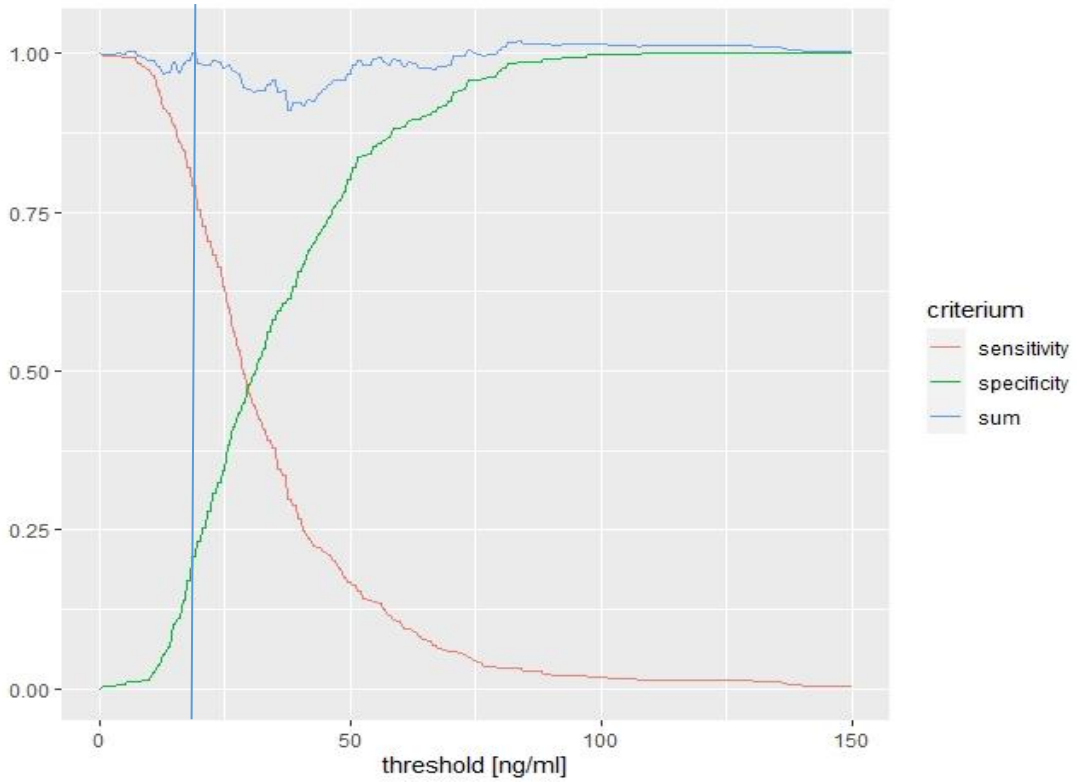


S7. Data olanzapine Sensitivity Specificity dataset

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
3	31	0	26	0	1,0000	0,0000	1,0000
3,5	31	0	26	0	1,0000	0,0000	1,0000
4	31	0	25	1	1,0000	0,0385	1,0385
4,5	31	0	25	1	1,0000	0,0385	1,0385
5	31	0	25	1	1,0000	0,0385	1,0385
5,5	29	2	22	4	0,9355	0,1538	1,0893
6	29	2	22	4	0,9355	0,1538	1,0893
6,5	28	3	22	4	0,9032	0,1538	1,0571
7	28	3	22	4	0,9032	0,1538	1,0571
7,5	28	3	22	4	0,9032	0,1538	1,0571
8	28	3	22	4	0,9032	0,1538	1,0571
8,5	28	3	22	4	0,9032	0,1538	1,0571
9	27	4	22	4	0,8710	0,1538	1,0248
9,5	26	5	20	6	0,8387	0,2308	1,0695
10	26	5	20	6	0,8387	0,2308	1,0695
10,5	26	5	20	6	0,8387	0,2308	1,0695
11	26	5	20	6	0,8387	0,2308	1,0695
11,5	25	6	19	7	0,8065	0,2692	1,0757
12	25	6	19	7	0,8065	0,2692	1,0757
12,5	25	6	19	7	0,8065	0,2692	1,0757
13	25	6	17	9	0,8065	0,3462	1,1526
13,5	25	6	17	9	0,8065	0,3462	1,1526
14	25	6	17	9	0,8065	0,3462	1,1526
14,5	25	6	17	9	0,8065	0,3462	1,1526
15	25	6	17	9	0,8065	0,3462	1,1526
15,5	25	6	17	9	0,8065	0,3462	1,1526
16	25	6	17	9	0,8065	0,3462	1,1526
16,5	25	6	17	9	0,8065	0,3462	1,1526
17	25	6	17	9	0,8065	0,3462	1,1526
17,5	25	6	17	9	0,8065	0,3462	1,1526
18	25	6	17	9	0,8065	0,3462	1,1526
18,5	25	6	15	11	0,8065	0,4231	1,2295
19	25	6	15	11	0,8065	0,4231	1,2295
19,5	25	6	15	11	0,8065	0,4231	1,2295
20	24	7	15	11	0,7742	0,4231	1,1973
20,5	23	8	13	13	0,7419	0,5000	1,2419
21	23	8	13	13	0,7419	0,5000	1,2419
21,5	23	8	13	13	0,7419	0,5000	1,2419
22	23	8	13	13	0,7419	0,5000	1,2419
22,5	23	8	13	13	0,7419	0,5000	1,2419
23	23	8	13	13	0,7419	0,5000	1,2419
23,5	23	8	12	14	0,7419	0,5385	1,2804
24	23	8	12	14	0,7419	0,5385	1,2804
24,5	22	9	12	14	0,7097	0,5385	1,2481
25	22	9	12	14	0,7097	0,5385	1,2481
25,5	22	9	11	15	0,7097	0,5769	1,2866
26	22	9	11	15	0,7097	0,5769	1,2866
26,5	21	10	11	15	0,6774	0,5769	1,2543
27	21	10	10	16	0,6774	0,6154	1,2928
27,5	21	10	9	17	0,6774	0,6538	1,3313
28	21	10	9	17	0,6774	0,6538	1,3313
28,5	20	11	9	17	0,6452	0,6538	1,2990
29	20	11	9	17	0,6452	0,6538	1,2990
29,5	20	11	9	17	0,6452	0,6538	1,2990
30	20	11	9	17	0,6452	0,6538	1,2990
30,5	16	15	9	17	0,5161	0,6538	1,1700
31	16	15	9	17	0,5161	0,6538	1,1700
31,5	16	15	9	17	0,5161	0,6538	1,1700
32	16	15	9	17	0,5161	0,6538	1,1700
32,5	16	15	9	17	0,5161	0,6538	1,1700
33	16	15	9	17	0,5161	0,6538	1,1700
33,5	16	15	9	17	0,5161	0,6538	1,1700
34	14	17	7	19	0,4516	0,7308	1,1824
34,5	14	17	7	19	0,4516	0,7308	1,1824
35	14	17	7	19	0,4516	0,7308	1,1824
35,5	14	17	7	19	0,4516	0,7308	1,1824
36	12	19	5	21	0,3871	0,8077	1,1948
36,5	12	19	5	21	0,3871	0,8077	1,1948
37	12	19	5	21	0,3871	0,8077	1,1948
37,5	12	19	5	21	0,3871	0,8077	1,1948
38	12	19	5	21	0,3871	0,8077	1,1948
38,5	12	19	3	23	0,3871	0,8846	1,2717
39	12	19	3	23	0,3871	0,8846	1,2717
39,5	12	19	3	23	0,3871	0,8846	1,2717
40	12	19	3	23	0,3871	0,8846	1,2717
40,5	12	19	3	23	0,3871	0,8846	1,2717
41	12	19	3	23	0,3871	0,8846	1,2717
41,5	12	19	3	23	0,3871	0,8846	1,2717
42	12	19	3	23	0,3871	0,8846	1,2717
42,5	12	19	3	23	0,3871	0,8846	1,2717
43	12	19	3	23	0,3871	0,8846	1,2717
43,5	12	19	3	23	0,3871	0,8846	1,2717
44	12	19	3	23	0,3871	0,8846	1,2717
44,5	12	19	3	23	0,3871	0,8846	1,2717
45	11	20	3	23	0,3548	0,8846	1,2395
45,5	11	20	2	24	0,3548	0,9231	1,2779
46	11	20	2	24	0,3548	0,9231	1,2779
46,5	11	20	2	24	0,3548	0,9231	1,2779
47	11	20	2	24	0,3548	0,9231	1,2779
47,5	11	20	2	24	0,3548	0,9231	1,2779
48	11	20	2	24	0,3548	0,9231	1,2779
48,5	10	21	2	24	0,3226	0,9231	1,2457
49	9	22	2	24	0,2903	0,9231	1,2134
49,5	9	22	2	24	0,2903	0,9231	1,2134
50	9	22	2	24	0,2903	0,9231	1,2134
50,5	7	24	1	25	0,2258	0,9615	1,1873
51	7	24	1	25	0,2258	0,9615	1,1873
51,5	7	24	1	25	0,2258	0,9615	1,1873
52	7	24	0	26	0,2258	1,0000	1,2258
52,5	7	24	0	26	0,2258	1,0000	1,2258
53	7	24	0	26	0,2258	1,0000	1,2258
53,5	7	24	0	26	0,2258	1,0000	1,2258
54	7	24	0	26	0,2258	1,0000	1,2258
54,5	7	24	0	26	0,2258	1,0000	1,2258
55	7	24	0	26	0,2258	1,0000	1,2258
55,5	7	24	0	26	0,2258	1,0000	1,2258
56	7	24	0	26	0,2258	1,0000	1,2258
56,5	7	24	0	26	0,2258	1,0000	1,2258
57	7	24	0	26	0,2258	1,0000	1,2258
57,5	7	24	0	26	0,2258	1,0000	1,2258
58	7	24	0	26	0,2258	1,0000	1,2258
58,5	7	24	0	26	0,2258	1,0000	1,2258
59	7	24	0	26	0,2258	1,0000	1,2258
59,5	7	24	0	26	0,2258	1,0000	1,2258
60	7	24	0	26	0,2258	1,0000	1,2258
60,5	7	24	0	26	0,2258	1,0000	1,2258
61	7	24	0	26	0,2258	1,0000	1,2258
61,5	7	24	0	26	0,2258	1,0000	1,2258
62	7	24	0	26	0,2258	1,0000	1,2258
62,5	6	25	0	26	0,1935	1,0000	1,1936
63	6	25	0	26	0,1935	1,0000	1,1936
63,5	6	25	0	26	0,1935	1,0000	1,1936
64	6	25	0	26	0,1935	1,0000	1,1936
64,5	6	25	0	26	0,1935	1,0000	1,1936
65	6	25	0	26	0,1935	1,0000	1,1936
65,5	6	25	0	26	0,1935	1,0000	1,1936
66	5	26	0	26	0,1613	1,0000	1,1613
66,5	5	26	0	26	0,1613	1,0000	1,1613
67	5	26	0	26	0,1613	1,0000	1,1613
67,5	5	26	0	26	0,1613	1,0000	1,1613
68	5	26	0	26	0,1613	1,0000	1,1613
68,5	5	26	0	26	0,1613	1,0000	1,1613
69	5	26	0	26	0,1613	1,0000	1,1613
69,5	4	27	0	26	0,1290	1,0000	1,1290
70	4	27	0	26	0,1290	1,0000	1,1290
70,5	4	27	0	26	0,1290	1,0000	1,1290
71	4	27	0	26	0,1290	1,0000	1,1290
71,5	4	27	0	26	0,1290	1,0000	1,1290
72	4	27	0	26	0,1290	1,0000	1,1290
72,5	4	27	0	26	0,1290	1,0000	1,1290

73	4	27	0	26	0,1290	1,0000	1,1290
73,5	4	27	0	26	0,1290	1,0000	1,1290
74	4	27	0	26	0,1290	1,0000	1,1290
74,5	4	27	0	26	0,1290	1,0000	1,1290
75	4	27	0	26	0,1290	1,0000	1,1290
75,5	4	27	0	26	0,1290	1,0000	1,1290
76	4	27	0	26	0,1290	1,0000	1,1290
76,5	4	27	0	26	0,1290	1,0000	1,1290
77	4	27	0	26	0,1290	1,0000	1,1290
77,5	4	27	0	26	0,1290	1,0000	1,1290
78	4	27	0	26	0,1290	1,0000	1,1290
78,5	4	27	0	26	0,1290	1,0000	1,1290
79	4	27	0	26	0,1290	1,0000	1,1290
79,5	4	27	0	26	0,1290	1,0000	1,1290
80	4	27	0	26	0,1290	1,0000	1,1290
80,5	4	27	0	26	0,1290	1,0000	1,1290
81	4	27	0	26	0,1290	1,0000	1,1290
81,5	4	27	0	26	0,1290	1,0000	1,1290
82	4	27	0	26	0,1290	1,0000	1,1290
82,5	4	27	0	26	0,1290	1,0000	1,1290
83	4	27	0	26	0,1290	1,0000	1,1290
83,5	4	27	0	26	0,1290	1,0000	1,1290
84	4	27	0	26	0,1290	1,0000	1,1290
84,5	4	27	0	26	0,1290	1,0000	1,1290
85	4	27	0	26	0,1290	1,0000	1,1290
85,5	4	27	0	26	0,1290	1,0000	1,1290
86	4	27	0	26	0,1290	1,0000	1,1290
86,5	4	27	0	26	0,1290	1,0000	1,1290
87	4	27	0	26	0,1290	1,0000	1,1290
87,5	4	27	0	26	0,1290	1,0000	1,1290
88	4	27	0	26	0,1290	1,0000	1,1290
88,5	4	27	0	26	0,1290	1,0000	1,1290
89	4	27	0	26	0,1290	1,0000	1,1290
89,5	4	27	0	26	0,1290	1,0000	1,1290
90	4	27	0	26	0,1290	1,0000	1,1290
90,5	4	27	0	26	0,1290	1,0000	1,1290
91	3	28	0	26	0,0968	1,0000	1,0968
91,5	3	28	0	26	0,0968	1,0000	1,0968
92	3	28	0	26	0,0968	1,0000	1,0968
92,5	3	28	0	26	0,0968	1,0000	1,0968
93	3	28	0	26	0,0968	1,0000	1,0968
93,5	3	28	0	26	0,0968	1,0000	1,0968
94	3	28	0	26	0,0968	1,0000	1,0968
94,5	3	28	0	26	0,0968	1,0000	1,0968
95	3	28	0	26	0,0968	1,0000	1,0968
95,5	3	28	0	26	0,0968	1,0000	1,0968
96	2	29	0	26	0,0645	1,0000	1,0645

S8. Plot escitalopram Sensitivity Specificity dataset (local maximum: 18.5)

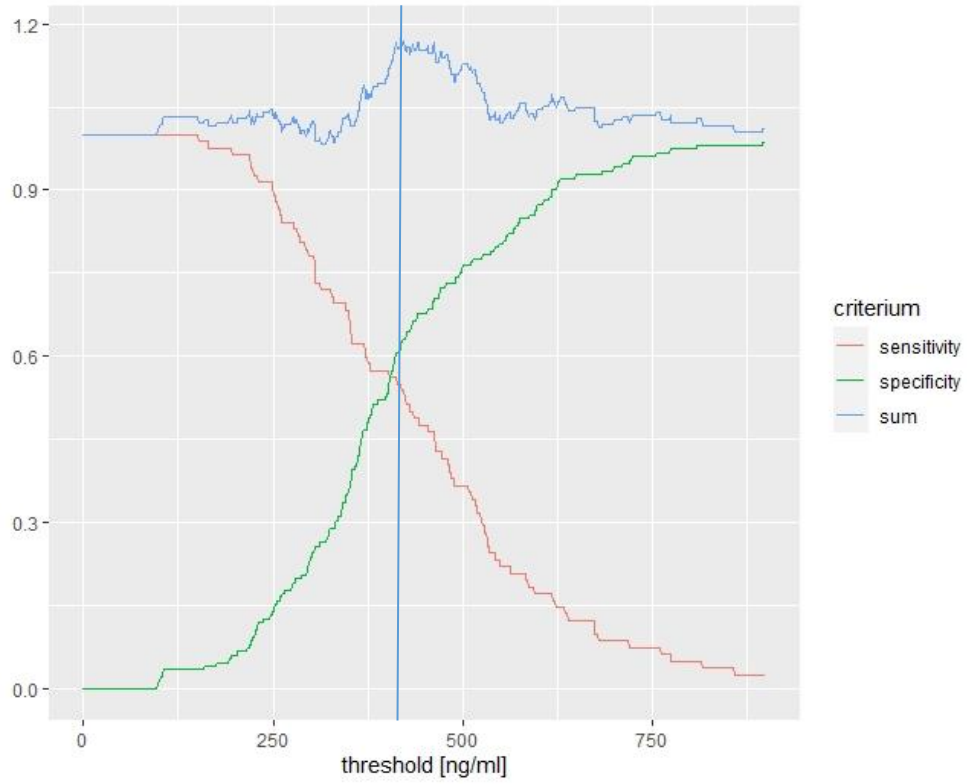


S9 Data escitalopram Sensitivity Specificity dataset

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
3	392	2	354	1	0,9949	0,0028	0,9977
3,5	392	2	353	2	0,9949	0,0056	1,0006
4	392	2	353	2	0,9949	0,0056	1,0006
4,5	391	3	353	2	0,9924	0,0056	0,9980
5	391	3	353	2	0,9924	0,0056	0,9980
5,5	391	3	351	4	0,9924	0,0113	1,0037
6	391	3	351	4	0,9924	0,0113	1,0037
6,5	391	3	351	4	0,9924	0,0113	1,0037
7	391	3	351	4	0,9924	0,0113	1,0037
7,5	388	6	351	4	0,9848	0,0113	0,9960
8	388	6	351	4	0,9848	0,0113	0,9960
8,5	386	8	350	5	0,9797	0,0141	0,9938
9	386	8	350	5	0,9797	0,0141	0,9938
9,5	384	10	350	5	0,9746	0,0141	0,9887
10	384	10	350	5	0,9746	0,0141	0,9887
10,5	380	14	346	9	0,9645	0,0254	0,9898
11	380	14	346	9	0,9645	0,0254	0,9898
11,5	371	23	342	13	0,9416	0,0366	0,9782
12	371	23	342	13	0,9416	0,0366	0,9782
12,5	360	34	336	19	0,9137	0,0535	0,9672
13	360	34	336	19	0,9137	0,0535	0,9672
13,5	357	37	332	23	0,9061	0,0648	0,9709
14	357	37	332	23	0,9061	0,0648	0,9709
14,5	349	45	319	36	0,8858	0,1014	0,9872
15	349	45	319	36	0,8858	0,1014	0,9872
15,5	339	55	316	39	0,8604	0,1099	0,9703
16	339	55	316	39	0,8604	0,1099	0,9703
16,5	333	61	306	49	0,8452	0,1380	0,9832
17	333	61	306	49	0,8452	0,1380	0,9832
17,5	323	71	295	60	0,8198	0,1690	0,9888
18	323	71	295	60	0,8198	0,1690	0,9888
18,5	312	82	281	74	0,7919	0,2085	1,0003
19	312	82	281	74	0,7919	0,2085	1,0003
19,5	297	97	273	82	0,7538	0,2310	0,9848
20	297	97	273	82	0,7538	0,2310	0,9848
20,5	287	107	265	90	0,7284	0,2535	0,9819
21	287	107	265	90	0,7284	0,2535	0,9819
21,5	277	117	256	99	0,7030	0,2789	0,9819
22	277	117	256	99	0,7030	0,2789	0,9819
22,5	269	125	246	109	0,6827	0,3070	0,9898
23	269	125	246	109	0,6827	0,3070	0,9898
23,5	261	133	240	115	0,6624	0,3239	0,9864
24	261	133	240	115	0,6624	0,3239	0,9864
24,5	249	145	233	122	0,6320	0,3437	0,9756
25	249	145	233	122	0,6320	0,3437	0,9756
25,5	236	158	219	136	0,5990	0,3831	0,9821
26	236	158	219	136	0,5990	0,3831	0,9821
26,5	221	173	209	146	0,5609	0,4113	0,9722
27	221	173	209	146	0,5609	0,4113	0,9722
27,5	210	184	201	154	0,5330	0,4338	0,9668
28	210	184	201	154	0,5330	0,4338	0,9668
28,5	195	199	195	160	0,4949	0,4507	0,9456
29	195	199	195	160	0,4949	0,4507	0,9456
29,5	183	211	185	170	0,4645	0,4789	0,9433
30	183	211	185	170	0,4645	0,4789	0,9433
30,5	175	219	179	176	0,4442	0,4958	0,9399
31	175	219	179	176	0,4442	0,4958	0,9399
31,5	167	227	171	184	0,4239	0,5183	0,9422
32	167	227	171	184	0,4239	0,5183	0,9422
32,5	160	234	165	190	0,4061	0,5352	0,9413
33	160	234	165	190	0,4061	0,5352	0,9413
33,5	154	240	156	199	0,3909	0,5606	0,9514
34	154	240	156	199	0,3909	0,5606	0,9514
34,5	149	245	149	206	0,3782	0,5803	0,9585
35	149	245	149	206	0,3782	0,5803	0,9585
35,5	136	258	144	211	0,3452	0,5944	0,9395
36	136	258	144	211	0,3452	0,5944	0,9395
36,5	132	262	140	215	0,3350	0,6056	0,9407
37	132	262	140	215	0,3350	0,6056	0,9407
37,5	117	277	137	218	0,2970	0,6141	0,9110
38	117	277	137	218	0,2970	0,6141	0,9110
38,5	114	280	130	225	0,2893	0,6338	0,9231
39	114	280	130	225	0,2893	0,6338	0,9231
39,5	105	289	122	233	0,2665	0,6563	0,9228
40	105	289	122	233	0,2665	0,6563	0,9228
40,5	98	296	118	237	0,2487	0,6676	0,9163
41	98	296	118	237	0,2487	0,6676	0,9163
41,5	93	301	110	245	0,2360	0,6901	0,9262
42	93	301	110	245	0,2360	0,6901	0,9262
42,5	88	306	106	249	0,2234	0,7014	0,9248
43	88	306	106	249	0,2234	0,7014	0,9248
43,5	87	307	101	254	0,2208	0,7155	0,9363
44	87	307	101	254	0,2208	0,7155	0,9363
44,5	85	309	97	258	0,2157	0,7268	0,9425
45	85	309	97	258	0,2157	0,7268	0,9425
45,5	83	311	92	263	0,2107	0,7408	0,9515
46	83	311	92	263	0,2107	0,7408	0,9515
46,5	79	315	86	269	0,2005	0,7577	0,9583
47	79	315	86	269	0,2005	0,7577	0,9583
47,5	74	320	82	273	0,1878	0,7690	0,9568
48	74	320	82	273	0,1878	0,7690	0,9568
48,5	69	325	77	278	0,1751	0,7831	0,9582
49	69	325	77	278	0,1751	0,7831	0,9582
49,5	66	328	71	284	0,1675	0,8000	0,9675
50	66	328	71	284	0,1675	0,8000	0,9675
50,5	64	330	64	291	0,1624	0,8197	0,9822
51	64	330	64	291	0,1624	0,8197	0,9822
51,5	60	334	58	297	0,1523	0,8366	0,9889
52	60	334	58	297	0,1523	0,8366	0,9889
52,5	56	338	57	298	0,1421	0,8394	0,9816
53	56	338	57	298	0,1421	0,8394	0,9816
53,5	55	339	56	299	0,1396	0,8423	0,9818
54	55	339	56	299	0,1396	0,8423	0,9818
54,5	54	340	52	303	0,1371	0,8535	0,9906
55	54	340	52	303	0,1371	0,8535	0,9906
55,5	53	341	50	305	0,1345	0,8592	0,9937
56	53	341	50	305	0,1345	0,8592	0,9937
56,5	49	345	49	306	0,1244	0,8620	0,9863
57	49	345	49	306	0,1244	0,8620	0,9863
57,5	45	349	47	308	0,1142	0,8676	0,9818
58	45	349	47	308	0,1142	0,8676	0,9818
58,5	43	351	42	313	0,1091	0,8817	0,9908
59	43	351	42	313	0,1091	0,8817	0,9908
59,5	42	352	42	313	0,1066	0,8817	0,9883
60	42	352	42	313	0,1066	0,8817	0,9883
60,5	37	357	41	314	0,0939	0,8845	0,9784
61	37	357	41	314	0,0939	0,8845	0,9784
61,5	37	357	38	317	0,0939	0,8930	0,9869
62	37	357	38	317	0,0939	0,8930	0,9869
62,5	35	359	37	318	0,0888	0,8958	0,9846
63	35	359	37	318	0,0888	0,8958	0,9846
63,5	32	362	37	318	0,0812	0,8958	0,9770
64	32	362	37	318	0,0812	0,8958	0,9770
64,5	30	364	35	320	0,0761	0,9014	0,9776
65	30	364	35	320	0,0761	0,9014	0,9776
65,5	29	365	34	321	0,0736	0,9042	0,9778
66	29	365	34	321	0,0736	0,9042	0,9778
66,5	27	367	33	322	0,0685	0,9070	0,9756
67	27	367	33	322	0,0685	0,9070	0,9756
67,5	25	369	30	325	0,0635	0,9155	0,9789
68	25	369	30	325	0,0635	0,9155	0,9789
68,5	24	370	29	326	0,0609	0,9183	0,9792
69	24	370	29	326	0,0609	0,9183	0,9792
69,5	23	371	27	328	0,0584	0,9239	0,9823
70	23	371	27	328	0,0584	0,9239	0,9823
70,5	23	371	22	333	0,0584	0,9380	0,9964

71	23	371	22	333	0,0584	0,9380	0,9964
71,5	22	372	21	334	0,0558	0,9408	0,9967
72	22	372	21	334	0,0558	0,9408	0,9967
72,5	21	373	20	335	0,0533	0,9437	0,9970
73	21	373	20	335	0,0533	0,9437	0,9970
73,5	19	375	15	340	0,0482	0,9577	1,0060
74	19	375	15	340	0,0482	0,9577	1,0060
74,5	17	377	15	340	0,0431	0,9577	1,0009
75	17	377	15	340	0,0431	0,9577	1,0009
75,5	16	378	15	340	0,0406	0,9577	0,9984
76	16	378	15	340	0,0406	0,9577	0,9984
76,5	14	380	14	341	0,0355	0,9606	0,9961
77	14	380	14	341	0,0355	0,9606	0,9961
77,5	14	380	13	342	0,0355	0,9634	0,9989
78	14	380	13	342	0,0355	0,9634	0,9989
78,5	14	380	13	342	0,0355	0,9634	0,9989
79	14	380	13	342	0,0355	0,9634	0,9989
79,5	13	381	10	345	0,0330	0,9718	1,0048
80	13	381	10	345	0,0330	0,9718	1,0048
80,5	13	381	8	347	0,0330	0,9775	1,0105
81	13	381	8	347	0,0330	0,9775	1,0105
81,5	13	381	6	349	0,0330	0,9831	1,0161
82	13	381	6	349	0,0330	0,9831	1,0161
82,5	13	381	6	349	0,0330	0,9831	1,0161
83	13	381	6	349	0,0330	0,9831	1,0161
83,5	13	381	5	350	0,0330	0,9859	1,0189
84	13	381	5	350	0,0330	0,9859	1,0189
84,5	11	383	5	350	0,0279	0,9859	1,0138
85	11	383	5	350	0,0279	0,9859	1,0138
85,5	11	383	5	350	0,0279	0,9859	1,0138
86	11	383	5	350	0,0279	0,9859	1,0138
86,5	11	383	5	350	0,0279	0,9859	1,0138
87	11	383	5	350	0,0279	0,9859	1,0138
87,5	11	383	5	350	0,0279	0,9859	1,0138
88	11	383	5	350	0,0279	0,9859	1,0138
88,5	9	385	3	352	0,0228	0,9915	1,0144
89	9	385	3	352	0,0228	0,9915	1,0144
89,5	9	385	3	352	0,0228	0,9915	1,0144
90	9	385	3	352	0,0228	0,9915	1,0144
90,5	8	386	3	352	0,0203	0,9915	1,0119
91	8	386	3	352	0,0203	0,9915	1,0119
91,5	8	386	3	352	0,0203	0,9915	1,0119
92	8	386	3	352	0,0203	0,9915	1,0119
92,5	8	386	2	353	0,0203	0,9944	1,0147
93	8	386	2	353	0,0203	0,9944	1,0147
93,5	8	386	2	353	0,0203	0,9944	1,0147
94	8	386	2	353	0,0203	0,9944	1,0147
94,5	8	386	2	353	0,0203	0,9944	1,0147
95	8	386	2	353	0,0203	0,9944	1,0147
95,5	8	386	2	353	0,0203	0,9944	1,0147
96	8	386	2	353	0,0203	0,9944	1,0147
96,5	8	386	2	353	0,0203	0,9944	1,0147
97	8	386	2	353	0,0203	0,9944	1,0147
97,5	7	387	1	354	0,0178	0,9972	1,0149
98	7	387	1	354	0,0178	0,9972	1,0149
98,5	7	387	1	354	0,0178	0,9972	1,0149
99	7	387	1	354	0,0178	0,9972	1,0149
99,5	7	387	1	354	0,0178	0,9972	1,0149
100	7	387	1	354	0,0178	0,9972	1,0149

S10. Plot venlafaxine Active Moiety Sensitivity Specificity dataset (local maximum: 419)



S11. Data venlafaxine Active Moiety Sensitivity Specificity dataset

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
95	82	0	152	0	1,0000	0,0000	1,0000
96	82	0	152	0	1,0000	0,0000	1,0000
97	82	0	151	1	1,0000	0,0066	1,0066
98	82	0	151	1	1,0000	0,0066	1,0066
99	82	0	150	2	1,0000	0,0132	1,0132
100	82	0	150	2	1,0000	0,0132	1,0132
101	82	0	149	3	1,0000	0,0197	1,0197
102	82	0	149	3	1,0000	0,0197	1,0197
103	82	0	149	3	1,0000	0,0197	1,0197
104	82	0	148	4	1,0000	0,0263	1,0263
105	82	0	147	5	1,0000	0,0329	1,0329
106	82	0	147	5	1,0000	0,0329	1,0329
107	82	0	147	5	1,0000	0,0329	1,0329
108	82	0	147	5	1,0000	0,0329	1,0329
109	82	0	147	5	1,0000	0,0329	1,0329
110	82	0	147	5	1,0000	0,0329	1,0329
111	82	0	147	5	1,0000	0,0329	1,0329
112	82	0	147	5	1,0000	0,0329	1,0329
113	82	0	147	5	1,0000	0,0329	1,0329
114	82	0	147	5	1,0000	0,0329	1,0329
115	82	0	147	5	1,0000	0,0329	1,0329
116	82	0	147	5	1,0000	0,0329	1,0329
117	82	0	147	5	1,0000	0,0329	1,0329
118	82	0	147	5	1,0000	0,0329	1,0329
119	82	0	147	5	1,0000	0,0329	1,0329
120	82	0	147	5	1,0000	0,0329	1,0329
121	82	0	147	5	1,0000	0,0329	1,0329
122	82	0	147	5	1,0000	0,0329	1,0329
123	82	0	147	5	1,0000	0,0329	1,0329
124	82	0	147	5	1,0000	0,0329	1,0329
125	82	0	147	5	1,0000	0,0329	1,0329
126	82	0	147	5	1,0000	0,0329	1,0329
127	82	0	147	5	1,0000	0,0329	1,0329
128	82	0	147	5	1,0000	0,0329	1,0329
129	82	0	147	5	1,0000	0,0329	1,0329
130	82	0	147	5	1,0000	0,0329	1,0329
131	82	0	147	5	1,0000	0,0329	1,0329
132	82	0	147	5	1,0000	0,0329	1,0329
133	82	0	147	5	1,0000	0,0329	1,0329
134	82	0	147	5	1,0000	0,0329	1,0329
135	82	0	147	5	1,0000	0,0329	1,0329
136	82	0	147	5	1,0000	0,0329	1,0329
137	82	0	147	5	1,0000	0,0329	1,0329
138	82	0	147	5	1,0000	0,0329	1,0329
139	82	0	147	5	1,0000	0,0329	1,0329
140	82	0	147	5	1,0000	0,0329	1,0329
141	82	0	147	5	1,0000	0,0329	1,0329
142	82	0	147	5	1,0000	0,0329	1,0329
143	82	0	147	5	1,0000	0,0329	1,0329
144	82	0	147	5	1,0000	0,0329	1,0329
145	82	0	147	5	1,0000	0,0329	1,0329
146	82	0	147	5	1,0000	0,0329	1,0329
147	82	0	147	5	1,0000	0,0329	1,0329
148	82	0	147	5	1,0000	0,0329	1,0329
149	82	0	147	5	1,0000	0,0329	1,0329
150	82	0	147	5	1,0000	0,0329	1,0329
151	82	0	147	5	1,0000	0,0329	1,0329
152	81	1	147	5	0,9878	0,0329	1,0207
153	81	1	147	5	0,9878	0,0329	1,0207
154	81	1	147	5	0,9878	0,0329	1,0207
155	81	1	147	5	0,9878	0,0329	1,0207
156	81	1	147	5	0,9878	0,0329	1,0207
157	81	1	147	5	0,9878	0,0329	1,0207
158	81	1	147	5	0,9878	0,0329	1,0207
159	81	1	147	5	0,9878	0,0329	1,0207
160	81	1	146	6	0,9878	0,0395	1,0273
161	81	1	146	6	0,9878	0,0395	1,0273
162	81	1	146	6	0,9878	0,0395	1,0273
163	81	1	146	6	0,9878	0,0395	1,0273
164	81	1	146	6	0,9878	0,0395	1,0273
165	80	2	146	6	0,9756	0,0395	1,0151
166	80	2	146	6	0,9756	0,0395	1,0151
167	80	2	146	6	0,9756	0,0395	1,0151
168	80	2	146	6	0,9756	0,0395	1,0151
169	80	2	146	6	0,9756	0,0395	1,0151
170	80	2	146	6	0,9756	0,0395	1,0151
171	80	2	146	6	0,9756	0,0395	1,0151
172	80	2	146	6	0,9756	0,0395	1,0151
173	80	2	146	6	0,9756	0,0395	1,0151
174	80	2	146	6	0,9756	0,0395	1,0151
175	80	2	146	6	0,9756	0,0395	1,0151
176	80	2	145	7	0,9756	0,0461	1,0217
177	80	2	145	7	0,9756	0,0461	1,0217
178	80	2	145	7	0,9756	0,0461	1,0217
179	80	2	145	7	0,9756	0,0461	1,0217
180	80	2	145	7	0,9756	0,0461	1,0217
181	80	2	145	7	0,9756	0,0461	1,0217
182	80	2	145	7	0,9756	0,0461	1,0217
183	80	2	145	7	0,9756	0,0461	1,0217
184	80	2	145	7	0,9756	0,0461	1,0217
185	80	2	145	7	0,9756	0,0461	1,0217
186	80	2	145	7	0,9756	0,0461	1,0217
187	80	2	145	7	0,9756	0,0461	1,0217
188	80	2	145	7	0,9756	0,0461	1,0217
189	80	2	145	7	0,9756	0,0461	1,0217
190	80	2	145	7	0,9756	0,0461	1,0217
191	80	2	145	7	0,9756	0,0461	1,0217
192	80	2	144	8	0,9756	0,0526	1,0282
193	80	2	144	8	0,9756	0,0526	1,0282
194	80	2	144	8	0,9756	0,0526	1,0282
195	80	2	143	9	0,9756	0,0592	1,0348
196	79	3	143	9	0,9634	0,0592	1,0226
197	79	3	143	9	0,9634	0,0592	1,0226
198	79	3	143	9	0,9634	0,0592	1,0226
199	79	3	143	9	0,9634	0,0592	1,0226
200	79	3	143	9	0,9634	0,0592	1,0226
201	79	3	143	9	0,9634	0,0592	1,0226
202	79	3	143	9	0,9634	0,0592	1,0226
203	79	3	142	10	0,9634	0,0658	1,0292
204	79	3	142	10	0,9634	0,0658	1,0292
205	79	3	142	10	0,9634	0,0658	1,0292
206	79	3	142	10	0,9634	0,0658	1,0292
207	79	3	142	10	0,9634	0,0658	1,0292
208	79	3	142	10	0,9634	0,0658	1,0292
209	79	3	142	10	0,9634	0,0658	1,0292
210	79	3	142	10	0,9634	0,0658	1,0292
211	79	3	142	10	0,9634	0,0658	1,0292
212	79	3	142	10	0,9634	0,0658	1,0292
213	79	3	142	10	0,9634	0,0658	1,0292
214	79	3	142	10	0,9634	0,0658	1,0292
215	79	3	142	10	0,9634	0,0658	1,0292
216	79	3	141	11	0,9634	0,0724	1,0358
217	79	3	141	11	0,9634	0,0724	1,0358
218	79	3	141	11	0,9634	0,0724	1,0358
219	79	3	140	12	0,9634	0,0789	1,0424
220	77	5	140	12	0,9390	0,0789	1,0180
221	77	5	139	13	0,9390	0,0855	1,0246
222	77	5	139	13	0,9390	0,0855	1,0246
223	77	5	138	14	0,9390	0,0921	1,0311
224	76	6	138	14	0,9268	0,0921	1,0189
225	76	6	138	14	0,9268	0,0921	1,0189
226	76	6	137	15	0,9268	0,0987	1,0255
227	76	6	136	16	0,9268	0,1053	1,0321
228	76	6	136	16	0,9268	0,1053	1,0321
229	76	6	135	17	0,9268	0,1118	1,0387
230	75	7	134	18	0,9146	0,1184	1,0331
231	75	7	134	18	0,9146	0,1184	1,0331
232	75	7	134	18	0,9146	0,1184	1,0331
233	75	7	134	18	0,9146	0,1184	1,0331
234	75	7	134	18	0,9146	0,1184	1,0331

235	75	7	134	18	0,9146	0,1184	1,0331
236	75	7	134	18	0,9146	0,1184	1,0331
237	75	7	133	19	0,9146	0,1250	1,0396
238	75	7	133	19	0,9146	0,1250	1,0396
239	75	7	133	19	0,9146	0,1250	1,0396
240	75	7	133	19	0,9146	0,1250	1,0396
241	75	7	133	19	0,9146	0,1250	1,0396
242	75	7	133	19	0,9146	0,1250	1,0396
243	75	7	133	19	0,9146	0,1250	1,0396
244	75	7	133	19	0,9146	0,1250	1,0396
245	75	7	133	19	0,9146	0,1250	1,0396
246	75	7	132	20	0,9146	0,1316	1,0462
247	75	7	132	20	0,9146	0,1316	1,0462
248	75	7	132	20	0,9146	0,1316	1,0462
249	74	8	131	21	0,9024	0,1382	1,0406
250	73	9	131	21	0,8902	0,1382	1,0284
251	73	9	130	22	0,8902	0,1447	1,0350
252	73	9	129	23	0,8902	0,1513	1,0416
253	72	10	129	23	0,8780	0,1513	1,0294
254	72	10	129	23	0,8780	0,1513	1,0294
255	72	10	128	24	0,8780	0,1579	1,0359
256	72	10	128	24	0,8780	0,1579	1,0359
257	71	11	128	24	0,8659	0,1579	1,0237
258	71	11	128	24	0,8659	0,1579	1,0237
259	71	11	128	24	0,8659	0,1579	1,0237
260	70	12	127	25	0,8537	0,1645	1,0181
261	70	12	127	25	0,8537	0,1645	1,0181
262	69	13	127	25	0,8415	0,1645	1,0059
263	69	13	126	26	0,8415	0,1711	1,0125
264	69	13	126	26	0,8415	0,1711	1,0125
265	69	13	126	26	0,8415	0,1711	1,0125
266	69	13	125	27	0,8415	0,1776	1,0191
267	69	13	125	27	0,8415	0,1776	1,0191
268	69	13	125	27	0,8415	0,1776	1,0191
269	69	13	125	27	0,8415	0,1776	1,0191
270	69	13	125	27	0,8415	0,1776	1,0191
271	69	13	125	27	0,8415	0,1776	1,0191
272	69	13	125	27	0,8415	0,1776	1,0191
273	69	13	125	27	0,8415	0,1776	1,0191
274	69	13	125	27	0,8415	0,1776	1,0191
275	69	13	124	28	0,8415	0,1842	1,0257
276	69	13	124	28	0,8415	0,1842	1,0257
277	69	13	123	29	0,8415	0,1908	1,0323
278	68	14	123	29	0,8293	0,1908	1,0201
279	68	14	123	29	0,8293	0,1908	1,0201
280	68	14	122	30	0,8293	0,1974	1,0266
281	68	14	122	30	0,8293	0,1974	1,0266
282	68	14	122	30	0,8293	0,1974	1,0266
283	67	15	122	30	0,8171	0,1974	1,0144
284	67	15	122	30	0,8171	0,1974	1,0144
285	67	15	122	30	0,8171	0,1974	1,0144
286	66	16	122	30	0,8049	0,1974	1,0022
287	66	16	122	30	0,8049	0,1974	1,0022
288	66	16	121	31	0,8049	0,2039	1,0088
289	66	16	121	31	0,8049	0,2039	1,0088
290	66	16	121	31	0,8049	0,2039	1,0088
291	66	16	121	31	0,8049	0,2039	1,0088
292	66	16	121	31	0,8049	0,2039	1,0088
293	65	17	121	31	0,7927	0,2039	0,9966
294	65	17	121	31	0,7927	0,2039	0,9966
295	65	17	120	32	0,7927	0,2105	1,0032
296	65	17	119	33	0,7927	0,2171	1,0098
297	64	18	118	34	0,7805	0,2237	1,0042
298	64	18	118	34	0,7805	0,2237	1,0042
299	64	18	116	36	0,7805	0,2368	1,0173
300	64	18	116	36	0,7805	0,2368	1,0173
301	64	18	116	36	0,7805	0,2368	1,0173
302	64	18	115	37	0,7805	0,2434	1,0239
303	64	18	115	37	0,7805	0,2434	1,0239
304	64	18	115	37	0,7805	0,2434	1,0239
305	63	19	114	38	0,7683	0,2500	1,0183
306	60	22	113	39	0,7317	0,2566	0,9883
307	60	22	113	39	0,7317	0,2566	0,9883
308	60	22	113	39	0,7317	0,2566	0,9883
309	60	22	113	39	0,7317	0,2566	0,9883
310	60	22	113	39	0,7317	0,2566	0,9883
311	60	22	113	39	0,7317	0,2566	0,9883
312	60	22	112	40	0,7317	0,2632	0,9949
313	59	23	112	40	0,7195	0,2632	0,9827
314	59	23	112	40	0,7195	0,2632	0,9827
315	59	23	112	40	0,7195	0,2632	0,9827
316	59	23	112	40	0,7195	0,2632	0,9827
317	59	23	112	40	0,7195	0,2632	0,9827
318	59	23	112	40	0,7195	0,2632	0,9827
319	59	23	112	40	0,7195	0,2632	0,9827
320	59	23	112	40	0,7195	0,2632	0,9827
321	59	23	111	41	0,7195	0,2697	0,9892
322	59	23	111	41	0,7195	0,2697	0,9892
323	59	23	110	42	0,7195	0,2763	0,9958
324	59	23	109	43	0,7195	0,2829	1,0024
325	59	23	108	44	0,7195	0,2895	1,0090
326	59	23	108	44	0,7195	0,2895	1,0090
327	58	24	108	44	0,7073	0,2895	0,9968
328	58	24	108	44	0,7073	0,2895	0,9968
329	58	24	108	44	0,7073	0,2895	0,9968
330	57	25	108	44	0,6951	0,2895	0,9846
331	57	25	108	44	0,6951	0,2895	0,9846
332	57	25	107	45	0,6951	0,2961	0,9912
333	57	25	106	46	0,6951	0,3026	0,9978
334	57	25	106	46	0,6951	0,3026	0,9978
335	57	25	106	46	0,6951	0,3026	0,9978
336	57	25	105	47	0,6951	0,3092	1,0043
337	57	25	105	47	0,6951	0,3092	1,0043
338	57	25	105	47	0,6951	0,3092	1,0043
339	57	25	105	47	0,6951	0,3092	1,0043
340	57	25	104	48	0,6951	0,3158	1,0109
341	57	25	102	50	0,6951	0,3289	1,0241
342	57	25	101	51	0,6951	0,3355	1,0306
343	57	25	101	51	0,6951	0,3355	1,0306
344	57	25	101	51	0,6951	0,3355	1,0306
345	57	25	101	51	0,6951	0,3355	1,0306
346	56	26	99	53	0,6829	0,3487	1,0316
347	56	26	99	53	0,6829	0,3487	1,0316
348	56	26	99	53	0,6829	0,3487	1,0316
349	56	26	98	54	0,6829	0,3553	1,0382
350	55	27	98	54	0,6707	0,3553	1,0260
351	54	28	96	56	0,6585	0,3684	1,0270
352	53	29	96	56	0,6463	0,3684	1,0148
353	52	30	94	58	0,6341	0,3816	1,0157
354	51	31	92	60	0,6220	0,3947	1,0167
355	51	31	92	60	0,6220	0,3947	1,0167
356	51	31	92	60	0,6220	0,3947	1,0167
357	51	31	92	60	0,6220	0,3947	1,0167
358	51	31	91	61	0,6220	0,4013	1,0233
359	51	31	91	61	0,6220	0,4013	1,0233
360	51	31	91	61	0,6220	0,4013	1,0233
361	51	31	89	63	0,6220	0,4145	1,0364
362	51	31	88	64	0,6220	0,4211	1,0430
363	51	31	88	64	0,6220	0,4211	1,0430
364	51	31	87	65	0,6220	0,4276	1,0496
365	51	31	84	68	0,6220	0,4474	1,0693
366	51	31	84	68	0,6220	0,4474	1,0693
367	51	31	83	69	0,6220	0,4539	1,0759
368	51	31	82	70	0,6220	0,4605	1,0825
369	51	31	81	71	0,6220	0,4671	1,0891
370	51	31	81	71	0,6220	0,4671	1,0891
371	50	32	81	71	0,6098	0,4671	1,0769
372	49	33	81	71	0,5976	0,4671	1,0647
373	49	33	81	71	0,5976	0,4671	1,0647
374	49	33	79	73	0,5976	0,4803	1,0778
375	48	34	79	73	0,5854	0,4803	1,0656
376	48	34	78	74	0,5854	0,4868	1,0722
377	48	34	78	74	0,5854	0,4868	1,0722
378	47	35	77	75	0,5732	0,4934	1,0666
379	47	35	77	75	0,5732	0,4934	1,0666
380	47	35	76	76	0,5732	0,5000	1,0732

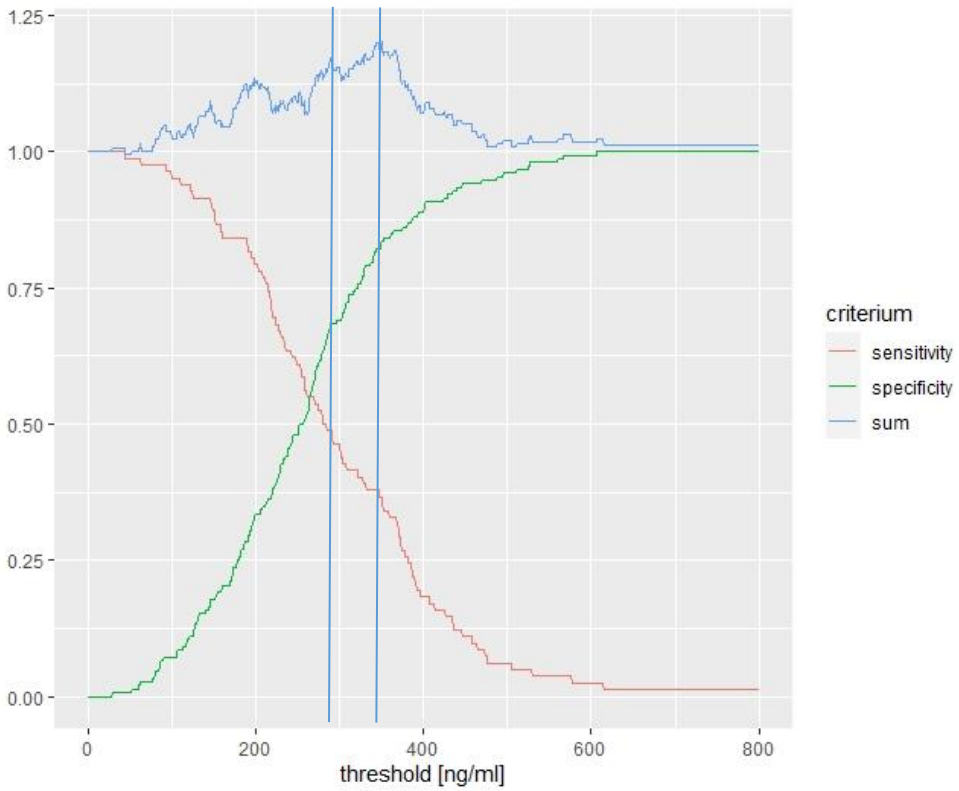
381	47	35	74	78	0,5732	0,5132	1,0863
382	47	35	74	78	0,5732	0,5132	1,0863
383	47	35	74	78	0,5732	0,5132	1,0863
384	47	35	74	78	0,5732	0,5132	1,0863
385	47	35	74	78	0,5732	0,5132	1,0863
386	47	35	74	78	0,5732	0,5132	1,0863
387	47	35	74	78	0,5732	0,5132	1,0863
388	47	35	73	79	0,5732	0,5197	1,0929
389	47	35	73	79	0,5732	0,5197	1,0929
390	47	35	73	79	0,5732	0,5197	1,0929
391	47	35	73	79	0,5732	0,5197	1,0929
392	47	35	73	79	0,5732	0,5197	1,0929
393	47	35	73	79	0,5732	0,5197	1,0929
394	47	35	73	79	0,5732	0,5197	1,0929
395	47	35	73	79	0,5732	0,5197	1,0929
396	47	35	73	79	0,5732	0,5197	1,0929
397	47	35	73	79	0,5732	0,5197	1,0929
398	47	35	72	80	0,5732	0,5263	1,0995
399	47	35	72	80	0,5732	0,5263	1,0995
400	47	35	72	80	0,5732	0,5263	1,0995
401	47	35	71	81	0,5732	0,5329	1,1061
402	47	35	71	81	0,5732	0,5329	1,1061
403	47	35	69	83	0,5732	0,5461	1,1192
404	46	36	67	85	0,5610	0,5592	1,1202
405	46	36	67	85	0,5610	0,5592	1,1202
406	46	36	65	87	0,5610	0,5724	1,1333
407	46	36	65	87	0,5610	0,5724	1,1333
408	46	36	65	87	0,5610	0,5724	1,1333
409	46	36	64	88	0,5610	0,5789	1,1399
410	46	36	62	90	0,5610	0,5921	1,1531
411	46	36	62	90	0,5610	0,5921	1,1531
412	46	36	60	92	0,5610	0,6053	1,1662
413	46	36	60	92	0,5610	0,6053	1,1662
414	45	37	60	92	0,5488	0,6053	1,1540
415	45	37	60	92	0,5488	0,6053	1,1540
416	45	37	60	92	0,5488	0,6053	1,1540
417	45	37	60	92	0,5488	0,6053	1,1540
418	45	37	59	93	0,5488	0,6118	1,1606
419	45	37	57	95	0,5488	0,6250	1,1738
420	44	38	57	95	0,5366	0,6250	1,1616
421	44	38	57	95	0,5366	0,6250	1,1616
422	44	38	56	96	0,5366	0,6316	1,1682
423	44	38	56	96	0,5366	0,6316	1,1682
424	43	39	56	96	0,5244	0,6316	1,1560
425	43	39	56	96	0,5244	0,6316	1,1560
426	42	40	55	97	0,5122	0,6382	1,1504
427	42	40	54	98	0,5122	0,6447	1,1569
428	42	40	54	98	0,5122	0,6447	1,1569
429	42	40	54	98	0,5122	0,6447	1,1569
430	42	40	54	98	0,5122	0,6447	1,1569
431	41	41	54	98	0,5000	0,6447	1,1447
432	41	41	53	99	0,5000	0,6513	1,1513
433	41	41	53	99	0,5000	0,6513	1,1513
434	41	41	52	100	0,5000	0,6579	1,1579
435	41	41	51	101	0,5000	0,6645	1,1645
436	40	42	51	101	0,4878	0,6645	1,1523
437	40	42	51	101	0,4878	0,6645	1,1523
438	40	42	51	101	0,4878	0,6645	1,1523
439	40	42	51	101	0,4878	0,6645	1,1523
440	40	42	50	102	0,4878	0,6711	1,1589
441	40	42	49	103	0,4878	0,6776	1,1654
442	40	42	49	103	0,4878	0,6776	1,1654
443	39	43	49	103	0,4756	0,6776	1,1532
444	39	43	49	103	0,4756	0,6776	1,1532
445	39	43	49	103	0,4756	0,6776	1,1532
446	39	43	49	103	0,4756	0,6776	1,1532
447	39	43	49	103	0,4756	0,6776	1,1532
448	39	43	49	103	0,4756	0,6776	1,1532
449	39	43	49	103	0,4756	0,6776	1,1532
450	39	43	49	103	0,4756	0,6776	1,1532
451	39	43	49	103	0,4756	0,6776	1,1532
452	39	43	49	103	0,4756	0,6776	1,1532
453	39	43	49	103	0,4756	0,6776	1,1532
454	39	43	48	104	0,4756	0,6842	1,1598
455	38	44	48	104	0,4634	0,6842	1,1476
456	38	44	48	104	0,4634	0,6842	1,1476
457	38	44	48	104	0,4634	0,6842	1,1476
458	38	44	48	104	0,4634	0,6842	1,1476
459	38	44	48	104	0,4634	0,6842	1,1476
460	38	44	48	104	0,4634	0,6842	1,1476
461	38	44	46	106	0,4634	0,6974	1,1608
462	38	44	46	106	0,4634	0,6974	1,1608
463	38	44	45	107	0,4634	0,7039	1,1674
464	35	47	45	107	0,4268	0,7039	1,1308
465	35	47	45	107	0,4268	0,7039	1,1308
466	35	47	45	107	0,4268	0,7039	1,1308
467	35	47	45	107	0,4268	0,7039	1,1308
468	35	47	45	107	0,4268	0,7039	1,1308
469	35	47	45	107	0,4268	0,7039	1,1308
470	35	47	42	110	0,4268	0,7237	1,1505
471	35	47	42	110	0,4268	0,7237	1,1505
472	35	47	42	110	0,4268	0,7237	1,1505
473	34	48	42	110	0,4146	0,7237	1,1383
474	34	48	42	110	0,4146	0,7237	1,1383
475	34	48	42	110	0,4146	0,7237	1,1383
476	34	48	42	110	0,4146	0,7237	1,1383
477	34	48	42	110	0,4146	0,7237	1,1383
478	34	48	41	111	0,4146	0,7303	1,1449
479	34	48	41	111	0,4146	0,7303	1,1449
480	34	48	41	111	0,4146	0,7303	1,1449
481	33	49	41	111	0,4024	0,7303	1,1327
482	33	49	41	111	0,4024	0,7303	1,1327
483	32	50	41	111	0,3902	0,7303	1,1205
484	32	50	41	111	0,3902	0,7303	1,1205
485	31	51	41	111	0,3780	0,7303	1,1083
486	31	51	41	111	0,3780	0,7303	1,1083
487	31	51	41	111	0,3780	0,7303	1,1083
488	31	51	41	111	0,3780	0,7303	1,1083
489	30	52	41	111	0,3659	0,7303	1,0961
490	30	52	41	111	0,3659	0,7303	1,0961
491	30	52	39	113	0,3659	0,7434	1,1093
492	30	52	39	113	0,3659	0,7434	1,1093
493	30	52	39	113	0,3659	0,7434	1,1093
494	30	52	39	113	0,3659	0,7434	1,1093
495	30	52	39	113	0,3659	0,7434	1,1093
496	30	52	38	114	0,3659	0,7500	1,1159
497	30	52	38	114	0,3659	0,7500	1,1159
498	30	52	38	114	0,3659	0,7500	1,1159
499	30	52	37	115	0,3659	0,7566	1,1224
500	30	52	36	116	0,3659	0,7632	1,1290
501	30	52	36	116	0,3659	0,7632	1,1290
502	30	52	36	116	0,3659	0,7632	1,1290
503	30	52	36	116	0,3659	0,7632	1,1290
504	30	52	36	116	0,3659	0,7632	1,1290
505	30	52	36	116	0,3659	0,7632	1,1290
506	30	52	36	116	0,3659	0,7632	1,1290
507	30	52	36	116	0,3659	0,7632	1,1290
508	29	53	36	116	0,3537	0,7632	1,1168
509	29	53	36	116	0,3537	0,7632	1,1168
510	29	53	36	116	0,3537	0,7632	1,1168
511	29	53	35	117	0,3537	0,7697	1,1234
512	28	54	35	117	0,3415	0,7697	1,1112
513	28	54	35	117	0,3415	0,7697	1,1112
514	28	54	34	118	0,3415	0,7763	1,1178
515	28	54	34	118	0,3415	0,7763	1,1178
516	28	54	34	118	0,3415	0,7763	1,1178
517	27	55	34	118	0,3293	0,7763	1,1056
518	27	55	34	118	0,3293	0,7763	1,1056
519	26	56	34	118	0,3171	0,7763	1,0934
520	26	56	34	118	0,3171	0,7763	1,0934
521	26	56	34	118	0,3171	0,7763	1,0934
522	26	56	34	118	0,3171	0,7763	1,0934
523	26	56	34	118	0,3171	0,7763	1,0934
524	25	57	34	118	0,3049	0,7763	1,0812
525	25	57	34	118	0,3049	0,7763	1,0812
526	24	58	33	119	0,2927	0,7829	1,0756

527	24	58	33	119	0,2927	0,7829	1,0756
528	24	58	33	119	0,2927	0,7829	1,0756
529	23	59	33	119	0,2805	0,7829	1,0634
530	23	59	33	119	0,2805	0,7829	1,0634
531	23	59	33	119	0,2805	0,7829	1,0634
532	22	60	33	119	0,2683	0,7829	1,0512
533	21	61	33	119	0,2561	0,7829	1,0390
534	21	61	33	119	0,2561	0,7829	1,0390
535	20	62	33	119	0,2439	0,7829	1,0268
536	20	62	33	119	0,2439	0,7829	1,0268
537	20	62	32	120	0,2439	0,7895	1,0334
538	20	62	32	120	0,2439	0,7895	1,0334
539	20	62	32	120	0,2439	0,7895	1,0334
540	20	62	32	120	0,2439	0,7895	1,0334
541	20	62	32	120	0,2439	0,7895	1,0334
542	20	62	31	121	0,2439	0,7961	1,0400
543	19	63	31	121	0,2317	0,7961	1,0278
544	19	63	31	121	0,2317	0,7961	1,0278
545	19	63	31	121	0,2317	0,7961	1,0278
546	19	63	31	121	0,2317	0,7961	1,0278
547	19	63	31	121	0,2317	0,7961	1,0278
548	19	63	31	121	0,2317	0,7961	1,0278
549	19	63	30	122	0,2317	0,8026	1,0343
550	18	64	30	122	0,2195	0,8026	1,0221
551	18	64	30	122	0,2195	0,8026	1,0221
552	18	64	30	122	0,2195	0,8026	1,0221
553	18	64	30	122	0,2195	0,8026	1,0221
554	18	64	30	122	0,2195	0,8026	1,0221
555	18	64	29	123	0,2195	0,8092	1,0287
556	18	64	29	123	0,2195	0,8092	1,0287
557	18	64	29	123	0,2195	0,8092	1,0287
558	18	64	28	124	0,2195	0,8158	1,0353
559	18	64	28	124	0,2195	0,8158	1,0353
560	18	64	28	124	0,2195	0,8158	1,0353
561	18	64	27	125	0,2195	0,8224	1,0419
562	18	64	27	125	0,2195	0,8224	1,0419
563	18	64	27	125	0,2195	0,8224	1,0419
564	17	65	27	125	0,2073	0,8224	1,0297
565	17	65	27	125	0,2073	0,8224	1,0297
566	17	65	27	125	0,2073	0,8224	1,0297
567	17	65	27	125	0,2073	0,8224	1,0297
568	17	65	26	126	0,2073	0,8289	1,0363
569	17	65	26	126	0,2073	0,8289	1,0363
570	17	65	26	126	0,2073	0,8289	1,0363
571	17	65	25	127	0,2073	0,8355	1,0428
572	17	65	25	127	0,2073	0,8355	1,0428
573	17	65	25	127	0,2073	0,8355	1,0428
574	17	65	24	128	0,2073	0,8421	1,0494
575	17	65	24	128	0,2073	0,8421	1,0494
576	17	65	23	129	0,2073	0,8487	1,0560
577	17	65	23	129	0,2073	0,8487	1,0560
578	17	65	23	129	0,2073	0,8487	1,0560
579	17	65	23	129	0,2073	0,8487	1,0560
580	17	65	23	129	0,2073	0,8487	1,0560
581	17	65	23	129	0,2073	0,8487	1,0560
582	17	65	23	129	0,2073	0,8487	1,0560
583	17	65	23	129	0,2073	0,8487	1,0560
584	16	66	23	129	0,1951	0,8487	1,0438
585	16	66	23	129	0,1951	0,8487	1,0438
586	16	66	23	129	0,1951	0,8487	1,0438
587	15	67	23	129	0,1829	0,8487	1,0316
588	15	67	23	129	0,1829	0,8487	1,0316
589	15	67	22	130	0,1829	0,8553	1,0382
590	15	67	22	130	0,1829	0,8553	1,0382
591	15	67	22	130	0,1829	0,8553	1,0382
592	15	67	22	130	0,1829	0,8553	1,0382
593	15	67	22	130	0,1829	0,8553	1,0382
594	15	67	22	130	0,1829	0,8553	1,0382
595	14	68	22	130	0,1707	0,8553	1,0260
596	14	68	21	131	0,1707	0,8618	1,0326
597	14	68	21	131	0,1707	0,8618	1,0326
598	14	68	20	132	0,1707	0,8684	1,0392
599	14	68	19	133	0,1707	0,8750	1,0457
600	14	68	19	133	0,1707	0,8750	1,0457
601	14	68	19	133	0,1707	0,8750	1,0457
602	14	68	19	133	0,1707	0,8750	1,0457
603	14	68	19	133	0,1707	0,8750	1,0457
604	14	68	19	133	0,1707	0,8750	1,0457
605	14	68	19	133	0,1707	0,8750	1,0457
606	14	68	19	133	0,1707	0,8750	1,0457
607	14	68	18	134	0,1707	0,8816	1,0523
608	14	68	18	134	0,1707	0,8816	1,0523
609	14	68	18	134	0,1707	0,8816	1,0523
610	14	68	18	134	0,1707	0,8816	1,0523
611	14	68	18	134	0,1707	0,8816	1,0523
612	14	68	18	134	0,1707	0,8816	1,0523
613	14	68	18	134	0,1707	0,8816	1,0523
614	14	68	18	134	0,1707	0,8816	1,0523
615	14	68	17	135	0,1707	0,8882	1,0589
616	14	68	17	135	0,1707	0,8882	1,0589
617	14	68	17	135	0,1707	0,8882	1,0589
618	14	68	15	137	0,1707	0,9013	1,0720
619	13	69	15	137	0,1585	0,9013	1,0599
620	13	69	15	137	0,1585	0,9013	1,0599
621	13	69	15	137	0,1585	0,9013	1,0599
622	13	69	15	137	0,1585	0,9013	1,0599
623	12	70	15	137	0,1463	0,9013	1,0477
624	12	70	15	137	0,1463	0,9013	1,0477
625	12	70	14	138	0,1463	0,9079	1,0542
626	12	70	13	139	0,1463	0,9145	1,0608
627	12	70	13	139	0,1463	0,9145	1,0608
628	12	70	13	139	0,1463	0,9145	1,0608
629	12	70	12	140	0,1463	0,9211	1,0674
630	12	70	12	140	0,1463	0,9211	1,0674
631	12	70	12	140	0,1463	0,9211	1,0674
632	12	70	12	140	0,1463	0,9211	1,0674
633	12	70	12	140	0,1463	0,9211	1,0674
634	12	70	12	140	0,1463	0,9211	1,0674
635	11	71	12	140	0,1341	0,9211	1,0552
636	11	71	12	140	0,1341	0,9211	1,0552
637	11	71	12	140	0,1341	0,9211	1,0552
638	11	71	12	140	0,1341	0,9211	1,0552
639	10	72	12	140	0,1220	0,9211	1,0430
640	10	72	12	140	0,1220	0,9211	1,0430
641	10	72	12	140	0,1220	0,9211	1,0430
642	10	72	12	140	0,1220	0,9211	1,0430
643	10	72	12	140	0,1220	0,9211	1,0430
644	10	72	12	140	0,1220	0,9211	1,0430
645	10	72	12	140	0,1220	0,9211	1,0430
646	10	72	12	140	0,1220	0,9211	1,0430
647	10	72	12	140	0,1220	0,9211	1,0430
648	10	72	12	140	0,1220	0,9211	1,0430
649	10	72	11	141	0,1220	0,9276	1,0496
650	10	72	11	141	0,1220	0,9276	1,0496
651	10	72	11	141	0,1220	0,9276	1,0496
652	10	72	11	141	0,1220	0,9276	1,0496
653	10	72	11	141	0,1220	0,9276	1,0496
654	10	72	11	141	0,1220	0,9276	1,0496
655	10	72	11	141	0,1220	0,9276	1,0496
656	10	72	11	141	0,1220	0,9276	1,0496
657	10	72	11	141	0,1220	0,9276	1,0496
658	10	72	11	141	0,1220	0,9276	1,0496
659	10	72	11	141	0,1220	0,9276	1,0496
660	10	72	11	141	0,1220	0,9276	1,0496
661	10	72	11	141	0,1220	0,9276	1,0496
662	10	72	11	141	0,1220	0,9276	1,0496
663	10	72	11	141	0,1220	0,9276	1,0496
664	10	72	11	141	0,1220	0,9276	1,0496
665	10	72	11	141	0,1220	0,9276	1,0496
666	10	72	11	141	0,1220	0,9276	1,0496
667	10	72	11	141	0,1220	0,9276	1,0496
668	10	72	11	141	0,1220	0,9276	1,0496
669	10	72	11	141	0,1220	0,9276	1,0496
670	10	72	11	141	0,1220	0,9276	1,0496
671	10	72	11	141	0,1220	0,9276	1,0496
672	10	72	11	141	0,1220	0,9276	1,0496

673	10	72	11	141	0,1220	0,9276	1,0496
674	8	74	11	141	0,0976	0,9276	1,0252
675	8	74	11	141	0,0976	0,9276	1,0252
676	8	74	11	141	0,0976	0,9276	1,0252
677	8	74	11	141	0,0976	0,9276	1,0252
678	8	74	11	141	0,0976	0,9276	1,0252
679	7	75	11	141	0,0854	0,9276	1,0130
680	7	75	11	141	0,0854	0,9276	1,0130
681	7	75	11	141	0,0854	0,9276	1,0130
682	7	75	11	141	0,0854	0,9276	1,0130
683	7	75	11	141	0,0854	0,9276	1,0130
684	7	75	10	142	0,0854	0,9342	1,0196
685	7	75	10	142	0,0854	0,9342	1,0196
686	7	75	10	142	0,0854	0,9342	1,0196
687	7	75	10	142	0,0854	0,9342	1,0196
688	7	75	10	142	0,0854	0,9342	1,0196
689	7	75	10	142	0,0854	0,9342	1,0196
690	7	75	10	142	0,0854	0,9342	1,0196
691	7	75	10	142	0,0854	0,9342	1,0196
692	7	75	10	142	0,0854	0,9342	1,0196
693	7	75	10	142	0,0854	0,9342	1,0196
694	7	75	10	142	0,0854	0,9342	1,0196
695	7	75	10	142	0,0854	0,9342	1,0196
696	7	75	10	142	0,0854	0,9342	1,0196
697	7	75	10	142	0,0854	0,9342	1,0196
698	7	75	10	142	0,0854	0,9342	1,0196
699	7	75	10	142	0,0854	0,9342	1,0196
700	7	75	9	143	0,0854	0,9408	1,0262
701	7	75	9	143	0,0854	0,9408	1,0262
702	7	75	9	143	0,0854	0,9408	1,0262
703	7	75	9	143	0,0854	0,9408	1,0262
704	7	75	9	143	0,0854	0,9408	1,0262
705	7	75	9	143	0,0854	0,9408	1,0262
706	7	75	9	143	0,0854	0,9408	1,0262
707	7	75	9	143	0,0854	0,9408	1,0262
708	7	75	9	143	0,0854	0,9408	1,0262
709	7	75	9	143	0,0854	0,9408	1,0262
710	7	75	9	143	0,0854	0,9408	1,0262
711	7	75	9	143	0,0854	0,9408	1,0262
712	7	75	8	144	0,0854	0,9474	1,0327
713	7	75	8	144	0,0854	0,9474	1,0327
714	7	75	8	144	0,0854	0,9474	1,0327
715	7	75	8	144	0,0854	0,9474	1,0327
716	7	75	8	144	0,0854	0,9474	1,0327
717	7	75	8	144	0,0854	0,9474	1,0327
718	7	75	8	144	0,0854	0,9474	1,0327
719	7	75	8	144	0,0854	0,9474	1,0327
720	6	76	8	144	0,0732	0,9474	1,0205
721	6	76	7	145	0,0732	0,9539	1,0271
722	6	76	7	145	0,0732	0,9539	1,0271
723	6	76	7	145	0,0732	0,9539	1,0271
724	6	76	6	146	0,0732	0,9605	1,0337
725	6	76	6	146	0,0732	0,9605	1,0337
726	6	76	6	146	0,0732	0,9605	1,0337
727	6	76	6	146	0,0732	0,9605	1,0337
728	6	76	6	146	0,0732	0,9605	1,0337
729	6	76	6	146	0,0732	0,9605	1,0337
730	6	76	6	146	0,0732	0,9605	1,0337
731	6	76	6	146	0,0732	0,9605	1,0337
732	6	76	6	146	0,0732	0,9605	1,0337
733	6	76	6	146	0,0732	0,9605	1,0337
734	6	76	6	146	0,0732	0,9605	1,0337
735	6	76	6	146	0,0732	0,9605	1,0337
736	6	76	6	146	0,0732	0,9605	1,0337
737	6	76	6	146	0,0732	0,9605	1,0337
738	6	76	6	146	0,0732	0,9605	1,0337
739	6	76	6	146	0,0732	0,9605	1,0337
740	6	76	6	146	0,0732	0,9605	1,0337
741	6	76	6	146	0,0732	0,9605	1,0337
742	6	76	6	146	0,0732	0,9605	1,0337
743	6	76	6	146	0,0732	0,9605	1,0337
744	6	76	6	146	0,0732	0,9605	1,0337
745	6	76	6	146	0,0732	0,9605	1,0337
746	6	76	6	146	0,0732	0,9605	1,0337
747	6	76	6	146	0,0732	0,9605	1,0337
748	6	76	6	146	0,0732	0,9605	1,0337
749	6	76	6	146	0,0732	0,9605	1,0337
750	6	76	6	146	0,0732	0,9605	1,0337
751	6	76	6	146	0,0732	0,9605	1,0337
752	6	76	6	146	0,0732	0,9605	1,0337
753	6	76	6	146	0,0732	0,9605	1,0337
754	6	76	6	146	0,0732	0,9605	1,0337
755	6	76	6	146	0,0732	0,9605	1,0337
756	6	76	5	147	0,0732	0,9671	1,0403
757	6	76	5	147	0,0732	0,9671	1,0403
758	6	76	5	147	0,0732	0,9671	1,0403
759	6	76	5	147	0,0732	0,9671	1,0403
760	6	76	5	147	0,0732	0,9671	1,0403
761	6	76	5	147	0,0732	0,9671	1,0403
762	5	77	5	147	0,0610	0,9671	1,0281
763	5	77	5	147	0,0610	0,9671	1,0281
764	5	77	5	147	0,0610	0,9671	1,0281
765	5	77	5	147	0,0610	0,9671	1,0281
766	5	77	5	147	0,0610	0,9671	1,0281
767	5	77	5	147	0,0610	0,9671	1,0281
768	5	77	5	147	0,0610	0,9671	1,0281
769	5	77	5	147	0,0610	0,9671	1,0281
770	5	77	5	147	0,0610	0,9671	1,0281
771	5	77	5	147	0,0610	0,9671	1,0281
772	5	77	5	147	0,0610	0,9671	1,0281
773	5	77	5	147	0,0610	0,9671	1,0281
774	5	77	4	148	0,0610	0,9737	1,0347
775	4	78	4	148	0,0488	0,9737	1,0225
776	4	78	4	148	0,0488	0,9737	1,0225
777	4	78	4	148	0,0488	0,9737	1,0225
778	4	78	4	148	0,0488	0,9737	1,0225
779	4	78	4	148	0,0488	0,9737	1,0225
780	4	78	4	148	0,0488	0,9737	1,0225
781	4	78	4	148	0,0488	0,9737	1,0225
782	4	78	4	148	0,0488	0,9737	1,0225
783	4	78	4	148	0,0488	0,9737	1,0225
784	4	78	4	148	0,0488	0,9737	1,0225
785	4	78	4	148	0,0488	0,9737	1,0225
786	4	78	4	148	0,0488	0,9737	1,0225
787	4	78	4	148	0,0488	0,9737	1,0225
788	4	78	4	148	0,0488	0,9737	1,0225
789	4	78	4	148	0,0488	0,9737	1,0225
790	4	78	4	148	0,0488	0,9737	1,0225
791	4	78	4	148	0,0488	0,9737	1,0225
792	4	78	4	148	0,0488	0,9737	1,0225
793	4	78	4	148	0,0488	0,9737	1,0225
794	4	78	4	148	0,0488	0,9737	1,0225
795	4	78	4	148	0,0488	0,9737	1,0225
796	4	78	4	148	0,0488	0,9737	1,0225
797	4	78	4	148	0,0488	0,9737	1,0225
798	4	78	4	148	0,0488	0,9737	1,0225
799	4	78	4	148	0,0488	0,9737	1,0225
800	4	78	4	148	0,0488	0,9737	1,0225
801	4	78	4	148	0,0488	0,9737	1,0225
802	4	78	4	148	0,0488	0,9737	1,0225
803	4	78	4	148	0,0488	0,9737	1,0225
804	4	78	4	148	0,0488	0,9737	1,0225
805	4	78	4	148	0,0488	0,9737	1,0225
806	4	78	4	148	0,0488	0,9737	1,0225
807	4	78	4	148	0,0488	0,9737	1,0225
808	4	78	4	148	0,0488	0,9737	1,0225
809	4	78	3	149	0,0488	0,9803	1,0290
810	4	78	3	149	0,0488	0,9803	1,0290
811	4	78	3	149	0,0488	0,9803	1,0290
812	4	78	3	149	0,0488	0,9803	1,0290
813	4	78	3	149	0,0488	0,9803	1,0290
814	4	78	3	149	0,0488	0,9803	1,0290
815	4	78	3	149	0,0488	0,9803	1,0290
816	3	79	3	149	0,0366	0,9803	1,0168
817	3	79	3	149	0,0366	0,9803	1,0168
818	3	79	3	149	0,0366	0,9803	1,0168

819	3	79	3	149	0,0366	0,9803	1,0168
820	3	79	3	149	0,0366	0,9803	1,0168
821	3	79	3	149	0,0366	0,9803	1,0168
822	3	79	3	149	0,0366	0,9803	1,0168
823	3	79	3	149	0,0366	0,9803	1,0168
824	3	79	3	149	0,0366	0,9803	1,0168
825	3	79	3	149	0,0366	0,9803	1,0168
826	3	79	3	149	0,0366	0,9803	1,0168
827	3	79	3	149	0,0366	0,9803	1,0168
828	3	79	3	149	0,0366	0,9803	1,0168
829	3	79	3	149	0,0366	0,9803	1,0168
830	3	79	3	149	0,0366	0,9803	1,0168
831	3	79	3	149	0,0366	0,9803	1,0168
832	3	79	3	149	0,0366	0,9803	1,0168
833	3	79	3	149	0,0366	0,9803	1,0168
834	3	79	3	149	0,0366	0,9803	1,0168
835	3	79	3	149	0,0366	0,9803	1,0168
836	3	79	3	149	0,0366	0,9803	1,0168
837	3	79	3	149	0,0366	0,9803	1,0168
838	3	79	3	149	0,0366	0,9803	1,0168
839	3	79	3	149	0,0366	0,9803	1,0168
840	3	79	3	149	0,0366	0,9803	1,0168
841	3	79	3	149	0,0366	0,9803	1,0168
842	3	79	3	149	0,0366	0,9803	1,0168
843	3	79	3	149	0,0366	0,9803	1,0168
844	3	79	3	149	0,0366	0,9803	1,0168
845	3	79	3	149	0,0366	0,9803	1,0168
846	3	79	3	149	0,0366	0,9803	1,0168
847	3	79	3	149	0,0366	0,9803	1,0168
848	3	79	3	149	0,0366	0,9803	1,0168
849	3	79	3	149	0,0366	0,9803	1,0168
850	3	79	3	149	0,0366	0,9803	1,0168
851	3	79	3	149	0,0366	0,9803	1,0168
852	3	79	3	149	0,0366	0,9803	1,0168
853	3	79	3	149	0,0366	0,9803	1,0168
854	3	79	3	149	0,0366	0,9803	1,0168
855	3	79	3	149	0,0366	0,9803	1,0168
856	3	79	3	149	0,0366	0,9803	1,0168
857	3	79	3	149	0,0366	0,9803	1,0168
858	3	79	3	149	0,0366	0,9803	1,0168
859	2	80	3	149	0,0244	0,9803	1,0047
860	2	80	3	149	0,0244	0,9803	1,0047
861	2	80	3	149	0,0244	0,9803	1,0047

S12. Plot o-desmethylvenlafaxine Sensitivity Specificity dataset (local maxima: 289, 344)



S13. Data o-desmethylvenlafaxine Sensitivity Specificity dataset

threshold	TP	FN	FP	TN	sensitivity	specificity	sum
28	82	0	152	0	1	0,0000	1,0000
29	82	0	151	1	1	0,0066	1,0066
30	82	0	151	1	1	0,0066	1,0066
31	82	0	151	1	1	0,0066	1,0066
32	82	0	151	1	1	0,0066	1,0066
33	82	0	151	1	1	0,0066	1,0066
34	82	0	151	1	1	0,0066	1,0066
35	82	0	151	1	1	0,0066	1,0066
36	82	0	151	1	1	0,0066	1,0066
37	82	0	151	1	1	0,0066	1,0066
38	82	0	151	1	1	0,0066	1,0066
39	82	0	151	1	1	0,0066	1,0066
40	82	0	151	1	1	0,0066	1,0066
41	82	0	151	1	1	0,0066	1,0066
42	82	0	151	1	1	0,0066	1,0066
43	82	0	151	1	1	0,0066	1,0066
44	81	1	151	1	0,98780488	0,0066	0,9944
45	81	1	151	1	0,98780488	0,0066	0,9944
46	81	1	151	1	0,98780488	0,0066	0,9944
47	81	1	151	1	0,98780488	0,0066	0,9944
48	81	1	151	1	0,98780488	0,0066	0,9944
49	81	1	151	1	0,98780488	0,0066	0,9944
50	81	1	151	1	0,98780488	0,0066	0,9944
51	81	1	151	1	0,98780488	0,0066	0,9944
52	81	1	150	2	0,98780488	0,0132	1,0010
53	81	1	150	2	0,98780488	0,0132	1,0010
54	81	1	150	2	0,98780488	0,0132	1,0010
55	81	1	150	2	0,98780488	0,0132	1,0010
56	81	1	150	2	0,98780488	0,0132	1,0010
57	81	1	150	2	0,98780488	0,0132	1,0010
58	81	1	150	2	0,98780488	0,0132	1,0010
59	81	1	150	2	0,98780488	0,0132	1,0010
60	81	1	149	3	0,98780488	0,0197	1,0075
61	81	1	149	3	0,98780488	0,0197	1,0075
62	81	1	148	4	0,98780488	0,0263	1,0141
63	80	2	148	4	0,97560976	0,0263	1,0019
64	80	2	148	4	0,97560976	0,0263	1,0019
65	80	2	148	4	0,97560976	0,0263	1,0019
66	80	2	148	4	0,97560976	0,0263	1,0019
67	80	2	148	4	0,97560976	0,0263	1,0019
68	80	2	148	4	0,97560976	0,0263	1,0019
69	80	2	148	4	0,97560976	0,0263	1,0019
70	80	2	148	4	0,97560976	0,0263	1,0019
71	80	2	148	4	0,97560976	0,0263	1,0019
72	80	2	148	4	0,97560976	0,0263	1,0019
73	80	2	148	4	0,97560976	0,0263	1,0019
74	80	2	148	4	0,97560976	0,0263	1,0019
75	80	2	148	4	0,97560976	0,0263	1,0019
76	80	2	148	4	0,97560976	0,0263	1,0019
77	80	2	147	5	0,97560976	0,0329	1,0085
78	80	2	147	5	0,97560976	0,0329	1,0085
79	80	2	146	6	0,97560976	0,0395	1,0151
80	80	2	145	7	0,97560976	0,0461	1,0217
81	80	2	145	7	0,97560976	0,0461	1,0217
82	80	2	145	7	0,97560976	0,0461	1,0217
83	80	2	145	7	0,97560976	0,0461	1,0217
84	80	2	145	7	0,97560976	0,0461	1,0217
85	80	2	144	8	0,97560976	0,0526	1,0282
86	80	2	143	9	0,97560976	0,0592	1,0348
87	80	2	142	10	0,97560976	0,0658	1,0414
88	80	2	142	10	0,97560976	0,0658	1,0414
89	80	2	142	10	0,97560976	0,0658	1,0414
90	80	2	141	11	0,97560976	0,0724	1,0480
91	80	2	141	11	0,97560976	0,0724	1,0480
92	80	2	141	11	0,97560976	0,0724	1,0480
93	79	3	141	11	0,96341463	0,0724	1,0358
94	79	3	141	11	0,96341463	0,0724	1,0358
95	79	3	141	11	0,96341463	0,0724	1,0358
96	79	3	141	11	0,96341463	0,0724	1,0358
97	79	3	141	11	0,96341463	0,0724	1,0358
98	79	3	141	11	0,96341463	0,0724	1,0358
99	78	4	141	11	0,95121951	0,0724	1,0236
100	78	4	141	11	0,95121951	0,0724	1,0236
101	78	4	141	11	0,95121951	0,0724	1,0236
102	78	4	141	11	0,95121951	0,0724	1,0236
103	78	4	141	11	0,95121951	0,0724	1,0236
104	78	4	141	11	0,95121951	0,0724	1,0236
105	78	4	141	11	0,95121951	0,0724	1,0236
106	78	4	140	12	0,95121951	0,0789	1,0302
107	78	4	139	13	0,95121951	0,0855	1,0367
108	78	4	139	13	0,95121951	0,0855	1,0367
109	78	4	139	13	0,95121951	0,0855	1,0367
110	77	5	139	13	0,93902439	0,0855	1,0246
111	77	5	139	13	0,93902439	0,0855	1,0246
112	77	5	139	13	0,93902439	0,0855	1,0246
113	77	5	138	14	0,93902439	0,0921	1,0311
114	77	5	138	14	0,93902439	0,0921	1,0311
115	77	5	138	14	0,93902439	0,0921	1,0311
116	77	5	138	14	0,93902439	0,0921	1,0311
117	77	5	138	14	0,93902439	0,0921	1,0311
118	77	5	136	16	0,93902439	0,1053	1,0443
119	77	5	136	16	0,93902439	0,1053	1,0443
120	77	5	136	16	0,93902439	0,1053	1,0443
121	77	5	135	17	0,93902439	0,1118	1,0509
122	76	6	135	17	0,92682927	0,1118	1,0387
123	76	6	135	17	0,92682927	0,1118	1,0387
124	76	6	135	17	0,92682927	0,1118	1,0387
125	75	7	135	17	0,91463415	0,1118	1,0265
126	75	7	135	17	0,91463415	0,1118	1,0265
127	75	7	132	20	0,91463415	0,1316	1,0462
128	75	7	132	20	0,91463415	0,1316	1,0462
129	75	7	132	20	0,91463415	0,1316	1,0462
130	75	7	131	21	0,91463415	0,1382	1,0528
131	75	7	130	22	0,91463415	0,1447	1,0594
132	75	7	129	23	0,91463415	0,1513	1,0659
133	75	7	129	23	0,91463415	0,1513	1,0659
134	75	7	129	23	0,91463415	0,1513	1,0659
135	75	7	129	23	0,91463415	0,1513	1,0659
136	75	7	129	23	0,91463415	0,1513	1,0659
137	75	7	129	23	0,91463415	0,1513	1,0659
138	75	7	129	23	0,91463415	0,1513	1,0659
139	75	7	129	23	0,91463415	0,1513	1,0659
140	75	7	128	24	0,91463415	0,1579	1,0725
141	75	7	128	24	0,91463415	0,1579	1,0725
142	75	7	128	24	0,91463415	0,1579	1,0725
143	75	7	128	24	0,91463415	0,1579	1,0725
144	75	7	127	25	0,91463415	0,1645	1,0791
145	75	7	127	25	0,91463415	0,1645	1,0791
146	75	7	125	27	0,91463415	0,1776	1,0923
147	74	8	125	27	0,90243902	0,1776	1,0801
148	73	9	125	27	0,8902439	0,1776	1,0679
149	73	9	125	27	0,8902439	0,1776	1,0679
150	73	9	125	27	0,8902439	0,1776	1,0679
151	72	10	125	27	0,87804878	0,1776	1,0557
152	71	11	124	28	0,86585366	0,1842	1,0501
153	71	11	124	28	0,86585366	0,1842	1,0501
154	71	11	123	29	0,86585366	0,1908	1,0566
155	71	11	123	29	0,86585366	0,1908	1,0566
156	71	11	123	29	0,86585366	0,1908	1,0566
157	71	11	123	29	0,86585366	0,1908	1,0566
158	70	12	123	29	0,85365854	0,1908	1,0444
159	70	12	122	30	0,85365854	0,1974	1,0510
160	69	13	121	31	0,84146341	0,2039	1,0454
161	69	13	121	31	0,84146341	0,2039	1,0454
162	69	13	121	31	0,84146341	0,2039	1,0454
163	69	13	121	31	0,84146341	0,2039	1,0454
164	69	13	121	31	0,84146341	0,2039	1,0454
165	69	13	121	31	0,84146341	0,2039	1,0454
166	69	13	121	31	0,84146341	0,2039	1,0454
167	69	13	121	31	0,84146341	0,2039	1,0454

168	69	13	121	31	0,84146341	0,2039	1,0454
169	69	13	120	32	0,84146341	0,2105	1,0520
170	69	13	120	32	0,84146341	0,2105	1,0520
171	69	13	120	32	0,84146341	0,2105	1,0520
172	69	13	117	35	0,84146341	0,2303	1,0717
173	69	13	116	36	0,84146341	0,2368	1,0783
174	69	13	116	36	0,84146341	0,2368	1,0783
175	69	13	116	36	0,84146341	0,2368	1,0783
176	69	13	116	36	0,84146341	0,2368	1,0783
177	69	13	114	38	0,84146341	0,2500	1,0915
178	69	13	114	38	0,84146341	0,2500	1,0915
179	69	13	114	38	0,84146341	0,2500	1,0915
180	69	13	113	39	0,84146341	0,2566	1,0980
181	69	13	113	39	0,84146341	0,2566	1,0980
182	69	13	112	40	0,84146341	0,2632	1,1046
183	69	13	111	41	0,84146341	0,2697	1,1112
184	69	13	111	41	0,84146341	0,2697	1,1112
185	69	13	111	41	0,84146341	0,2697	1,1112
186	69	13	110	42	0,84146341	0,2763	1,1178
187	69	13	109	43	0,84146341	0,2829	1,1244
188	69	13	109	43	0,84146341	0,2829	1,1244
189	69	13	109	43	0,84146341	0,2829	1,1244
190	67	15	109	43	0,81707317	0,2829	1,1000
191	67	15	107	45	0,81707317	0,2961	1,1131
192	67	15	107	45	0,81707317	0,2961	1,1131
193	67	15	106	46	0,81707317	0,3026	1,1197
194	67	15	106	46	0,81707317	0,3026	1,1197
195	66	16	105	47	0,80487805	0,3092	1,1141
196	66	16	104	48	0,80487805	0,3158	1,1207
197	66	16	102	50	0,80487805	0,3289	1,1338
198	65	17	102	50	0,79268293	0,3289	1,1216
199	65	17	101	51	0,79268293	0,3355	1,1282
200	65	17	101	51	0,79268293	0,3355	1,1282
201	65	17	101	51	0,79268293	0,3355	1,1282
202	65	17	101	51	0,79268293	0,3355	1,1282
203	64	18	101	51	0,7804878	0,3355	1,1160
204	64	18	101	51	0,7804878	0,3355	1,1160
205	64	18	101	51	0,7804878	0,3355	1,1160
206	64	18	100	52	0,7804878	0,3421	1,1226
207	64	18	100	52	0,7804878	0,3421	1,1226
208	63	19	99	53	0,76829268	0,3487	1,1170
209	63	19	99	53	0,76829268	0,3487	1,1170
210	63	19	99	53	0,76829268	0,3487	1,1170
211	63	19	99	53	0,76829268	0,3487	1,1170
212	62	20	98	54	0,75609756	0,3553	1,1114
213	62	20	98	54	0,75609756	0,3553	1,1114
214	62	20	97	55	0,75609756	0,3618	1,1179
215	61	21	97	55	0,74390244	0,3618	1,1057
216	61	21	97	55	0,74390244	0,3618	1,1057
217	59	23	97	55	0,7195122	0,3618	1,0814
218	59	23	97	55	0,7195122	0,3618	1,0814
219	57	25	95	57	0,69512195	0,3750	1,0701
220	57	25	94	58	0,69512195	0,3816	1,0767
221	57	25	94	58	0,69512195	0,3816	1,0767
222	57	25	94	58	0,69512195	0,3816	1,0767
223	57	25	93	59	0,69512195	0,3882	1,0833
224	56	26	93	59	0,68292683	0,3882	1,0711
225	56	26	92	60	0,68292683	0,3947	1,0777
226	56	26	91	61	0,68292683	0,4013	1,0842
227	55	27	91	61	0,67073171	0,4013	1,0720
228	55	27	89	63	0,67073171	0,4145	1,0852
229	55	27	88	64	0,67073171	0,4211	1,0918
230	54	28	87	65	0,65853659	0,4276	1,0862
231	54	28	87	65	0,65853659	0,4276	1,0862
232	54	28	87	65	0,65853659	0,4276	1,0862
233	54	28	86	66	0,65853659	0,4342	1,0927
234	53	29	86	66	0,64634146	0,4342	1,0806
235	53	29	86	66	0,64634146	0,4342	1,0806
236	52	30	85	67	0,63414634	0,4408	1,0749
237	52	30	85	67	0,63414634	0,4408	1,0749
238	52	30	83	69	0,63414634	0,4539	1,0881
239	52	30	83	69	0,63414634	0,4539	1,0881
240	52	30	83	69	0,63414634	0,4539	1,0881
241	52	30	82	70	0,63414634	0,4605	1,0947
242	52	30	82	70	0,63414634	0,4605	1,0947
243	51	31	81	71	0,62195122	0,4671	1,0891
244	51	31	80	72	0,62195122	0,4737	1,0956
245	51	31	79	73	0,62195122	0,4803	1,1022
246	51	31	79	73	0,62195122	0,4803	1,1022
247	51	31	79	73	0,62195122	0,4803	1,1022
248	50	32	79	73	0,6097561	0,4803	1,0900
249	50	32	79	73	0,6097561	0,4803	1,0900
250	50	32	79	73	0,6097561	0,4803	1,0900
251	50	32	77	75	0,6097561	0,4934	1,1032
252	50	32	76	76	0,6097561	0,5000	1,1098
253	50	32	76	76	0,6097561	0,5000	1,1098
254	49	33	76	76	0,59756098	0,5000	1,0976
255	48	34	76	76	0,58536585	0,5000	1,0854
256	48	34	76	76	0,58536585	0,5000	1,0854
257	48	34	75	77	0,58536585	0,5066	1,0919
258	46	36	75	77	0,56097561	0,5066	1,0676
259	46	36	74	78	0,56097561	0,5132	1,0741
260	46	36	74	78	0,56097561	0,5132	1,0741
261	45	37	73	79	0,54878049	0,5197	1,0685
262	45	37	73	79	0,54878049	0,5197	1,0685
263	45	37	72	80	0,54878049	0,5263	1,0751
264	45	37	69	83	0,54878049	0,5461	1,0948
265	45	37	68	84	0,54878049	0,5526	1,1014
266	45	37	66	86	0,54878049	0,5658	1,1146
267	45	37	66	86	0,54878049	0,5658	1,1146
268	45	37	65	87	0,54878049	0,5724	1,1211
269	45	37	65	87	0,54878049	0,5724	1,1211
270	44	38	63	89	0,53658537	0,5855	1,1221
271	44	38	62	90	0,53658537	0,5921	1,1287
272	44	38	60	92	0,53658537	0,6053	1,1418
273	44	38	60	92	0,53658537	0,6053	1,1418
274	44	38	60	92	0,53658537	0,6053	1,1418
275	43	39	60	92	0,52439024	0,6053	1,1297
276	43	39	58	94	0,52439024	0,6184	1,1428
277	43	39	58	94	0,52439024	0,6184	1,1428
278	43	39	57	95	0,52439024	0,6250	1,1494
279	42	40	56	96	0,51219512	0,6316	1,1438
280	41	41	56	96	0,5	0,6316	1,1316
281	41	41	55	97	0,5	0,6382	1,1382
282	41	41	55	97	0,5	0,6382	1,1382
283	41	41	54	98	0,5	0,6447	1,1447
284	41	41	53	99	0,5	0,6513	1,1513
285	40	42	52	100	0,48780488	0,6579	1,1457
286	40	42	50	102	0,48780488	0,6711	1,1589
287	40	42	50	102	0,48780488	0,6711	1,1589
288	40	42	49	103	0,48780488	0,6776	1,1654
289	40	42	48	104	0,48780488	0,6842	1,1720
290	40	42	48	104	0,48780488	0,6842	1,1720
291	39	43	48	104	0,47560976	0,6842	1,1598
292	38	44	48	104	0,46341463	0,6842	1,1476
293	38	44	48	104	0,46341463	0,6842	1,1476
294	38	44	48	104	0,46341463	0,6842	1,1476
295	38	44	48	104	0,46341463	0,6842	1,1476
296	38	44	47	105	0,46341463	0,6908	1,1542
297	38	44	47	105	0,46341463	0,6908	1,1542
298	38	44	47	105	0,46341463	0,6908	1,1542
299	38	44	47	105	0,46341463	0,6908	1,1542
300	38	44	47	105	0,46341463	0,6908	1,1542
301	36	46	47	105	0,43902439	0,6908	1,1298
302	36	46	47	105	0,43902439	0,6908	1,1298
303	36	46	46	106	0,43902439	0,6974	1,1364
304	35	47	45	107	0,42682927	0,7039	1,1308
305	35	47	45	107	0,42682927	0,7039	1,1308
306	35	47	43	109	0,42682927	0,7171	1,1439
307	35	47	43	109	0,42682927	0,7171	1,1439
308	34	48	42	110	0,41463415	0,7237	1,1383
309	34	48	42	110	0,41463415	0,7237	1,1383
310	34	48	42	110	0,41463415	0,7237	1,1383
311	34	48	40	112	0,41463415	0,7368	1,1515
312	34	48	40	112	0,41463415	0,7368	1,1515
313	34	48	40	112	0,41463415	0,7368	1,1515

314	34	48	40	112	0,41463415	0,7368	1,1515
315	34	48	40	112	0,41463415	0,7368	1,1515
316	34	48	39	113	0,41463415	0,7434	1,1581
317	34	48	39	113	0,41463415	0,7434	1,1581
318	34	48	39	113	0,41463415	0,7434	1,1581
319	34	48	38	114	0,41463415	0,7500	1,1646
320	34	48	38	114	0,41463415	0,7500	1,1646
321	34	48	38	114	0,41463415	0,7500	1,1646
322	33	49	37	115	0,40243902	0,7566	1,1590
323	33	49	37	115	0,40243902	0,7566	1,1590
324	33	49	37	115	0,40243902	0,7566	1,1590
325	33	49	37	115	0,40243902	0,7566	1,1590
326	33	49	35	117	0,40243902	0,7697	1,1722
327	33	49	35	117	0,40243902	0,7697	1,1722
328	32	50	35	117	0,3902439	0,7697	1,1600
329	32	50	33	119	0,3902439	0,7829	1,1731
330	32	50	32	120	0,3902439	0,7895	1,1797
331	32	50	32	120	0,3902439	0,7895	1,1797
332	31	51	32	120	0,37804878	0,7895	1,1675
333	31	51	32	120	0,37804878	0,7895	1,1675
334	31	51	32	120	0,37804878	0,7895	1,1675
335	31	51	32	120	0,37804878	0,7895	1,1675
336	31	51	32	120	0,37804878	0,7895	1,1675
337	31	51	31	121	0,37804878	0,7961	1,1741
338	31	51	31	121	0,37804878	0,7961	1,1741
339	31	51	31	121	0,37804878	0,7961	1,1741
340	31	51	30	122	0,37804878	0,8026	1,1807
341	31	51	28	124	0,37804878	0,8158	1,1938
342	31	51	28	124	0,37804878	0,8158	1,1938
343	31	51	28	124	0,37804878	0,8158	1,1938
344	31	51	27	125	0,37804878	0,8224	1,2004
345	31	51	27	125	0,37804878	0,8224	1,2004
346	31	51	27	125	0,37804878	0,8224	1,2004
347	30	52	27	125	0,36585366	0,8224	1,1882
348	30	52	27	125	0,36585366	0,8224	1,1882
349	30	52	26	126	0,36585366	0,8289	1,1948
350	30	52	25	127	0,36585366	0,8355	1,2014
351	29	53	25	127	0,35365854	0,8355	1,1892
352	28	54	25	127	0,34146341	0,8355	1,1770
353	28	54	24	128	0,34146341	0,8421	1,1836
354	28	54	24	128	0,34146341	0,8421	1,1836
355	28	54	24	128	0,34146341	0,8421	1,1836
356	28	54	24	128	0,34146341	0,8421	1,1836
357	28	54	24	128	0,34146341	0,8421	1,1836
358	28	54	24	128	0,34146341	0,8421	1,1836
359	27	55	24	128	0,32926829	0,8421	1,1714
360	27	55	24	128	0,32926829	0,8421	1,1714
361	27	55	23	129	0,32926829	0,8487	1,1780
362	27	55	23	129	0,32926829	0,8487	1,1780
363	27	55	23	129	0,32926829	0,8487	1,1780
364	27	55	22	130	0,32926829	0,8553	1,1845
365	27	55	22	130	0,32926829	0,8553	1,1845
366	27	55	22	130	0,32926829	0,8553	1,1845
367	27	55	22	130	0,32926829	0,8553	1,1845
368	26	56	22	130	0,31707317	0,8553	1,1723
369	26	56	22	130	0,31707317	0,8553	1,1723
370	25	57	22	130	0,30487805	0,8553	1,1601
371	24	58	22	130	0,29268293	0,8553	1,1479
372	24	58	22	130	0,29268293	0,8553	1,1479
373	23	59	22	130	0,2804878	0,8553	1,1358
374	22	60	22	130	0,26829268	0,8553	1,1236
375	22	60	21	131	0,26829268	0,8618	1,1301
376	22	60	21	131	0,26829268	0,8618	1,1301
377	22	60	21	131	0,26829268	0,8618	1,1301
378	21	61	21	131	0,25609756	0,8618	1,1179
379	21	61	21	131	0,25609756	0,8618	1,1179
380	21	61	21	131	0,25609756	0,8618	1,1179
381	21	61	20	132	0,25609756	0,8684	1,1245
382	20	62	20	132	0,24390244	0,8684	1,1123
383	20	62	20	132	0,24390244	0,8684	1,1123
384	20	62	20	132	0,24390244	0,8684	1,1123
385	20	62	20	132	0,24390244	0,8684	1,1123
386	18	64	19	133	0,2195122	0,8750	1,0945
387	18	64	19	133	0,2195122	0,8750	1,0945
388	18	64	18	134	0,2195122	0,8816	1,1011
389	18	64	18	134	0,2195122	0,8816	1,1011
390	17	65	18	134	0,20731707	0,8816	1,0889
391	17	65	18	134	0,20731707	0,8816	1,0889
392	16	66	18	134	0,19512195	0,8816	1,0767
393	16	66	18	134	0,19512195	0,8816	1,0767
394	16	66	17	135	0,19512195	0,8882	1,0833
395	16	66	17	135	0,19512195	0,8882	1,0833
396	15	67	17	135	0,18292683	0,8882	1,0711
397	15	67	17	135	0,18292683	0,8882	1,0711
398	15	67	17	135	0,18292683	0,8882	1,0711
399	15	67	17	135	0,18292683	0,8882	1,0711
400	15	67	17	135	0,18292683	0,8882	1,0711
401	15	67	16	136	0,18292683	0,8947	1,0777
402	15	67	15	137	0,18292683	0,9013	1,0842
403	15	67	14	138	0,18292683	0,9079	1,0908
404	15	67	14	138	0,18292683	0,9079	1,0908
405	15	67	14	138	0,18292683	0,9079	1,0908
406	15	67	14	138	0,18292683	0,9079	1,0908
407	14	68	14	138	0,17073171	0,9079	1,0786
408	14	68	14	138	0,17073171	0,9079	1,0786
409	14	68	14	138	0,17073171	0,9079	1,0786
410	14	68	14	138	0,17073171	0,9079	1,0786
411	14	68	14	138	0,17073171	0,9079	1,0786
412	14	68	14	138	0,17073171	0,9079	1,0786
413	14	68	14	138	0,17073171	0,9079	1,0786
414	13	69	14	138	0,15853659	0,9079	1,0664
415	13	69	14	138	0,15853659	0,9079	1,0664
416	13	69	14	138	0,15853659	0,9079	1,0664
417	13	69	14	138	0,15853659	0,9079	1,0664
418	13	69	14	138	0,15853659	0,9079	1,0664
419	13	69	14	138	0,15853659	0,9079	1,0664
420	13	69	14	138	0,15853659	0,9079	1,0664
421	13	69	14	138	0,15853659	0,9079	1,0664
422	13	69	14	138	0,15853659	0,9079	1,0664
423	13	69	14	138	0,15853659	0,9079	1,0664
424	13	69	13	139	0,15853659	0,9145	1,0730
425	13	69	13	139	0,15853659	0,9145	1,0730
426	12	70	13	139	0,14634146	0,9145	1,0608
427	12	70	13	139	0,14634146	0,9145	1,0608
428	12	70	13	139	0,14634146	0,9145	1,0608
429	12	70	12	140	0,14634146	0,9211	1,0674
430	12	70	12	140	0,14634146	0,9211	1,0674
431	12	70	12	140	0,14634146	0,9211	1,0674
432	12	70	12	140	0,14634146	0,9211	1,0674
433	12	70	12	140	0,14634146	0,9211	1,0674
434	12	70	12	140	0,14634146	0,9211	1,0674
435	10	72	11	141	0,12195122	0,9276	1,0496
436	10	72	11	141	0,12195122	0,9276	1,0496
437	10	72	11	141	0,12195122	0,9276	1,0496
438	10	72	11	141	0,12195122	0,9276	1,0496
439	10	72	11	141	0,12195122	0,9276	1,0496
440	10	72	10	142	0,12195122	0,9342	1,0562
441	10	72	10	142	0,12195122	0,9342	1,0562
442	10	72	10	142	0,12195122	0,9342	1,0562
443	10	72	10	142	0,12195122	0,9342	1,0562
444	10	72	10	142	0,12195122	0,9342	1,0562
445	10	72	10	142	0,12195122	0,9342	1,0562
446	9	73	9	143	0,1097561	0,9408	1,0505
447	9	73	9	143	0,1097561	0,9408	1,0505
448	9	73	9	143	0,1097561	0,9408	1,0505
449	9	73	9	143	0,1097561	0,9408	1,0505
450	9	73	9	143	0,1097561	0,9408	1,0505
451	9	73	9	143	0,1097561	0,9408	1,0505
452	9	73	9	143	0,1097561	0,9408	1,0505
453	9	73	9	143	0,1097561	0,9408	1,0505
454	9	73	9	143	0,1097561	0,9408	1,0505
455	9	73	9	143	0,1097561	0,9408	1,0505
456	9	73	9	143	0,1097561	0,9408	1,0505
457	9	73	9	143	0,1097561	0,9408	1,0505
458	8	74	9	143	0,09756098	0,9408	1,0384
459	8	74	9	143	0,09756098	0,9408	1,0384

460	8	74	9	143	0,09756098	0,9408	1,0384
461	8	74	9	143	0,09756098	0,9408	1,0384
462	8	74	9	143	0,09756098	0,9408	1,0384
463	8	74	9	143	0,09756098	0,9408	1,0384
464	7	75	9	143	0,08536585	0,9408	1,0262
465	7	75	9	143	0,08536585	0,9408	1,0262
466	7	75	9	143	0,08536585	0,9408	1,0262
467	7	75	9	143	0,08536585	0,9408	1,0262
468	7	75	9	143	0,08536585	0,9408	1,0262
469	7	75	9	143	0,08536585	0,9408	1,0262
470	7	75	8	144	0,08536585	0,9474	1,0327
471	7	75	8	144	0,08536585	0,9474	1,0327
472	7	75	8	144	0,08536585	0,9474	1,0327
473	6	76	8	144	0,07317073	0,9474	1,0205
474	6	76	8	144	0,07317073	0,9474	1,0205
475	6	76	8	144	0,07317073	0,9474	1,0205
476	5	77	8	144	0,06097561	0,9474	1,0083
477	5	77	8	144	0,06097561	0,9474	1,0083
478	5	77	8	144	0,06097561	0,9474	1,0083
479	5	77	8	144	0,06097561	0,9474	1,0083
480	5	77	8	144	0,06097561	0,9474	1,0083
481	5	77	8	144	0,06097561	0,9474	1,0083
482	5	77	8	144	0,06097561	0,9474	1,0083
483	5	77	8	144	0,06097561	0,9474	1,0083
484	5	77	8	144	0,06097561	0,9474	1,0083
485	5	77	8	144	0,06097561	0,9474	1,0083
486	5	77	8	144	0,06097561	0,9474	1,0083
487	5	77	8	144	0,06097561	0,9474	1,0083
488	5	77	7	145	0,06097561	0,9539	1,0149
489	5	77	7	145	0,06097561	0,9539	1,0149
490	5	77	7	145	0,06097561	0,9539	1,0149
491	5	77	7	145	0,06097561	0,9539	1,0149
492	5	77	7	145	0,06097561	0,9539	1,0149
493	5	77	7	145	0,06097561	0,9539	1,0149
494	5	77	7	145	0,06097561	0,9539	1,0149
495	5	77	6	146	0,06097561	0,9605	1,0215
496	5	77	6	146	0,06097561	0,9605	1,0215
497	5	77	6	146	0,06097561	0,9605	1,0215
498	5	77	6	146	0,06097561	0,9605	1,0215
499	5	77	6	146	0,06097561	0,9605	1,0215
500	5	77	6	146	0,06097561	0,9605	1,0215
501	5	77	6	146	0,06097561	0,9605	1,0215
502	5	77	6	146	0,06097561	0,9605	1,0215
503	5	77	6	146	0,06097561	0,9605	1,0215
504	5	77	6	146	0,06097561	0,9605	1,0215
505	4	78	6	146	0,04878049	0,9605	1,0093
506	4	78	6	146	0,04878049	0,9605	1,0093
507	4	78	6	146	0,04878049	0,9605	1,0093
508	4	78	6	146	0,04878049	0,9605	1,0093
509	4	78	6	146	0,04878049	0,9605	1,0093
510	4	78	6	146	0,04878049	0,9605	1,0093
511	4	78	6	146	0,04878049	0,9605	1,0093
512	4	78	5	147	0,04878049	0,9671	1,0159
513	4	78	5	147	0,04878049	0,9671	1,0159
514	4	78	5	147	0,04878049	0,9671	1,0159
515	4	78	5	147	0,04878049	0,9671	1,0159
516	4	78	5	147	0,04878049	0,9671	1,0159
517	4	78	5	147	0,04878049	0,9671	1,0159
518	4	78	5	147	0,04878049	0,9671	1,0159
519	4	78	5	147	0,04878049	0,9671	1,0159
520	4	78	5	147	0,04878049	0,9671	1,0159
521	4	78	5	147	0,04878049	0,9671	1,0159
522	4	78	5	147	0,04878049	0,9671	1,0159
523	4	78	5	147	0,04878049	0,9671	1,0159
524	4	78	5	147	0,04878049	0,9671	1,0159
525	4	78	4	148	0,04878049	0,9737	1,0225
526	4	78	4	148	0,04878049	0,9737	1,0225
527	4	78	3	149	0,04878049	0,9803	1,0290
528	4	78	3	149	0,04878049	0,9803	1,0290
529	4	78	3	149	0,04878049	0,9803	1,0290
530	3	79	3	149	0,03658537	0,9803	1,0168
531	3	79	3	149	0,03658537	0,9803	1,0168
532	3	79	3	149	0,03658537	0,9803	1,0168
533	3	79	3	149	0,03658537	0,9803	1,0168
534	3	79	3	149	0,03658537	0,9803	1,0168
535	3	79	3	149	0,03658537	0,9803	1,0168
536	3	79	3	149	0,03658537	0,9803	1,0168
537	3	79	3	149	0,03658537	0,9803	1,0168
538	3	79	3	149	0,03658537	0,9803	1,0168
539	3	79	3	149	0,03658537	0,9803	1,0168
540	3	79	3	149	0,03658537	0,9803	1,0168
541	3	79	3	149	0,03658537	0,9803	1,0168
542	3	79	3	149	0,03658537	0,9803	1,0168
543	3	79	3	149	0,03658537	0,9803	1,0168
544	3	79	3	149	0,03658537	0,9803	1,0168
545	3	79	3	149	0,03658537	0,9803	1,0168
546	3	79	3	149	0,03658537	0,9803	1,0168
547	3	79	3	149	0,03658537	0,9803	1,0168
548	3	79	3	149	0,03658537	0,9803	1,0168
549	3	79	3	149	0,03658537	0,9803	1,0168
550	3	79	3	149	0,03658537	0,9803	1,0168
551	3	79	3	149	0,03658537	0,9803	1,0168
552	3	79	3	149	0,03658537	0,9803	1,0168
553	3	79	3	149	0,03658537	0,9803	1,0168
554	3	79	3	149	0,03658537	0,9803	1,0168
555	3	79	3	149	0,03658537	0,9803	1,0168
556	3	79	3	149	0,03658537	0,9803	1,0168
557	3	79	3	149	0,03658537	0,9803	1,0168
558	3	79	3	149	0,03658537	0,9803	1,0168
559	3	79	3	149	0,03658537	0,9803	1,0168
560	3	79	3	149	0,03658537	0,9803	1,0168
561	3	79	2	150	0,03658537	0,9868	1,0234
562	3	79	2	150	0,03658537	0,9868	1,0234
563	3	79	2	150	0,03658537	0,9868	1,0234
564	3	79	2	150	0,03658537	0,9868	1,0234
565	3	79	2	150	0,03658537	0,9868	1,0234
566	3	79	2	150	0,03658537	0,9868	1,0234
567	3	79	1	151	0,03658537	0,9934	1,0300
568	3	79	1	151	0,03658537	0,9934	1,0300
569	3	79	1	151	0,03658537	0,9934	1,0300
570	3	79	1	151	0,03658537	0,9934	1,0300
571	3	79	1	151	0,03658537	0,9934	1,0300
572	3	79	1	151	0,03658537	0,9934	1,0300
573	3	79	1	151	0,03658537	0,9934	1,0300
574	3	79	1	151	0,03658537	0,9934	1,0300
575	3	79	1	151	0,03658537	0,9934	1,0300
576	3	79	1	151	0,03658537	0,9934	1,0300
577	2	80	1	151	0,02439024	0,9934	1,0178
578	2	80	1	151	0,02439024	0,9934	1,0178
579	2	80	1	151	0,02439024	0,9934	1,0178
580	2	80	1	151	0,02439024	0,9934	1,0178
581	2	80	1	151	0,02439024	0,9934	1,0178
582	2	80	1	151	0,02439024	0,9934	1,0178
583	2	80	1	151	0,02439024	0,9934	1,0178
584	2	80	1	151	0,02439024	0,9934	1,0178
585	2	80	1	151	0,02439024	0,9934	1,0178
586	2	80	1	151	0,02439024	0,9934	1,0178
587	2	80	1	151	0,02439024	0,9934	1,0178
588	2	80	1	151	0,02439024	0,9934	1,0178
589	2	80	1	151	0,02439024	0,9934	1,0178
590	2	80	1	151	0,02439024	0,9934	1,0178
591	2	80	1	151	0,02439024	0,9934	1,0178
592	2	80	1	151	0,02439024	0,9934	1,0178
593	2	80	1	151	0,02439024	0,9934	1,0178
594	2	80	1	151	0,02439024	0,9934	1,0178
595	2	80	1	151	0,02439024	0,9934	1,0178
596	2	80	1	151	0,02439024	0,9934	1,0178
597	2	80	1	151	0,02439024	0,9934	1,0178
598	2	80	1	151	0,02439024	0,9934	1,0178
599	2	80	1	151	0,02439024	0,9934	1,0178
600	2	80	1	151	0,02439024	0,9934	1,0178
601	2	80	1	151	0,02439024	0,9934	1,0178
602	2	80	1	151	0,02439024	0,9934	1,0178

2. ETHICAL VOTE FOR PATIENT DATA COLLECTION AT THE CIMH

UMM
UNIVERSITÄTSMEDIZIN
MANNHEIM

Medizinische Fakultät Mannheim
der Universität Heidelberg

Universitätsklinikum Mannheim

UMM Universitätsmedizin Mannheim
Med. Ethik-Kommission II, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim

Herrn
Prof. Dr. med. Gerhard Gründer
Abteilung Molekulares Neuroimaging
Zentralinstitut für Seelische Gesundheit
J 5

68159 Mannheim

Ethik-Kommission II der Universität Heidelberg
Medizinische Fakultät Mannheim

Vorsitzender: **Prof. Dr. med. Jens P. Striebel**
Geschäftsstelle: S. Cao, M. Goerner, K. Heberlein

Haus 42 – Ebene 3
Theodor-Kutzer-Ufer 1-3
68167 Mannheim

Telefon: +49 621 383 - 71770 / - 71776 / - 71777
Telefax: +49 621 383 - 71772
ethikkommission-II@medma.uni-heidelberg.de
www.umm.uni-heidelberg.de/forschung/medizinische-ethikkommission-ii

Mannheim, 11.12.2018 / MG
Unser Zeichen: **2018-890R-MA**

Studientitel: Anonymisierte Datenverarbeitung von Patientendaten aus dem Therapeutischen Drug Monitoring am Zentralinstitut für Seelische Gesundheit
Studienleiter: **Prof. Dr. med. Gerhard Gründer**
Prüfstelle: Abteilung Molekulares Neuroimaging, Zentralinstitut für Seelische Gesundheit, J 5, 68159 Mannheim, Eingang 10.12.2018

Berufsrechtliche Beratung

Sehr geehrter Herr Professor Gründer,

die Ethik-Kommission II ist nach Durchsicht der Antragsunterlagen der Auffassung, dass gegen die Durchführung der o. g. wissenschaftlichen Studie **keine ethischen und berufsrechtlichen Bedenken** bestehen, sofern nachfolgende Bedingungen uneingeschränkt eingehalten werden:

1. Es handelt sich um die retrospektive Auswertung von vorhandenem Datenmaterial.
2. Es finden weder Untersuchungen noch Befragungen oder sonstige Kontaktierungen der Patienten statt, auf die sich die auszuwertenden Daten beziehen.
3. Es werden keine zusätzlichen Untersuchungen oder Bestimmungen gemacht.
4. Die Datenauswertung erfolgt in anonymisierter bzw. pseudonymisierter Weise.
5. Alle an der Datenverarbeitung beteiligten Personen sind über ihre Schweigepflicht belehrt und auf die bei ihrer Verletzung drohenden Sanktionen hingewiesen worden.
6. Die Vorgaben der EU-DSGVO werden eingehalten.

Hinweis:

Die Ethik-Kommission II macht darauf aufmerksam, dass bei Verwendung von Patientendaten aus der UMM die besonderen Vorgaben bezüglich der Speicherung, der Datenanonymisierung und des Datentransfers der UMM beachtet werden müssen.

Mit freundlichen Grüßen

Prof. Dr. med. Jens-Peter Striebel

Eingereichte Unterlagen:
- Ethikantrag vom 05.12.2018

Zentralinstitut für Seelische Gesundheit
Landesstiftung
des öffentlichen Rechts

Zentralinstitut für Seelische Gesundheit | Postfach 12 21 20 | D-68072 Mannheim

Ethik-Kommission II
Medizinische Fakultät Mannheim
der Ruprecht-Karls-Universität Heidelberg
Theodor-Kutzer-Ufer 1-3
68167 Mannheim

Abteilung Molekulares Neuroimaging
Leitung:
Prof. Dr. Gerhard Gründer
Universität Heidelberg
Medizinische Fakultät Mannheim

Telefon +49 621 1703-1900
Telefax +49 621 1703-801900

gerhard.gruender@zi-mannheim.de
www.zi-mannheim.de

26.07.2019

Amendement zu der zustimmenden Bewertung des Forschungsvorhabens: „Anonymisierte Datenverarbeitung von Patientendaten aus dem Therapeutischen Drug Monitoring am Zentralinstitut für Seelische Gesundheit" Zeichen: 2018-890R-MA

Sehr geehrter Herr Professor Striebel,
sehr geehrte Damen und Herren,

hiermit bitte ich Sie um Kenntnisnahme einer nachträglichen Änderung unseres Forschungsvorhabens „Anonymisierte Datenverarbeitung von Patientendaten aus dem Therapeutischen Drug Monitoring am Zentralinstitut für Seelische Gesundheit".

Die Bedingungen, welche aus der berufsrechtlichen Beratung hervorgingen, werden weiterhin uneingeschränkt eingehalten. Die allgemeinen Grundsätze für die Zulässigkeit der Datenverarbeitung sind zu jedem Zeitpunkt der Studie erfüllt.

Die Änderung betrifft den Umfang der erhobenen Daten, welcher im Rahmen des eingereichten Antrages zur Beurteilung eines Forschungsvorhabens Patientendaten aus dem Therapeutischen Drug Monitoring umschließt. Das Forschungsvorhaben soll nun auch Daten aus klinischen Routineuntersuchungen einschließen, welche im Krankenhausinformationssystem erfasst wurden. Dies schließt Daten aus dem Laborbefund, dem medizinischen Stammblatt, Daten zum Verlauf und der Diagnose vorliegender Erkrankungen, der medikamentösen Behandlung sowie klinisch indizierter EEG- und MRT-Untersuchungen ein.

Ziel der Erweiterung unserer Datenerhebung ist es, den Einfluss o.g. Parameter auf die entstehenden Medikamentenspiegel im Blut von Patienten sowie auf die klinische Wirksamkeit dieser Medikamente zu untersuchen, um neue Einblicke in die klinische Psychopharmakologie zu erlangen.

Für Rückfragen stehe ich Ihnen gerne zur Verfügung.

Mit freundlichen Grüßen

Prof. Dr. med. Gerhard Gründer

3. PUBLICATION “THERAPEUTIC REFERENCE RANGES FOR PSYCHOTROPIC DRUGS: A PROTOCOL FOR SYSTEMATIC REVIEWS”

frontiers in Psychiatry

METHODS
published: 24 November 2021
doi: 10.3389/fpsyt.2021.787043

Therapeutic Reference Ranges for Psychotropic Drugs: A Protocol for Systematic Reviews

Xenia M. Hart[1], Luzie Eichentopf[1], Xenija Lense[1], Thomas Riemer[2], Katja Wesner[1], Christoph Hiemke[3] and Gerhard Gründer[1]*

[1] Department of Molecular Neuroimaging, Medical Faculty Mannheim, Central Institute of Mental Health, Heidelberg University, Mannheim, Germany, [2] Berlin Institute of Health, Institute of Clinical Pharmacology and Toxicology, Charité - Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany, [3] Department of Psychiatry and Psychotherapy, Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center of Mainz, Mainz, Germany

Background: For many psychotropic drugs, monitoring of drug concentrations in the blood (Therapeutic Drug Monitoring; TDM) has been proven useful to individualize treatments and optimize drug effects. Clinicians hereby compare individual drug concentrations to population-based reference ranges for a titration of prescribed doses. Thus, established reference ranges are pre-requisite for TDM. For psychotropic drugs, guideline-based ranges are mostly expert recommendations derived from a conglomerate of cohort and cross-sectional studies. A systematic approach for identifying therapeutic reference ranges has not been published yet. This paper describes how to search, evaluate and grade the available literature and validate published therapeutic reference ranges for psychotropic drugs.

Methods/Results: Following PRISMA guidelines, relevant databases have to be systematically searched using search terms for the specific psychotropic drug, blood concentrations, drug monitoring, positron emission tomography (PET) and single photon emission computed tomography (SPECT). The search should be restricted to humans, and diagnoses should be pre-specified. Therapeutic references ranges will not only base upon studies that report blood concentrations in relation to clinical effects, but will also include implications from neuroimaging studies on target engagement. Furthermore, studies reporting concentrations in representative patient populations are used to support identified ranges. Each range will be assigned a level of underlying evidence according to a systematic grading system.

Discussion: Following this protocol allows a comprehensive overview of TDM literature that supports a certain reference range for a psychotropic drug. The assigned level of evidence reflects the validity of a reported range rather than experts' opinions.

Keywords: psychotropic drugs, drug monitoring, therapeutic reference range, concentration/effect relationship, systematic review

OPEN ACCESS

Edited by:
Laura Mercolini,
University of Bologna, Italy

Reviewed by:
Lucie Bartova,
Medical University of Vienna, Austria
Paul Glue,
University of Otago, New Zealand

****Correspondence:***
Xenia M. Hart
xenia.hart@zi-mannheim.de

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *30 September 2021*
Accepted: *26 October 2021*
Published: *24 November 2021*

Citation:
Hart XM, Eichentopf L, Lense X, Riemer T, Wesner K, Hiemke C and Gründer G (2021) Therapeutic Reference Ranges for Psychotropic Drugs: A Protocol for Systematic Reviews. Front. Psychiatry 12:787043. doi: 10.3389/fpsyt.2021.787043

INTRODUCTION

Many psychotropic drugs have been in use for over 60 years. Great efforts have been made to individualize treatment with the available compounds (1). The only tool for such a personalization, which is now widely used in psychiatric clinical practice, is therapeutic drug monitoring (TDM). TDM-guided therapies aim at titrating drug levels in the blood within a range that is clinically helpful without causing harm. A key principle of TDM is the comparison of individual drug concentrations in the blood to a population-based reference range, the drug-specific therapeutic reference range. At concentrations below the lower limit of this range, a drug-induced response is unlikely to occur. Tolerability is expected to decrease at concentrations above the upper limit. Lower and upper limit of a reference range, respectively, should derive from well-designed clinical studies that relate measured drug concentrations to treatment response or specific adverse drug reactions. For many psychotropic drugs relationships between target engagement (TE) and drug blood concentrations on the one hand and clinical effects and side effects on the other hand are well-documented (2–4). TE by the respective drug (usually occupancy of neuroreceptors or transporters) can be quantified using molecular neuroimaging techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT). These studies supplement data from clinical studies in a meaningful manner. An overview of systematic reviews which aimed at finding therapeutic reference ranges, stated: "[W]e were not aware of a consensus on the optimum methodology for a systematic review that aims to determine upper and lower limits of the therapeutic range for a particular drug" (5). Inconsistent methodologies concerning the way that reference ranges were found have led to a high variation of ranges reported in the literature. In addition, current rating instruments are not designed to rate the quality of TDM studies. Understandably, this has led to criticism among clinicians, and reported ranges are more or less considered experts' opinions. As pointed out in a critical commentary, this holds also true for previously published TDM Consensus Guidelines that report therapeutic reference ranges for 154 neuropsychiatric drugs along with levels of recommendation for their clinical use (6–9).

MATERIALS AND METHODS

Objective and Research Questions

This research protocol provides a tool for searching, evaluating and grading available literature in order to validate published therapeutic reference ranges for psychotropic drugs. Particular emphasis will be given to studies which investigate blood levels and clinical outcomes, such as response to drug treatment or adverse drug reactions. Studies on target engagement (usually receptor/transporter occupancy) from molecular neuroimaging can supplement the clinical evidence. The following research questions are addressed: Is there evidence for a concentration/response relationship and for a concentration/side effect relationship for a certain drug? Is there evidence that supports a lower or upper limit of a therapeutic reference range? How does the drug concentration relate to target engagement (usually receptor/transporter occupancy); and are these findings in line with the concentration/effect relationships and drug concentrations found in patients with psychiatric disorders receiving therapeutically effective doses? The authors may furthermore compute preliminary reference ranges from relevant studies, such as mean or median concentration ranges in patients with psychiatric disorders. This systematic review protocol follows the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) (10) statement. Corresponding systematic reviews for four individual psychotropic drugs have been registered at the International Prospective Register of Systematic Reviews (PROSPERO; CRD42020215873, CRD42021216182, CRD42020218248, CRD42020215872).

Search Strategy

The first step is a systematic search for relevant literature using established databases, such as MEDLINE, Web of Science, PsycINFO, and the Cochrane Library. Search terms for the relevant drug, blood concentrations, drug monitoring, PET and SPECT are helpful. No preset database search filters and no restrictions in regard to the publication date are to be applied. The search is complemented by a hand search in the reference lists of the included publications and in former published guidelines. An example of a search strategy for the antidepressant drug escitalopram is provided in the **Supplementary Material**.

Eligibility Criteria

There are no restrictions in regard to the study design, e.g., both observational and interventional studies are included. Case reports and case series, however, are excluded. The search is restricted to humans, and relevant diagnoses have to be pre-specified, assuming that a specific reference range will only be valid for a particular indication. In order to be included in the evaluation of a certain concentration/effect relationship, studies must refer to patients with psychiatric disorders under monotherapy of the respective drug, meaning no other drug that mediates the relevant treatment effect should be administered concurrently. If at least one measurement was performed before the start of the new medication, the study will be considered for the computation of preliminary ranges only. Drug concentrations in blood should be measured after intake of the respective drug under steady-state conditions. Exceptions are made for molecular neuroimaging studies, which will be considered independent of the dosing period and diagnosis (studies with healthy volunteers included). Since studies investigating long-acting depot formulations are scarce, these studies will also be evaluated without regard to steady state conditions.

Study Selection

After the removal of duplicates, screening of the literature has to be performed by two independent reviewers according to PRISMA guidelines. In cases where a final decision on the inclusion cannot be made based on the abstract alone, the full article must be reviewed. Any disagreements between the two reviewers must be resolved in a subsequent discussion. Inclusion and exclusion criteria are presented in **Table 1**. All studies that examine the drug blood concentrations in

TABLE 1 | Inclusion and exclusion criteria for study eligibility.

	Inclusion criteria	Exclusion criteria
Population	- Psychiatric patients treated with the respective psychotropic drug (not applicable for neuroimaging studies) - Main drug indications, which are specific to each drug, will be defined before the start of the review	- Non-human subjects, healthy volunteers, non-psychiatric patients - Post-mortem studies - Maternal use during pregnancy or lactation
Intervention	- Psychotropic monotherapy arm or period of observation (at least one blood level measurement before add-on therapy) - Treatment duration long enough to reach steady state (not applicable for neuroimaging studies and studies with depot formulations)	- Blood level is not measured in the steady state - Studies primarily comparing blood analysis techniques
Outcome(s)	- Drug concentrations measured in the blood (serum or plasma) - For concentration/effect studies: direct clinical outcome measures, i.e., safety or efficacy using a standardized rating scale (e.g., HAMD, MADRS, CGI)* - For neuroimaging studies: target engagement, usually by receptor or transporter occupancy	- No mean or median blood level reported
Study Design	- Observational and interventional studies are included - Reviews and meta-analyses investigating a concentration/effect relationship for the relevant drug	- Reviews and experts' opinions - Gray literature - Case reports and case series
Other		- Papers containing the same data - No abstract available - Data from simulation studies

**Biomarkers (e.g., QTc-time) are not regarded a direct clinical outcome measure.*

relation to clinical effect (without concomitant psychiatric medication), dose or target engagement have to be identified. Studies that did not ensure steady-state must be excluded (not necessarily applicable for imaging studies and studies with depot formulations). Studies performing population pharmacokinetic modeling analyses should be identified in the systematic review in order to discuss moderating factors on drug concentrations.

Data Extraction

Both reviewers have to independently extract the following information from each study: lead author, year, title, country, study design, number and details of subjects, diagnosis, mean dose ± standard deviation (SD), mean blood concentration ± SD, concentration range, clinical efficacy or side effect measures, and main outcomes. Any disagreements between the reviewers have to be resolved in a subsequent discussion. Finally, if necessary, the authors of the original papers will also be contacted if further data is necessary for their interpretation.

Quality Assessment

Reviewers have to independently (i) rate internal quality of included studies dependent of the study design (ii) assess the quality and reporting of TDM components of the studies. To date, there are no standardized quality tools for studies specifically investigating TDM or concentration/effect relationships. Therefore, we adjusted the quality criteria in a recent review by Kloosterboer et al. on the concentration/effect relationship of psychotropic drugs in minors (11), which were modified from a previously published meta-analysis by Ulrich et al. for haloperidol (12). A detailed description of the individual items can be found in the **Supplementary Material**. If a study does not completely report or implement an item, that item is rated insufficient. The TDM quality score ranges from 0 to 10 [selection (scale 0–3), comparability (scale 0–2), and drug monitoring (scale 0–5)]. For the quality assessment of cohort studies and cross-sectional studies, an adapted version of the Newcastle–Ottawa Scale (13) is used. The quality score ranges from 0 to 10 [selection (scale 0–4), comparability (scale 0–2), and outcome (scale 0–4)] for cohort studies and from 0 to 8 [selection (scale 0–4), comparability (scale 0–2), and outcome (scale 0–2)] for cross-sectional studies. Likewise, reviewers rate the quality of the relevant efficacy cohort of randomized controlled clinical trials separately using the Cochrane risk-of-bias tool for randomized trials (14). Any disagreements are resolved through discussion. Authors of the original papers will be contacted if further information is required.

Considerations for the Quality Assessment of TDM Studies

Representativeness of the Patient Sample

For the study results to be applied in a generalized manner, it is important to have a representative sample, which reflects the target population of the resulting reference range. A study population only comprising of treatment-resistant patients or patients with side effects to another treatment does not reflect the general patient population and a resulting range is not transferable to "normal" patients. Likewise, a study population drawn from patients for whom genotyping has been demanded by the clinician will not reflect the target population. Patients 18 years and younger or 65 years and older should be compared with the average adult population. For some psychotropic drugs, ethnic variation in distribution in CYP expression patterns is relevant for the metabolism of the administered drug. This is especially important, if the main metabolite of the drug contributes to the pharmacologic action. A variation in the metabolite-to-parent compound ratio and thus, the sum of active and parent compound, may possibly influence clinical effects in these drugs. Since the evidence on this phenomenon is still very small, its clinical relevance should be revised for every substance individually. If an influence has been shown, studies must be evaluated in regard to the factor ethnicity. This holds also true for

studies using variations in drug formulations or chemical forms (prodrugs). References ranges may not easily be transferred from originator products.

Diagnosis

To ensure comparability between studies, patients should be selected patients should be selected according to psychiatric and associated classification systems [of which the latest versions are the 5th edition of the American Psychiatric Association's (15) and the 11th edition of the World Health Organization's (16), which comes into effect in 2022]. Ideally, a homogeneous sample of patients according to one main diagnosis should be investigated. With a heterogeneous sample, a sub-analysis per relevant category should be provided. Differences in reference ranges across, usually related but also across unrelated, diagnosis should be emphasized in the final review.

Comedication

To avoid clinical effect bias, no drugs that potentially affect the treatment outcome should have been taken concomitantly during the study period. If detailed information on comedication was not provided, the study is rated as insufficient. The use of on-demand medication such as benzodiazepines or sleep medication must be considered adequate. Pre-medication should be registered as study characteristic and not be scored. For reviews about reference ranges of substances in which the active metabolite contributes to clinical efficacy and an altered metabolite to parent compound ratio might lead to a change in clinical efficacy, studies allowing concomitant drugs that interfere with the metabolism of the target drug should be identified.

Dose Design

The clinical status of a subject determines the amount of dose administered and thus the drug concentration. To avoid a possible reversal of a causal relationship resulting from such an effect, a study design with a fixed dose should be preferred over a design with a flexible dose (17). Flexible dosing is usually insufficient, since it may give rise to artificially negative correlations between concentrations and clinical effects (10).

Analytical Method for the Assay of Drug Concentration in Serum or Plasma

An analytical method is considered valid if it accurately, precisely, selectively, sensitively, reproducibly, and stably measures the concentration of the substance (9). In general, chromatographic methods, such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS), are selective and sensitive measurement methods. Immunoassays are considered low specific. The lower detection limit of the chosen analytical method should allow drug concentration measurements below the lower limit of currently recommended therapeutic reference ranges. Double measurements of samples are preferred, but they are not performed in clinical routine practice.

Blood Sample Collection

The time of sample collection affects the blood concentration of the drug. Sampling should be performed at steady-state, preferably at trough level since TDM-guided pharmacotherapy usually relies on minimal drug concentration, if not indicated otherwise. In clinical routine, blood withdrawal in the morning, before the first dose has been recommended (12–16 or 24 h after last dose) (9). Inconsistent sampling time points introduce bias; however considerably less likely for substances with long half-lives than for those with short elimination half-lives. Drug concentration of substances with long elimination half-lives (e.g., fluoxetine and aripiprazole), extended-release and depot formulations remain relatively stable over the day (18) and allow sampling within 12–24 h after the last drug intake. Sampling times should be described in publications when reporting drug concentrations. It is generally assumed that the steady-state condition is reached after 5 times the half-life of a drug. Drug sampling before the steady-state is reached, however, may result in an underestimation of clinical efficacy. This also holds true for long-acting depot medication.

Concentration Design

Correlations of measured serum concentrations with early response (e.g., after 1 week) is problematic, because of the well-described time lag between treatment initiation and onset of antidepressant/antipsychotic effects. The sampling schedule should include repeated sampling (at least two samples) in a patient over several weeks, ideally at different doses. In order to reflect a representative distribution of drug concentrations, a study's dose regimen should result in a sufficiently wide drug concentration range, with data of sub- and/or supratherapeutic drug concentrations.

RESULTS

Reporting of Results

Results must be reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The characteristics of all included studies (author/s, year, country, study design, intervention details, and study population details) must be displayed in a tabular summary.

Grading of Evidence

The strength of available evidence for that supports a concentration/response relationship or concentration/ side effect relationship for a drug will be reflected by the assignment of a certain level. Grading into levels of evidence will be performed following the recommendations of the WFSBP guidelines for clinical guideline development (19). (i) Prioritize and evaluate (risk-of-bias assessment) single RCTs: when sufficient RCTs exist that support a certain concentration/effect relationship and these are of high quality and do not contradict each other, this approach is preferred. (ii) Evaluate meta-analyses (risk-of-bias assessment): when there are at least three RCTs for one treatment and these are inconsistent—meaning that some studies show a difference to placebo and others do not—meta-analyses of high quality should be used. (iii) Evaluate systematic reviews without meta-analysis (risk-of-bias assessment). This source of evidence should only be used if no recommendations can be generated from (1) and (2). It is not recommended to base the evidence grading on non-systematic reviews. Levels of evidence relating to the published literature are documented in **Table 2**. If evidence is

TABLE 2 | Grading into levels of evidence for a concentration/effect relationship following the recommendations of the WFSBP guidelines for clinical guideline development.

Levels of evidence for concentration/effect relationship		
Evidence for a certain effect is:	**Grade**	**Explanation**
Strong	A	At least two independent randomized clinical trials with a low risk of bias show a concentration/effect relationship AND No negative randomized clinical trials with a low risk of bias exist. If there are contradicting results from randomized clinical trials, the majority of randomized clinical trials AND/OR a meta-analysis with low risk of bias shows a relationship.
Limited	B	One randomized clinical trials with a moderate risk of bias showing a concentration/effect relationship AND No negative studies exist OR Meta-analyses with a moderate risk of bias that show a relationship.
Low	C	One or more prospective open studies (with a minimum of 10 evaluable patients per group) using a control group, but no randomization, or using no control group, show concentration/effect relationships. OR One or more well-conducted case control or cohort studies (with a minimum of 10 evaluable patients) with a moderate probability that the concentration/effect relationship is causal. OR Randomized clinical trials AND/OR meta-analyses with a high risk of bias show concentration/effect relationships.
No evidence	D	Insufficient data do not allow evaluation if a concentration/effect relationship exists OR Evidence is given that a concentration/effect relationship does not exist (e.g., tranylcypromine, agomelatine)

found to support the relationship between drug concentration and therapeutic response (level A, strong or level B, limited), a valid therapeutic reference range, at least the lower limit, is likely to be found by an evaluation of the available data. The overall quality of evidence is reported as "strong," "limited," "low," or "no evidence."

Data Synthesis

Concentration data must be pooled in order to find mean concentration ranges across studies. The theoretically expected concentration range in a patient population is estimated using data from a reference sample of patients, preferentially without co-medication or pharmacogenetic abnormalities. The pooled concentration, daily dose and C/D have to be combined and calculated using random-effect and fixed-effect models based on the I^2 statistic. The I^2 statistic has to be used to examine to presence of substantial heterogeneity between studies, with I^2-values $> 50\%$ indicating heterogeneity. Subgroup analyses might be appropriate to examine the impact of moderating factors on concentration, such as patient populations with differing CYP expression patterns, age, sex or concomitant medications. In the next step, ranges of blood concentrations from only responders to a drug are computed to obtain a preliminary responder reference range for the psychotropic drug. There is no consistent method for calculating these ranges. We propose the use of mean $\pm$ one standard deviation (SD) or interquartile ranges (25th−75th percentiles) of drug concentrations in the blood.

DISCUSSION

Our strategy, on how to search and grade TDM-related literature, aims at finding therapeutic reference ranges for psychotropic drugs that are objectively evaluated. Each drug has to be assigned to a level according to the strength of evidence which refers to the underlying concentration/effect relationship. Methodology that has been used to uncover clinical response of psychotropic drugs in relation to blood concentration, however, is highly prone to failure (20). Concentration/response relationships are not well-established for most psychotropic drugs. As a consequence, many published ranges must be regarded as preliminary. In addition, published studies strongly differ in design and quality; their critical evaluation, as described here, is mandatory. This protocol introduces a standard on how to identify and grade evidence underlying therapeutic reference ranges. The methodology may be extended to other drug classes, since the lack of evaluated therapeutic reference ranges is not restricted to TDM in psychiatry.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XH developed the first draft of the protocol. CH and GG supervised the entire manuscript writing and contributed to the revision of the protocol. XL, KW, LE, and TR have contributed to the development of the search strategy and quality assessment criteria. All authors have read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.787043/full#supplementary-material

REFERENCES

1. Lloret-Linares C, Bellivier F, Haffen E, Aubry JM, Daali Y, Heron K. Markers of individual drug metabolism: towards the development of a personalized antidepressant prescription. *Curr Drug Metab.* (2015) 16:17–45. doi: 10.2174/1389200216011507021607 28
2. Gründer G, Hiemke C, Paulzen M, Veselinovic T, Vernaleken I. Therapeutic plasma concentrations of antidepressants and antipsychotics: lessons from PET imaging. *Pharmacopsychiatry.* (2011) 44:236–48. doi: 10.1055/s-0031-1286282
3. Cumming P, Abi-Dargham A, Gründer G. Molecular imaging of schizophrenia: neurochemical findings in a heterogeneous and evolving disorder. *Behav Brain Res.* (2021) 398:113004. doi: 10.1016/j.bbr.2020.113004
4. Eap CB, Gründer G, Baumann P, Ansermot N, Conca A, Corruble E. Tools for optimising pharmacotherapy in psychiatry (therapeutic drug monitoring, molecular brain imaging and pharmacogenetic tests): focus on antidepressants. *World J Biol Psychiatry.* (2021) 22:561–628. doi: 10.1080/15622975.2021.1878427
5. Cooney L, Loke YK, Golder S, Kirkham J, Jorgensen A, Sinha I. Overview of systematic reviews of therapeutic ranges: methodologies and recommendations for practice. *BMC Med Res Methodol.* (2017) 17:84. doi: 10.1186/s12874-017-0363-z
6. Baumann P, Hiemke C, Ulrich S, Eckermann G, Gaertner I, Gerlach M. The AGNP-TDM expert group consensus guidelines: therapeutic drug monitoring in psychiatry. *Pharmacopsychiatry.* (2004) 37:243–65. doi: 10.1055/s-2004-832687
7. Hiemke C, Baumann P, Bergemann N, Conca A, Dietmaier O, Egberts K. AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. *Pharmacopsychiatry.* (2011) 44:195–235. doi: 10.1055/s-0031-1286287
8. de Leon J. A critical commentary on the 2017 AGNP consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology. *Pharmacopsychiatry.* (2018) 51:63–8. doi: 10.1055/s-0043-117891
9. Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. *Pharmacopsychiatry.* (2018) 51:9–62. doi: 10.1055/s-0043-116492
10. Hiemke C. Concentration-effect relationships of psychoactive drugs and the problem to calculate therapeutic reference ranges. *Ther Drug Monit.* (2019) 41:174–9. doi: 10.1097/FTD.0000000000000582
11. Kloosterboer SM, Vierhout D, Stojanova J, Egberts KM, Gerlach M, Dieleman GC. Psychotropic drug concentrations and clinical outcomes in children and adolescents: a systematic review. *Expert Opin Drug Saf.* (2020) 19:873–90. doi: 10.1080/14740338.2020.1770224
12. Ulrich S, Wurthmann C, Brosz M, Meyer FP. The relationship between serum concentration and therapeutic effect of haloperidol in patients with acute schizophrenia. *Clin Pharmacokinet.* (1998) 34:227–63. doi: 10.2165/00003088-199834030-00005
13. Wells GA, Shea B, O'Connell D, Welch PJV, Losos M. The newcastle-ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. *Ottawa Health Research Institute Web site 7.* (2014).
14. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* (2019) 366:l4898. doi: 10.1136/bmj.l4898
15. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* 5th ed. Arlington, VA: American Psychiatric Association (2013).
16. World Health Organization. *International Statistical Classification of Diseases and Related Health Problems.* 11th ed. Available online at: https://icd.who.int/ (accessed October 21 2021).
17. Ulrich S, Lauter J. Comprehensive survey of the relationship between serum concentration and therapeutic effect of amitriptyline in depression. *Clin Pharmacokinet.* (2002) 41:853–76. doi: 10.2165/00003088-200241110-00004
18. Sheehan JJ, Reilly KR, Fu DJ, Alphs L. Comparison of the peak-to-trough fluctuation in plasma concentration of long-acting injectable antipsychotics and their oral equivalents. *Innov Clin Neurosci.* (2012) 9:17–23.
19. Hasan A, Bandelow B, Yatham LN, Berk M, Falkai P, Möller HJ, et al. WFSBP guideline task force chairs. WFSBP guidelines on how to grade treatment evidence for clinical guideline development. *World J Biol Psychiatry.* (2019) 20:2–16. doi: 10.1080/15622975.2018.1557346
20. Bengtsson F. Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit.* (2004) 26:145–51. doi: 10.1097/00007691-200404000-00010

Conflict of Interest: CH has received speaker's fees from Otsuka. He is editor of PSIAC, a web-based platform analyzing pharmacokinetic and –dynamic drug interactions. The software is distributed by Springer Nature, Heidelberg, Germany. GG has served as a consultant for Allergan, Boehringer Ingelheim, Institute for Quality and Efficiency in Health Care (IQWiG), Janssen-Cilag, Lundbeck, Otsuka, Recordati, ROVI, Sage, and Takeda. He has served on the speakers' bureau of Gedeon Richter, Janssen Cilag, Lundbeck, Otsuka, Recordati. He has received grant support from Boehringer Ingelheim, Lundbeck and Saladax. He is co-founder and/or shareholder of Mind and Brain Institute GmbH, Brainfoods GmbH, OVID Health Systems GmbH and MIND Foundation gGmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

4. ACCEPTED MANUSCRIPT: “THERAPEUTIC REFERENCE RANGE FOR ARIPIPRAZOLE IN SCHIZOPHRENIA REVISED: A SYSTEMATIC REVIEW AND METAANALYSIS”

Psychopharmacology
https://doi.org/10.1007/s00213-022-06233-2

REVIEW

Therapeutic Reference Range for Aripiprazole in Schizophrenia Revised: a Systematic Review and Metaanalysis

Xenia M. Hart[1,13] · Christoph Hiemke[2,13] · Luzie Eichentopf[1] · Xenija M. Lense[1] · Hans Willi Clement[3,13] · Andreas Conca[4,13] · Frank Faltraco[5,13] · Vincenzo Florio[4] · Jessica Grüner[3] · Ursula Havemann-Reinecke[6,13] · Espen Molden[7] · Michael Paulzen[8,13] · Georgios Schoretsanitis[9,10,11,13] · Thomas G. Riemer[12] · Gerhard Gründer[1,13]

Received: 20 April 2022 / Accepted: 1 September 2022

Abstract

Rationale While one of the basic axioms of pharmacology postulates that there is a relationship between the concentration and effects of a drug, the value of measuring blood levels is questioned by many clinicians. This is due to the often-missing validation of therapeutic reference ranges.

Objectives Here, we present a prototypical meta-analysis of the relationships between blood levels of aripiprazole, its target engagement in the human brain, and clinical effects and side effects in patients with schizophrenia and related disorders.

Methods The relevant literature was systematically searched and reviewed for aripiprazole oral and injectable formulations. Population-based concentration ranges were computed ($N = 3{,}373$) and pharmacokinetic influences investigated.

Results Fifty-three study cohorts met the eligibility criteria. Twenty-nine studies report blood level after oral, 15 after injectable formulations, and nine were positron emission tomography studies. Conflicting evidence for a relationship between concentration, efficacy, and side effects exists (assigned level of evidence low, C; and absent, D). Population-based reference ranges are well in-line with findings from neuroimaging data and individual efficacy studies. We suggest a therapeutic reference range of *120–270* ng/ml and *180–380* ng/ml, respectively, for aripiprazole and its active moiety for the treatment of schizophrenia and related disorders.

Conclusions High interindividual variability and the influence of CYP2D6 genotypes gives a special indication for Therapeutic Drug Monitoring of oral and long-acting aripiprazole. A starting dose of 10 mg will in most patients result in effective concentrations in blood and brain. 5 mg will be sufficient for known poor metabolizers.

Keywords Aripiprazole · Reference range · Blood level · Therapeutic Drug Monitoring · Clinical effects · Adverse drug reaction · Dopamine receptor occupancy

Abbreviations

AIMS	Abnormal Involuntary Movement Scale
AM	Active moiety, sum of ARI and DARI
ARI	Aripiprazole
BARS	Barnes Akathisia Rating Scale
BD	Bipolar disorders
BL	Blood level
C/D	Concentration to dose (mean C / mean D)
CGI	Clinical Global Impression
CGI-I	Clinical Global Impression—Improvement
CGI-S	Clinical Global Impression—Severity
CS	Cohort study
CSS	Cross-sectional study
CYP	Cytochrome P450
d	Day
D-ARI	Dehydroaripiprazole
DIEPS	Drug-induced extrapyramidal symptoms
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th edition
EPS	Extrapyramidal side effects

✉ Xenia M. Hart
xenia.hart@zi-mannheim.de

Extended author information available on the last page of the article

HPLC with UV detection	High-performance liquid chromatography method with UV-absorbance detection
HV	Healthy volunteers
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th edition
LC/MS/MS	Liquid chromatography/ tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
m	Month
MPR	Metabolite to parent ratio
NA	Not available
PANSS	Positive and Negative Syndrome Scale
PD Comedication	Concomitant psychotropic medication with antipsychotic efficacy
PM	Poor metabolizers
QA	Result of the study-type specific quality assessment
RCT	Randomized controlled trial
SAD	Schizoaffective disorder
SAS	Simpson-Angus Extrapyramidal Symptoms Scale
SC	Serum concentration
SCZ	Schizophrenia
SD	Standard deviation
ST score	Study-specific quality assessment score
TDM	Therapeutic Drug Monitoring
TDM score	Quality assesssment score of the Therapeutic Drug Monitoring component
UKU	UKU side effect rating scale
UPLC-MS/MS	Ultra-performance liquid chromatography–tandem mass spectrometry
w	Week

Introduction

One of the fundamental principles of pharmacology is the existence of a relationship between the dose (or concentration) of a drug and the organism's (patient's) response to that drug. For drugs that exert their clinical effect by binding to a receptor (or transporter), the dose–response relationship is closely related to the drug–receptor binding relationship. Since the blood levels (BLs) of orally administered drugs are extremely variable at a given dose (Gründer et al. 2008), the BL of a drug is usually a much more accurate indicator of the extent to which the molecular target is occupied by the substance. Despite the fundamental validity of these basic principles of pharmacology, *therapeutic reference ranges* for BLs of drugs are still considered by many clinicians to be insufficiently valid to guide therapy with psychotropic drugs. *Therapeutic Drug Monitoring (TDM)*, the assessment of medication BLs for personalized treatment, is primarily used as a tool to identify adherence problems or for problem solving. Here, we present a prototypic systematic review and metaanalysis on the relationship between BLs of aripiprazole (ARI), and first, clinical outcome, and second, dopamine receptor occupancy, with the aim of establishing a definitive reference range for ARI in patients with schizophrenia and related disorders.

Aripiprazole attracted particular interest when it appeared on the market because of its novel mechanism of action (Gründer et al. 2003). ARI acts as a partial agonist at $D_{2/3}$ and 5-HT_{1A} receptors, and as an antagonist at serotonin 5-HT_{2A} receptors (Gründer et al. 2006). Its active metabolite, dehydroaripiprazole (D-ARI) has a similar pharmacological profile to its parent compound, thus is a relevant mediator for treatment outcome. ARI's antipsychotic efficacy is comparable to that of antagonist antipsychotics. Extrapyramidal side effects and weight gain are rare, and prolactin is decreased rather than increased (Huhn et al. 2019). Clinically used ARI doses range from 10 to 30 mg daily (Otsuka Pharmaceutical Co. 2016). A recent work, however, revealed that a dose of around 12 mg/day is sufficient to produce 95% of the maximum effect of ARI in patients with schizophrenia (Leucht et al. 2020). The authors concluded that patients usually do not benefit from higher doses.

International guidelines for Therapeutic Drug Monitoring (TDM) propose a therapeutic reference range of *100–350* ng/ml for ARI and *150–500* ng/ml for the active moiety (Hiemke et al. 2018; Schoretsanitis et al. 2021). While TDM is recommended for dose titration in some patients treated with ARI, the evidence for a relationship between BLs and clinical efficacy and side effects is sparse (Sparshatt et al. 2010; Lopez and Kane 2013; Mauri et al. 2018). However, the fact that a relationship between BLs and clinical effects has not been convincingly demonstrated to date does not mean that it does not exist. The available studies may simply be methodologically inadequate (Preskorn 2013; Hiemke 2019). We consider the methodology proposed here as a prototype for establishing therapeutic reference ranges for antipsychotic drugs.

Methods

Inclusion Criteria

Both randomized controlled trials (RCTs) and uncontrolled studies reporting ARI blood concentrations in humans (serum or plasma), referred to herein as BLs, were eligible for inclusion, especially those investigating relationships with clinical effects or $D_{2/3}$ receptor occupancy (suppl. table S2). Reviews and meta analyses investigating a concentration/efficacy-relationship for ARI were also included. Studies were included regardless of ARI dosage forms. The indications were restricted to schizophrenia, schizophrenia spectrum disorders, and bipolar disorder.

Study selection process

We followed our previously published protocol and relevant guidelines (Page et al. 2021; Hart et al. 2021) including a quality control of publications (Hart et al. 2021) and grading of available evidence (Hasan et al. 2019) (for complete search terms see suppl. S1). Risk of bias was assessed with the Cochrane risk-of-bias tool 2.0 (Sterne et al. 2019) and a previously reported rating instrument (Hart et al. 2021). Four electronic databases were systematically searched on February 16, 2021 without restriction of language or publication date (PsycINFO, Medline via PubMed, Cochrane CENTRAL, Web of Science; last updated January 31, 2022). Search terms for aripiprazole, blood concentrations, drug monitoring, PET, and SPECT were used. See supplemental material S1 for full database search strings. No preset database search filters and no restrictions regarding the publication date were applied. The search was complemented by a hand search in the reference lists of the included publications and in former published guidelines. After the removal of duplicates, screening of the literature was performed by two independent reviewers (LE, XH) according to PRISMA guidelines. In cases where a final decision on the inclusion could not be made based on the abstract alone, the full article was reviewed. Both reviewers independently extracted the following information from each study: lead author, year, title, country, study design, number and details of subjects, diagnosis, mean dose ± standard deviation (SD), mean blood concentration ± SD, concentration range, clinical efficacy or side effect measures, and main outcomes. Any disagreements between the reviewers were resolved in a subsequent discussion. Additional data were requested from the authors, whenever concentration data were not complete. This study is registered under PROSPERO number CRD42020215872.

Qualitative and quantitative synthesis

Outcomes of interest for the qualitative synthesis were reports of an association between ARI and/or D-ARI BLs and clinical effect, either efficacy or side effects. Eligible reports could be qualitative or quantitative, continuous or categorical but required a structured clinical assessment by a rating scale. Factors influencing ARI and D-ARI BL among patients were extracted. Studies reporting $D_{2/3}$ receptor occupancy in relation to the participants' BLs were extracted, and 90% effective concentrations (EC_{90} values) were computed from EC_{50} as previously described (Hart et al. 2022). For the quantitative synthesis, means, standard deviations, medians, and interquartile ranges of relevant BLs were assessed. Means and standard deviations of the C/D ratio were selected. Data were either extracted from the manuscript or, if numbers for the whole sample were given, calculated manually.

Statistical Analysis

A combined metaanalysis was performed using the R (Version 4.0.3) "metafor" and "meta" package. I^2 statistic was used to evaluate heterogeneity of the studies, with I^2 values > 50% indicating heterogeneity. Ninety-five percent confidence intervals (CIs) were calculated from mean concentrations and C/D value, and data were combined using random-effect models based on the I^2 statistic. Four quality assessment criteria that could have a potential influence on the clinical validity of a therapeutic reference range were identified a priory (Q1 "ethnic group Caucasian," Q2b "diagnosis schizophrenia," Q4 "dose design," and Q6a "sampling at trough"). Their impact as moderating factors on mean BLs was investigated by subgroup analyses of studies rated sufficient or insufficient on these criteria if a minimum of three records per group were available. Forest plots of subgroup differences identified as significant ($p \leq 0.05$) were retrieved for visualization of subgroup differences. Linear regression analysis was used to display the relationship between ARI dose and ARI and D-ARI BLs.

Results

Study overview

From the 715 articles initially identified, a total of 51 articles comprising of 53 studies (Fig. 1) published from 2002 to 2021 were selected (for study details see suppl. table S3–S5). Four articles reported results from two or more separate patient samples including one article that developed a population-based pharmacokinetic model. In total, 29 studies were identified that report BL after oral

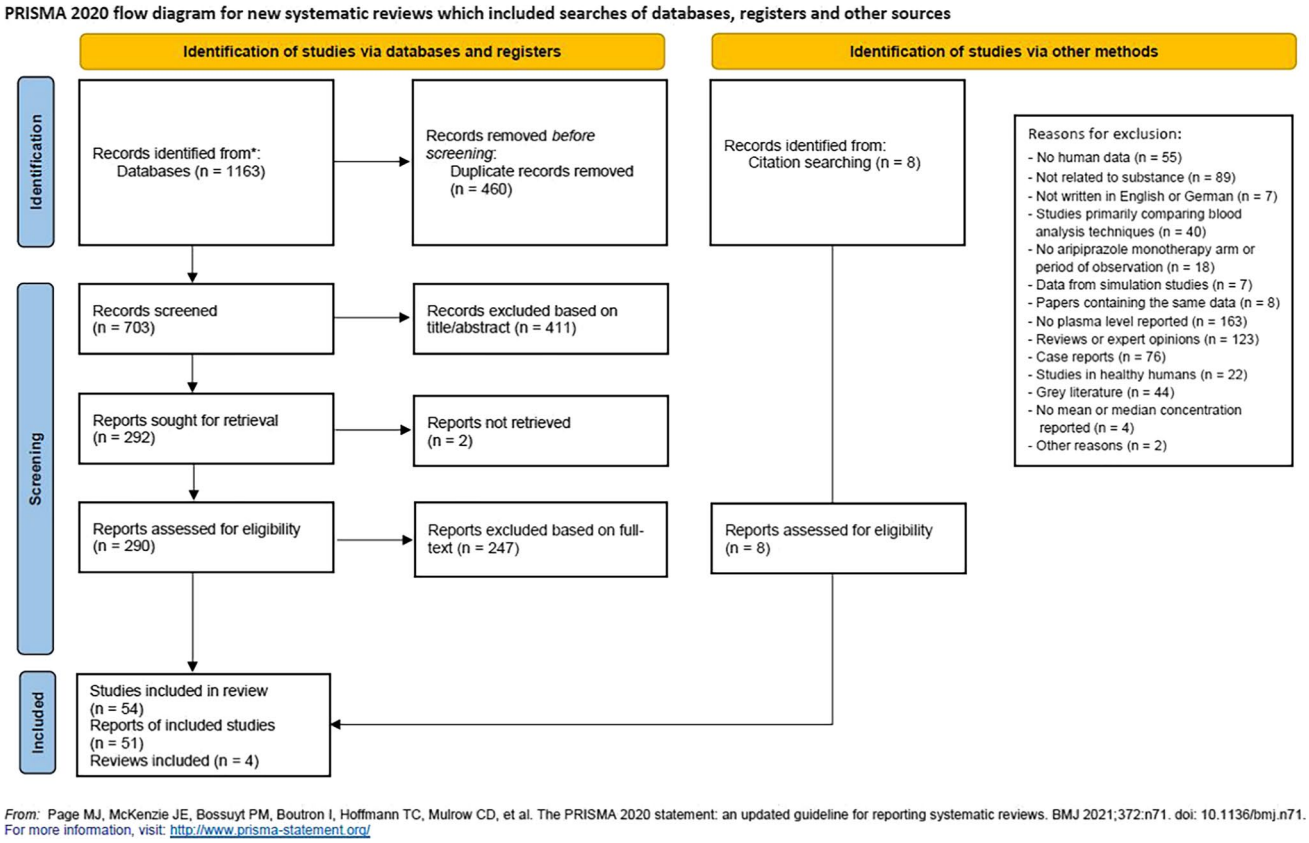


Fig. 1 Study Overview according to PRISMA

ARI administration. Thirteen of them additionally reported results from clinical efficacy or side effect assessments. Of 15 studies that reported BL after ARI injections (13 LAI, 2 acute), nine studies reported clinical efficacy measures. Nine neuroimaging studies on (striatal) $D_{2/3}$ receptor occupancy were found. Rating results are presented in the supplemental material S6-S11.

Risk of bias rating for TDM component

See suppl. fig. S6 and S11 for results. The most frequently missed TDM criterion was Q1 "study population," since the majority of studies did not solely include Caucasian patients. The second most frequently missed criteria were comedication (Q3) and dose design (Q4) followed by an inhomogeneous diagnosis (Q2b). More than half of the studies used a naturalistic design allowing for flexible dosing or administered single doses. As a result of a high percentage of uncontrolled cohort and retrospective TDM studies, comedication with psychotropic and pharmacokinetically interfering drugs was common among studies. Studies with retrospective data collection, such as cross-sectional studies, could usually not fulfill the criterion of a predefined sampling schedule (Q7a). However, even among cohort studies, single sampling was common. Nevertheless, most studies reported sufficiently broad concentration ranges for ARI (Q7b), a crucial qualification to find a concentration/efficacy-relationship. Most studies selected patients according to the psychiatric classification system "Diagnostic and Statistical Manual of Mental Disorders version IV or 5" (Q2a). However, studies often did not distinguish between patients with a diagnosis of schizophrenia and with other psychotic disorders (Q2b). The analytical method (Q5) was rated as insufficient in 16 studies because precise information on the detection limit was missing. Sampling time (Q6b) and steady state (Q6a) were given in the majority of selected studies.

Concentration/efficacy-relationship

In general, we found highly heterogeneous reports of clinical efficacy/concentration-relationships (Table 1). A clear relationship between ARI BL and antipsychotic effects was reported by two prospective cohort studies, both considered of having moderate risk of bias (TDM score; 4/10 and 8/10, ST score; both 6/10) (Lin et al. 2011; Nemoto et al. 2012). One study, however, introduced a considerable amount of bias by add-on therapy with the antidepressant and CYP2D6 inhibitor paroxetine (Nemoto et al. 2012). Another study

Table 1 Level of evidence; Summarized results of the qualitative synthesis. Studies reporting a concentration/efficacy- or side effect-relationship

Reference	Design and subjects	Efficacy	Side effects	BL range	Psyc. Comed	TDM Score	Study score	Comment and risk for Bias
Nemoto et al. 2012	Prospective CS; paroxetine add-on; fixed ARI doses (mean 14.6 mg); SCZ; $N=14$	Positive (CGI)	Not found (DIEPS)	Yes	Yes	8/10	6/10	CGI decreased with increasing ARI BL; CAVE: add-on
Lin et al. 2011	Prospective CS with flexible doses (mean 15 mg/day); SCZ or SAD; $N=45$	Positive (PANSS)	Not found (AIMS, BARS, SAS)	Yes	No	6/10	4/10	Higher ARI BL in responders (20% decrease in PANSS score)
Nakamura et al. 2009	Prospective CS with fixed doses (mean 22 mg/day); carbamazepine add-on; SCZ; $N=18$	Negative (PANSS)	Positive (UKU)	Yes	Yes	8/10	4/10	Higher response and less neurological AEs with decreasing ARI BL; CAVE: add-on
Hwang et al. 2015	Cluster RCT with fixed doses (15 mg/day); SCZ or SAD; $N=79$	Not available	Negative (BARS; Akathisia)	No	Yes	7/10	Moderate	Higher ARI BL correlated with greater reduction in BARS on day 56
Steen et al. 2017	CSS; flexible doses, multiple diagnoses; $N=373$	Not available	Not available	Yes	No	5/10	6/9	Better attention and working memory nominally associated with higher ARI BL
Veselinovic et al. 2019	Cohort nested in RCT; flexible design (mean 15.5 mg/day); SCZ; $N=11$	Not available	Not found (SAS, BARS, AIMS)	Yes	Yes	8/10	6/10	Physical and mental well-being correlated negatively with estimated D_2 receptor occupancy

BL = blood level, CS = cohort study, CSS = cross-sectional study, Psyc. Comed. = psychiatric comedication, RCT = randomized controlled trial, SCZ = schizophrenia, SAD = schizoaffective disorder

by Lin et al (2011) in patients with schizophrenia or schizoaffective disorder with an acute exacerbation, the only study that a priori aimed at finding a concentration efficacy relationship, did not allow for relevant psychiatric comedication (Lin et al. 2011). After six weeks of treatment under flexible dosing, responders, defined by at least 20% decrease in PANSS total score, had higher D-ARI and AM BLs than nonresponders (however not significant for ARI alone). Nakamura and colleagues (2009) reported conflicting results in patients with SCZ, which should, however, also be regarded with caution due to the combination with low doses of the anticonvulsant drug carbamazepine (which lowers ARI levels by inducing CYP3A4) (Nakamura et al. 2009). In addition, one study in patients with schizophrenia, other psychotic disorders or bipolar disorder, reported better attention and working memory in patients with higher ARI BLs (Steen et al. 2017). Another study reported a negative association between patient-reported physical well-being and very high $D_{2/3}$ receptor occupancy, estimated from ARI BLs in patients with schizophrenia (Veselinović et al. 2019). No metaanalysis on the concentration/effect-relationship of ARI is available. None of the LAI studies has aimed at or described a correlation between ARI BLs, response, or side effects. Concomitant oral antipsychotic treatment was given in all LAI studies that included patients with schizophrenia. To sum up, despite conflicting results from pharmacokinetic studies, one study at moderate risk of bias was able to report a positive association between ARI concentration and clinical efficacy, which justifies the classification of the evidence as "low" for the concentration/efficacy-relationship after oral administration (Level C; low) (Hart et al. 2021).

Concentration/side effect-relationship

A total of ten studies measured general or specific motor side effects using a structured clinical rating scale. Five studies did not detect an association between BLs and side effects. One study found a general decrease in neurological side effects (assessed by the UKU side effect rating scale) when ARI BLs decreased after carbamazepine add-on therapy in patients with schizophrenia (Nakamura et al. 2009). As discussed above, this finding should be treated with care, because carbamazepine exerts psychotropic effects itself. In a cluster RCT, Hwang et al. (2015) observed that after 56 days of treatment, the sample of schizophrenia and schizoaffective disorder patients with higher ARI BLs scored lower on an akathisia scale (Hwang et al. 2015). This counterintuitive result, however, could also be interpreted as a manifestation of the positive effect of ARI on psychomotor agitation with continued therapy. Of note, all patients had BLs within the currently recommended reference range of ARI (*100–350* ng/ml). The study was rated with a moderate risk of bias (TDM score; 4/10, RoB some concerns). No systematic review or metaanalysis on the concentration/side effect-relationship is available. Overall, the available evidence on side effects caused by ARI treatment, i.e., mainly psychomotor related events such as akathisia, does not support a causal relationship with BLs. A possible relationship could, however, been obscured by rather unspecific instruments that were used to assess potential medication-related side effects. The existing studies do not allow for an evaluation (Level D; no evidence).

Dopamine receptor occupancy

Five positron emission tomography studies were identified that provide valuable insights into the association between ARI blood concentrations and striatal $D_{2/3}$ receptor occupancy (Table 2 (Hart et al. 2022)). Three out of four studies that included patients with schizophrenia additionally measured clinical effects (Mamo et al. 2007; Kegeles et al. 2008; Shin et al. 2018). Overall, a high target engagement of $D_{2/3}$ receptors (> 90%), a prerequisite for partial agonist antipsychotic efficacy (Hart et al. 2022), was reached with ARI BLs of 90 ng/ml (putamen; patients with schizophrenia) (Gründer et al. 2008), 100 ng/ml (striatum; healthy volunteers) (Kim et al. 2012), and 110 ng/ml (putamen; healthy volunteers) (Takahata et al. 2012), and 180 ng/ml for the AM (putamen; patients with schizophrenia) (Gründer et al. 2008). After fixed doses of ARI, one study reported an ED_{80} value of 6 mg (Kegeles et al. 2008). The authors found a decrease in PANSS positive subscale scores with higher target engagement ($N = 7$). Another study could not confirm this finding but reported extrapyramidal side effects (EPS) in two patients with very high BLs and a D_2 receptor occupancy > 90% (Mamo et al. 2007; Mizrahi et al. 2009). To sum up, PET studies suggest a strong relationship between target engagement and BL with ARI concentrations above 90 ng/ml resulting in clinically effective target engagement.

Population-based target concentration range

Blood level after fixed and flexible dosing

Studies were excluded in case of insufficient data reports, and one study each due to i) sole inclusion of patients with bipolar disorder, ii) sampling at peak, and iii) unusual dose regimen. Linear regression analysis of mean concentrations across 17 and 10 studies show a strong relationship between dose and ARI concentration (N= 3,778, $r = 0.85$, $P < 0.0001$, Fig. 2) and between dose and the AM concentration (N= 3,280, $r = 0.79$, $p = 0.007$, suppl. fig. S12).

Table 2 Selected dopamine receptor occupancy studies that report a relationship between ARI BL or dose and D_2 occupancy. EC_{90} estimated from EC_{50}

Reference	Design and subjects	PET tracer	Mean ARI dose (range) [mg/day]	Mean ARI BL (range) [ng/ml]	Mean receptor occupancy (%)	EC_{50} [ng/ml]	EC_{90} [ng/ml]	Comment
Kim et al. 2012	RCT; *N* = 18; healthy volunteers; mean age 23; 100% males	[^{11}C]raclopride	13 ± 12 (2–30)	Peak: 3.4 ± 0.9 per mg	$D_{2/3}$: 62 ± 21 (s)	11.1 (s)	100 (s)	Values reported for PK model; PK/PD model estimates EC_{90} of 77 ng/ml (s).
Gründer et al. 2008	CS; *N* = 16/8 (medicated/ medication-free); SCZ or SAD (DSM-4); mean age 30; 94% males	[^{18}F]fallypride	19 ± 7 (5–30)	245 ± 307	$D_{2/3}$: 83 ± 1 (p), 84 ± 1 (c)	10 ± 4 (p) 9 ± 4 (c)	90 (p), 81 (c)	Complete occupancy with ARI BL > 100–150 ng/ml. EC_{90} for AM is 180 ng/ml.
Takahata et al. 2012	CS; *N* = 11; healthy volunteers; mean age 24 ± 4; 100% males	[^{11}C]raclopride, [11C] FLB457	6	29 ± 5	$D_{2/3}$: 74 ± 7 (c), 70.1 ± 6.3 (p)	9.9 (s), 12.2 (p)	89 (s), 110 (p)	Concentration reported for raclopride scans; lower in FLB457. No preferential extrastriatal binding of ARI.
Mamo et al. 2007 ; Mizrahi et al. 2009	RCT; *N* = 12; SCZ or SAD; mean age 31; 75% males	[^{11}C]raclopride, [18F] setoperone, [^{11}C] WAY100635	19 ± 8 (10–30)	221 ± 179	$D_{2/3}$: 87 ± 4 (p), 92.9 ± 5.7 (c)	NA	NA	ARI and DARI BL correlated with D_2 occup. (p and s). No corr. between occup. And clinical or well-being scores. EPS in 2 patients with occupancy > 90%.
Kegeles et al. 2008	CS; *N* = 19; SCZ or SAD (DSM-4); mean age 29; 79% males	[^{18}F]fallypride	14 ± 11 (2–40)	NA	$D_{2/3}$: NA 80 ± 15 (s) in 15 mg	ED_{80} 5.6 ± 1.0 (s) ~ 100	NA	Dose correlated with ARI BL, PANSS positive scale correlated with D_2 occup. (s). No EPS occured.

c = caudate, CS = cohort study, EPS = extrapyramidal side effects, NA =. Not available, p = putamen, RCT = randomized controlled trial, SCZ = schizophrenia, SAD = schizoaffective disorder, s = striatum

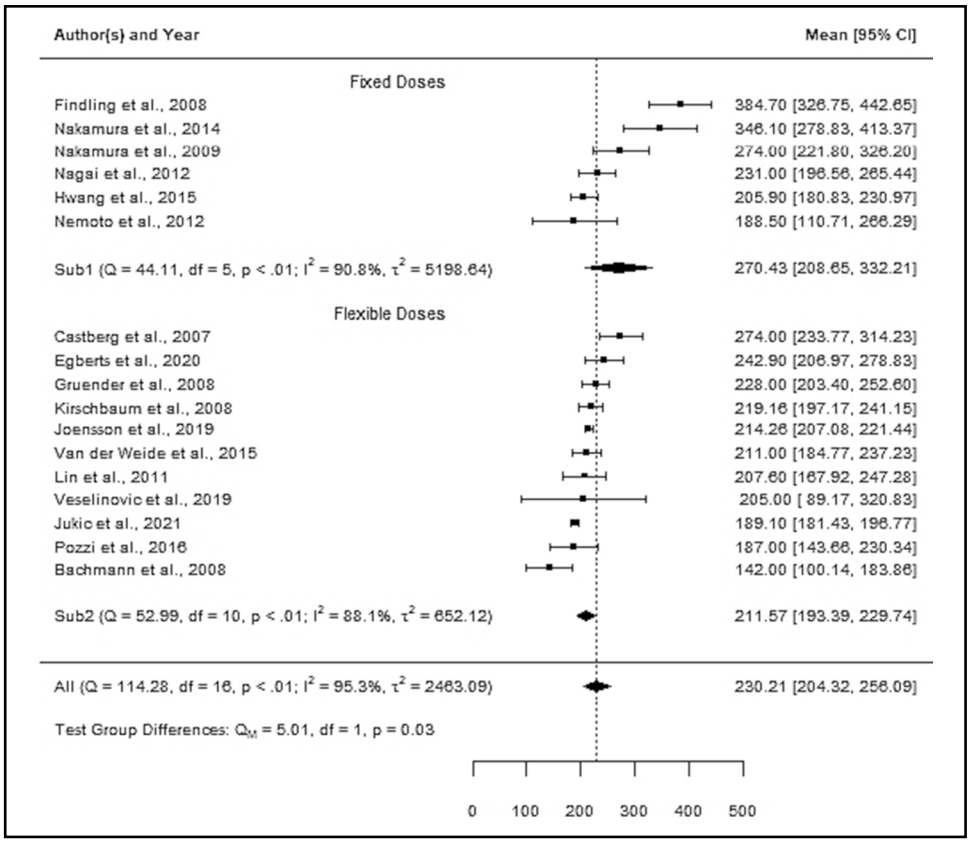


Fig. 2 Mean Aripiprazole Dose [mg/day] Versus Mean Aripiprazole blood concentration [ng/ml] (β-coefficient = 12.205 (8.007–16.403), r2 = 0.719, P < .0001, y = 25.612 + 12.205 * x) N= 3,778

The combined mean C/D ratio across seven and six studies was 13.8 (ng/ml)/(mg/day) [12.4, 15.3] ($Q = 38.1$, $df = 6$, $p \leq 0.05$, $I^2 = 88\%$, $T^2 = 2.96$) and 18.2 [16.6, 19.7] ($Q = 29.3$, $df = 5$, $p < 0.0001$, $I^2 = 84\%$, $T^2 = 2.81$) for ARI and the AM, respectively (Table 3). The combined mean concentration across 17 and nine studies was 230 ng/ml [204, 256] ($n = 3778$) and 305 [257, 353] ($N = 3332$, $Q = 84.8$, $df = 9$, $p < 0.01$, $I^2 = 98\%$, $T^2 = 5205$) for ARI and the AM, respectively (suppl. fig. S13). Mean doses were 17 and 16 mg/day. Subgroup analysis could be performed accordingly with all four predefined quality assessment criteria, since at least three studies per subgroup were available (suppl. table S14). One subgroup comparison "dose design" revealed significantly differing mean drug concentrations between both groups ($Chi^2 = 5.0$, $df = 1$, $p = 0.03$, $I^2 = 94\%$). Studies using fixed dose designs used higher doses resulting in higher drug concentrations compared to studies comprising real-world patients from psychiatric clinics (Fig. 3).

Concentration range from real-world patients

Data from 3,373 patients with schizophrenia and schizophrenia spectrum disorders that were treated with oral ARI under flexible dosing were derived from eight studies using a naturalistic design. Two preliminary ranges were computed i) a mean ± standard deviation (SD) range of *71–358* ng/ml and ii) a 25^{th}–75^{th} interquartile range of *120–273* ng/ml (Fig. 4). Two studies in children and/or adolescents discussed the comparability of the results with those obtained from adults (Bachmann et al. 2008; Egberts et al. 2020).

Table 3 Expected concentration ranges from approved doses based on our findings (C/D ratios) and based on ratios from TDM Guidelines

Administered Dose [mg/day]	Expected ARI BL [ng/ml] based on C/D ratio 13.82	Dose-related range based on TDM Guidelines 11.72	Expected ARI + D-ARI BL [ng/ml] based on C/D ratio 18.18	Dose-related range based on TDM Guidelines 16.45
5	69.1 [62.0, 76.3]	58.6 [41.8–76.5]	90.9 [83.21, 98.7]	82.3 [56.0–109.5]
10	138.2 [123.9, 152.5]	117.2 [81.5–152.9]	181.8 [166.3, 197.3]	164.5 [111.9–218.9]
20	276.4 [247.8, 305]	234.4 [163.0–305.8]	363.6 [332.6, 394.6]	329 [223.8–437.8]
30	414.6 [371.7, 457.5]	351.6 [244.5–458.7]	545.4 [498.9, 591.9]	493.5 [335.7–656.7]

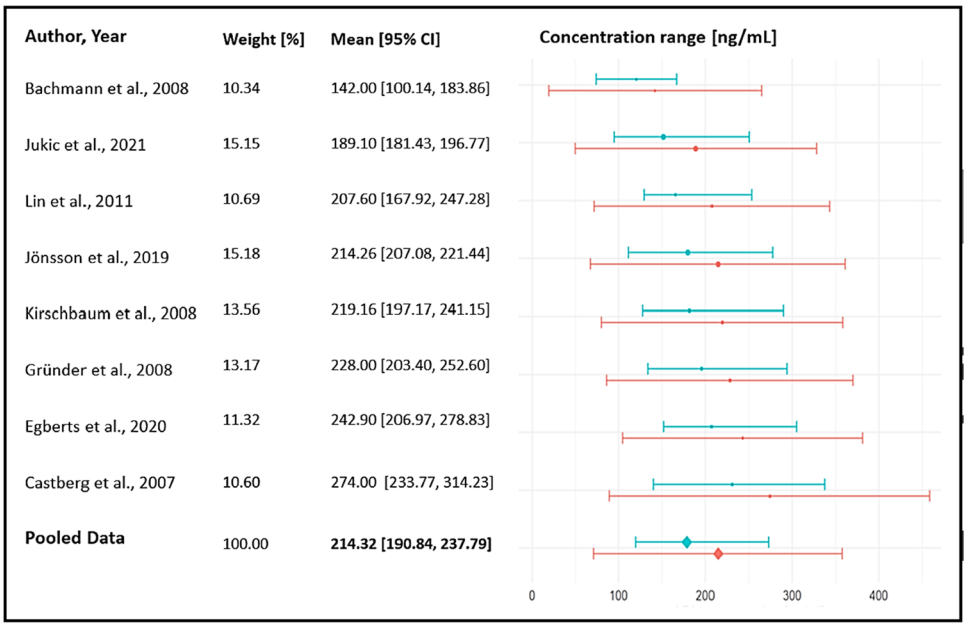


Fig. 3 Overall mean ARI concentration estimate [ng/ml] with subgroup analysis „dose design,"($N = 3,778$)

Factors influencing ARI blood levels

Sex, age, and body weight Three studies reported significantly higher BLs in females compared to males (Table 4). Linear regression analysis with correction for dose, weight, age, and comedication revealed that girls had about 41% higher BLs than boys (Egberts et al. 2020). Another study found dose-corrected BLs about 10% higher in women (Jönsson et al. 2019). One conflicting result was reported by a study that found 28% higher mean ARI concentrations corrected for defined daily doses (DDD) in men than in woman (Hoekstra et al. 2021). Five studies, including two studies that used advanced modeling techniques, did not find sex-related differences in BLs. Of eight studies that investigated age or age groups in relationship to BLs, only two studies found a weak correlation. In a large naturalistic dataset (*N = 1,610, age 8–92 years*), 16% higher dose-corrected concentrations were noted in patients older than 65 years. Most of the remaining studies did not include patients older than 65 years. Four studies consistently found no association between body weight and ARI BLs.

Concomitant Medication Most studies that were interested in the effect of comedication measured drug concentrations

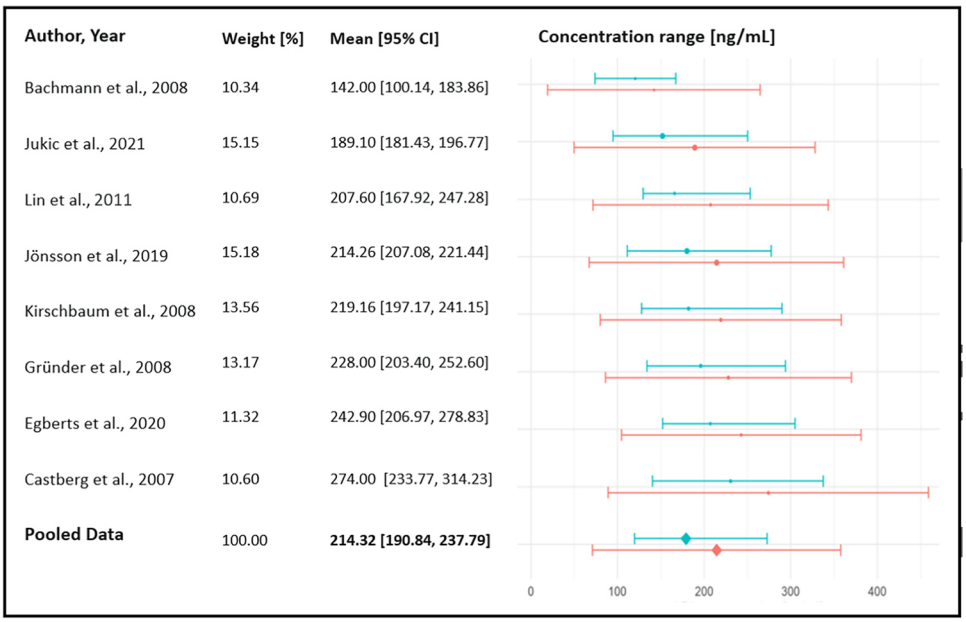


Fig. 4 Target ranges for ARI [ng/ml] (N= 3,778, Combined range mean ± SD: 71–358, combined interquartile range: 120–273, mean concentration 214 [191, 238] ($Q = 52.12$, $df = 7$, $p < .0001$, $I^2 = 93.2$, $T^2 = 932.1$))(Mean ± SD ranges of studies depicted as red lines, 25^{th}–75^{th} interquartile ranges of studies depicted as blue lines.)

Table 4 Factors influencing ARI blood levels after oral administration (Y = correlation found * < .05, ** < .001, p < 0.0001***; (Y) = trend found, not significant or only in discussion; N = no correlation or trend found; blank = not reported)

No	Reference	Dose (linear)	CYP2D6 Genotype	Sex (higher in female)	Age	Body weight	Comedication (CYP2D6 or − 3A4)	
1	Pozzi et al. 2016	**Y****			N	N	**Y****	r = 0.37 (Number)
2	Egberts et al. 2020	**Y****		**Y****	**(Y*)**	N	(Y)	
3	Kirschbaum et al. 2008	**Y****					**Y***	CYP2D6
4	Lin et al. 2011	**Y*****						
5	Molden et al. 2006	**Y*****		N			N	
6	Jönsson et al. 2019	(Y)		**Y*****	**Y***			
7	Veselinovic et al. 2019	**(Y*)**		(Y)				
8	Steen et al. 2017	**Y****						
9	Gründer et al. 2008	**Y****						
10	Van der Weide et al. 2015	**Y***	**Y***	N	N			
11	Kim et al. 2008		**Y****	N	N	N		
12	Hwang et al. 2015		**Y***					
13	Nemoto et al. 2012		**Y***				**Y***	Paroxetine
14	Nagai et al. 2012		**Y****	**Y****	N			
15	Nakamura et al. 2014		**Y***				N	Haloperidol
16	Hendset et al. 2007		**Y***					
17	Jukic et al. 2019		**Y***					
18	Nakamura et al. 2009		N				**Y****	Carbamazepine
19	Nemoto et al. 2014		N				**Y***	Paroxetine
20	Hoekstra et al. 2021			**Y***				
21	Bachmann et al. 2008			N	N	N	N	
22	Zuo et al. 2006						N	Clozapine
23	Castberg et al. 2007			N	N		(Y)	
24	Eryilmaz et al. 2014						**Y***	Valproate
25	Waade et al. 2009						**Y***	CYP2D6, CYP3A4

before and after the add-on of a pharmacokinetically relevant drug. Two studies showed an increase of ARI BLs after the administration of paroxetine (Nemoto et al. 2012, 2014) (Table 4). The mood stabilizers carbamazepine and valproate were found to decrease ARI (AM) BLs by 65% and 23%, respectively (Nakamura et al. 2009; Eryilmaz et al. 2014). No influence of escitalopram (Nemoto et al. 2014), haloperidol (Nakamura et al. 2014) or clozapine (Zuo et al. 2006) coadministration was found. Concurrent treatment with *CYP3A4* inducers, *CYP2D6* inhibitors, alimemazine, or lithium changed BLs by 40%–60%, which has to be considered clinically relevant (Waade et al. 2009). Similar effects were shown in children and adolescents (Kirschbaum et al. 2008; Pozzi et al. 2016).

CYP2D6 Genotyping Ten studies investigated whether the relationships of the genetic variants of *CYP2D6* with ARI BLs are consistent with known functions (phenotypes) (Table 4; supplemental S15 for phenotype classifications). Eight studies reported an association of *CYP2D6* phenotypes with BLs whereas two studies could not confirm these findings. One study in Asian patients reported lower ARI BL with *CYP2D6*10 (vt)* alleles (intermediate metabolizers, IM) (Hwang et al. 2015). This finding was confirmed in another study (Nemoto et al. 2012). The same group was not able to replicate this result (Nemoto et al. 2014). A Japanese study found that dose-corrected ARI and AM concentrations increased with a general increase in the number of the mutated *CYP2D6 alleles *5, *10, and *14* (Nagai et al. 2012). A Norwegian study reported 50% higher median BLs in *CYP2D6* poor metabolizers (PM) than in extensive metabolizers (EM) (Hendset et al. 2007). Two studies performed more comprehensive classifications of phenotypes with subjects classified into four groups. A Swedish study found an increase in AM concentrations by about 40% in PMs and IMs (Jukic et al. 2019). A Dutch study performed a multiple regression analysis and found dose and predicted CYP2D6 phenotype as influencing factors on ARI and D-ARI BLs ($r^2 = 0.01$) (van der Weide and van der Weide 2015). Dose-corrected concentrations were 56% higher in predicted PMs, 4% higher in IMs and 11% lower in ultrarapid metabolizers (UMs) compared to EMs. A similar result was replicated in

a study that has used pharmacokinetic modeling methods to explain interindividual variance in BLs (Kim et al. 2008). *CYP2D6* genotype, but not sex, age or bodyweight, remained a significant covariate in the final model. 1.5–1.7-fold higher BLs in PMs and IMs were also found in patients after LAI treatment (Tveito et al. 2020).

TDM for long-acting injectable (LAI) aripiprazole

Aripiprazole lauroxil (AL) Three randomized studies assessed pharmacokinetic profiles after single injections of AL and two studies applied multiple injections. As described previously, higher peak plasma concentrations were found following administration to the deltoid site when compared with the gluteal site (Hard et al. 2019; Schoretsanitis et al. 2021). All patients were stabilized on oral antipsychotic treatment; clinical ratings remained stable. After five gluteal injections of 441 (q4wk), 882 (q6wk), or 1064 (q8wk) mg, patients showed quite similar average ARI concentrations (*126–141* ng/ml). Maximum concentrations were below 200 ng/ml for all dosages (Hard et al. 2017). Before reaching steady state, after 12 weeks, the median BLs only exceeded the 120 ng/ml threshold at the high dosages of 662 and 882 mg (q4wk), not at the 441 mg dosage nor at longer application periods (Hard et al. 2018). However, over the time course of a year, simulated median BLs in all dosage regimens would hit the threshold.

Aripiprazole monohydrate (AM) Three studies (two RCTs, one observational study) report ARI BLs after multiple injections of AM 200, 300, or 400 mg (q4wk) for up to one year. In patients with schizophrenia, clinical scale scores remained stable under oral antipsychotic treatment. After five injections of 400 mg, 300 mg, and 200 mg, trough BLs were 212 ± 113/ 239 ± 133 ng/ml, 156 ± 68 ng/ml, and 95 ± 86 ng/ml (Mallikaarjun et al. 2013; Raoufinia et al. 2017). Lower BLs were found in patients with bipolar disorder after doses of 300 and 400 mg (113–132 ng/ml) (Mauri et al. 2020). The authors discussed a limit below *150 ng/ml* as therapeutic threshold for depressive and positive symptoms. In conclusion, monthly injections (e.g., five or more) of 300 mg and more will most likely result in BL above *120 ng/ml*.

Discussion

Aripiprazole has been proven effective for the treatment of schizophrenia (Leucht et al. 2012). However, our qualitative synthesis revealed a low quality of evidence for an association between drug blood concentration and efficacy. We identified various reasons why trials were not able to find a relationship between drug concentrations and antipsychotic treatment efficacy (i.e., psychiatric comedication and flexible dose design). Only one study was able to find a clear relationship between increasing AM concentrations and antipsychotic response (PANSS scores) in patients with schizophrenia or schizoaffective disorders (Lin et al. 2011). Controlled randomized studies that aimed at finding a concentration/ efficacy-relationship for ARI are almost missing. The few controlled studies that are available are of moderate to high risk for bias.

In agreement with previous reports (Citrome 2006), the present work also shows that there is no evidence for concentration-dependent side effects. There is some evidence to suggest a link between BLs and neurological side effects, particularly akathisia. However, the available clinical instruments (e.g., Barnes Akathisia Rating Scale, BARS) do not appear to be sensitive enough to distinguish between positive treatment effects (reduction in psychomotor agitation) and reduction in true akathisia (Hwang et al. 2015). The low incidence of EPS and other side effects despite high striatal D_2 receptor occupancy in PET studies is fully consistent with the mechanism of action of ARI (Grunder et al. 2003). Even with 100% receptor occupancy, the postsynaptic signal will be sufficient to limit neurological side effects in most patients (Mizrahi et al. 2009). When reports on clinical efficacy are rare, a point of futility, meaning a concentration threshold above which a further increase in clinical efficacy cannot be expected, has been suggested as upper orienting limit for a therapeutic reference range (Meyer and Stahl 2021). To date, a clear cutoff for the onset of therapeutic response or side effects has not been shown for ARI. The present work demonstrates how population-based ranges can be used to supplement clinical efficacy data in a meaningful manner and how to identify a therapeutic reference range for a psychotropic drug from manifold types of studies, despite a low grade of first level evidence.

Therapeutic reference range for aripiprazole Fifty percent of patients with schizophrenia and related disorders treated under effective doses present ARI concentrations between *120* and *273* ng/ml, which is quite consistent with previously reported ranges from responders in single studies (*134–271* ng/ml based upon PANSS scores (Lin et al. 2011) and *124–286* ng/ml based upon CGI assessments (Kirschbaum et al. 2008)). In support, PET studies demonstrate consistently that therapeutically effective target engagement can be already reached with BLs around *90–110* ng/ml (*180* ng/ml for the AM) (Hart et al. 2022). The "average" patient will attain the efficacy threshold of 120 ng/ml with a dose of 9 mg once daily. The upper limit of *270* ng/ml will be reached with a dose of 20 mg/day (Table 3). For LAI formulations, AM and AL, doses of at least 300 mg and 463 mg are expected to lead to BLs within the proposed range.

Moderating factors and implications for TDM As a prerequisite for dose titration, the present work confirms a linear dose/concentrationrelationship for ARI within the common dosing range of 5–30 mg daily. The steady-state concentration of the major active metabolite, D-ARI, represents about 40% of the parent drug (metabolite-to-parent compound ratio (MPR); *0.40 = (304.6–218.1)/ 218.1 ng/ml*; suppl. fig. S13). Current guidelines report dose-corrected concentration values of *11.7* and *16.5 (ng/mg)/(mg/day)* for ARI and the AM, respectively. We found somewhat higher mean C/D ratios of *13.8* and *18.2*, respectively. The findings of higher dose-corrected concentrations in our study might be explained by a higher percentage of female patients, a higher mean age, and the permission for using potentially CYP-inhibiting comedication in the included studies compared to, e.g., phase-I studies. Future research is needed to evaluate sex- and age-specific dosing. Body weight is frequently discussed in studies to explain BL differences between Asian and European study populations. However, while CYP expression patterns are certainly different among Asian and European populations, no study has systematically explored ethnic differences in ARI's metabolism. Also, it is not clear yet, whether a different proportion of the AM relative to the parent compound leads to a change in pharmacodynamics of the drug. More eminent, higher mean BLs have consistently found in *CYP2D6* poor metabolizers. The evidence across the genetic variants of CYP2D6 is striking and calls for a dose adaption of at least 50%, which is currently not taken into account in relevant guidelines (recommended starting dose 10 mg/day for PMs) (Swen et al. 2011). Regarding clinical TDM practice, the evidence suggests that small differences in sampling time points of a few hours (i.e., 9–14 h vs. 20–24 h) may only marginally change the expected ARI blood concentration (Korell et al. 2018). An efficacy of lower doses in maintenance treatment compared to acute therapy has been discussed by dose/efficacy-metaanalysis for antipsychotic drugs (Uchida et al. 2011; Leucht et al. 2021). In the present work, studies have been included irrespective of former treatment duration. It remains unclear, if this may affect the clinical transferability of the suggested reference range.

Conclusion

We suggest a therapeutic reference range of *120–270 ng/ml* and *180–380 ng/ml*, respectively, for ARI and its AM for the treatment of schizophrenia and related disorders. Based on the available data, the evidence for a concentration/effect-relationship is low, which results in limited implications for dose titration within the presented reference range. However, concentrations above the lower limit of the therapeutic reference range seem likely to increase treatment response. Concentrations above the upper limit are unlikely to further improve treatment response, but the incidence of adverse events seems equally unlikely to increase. A starting dose of 10 mg/day will result in effective concentrations in blood and brain of most patients. High interindividual variability and the influence of *CYP2D6* genotypes represents a special indication for TDM of oral and long-acting ARI. A starting dose of 5 mg/day might be sufficient in known *CYP2D6* PM.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00213-022-06233-2.

Author contributions XH developed the first draft of the protocol. CH and GG supervised the entire manuscript writing and contributed to the revision of the protocol. XL, JG, LE, and TR have contributed to the development of the search strategy and quality assessment. XH, CH, GG, HWC, AC, FF, VF, UHR, MP, EM, TR, and GS confirmed grading of the level of revealed evidence. All authors have read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability statement Generated Statement: The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author/s.

Declarations

Conflicts of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. GG has served as a consultant for Allergan, Boehringer Ingelheim, Institute for Quality and Efficiency in Health Care (IQWiG), Janssen-Cilag, Lundbeck, Otsuka, Recordati, Roche, ROVI, Sage, and Takeda. He has served on the speakers' bureau of Gedeon Richter, Janssen Cilag, Lundbeck, Otsuka, and Recordati. He has received grant support from Boehringer Ingelheim, Lundbeck, and Saladax. He is co-founder and/or shareholder of Mind and Brain Institute GmbH, Brainfoods GmbH, OVID Health Systems GmbH and MIND Foundation gGmbH. CH has served on the speakers' bureau of Otsuka. GS has served as a consultant and has received speaker fees from HLS Therapeutics. MP has received speaker's fees from Janssen, ROVI, Neuraxpharm, Lundbeck, and Otsuka. He has served as a consultant for Novartis, Otsuka, and ROVI. MP is an editor of an internet-based drug–drug interaction program (www.psiac.de).

References

Bachmann CJ, Rieger-Gies A, Heinzel-Gutenbrunner M, Hiemke C, Remschmidt H, Theisen FM (2008) Large variability of aripiprazole and dehydroaripiprazole serum concentrations in adolescent patients with schizophrenia. Ther Drug Monit 30(4):462–466. https://doi.org/10.1097/FTD.0b013e318178e18d

Citrome L (2006) A review of aripiprazole in the treatment of patients with schizophrenia or bipolar I disorder. Neuropsychiatr Dis Treat 2(4):427–443. https://doi.org/10.2147/nedt.2006.2.4.427

Egberts K, Reuter-Dang SY, Fekete S, Kulpok C, Mehler-Wex C, Wewetzer C, Karwautz A, Mitterer M, Holtkamp K, Boege I, Burger R, Romanos M, Gerlach M, Taurines R (2020) Therapeutic drug monitoring of children and adolescents treated with aripiprazole: observational results from routine patient care. J Neural Transm (Vienna) 127(12):1663–1674. https://doi.org/10.1007/s00702-020-02253-4

Eryilmaz G, HizliSayar G, Özten E, Gül IG, Karamustafalioğlu O, Yorbik Ö (2014) Effect of valproate on the plasma concentrations of aripiprazole in bipolar patients. Int J Psychiatry Clin Pract 18(4):288–292. https://doi.org/10.3109/13651501.2014.941879

Grunder G, Carlsson A, Wong DF (2003) Mechanism of new antipsychotic medications: occupancy is not just antagonism. Arch Gen Psychiatry 60(10):974–977. https://doi.org/10.1001/archpsyc.60.10.974

Gründer G, Fellows C, Janouschek H, Veselinovic T, Boy C, Bröcheler A, Kirschbaum KM, Hellmann S, Spreckelmeyer KM, Hiemke C, Rösch F, Schaefer WM, Vernaleken I (2008) Brain and plasma pharmacokinetics of aripiprazole in patients with schizophrenia: an [18F]fallypride PET study. Am J Psychiatry 165(8):988–995. https://doi.org/10.1176/appi.ajp.2008.07101574

Gründer G, Kungel M, Ebrecht M, Göröcs T, Modell S (2006) Aripiprazole: pharmacodynamics of a dopamine partial agonist for the treatment of schizophrenia. Pharmacopsychiatry 39(1):21–25. https://doi.org/10.1055/s-2006-931485

Hard ML, Mills RJ, Sadler BM, Wehr AY, Weiden PJ, von Moltke L (2017) Pharmacokinetic Profile of a 2-Month Dose Regimen of Aripiprazole Lauroxil: A Phase I Study and a Population Pharmacokinetic Model. CNS Drugs 31(7):617–624. https://doi.org/10.1007/s40263-017-0447-7

Hard ML, Wehr A, von Moltke L, Du Y, Farwick S, Walling DP, Sonnenberg J (2019) Pharmacokinetics and safety of deltoid or gluteal injection of aripiprazole lauroxil NanoCrystalÂ® Dispersion used for initiation of the long-acting antipsychotic aripiprazole lauroxil. Ther Adv Psychopharmacol 9:2045125319859964. https://doi.org/10.1177/2045125319859964

Hard ML, Wehr AY, Sadler BM, Mills RJ, von Moltke L (2018) Population Pharmacokinetic Analysis and Model-Based Simulations of Aripiprazole for a 1-Day Initiation Regimen for the Long-Acting Antipsychotic Aripiprazole Lauroxil. Eur J Drug Metab Pharmacokinet 43(4):461–469. https://doi.org/10.1007/s13318-018-0488-4

Hart XM, Eichentopf L, Lense X, Riemer T, Wesner K, Hiemke C, Gründer G (2021) Therapeutic Reference Ranges for Psychotropic Drugs: A Protocol for Systematic Reviews. Front Psych 12(2071):787043. https://doi.org/10.3389/fpsyt.2021.787043

Hart XM, Schmitz CN, Gründer G (2022) Molecular Imaging of Dopamine Partial Agonists in Humans: Implications for Clinical Practice. Front Psych 13:832209. https://doi.org/10.3389/fpsyt.2022.832209

Hasan A, Bandelow B, Yatham LN, Berk M, Falkai P, Moller HJ, Kasper S (2019) WFSBP guidelines on how to grade treatment evidence for clinical guideline development. World J Biol Psychiatry 20(1):2–16. https://doi.org/10.1080/15622975.2018.1557346

Hendset M, Hermann M, Lunde H, Refsum H, Molden E (2007) Impact of the CYP2D6 genotype on steady-state serum concentrations of aripiprazole and dehydroaripiprazole. Eur J Clin Pharmacol 63(12):1147–1151. https://doi.org/10.1007/s00228-007-0373-6

Hiemke C (2019) Concentration-Effect Relationships of Psychoactive Drugs and the Problem to Calculate Therapeutic Reference Ranges. Ther Drug Monit 41(2):174–179. https://doi.org/10.1097/ftd.0000000000000582

Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K, Eckermann G, Egberts K, Gerlach M, Greiner C, Grunder G, Haen E, Havemann-Reinecke U, Hefner G, Helmer R, Janssen G, Jaquenoud E, Laux G, Messer T, Mossner R, Muller MJ, Paulzen M, Pfuhlmann B, Riederer P, Saria A, Schoppek B, Schoretsanitis G, Schwarz M, Gracia MS, Stegmann B, Steimer W, Stingl JC, Uhr M, Ulrich S, Unterecker S, Waschgler R, Zernig G, Zurek G, Baumann P (2018) Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry 51(1–02):9–62. https://doi.org/10.1055/s-0043-116492

Hoekstra S, Bartz-Johannessen C, Sinkeviciute I, Reitan SK, Kroken RA, Løberg EM, Larsen TK, Rettenbacher M, Johnsen E, Sommer IE (2021) Sex differences in antipsychotic efficacy and side effects in schizophrenia spectrum disorder: results from the BeSt InTro study. NPJ Schizophr 7(1):39. https://doi.org/10.1038/s41537-021-00170-3

Huhn M, Nikolakopoulou A, Schneider-Thoma J, Krause M, Samara M, Peter N, Arndt T, Bäckers L, Rothe P, Cipriani A, Davis J, Salanti G, Leucht S (2019) Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. Lancet 394(10202):939–951. https://doi.org/10.1016/s0140-6736(19)31135-3

Hwang TJ, Lo WM, Chan HY, Lin CF, Hsieh MH, Liu CC, Liu CM, Hwu HG, Kuo CH, Chen WJ (2015) Fast Versus Slow Strategy of Switching Patients With Schizophrenia to Aripiprazole From Other Antipsychotics. J Clin Psychopharmacol 35(6):635–644. https://doi.org/10.1097/JCP.0000000000000426

Jönsson AK, Spigset O, Reis M (2019) A Compilation of Serum Concentrations of 12 Antipsychotic Drugs in a Therapeutic Drug Monitoring Setting. Ther Drug Monit 41(3):348–356. https://doi.org/10.1097/ftd.0000000000000585

Jukic MM, Smith RL, Haslemo T, Molden E, Ingelman-Sundberg M (2019) Effect of CYP2D6 genotype on exposure and efficacy of risperidone and aripiprazole: a retrospective, cohort study. Lancet Psychiatry 6(5):418–426. https://doi.org/10.1016/s2215-0366(19)30088-4

Kegeles LS, Slifstein M, Frankle WG, Xu X, Hackett E, Bae S-A, Gonzales R, Kim J-H, Alvarez B, Gil R, Laruelle M, Abi-Dargham A (2008) Dose–occupancy study of striatal and extrastriatal dopamine D_2 receptors by aripiprazole in schizophrenia with PET and [^{18}F]fallypride. Neuropsychopharmacol 33(13):3111–3125. https://doi.org/10.1038/npp.2008.33

Kim E, Howes OD, Kim BH, Jeong JM, Lee JS, Jang IJ, Shin SG, Turkheimer FE, Kapur S, Kwon JS (2012) Predicting brain occupancy from plasma levels using PET: superiority of combining pharmacokinetics with pharmacodynamics while modeling the relationship. J Cereb Blood Flow Metab 32(4):759–768. https://doi.org/10.1038/jcbfm.2011.180

Kim JR, Seo HB, Cho JY, Kang DH, Kim YK, Bahk WM, Yu KS, Shin SG, Kwon JS, Jang IJ (2008) Population pharmacokinetic modelling of aripiprazole and its active metabolite, dehydroaripiprazole, in psychiatric patients. Br J Clin Pharmacol 66(6):802–810. https://doi.org/10.1111/j.1365-2125.2008.03223.x

Kirschbaum KM, Müller MJ, Malevani J, Mobascher A, Burchardt C, Piel M, Hiemke C (2008) Serum levels of aripiprazole and dehydroaripiprazole, clinical response and side effects. World J Biol Psychiatry 9(3):212–218. https://doi.org/10.1080/15622970701361255

Korell J, Green B, Rae A, Remmerie B, Vermeulen A (2018) Determination of plasma concentration reference ranges for oral

aripiprazole, olanzapine, and quetiapine. Eur J Clin Pharmacol 74(5):593–599. https://doi.org/10.1007/s00228-018-2419-3

Leucht S, Bauer S, Siafis S, Hamza T, Wu H, Schneider-Thoma J et al (2021) Examination of Dosing of Antipsychotic Drugs for Relapse Prevention in Patients With Stable Schizophrenia: A Meta-analysis. JAMA Psychiatry 78(11):1238–48. https://doi.org/10.1001/jamapsychiatry.2021.2130

Leucht S, Crippa A, Siafis S, Patel MX, Orsini N, Davis JM (2020) Dose-Response Meta-Analysis of Antipsychotic Drugs for Acute Schizophrenia. Am J Psychiatry 177(4):342–353. https://doi.org/10.1176/appi.ajp.2019.19010034

Leucht S, Tardy M, Komossa K, Heres S, Kissling W, Salanti G, Davis JM (2012) Antipsychotic drugs versus placebo for relapse prevention in schizophrenia: a systematic review and meta-analysis. Lancet 379(9831):2063–2071. https://doi.org/10.1016/s0140-6736(12)60239-6

Lin SK, Chen CK, Liu YL (2011) Aripiprazole and dehydroaripiprazole plasma concentrations and clinical responses in patients with schizophrenia. J Clin Psychopharmacol 31(6):758–762. https://doi.org/10.1097/JCP.0b013e3182356255

Lopez LV, Kane JM (2013) Plasma levels of second-generation antipsychotics and clinical response in acute psychosis: a review of the literature. Schizophr Res 147(2–3):368–374. https://doi.org/10.1016/j.schres.2013.04.002

Mallikaarjun S, Kane JM, Bricmont P, McQuade R, Carson W, Sanchez R, Forbes RA, Fleischhacker WW (2013) Pharmacokinetics, tolerability and safety of aripiprazole once-monthly in adult schizophrenia: an open-label, parallel-arm, multiple-dose study. Schizophr Res 150(1):281–288. https://doi.org/10.1016/j.schres.2013.06.041

Mamo D, Graff A, Mizrahi R, Shammi CM, Romeyer F, Kapur S (2007) Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. Am J Psychiatry 164(9):1411–1417. https://doi.org/10.1176/appi.ajp.2007.06091479

Mauri MC, Paletta S, Di Pace C, Reggiori A, Cirnigliaro G, Valli I, Altamura AC (2018) Clinical Pharmacokinetics of Atypical Antipsychotics: An Update. Clin Pharmacokinet 57(12):1493–1528. https://doi.org/10.1007/s40262-018-0664-3

Mauri MC, Reggiori A, Minutillo A, Franco G, Pace CD, Paletta S, Cattaneo D (2020) Paliperidone LAI and Aripiprazole LAI Plasma Level Monitoring in the Prophylaxis of Bipolar Disorder Type I with Manic Predominance. Pharmacopsychiatry 53(5):209–219. https://doi.org/10.1055/a-1113-7862

Meyer JM, Stahl SM (2021) The Clinical Use of Antipsychotic Plasma Levels: Stahl's Handbooks. Cambridge University Press, Cambridge

Mizrahi R, Mamo D, Rusjan P, Graff A, Houle S, Kapur S (2009) The relationship between subjective well-being and dopamine D2 receptors in patients treated with a dopamine partial agonist and full antagonist antipsychotics. Int J Neuropsychopharmacol 12(5):715–721. https://doi.org/10.1017/s1461145709000327

Nagai G, Mihara K, Nakamura A, Suzuki T, Nemoto K, Kagawa S, Ohta I, Arakaki H, Kondo T (2012) Prolactin concentrations during aripiprazole treatment in relation to sex, plasma drugs concentrations and genetic polymorphisms of dopamine D2 receptor and cytochrome P450 2D6 in Japanese patients with schizophrenia. Psychiatry Clin Neurosci 66(6):518–524. https://doi.org/10.1111/j.1440-1819.2012.02391.x

Nakamura A, Mihara K, Nagai G, Suzuki T, Kondo T (2009) Pharmacokinetic and pharmacodynamic interactions between carbamazepine and aripiprazole in patients with schizophrenia. Ther Drug Monit 31(5):575–578. https://doi.org/10.1097/FTD.0b013e3181b6326a

Nakamura A, Mihara K, Nemoto K, Nagai G, Kagawa S, Suzuki T, Kondo T (2014) Lack of correlation between the steady-state plasma concentrations of aripiprazole and haloperidol in Japanese patients with schizophrenia. Ther Drug Monit 36(6):815–818. https://doi.org/10.1097/ftd.0000000000000082

Nemoto K, Mihara K, Nakamura A, Nagai G, Kagawa S, Suzuki T, Kondo T (2012) Effects of paroxetine on plasma concentrations of aripiprazole and its active metabolite, dehydroaripiprazole, in Japanese patients with schizophrenia. Ther drug Monit 34(2):188–192. https://doi.org/10.1097/FTD.0b013e31824a31e6

Nemoto K, Mihara K, Nakamura A, Nagai G, Kagawa S, Suzuki T, Kondo T (2014) Effects of escitalopram on plasma concentrations of aripiprazole and its active metabolite, dehydroaripiprazole, in Japanese patients. Pharmacopsychiatry 47(3):101–104. https://doi.org/10.1055/s-0034-1372644

Otsuka Pharmaceutical Co. L (2016) "Aripiprazole: highlights of prescribing information." https://www.otsukaus.com/sites/g/files/qhldwo5616/files/media/static/Abilify-PI.pdf. Accessed 14 Sep 2022

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Bmj 372:n71. https://doi.org/10.1136/bmj.n71

Pozzi M, Cattaneo D, Baldelli S, Fucile S, Capuano A, Bravaccio C, Sportiello L, Bertella S, Auricchio F, Bernardini R, Ferrajolo C, Guastella G, Mani E, Carnovale C, Pisano S, Rafaniello C, Riccio MP, Rizzo R, Scuderi MG, Sperandeo S, Villa L, Pascotto A, Molteni M, Rossi F, Radice S, Clementi E (2016) Therapeutic drug monitoring of second-generation antipsychotics in pediatric patients: an observational study in real-life settings. Eur J Clin Pharmacol 72(3):285–293. https://doi.org/10.1007/s00228-015-1982-0

Preskorn SH (2013) Outliers on the dose-response curve: if the problem is not concentration, then what? J Psychiatr Pract 19(6):490–494. https://doi.org/10.1097/01.pra.0000438188.66735.68

Raoufinia A, Peters-Strickland T, Nylander A-G, Baker RA, Eramo A, Jin N, Bricmont P, Repella J, McQuade RD, Hertel P, Larsen F (2017) Aripiprazole once-monthly 400 mg: Comparison of pharmacokinetics, tolerability, and safety of deltoid versus gluteal administration. Int J Neuropsychopharmacol 20(4):295–304. https://doi.org/10.1093/ijnp/pyw116

Schoretsanitis G, Baumann P, Conca A, Dietmaier O, Giupponi G, Gründer G, Hahn M, Hart X, Havemann-Reinecke U, Hefner G, Kuzin M, Mössner R, Piacentino D, Steimer W, Zernig G, Hiemke C (2021) Therapeutic Drug Monitoring of Long-Acting Injectable Antipsychotic Drugs. Ther Drug Monit 43(1):79–102. https://doi.org/10.1097/ftd.0000000000000830

Shin S, Kim S, Seo S, Lee JS, Howes OD, Kim E, Kwon JS (2018) The relationship between dopamine receptor blockade and cognitive performance in schizophrenia: a [(11)C]-raclopride PET study with aripiprazole. Transl Psychiatry 8(1):87. https://doi.org/10.1038/s41398-018-0134-6

Sparshatt A, Taylor D, Patel MX, Kapur S (2010) A systematic review of aripiprazole–dose, plasma concentration, receptor occupancy, and response: implications for therapeutic drug monitoring. J Clin Psychiatry 71(11):1447–1456. https://doi.org/10.4088/JCP.09r05060gre

Steen NE, Aas M, Simonsen C, Dieset I, Tesli M, Nerhus M, Gardsjord E, Mørch R, Agartz I, Melle I, Ueland T, Spigset O, Andreassen OA (2017) Serum levels of second-generation antipsychotics are associated with cognitive function in psychotic disorders. World J Biol Psychiatry 18(6):471–482. https://doi.org/10.1080/15622975.2016.1245441

Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni

P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. Bmj 366:l4898. https://doi.org/10.1136/bmj.l4898

Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, Rongen GA, van Schaik RH, Schalekamp T, Touw DJ, van der Weide J, Wilffert B, Deneer VH, Guchelaar HJ (2011) Pharmacogenetics: from bench to byte–an update of guidelines. Clin Pharmacol Ther 89(5):662–673. https://doi.org/10.1038/clpt.2011.34

Takahata K, Ito H, Takano H, Arakawa R, Fujiwara H, Kimura Y, Kodaka F, Sasaki T, Nogami T, Suzuki M et al (2012) Striatal and extrastriatal dopamine D_2 receptor occupancy by the partial agonist antipsychotic drug aripiprazole in the human brain: a positron emission tomography study with [^{11}C]raclopride and [^{11}C]FLB457. Psychopharmacol 222(1):165–172. https://doi.org/10.1007/s00213-011-2633-5

Tveito M, Molden E, Høiseth G, Correll CU, Smith RL (2020) Impact of age and CYP2D6 genetics on exposure of aripiprazole and dehydroaripiprazole in patients using long-acting injectable versus oral formulation: relevance of poor and intermediate metabolizer status. Eur J Clin Pharmacol 76(1):41–49. https://doi.org/10.1007/s00228-019-02768-0

Uchida H, Suzuki T, Takeuchi H, Arenovich T, Mamo DC (2011) Low dose vs standard dose of antipsychotics for relapse prevention in schizophrenia: meta-analysis. Schizophr Bull 37(4):788–99. https://doi.org/10.1093/schbul/sbp149

van der Weide K, van der Weide J (2015) The Influence of the CYP3A4*22 Polymorphism and CYP2D6 Polymorphisms on Serum Concentrations of Aripiprazole, Haloperidol, Pimozide, and Risperidone in Psychiatric Patients. J Clin Psychopharmacol 35(3):228–236. https://doi.org/10.1097/jcp.0000000000000319

Veselinović T, Scharpenberg M, Heinze M, Cordes J, Mühlbauer B, Juckel G, Rüther E, Paulzen M, Haen E, Hiemke C et al (2019) Dopamine D2 Receptor Occupancy Estimated From Plasma Concentrations of Four Different Antipsychotics and the Subjective Experience of Physical and Mental Well-Being in Schizophrenia: results From the Randomized NeSSy Trial. J Clin Psychopharmacol 39(6):550–560. https://doi.org/10.1097/JCP.0000000000001131

Waade RB, Christensen H, Rudberg I, Refsum H, Hermann M (2009) Influence of comedication on serum concentrations of aripiprazole and dehydroaripiprazole. Ther Drug Monit 31(2):233–238. https://doi.org/10.1097/FTD.0b013e3181956726

Zuo XC, Liu SK, Yi ZY, Xie ZH, Li HD (2006) Steady-state pharmacokinetic properties of aripiprazole 10 mg PO g12h in Han Chinese adults with schizophrenia: A prospective, open-label, pilot study. Curr Ther Res Clin Exp 67(4):258–269. https://doi.org/10.1016/j.curtheres.2006.08.003

Authors and Affiliations

Xenia M. Hart[1,13] · Christoph Hiemke[2,13] · Luzie Eichentopf[1] · Xenija M. Lense[1] · Hans Willi Clement[3,13] · Andreas Conca[4,13] · Frank Faltraco[5,13] · Vincenzo Florio[4] · Jessica Grüner[3] · Ursula Havemann-Reinecke[6,13] · Espen Molden[7] · Michael Paulzen[8,13] · Georgios Schoretsanitis[9,10,11,13] · Thomas G. Riemer[12] · Gerhard Gründer[1,13]

1 Department of Molecular Neuroimaging, Medical Faculty Mannheim, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

2 Department of Psychiatry and Psychotherapy and Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center of Mainz, Mainz, Germany

3 Department of Child and Adolescent Psychiatry, University of Freiburg, Freiburg, Germany

4 Sanitario Di Bolzano, Servizio Psichiatrico del Comprensorio, Bolzano, Italy

5 Department of Psychiatry and Psychotherapy, University of Rostock, Rostock, Germany

6 Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Göttingen, Germany

7 Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway

8 Department of Psychiatry, Psychotherapy and Psychosomatics, Alexianer Hospital Aachen, Aachen, Germany

9 Department of Psychiatry, Psychotherapy and Psychosomatics, University of Zurich, Psychiatric University Hospital Zurich, Zurich, Switzerland

10 Department of Psychiatry, The Zucker Hillside Hospital, Northwell Health, New York, NY, USA

11 Zucker School of Medicine at Northwell/Hofstra, Department of Psychiatry, Hempstead, NY, USA

12 Institute of Clinical Pharmacology and Toxicology, Berlin Institute of Health, Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität Zu Berlin, Berlin, Germany

13 Arbeitsgemeinschaft Für Neuropsychopharmakologie Und Pharmakopsychiatrie (AGNP), Work group Therapeutic Drug Monitoring, München, Germany

5. ACCEPTED MANUSCRIPT “CONCENTRATIONS OF ESCITALOPRAM IN BLOOD OF PATIENTS TREATED IN A NATURALISTIC SETTING: FOCUS ON PATIENTS WITH ALCOHOL AND BENZODIAZEPINE USE DISORDER”

European Archives of Psychiatry and Clinical Neuroscience
https://doi.org/10.1007/s00406-022-01491-9

ORIGINAL PAPER

Concentrations of escitalopram in blood of patients treated in a naturalistic setting: focus on patients with alcohol and benzodiazepine use disorder

X. M. Hart[1] · S. Heesen[1] · C. N. Schmitz[1] · S. Dörfler[2] · D. Wedekind[2] · G. Gründer[1] · C. Hiemke[3] · U. Havemann-Reinecke[2]

Received: 17 March 2022 / Accepted: 13 September 2022

Abstract

The selective serotonin reuptake inhibitor escitalopram (ESC) is indicated for the treatment of major depressive disorder (MDD) and of generalized anxiety disorder (GAD). Monitoring of blood levels (BLs) is strongly indicated due to ESC's high interindividual pharmacokinetic variability. The aim of this study was to analyse clinical efficacy and pharmacokinetic influences on ESC BLs, in patients with depressive disorder alone and with comorbid alcohol or benzodiazepine use disorder. Data were collected from patients treated under naturalistic conditions for whom Therapeutic Drug Monitoring (TDM) was requested to guide antidepressant drug therapy and analysed retrospectively. Particular emphasis was given to patients with alcohol or benzodiazepine use disorder. Responders according to the clinical global impression (CGI) scale were compared with nonresponders for their ESC blood level (BL). The patient sample included 344 patients from 16 psychiatric hospitals in Germany. Influencing factors that could explain 22% of ESC BLs were dose, sex and age. Variability was high between individuals, and doses up to 40 mg were common in real-world settings. Patients treated with ESC monotherapy who responded showed a trend towards higher BLs compared to nonresponders with a concentration of 15 ng/mL separating both groups. Pathological changes in liver function (alcoholic liver disease indicated by elevated GGT in combination with an AST/ALT ratio ≥ 1) resulted in higher dose-corrected ESC concentrations. Influencing factors that could explain 22% of ESC blood levels were dose, sex, age and liver function. Our findings confirm the currently recommended lower threshold level and support the need for standard TDM analyses in everyday clinical practice. The ICD 10 diagnosis alcohol dependence alone does not lead to pharmacokinetic changes in the metabolism of ESC, but altered liver function does.

Keywords SSRI · Escitalopram · Depressive disorder · Depression · Pharmacokinetics · Alcohol use disorder · Benzodiazepine use disorder

Background

Prescription rates of citalopram's racemic S-isomer escitalopram (ESC) has been forged ahead in the past years [1]. ESC has become a popular alternative to its precursor citalopram owed to ESC's convincingly proven antidepressant effect and tolerability profile. The selective serotonin reuptake inhibitor (SSRI) is indicated for the treatment of major depressive disorder (MDD) and of generalized anxiety disorder (GAD). It is also approved for the treatment of obsessive compulsive disorder (OCD) in the EU, but not in the USA. The approved ESC doses range from 10 to 20 mg per day. Under naturalistic conditions, however, up to 40 mg/day are common accounting for a high interindividual pharmacokinetic variability [2–5]. Despite the manufacturer's

C. Hiemke and U. Havemann-Reinecke contributed equally to this manuscript.

✉ X. M. Hart
xenia.hart@zi-mannheim.de

[1] Department of Molecular Neuroimaging, Medical Faculty Mannheim, Central Institute of Mental Health, Heidelberg University, J5, 68159 Mannheim, Germany

[2] Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, University of Göttingen, Göttingen, Germany

[3] Institute of Clinical Chemistry and Laboratory Medicine, Department of Psychiatry and Psychotherapy, University Medical Center of Mainz, Mainz, Germany

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

notifications about the influence of population-dependent influences such as older age and hepatic dysfunction on ESC pharmacokinetics [6], data from naturalistic patient populations is surprisingly rare. Among previously published studies, two important factors on ESC drug concentrations, age [2–4, 7] and *sex* [3, 7–9], have been frequently discussed. However, findings are inconsistent [2, 5]. ESC is primarily metabolized by the cytochrome P450 (CYP) isoenzymes CYP2C19 (36%), CYP2D6 (30%) and CYP3A4 (34%). Two major metabolites, S-desmethylescitalopram (S-DCT) and S-didesmethylescitalopram (S-DDCT), have been identified, which weakly contribute to the pharmacologic activity of ESC. On this account, CYP2C19 genotypes have been shown to substantially impact ESC levels [4, 7, 8, 10, 11]. Little information is known about the influence of prescribed comedication [2]. No information is available on the influence of liver abnormalities e.g. caused by alcohol abuse, a common comorbidity in patients treated with ESC for MDD, GAD or OCD. Furthermore, very few studies systematically investigated antidepressant effects or side effects of ESC in relation to drug levels [12, 13]. In a naturalistic setting, only one TDM study reported drug effects from a small sample of ten ESC treated patients [14]. Overall, limited data are available describing the relationship between ESC BLs, medication efficacy and tolerability. Nevertheless, current guidelines recommend BL monitoring for ESC for dose titration, special indications and for problem solving, and they suggest a reference range between 15 and 80 ng/mL [15]. This is the first study that investigates drug levels and clinical efficacy in a large sample of patients treated with ESC in a naturalistic setting. The aim of our study was to investigate an optimal concentration range for ESC and identify influences on ESC BLs.

Material and methods

Patient sample

Influencing factors like age, sex, comedication, liver function (AST/ALT ratio), comorbid alcohol-related disorder (International Classification of Diseases (ICD) 10 F10.2; F10.3) and benzodiazepine-related disorder (ICD 10 F 13.2.; 13.3) affecting the pharmacokinetics of ESC were studied in a naturalistic design. Data were collected between 08 January 2004 to 07 September 2009 from patients for whom TDM was requested to guide the antidepressant drug therapy in sixteen Departments of Psychiatry and Psychotherapy in Germany (Aachen, Augsburg, Bad Soden, Dresden, Göttingen, Gummersbach, Heidelberg, Karlsbach-Langensteinbach, Kiedrich, Königstein im Taunus, Mainz, Marienheide, München, Nürnberg, Ulm, Wasserburg). Alcohol- and benzodiazepine-dependent patients were inpatients for a qualified withdrawal treatment for at least three weeks. They were treated for a psychiatric disorder, for which treatment with ECS was indicated. Drug levels, demographic data, daily dose, diagnoses (according to the 10th edition of the International Classification of Diseases (ICD-10) [16]), comedication, laboratory results, the reason for therapeutic drug monitoring (TDM), the severity of illness, therapeutic effects and side effects were registered on the request form by the requesting physician. Side effects were rated using a short version of the Utvalg for Kliniske Undersogelser (UKU [17]) rating scale with a four-point global scale (0, absent; 1, mild; 2, moderate; 3, severe) for severity on the day of blood withdrawal. Severity of illness and the patient's response were assessed on the day of blood withdrawal with the Clinical Global Impressions Scale (CGI-I [18]), item 1 for evaluation of severity of illness (from score 2–8) and item 2 as global improvement rating (1, very much improved; 2, much improved; 3, slightly improved; 4, unchanged or worse). Minimal drug concentrations (trough levels) of ESC and two metabolites were measured under steady-state conditions from patients whose treatment was guided by TDM. Patients with doses ranging from 5–40 mg per day were eligible for analysis. Only one level per patient was selected, the last sample for which the daily dose was given on the request form. Reasons for exclusion of individual data were: i) missing information on administered ESC dose, ii) no escitalopram was detectable (0 ng/ml), iii) citalopram noted as comedication, iv) drug concentration was not in the steady-state, v) sample not taken at trough vi) chromatographic interferences, vii) noncompliance was reported by the clinician on the request form and viii) questionable compliance documented by the clinician and patients below individual dose-related reference range for both ESC and D-ESC.

Determination of blood levels

ESC and two major metabolites D-ESC and DD-ESC were determined in serum by high performance liquid chromatography (HPLC) as described previously for mirtazapine (Shams et al. 2004) with slight modifications in the Neurochemical Laboratory of the Department of Psychiatry and Psychotherapy University Medical Center at Mainz, Germany. An HPLC system (Agilent 1100 obtained from Bio-Rad, Munich, Germany) with column-switching was used consisting of an autosampler, a thermostated column set at 25 °C with an electric six-port switching valve, two HPLC pumps and a fluorescence detector. For online sample clean-up, 0.1 ml serum was injected on a pre-column (10×4.0 mm i.d.) filled with LiChrospher CN material of 20 µm particle size (MZ-Analysentechnik, Mainz, Germany). The pre-column was washed with deionized water containing 8% (V/V) acetonitrile to remove proteins and other interfering

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

compounds for five minutes. Drugs were eluted and separated on LiChrospher CN material (5 µm; column size 250 × 4.6 mm i.d., MZ-Analysentechnik) using 50% (V/V) acetonitrile and phosphate buffer (8 mM, pH 6.4) and quantified by fluorescence detection. The excitation wavelength was set at 290 nm, and the emission wavelength at 350 nm. HPLC analysis of a single sample was completed within 20 min. Each analytical series included at least two control samples containing a low or high concentration of ESC and D-ESC, respectively. There was linear relation between drug concentration and detector signal from 2 to at least 200 ng/mL. The lower limit of quantification was 2 ng/mL. The intra- and inter-assay reproducibility of quality control samples were below 10% for all analyses. For calculations, results reported as < 5 ng/mL and < 10 ng/mL ($n = 16$) were set to 2.5 and 5 ng/mL.

Statistical analysis

Antidepressant effects and side effects

Escitalopram medication effects were investigated i) in a sample of patients with depressive disorder under CNS-relevant comedication and ii) in a sample of patients without CNS-relevant comedication. Responders were identified as patients with CGI-Improvement score ≤ 2. Nonresponders were characterized as patients with CGI-Improvement score > 2 or nonresponse noted as reason for TDM on the request form. A Kruskal–Wallis test was applied to compare drug levels among patient groups. Receiver operating characteristic (ROC) analysis was used to define a drug level threshold that is able to distinguish responders from nonresponders. Calculations were carried out using SPSS (version 26) and R 2.10.1. For all analyses, $p \leqslant 0.05$ was defined as statistically significant.

Identification of factors influencing ESC blood levels

Pharmacokinetic variability of ESC was expressed as the range in dose-adjusted serum concentrations (C/D ratios; ng/mL/mg/day). As an in vivo measure of CYP activities, the metabolic ratios D-ESC/ESC and DD-ESC/ESC were calculated. For descriptive analyses, mean, median, standard deviation and interquartile range were calculated. Differences between males and females and different age groups were tested by a two-tailed, nonparametric Mann–Whitney test, and for multiple comparisons, the Kruskal–Wallis test with Dunn's post hoc test was performed. Correlation coefficients (Spearman-rho) were calculated to determine the relation between drug serum levels, daily doses, age and liver function (estimated by γ-glutamyltransferase (GGT), alanine aminotransferase (ALT) and ratio of aspartate aminotransferase (AST)/ALT). An AST/ALT ratio ≥ 1 has been associated with the incidence of liver cirrhosis. Together with an elevated GGT, it has been found a quite selective parameter indicating an alcoholic liver disease [19]. In this study, alcohol-related liver dysfunctions were assumed in patients that showed a GGT (66 U/l for men, 39 U/l for women) value above the recommended reference range and additionally an AST/ALT ratio ≥ 1.The comparison group comprised patients with GGT values within the recommended reference range for women and men. In a similar manner, patients with an alcohol or benzodiazepine dependence were compared to a control group in order to determine a possible role of liver dysfunctions on the pharmacokinetics of ESC. We then used a multivariate modelling approach to predict ESC concentration based on clinical parameters. Pharmacokinetically relevant variables such as ESC dose, age, sex and comedication with cytochrome CYP2D6 inhibitors were used to predict the ESC concentration of each subject. For this analysis, we used generalized linear models (GLM) with a linear link-function and a gamma distribution underlying the response variable. Dose, age and sex were selected as predictors for the GLM since patients with CYP altering comedication were sparse. The modelling was performed using the custom written python-code as well as the sklearn-toolbox [20].

Results

Patient sample characteristics

Of 344 patients, 44 were excluded (39 patients without indication of doses, 2 outliers excluded, 1 patient with citalopram comedication and 2 patients with doses more than twice above the approved maximum daily dosage (> 40 mg)). The final sample comprised 300 patients that were included in the analysis (female: $n = 180$, 60%; male: $n = 119$, 39.7%, unknown $n = 1$) aged from 18 to 86 years (mean 48.6 ± 16.5 years). Most patients were from the University Medicine of Göttingen (36.3%), followed by Mainz (24.3%), Ulm (15.7%) and Augsburg (14.0%). For 178 patients, information on patient setting was available with most of patients staying in a psychiatric hospital at the time of inclusion (89.9%). More than half of all patients treated with ESC was diagnosed with a depression as primary diagnosis ($n = 157$, 52.3%). The remaining patients were either diagnosed with other diagnosis than depression ($n = 92$, 30.7%), or no information on diagnosis was available ($n = 51$, 17.0%). For about every second patient, only one diagnosis was noted on the request form (53%, $n = 132$ of 249). The other half of the patients was diagnosed with a minimum of one and up to ten additional comorbid psychiatric and/or somatic conditions ($n = 117$ of 249). From the sample of depressed patients ($n = 157$), 42.7% of patients

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

had a comorbid psychiatric diagnosis ($n=67$). Frequent additional comorbid diagnoses were alcohol- and substance-related disorders (ICD F10.2; F10.3 $n=36$, ICD F13.2 $n=8$), anxiety and related disorders (ICD F40, 41, 42, 43, $n=21$) and personality disorders (ICD F60, $n=9$). Patients without depression ($n=92$) were either treated/cotreated with ESC for an anxiety or related disorder (37.0%, ICD F40/41/42/43), schizophrenic spectrum disorder (21.7%, ICD F20-F29), or bipolar disorder (15.2%, ICD F30/31). The reason for TDM was reported in 190 patients. In 43.2% of all requests, the reason for TDM was follow-up control. Additional reasons for TDM were start of medication (26.3%), compliance control (21.1%), change in medication (11.1%), nonresponse (3.7%) and side effects (1.6%). The majority included request forms had rather been a repeated measure of the drug level than first monitoring (18% "first"). Concomitant medication was frequent, and it was reported in 72.3% of patients with up to 10 additional drugs and 2.0 concomitant drugs on average (for full list, see Supplementary Table 1). Other CNS-relevant drugs were given in 187 of all patients. An additional antidepressant drug was given to 121 (40.3%) of them; most preferred was mirtazapine (68.6%), followed by trimipramine (10.7%). Overall, benzodiazepines were given in 26 patients in addition to their treatment with ESC. 83 patients were treated with ESC monotherapy.

For more than half of the patients, the CGI severity score was available ($n=165$). Most patients were classified as markedly ill (CGI.S; 5, 32.1%, $n=53$) and severely ill (CGI-S; 6, 40.6%, $n=67$). The CGI-improvement score was noted for 154 patients with most patients classified as much improved (CGI-I; 2, 45.5%) or minimally improved (CGI-I; 3, 23.4%). Overall, the response rate was 59.5% in 163 patients for whom information on response was available. The UKU scale was available for 148 patients. 25 patients experienced side effects with most of them experiencing tension/inner restlessness ($n=18$).

The mean ($\pm$SD) ESC dose was 16.9 mg $\pm$ 7.3 mg/day in all 300 patients. In total, most common doses were 10 mg (31.3%), 15 mg (17.3%) and 20 mg (36.0%). 3.3% of patients had doses lower than 10 mg, and 12% of patients were treated with doses above 20 mg. The mean serum concentration of ESC was 28.3 $\pm$ 20.6 ng/mL (2.5–105.0 ng/mL, $n=300$), the mean serum concentration of D-ESC was 13.1 $\pm$ 9.5 ng/mL (2.5–78.0 ng/mL, $n=297$), and the mean DD-ESC concentration was 4.5 $\pm$ 8.2 ng/mL (0.0–76.0, $n=83$).

Clinical effects for depressive patients treated with ESC

A total of 157 patients were treated with ESC for depressive disorder (ICD 10 F32/F33). Of those, the majority received additional CNS-relevant medications ($n=103$). Detailed information on patients with depression with and without CNS-relevant comedication can be found in Table 1. Mean doses of patients treated with ESC for depression AQ2 were 17.0 $\pm$ 6.8 mg/day (5–40). Mean ESC and S-DCT serum concentrations were 29.7 $\pm$ 21.0 ng/mL (median 23.0, IQR 16.0–41.5) and 13.4 $\pm$ 10.6 ng/mL (median 11.0, IQR 6.0–17.0). For the majority of patients with depression, ESC serum levels within the therapeutic reference range of 15–80 ng/mL were detected (73.3%, $n=115$). 23.6% of patients had levels below and 3.2% of patients had concentrations above this range.

Antidepressant efficacy of ESC alone was assessed in 51 patients independent from diagnosis (shown in supplemental Table II). ESC concentrations were higher in responders (median 17.0; $n=30$) than in nonresponders (12.0; $n=21$) (not significant). The ROC curve identified a cut-off point of 14.5 ng/mL that discriminates responders from nonresponders (AUC 0.652, p 0.066, shown in supplemental Fig. 2). 64.1% of patients with a drug level above 14.5 ng/mL responded to the ESC treatment. The response rate below this threshold was 41.7%. When selecting patients with ESC as the only antidepressant and without other CNS-relevant comedication, 50 patients with information on side effects were available. Specific side effects were reported in 12 patients. The most frequently reported side effect was tension/unrest in 8 cases. Their mean ESC BL was 36.0 $\pm$ 33.5, and the mean dose was 15.6 mg $\pm$ 5.0.

Influencing factors on ESC, S-DCT and DS-DCT blood levels in patients treated with ESC

The total sample showed a good correlation between BL and applied ESC doses ($n=300$, $r=0.52$; $P<0.0001$), S-DCT ($n=297$, $r=0.63$; $P<0.0001$) and DD-ESC ($n=83$, $r=0.26$, $p=0.0018$). Figure 1 illustrates a high inter-individual variation in ESC BLs among all dosage levels. Mean C/D ratios and MPRs for men and women and for different age groups are presented in Table 2. C/D ratios of the total sample were 1.72 $\pm$ 1.11 for ESC and 0.79 $\pm$ 0.61 for S-DCT. Mean MPRs were 0.58 $\pm$ 0.36 and 0.21 $\pm$ 0.25 for D-ESC/ESC and DD-ESC/ ESC.

ESC and D-ESC BLs showed a good correlation with sex ($n=299$, $r=0.16$, $p=0.006$ and $n=296$, $r=0.15$, $p=0.010$). This correlation could not be observed for DD-ESC drug levels ($n=83$). Men showed 20% (C/D; 1.54, $n=119$) lower dose-corrected concentrations than woman (C/D; 1.84, $n=180$). This difference was statistically significant, also for the metabolite (p 0.03 and p 0.010). As a consequence, men in general had lower mean ESC and D-ESC concentrations compared to women (23.6 $\pm$ 15.5 and 31.5 $\pm$ 22.9 ng/mL p 0.006; 11.4 $\pm$ 7.6 and 14.2 $\pm$ 10.5 ng/mL p 0.012).

Furthermore, age positively correlated with the ESC concentration ($n=299$, $r=0.11$, $p=0.05$) and with the

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

Table 1 Demographic data, CGI scores, daily doses and serum concentrations of escitalopram and its active metabolites in patients with major depression

Sample (*n*) (male/female/unknown)		300	(119/180/1)
Patients with depression (*n*) (male/female)		157	(55/102)
Patients with depression under S-CT monotherapy (*n*) (male/female)		53	(19/34)
Patients with S-CT monotherapy (*n*) (male/female)		109	(48/61)
Patients with depression ($n=157$)			
Age, years	Mean ± SD (range)	52.6 ± 16.2	(18–86)
No. of Comedication	Mean ± SD	2.1 ± 2.1	
CGI severity score			
–of all depressive patients ($n=91$)	Mean ± SD (range)	5.8 ± 1.0	(2–8)
–of all depressive patients under S-CT monotherapy ($n=26$)	Mean ± SD (range)	5.8 ± 0.9	(4–8)
CGI-improvement score			
–of all depressive patients ($n=84$)	Mean ± SD (range)	2.3 ± 1.0	(1–5)
–of all depressive patients under S-CT monotherapy ($n=23$)	Mean ± SD (range)	2.1 ± 1.1	(1–5)
S-CT dose, mg/d			
–of all depressive patients ($n=157$),	Mean ± SD (range)	17.0 ± 6.8	(5–40)
–of all depressive patients under S-CT monotherapy ($n=53$), mean ± SD (range)	Mean ± SD (range)	15.5 ± 5.1	(5–25)
Serum concentrations, ng/mL			
–S-CT ($n=157$)	Mean ± SD (range)	29.7 ± 21.0	(2.5–99.0)
	Median (IQR)	23.0	(16.0–41.5)
–D-SCT ($n=156$)	Mean ± SD (range)	13.4 ± 10.6	(2.5–78.0)
	Median (IQR)	11.0	(6.0–17.0)
–DD-SCT (n = 46)	Mean ± SD (range)	5.2 ± 11.0	(0.0–76.0)
	Median (IQR)	2.5	(2.5–5.0)
Metabolite-to-parent compound ratio (MPR)			
–D-SCT/S-CT) ($n=156$)	Mean ± SD (range)	0.6 ± 0.4	(0.1–2.2)
–DD-SCT/ S-CT) ($n=46$)	Mean ± SD (range)	0.2 ± 0.3	(0.0–1.9)
Dose-corrected serum concentrations (C/D), ng/mL/mg			
–S-CT/Dose ($n=157$)	Mean ± SD (range)	1.8 ± 1.2	(0.3–6.8)
–D-SCT/Dose ($n=156$)	Mean ± SD (range)	0.8 ± 0.8	(0.1–8.0)

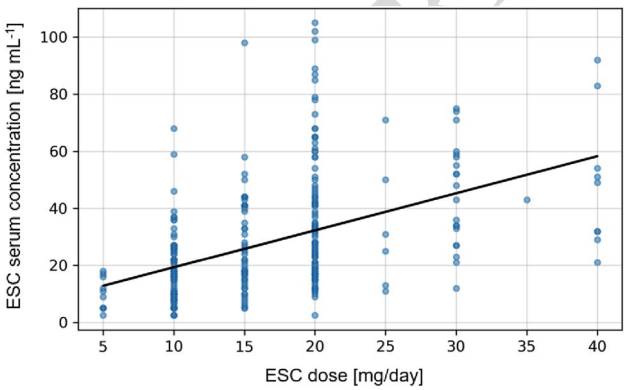


Fig. 1 Linear regression of ESC dose and serum concentration ($n=300$, $r=0.52$; $P<.0001$)

dose-corrected ESC ($n=299$, $r=0.130$, $p=0.025$) and D-ESC concentrations ($n=296$, $r=0.124$, $p=0.033$). Drug levels increased with age, especially in patients 60 years and older.

A multivariate regression analysis using threefold cross-validation (permutated 1000 times, $n=299$) was performed including ESC concentrations and the variables dose, age and sex. The accuracy of the prediction was averaged over all cross-validations and permutations. Based on dose, age and sex, the models could predict ESC BLs with an average generalized coefficient of determination of $D^2=0.22\pm0.059$ (shown in supplemental Table II).

Influences of pathological liver alterations, alcohol and benzodiazepine dependence

Laboratory markers GGT and the AST/ ALT ratio were available for 68 patients (50% of them diagnosed with a depression). Of these, 15 patients were classified as patients with liver dysfunctions (22%). Dose-corrected ESC concentrations were higher in patients with ($n=15$) compared to patients without ($n=51$) liver dysfunctions identified by clinical relevant laboratory markers ($p=0.013$; mean 2.23 ± 0.27 vs. 1.51 ± 0.12 ng/mL/ mg/

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

day). Of all patients in which the liver values were available, 39 and 33% of patients with alcohol or benzodiazepine dependence showed a potential alcoholic liver disease by clinical relevant markers. C/D ratios and MPRs did not considerably vary in patients with alcohol dependence and patients without this diagnosis (shown in Table 3).

When compared to a control group, a higher number of men constituted the patient group suffering from alcohol use disorder (shown in Table 4). Patients with the disorder were

Table 2 Metabolic ratios in men and women and different age groups (Mann–Whitney/Kruskal–Wallis)

	C/D S-CT ng/ml/mg	*n*	C/D D-SCT ng/ml/mg	*n*	MPR (D-SCT/S-CT)	*n*	MPR (DD-SCT/S-CT)	*n*
Male	**1.54 ± 0.94**	119	**0.71 ± 0.39**	119	0.58 ± 0.34	119	0.19 ± 0.16	34
Female	**1.84 ± 1.19**	180	**0.84 ± 0.72**	177	0.58 ± 0.38	177	0.22 ± 0.30	49
< 20	**1.73 ± 0.96**	10	**1.37 ± 2.28**	10	0.71 ± 0.61	10	NA	0
20–29	**1.85 ± 1.05**	30	**0.71 ± 0.36**	30	0.48 ± 0.27	30	0.11 ± 0.06	11
30–39	**1.47 ± 0.76**	52	**0.78 ± 0.40**	52	0.64 ± 0.38	52	0.19 ± 0.16	12
40–49	**1.46 ± 0.97**	74	**0.67 ± 0.33**	73	0.63 ± 0.43	73	0.21 ± 0.15	26
50–59	**1.79 ± 1.28**	55	**0.79 ± 0.34**	54	0.61 ± 0.34	54	0.24 ± 0.43	18
60–69	**1.89 ± 1.23**	40	**0.79 ± 0.81**	39	0.46 ± 0.29	39	0.28 ± 0.28	13
70–79	**1.98 ± 1.08**	29	**0.92 ± 0.37**	29	0.55 ± 0.23	29	0.07	2
> 80	**2.85 ± 1.7**	9	**0.98 ± 0.54**	9	0.41 ± 0.24	9	0.07	1
Total	1.72 ± 1.11	299	0.79 ± 0.61	296	0.58 ± 0.36	296	0.21 ± 0.25	83

Significant differences between groups in bold

C/D ratios between gender groups (S-CT *p* .03, D-SCT *p* .02) and age groups (S-CT *p* .024, D-SCT *p* .031). MPRs between gender groups (D-SCT/S-CT *p* .70, DD-SCT/S- CT *p* .75) and age groups (D-SCT/S-CT *p* .13, DD-SCT/S-CT *p* .37)

Table 3 Metabolic ratios in patients with alcohol (F10) or substance use disorder (F13) and under benzodiazepine use compared to control group (Mann–Whitney/Kruskal–Wallis)

	C/D-ESC ng/ml/mg	*n*	C/D D-ESC ng/ml/mg	*n*	MPR (D-ESC/ESC)	*n*	MPR (DD-ESC/ESC)	*n*
Alcohol use disorder (F10)	1.73 ± 1.11	68	0.78 ± 0.36	67	0.61 ± 0.40	67	0.19 ± 0.15	39
Substance use disorder (F13)	1.88 ± 1.35	15	0.78 ± 0.61	15	0.58 ± 0.37	15	0.20 ± 0.26	9
Benzodiazepine use	1.87 ± 1.09	26	**0.89 ± 0.29**	26	0.59 ± 0.26	26	0.54 ± 0.88	4
Liver abnormalities from lab results and sonography	1.56 ± 0.68	8	0.77 ± 0.31	7	0.57 ± 0.22	7	0.27 ± 0.14	5

Significant differences between groups in bold

D-ESC C/D ratio between patients with benzodiazepine use (*p* .009)

Table 4 Patients with and without alcohol use disorder (F10) (Mann–Whitney/Kruskal–Wallis)

	Patients with alcohol use disorder	All Patients without alcohol use disorder	All	
Sample size	68	179	247	
–with depression	37 (54.5%)	120 (67%)	157 (63.6%)	
Age (years)	47.6 ± 10.4	50.5 ± 17.7	49.7 ± 16.0	0.207
Sex % female	**44.1%**	**63.1%**	**60.2%**	**0.007**
CGI-S	**5.3 ± 0.82**	**5.8 ± 0.95**	**5.7 ± 0.94**	**0.016**
Dose (mg/day)	**14.85 ± 6.6**	**17.5 ± 7.2**	**16.7 ± 7.1**	**0.007**
S-CT concentration (ng/mL)	23.8 ± 14,9	29.8 ± 21,9	28.1 ± 20.3	0.131
S-DCT concentration (ng/mL)	11.4 ± 7.0	13.9 ± 10.7	13.3 ± 9.9	0.197
C/D ratio	1.73 ± 1.11	1.71 ± 1,13	1.72 ± 1.12	0.916
MPR D-SCT/S-CT	0.61 ± 0.40	0.57 ± 0.35	0.58 ± 0.36	0.759

Significant differences between groups in bold

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

less severely ill (CGI-S), and they were treated with lower doses resulting in lower ESC concentrations. The interquartile concentration range was 11–34 ng/mL. 69.1% of patients had concentrations within the recommended reference range for antidepressant treatment with ESC, and 30.9% had concentrations below this range. No concentration above this range was detected. With comparable doses, patients with acute benzodiazepine use ($n = 26$) and patients with benzodiazepine use disorder ($n = 15$) showed a trend towards higher dose-corrected concentrations compared to patients without these disorders. This effect did, however, only reach significance for D-ESC in the subgroup with acute benzodiazepine use (p 0.009). Of note, a small sample of patients with documented liver abnormalities confirmed by sonography (e.g. K76.0, K70.0) had lower dose-corrected concentrations compared to controls ($n = 8$, C/D 1.56 ± 0.68, not significant).

Discussion

This study presents an overview of the treatment effects and pharmacokinetics of ESC in patients treated in a naturalistic setting, including the interaction potential of comorbidities such as alcohol and substance use disorders. An optimal antidepressant effect for ESC is expected within a recommended target range of 15–80 ng/mL [15]. The majority of our patients (72%) had serum concentrations within this range, and they were treated within the approved dosage range of 10–20 mg. However, 11% of patients required doses above 20 mg to reach drug levels within the recommended therapeutic reference range. More concerning is that every fourth patient (25.6%) treated with an approved dosage did not reach the target threshold concentration of 15 ng/mL. The results of our efficacy analysis confirm the recommended threshold of 15 ng/mL, above which antidepressant response becomes more likely, in a sample of patients treated with ESC monotherapy [15]. The interquartile range from patients with depression was 16.0–41.5 ng/mL, and with 13.5–25.3 ng/mL it was somewhat lower in responders. The overall response rate of 59.5% was in line with previous studies [21]. The majority of samples included in this study were follow-up measurements. As an explanation for follow-up concentrations below the therapeutic reference range, placebo response under antidepressant drug treatment has been frequently discussed in drug monitoring trials [22].

Patients with alcohol use disorders were prescribed lower ESC doses, resulting in lower drug concentrations. Less severe depressive symptoms (according to CGI-S) in this population might have led to prescription of lower ESC doses. However, a different response pattern to antidepressant treatment in patients with alcohol use disorder remains a possibility. Patients with alcohol dependence did not show considerably differing metabolic ratios compared to patients without this comorbidity. An effect on drug levels could more likely be explained by other factors such as female sex, higher age and pathological liver function. The relationship of applied doses, age and sex with the ESC serum concentrations could be partially described by a linear function. However, most of the variation of ESC serum concentrations could not be predicted by these variables and, thus, highlights the necessity of clinical measurements of serum concentrations in case of insufficient response.

Reasons why gender may affect pharmacokinetics are molecular as well as physiological factors. Men are supposed to have a higher activity of CYP1A2, P-glycoprotein and some isoforms of glucuronosyltransferases and sulfotransferases. In women, CYP2D6 activity is higher. Physiological factors are women's generally lower body weight and organ size, higher percentage of body fat, lower glomerular filtration rate and different involvement of steroid hormones that may influence the activity of all three CYP isoenzymes metabolizing ESC and citalopram [23]. The univariate correlation of sex and ESC serum concentration can be attributed to multifarious potential covariates such as body weight, body composition or metabolic properties. In our study, mean serum concentration and C/D ratio were in line with values previously reported [13, 24, 25], however, higher than those indicated in the TDM guidelines [15]. Our findings confirm the results of Waade et al., 2014 [7], who reported 15% lower metabolic ratios in women compared to men. In line with other studies, we found increasing C/D ratios with age [2].

The results of this study should be interpreted cautiously. First, the routine TDM setting did not allow us to control patient adherence to the treatment, nor to control for other influences on antidepressant responses. Not only psychological interventions (e.g. psychotherapy) and psychosocial factors (e.g. stress levels and social support), but also a series of other factors like hypothyroidism, hormonal changes, nutrition deficiencies, or sleep disorders (e.g. insomnia and obstructive sleep apnoea) might be relevant in this context.

Second, the patients included in the study were not genotyped, altered C/D ratios may be a result of CYP2D6 and CYP2C19 genetic variability. The activity of both isoenzymes is of major importance in the biotransformation of ESC and many other drugs. The relatively high extent of polypharmacy of on average two co-administered drugs may have contributed to this effect. Co-prescription of potent CYP2D6 inhibitors, CYP3A4 inhibitors/inducers or CYP2C19 inhibitors/inducers, was identified from the requisition forms. Since less than 2% of patients per group were co-administered with relevant comedication, the effects of comedication were considered negligible. However, a potential influence of comedication cannot be ruled out, especially in subgroups of older patients with increasing polypharmacy.

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

The diagnosis of alcohol or benzodiazepine dependence alone may not affect ESC BLs, but liver dysfunction does. Reduced liver function in alcoholic liver disease, indicated by elevated GGT and AST/ALT ratio, resulted in higher dose-corrected ESC concentrations. Previous studies could not find clinically relevant differences in ESC, D-ESC and DD-ESC levels in patients with hepatic impairment compared to healthy adults [26].

To sum up, the present study strongly supports a target concentration of 15 ng/mL for antidepressant response. 75% of all patients with depression had BLs below 42 ng/mL. Patients with comorbid alcohol use disorder in treatment might require even lower concentrations (interquartile concentration 11–34 ng/mL). Clearly, further prospective studies are needed to confirm our findings.

Conclusion

This study adds evidence to the results from previous studies indicating that age, sex and liver function affect the serum levels of ESC and its metabolite D-ESC. Pronounced pharmacokinetic variability requires dosages above the approved maximum daily dosage in a relevant number of patients and supports the level 2 ("recommended") recommendation of the AGNP expert group [15] to monitor ESC serum levels for treatment optimization.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00406-022-01491-9.

Acknowledgements We thank the laboratory team of the Neurochemical Laboratory of the Department of Psychiatry and Psychotherapy, University Medical Center of Mainz, who provided the TDM results. We also kindly thank Dr. Anne Brückner, Department of Psychiatry and Psychotherapy, University Medical Center of Mainz, and Biologist Cornelia Genée, Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, for the preparation of first datasets and preliminary analysis of the data.

Author contributions XH developed the first draft of the manuscript. CH, UHR and GG supervised the entire manuscript writing and contributed to the revision of the manuscript. CH and UHR participated in the research design of the study. DW recruited patients and collected TDM samples. XH, CS and SH performed the statistical analysis.

Funding Open Access funding enabled and organized by Projekt DEAL. The authors declare that the study did not receive any funding.

Data availability The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest CH has received speaker's fees from Otsuka. He is editor of PSIAC, a web-based platform analysing pharmacokinetic and dynamic drug interactions. The software is distributed by Springer Nature, Heidelberg, Germany. GG has served as a consultant for Allergan, Boehringer Ingelheim, Institute for Quality and Efficiency in Health Care (IQWiG), Janssen-Cilag, Lundbeck, Otsuka, Recordati, ROVI, Sage, and Takeda. He has served on the speakers' bureau of Gedeon Richter, Janssen Cilag, Lundbeck, Otsuka, Recordati. He has received grant support from Boehringer Ingelheim, Lundbeck and Saladax. He is co-founder and/or shareholder of Mind and Brain Institute GmbH, Brainfoods GmbH, OVID Health Systems GmbH and MIND Foundation gGmbH. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval This study was conducted in accordance with the World Medical Association Declaration of Helsinki. Ethics approval and written informed consent were not required for this study.

References

1. Schwabe U, Ludwig W-D. Arzneiverordnungs-Report 2020
2. Reis M, Chermá MD, Carlsson B, Bengtsson F (2007) Therapeutic drug monitoring of escitalopram in an outpatient setting. Ther Drug Monit 29(6):758–766
3. Reis M, Aamo T, Spigset O, Ahlner J (2009) Serum concentrations of antidepressant drugs in a naturalistic setting: compilation based on a large therapeutic drug monitoring database. Ther Drug Monit 31(1):42–56
4. Tsuchimine S, Ochi S, Tajiri M, Suzuki Y, Sugawara N, Inoue Y et al (2018) Effects of Cytochrome P450 (CYP) 2C19 Genotypes on Steady-State Plasma Concentrations of Escitalopram and its Desmethyl Metabolite in Japanese Patients With Depression. Ther Drug Monit 40(3):356–361
5. Unterecker S, Riederer P, Proft F, Maloney J, Deckert J, Pfuhlmann B (2013) Effects of gender and age on serum concentrations of antidepressants under naturalistic conditions. J Neural Transm (Vienna) 120(8):1237–1246
6. Lundbeck Canada Inc. Product Monograph including patient medication information. Available from: https://www.lundbeck.com/content/dam/lundbeck-com/americas/canada/products/files/cipralex_product_monograph_english.pdf. [Accessed 18.02.2022]
7. Waade RB, Hermann M, Moe HL, Molden E (2014) Impact of age on serum concentrations of venlafaxine and escitalopram in different CYP2D6 and CYP2C19 genotype subgroups. Eur J Clin Pharmacol 70(8):933–940
8. Jukić MM, Haslemo T, Molden E, Ingelman-Sundberg M (2018) Impact of CYP2C19 genotype on escitalopram exposure and therapeutic failure: a retrospective study based on 2,087 patients. Am J Psychiatry 175(5):463–470

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

9. Scherf-Clavel M, Deckert J, Menke A, Unterecker S (2019) Smoking Is associated with lower dose-corrected serum concentrations of escitalopram. J Clin Psychopharmacol 39(5):485–488
10. Rudberg I, Hendset M, Uthus LH, Molden E, Refsum H (2006) Heterozygous mutation in CYP2C19 significantly increases the concentration/dose ratio of racemic citalopram and escitalopram (S-citalopram). Ther Drug Monit 28(1):102–105
11. Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E (2008) Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. Clin Pharmacol Ther 83(2):322–327
12. Florio V, Porcelli S, Saria A, Serretti A, Conca A (2017) Escitalopram plasma levels and antidepressant response. Eur Neuropsychopharmacol : J European Coll Neuropsychopharmacol 27(9):940–944
13. Leuchter AF, Cook IA, Marangell LB, Gilmer WS, Burgoyne KS, Howland RH et al (2009) Comparative effectiveness of biomarkers and clinical indicators for predicting outcomes of SSRI treatment in major depressive disorder: results of the BRITE-MD study. Psychiatry Res 169(2):124–131
14. Lloret-Linares C, Bosilkovska M, Daali Y, Gex-Fabry M, Heron K, Bancila V et al (2018) Phenotypic assessment of drug metabolic pathways and P-glycoprotein in patients treated with antidepressants in an ambulatory setting. J Clini Psychiatry. 79(2):14196
15. Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K et al (2018) Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. Pharmacopsychiatry 51(1–02):9–62
16. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th ed. Available online at: https://icd.who.int/browse10/2010/en (accessed July 13 2022)
17. Lingjaerde O, Ahlfors UG, Bech P, Dencker SJ, Elgen K (1987) The UKU side effect rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. Acta Psychiatr Scand Suppl 334:1–100
18. Guy W. ECDEU assessment manual for psychopharmacology. Rockville, Md.: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs. 1976
19. Baral N, Pokhrel S, Lamsal M, Yadav BN, Sah SP (2005) Utility of gamma-glutamyl transpeptidase and mean corpuscular volume in alcoholic liver disease. Southeast Asian J Trop Med Public Health 36(4):1007–1010
20. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. 2012 Scikit-learn: Machine Learning in Python. Journal of Machine Learning Research. 12
21. Yang LP, Scott LJ (2010) Escitalopram: in the treatment of major depressive disorder in adolescent patients. Paediatr Drugs 12(3):155–163
22. Hiemke C (2019) Concentration-effect relationships of psychoactive drugs and the problem to calculate therapeutic reference ranges. Ther Drug Monit 41(2):174–179
23. Meibohm B, Beierle I, Derendorf H (2002) How important are gender differences in pharmacokinetics? Clin Pharmacokinet 41(5):329–342
24. Engelmann J, Wagner S, Solheid A, Herzog DP, Dreimüller N, Müller MB et al (2021) Tolerability of high-dose venlafaxine after switch from escitalopram in nonresponding patients with major depressive disorder. J Clin Psychopharmacol 41(1):62–66
25. Florio V, Porcelli S, Saria A, Serretti A, Conca A (2017) Escitalopram plasma levels and antidepressant response. Eur Neuropsychopharmacol 27(9):940–944
26. Rao N (2007) The clinical pharmacokinetics of escitalopram. Clin Pharmacokinet 46(4):281–290

Journal : Large 406 | Article No : 1491 | Pages : 9 | MS Code : 1491 | Dispatch : 19-9-2022

6. PUBLICATION “MOLECULAR IMAGING OF DOPAMINE PARTIAL AGONISTS IN HUMANS: IMPLICATIONS FOR CLINICAL PRACTICE”

frontiers | Frontiers in Psychiatry

REVIEW
published: 06 April 2022
doi: 10.3389/fpsyt.2022.832209

Molecular Imaging of Dopamine Partial Agonists in Humans: Implications for Clinical Practice

Xenia M. Hart[1], Christian N. Schmitz[1,2] and Gerhard Gründer[1]**

[1] *Department of Molecular Neuroimaging, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany,* [2] *Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany*

Positron emission tomography (PET) has been used since the late 1980s for the assessment of relationships between occupancy of $D_{2/3}$ receptors by antipsychotic drugs in the human brain and the clinical effects and side effects of these compounds in patients. It is now well established for most $D_{2/3}$ antagonists, both of the first and the second generation, that the ideal occupancy of their target receptors is between approximately 65 and 80%. If the occupancy is below 65%, the probability of treatment response is reduced, if the occupancy is higher than 80%, the risk for extrapyramidal side-effects increases substantially. However, partial agonist antipsychotics behave different from these rules. It has been shown for all three available drugs of this class (aripiprazole, brexpiprazole, cariprazine) that, due to their special pharmacology, a very high target engagement (>90%) not only is not harmful but represents a prerequisite for antipsychotic efficacy. The available PET studies for these drugs are reviewed in this work. It is demonstrated that optimal plasma levels for partial agonist antipsychotics can be derived from these studies, which can guide individual treatment in routine patient care.

Keywords: dopamine partial agonists, brexpiprazole, cariprazine, aripiprazole, positron emission tomography, molecular neuroimaging

OPEN ACCESS

Edited by:
György Németh,
Gedeon Richter, Hungary

Reviewed by:
Uma Suryadevara,
University of Florida, United States
Pal Czobor,
Semmelweis University, Hungary

****Correspondence:***
Xenia M. Hart
xenia.hart@zi-mannheim.de
Gerhard Gründer
gerhard.gruender@zi-mannheim.de

Specialty section:
This article was submitted to Psychopharmacology, a section of the journal Frontiers in Psychiatry

Received: *09 December 2021*
Accepted: *11 March 2022*
Published: *06 April 2022*

Citation:
Hart XM, Schmitz CN and Gründer G (2022) Molecular Imaging of Dopamine Partial Agonists in Humans: Implications for Clinical Practice. Front. Psychiatry 13:832209. doi: 10.3389/fpsyt.2022.832209

INTRODUCTION

Determination of clinically useful and rational doses of antipsychotics represents the application of neuroimaging that has had the largest impact on clinical practice in psychiatry (1–3). Molecular imaging with positron emission tomography (PET) is now a routine tool for development of new compounds of this class (3). All antipsychotic agents that are currently in use for the treatment of psychotic disorders, such as schizophrenia, are either antagonists or partial agonists at dopamine $D_{2/3}$ receptors. Assessment of occupancy (target engagement, TE) of these receptors by antipsychotics helped in establishing relationships between TE and antipsychotic doses and their respective plasma concentrations. Studies of the clinical effects and side effects as a function of TE facilitated not only the understanding of antipsychotic drug action, but also the rational dosing of these compounds, which can be further improved when dosing is guided by Therapeutic Drug Monitoring [TDM; (2)]. Assessment of TE with PET or single photon emission computed tomography (SPECT) is based on the concept that the experimental pharmaceutical displaces the radioligand, which binds to the target at trace concentrations. The extent of this displacement is

related to the baseline binding of the radioligand in its unblocked state. Because it is often not feasible to study patients with schizophrenia in medication-free state, patients are usually studied in blocked state only (which means that they are treated with the experimental drug). Unblocked baseline data are taken from healthy volunteers, assuming that patients in the untreated state and controls differ only marginally in receptor availability. The radioactivity in the region of interest in the blocked vs. the unblocked state then. provides the target occupancy (in%) as follows (2):

$$\text{Occupancy}\,[\%] = 100 - [(\text{Tracer binding}_{\text{blocked}}/\text{Tracer Binding}_{\text{unblocked}}) \times 100] \quad (1)$$

Farde et al. in their pioneering early PET studies from the late 1980s demonstrated that clinically effective doses of first-generation antipsychotics (e.g., haloperidol) occupy $D_{2/3}$ dopamine receptors in the striatum of patients with schizophrenia in the range between 65 and 90% (4). These authors also suggested a "therapeutic window" between 65 and 80% striatal dopamine $D_{2/3}$ receptor occupancy for antipsychotic drug action, implying a "ceiling" of about 65% occupancy for sufficient treatment response, although such a high occupancy does not necessarily mean that every patient sufficiently improves. The risk for extrapyramidal side-effects (EPS) increases above a striatal $D_{2/3}$ receptor occupancy of 80%. These relationships also apply to most of the second-generation antipsychotics (5). However, there are certain exceptions to those general rules (6). Antipsychotics with low affinity for D_2-like dopamine receptors such as clozapine and quetiapine even at very high doses or plasma concentrations practically never occupy striatal $D_{2/3}$ receptors to an extent that is associated with EPS (7, 8). Partial agonists at $D_{2/3}$ receptors, on the other hand, have a completely different binding pattern at their main targets. At clinically effective doses, they almost completely occupy $D_{2/3}$ receptors, an observation that has been made first for aripiprazole (9). This unique feature is explained by the pharmacological properties of partial agonists with low intrinsic activity (10). **Figure 1** depicts the different prototypic patterns of target engagement of the available antipsychotic drugs at striatal $D_{2/3}$ dopamine receptors as a function of their plasma concentrations.

Here, we summarize the literature on molecular imaging studies with the available partial agonists, aripiprazole, brexpiprazole, and cariprazine. We show that these studies, especially when target engagement is related to plasma concentrations of the respective drug, can guide rational dosing and Therapeutic Drug Monitoring of these compounds.

Aripiprazole was the first $D_{2/3}$ dopamine partial agonist that was approved for the treatment of schizophrenia (United States: 2002). It was later approved for various other indications including mania and major depression (adjunctive treatment). Aripiprazole binds with very high affinity (in the low nanomolar range) to D_2 and somewhat lesser affinity to D_3 receptors. At both receptors it acts as a partial agonist with low intrinsic activity. Aripiprazole is also a partial agonist at the 5-HT_{1A} and an antagonist at the 5-HT_{2A} serotonin receptor. It has an elimination half-life of 60–80 h. Its main active metabolite, dehydroaripiprazole, has a similar receptor binding profile, and it amounts to up to 40% of the parent concentrations (11).

Brexpiprazole is approved for the treatment of schizophrenia (United States: 2015) and as an adjunctive treatment for major depression. It has a binding profile very similar to the one of its predecessor aripiprazole, with somewhat lower intrinsic activity at D_2 and D_3 receptors. Brexpiprazole has an elimination half-life of approximately 90 h. Its main metabolite (DM-3411) amounts to 23–48% of the parent compound, but it does not contribute to the pharmacodynamic effects, because it does not pass the blood-brain barrier (12).

Cariprazine received FDA approval for the treatment of schizophrenia in 2015. It has partial agonist activity at dopamine $D_{2/3}$ receptors, with and six- to eightfold higher affinity for human dopamine D_3 over D_2 receptors. Like aripiprazole and brexpiprazole, cariprazine is a partial agonist at the 5-HT_{1A} and an antagonist at the 5-HT_{2A} serotonin receptor. The elimination half-life of the parent compound is 50–120 h. However, cariprazine has two active metabolites, N-desmethyl cariprazine (DCAR) and NN-didesmethyl cariprazine (DDCAR). DDCAR is eliminated with a half-life of 2–3 weeks. At steady-state, it significantly contributes to the antipsychotic activity of the drug (13, 14).

METHODS

Search Strategy

In September 2021 (last updated 08.12.2021), four electronic databases (PsycINFO, Medline via PubMed, Cochrane CENTRAL, Web of Science) were systematically searched for relevant articles without restrictions in language or publication date. Keywords included the respective psychotropic drug (aripiprazole, brexpiprazole or cariprazine) and PET/SPECT. Studies in humans and non-human primates were included. Only full-text articles were taken into consideration, abstracts were excluded.

Calculation of EC_{90} Values

The available literature was screened for papers that reported $D_{2/3}$ dopamine receptor occupancy values of the respective drug in relation to administered doses. Both studies in healthy volunteers and in patients were acceptable. Special emphasis was put on studies that also reported plasma or serum drug concentrations, because they usually allow the calculation of an "effective concentration 50" (EC_{50}), which is the concentration predicted to provide 50% of the maximum attainable receptor occupancy. This is a constant characterizing an individual drug. It is related to the maximum attainable receptor occupancy (E_{max}) and the plasma concentration of the drug (C) that is associated with a measured receptor occupancy according to the law of mass action (Michaelis-Menten kinetics):

$$\text{Occupancy}[\%] = (E_{\max} \times [C])/(EC_{50} + [C]) \quad (2)$$

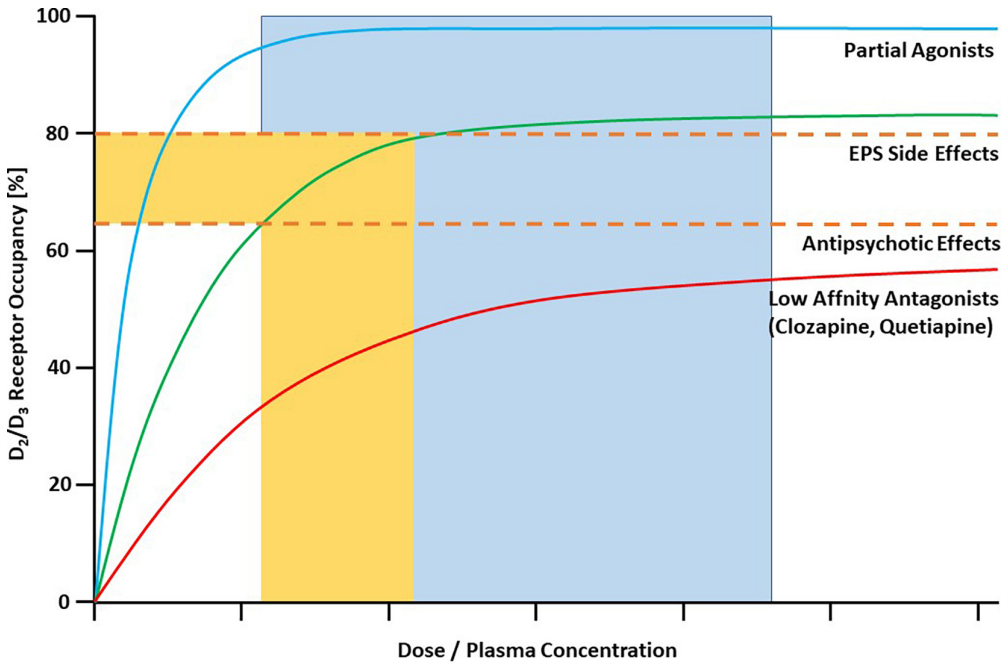


FIGURE 1 | Characteristic binding curves of antipsychotic drugs in human striatum as measured with PET. Dashed lines represent threshold occupancy values for EPS (80%) and antipsychotic effects (65%). Most antipsychotics, including most of the SGAs, are characterized by the green line. They reach optimal occupancy (65–80%) in a "therapeutic window" of corresponding plasma concentrations. Antipsychotics with low affinity for D_2/D_3 receptors are described by the red line. Even at very high plasma concentrations they usually do not cross the 80% threshold for EPS. They exert antipsychotic effects despite relatively low occupancy in the striatum. All clinically available partial agonist antipsychotics are characterized by the blue binding curve. They have antipsychotic effects only at almost total saturation of D_2/D_3 receptors (in the flat part of the curve), represented by the blue area. The upper threshold is not sharply defined. Copyright © 1969, Elsevier. From (1).

From the experimentally determined EC_{50} values, an EC_{90} value can be calculated according to the following equations (maximum attainable receptor occupancy is less than 100%; unconstrained model):

$$90 \times (EC_{50} + [C]) = E_{max} \times [C] \quad (3)$$

$$90 \times EC_{50} + 90[C] = E_{max} \times [C] \quad (4)$$

$$90 \times EC_{50} = E_{max} \times [C] - 90[C] \quad (5)$$

Assuming that the maximum attainable receptor occupancy is 100% (i.e., all available receptors can be occupied by the drug; constrained model), EC_{90} is then:

$$EC_{90} = (90 \times EC_{50})/10 \quad (6)$$

Uchida et al. (15) demonstrated that the relationship between $D_{2/3}$ dopamine receptor occupancy and the respective plasma levels are in some cases better described by an unconstrained model. The constrained model assumes that all dopamine $D_{2/3}$ receptors (100%) can be occupied by the antipsychotic. For most antipsychotics, E_{max} values derived with an unconstrained model are close to 100%, and therefore EC_{50} values estimated from the constrained and the unconstrained model do not substantially differ. For example, for haloperidol the EC_{50} estimated from the unconstrained model was 0.32 and 0.70 ng/ml, when E_{max} was constrained to 100% (15). For olanzapine, the respective values are 7 and 10 ng/ml, and for risperidone 5 and 8 ng/ml. For compounds with a low affinity to $D_{2/3}$ receptors such as clozapine, the situation is more complicated. Here, the experimentally determined E_{max} values are far below 100%. Using an unconstrained model, Uchida et al. (15) calculated a maximum attainable receptor occupancy for clozapine of only 60%, with a respective EC_{50} of 105 ng/ml. The constrained model provided an EC_{50} value of 483 ng/ml. Biologically, it makes no sense to believe that clozapine does not occupy more than 60% of striatal $D_{2/3}$ dopamine receptors. In monkeys, high doses of clozapine occupy more than 80% of $D_{2/3}$ receptors (16). Almost all PET studies that determined $D_{2/3}$ dopamine receptor occupancy by clozapine used [^{11}C]raclopride as the radiotracer (15). In our own study with [^{18}F]fallypride as the radiotracer, we calculated, using an unconstrained model, an E_{max} close to complete receptor saturation, and respective EC_{50} values of 950 ng/mL for the putamen and 582 ng/ml for the caudate (7). These values seem to be biologically and especially clinically more meaningful, since the therapeutic reference range for clozapine is 350 – 600 ng/ml (17), and even much higher plasma concentrations are tolerated without extrapyramidal side-effects (7).

For the purpose of this paper, it seems feasible to work with EC_{90} values that are derived from a constrained

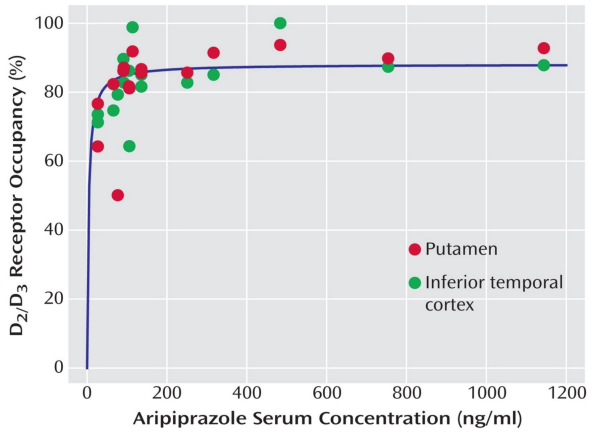


FIGURE 2 | Relationship between aripiprazole serum levels and dopamine D2/D3 receptor occupancy in the putamen and the inferior temporal cortex (representative of cortical binding due to high D2/D3 receptor density) in 16 patients with schizophrenia and schizoaffective disorder receiving therapeutic doses of aripiprazole. Copyright © American Psychiatric Association. From (18).

model. All available $D_{2/3}$ partial agonist antipsychotics are high affinity compounds that occupy their main molecular target close to saturation at doses used in clinical practice. Differences in EC_{90} values calculated from constrained versus unconstrained models might therefore be negligible. It is proposed here that the EC_{90} values determined experimentally with molecular (in almost all cases PET) imaging represent the lower threshold of a therapeutic reference range to be used for TDM.

MOLECULAR IMAGING OF DOPAMINE PARTIAL AGONISTS

Aripiprazole

For aripiprazole, nine PET studies in human subjects are available that report $D_{2/3}$ receptor occupancy values (9, 18–26) (**Table 1**). However, only two of them report ED_{50} values [or individual plasma concentrations, from which an ED_{50} value was derived: (18, 26); **Figure 2**].

Yokoi et al. (9) published the first PET occupancy study with aripiprazole in 15 healthy volunteers, who were treated with fixed aripiprazole doses for a duration of 14 days. They documented a dose-dependent increase of $D_{2/3}$ dopamine receptor occupancy, with a mean occupancy of 30% (caudate) and 34% (putamen) at a dose as low as 0.5 mg, that increased to 49 and 57% at 1 mg, 74 and 72% at 2 mg, 86 and 85% at 10 mg, and 92 and 86% at 30 mg. These authors measured plasma levels, but they did not calculate EC_{50} values. However, the plasma concentration/occupancy curve reported by Yokoi et al. (9) is very similar to the one published by Gründer et al. (18), indicating that the flat part of the curve begins at around 100 ng/ml.

Mamo et al. (23) quantified aripiprazole binding to three different receptor types in 12 patients with schizophrenia, who were treated with aripiprazole doses between 10 and 30 mg daily: $D_{2/3}$ dopamine (with [^{11}C]raclopride), 5-HT_2 serotonin (with [^{18}F]setoperone), and 5-HT_{1A} (with [^{11}C]WAY100635). Even the lowest dose was associated with 85% $D_{2/3}$ dopamine receptor occupancy, and the higher doses led to occupancies above 90%. Extrapyramidal side-effects were documented in two patients (with occupancy > 90%) in whom plasma levels were far above the mean for their dose (442 ng/ml and 663 ng/ml, respectively). 5-HT_2 serotonin occupancy was in the medium range (54–60%), while 5-HT_{1A} receptors were occupied by less than 20% (23). The authors measured aripiprazole and dehydroaripiprazole plasma levels, but EC_{50} values were not reported. However, at the (lowest) 10 mg dose the mean aripiprazole level was 126 ng/ml (dehydroaripiprazole 35 ng/ml); later PET studies [(18, 26), see below] have consistently shown that at these plasma levels $D_{2/3}$ dopamine receptor occupancy is close to 90%. Mizrahi et al. (24) described the same patient sample that Mamo et al. (23) have been investigating. These patients with schizophrenia were switched from olanzapine or risperidone to aripiprazole and both $D_{2/3}$ receptor occupancy and subjective well-being (with the Subjective Wellbeing under Neuroleptics Scale, SWN) were measured. Although receptor occupancy was very high under aripiprazole treatment (82–99%), the SWN score increased significantly after switch from an antagonist to the partial agonist antipsychotic. Plasma levels were not reported (24).

$D_{2/3}$ dopamine receptor occupancy was measured in 16 patients with schizophrenia or schizoaffective disorder on steady-state treatment with aripiprazole at doses ranging from 5 to 30 mg daily by Gründer et al. (18). $D_{2/3}$ receptor occupancy was high already at 5 mg/day, and receptors were almost completely occupied above plasma levels of 100–150 ng/ml (**Figure 2**). EC_{50} values for the various brain regions examined ranged from 4 to 10 ng/ml, with 10 ng/ml for the putamen and 9 ng/ml for the caudate. This study is also the only one that reports EC_{50} estimates that are based on active moiety (aripiprazole + dehydroaripiprazole) concentrations of the drug (putamen 20 ng/ml, caudate 18 ng/ml). Aripiprazole's main (active) metabolite, dehydroaripiprazole, also occupies the $D_{2/3}$ receptor. Thus, a not negligible fraction of total occupancy (usually 20–30%) is attributable to dehydroaripiprazole binding. When one calculates EC_{90} values based on an EC_{50} value of 10 ng/ml for aripiprazole alone and 20 ng/ml for the active moiety, these values are 90 and 180 ng/ml, respectively (18).

Kegeles et al. (20) measured $D_{2/3}$ dopamine receptor occupancy in 19 patients with schizophrenia or schizoaffective disorder, who were subchronically (minimum of steady dose: 10 days) treated with aripiprazole doses between 2 and 40 mg daily. Occupancy values were very high, ranging from a mean of 72% at 2 mg/day to 97% at 40 mg/day. Changes in the PANSS positive symptom subscale correlated positively with receptor occupancy in the striatum, but not in extrastriatal brain regions. Unfortunately, since plasma levels were not measured in two patients, these authors related occupancy values to doses rather than plasma levels. Thus, EC_{50} values are not reported. Instead, they calculated ED_{80} values (effective dose 80: the dose, that is associated with 80% occupancy). The mean ED_{80} from striatal regions was 5.6 mg and the mean ED_{80} from extrastriatal

TABLE 1 | PET studies reporting D_2 receptor occupancy and aripiprazole (ARI) blood concentrations.

No	Author, year	PET tracer	Design	Subjects	*Mean Dose (range) [mg/day]*	Mean ARI Conc. (range) [ng/ml]	Mean Receptor occupancy (%)	EC_{50} [ng/ml]	EC_{90} (estimated from EC_{50}) [ng/ml]	Comment
1	(9)	[^{11}C]raclopride	Cohort study, dose response PET scans of fixed doses of ARI taken for 14 days, trough samples analyzed by HPLC with UV detection	*N* = 15; healthy volunteers; age 32 ± 9; 100% males	10 ± 12.8 (0.5–30)	NA (only in diagram)	$D_{2/3}$: 66.8 ± 25.0 (c); 66.9 ± 21.59 (p)	NA	NA	Hyperbolic relation between peak ARI conc. and D_2 occup. (p)
2	(23); (24) (same cohort)	[^{11}C]raclopride, [^{18}F]setoperone, [^{11}C]WAY100635	RCT, 3 PET scans after ARI taken for 14 days; diagnosis acc. to DSM-4. Peak levels measured with LC/MS, clinical efficacy assessments	*N* = 12; SCZ or SD; age 31 ± 7; 75% males	18.8 ± 7.7 (10–30)	220.8 ± 179.0	$D_{2/3}$: 86.6 ± 3.7 (p), 92.9 ± 5.7 (c), 91.0 ± 4.0 (cs); 5-HT_2: 54.0 ± 15.3 (tc), 59.4 ± 12.9 (fc); 5-HT_{1A}: 16.2 ± 14.3 (tc), 16.5 ± 13.8 (fc)	NA	NA	ARI and DARI conc. correlated with D_2 occup. (p and s). No corr. between occup. and clinical or well-being scores. EPS in 2 patients with occup. >90%
3	(18)	[^{18}F]fallypride	Cohort study with unmedicated vs. medicated patients, trough serum concentrations in steady-state measured with HPLC	*N* = 16/8 (medicated/ unmedicated); SCZ or SD (DSM-4); age 30; 94% males	18.8 ± 7.2 (5–30)	245 ± 307	$D_{2/3}$: 83 ± 1 (p), 84 ± 1 (c), 85 ± 7 (t)	10 ± 4 (p) 9 ± 4 (c)	90 (p), 81 (c)	Complete occup. with ARI conc. >100–150 ng/ml. Lower EC_{50} in thalamus (6 ± 2 ng/mL)
4	(20)	[^{18}F]fallypride	Cohort study, fixed doses of ARI taken for min. 14 days, serum conc. measured with RP LC with UV, clinical efficacy assessments	*N* = 19; SCZ or SD (DSM-4); age 29; 79% males	13.9 ± 11 (2–40)	NA (excl. in analysis)	$D_{2/3}$: NA 79.8 ± 14.8 (s) in 15 mg	ED_{80} 5.63 ± 1.0 (s) approx. 100 ng/ml	NA	Dose correlated with ARI conc., PANSS positive scale corr. with D_2 occup. (s). No EPS.
5	(19)	[^{11}C]raclopride, L-[β-^{11}C]DOPA	Cohort study on dopamine synthesis capacity, PET scans after single dose of ARI, serum conc. measured with LC/MS	*N* = 12; healthy volunteers; age 24.1 ± 3.2; 100% males	5.3 ± 2.3 (3–9)	23.8 ± 11.3	$D_{2/3}$: 67.2 ± 9.7 (c), 64.3 ± 8.9 (p)	NA	NA	No changes in dopamine synthesis capacity.
6	(21)	[^{11}C]raclopride	RCT, single dose of aripiprazole after fasting, sampling up to 120 h	*N* = 18; healthy volunteers; age 22.9 ± 2.4; 100% males	12.7 ± 11.5 (2–30)	Peak: 3.4 ± 0.9 per mg	$D_{2/3}$: 61.7 ± 21.2 (s)	11.1 (s)	99.9 (s)	Values reported for PK model; PK/PD model estimates EC_{90} of 77.4 ng/mL (s)
7	(26)	[^{11}C]raclopride, [^{11}C]FLB457	Cohort single dose study on extrastriatal binding of ARI, peak conc. measured with LC/MS	*N* = 11; healthy volunteers; age 23.7 ± 4.0; 100% males	6	29.4 ± 4.8	$D_{2/3}$: 74.1 ± 6.7 (c), 70.1 ± 6.3 (p), 57.6 ± 6.7 (t), 51.3 ± 9.2 (fc), 58.4 ± 3.0 (tc)	9.9 (s), 12.2 (p), 18.9 (t), 24.3 (fc), 18.2 (tc)	89.1 (s), 109.8 (p)	Concentration reported for raclopride scans; lower in FLB457. No preferential extrastriatal binding of ARI
8	(22)	[^{11}C]raclopride and [^{18}F]FDG	RCT, PET and fMRI study with single dose of aripiprazole after fasting, sampling before scans	*N* = 15; healthy volunteers; age 23.1 ± 2.4; 100% males	12.4 ± 11.4 (2–30)	15.0 ± 14.3	$D_{2/3}$: 50.2 ± 22.0 (s)	NA	NA	Reaction times in working memory task and metabolic change in frontal lobe pos. corr. with D_2 occup.
9	(25)	[^{11}C]raclopride	Cohort study; PET and fMRI scans performed after flexible ARI; trough samples in the steady-state	*N* = 7; SCZ (DSM-4); age 32; 28.6% males	14.2 ± 12 (2–30)	289.9 ± 325.2	$D_{2/3}$: 65.0 ± 8.6 (s)	NA	NA	Error rates and reaction time in working memory task pos. corr. with D_2 occup.

c, cortex; fc, frontal cortex; p, putamen; s, striatum; tc, temporal cortex; t, thalamus; NA, no information available; RCT, randomized-controlled trial; SCZ, Schizophrenia; SD, schizoaffective disorder.

TABLE 2 | PET studies reporting D_2 receptor occupancy and brexpiprazole (BXP) blood concentrations.

No	Author, year	PET Tracer	Design	Subjects	*Mean Dose (range) [mg/day]*	Mean BXP Conc. (range) [ng/ml]	Mean Receptor Occupancy (%)	EC_{50} [ng/ml]	EC_{90} (estimated from EC_{50}) [ng/ml]	Comment
1	(28)	[^{11}C]raclopride	Cohort study with dose response PET of BXP after single doses (phase 1). Plasma samples measured with HPLC	*N* = 15; healthy volunteers; age 33.9 ± 6.8; 93.3% males	2.68 (0.25–6)	32.5 ± 25.8	$D_{2/3}$ (p and c): 0.25 mg: < 20; 2–4 mg: 59–75; 5–6 mg: 77–88	7.75 (c), 8.13 (p)	69.8 (c), 73.2 (p)	BXP AUC and c_{max} increased with dose, no ADR observed in study.
2	(36)	[^{11}C]-(+)-PHNO, [^{11}C]CUMI101, [^{11}C]MDL100907, [^{11}C]DASB	Cohort study comparing patients at baseline (unmedicated) and medicated, trough serum conc. at steady-state measured with HPLC	*N* = 12; SCZ (DSM-4); age 42 ± 8; 58.3% males	3.0 (1–4), at day 4–10	82 ± 59 (*N* = 7 from D_2 diagram)	$D_{2/3}$: 47.7 ± 38.5 SERT: −3 ± 15 5-HT_{1A}: 4 ± 6 5-HT_{2A}: 36.5 ± 20.9	22 (s)	198 (s)	Dose dependent binding for D_2 and 5-HT_{2A} receptors, not detectable for D_3. EC_{50} from non-linear model. Values for other models ranged up to 52 ng/ml.

c, cortex; p, putamen; s, striatum; t, thalamus; SCZ, Schizophrenia.

TABLE 3 | PET studies reporting D_2 receptor occupancy and cariprazine (CP) blood concentrations (*converted; conversion factor 2.34).

No	Author, year	PET tracer	Design	Subjects	*Mean Dose (range) [mg/day]*	Mean CP conc. (range) [ng/ml]	Mean receptor Occupancy (%)	EC_{50} [ng/mL] (*converted; conversion factor 2.34)	EC_{90} (estimated from EC_{50}) [ng/ml] (*converted; conversion factor 2.34)	Comment
1	(30)	[^{11}C]raclopride, [^{11}C]MNPA, [^{11}C]WAY-100635	Animal PET study after single doses of CP, plasma samples measured with HPLC	*N* = 3; healthy monkeys (macaca fascicularis) 3–4 kg weight	(a) 1–5 μg/kg (b) 30–300 μg/kg	(a) < 1.0 (b) 3.1–34.1	$D_{2/3}$: 5–94% (antagonist); $D_{2/3}$: 45–80% (agonist); 5-HT_{1A}: 18–30%	NA	NA	Dose dependent occupancy of 5–90% of D_2/D_3 receptors in striatum of monkeys
2	(13)	[^{11}C]-(+)-PHNO	Cohort study after single doses of CP, plasma samples measured with HPLC	*N* = 9; SCZ; age 42 ± 8; 58.3% males	4.5 (1–12), at day 5–15	12.4 ± 13.1	D_2: 0.91; D_3: 0.78; (regions accounted for: c, p, vs, t, globus pallidus, substantia nigra/ventral tegmental area)	D_2: 4.14 ± 0.91*; D_3: 3.32 ± 0.87*	D_2: 37.26*; D_3: 29.88*	Near complete D_2 and D_3 occup. after 12 mg for 2 weeks. One patient withdrew due to emesis. PK-PD analysis reports higher EC_{50} values of 9.0 (D_3) and 30.5 (D_2).

c, cortex; p, putamen; t, thalamus; vs, ventral striatum; NA, no information available.

regions 3.9 mg. While this significant difference indicates a high binding in extrastriatal brain regions, the 1.7 mg difference is clinically meaningless. The study is in line with the one by Gründer et al. (18) insofar as it indicates that $D_{2/3}$ receptors are almost completely occupied by aripiprazole at doses as low as 10 mg/day (20).

Takahata et al. (26) assessed striatal $D_{2/3}$ receptor occupancy with [^{11}C]raclopride and extrastriatal occupancy with [^{11}C]FLB457. They administered single oral doses of 6 mg aripiprazole to 11 healthy male volunteers 150 min prior to the PET scan. While they could not find differential binding in striatal and extrastriatal regions, $D_{2/3}$ occupancy was 74% in the caudate and 70% in the putamen. The corresponding mean plasma concentrations were 29.4 ng/ml for aripiprazole and 1.4 ng/ml for dehydroaripiprazole. Based on these values, the calculated EC_{50} values were 9.9 ng/ml for the striatum and 12.2 ng/ml for the putamen. However, Takahata et al. (26) based the calculation of their EC_{50} values on plasma concentrations of the parent (aripiprazole) compound only (K. Takahata, personal communication). Because the concentrations of the metabolite were so low in that study (the PET scan was started 150 min after administration of the drug), its contribution to total occupancy was most likely very small. With prolonged treatment, the effect of dehydroaripiprazole on EC_{50} estimates is substantial (18).

Ito et al. (19) administered single oral aripiprazole doses in the range between 3 and 9 mg to twelve healthy men. They measured $D_{2/3}$ receptor occupancy with [^{11}C]raclopride PET and dopamine synthesis capacity with L-[β-^{11}C]DOPA. The mean striatal $D_{2/3}$ occupancies were 55% (putamen) and 57% (caudate) at 3 mg, 69 and 73% at 6 mg, and 76 and 78% at 9 mg. Plasma concentrations of aripiprazole and dehydroaripiprazole were assessed separately. They were 12 + 0.4 ng/ml at 3 mg, 29 + 0.9 ng/ml at 6 mg, and 40 + 1.4 ng/ml at 9 mg. EC_{50} values are not reported by Ito et al. (19). However, from the reported data a value of approximately 10 ng/ml can be roughly estimated.

Kim et al. (22) assessed $D_{2/3}$ receptor occupancy with [^{11}C]raclopride PET in 15 healthy volunteers after administration of single oral aripiprazole doses. In addition, they measured glucose metabolism with [^{18}F]FDG and assessed cognitive performance. Mean $D_{2/3}$ receptor occupancy was 16% after 2 mg aripiprazole, 36% after 5 mg, 63% after 10 mg and 73% after 30 mg. The corresponding aripiprazole plasma concentrations (there is no information in the paper on determination of metabolites) were 2.6, 5.8, 13.2, and 35.4 ng/ml. Although these values were determined after single doses in healthy subjects, they are in line with the EC_{50} values of approximately 10 ng/ml determined after chronic treatment in patients with schizophrenia (18, 26). Greater striatal $D_{2/3}$ receptor occupancy was associated with lower frontal glucose metabolism, and greater reduction in frontal metabolism corresponded to longer reaction times (22).

The same authors compared two different analytical approaches on data from 18 healthy subjects (21), who received the same single aripiprazole doses as those applied in Kim et al. (22). It has to be assumed that the subject samples in these two studies are overlapping. The mean $D_{2/3}$ receptor occupancy in this somewhat larger sample was 30% after 2 mg aripiprazole, 54% after 5 mg, 72% after 10 mg and 82% after 30 mg. The authors calculated an EC_{50} of 11.1 ng/ml with the conventional pharmacodynamic model. When they applied a novel PK-PD model, they found a slightly lower EC_{50} of 8.6 ng/ml. This difference might be considered negligible for clinical purposes, and when taking into account that these values are omitting the contribution of the metabolite to total aripiprazole occupancy.

Shin et al. (25) measured $D_{2/3}$ receptor occupancy in seven patients with schizophrenia and related striatal occupancy to cognitive performance. They found that patients with higher occupancy performed better in certain cognitive dimensions such as working memory and reaction time (25). While these authors determined aripiprazole plasma levels at times of the PET scans, they did not report EC_{50} values.

Conclusion for Clinical Practice

Among the three available partial dopamine agonist antipsychotics, by far the broadest molecular imaging database exists for aripiprazole. Nine PET studies have been conducted over the last 20 years. Although only two of them estimated EC_{50} values (18, 26), the evidence regarding a therapeutic reference range that can be derived from those studies is appealingly consistent. Above a threshold of approximately 100 ng/ml aripiprazole (parent compound only) $D_{2/3}$ receptors are close to being completely occupied. When the active moiety (aripiprazole + dehydroaripiprazole) is considered, this value is 180 ng/ml.

The "Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017" (17) reports a therapeutic reference range of 100 – 350 ng/ml for the parent compound and 150 – 500 ng/ml for the active moiety. The lower thresholds are in good agreement with the imaging-based values. The upper thresholds are somewhat arbitrary in nature, since much higher values are tolerated by many patients in clinical practice. However, there are hints in the literature that point to an increased EPS risk at higher plasma concentrations (20).

Brexpiprazole

Two PET studies that measured $D_{2/3}$ receptor occupancy are available for brexpiprazole (27, 28) (**Table 2**). One study was conducted in healthy subjects after the administration of single oral brexpiprazole doses (28), the second study assessed D_2/D_3 receptor occupancy as well as 5-HT_{1A}, 5-HT_{2A} and serotonin transporter (SERT) occupancies in a total of 12 patients with schizophrenia after 10 days treatment (27).

Wong et al. (28) administered single brexpiprazole doses in the range between 0.5 and 6 mg to 15 healthy subjects and determined $D_{2/3}$ receptor occupancy with [^{11}C]raclopride at two different time points post-dose (4 h and 23.5 h). The mean $D_{2/3}$ receptor occupancy in putamen and caudate nucleus increased with increasing doses, with less than 20% at the 0.25 mg dose and values above 80% at the 6 mg dose. Receptor occupancy remained in the similar range 23.5 h after drug administration. At the clinically recommended brexpiprazole doses of 2–4 mg/day, $D_{2/3}$ receptor occupancies ranged from 59 to 75% at 4 h and from 53 to 74% at 23.5 h post-dose. When the estimated attainable maximum occupancy E_{max} was unconstrained, it was 89% for the

TABLE 4 | Main pharmacokinetic parameters derived from PET studies of aripiprazole, brexpiprazole and cariprazine.

Partial agonists and active metabolites	Recommendation to use TDM	Half-live ($t_{1/2}$)	Therapeutic reference range	Laboratory alert level
Aripiprazole	Recommended	60–80 h	100–350 ng/mL	1,000 ng/mL
Aripiprazole plus dehydroaripiprazole		30–47 days	150–500 ng/mL	
Brexpiprazole	Useful	90 h	40–140 ng/mL	280 ng/mL
Cariprazine	Useful	50–120 h	10–20 ng/mL	40 ng/mL
N-desmethyl cariprazine				
N,N-didesmethyl cariprazine		2–3 weeks		

putamen and 95% for the caudate, with the corresponding EC_{50} values being 8.1 and 7.8 ng/ml, respectively (28). When E_{max} was constrained to 100%, EC_{50} was 11.5 and 9.0 ng/ml, respectively.

When the estimation of an EC_{90} value is conducted based on an EC_{50} of 10 ng/ml, EC_{90} is 90 ng/ml, with an EC_{50} of 9 ng/ml the estimated EC_{90} is 81 ng/ml, and with an EC_{50} of 11 ng/ml the estimated EC_{90} is 99 ng/ml. Thus, the study suggests that at brexpiprazole plasma concentrations of 80–100 ng/ml striatal D_2/D_3 receptors are almost completely occupied by the drug.

The second PET study with brexpiprazole was a multi-tracer study to characterize the compound's binding to four different molecular targets: dopamine D_2/D_3, serotonin 5-HT_{1A} and 5-HT_{2A} receptors, and the serotonin transporter (SERT) (27). While D_2/D_3 receptor occupancy is usually measured with antagonist radiotracers like [^{11}C]raclopride or [^{18}F]fallypride, this study applied the agonist tracer [^{11}C]-(+)-PHNO. [^{11}C]-(+)-PHNO allows the differentiation of binding to D_2 and D_3 receptors, but it systematically underestimates D_2 occupancy by about 20% compared to assessment with antagonist radiotracers (29). After 10 days of treatment of patients with schizophrenia with brexpiprazole, the mean D_2 receptor occupancy was 64% following 1 mg/day and 80% following 4 mg/day. The corresponding estimated EC_{50} values were, depending on the brain region, between 22 and 52 ng/ml (27). From these numbers an EC_{90} value between 198 and 495 ng/ml can be derived. Thus, in this study, at the same plasma concentrations the measured D_2 receptor occupancies are substantially lower than in the study published by Wong et al. (28). While brexpiprazole did not significantly occupy the 5-HT_{1A} receptor and the SERT, 5-HT_{2A} receptor occupancy was 28% following 1 mg and 45% following 4 mg brexpiprazole (27).

Conclusion for Clinical Practice

The two available molecular imaging studies are inconclusive with regard to their clinical implications. One study determined D_2/D_3 receptor occupancy after single brexpiprazole doses (28); the second study used an agonist radiotracer that systematically underestimates D_2 receptor occupancy (27, 29). Taking this underestimation into account, it seems reasonable to believe that striatal D_2/D_3 receptors are almost or completely saturated at 80–100 ng/ml brexpiprazole in plasma, and probably even at lower concentrations. However, this has to be confirmed in a study in patients treated with multiple doses and with an antagonist radiotracer.

The "Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017" (17) reports a therapeutic reference range of 40 – 140 ng/ml for brexpiprazole. Based on the available PET studies, the lower limit value would tend to be too low, while the upper limit value could also be exceeded in clinical practice.

Cariprazine

Two PET studies quantified D_2/D_3 receptor occupancy under treatment with cariprazine, one in monkeys (30) and one in humans (13) (**Table 3**). Seneca et al. (30) studied the occupancy of D_2 and D_3 dopamine receptors and 5-HT_{1A} serotonin receptors after a single low and a single high cariprazine dose, respectively, in three monkeys. Girgis et al. (13) assessed the occupancy of D_2/D_3 receptors by cariprazine in eight patients with schizophrenia at various doses and time-points post-dose.

Seneca et al. (30) in their study in three monkeys applied three different radiotracers: D_2/D_3 receptor occupancy was quantified both with an agonist ([^{11}C]MNPA) and an antagonist tracer ([^{11}C]raclopride), and [^{11}C]WAY-100635 was used for assessment of 5-HT_{1A} receptor occupancy. A total of 15 PET examinations were carried out. Each monkey was subjected to a baseline examination and then scanned again after intravenous administration of either a low (1–5 μg/kg body weight) or a high (30–300 μg/kg) dose of cariprazine. Blood samples for determination of the plasma concentrations of cariprazine and its two main metabolites desmethyl- (DCAR) and didesmethyl cariprazine (DDCAR) were taken at prespecified time-points. At doses of 5 and 30 μg/kg cariprazine caused a dose-dependent D_2/D_3 receptor occupancy of approximately 45 and 80%, while the highest dose (300 μg/kg) was associated with 94% occupancy. Occupancy values did not differ for agonist and antagonist radiotracers. Occupancy of 5-HT_{1A} receptors was 10–20% at the lower doses, and it plateaued at 30% with the highest dose (30). Although the authors measured plasma levels of cariprazine and its metabolites, they did not calculate EC_{50} values. Therefore, an EC_{90} value cannot be calculated based on that study.

The second study assessed cariprazine's occupancy of D_2/D_3 receptors in patients with schizophrenia (13). The radioligand used was the agonist tracer [^{11}C]-(+)-PHNO, and the patients were scanned at baseline and on days 1, 4, and 15 of treatment with cariprazine between 1 and 12 mg/day. Plasma (and cerebrospinal fluid) samples were analyzed for concentrations of cariprazine, DCAR, and DDCAR. After treatment with the lowest cariprazine dose (1 mg/day), D_3 occupancy was 76% (range 58–89%) and D_2 occupancy 45% (range 14–64%). At the dose of 3 mg/day, the mean D_3 and D_2 receptor occupancies were 92% (range 86–96%) and 79% (range 68–88%), respectively. Thus, at

those lower doses, cariprazine binding was more selective for D_3 over D_2 receptors. At higher doses, this selectivity is lost. The dose of 12 mg/day led to complete saturation of both receptor subtypes. Since both metabolites are pharmacologically active, estimation of EC_{50} values were carried out with active moiety values (cariprazine + DCAR + DDCAR). Also, EC_{50} estimation was conducted separately for D_2 and D_3 receptors and for acute (occupancy estimation on days 1 and 4) and for subchronic treatment (occupancy estimation on day 15).

After acute dosing, the EC_{50} was 0.61 ng/ml for the D_3 and 0.76 ng/ml for the D_2 receptor. After 15 days treatment, when more of the slow-forming active metabolites, especially DDCAR, have accumulated, the EC_{50} values were 1.64 ng/ml for the D_3 and 5.56 ng/ml for the D_2 receptor. This suggests greater D_3 selectivity of cariprazine with longer treatment, which is most likely explained by the grater D_3 selectivity of DDCAR. DDCAR, which has a very long half-life, develops very slowly during treatment. While cariprazine is the dominant compound during the first few days of treatment, the active moiety mainly consists of DDCAR and cariprazine during chronic treatment (13). From the EC_{50} values estimated at day 15, the corresponding EC_{90} values are 14.8 ng/ml for the D_3 receptor and 50.0 ng/ml for the D_2 receptor.

Conclusion for Clinical Practice

Only one human PET study that provides EC_{50} estimates has been published, and this was conducted with the agonist radiotracer [^{11}C]-(+)-PHNO. PET studies with the antagonist radiotracers [^{11}C]raclopride and [^{18}F]fallypride have been published as abstracts only. While the available PET study in monkeys suggests that $D_{2/3}$ receptor occupancy is similarly high when assessed with the agonist [^{11}C]MNPA and the antagonist [^{11}C]raclopride, the D_3-preferring agonist [^{11}C]-(+)-PHNO might still underestimate D_2 occupancy (29). The study by Girgis et al. (13) suggests that D_3 and D_2 receptors are almost completely saturated at approximately 15 and 50 ng/ml. The "Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017" (17) reports a therapeutic reference range of 10 – 20 ng/ml for cariprazine. However, the latter range is based on cariprazine levels only, while the EC_{50} values estimated by Girgis et al. (13) are based on active moiety values. A therapeutic reference range for the active moiety (cariprazine + DCAR + DDCAR) will be necessarily higher than one for the parent compound only (see discussion of aripiprazole above). However, due to a lack of data, such a reference range has not been defined yet.

DISCUSSION

Molecular imaging, especially with PET, has been used since the late 1980s for determination of rational antipsychotic dosing. These studies did not only demonstrate that the doses of some of the classical antipsychotics such as haloperidol over the first decades of their clinical use were irrationally high (31). They also showed that some of the newer (second-generation) antipsychotics were initially not dosed correctly. The best example is risperidone. This compound was approved and marketed for the treatment of schizophrenia in the United States in 1993 and soon thereafter throughout the world. The highest approved dose was 16 mg, and two-digit doses were quite commonly used during the first several years after market access (32). The first PET study with risperidone was published in the year of market entry (33). Three healthy volunteers were administered a single 1 mg oral dose of risperidone. The determined $D_{2/3}$ receptor occupancy was approximately 50% even at this very low dose. Subsequent studies showed that the incidence of EPS rises at doses above 6 mg risperidone daily, the dose at which $D_{2/3}$ occupancy crosses the 80% threshold in most patients (34). It took years for the results of these PET studies to change clinical practice of excessive doses, years in which many patients suffered unnecessary side effects due to incorrect dosages. Thus, since the mid-1990s at the latest, the characterization of target engagement of new antipsychotics has been part of their development program.

This is also true for the class of dopamine partial agonists. Aripiprazole was the prototype of this class of new drugs, it entered the market in 2002 in the United States. With the publication of the first PET study on this compound (9), it became immediately clear that the magnitude of its target engagement has to be interpreted differently from antagonist antipsychotics, and that it does not follow the "65 – 80% therapeutic window" rule for D_2 antagonists (10) (**Figure 1**). Aripiprazole is still by far the most extensively studied partial agonist antipsychotic, and – as demonstrated in this paper – the data are very consistent in showing that more than 90% of all $D_{2/3}$ dopamine receptors are occupied above a plasma concentration of approximately 100 ng/ml of the parent compound. Theoretically, substantially increasing the plasma concentration above this value is probably of no benefit to the patient. This is underlined by a recent dose-response meta-analysis that demonstrated that the 95% effective dose of aripiprazole is 11.5 mg/day and that its antipsychotic efficacy does not increase above this dose (35). The plasma concentration, however, can substantially vary at a given dose (18). Thus, monitoring of the plasma concentration is certainly a better tool for tailoring treatment to the individual patient. Although factors that characterize a patient individually, e.g., his psychopathology, are likely to influence the measurement of receptor availability, these influences are small and negligible compared to the effects of pharmacological treatment *per se*.

The situation is much less clear for the other two available dopamine partial agonist, brexpiprazole and cariprazine. As outlined in this paper, the few PET studies that have been published with these compounds, are somewhat inconclusive with regard to a therapeutic reference range. Specifically, a lower threshold at which almost complete occupancy of $D_{2/3}$ receptors can be assumed, cannot be derived from these studies with sufficient certainty. It would be desirable if at least one PET study that met certain methodological standards were carried out when a new antipsychotic is launched on the market, or even before it is launched. A methodological standard procedure for PET studies aiming at supporting therapeutic concentration ranges has not been specified yet. Certainly, such investigations

should be performed in a minimum number of patients (n = 15 or larger) who have been treated for a sufficient period of time (minimum steady-state) over the entire dose range. An antagonist should be used as the radiotracer ([^{11}C]raclopride or [^{18}F]fallypride), as extensive reference data are available for these. Studies with agonists as radioligands or those with preferential binding to D_3 receptors could supplement the characterization in individual cases. Not only a large variance in reporting the results across studies, but also a considerable heterogeneity in the study populations (i.e., healthy volunteers vs. patients; dose and blood sampling designs; measurement of solely the major analyte vs. the analyte plus active metabolites) impede a comparability of the results. In terms of design, it has to be differentiated between studies that do or do not aim at linking PET findings with clinical effects. In order to be able to report a reliable relationship between receptor occupancy and clinical effects, the study designs have to be far more complex than most of the studies reviewed in this work (i.e., including a randomized, double-blind study phase).

In summary, this overview shows that molecular imaging is an excellent tool for characterizing antipsychotics in general and partial dopamine agonists in particular (**Table 4**). This is not just an academic exercise. Once the relationship between plasma concentrations of a substance and its binding to the molecular target in the brain has been clarified (which can be done with little effort), the determination of the plasma concentration in the individual patient allows for tailor-made treatment at the lowest possible cost.

AUTHOR CONTRIBUTIONS

GG developed the first draft of the protocol. XH contributed to the writing of the manuscript, to the development of the search strategy, and critical appraisal. CS contributed with writing and critical appraisal. All authors have read and approved the final manuscript.

REFERENCES

1. Cumming P, Abi-Dargham A, Gründer G. Molecular imaging of schizophrenia: neurochemical findings in a heterogeneous and evolving disorder. *Behav Brain Res.* (2021) 398:113004. doi: 10.1016/j.bbr.2020.113004
2. Gründer G, Hiemke C, Paulzen M, Veselinovic T, Vernaleken I. Therapeutic plasma concentrations of antidepressants and antipsychotics: lessons from PET imaging. *Pharmacopsychiatry.* (2011) 44:236–48. doi: 10.1055/s-0031-1286282
3. Wong DF, Tauscher J, Gründer G. The role of imaging in proof of concept for CNS drug discovery and development. *Neuropsychopharmacology.* (2009) 34:187–203. doi: 10.1038/npp.2008.166
4. Farde L, Nordström AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch Gen Psychiat.* (1992) 49:538–44. doi: 10.1001/archpsyc.1992.01820070032005
5. Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L. Suggested minimal effective dose of risperidone based on PET-measured D2 and 5-HT2A receptor occupancy in schizophrenic patients. *Am J Psychiat.* (1999) 156:869–75. doi: 10.1176/ajp.156.6.869
6. Gründer G, Hippius H, Carlsson A. The 'atypicality' of antipsychotics: a concept re-examined and re-defined. *Nat Rev Drug Discov.* (2009) 8:197–202. doi: 10.1038/nrd2806
7. Gründer G, Landvogt C, Vernaleken I, Buchholz HG, Ondracek J, Siessmeier T, et al. The striatal and extrastriatal D2/D3 receptor-binding profile of clozapine in patients with schizophrenia. *Neuropsychopharmacology.* (2006) 31:1027–35. doi: 10.1038/sj.npp.1300931
8. Vernaleken I, Janouschek H, Raptis M, Hellmann S, Veselinovic T, Bröcheler A, et al. Dopamine D2/3 receptor occupancy by quetiapine in striatal and extrastriatal areas. *Int J Neuropsychopharmacol.* (2010) 13:951–60. doi: 10.1017/S1461145710000374
9. Yokoi F, Gründer G, Biziere K, Stephane M, Dogan AS, Dannals RF, et al. Dopamine D2 and D3 receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [11C]raclopride. *Neuropsychopharmacology.* (2002) 27:248–59. doi: 10.1016/S0893-133X(02)00304-4
10. Gründer G, Carlsson A, Wong DF. Mechanism of new antipsychotic medications: occupancy is not just antagonism. *Arch Gen Psychiatry.* (2003) 60:974–7.
11. Gründer G, Kungel M, Ebrecht M, Görögcs T, Modell S. Aripiprazole: pharmacodynamics of a dopamine partial agonist for the treatment of schizophrenia. *Pharmacopsychiatry.* (2006) 39:S21–5. doi: 10.1055/s-2006-931485
12. Citrome L. Brexpiprazole: a new dopamine D2 receptor partial agonist for the treatment of schizophrenia and major depressive disorder. *Drugs Today.* (2015) 51:397–414. doi: 10.1358/dot.2015.51.7.2358605
13. Girgis RR, Slifstein M, D'Souza D, Lee Y, Periclou A, Ghahramani P, et al. Preferential binding to dopamine D3 over D2 receptors by cariprazine in patients with schizophrenia using PET with the D3/D2 receptor ligand [(11)C]-(+)-PHNO. *Psychopharmacology.* (2016) 233:3503–12. doi: 10.1007/s00213-016-4382-y
14. Veselinović T, Paulzen M, Gründer G. Cariprazine, a new, orally active dopamine D2/3 receptor partial agonist for the treatment of schizophrenia, bipolar mania and depression. *Expert Rev Neurother.* (2013) 13:1141–59. doi: 10.1586/14737175.2013.853448
15. Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC. Predicting dopamine D2 receptor occupancy from plasma levels of antipsychotic drugs: a systematic review and pooled analysis. *J Clin Psychopharmacol.* (2011) 31:318–25. doi: 10.1097/JCP.0b013e318218d339
16. Suhara T, Okauchi T, Sudo Y, Takano A, Kawabe K, Maeda J, et al. Clozapine can induce high dopamine D(2) receptor occupancy in vivo. *Psychopharmacology.* (2002) 160:107–12. doi: 10.1007/s00213-001-0967-0
17. Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K, et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. *Pharmacopsychiatry.* (2018) 51:9–62. doi: 10.1055/s-0043-116492
18. Gründer G, Fellows C, Janouschek H, Veselinovic T, Boy C, Bröcheler A, et al. Brain and plasma pharmacokinetics of aripiprazole in patients with schizophrenia: an [18F]fallypride PET study. *Am J Psychiat.* (2008) 165:988–95. doi: 10.1176/appi.ajp.2008.07101574
19. Ito H, Takano H, Arakawa R, Takahashi H, Kodaka F, Takahata K, et al. Effects of dopamine D2 receptor partial agonist antipsychotic aripiprazole on dopamine synthesis in human brain measured by PET with L-[beta-11C]DOPA. *PLoS One.* (2012) 7:e46488. doi: 10.1371/journal.pone.0046488
20. Kegeles LS, Slifstein M, Frankle WG, Xu X, Hackett E, Bae SA, et al. Dose-occupancy study of striatal and extrastriatal dopamine D2 receptors by aripiprazole in schizophrenia with PET and [18F]fallypride. *Neuropsychopharmacology.* (2008) 33:3111–25. doi: 10.1038/npp.2008.33
21. Kim E, Howes OD, Kim BH, Jeong JM, Lee JS, Jang IJ, et al. Predicting brain occupancy from plasma levels using PET: superiority of combining pharmacokinetics with pharmacodynamics while modeling the relationship. *J Cereb Blood Flow Metab.* (2012) 32:759–68. doi: 10.1038/jcbfm.2011.180
22. Kim E, Howes OD, Turkheimer FE, Kim BH, Jeong JM, Kim JW, et al. The relationship between antipsychotic D2 occupancy and change in frontal metabolism and working memory: a dual [(11)C]raclopride and [(18) F]FDG imaging study with aripiprazole. *Psychopharmacology.* (2013) 227:221–9. doi: 10.1007/s00213-012-2953-0

23. Mamo D, Graff A, Mizrahi R, Shammi CM, Romeyer F, Kapur S. Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. *Am J Psychiat.* (2007) 164:1411–7. doi: 10.1176/appi.ajp.2007.06091479
24. Mizrahi R, Mamo D, Rusjan P, Graff A, Houle S, Kapur S. The relationship between subjective well-being and dopamine D2 receptors in patients treated with a dopamine partial agonist and full antagonist antipsychotics. *Int J Neuropsychopharmacol.* (2009) 12:715–21. doi: 10.1017/S1461145709000327
25. Shin S, Kim S, Seo S, Lee JS, Howes OD, Kim E, et al. The relationship between dopamine receptor blockade and cognitive performance in schizophrenia: a [(11)C]-raclopride PET study with aripiprazole. *Transl Psychiat.* (2018) 8:87. doi: 10.1038/s41398-018-0134-6
26. Takahata K, Ito H, Takano H, Arakawa R, Fujiwara H, Kimura Y, et al. Striatal and extrastriatal dopamine D2 receptor occupancy by the partial agonist antipsychotic drug aripiprazole in the human brain: a positron emission tomography study with [^{11}C]raclopride and [^{11}C]FLB457. *Psychopharmacology.* (2012) 222:165–72. doi: 10.1007/s00213-011-2633-5
27. Girgis RR, Forbes A, Abi-Dargham A, Slifstein M. A positron emission tomography occupancy study of brexpiprazole at dopamine D(2) and D(3) and serotonin 5-HT(1A) and 5-HT(2A) receptors, and serotonin reuptake transporters in subjects with schizophrenia. *Neuropsychopharmacology.* (2020) 45:786–92. doi: 10.1038/s41386-019-0590-6
28. Wong DF, Raoufinia A, Bricmont P, Brašič JR, McQuade RD, Forbes RA, et al. An open-label, positron emission tomography study of the striatal D(2)/D(3) receptor occupancy and pharmacokinetics of single-dose oral brexpiprazole in healthy participants. *Eur J Clin Pharmacol.* (2021) 77:717–25. doi: 10.1007/s00228-020-03021-9
29. Graff-Guerrero A, Mamo D, Shammi CM, Mizrahi R, Marcon H, Barsoum P, et al. The effect of antipsychotics on the high-affinity state of D2 and D3 receptors: a positron emission tomography study with [11C]-(+)-PHNO. *Arch Gen Psychiat.* (2009) 66:606–15. doi: 10.1001/archgenpsychiatry.2009.43
30. Seneca N, Finnema SJ, Laszlovszky I, Kiss B, Horváth A, Pásztor G, et al. Occupancy of dopamine D2 and D3 and serotonin 5-HT1A receptors by the novel antipsychotic drug candidate, cariprazine (RGH-188), in monkey brain measured using positron emission tomography. *Psychopharmacology.* (2011) 218:579–87. doi: 10.1007/s00213-011-2343-z
31. Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiat.* (2000) 157:514–20. doi: 10.1176/appi.ajp.157.4.514
32. Chouinard G, Jones B, Remington G, Bloom D, Addington D, MacEwan GW, et al. A Canadian multicenter placebo-controlled study of fixed doses of risperidone and haloperidol in the treatment of chronic schizophrenic patients. *J Clin Psychopharmacol.* (1993) 13:25–40.
33. Nyberg S, Farde L, Eriksson L, Halldin C, Eriksson B. 5-HT2 and D2 dopamine receptor occupancy in the living human brain. A PETstudy with risperidone. *Psychopharmacology.* (1993) 110:265–72. doi: 10.1007/BF02251280
34. Kapur S, Remington G, Zipursky RB, Wilson AA, Houle S. The D2 dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. *Life Sci.* (1995) 57:L103–7. doi: 10.1016/0024-3205(95)02037-j
35. Leucht S, Crippa A, Siafis S, Patel MX, Orsini N, Davis JM. Dose-response meta-analysis of antipsychotic drugs for acute schizophrenia. *Am J Psychiat.* (2020) 177:342–53. doi: 10.1176/appi.ajp.2019.19010034
36. Girgis RR, Forbes A, Abi-Dargham A, Slifstein M. A positron emission tomography occupancy study of brexpiprazole at dopamine D(2) and D(3) and serotonin 5-HT(1A) and 493 5-HT(2A) receptors, and serotonin reuptake transporters in subjects with schizophrenia. *Neuropsychopharmacology.* (2020) 45:786–92. doi: 10.1038/s41386-019-0590-6

Conflict of Interest: GG has served as a consultant for Allergan, Boehringer Ingelheim, Institute for Quality and Efficiency in Health Care (IQWiG), Janssen-Cilag, Lundbeck, Otsuka, Recordati, ROVI, Sage, and Takeda. He has served on the speakers' bureau of Gedeon Richter, Janssen Cilag, Lundbeck, Otsuka, Recordati. He has received grant support from Boehringer Ingelheim, Lundbeck and Saladax. He is co-founder and/or shareholder of Mind and Brain Institute GmbH, Brainfoods GmbH, OVID Health Systems GmbH and MIND Foundation gGmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.