Pharmacokinetics of Antidepressants and Lithium

Variability and Clinical Implication for Individual Dose Adaptation

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Katja Grasmäder

aus

Kassel

Bonn, Dezember 2003

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der

Rheinischen-Friedrich-Wilhelms-Universität Bonn

1. Referentin:	Prof. Dr. Marie Luise Rao Klinik und Poliklinik für Psychiatrie und Psychotherapie der Universität Bonn Neurochemisches Labor
2. Referent:	Prof. Dr. Ulrich Jaehde Pharmazeutisches Institut der Universität Bonn Klinische Pharmazie

Tag der Promotion:

List of contents

1 Introduction 1
1.1 Treatment of depression 1
1.2 Mechanism of action of antidepressants and mood stabilizers 3
1.3 Pharmacokinetics 6
1.3.1 Definition of pharmacokinetic parameters 6
1.3.2 Pharmacokinetics of antidepressants and lithium 7
1.4 Factors influencing pharmacokinetics 8
1.4.1 Determination of factors influencing pharmacokinetics 8
1.4.2 Factors influencing the pharmacokinetics of antidepressants and lithium 10
1.5 Relevance of therapeutic drug monitoring of antidepressants and lithium 13
2 Aims of the thesis 19
3 Patients and methods 21
3.1 Study design 21
3.1.1 Kompetenznetz Depression: Therapeutic drug monitoring and genotyping 21
3.1.1.1 Evaluation of the impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome 22
3.1.1.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine 24
3.1.2 A computer-assisted method for lithium dose individualisation 25
3.2 Ratings 26
3.2.1 Hamilton Depression Rating Scale 26
3.2.2 Clinical Global Impression 26
3.2.3 UKU side effect rating scale 27
3.3 Blood sampling 27

3.4 Analytical methods ... 28
3.4.1 Antidepressants ... 28
3.4.2 Lithium ... 32
3.5 Genotyping ... 33
3.5.1 CYP2C9 ... 33
3.5.2 CYP2C19 ... 34
3.5.3 CYP2D6 ... 35
3.6 Pharmacokinetic analysis ... 37
3.6.1 Pharmacokinetic models ... 37
3.6.1.1 One-compartment model ... 37
3.6.1.2 Two-compartment model ... 38
3.6.2 Population pharmacokinetic analysis ... 39
3.6.2.1 Basic model building ... 40
3.6.2.2 Final model building ... 41
3.6.2.3 Model check ... 42
3.6.3 Bayesian curve fitting ... 42
3.6.3.1 Establishment of a service for lithium dose individualisation by Bayesian curve fitting ... 43
3.6.3.2 Predictive performance ... 44
3.6.3.3 Method of Pepin and colleagues (1980) ... 45
3.6.3.4 Method of Yukawa and colleagues (1993) ... 46
3.6.3.5 Method of Jermain and colleagues (1991) ... 47
3.7 Statistical analysis ... 48
3.7.1 Descriptive statistics and hypothesis testing ... 48
3.7.2 Correlation and regression ... 48
3.7.3 Receiver operating curve ... 50
3.7.4 Goodness-of-fit ... 50
4 Results ... 51

4.1 Impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome51
4.1.1 Patient characteristics 51
4.1.2 Genotype distributions.....54
4.1.3 Relationship between genotype and trough plasma concentrations 57
4.1.4 Relationship between genotype and treatment response 58
4.1.5 Relationship between genotype and side effects 59
4.1.6 Plasma concentrations and clinical outcome of poor metabolizers and ultrarapid metabolizers 60
4.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine .62
4.2.1 Patient characteristics 62
4.2.2 Therapeutic range 63
4.2.3 Population pharmacokinetic analysis 68
4.3 Computer-assisted dose individualisation of lithium 76
4.3.1 Patients characteristics.....76
4.3.2 Validation of the method.....76
5 Discussion.....81
5.1 Impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome 81
5.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine .88
5.3 Dose individualisation of lithium.....92
6 Conclusion 97
7 Summary.....99
8 References.....103
9 Publications originating from this thesis.....119
10 Acknowledgements 121
11 Erklärung.....129
12 Curriculum Vitae.....131

Abbreviations

a	Intercept in regression analysis and constant in residual error models of population pharmacokinetics
AGNP	Arbeitsgemeinschaft für Neuropsychologie und Psychopharmakologie
AIC	Akaike Information Criterion
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APA	American Psychiatric Association
AST	Aspartate aminotransferase
b	Regression coefficient in multiple linear regression analysis and constant in residual error models of population pharmacokinetics
BMI	Body mass index
bp	Base pairs
CGI	Clinical Global Impression
CIPS	Collegium Internationale Psychiatriae Scalarum
CL	Clearance
CL_{crea}	Creatinine clearance
CL/F	Clearance scaled by bioavailability
c_{max}	Plasma peak concentration
CYP	Cytochrome P450 enzyme system
CYP1A2	Cytochrome P450 isoenzyme 1A2
CYP2C9	Cytochrome P450 isoenzyme 2C9
CYP2C19	Cytochrome P450 isoenzyme 2C19
CYP2D6	Cytochrome P450 isoenzyme 2D6
CYP3A4	Cytochrome P450 isoenzyme 3A4
D	Dose
d	Day
df	Degree of freedom
DGPPN	Deutsche Gesellschaft für Psychiatrie, Psychotherapie und Nervenheilkunde
dL	Decilitre
DNA	Desoxyribonucleic acid
EDTA	Ethylene diamino tetra acetic acid
EM	Extensive metabolizer
F	Bioavailability; statistical test parameter in ANOVA
FDA	Food and Drug Administration

FOCE	First order conditional estimation method in WinNonMix™
GABA	γ-Aminobutyric acid
γ-GT	Gamma-glutamyl transferase
hr	Hour
HAMD	Hamilton Depression Rating Scale
HPLC	High-performance liquid chromatography
5-HT	Serotonin
IBW	Ideal body weight
ICD-10	International Classification of Diseases and Related Health Problems, 10th edition
IM	Intermediate metabolizer
k_{01}	Absorption rate constant
k_{10}	Elimination rate constant
k_{12}, k_{21}	Distribution rate constants from the central to the peripheral compartment in a two-compartment model
kg	Kilogram
L	Litre
LBW	Lean body weight
mACh	Muscarinic cholinergic
MAOI	Monoamine oxidase inhibitor
mg	Milligram
mL	Millilitre
mm HG	Millimetres of mercury, measurement of blood pressure
mmol	Millimol
MPE	Mean prediction error
MSE	Mean squared error
NA	Noradrenaline
n	Number
ng	Nanogram
NSAID	Nonsteroidal antiinflammatory drugs
NSSA	Noradrenergic and specific serotonergic antidepressant
OFV	Objective function value
OR	Odds Ratio
p	Significance level in statistical tests (probability of a type I error)
P	Probability
PCR	Polymerase chain reaction
PM	Poor metabolizer

r^2	Coefficient of determination in multiple linear and logistic regression analysis
r_S	Spearman's correlation coefficient
RFLP	Restriction fragment length polymorphism analysis
RIMA	Reversible inhibitor of monoamine oxidase A
S_{crea}	Serum creatinine concentration
SD	Standard deviation
SE	Standard error
SNRI	Selective noradrenaline reuptake inhibitor
ss	Sum of least squares
SSNRI	Selective serotonin and noradrenaline reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
T	Statistical test parameter in a t-test
TCA	Tri- or tetracyclic antidepressant
$t_{1/2}$	Half-life
t_{max}	Time to reach plasma peak concentrations
U	Unit
UKU	UKU side effec rating scale
UM	Ultrarapid metabolizer
V_1	Peripheral volume of distribution (two-compartment model)
V_c	Central volume of distribution (two-compartment model)
V_d	Volume of distribution (one-compartment model)
V_d/F	Volume of distribution (one-compartment model) scaled by bioavailability
WHO	World Health Organization
α	Hybrid constant in two-compartment models
β	Hybrid constant in two-compartment models
ε	Residual error of population pharmacokinetic models
η	Interindividual variability of population pharmacokinetic parameters
Θ_p	Population mean of pharmacokinetic parameters
Θ_i	Individual pharmacokinetic parameters in population pharmacokinetic analysis
Θ_1, Θ_2	Shift parameter or multiplier associated with a specific covariate in a population pharmacokinetic model
σ^2	Variance
χ^2	Statistical test parameter in χ^2-testing

1 Introduction

1.1 Treatment of depression

Depression occurs in 17% of the European population (Lecrubier 2001) and its global burden is expected to increase over the next decades. With respect to the extent of disability experienced by patients depression is estimated to become second among all diseases causing disability by the year 2020 (WHO 1996). In spite of this, the diagnosis of depression is often overlooked because it is masked by somatic symptoms and has the stigma of a mental disease. In Germany, recognition and treatment of depression still remains deficient: the diagnosis of depression is not recognised in over 30% of depressive patients and in about 50% treatment is inadequate (Sachverständigenrat für die Konzertierte Aktion im Gesundheitswesen 2001). These deficiencies are most obvious in primary care and elderly patients. Thus, the German Ministry of Education and Research initialised the “Kompetenznetz Depression” that promotes joint research between universities, state hospitals, psychiatrists and general practitioners to improve the treatment of depression.

So far, the cause of depression is not well understood but genetic, social, cognitive, psychodynamic as well as biologic factors are discussed to trigger its occurence within a vulnerability-stress-model. The German guideline for the treatment of affective disorders (Deutsche Gesellschaft für Psychiatrie, Psychotherapie und Nervenheilkunde (DGPPN) 2001) includes both, neurobiological and social factors when recommending antidepressant drug treatment and psychotherapy alone or in combination as first choice to treat unipolar depression. For bipolar depressive disorder, lithium, carbamazepine, and valproic acid are administered as mood stabilisers and antidepressants serve as additional medication in case of initial treatment failure. Initially, antidepressive medication should be given over a time period of at least three weeks before adapting the dose, changing medication or combining several antidepressants (Figure 1).

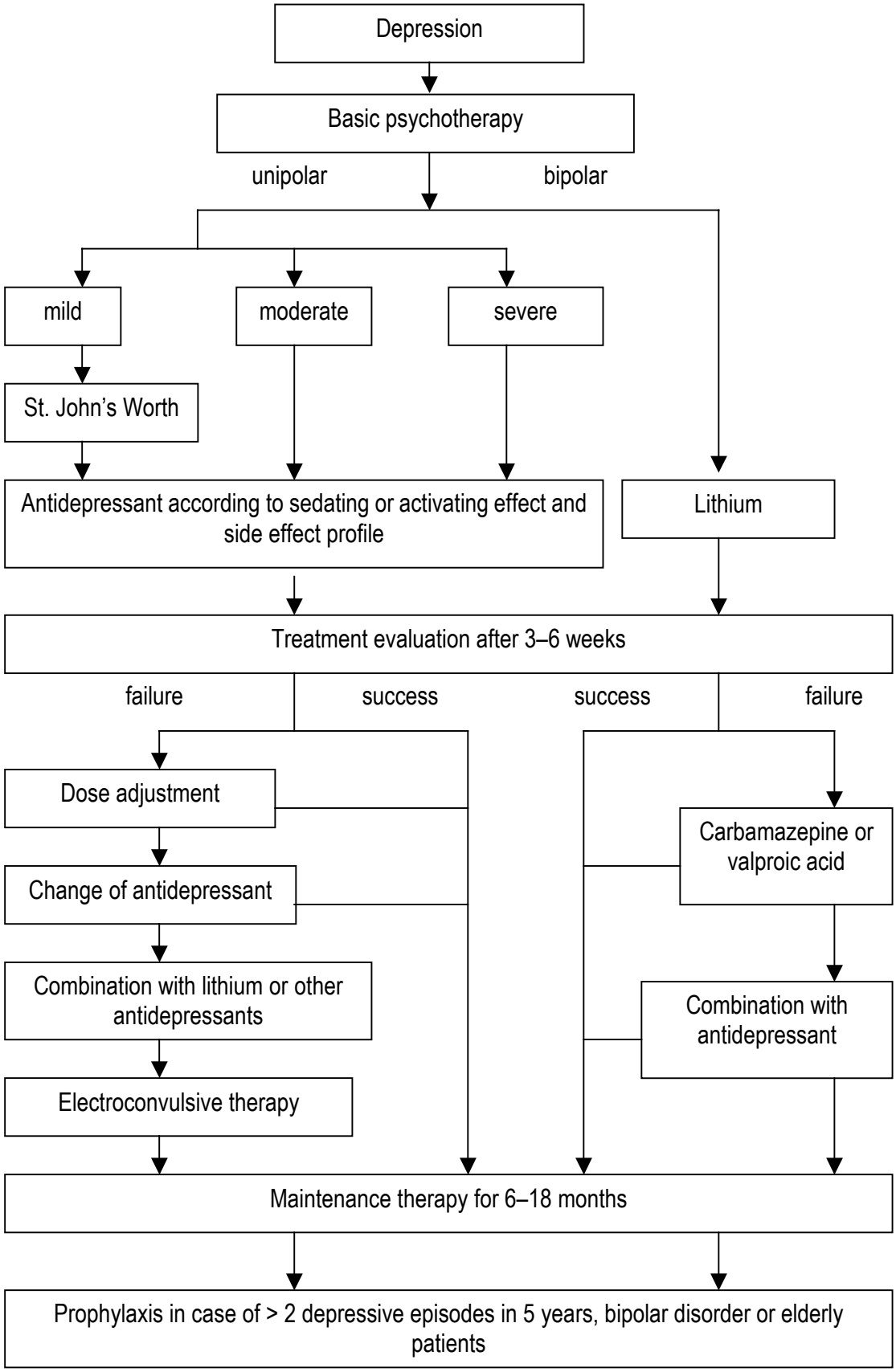
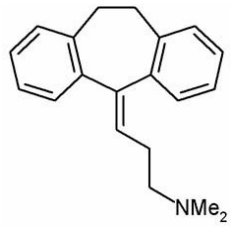


Figure 1: Treatment of depression according to the German guideline (DGPPN 2001)

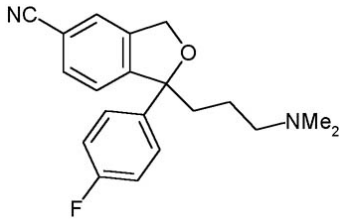
The choice of a specific antidepressant depends on the individual patient's clinical presentation, the predicted response and the antidepressant's side effect profile. Tricyclic antidepressants (TCAs) represent the first generation of antidepressants and are characterized by good clinical efficacy but high risk for vegetative side effects and life-threatening intoxications, such as arrythmia, seizures or delusion. Irreversible monoamine oxidase inhibitors (MAOI) require a special diet because of the risk of a hypertensive crisis in combination with dietary tyramine. This led to the market introduction of drugs that are better tolerated and have a low risk of toxic effects such as selective serotonin reuptake inhibitors (SSRIs), reversible monoamine oxidase A inhibitors (RIMA), selective noradrenaline reuptake inhibitors (SNRI), or new antidepressants with dual principle of action such as mirtazapine (noradrenergic and specific serotonergic antidepressant, NSSA) or venlafaxine (selective serotonin and noradrenaline reuptake inhibitor, SSNRI). These substances are equally effective and easier to handle because their side effect profile does not require careful dose increase when starting antidepressant treatment. The structures of the antidepressants studied in this thesis are given in Figure 2.

1.2 Mechanism of action of antidepressants and mood stabilizers

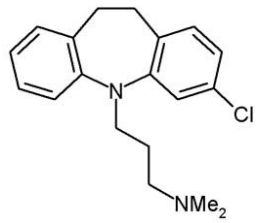
Antidepressants enhance the serotonergic, noradrenergic and dopaminergic transmission in the brain. These effects are achieved by inhibition of serotonin or noradrenaline reuptake from the synpatic cleft into the neuron (TCAs, SSRIs, SNRIs, NSSRIs), by inhibition of the monoamine metabolism via the monoamine oxidase (tranylcypromine, moclobemide) or by blocking specific receptors, for example presynaptic noradrenaline-α_2 receptors and serotonergic 5-HT_2- and 5-HT_3 receptors thus enhancing the stimulation of 5-HT_{1A} autoreceptors (mirtazapine). With chronic administration of antidepressants the monoamine receptors adapt their responsiveness by down-regulation of noradrenaline-β receptors, noradrenaline-α_2 receptors and serotonin-5-HT_{1A} autoreceptors and by up-regulation of noradrenaline-α_1 receptors and dopamine-D_2 receptors (Ebert 2001). Altogether these effects lead to a modulation of



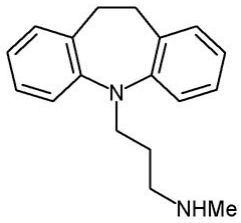
Amitriptyline



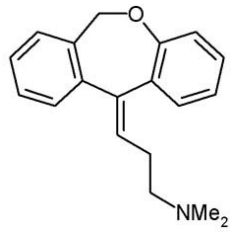
Citalopram



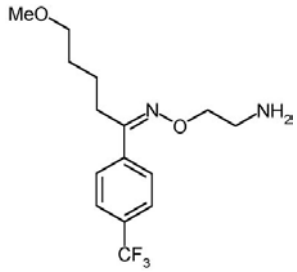
Clomipramine



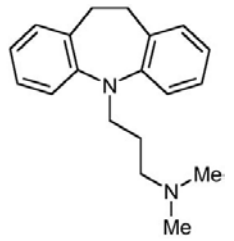
Desipramine



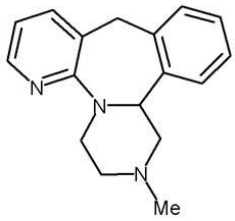
Doxepin



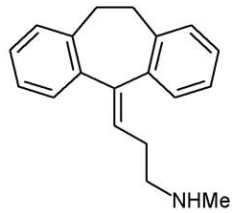
Fluvoxamine



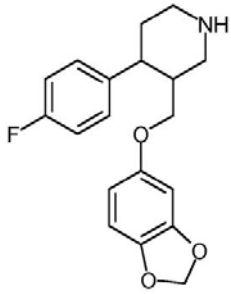
Imipramine



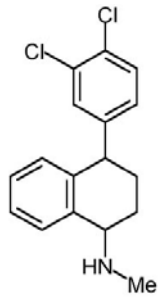
Mirtazapine



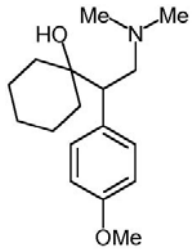
Nortriptyline



Paroxetine



Sertraline



Venlafaxine

Figure 2. Structures of antidepressants evaluated in this thesis

second messenger systems and gene expression that causes the antidepressive effect with a delay of 3-6 weeks.

The antidepressants' side effect profiles are based on their activity on specific receptor sites and varies among the antidepressant agents (Table 1). For example, inhibition of

Table 1. Receptor binding profiles of antidepressants*

Antidepressant	Class	5-HT re-uptake	NA re-uptake	α_1 inhi-bition	mACh inhi-bition	H_1 inhi-bition	5-HT_{2A} inhi-bition
Amitriptyline	TCA	++	++	++	++	+++	++
Citalopram	SSRI	+++	-	n.a.	n.a.	n.a.	n.a.
Clomipramine	TCA	+++	++	++	++	++	++
Desipramine	TCA	+	+++	+	+	+	(+)
Doxepin	TCA	(+)	++	++	++	+++	++
Fluoxetine	SSRI	++	+	-	-	-	-
Fluvoxamine	SSRI	+++	-	-	-	-	(+)
Imipramine	TCA	++	++	+	++	++	+
Maprotiline	TCA	-	+++	++	(+)	+++	+
Mianserine	TCA	-	+	(+)	(+)	+++	+++
Mirtazapine	NSSA	-	-	(+)	(+)	++	+++
Moclobemide	RIMA	-	-	-	-	-	n.a.
Nortriptyline	TCA	+	+++	++	+	+++	++
Paroxetine	SSRI	+++	(+)	-	+	-	-
Reboxetine	SNRI	-	+++	-	-	-	-
Sertraline	SSRI	+++	-	(+)	(+)	-	-
Tranylcypromine	MAOI	+	(+)	-	-	-	-
Venlafaxine	SSNRI	++	(+)	-	-	-	n.a.
Viloxazine	SNRI	-	++	n.a.	-	n.a.	n.a.

** adapted according to Möller 2000b*

Inhibition constant K_i: +++ < 10; ++ 11-100; + 100-200; (+) 200-1000;(-) > 1000 nmol/L

5-HT: serotonin; NA: noradrenaline; α_1: noradrenergic; mACh: muscarinic cholinergic; H_1: histaminergic; TCA: tri- or tetracyclic antidepressant; SSRI: selective serotonin reuptake inhibitor; NSSA: Noradrenergic and specific serotonergic antidepressant; RIMA: reversible inhibitor of monoamine oxidase A; SNRI: selective noradrenaline reuptake inhibitor; MAOI: monoamine oxidase inhibitor; SSNRI: selective serotonin and noradrenaline reuptake inhibitor; n.a.: data not available

histamine-H_1 receptors leads to weight gain and drowsiness; anticholinergic effects cause constipation, blurred vision and dry mouth; antagonism of noradrenaline-α_1 receptors results in dizziness, decreased blood presure or drowsiness; serotonin-5-HT_2 receptor stimulation leads to agitation, akathisia, anxiety, panic attacs, insomnia and sexual dysfunction and agonism of serotonin-5-HT_3 receptors causes nausea, gastrointestinal distress, diarrhea, and headache (Möller 2000a).

Bipolar depressive disorder is characterized by both depressive and manic episodes and is treated with mood stabilisers such as lithium, carbamazepine or valproate as first choice. Antidepressants represent an additional treatment option for depressive episodes but are second in line due to their potential to induce mania. So far, the mechanisms underlying the mood-stabilising effect are not well understood but the mechanisms of action of the medications are. Lithium inhibits the inositol-monophosphatase and influences other second-messenger systems such as the intracellular calcium concentration, adenylat cyclases, G-proteins or proteinkinase C. It activates the serotonergic transmission and modulates dopaminergic, noradrenergic, and cholinergic systems as well as the transmission of γ-aminobutyric acid (GABA). It modulates the circadian rhythm and gene expression of G-proteins, adenylate-cyclases or peptide hormones (Benkert and Hippius 2000). Valproate and carbamazepine directly reduce neuronal conduction by inhibiting sodium ion channels. They also modulate GABAergic and dopaminergic effects as well as other second messenger systems.

1.3 Pharmacokinetics

1.3.1 Definition of pharmacokinetic parameters

Pharmacokinetics describe mathematically the fate of a drug in an organism over time. The organism is often characterized as a system of compartments into which a drug is absorbed, distributed and from which it is eliminated. Orally administered drugs penetrate from an absorption compartment such as the gastro-intestinal system into a

central compartment, for example the systemic blood circulation. Analysing the plasma concentration of a drug, the time (t_{max}) to reach plasma peak concentrations (c_{max}) is linked to the absorption rate that is determined by the absorption rate constant (k_{01}) when the process follows first-order kinetics. The c_{max} depends on the bioavailability (F) of a drug, that describes the fraction of the administered dose reaching the systemic blood circulation. The drug is distributed in the body according to its physico-chemical properties and physiological factors, e.g. blood flow, concentration of plasma proteins, or proportion of extracellular fluid in the body. The extent of distribution is determined by the volume of distribution (V_d). The concentration of a drug may be higher in some physiological systems than in others, thus the body compartment may be divided in central and peripheral compartments, described by different volumes of distribution (e.g. V_c, V_1) and distribution rate constants (e.g. k_{12}, k_{21}). The half-life ($t_{1/2}$) is the time needed to reduce the plasma concentration of a drug by 50%. This parameter is linked to the elimination rate constant (k_{10}), to the clearance (CL) and to the volume of distribution, as clearance determines the volume of the central compartment (V_c) that is cleared of a drug in a certain time.

1.3.2 Pharmacokinetics of antidepressants and lithium

The pharmacokinetics of antidepressants are often described by a two-compartment model. These substances have to be lipophilic in order to pass the blood-brain barrier and thus are likely to distribute into peripheral compartments. This lipophilic property may be one of the reasons why they undergo extensive metabolism in the liver and show a first-pass effect, leading to variable bioavailability ranging from 30 to 80% (Table 2). The time until peak plasma concentration is reached varies between 1 and 8 hours. These drugs are mainly metabolized by the liver via oxidation by the cytochrome P450 enzyme system (CYP) and glucuronidation. In general, their half-life ranges from 9-40 hours. Most antidepressants are highly bound to plasma proteins. The variation of protein binding may also affect clearance and volume of distribution. A linear relationship between dose and plasma concentrations exists for most antidepressants,

Table 2. Pharmacokinetic parameters of antidepressants*

Antidepressant	Bioavailability [%]	Half-life [hr]	Time to peak concentration [hr]	Protein binding [%]
Amitriptyline	33-64	15-47	1-5	94-97
Citalopram	80	23-45	2-4	< 80
Clomipramine	50	12-36	3-8	98
Desipramine	50-68	15-25	2-6	73-92
Doxepin	15-45	8-25	2-4	80
Fluvoxamine	> 53	9-28	2-8	70
Imipramine	22-77	4-18	2	90
Mirtazapine	48	20-40	2	85
Nortriptyline	46-59	18-56	4-6	93-95
Paroxetine	> 64	8-44	1-11	95
Sertraline	> 44	22-36	4-8	99
Venlafaxine	40-45	5	2-4	27-30

** derived from the manufacturers' product information*

except for paroxetine, fluvoxamine and clomipramine. The pharmacokinetic parameters of the drugs that were analysed in this work are detailed in Table 2 according to the manufacturers' information.

The pharmacokinetics of lithium differs significantly from that of the antidepressants because it is exclusively eliminated by the kidney. The elimination is linked to the excretion and reabsorption of sodium ions in the proximal tubules of the kidney. This variability results in an unpredictable half-life ranging from 18 to 36 hours in patients with normal renal function. Its bioavailability is 80-100% and depends on the type of the administered lithium salt. The time to reach peak plasma concentrations ranges from 1-3 hours for lithium acetate and from 4 to 4.5 hours for lithium carbonate.

1.4 Factors influencing pharmacokinetics

1.4.1 Determination of factors influencing pharmacokinetics

The pharmacokinetic behaviour of a drug is altered by factors affecting the absorption, distribution or elimination process. Food intake or the pH value of the gastro-intestinal system can affect drug absorption. The distribution can vary according to the

individual's height, weight, sex, age or protein binding and elimination is altered by liver or renal function or the activity of metabolizing enzymes or transporters. Co-medication can influence every pharmacokinetic process for example by building complexes with the drug in the intestine, by replacing drugs at protein binding sites and by inhibiting or inducing metabolic enzymes or transporters. Nowadays, controlled clinical studies evaluate the influence of factors that are likely to be relevant in patients where the drug will be administered. The typical study design includes 10-20 subjects per study arm and 8-20 plasma concentration measurements per patient and dosing interval (Hildebrand 2003). These dense data allow the determination of the individual's pharmacokinetic parameters from plasma concentration-time profiles. The parameters are then summarized for every study arm and compared for statistically significant differences. Covariates that are often evaluated during drug development are the effect of food, age, gender, renal or hepatic impairment or co-medication that is known to alter the plasma concentration of many other drugs. However, these studies consist of a small number of carefully selected participants and except for one particular factor all others influencing the pharmacokinetics are excluded. As the mechanisms underlying pharmacokinetic variability have been intensively studied over the last twenty years, this knowledge is now included in drug development. Nevertheless, old drugs still remain less well studied.

Studies evaluating the influence of several covariates in a naturalistic clinical setting are rare because of the lack of dense pharmacokinetic data, but over the years new pharmacokinetic methods were developed that are based on a population approach rather than modeling individual pharmacokinetics. Nonlinear mixed-effects modeling can be used to describe and quantify the mean and variability of pharmacokinetic or pharmacodynamic parameters in a population. When analysing dense data, this method is applied to generate more accurate estimates of the variability within a population and to identify covariates explaining this variability (Food and Drug Administration 1999). However, it is also useful for pharmacokinetic or

pharmacodynamic analysis of sparse, unbalanced and heterogenous data (Sheiner et al. 1977).

1.4.2 Factors influencing the pharmacokinetics of antidepressants and lithium

The pharmacokinetics of antidepressants are altered by drug interaction, patients' age, sex and weight, renal and hepatic function as well as smoking, alcohol and food. The ingestion of food increases the bioavailability of sertraline by 40% (Goodnick 1994). Chronic alcohol abuse induces metabolic liver enzymes and therefore increases clearance and first pass-effect. On the other hand alcohol may cause liver cirrhosis that results in impaired elimination. In the elderly or in females, plasma concentrations of antidepressants are often higher. The gender effect has been reported for amitriptyline, clomipramine, imipramine, nortriptyline, trazodone and mirtazapine (Frackiewicz et al. 2000, Timmer et al. 2000). The influence of age has been found for most of the TCAs, SSRIs and mirtazapine (Preskorn 1993, Timmer et al. 2000, Bazire 2000).

Oxidative drug metabolism is catalysed by the hepatic cytochrome P450 (CYP) enzyme system. Drug-drug interactions can be explained by inhibition or induction of these isoenzymes and mutations in the genetic code of some of these isoenzymes cause high variability in the elimination of antidepressants. Five enzymes (CYP3A4, CYP1A2, CYP2C9, CYP2C19, CYP2D6) account for the metabolism of the majority of commonly used drugs. CYP3A4 is the most frequent enzyme in the liver followed by CYP1A2, CYP2C9, CYP2C19 and CYP2D6.

CYP3A4 catalyses the metabolism of many drugs such as carbamazepine, protease inhibitors, oral contraceptives and antipsychotics. This isoenzyme partly mediates the metabolism of all antidepressants to a varying degree as can be seen by reduced plasma concentrations when carbamazepine or barbiturates, potent inducers of CYP3A4, are co-administered (Goodnick 1994).

Smoking and omeprazole induce CYP1A2 thus enhancing the metabolism of drugs that are substrates of this isoenzyme. This effect has been reported for most of the TCAs (Goodnick 1994). On the other hand, CYP1A2 is inhibited by fluvoxamine,

ciprofloxacine or nutritional flavonoides (Bazire 2000). Similarly to CYP3A4 the metabolism of antidepressants is mediated partly by CYP1A2.

Other metabolic enzymes, for example the closely related CYP2C9 and CYP2C19 isoenzymes, are involved to a variable extent in the metabolism of TCAs, citalopram, fluoxetine, sertraline and moclobemide. Drug interactions have been reported with fluvoxamine, an inhibitor of CYP2C9 and CYP2C19. This increases the plasma concentrations of amitriptyline, clomipramine, mirtazapine and warfarin (van Harten 1993, Anttila et al. 2001). In addition, the CYP2C9 and CYP2C19 genes are polymorph and the genotype is linked to enzyme activity.

Variations due to genetic polymorphism range from the complete loss of enzyme activity (poor metabolizer), decreased (intermediate metabolizer) to normal enzyme activity (extensive metabolizer). Poor metabolizers are homozygous or heterozygous carriers of two defective alleles, intermediate metabolizers possess one functional and one defective allele and extensive metabolizers are carriers of two functional wild type alleles. Twelve CYP2C9 alleles causing different enzyme activity are known, but only the defective alleles *CYP2C9 *2* and *CYP2C9 *3* are of clinical importance (Aynacioglu et al. 1999). 1-3% of Caucasians are poor metabolizers, whereas the genotype intermediate metabolizer occurs in up to 35% (Wormhoudt 1999, de Morais 1994). The potential impact of being a poor metabolizer can be seen when receiving standard doses of warfarine: poor metabolizers develop high warfarine plasma concentrations and are therefore at risk of bleeding complications (Aithal et al. 1999, Steward et al. 1997, van der Weide et al. 2001). Another example is the clearance of phenytoin which is decreased in poor metabolizers (Kidd et al. 1999) and intermediate metabolizers (Ninomiya et al. 2000).

Nine alleles have been identified resulting in reduced or defective enzyme activity of the CYP2C19 isoenzyme. Out of these only one defective allele *(CYP2C19*2)* occurs frequently in Caucasians. Pronounced ethnic differences exist with respect to the frequency of CYP2C19 deficiency: 12-23% Orientals but only 2-5% Caucasians are

poor metabolizers of CYP2C19. In contrast, intermediate metabolizers are seen more frequently in Caucasians (25%) (Xie et al. 1999). Reduced clearance in poor metabolizers of CYP2C19 has been shown with omeprazole (Leiri et al. 1996), lansoprazole (Furuta et al. 2001) and diazepam (Meyer 2000).

Out of all cytochrome P450 isoenzymes CYP2D6 is the most active isoenzyme for the metabolism of antidepressants and thus most likely the cause of altered plasma concentrations due to drug interactions or genetic polymorphism of metabolising enzymes in antidepressive therapy. Antidepressant plasma concentrations rise when inhibitors of CYP2D6 such as cimetidine, paroxetine or fluoxetine are co-administered or when several substrates of CYP2D6, such as antidepressants, antipsychotics or β-adrenoreceptor blockers are administered in combination (Goodnick 1994, Bazire 2000).

Genetic polymorphism of the CYP2D6 gene is associated with the extent of oxidative metabolism in the liver (Coutts and Urichuk 1999; Eichelbaum and Gross 1990). At least fifteen out of more than 50 mutations account for CYP2D6 deficiency but detection of CYP2D6 *3, *4, *5, *6 alleles and gene duplication is sufficient for a highly reliable prediction of the CYP2D6 phenotype (Griese et al. 1998; Sachse et al. 1997).

Among Caucasians, 5-10% are poor metabolizers of CYP2D6 and deficient in their capacity to metabolize CYP2D6 substrates. An example for this is the metabolism of desipramine which is decreased in poor metabolizers resulting in high serum concentrations and adverse drug effects (Bluhm et al. 1993; Spina et al. 1997). Similarly, the metabolism of venlafaxine is also reduced in poor metabolizers (Lessard et al. 1999). Due to polymorphism of the CYP2D6 gene, the dose needed to cause an antidepressant effect of nortriptyline ranges from 10 to 500 mg/d (Bertilsson et al. 1985, Dahl et al. 1996). Intermediate metabolizers of CYP2D6 or carriers of the CYP2D6*9 allele show reduced enzyme activity (Raimundo et al. 2000, Griese et al. 1998). Duplication of the CYP2D6 gene may cause high enzyme activity and occurs in 1-10% Caucasians that are called ultrarapid metabolizers. Duplication of other CYP

isoenzymes has not been observed so far. Nevertheless, investigation of the lack of therapeutic response after intake of CYP2D6 substrates showed that gene duplication predicts high clearance in only 20-25% but high clearance does not predict duplication of CYP2D6 (Bergmann et al. 2001, Johansson et al. 1993).

Population pharmacokinetic studies exist for nortriptyline and doxepin. Nortriptyline clearance is altered by the CYP2D6 genotype (Kvist et al. 2001) or inhibitors of CYP2D6 (Jerling et al. 1994) and relevant covariates of doxepin pharmacokinetics are age and weight (Meyer-Barner et al. 2002).

The clearance of lithium is not affected by variations of hepatic metabolizing enzymes but highly variable because of its linkage to renal function and to the balance between sodium excretion and reabsorption in the proximal tubules of the kidney. This balance is influenced by the patient's state of hydratation, sodium intake fever, pregnancy, old age or diseases that cause changes in renal perfusion or excretion capacity. Renal excretion of lithium is also affected by co-medication with diuretics, antihypertensives, corticoides or nonsteroidal antiinflammatory drugs (Michell 2000, Benkert and Hippius 2000).

There are three population pharmacokinetic studies evaluating covariates on lithium pharmacokinetics. One study detected creatinine clearance and lean body weight to alter the lithium clearance (Jermain et al. 1991); another study found that age, total body weight, height and serum creatinine are significant covariates (Taright et al. 1994). A third study states a relationship between the lithium clearance and total body weight, age and serum creatinine (Yukawa et al. 1992).

1.5 Relevance of therapeutic drug monitoring of antidepressants and lithium

Therapeutic drug monitoring means to optimise individual dosing schedules by measuring the plasma concentrations of a drug. It is applied to drugs that are characterized by high interindividual pharmacokinetic variability, a narrow therapeutic range and a known relationship between plasma concentration and clinical effect.

With respect to psychopharmacotherapy, mood stabilisers, some antidepressants and some antipsychotics fulfill these criteria. Therapeutic drug monitoring is the standard of care in the treatment of lithium and anticonvulsants and recommended for tricyclic antidepressants, haloperidol and clozapine (Michell 2000) since blood concentrations of these drugs are highly variable. Thus, standard doses may cause subtherapeutic or toxic blood concentrations.

Therapeutic drug monitoring of lithium is mandatory to avoid toxicity and to detect nonresponders and noncompliance. For these reasons it is recommended weekly in the first month of treatment, than monthly for five months and afterwards every three months (Benkert and Hippius 2000). Therapeutic serum concentrations range from 0.4 to 1.2 mmol/L, whereas lower serum concentrations are desired for treatment augmentation; 0.6-1.2 mmol/L are recommended in the treatment of bipolar affective disorder or mania. Toxicity occurs at serum concentrations greater than 1.5-2.0 mmol/L and is characterized by coarse tremor, apathy, hyperreflexia, hypertonia, nausea, diarrhea, myoclonus, seizures, acute renal failure, cardiac dysrythmia and coma. Serum concentrations greater than 3.5 mmol/L are potentially lethal and necessitate hemodialysis (Michell 2000). Different methods exist for dose individualisation of lithium. Linear regression equations are used for estimation of initial lithium doses (Jermain et al. 1991, Pepin et al. 1980, Yukawa et al. 1993, Zetin et al. 1983). Doses can be individualised by nomographs and by serum concentration measurement after administration of a test dose (Gaillot et al. 1979, Cooper and Simpson 1982, Perry et al. 1986). Most of these methods take into account the creatinine clearance as well as weight and age and are only applicable to predefined dosing schemes. Thus, the physician needs to know all factors influencing the pharmacokinetics of lithium in order to plan dose individualisation before treatment initialisation.

Computer-assisted methods that apply the theorem of Bayes allow the use of every dosing scheme and serum concentration measurement that may occur in clinical routine. This method estimates individual pharmacokinetic parameters based on

Table 3. Therapeutic ranges of tricyclic antidepressants

	Minimum effective concentra-tion [ng/mL]	Minimum toxic concentra-tion [ng/mL]	Conentration – response relationship	References
Amitriptyline*	100	220	Bisigmoidal	Hiemke et al. 2000 Ulrich and Läuter 2002
Clomipramine*	175	450	Curvilinear	Hiemke et al. 2000 DUAG 1999
Desipramine	100	150	Curvilinear	Hiemke et al. 2000 APA Task Force 1985
Doxepin*	20	150	Not known	Hiemke et al. 2000 Leucht et al. 2001
Imipramine*	175	350	Linear	Hiemke et al. 2000 APA Task Force 1985
Nortriptyline	70	170	Curvilinear	Hiemke et al. 2000 APA Task Force 1985

** sum of drug and active demethylated metabolite*

population pharmacokinetic parameters and their variability in combination with individual serum concentrations (Jaehde 2003).

Therapeutic drug monitoring of tricyclic antidepressants is established to avoid subtherapeutic or toxic plasma concentrations and to check treatment compliance (American Psychiatric Association Task Force 1985). Therapeutic ranges are defined by a lower threshold for minimum effective plasma concentrations and an upper theshold for maximum effective or minimum toxic plasma concentrations. High plasma concentrations of TCA may be less effective in case of a curvilinear or bisigmoidal concentration- resonse relationship, and have the risk of cardiac and brain toxicity resulting in arrythmia, seizures or delirium (Table 3).

An American cost-benefit calculation states that the costs associated with TCA brain toxicity overweight the costs of a single plasma concentration measurement at the start of antidepressive therapy. Assuming that sixty out of thousand depressed patients develop delirium because of high TCA plasma concentrations, the savings of therapeutic drug monitoring are estimated at about 350 $ per patient (Preskorn 1989). Although antidepressant doses given in the United States tend to be higher than in Germany, a

recent German study stresses the importance of therapeutic drug monitoring of TCAs. In this study including 108 patients, therapeutic plasma concentrations were associated with improved clinical response. Patients without therapeutic drug monitoring were more likely to develop plasma concentrations outside the therapeutic range and experienced more side effects. These beneficial outcomes were noted even in spite of a poor compliance of physicians to follow the recommendations of the therapeutic drug monitoring service during this study (Müller et al. 2003).

In contrast to TCAs, the benefit of therapeutic drug monitoring of second generation antidepressants is controversely discussed. Different levels of evidence exist about the relevance of therapeutic drug monitoring: therapeutic drug monitoring appears to be useful in clinical routine for some of the newer antidepressants, but for most of the new antidepressants the clinical benefit remains unclear and therapeutic drug monitoring should therefore be reserved to particular clinical situations until more information is available. In general, the toxicity of antidepressants such as maprotiline and trimipramine is comparable to TCAs and thus therapeutic drug monitoring may be useful. New drugs are better tolerated, therapeutic ranges are mostly not well established and therapeutic drug monitoring should only be considered in cases of nonresponse, severe side effects or to check compliance (Table 4).

The present thesis evaluates the benefit of therapeutic drug monitoring for venlafaxine, mirtazapine and the SSRI citalopram, fluvoxamine, paroxetine and sertraline.

So far, venlafaxine is the only new antidepressant for which an association between plasma concentration, CYP2D6 genotype and clinical effect was demonstrated. Plasma concentrations were correlated to the decrease in the Montgomery and Åsberg Depression Rating Scale after 3 to 6 weeks of treatment and were significantly higher in responders than in nonresponders (Charlier et al. 2002). Additionally, poor metabolizers of CYP2D6 had higher plasma concentrations of the sum of venlafaxine and O-desmethylvenlafaxine and the ratio of the drug to its main metabolite was greater than one (Veefkind et al. 2000, Lessard et al. 1999).

Table 4. Proposed plasma concentration ranges of new antidepressants

Antidepressant	Daily dose [mg]	Proposed concentration range* [ng/mL]	Routine TDM useful	Additional references
Citalopram	20-60	50-130	Unclear	Bjerkenstedt et al. 1985
Fluoxetine**	20-80	100-400	Unclear	Amsterdam et al. 1997
Fluvoxamine	50-300	20-300	Unclear	Härtter et al. 1998
Maprotiline	25-225	125-200	Yes	Kasper et al. 1993
Mianserine	30-90	15-70	Unclear	
Mirtazapine	15-60	10-80	Unclear	Timmer et al. 2000
Moclobemide	150-600	300-1000	Unclear	Gex-Fabry et al. 1995
Paroxetine	20-60	40-120	Unclear	Rao et al. 1999
Reboxetine	2-12	10-100	Unclear	
Sertraline	50-200	20-50	Unclear	Lundmark et al. 2000
Trimipramine	25-150	150-350	Yes	Isacsson et al. 1997
Venlafaxine**	75-375	200-400	Yes	Charlier et al. 2002, Veefkind et al. 2000

** according to Hiemke et al. 2000*
*** Sum of drug and active demethylated metabolite*

Studies evaluating a concentration-response relationship are lacking for mirtazapine. There is a linear relationship between dose and plasma concentration over a dosing range of 15 – 80 mg/day (Timmer et al. 1995). Effective doses range from 5 to 60 mg/day and result in plasma concentrations of 5 to 100 ng/mL (Timmer et al. 2000). Its sedative effect was found to be more prominent in low-dose treatment of mirtazapine (< 15 mg/day) (Kasper et al. 1997).

SSRIs are characterized by a flat dose-response relationship as different doses in clinical trials are found to be equally effective. Thus, recommended therapeutic plasma concentrations are often estimated from the minimum dose in clinical trials (Preskorn 1997). Studies evaluating concentration-response relationships of SSRIs were mainly

carried out in small patient groups and the results remain conflicting: most studies failed to show a correlation between plasma concentrations and the severity of depression but comparison between nonresponders and responders in some studies indicate minimum effective concentrations (Rasmussen and Brøsen 2000, Härtter et al. 1998, Rao et al. 1999). However, a Scandinavian study in elderly depressed patients found that therapeutic drug monitoring helps to avoid unnecessary dose increases and thus drug costs were reduced by 10.2% (Lundmark et al. 2000).

2 Aims of the thesis

The pharmacokinetics of antidepressants and lithium is highly variable among patients, thus considerable effort is made to contol this variablilty, e.g. by therapeutic drug monitoring, phenotyping or genotyping. Nevertheless, the benefit of plasma concentration control still remains unclear, especially with respect to new antidepressants.

This thesis aims to provide basic information and tools to improve treatment with psychoactive drugs by therapeutic drug monitoring in a routine clinical setting.

Three aspects were evaluated in detail from routine drug monitoring data and from a naturalistic clinical study in psychiatry:

- The impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on antidepressants' plasma concentrations, side effects and treatment response.
- The concentration-effect relationship for mirtazapine and factors influencing its pharmacokinetics applying population pharmacokinetic methods.
- The use of population pharmacokinetic data to establish a computer-assisted service for dose individualisation of lithium.

The applied statistical and pharmacokinetic methods were able to control multiple influencing factors occurring in clinical routine and focussed on linear and logistic regression as well as population pharmacokinetic analysis.

3 Patients and methods

3.1 Study design

3.1.1 Kompetenznetz Depression: Therapeutic drug monitoring and genotyping

In 1998 a network was initiated in Germany to promote interdisciplinary research to better understand the etiology of depression and to improve antidepressive treatment. This network, the “Kompetenznetz Depression”, is sponsored by the German Ministry of Education and Research and includes six main multicentre projects that are further divided into subprojects. The subproject 3.8 was designed to answer questions about the potential of therapeutic drug monitoring of antidepressants to reduce side effects, the length of the patients’ stay in the hospital, and treatment costs. Four centres were involved: the Departments of Psychiatry of the Universities of Bonn and Mainz and the State Hospitals of Gabersee and Kiedrich. Patients were recruited from 2000 to 2003. Inclusion criteria were: (1) ICD-10 diagnosis F 3 (WHO 1992); (2) at least moderately ill according to the Clinical Global Impression Item Severity of illness (CGI > 4) (National Institute of Mental Health 1976, see Appendix 4); (3) start of an antidepressive monotherapy with amitriptyline, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, imipramine, mirtazapine, nortriptyline, paroxetine, sertraline or venlafaxine. No restriction was made with respect to other drugs and a change of antidepressant therapy during the study. Exclusion criteria were: (1) Substance dependency or drug abuse within the last 3 months; (2) prior treatment with fluoxetine; (3) acute suicidal tendency; (4) pregnancy, (5) admission to the hospital by legal commitment or for crisis intervention. Patients gave their written informed consent for weekly antidepressant plasma concentration measurements and a clinical interview to assess treatment response and side effects during their stay in the hospital. This informed consent covered a maximum of ten weeks. Additional questionnaires assessed the direct treatment costs and the patient’s quality of life at the beginning of

the study, at discharge from hospital, and at three and six months post inclusion into the study.

The subproject 3.8 co-operated with the project 5 of the Kompetenznetz Depression, that evaluated the molecular genetics of depression. Therefore, patients were also asked for their informed consent for a genetic analysis of factors underlying the response to the treatment of depression; therefore an additional blood sample was drawn. The studies were approved by the local Ethics Committees and were conducted according to the declaration of Helsinki. The data of patients who agreed to genotyping were analysed for the impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome.

An amendment was approved by the Ethics Committee of the Medical Faculty of the University of Bonn to include also patients treated with mirtazapine. These data were evaluated for a therapeutic range and factors influencing the pharmacokinetics of mirtazapine.

3.1.1.1 Evaluation of the impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome

Subgroups were selected from the entire patient group that gave informed consent for genotyping. Patients were selected according to defined criteria to evaluate the relationship between genotype, trough plasma concentrations, response, and side effects.

To evaluate the relationship between genotype and trough plasma concentration we calculated the mean dose-corrected plasma concentration of each antidepressant that was administered within the course of the study. For each antidepressant the median dose-corrected plasma concentration should represent the plasma oncentration of a typical extensive metabolizer and was calculated of all samples that were available for one drug; then patients' mean dose-corrected plasma concentration was estimated as relative deviation with respect to the drug-specific median. Co-medication was stratified for substrates as well as inhibitors or inducers of CYP1A2, CYP2C9, CYP2C19,

CYP2D6 and CYP3A4. Mean coffee, cigarette and alcohol consumption was recorded on a 3-item scale with 0 = no consumption, 1 = up to 5 cups of coffee, 10 cigarettes or one glass of alcohol corresponding to 200 mL wine per day and 2 = consumption exeeding 1. Observations were excluded if plasma concentrations or doses were missing or steady-state was not reached. When more than one antidepressant was administered within the course of the study, only the antidepressant with the most observations was carried forward for analysis.

Patients were selected for evaluation of a genotype-response relationship according to the following criteria: (1) at least three weekly observations carried out on the same antidepressant and (2) no change of diagnosis within the course of the study. The severity of depression was assessed by the Hamilton Depression Rating Scale (HAMD) and the Clinical Global Impression (CGI) (see 3.2.1, 3.2.2 and Appendix 1). Treatment response was defined according to the HAMD as 40% reduction in the total score from the first to the last observation. We chose 40% since, as a rule, the first observation was carried out after one week of drug treatment, thus excluding spontaneous remission. Response according to CGI was defined as a CGI 1 reduction of at least two points between the first and the last observation when CGI 2 was rated less than 4 at the last observation. Co-medication was stratified for benzodiazepines, antipsychotics, mood stabilisers, hypnotics and other antidepressants to control for putative interferences.

The evaluation of the relationship between genotype and side effects was carried out on the side effects reported at the patients' first observation after the start of antidepressive treatment. Side effects were assessed by the UKU side effect rating scale (see 3.2.3 and Appendix 2). Patients were divided into two groups according to the relevance of side effects at their first observation. Each UKU symptom item assesses the severity of side effects (*not present, present to a mild, moderate, or severe degree*) and the relationship to the drug (*improbable, possible, probable*). Side effects were judged relevant when at least four symptom items were rated *moderate* or

severe and *possibly* caused by the drug or when at least two symptom items were rated *moderate* or *severe* and *probably* caused by the drug. To control for putative interferences, co-medication was stratified for causing sedation or agitation, disturbing the gastro-intestinal system, influencing blood pressure, or provoking serotonergic or anticholinergic effects.

3.1.1.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine

Patients treated with mirtazapine were carried forward for two evaluations: the analysis of a relationship between trough plasma concentration, response and side effects and the evaluation of factors influencing mirtazapine pharmacokinetics applying population pharmacokinetic methods.

The evaluation of the the relationship between mirtazapine trough plasma concentrations and side effects was carried out on the entire mirtazapine data. The main side effects of mirtazapine are sedation and weight gain. Thus, the UKU items “sleepiness/sedation”, “increased duration of sleep”, “weight gain” and “global assessment of the patient’s performance” were evaluated separately to assess the relationship between trough plasma concentrations and side effects. Logistic regression controlled for the putative influence of co-medication with benzodiazepines, hypnotics or other sedative medication. In addition, observations that took place in the first week of mirtazapine treatment when side effects are generally more pronounced were analysed separately.

To evaluate the relationship between mirtazapine trough plasma concentrations on treatment response, patients were selected according to the following criteria: (1) at least fourteen days of therapy with mirtazapine; (2) no co-medication with lithium, carbamazepine or other antidepressants; (3) no co-diagnosis of personality disorder. When treatment with mirtazapine was started 6-7 days before the first observation, pivotal efficacy trials showed a mean reduction of the HAMD score of about 25% (Bremner 1995). Therefore, we defined the response to mirtazapine as a reduction in the HAMD score of 40% or more from the first to the last observation.

The evaluation of factors influencing the pharmacokinetics of mirtazapine was performed by population pharmacokinetic analysis. A number of factors with possible impact on the pharmacokinetics of mirtazapine were recorded weekly: Co-medication, weight, height, age, gender, weekly AST-, ALT-, and γ-GT activity, serum creatinine concentration, smoking habits, coffee and alcohol consumption, blood pressure and pulse. We noted the time of ingestion of the last dose and of blood withdrawal reported by the patients. Co-medication was categorized into substrates, inhibitors or inducers of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4. Plasma concentrations were excluded from analysis when dosing schedules were missing, and when noncompliance or interference in the analytical assay was documented. For population pharmacokinetic analysis of mirtazapine, only observations with constant co-medication were selected from every patient.

3.1.2 A computer-assisted method for lithium dose individualisation

The computer-assisted dose individualisation was established by specifying the population characteristics of lithium according to the current literature in tha Abbottbase pharmacokinetic software® for Bayesian curve fitting. This specification included population pharmacokinetic parameters and their variability as well as covariates with influence on lithium pharmacokinetics.

The performance of the software that was extended by the lithium specification was validated with serum concentrations from routinely monitored inpatients that were retrospectively evaluated. Patient's age, height, weight, serum creatinine, co-medication and dosing schedule were noted from the patients' charts.

Each patient's data were fitted by the extended software. Covariates were included if they reduced the residual sum of squares of the fit. To evaluate the predictive performance of the software, the accuracy and precision to predict the last observation of every patient was compared with three a-priori methods for dose individualisation (Pepin et al. 1980; Jermain et al. 1991; Yukawa et al. 1993).

3.2 Ratings

3.2.1 Hamilton Depression Rating Scale

The Hamilton Depression Rating Scale (HAMD) is the most common rating scale to measure the severity of depression. In this study the 17-item version was applied (Hamilton 1960, Appendix 1). The 17 items consist ot three or four grades scoring from 0 to 2 or 3, respectively, with increasing severity of depressive symptoms. The single item scores are then summarized to the total score reflecting the severity of depression.

The rater refers to the patient's state of the preceding week taking into account the information she or he gets from the clinical interview as well as information from the hospital staff or from other persons who are in contact with the patient.

The interrater reliability was found to range from 0.73 to 0.91. The validity was proven by its wide use in clinical studies (Collegium Internationale Psychiatriae Scalarum 1981). Within the subproject 3.8, annual rater-trainings were performed. The intraclass correlation ranged from 0.71 to 0.83 calculated from the rating of three recorded clinical interviews.

3.2.2 Clinical Global Impression

The Clinical Global Impression (CGI) consists of three items that are evaluated separately: Severity of illness (CGI1), Global improvement (CGI2) and the Efficacy index (CGI3). The CGI can be applied to assess the treatment of every illness (Appendix 3 and 4). The rating is based on the rater's experience with the specific disease. Similar to the HAMD, the CGI refers to the patients' state of the preceding week.

The inter-rater reliability was found to depend on the rater's education (medical or nursing staff) and ranged from 0.35 to 0.66, the re-test reliability over 8 weeks ranged from 0.15 to 0.81 (Collegium Internationale Psychiatriae Scalarum 1981). Rater trainings among the study centres involved in this study revealed intraclass correlation

coefficients for the CGI1 of 0.85 and 0.86. The CGI is a quick and simple scale to measure treatment efficacy, and is widely used in psychiatric research although its validity is controversial (Beneke and Rasmus 1992).

3.2.3 UKU side effect rating scale

The UKU side effect rating scale was developed by Lingjaerde and colleagues from 1981 to 1986 to assess the side effects of psychotropic drugs including antidepressants, antipsychotics and mood stabilisers (Lingjaerde et al. 1987). The scale contains 48 items assessing the severity of specific symptoms and their relationship to the analysed drug, a global assessment of the patient's daily performance, and a statement of the consequences that side effects have on continuing medication. In the present study antidepressant side effects were assessed by an abridged version containing 30 symptom items, the global assessment and the statement of consequences (Appendix 2). Only those symptoms were assessed that occured during two days prior to the interview.

The items can be either evaluated separately, in clusters of psychic, neurological, autonomic and other side effects, or in total. The inter-rater reliability was reported to range from 0.37 to 0.96 (Lingjaerde et al. 1987).

3.3 Blood sampling

Weekly venous puncture for antidepressant plasma concentration measurement was performed in the morning of the clinical interview. Blood samples were collected into 10 mL tubes containing 0.2 mg EDTA as anticoagulant before administration of the antidepressants' morning dose. In Bonn and Mainz, samples were transferred to the laboratories within three hours, centrifuged at 13000 x g for 10 min at 4°C or at 10000 X g for 5 min, respectively, and stored at –20°C until analysis. Blood samples of Gabersee were sent to Mainz for analysis. All plasma concentration measurements were performed within three days.

Venous puncture for routine monitoring of lithium serum concentrations was performed in the morning before administration of the morning dose. Blood samples were collected in 10 mL tubes, transferred to the laboratory within three hours, clotted for 30 min and then centrifuged at 2000 x g for 10 minutes at 4°C to obtain patients' serum. The supernatant was stored at –20°C until analysis.

3.4 Analytical methods

3.4.1 Antidepressants

The determination of antidepressant plasma concentrations was carried out in the laboratories of Bonn and Mainz by reversed-phase high-performance-liquid-chromatography (HPLC) with ultra-violet detection. The method used in Bonn was described in detail by Frahnert et al. (2003). It includes solid phase extraction on 3 ml 3M-Empore high performance extraction disk cartridges (Varian, Darmstadt, Germany) with the help of a Baker spe-12G vacuum instrument and was carried out according to the manufacturer's instructions: the mixed-phase sorbent was conditioned with one ml methanol followed by one ml water; then 0.9 ml supernatant, 0.1 ml melperone (3000 ng/ml) as internal standard and 2.0 ml 0.1 M potassium dihydrogenphosphate buffer (pH 6.0) were mixed in 16 x 100 mm polypropylene tubes (Sarstedt, Nymbrecht, Germany). The sample was transfered and passed through the extraction disk cartridge. To eliminate interferences, the cartridge was washed with one ml water, one ml 1 M acetic acid, one ml n-hexane, two ml n-hexane:ethyl acetate (1:1) and one ml methanol. The antidepressants and atypical antipsychotics were eluted with one ml 2-propanol : ammonia solution (25%) : dichloromethane (20:2:78). The eluent was evaporated to dryness, the residue dissolved in 250 µl acetonitril:water (3:7) and 100 µl was injected in a HPLC system consisting of a Bischoff 2200 high-performance liquid chromatography pump (Bischoff, Leonberg, Germany), a solvent degasser unit SDU 2003 (Bischoff, Leonberg, Germany) and a Waters Intelligent Sample Processor (WISP 717) equipped with a cooling module at 4°C (Millipore-Waters, Eschborn, Germany).

Table 5. Assay specification for measuring antidepressants in Bonn*

Antidepressant	Validation range [ng/mL]	Plasma concen-tration [ng/mL]	Relative error [%] (n = 10)	Intra-assay coefficient of variation [%] (n = 10)	Inter-assay coefficient of variation [%] (n > 10)
Amitriptyline	10-500	75	1.9	1.7	1.9
		100	2.6	2.6	7.2
		200	3.4	2.6	6.3
Citalopram	5-300	10	18.7	5.9	8.7
		50	-2.2	1.5	1.3
		100	0.4	1.3	2.6
Clomipramine	10-750	75	-2.8	3.1	2.9
		100	-0.4	5.2	7.6
		300	-0.8	4.6	5.9
Norclomipramine	10-750	75	-1.2	3.2	3.7
		100	2.1	5.7	5.8
		300	-0.3	1.5	3.7
Desipramine	10-500	75	0.1	4.2	2.5
		100	-2.9	4.6	8.7
		200	-0.4	2.1	6.5
Doxepin	5-500	75	0.8	2.2	1.9
		100	2.0	2.7	8.3
		200	-1.3	3.0	7.3
Nordoxepin	5-500	75	0.5	3.0	2.5
		100	7.0	2.8	7.2
		200	0.9	2.3	7.2
Fluvoxamine	5-500	10	1.7	8.7	5.8
		50	7.2	4.9	4.8
		150	6.8	3.6	3.3
Imipramine	10-500	75	0.5	1.6	3.1
		100	3.8	2.6	8.7
		200	4.2	5.4	7.8
Mirtazapine	5-300	10	1.0	5.6	7.7
		50	-2.0	4.6	2.5
		100	-5.3	5.2	3.9
Nortriptyline	10-500	75	-2.1	2.0	1.7
		100	3.5	3.9	8.1
		200	3.9	3.2	7.2
Paroxetine	5-500	10	-1.4	4.7	6.3
		50	7.0	6.2	4.5
		150	2.5	4.3	3.2
Sertraline	5-300	10	11.1	1.4	7.4
		50	0.6	3.6	1.6
		100	-1.4	1.9	4.2

**adapted from Frahnert et al. (2003)*

The analytical column (250 x 4.6 mm I.D.) containing Nucleosil 100-5-Protect 1 (endcapped), particle size 5 µm (Macherey & Nagel, Düren, Germany) was kept in a column oven (EchoTherm CO30, Torrey Pines Scientific LLC, Solana Beach, USA) maintained at 25°C. The mobile phase consisted of 25 mM potassium dihydrogenphosphate (pH 7.0) : acetonitrile (60:40) at a flow rate of one ml/min. The eluted substances were detected by a Shimadzu SPD-10AVP UV-detector (Shimadzu, Duisburg, Germany) at 230 nm. The acquisition and integration was performed by McDacq32 Software, version 1.51 (Bischoff, Leonberg, Germany). The accuracy and precision of the HPLC method with solid phase extraction are presented in Table 5 (Frahnert et al. 2003).

In Mainz plasma samples were directly injected in a pre-column for purification before separation by the analytical column (Härtter and Hiemke 1992a, Härtter et al. 1992b, Härtter et al. 1994). The chromatographic system consisted of an autosampler 231 XL (Gilon, Villiers Le Bel, France) equipped with a 7010 Rheodyne injection valve and a 100 µL sample loop, a Bischoff HPLC pump 2250 (Bischoff, Leonberg, Germany), and an automated six-port swiching valve Rheodyne 7000 (Besta, Wilhelmsfeld, Germany). Detection of sertraline and paroxetine was performed with a UV detector SPD-10A (Shimadzu, Duisburg, Germany) at a wave length of 210 nm and with a fluorescence detector Shimadzu RF-10A XL (Shimadzu, Duisburg, Germany) for venlafaxine and desmethylvenlafaxine at 220 nm and 305 nm. Recording and integration was performed with the Kontron Integration Pack 3.9 (Kontron, Milano, Italy). Patients' plasma samples were directly injected onto a 10 x 2.0 mm clean-up column filled with 20 µg CN-bonded silica (MZ-Analysentechnik, Mainz, Germany) and washed with deionized water containing 5 % acetonitrile. The mobile phase for separation of sertraline and paroxetine consisted of 0.01 M dipotassium-hydrogenphosphate buffer (adjusted with 85% phosphoric acid to pH 6.4) : acetonitrile (1:1 vol/vol); the mobile phase for determination of venlafaxine an O-desmethylvenlafaxine consisted of triethylamine buffer (2.5 mL in 1700 mL, adjusted with 85% phosphoric acid to pH 2.5) :

acetonitrile (85:15 vol/vol) (Dr. Sebastian Härtter, oral communication). Patients evaluated with the HPLC method with direct injection received paroxetine, sertraline or venlafaxine. The limit of quantification was 5 ng/mL for paroxetine and sertraline and 10 ng/mL for venlafaxine and O-desmethylvenlafaxine (Dr. Sebastian Härtter, oral communication).

Internal quality control criteria of both laboratories were taken from the Guideline of the Bundesärztekammer (Bundesärztekammer 2002). Thus, the measured concentration of quality control samples should not deviate more than three times the standard deviation from the true concentration.

Quality was assured follows: the chromatographic system was calibrated with standard curves for every antidepressant consisting of six quality controls samples prepared in the laboratory; in Bonn, two commerially available quality control samples (Lyphochek Benzo/TCA Control-Set, Bio-rad, München, Germany and ClinChek Control for Tricyclic Antidepressants, Recipe, München, Germany) were carried forward in addition. All stock solutions for calibration standards and quality control were prepared by dissolving 10 mg of the respective drug in 10 ml methanol. Pooled drug-free serum from healthy volunteers was spiked with stock solution of the drug in water (HPLC-grade, 1:10) to achieve calibration standard concentrations. Quality control samples that were run in each assay, were prepared in the same way. All serum standards, quality control samples and stock solutions were stored in aliquots at -20°C and were stable for at least 3 months. In Bonn the internal standard melperone was diluted with serum to a concentration of 3000 ng/ml.

When analysing patient samples, internal quality control was assured by two quality control samples (see high and low concentration in Table 5 and Table 6).

In addition to internal quality control, both laboratories participated in external quality controls of Health Control, Cardiff. United Kingdom. It was carried out every month for amitriptyline, nortriptyline, imipramine, desipramine, clomipramine and norclomipramine, and every three months for doxepine, nordoxepine, fluvoxamine,

Table 6. Internal quality control data for measuring antidepressants in Mainz*

Antidepressant	Plasma concentration [ng/mL]	Relative error [%] (n = 20)	Inter-day coefficient of variation [%] (n = 20)
Paroxetine	22	-13.6	10.1
	105	-1.9	10.5
Sertaline	21	-2.9	11.7
	101	3.3	9.1
Venlafaxine	23	10.4	10.8
	244	2.5	6.3
O-desmethylvenlafaxine	50	3.8	7.7
	499	0.5	4.1

** based on oral communication with Dr. Sebastian Härtter*

paroxetine, sertraline and citalopram. A comparison of the external quality control results showed that more than 80% of the samples did not deviate more than 20% from the consensus mean of all laboratories participating at Health Control.

For mirtazapine, no external quality control was available, thus plasma concentrations were analysed in duplicate for this thesis.

3.4.2 Lithium

Lithium serum concentrations were determined by flame emission spectroscopy. This assay is based on thermic excitation of valence electrons and measures the photoenergy that is set free when returning to the ground state at a wave lengh of 680.7 nm (Amdisen 1975).

The flame photometer (FMC 6341 with compressor 5240, Eppendorf, Hamburg) was heated for 15 min before analysis and calibrated with 2 mL destilled water and 2mL lithium standard solution (2 mM, Eppendorf, Hamburg). Patient samples were prepared by mixing 100µL of plasma and 2 mL destilled water and were analysed in duplicate. In addition to the patient samples, two external quality control samples (Lyphocheck Assayed Chemistry Control Level 1, BioRad, München and Precinorm U Universal-Kontrollserum für Lithium, Roche Molecular Biochemicals, Mannheim), and two internal quality control samples were analysed in duplicate. Internal quality control was carried

out according to the Guideline of the Bundesärztekammer (Bundesärztekammer 2002); measured concentrations of quality control samples should not deviate more than three times the standard deviation from the true concentration. Every third month, the laboratory participated in external quality controls of INSTAND e.V., Düsseldorf, Germany.

Applying this method, the coefficient of variation within and between days was 0.8% and 4%, respectively, and the relative error was 3.3%.

3.5 Genotyping

Genomic DNA was prepared from leukocytes of 10 ml whole blood samples (Lewin and Steward-Haynes 1992) with a DNA Blood Isolation Kit QIAGEN-tip 500 (Qiagen GmbH, Germany) according to the manufacturer's instructions. Isolated DNA was stored at -20°C until genotyping was begun.

Genotyping for CYP2C9, CYP2C19, and CYP2D6 was performed with patients' genomic DNA. Amplification of the gene sequences studied was carried out by polymerase chain reaction (PCR, Saiki et al. 1988). Defective alleles of CYP2C9 and CYP2C19 were detected by restriction fragment length polymorphism (RFLP, Brockmöller et al. 1995). Furthermore, for CYP2D6 genotyping automated sequencing analysis (Cycle Sequencing, Wen 2001) was used.

3.5.1 CYP2C9

CYP2C9 and was done by RFLP analysis that included screening for the major defective alleles CYP2C9*2 and CYP2C9*3 (de Morais et al. 1994). Genomic DNA (300 ng) was used to amplify the CYP2C9 gene with the PCR engine T Gradient (Biometra, Göttingen, Germany), 1 U Taq DNA polymerase (Amersham Bioscience, Freiburg, Germany), 200 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 200 nmol forward primer and 200 nmol reverse primer (Interaktiva, Ulm, Germany) as stated by de Morais et al. (1994).

The detection of the CYP2C9*2 mutation was carried out as follows: the amplified fragment [375 bp] was incubated at 37°C for 18 hrs with the restriction enzyme Sau 96I (New England Biolabs, Frankfurt, Germany) that was specific for the sequence of the analysed mutation. The fragments' size was then controlled by horizontal agarose gel electrophoresis (80 V, 60 min) (Meyers et al. 1976) with the Sub Cell GT gel chamber and the Power Pack 3000 electrophoresis system (Bio-Rad, München, Germany). The gel consisted of 2% peq Gold Universal Agarose (peq Lab, Erlangen, Germany). The comparison with a specific control DNA (Roche, Mannheim, Germany) allowed the detection of three fragments [177 bp, 119 bp, 79 bp] in carriers of two wild type alleles, two fragments [256 bp, 119 bp] in carriers of two CYP2C9*2 alleles, and four fragments in carriers of one wildtype and one CYP2C9*2 allele [256 bp, 177 bp, 119 bp, 79 bp]. For detection of the CYP2C9*3 mutation the PCR product [175] was incubated at 37 °C for 18 hrs with the restriction enzyme StyI (New England Biolabs, Frankfurt, Germany). Agarose gel electrophoresis (80 V, 90 min) as described for CYP2C9*2 reveiled one fragment for carriers of two wildtype alleles [137 bp], two fragments for carriers of two CYP2C9*3 alleles [104 bp, 33 bp], and three fragments for carriers of one wildtype and one defective allele [137 bp, 104 bp, 33 bp].

Patients were divided into carriers of none, one, or two functional alleles of CYP2C9.

3.5.2 CYP2C19

Genotyping of CYP2C19 screened for the major defective alleles CYP2C9*2 (Brockmöller et al. 1995). Amplification of the CYP2C19 gene was carried out with 600 ng genomic DNA with the PCR engine T Gradient (Biometra, Göttingen, Germany), 1 U Taq DNA polymerase (Amersham Bioscience , Freiburg, Germany), 100 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 300 nmol forward primer and 300 nmol reverse primer (Interaktiva, Ulm, Germany) as stated by Brockmöller et al. (1995).

The amplified fragment [168 bp] was incubated at 37°C for 18 hrs with the restriction enzyme SmaI (New England Biolabs, Frankfurt, Germany) that was specific for the

sequence of the wildtype allele. The fragments' size was then controlled by horizontal agarose gel electrophoresis (80 V, 90 min) (Meyers et al. 1976) with the Sub Cell GT gel chamber and the Power Pack 3000 electrophoresis system (Bio-Rad, München, Germany). The gel consisted of 2% peq Gold Universal Agarose (peq Lab, Erlangen, Germany). The comparison with a specific control DNA (Roche, Mannheim, Germany) allowed the detection of two fragments [118 bp, 50 bp] in carriers of two wild type alleles, one fragment [168 bp] in carriers of two CYP2C19*2 alleles and three fragments in carriers of one wildtype and one CYP2C19*2 allele [168 bp, 118 bp, 50 bp].

3.5.3 CYP2D6

CYP2D6 genotyping screened for the functional wildtype alleles CYP2D6*1 and CYP2D6*2, for the major defective alleles CYP2D6 *3, *4, *5, *6 as well as for the rare defective alleles CYP2D6 *7 and *8, the CYP2D6 *9 allele, that shows reduced enzyme activity, and gene duplication (Daly et al. 1996).

First, a PCR from DNA samples was performed to generate a large fragment of the entire CYP2D6 gene [4414 bp] with the PCR engine T Gradient (Biometra, Göttingen, Germany), 2.63 U Expand Long Template (Roche, Mannheim, Germany), 500 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 300 nmol forward primer and 300 nmol reverse primer (Interaktiva, Ulm, Germany). Then, this fragment was used as a template to amplify four of the nine exons of the CYP2D6 gene (exon 3 [192 bp], exon 4 [202 bp], exon 5 [218 bp] and exon 6 [186 bp]) applying a set of nested PCRs with 1 U Taq DNA polymerase (Amersham Bioscience , Freiburg, Germany), 100 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 200 nmol forward primer and 200 nmol reverse primer (Interaktiva, Ulm, Germany) as previously described (Broly et al. 1995). Mutations in these amplified exons were screened using the cycle sequencing technique on an automated DNA sequencer (Model ABI 310, Applied Biosystems Inc. California, USA) under the following conditions: heating at 96°C for 2 min and 25 cycles (96°C for 10 sec, 60°C for

245 sec); the sequencing primers were those used for exon PCR, the didesoxyribonucleic triphosphates originated from the BigDye Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany).

The complete allele deletion (CYP2D6*5) was detected by PCR from genomic DNA according to the method of Steen et al. (1995). A long-PCR was carried out of 600 ng genomic DNA with the PCR engine T Gradient (Biometra, Göttingen, Germany), 1 U Expand Long Template (Roche, Mannheim, Germany), 400 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 200 nmol forward primer, 200 nmol reverse primer and 200 nmol of a third primer (Interaktiva, Ulm, Germany) producing specific fragments of 4500 bp and 3500 bp in case of deletion of the CYP2D6 gene. Separation of the PCR products were achieved by horizontal agarose gel electrophoresis (80 V, 120 min) (Meyers et al. 1976) with the Sub Cell GT gel chamber and the Power Pack 3000 electrophoresis system (Bio-Rad, München, Germany). The gel consisted of 1% peq Gold Universal Agarose (peq Lab, Erlangen, Germany) and ethidiumbromide (Merck, Darmstadt, Germany). The fragments' length was determined in comparison with a specific control DNA (Roche, Mannheim, Germany).

Detection of CYP2D6 gene duplication was carried out by long-PCR from 750 ng genomic DNA. The PCR conditions were chosen as described by Løvlie et al. (1996), applying 1 U Expand Long Template (Roche, Mannheim, Germany), 400 µM desoxyribonucleic triphosphates (Applied Biosystems, Darmstadt, Germany), 300 nmol forward primer and 300 nmol reverse primer (Interaktiva, Ulm, Germany). Specific fragments of 5200 bp and 3600 bp allowed the detection of CYP2D6 gene duplication by agarose gel electrophoresis that was carried out as described for the detection of gene deletion.

Patients were divided in carriers of none (poor metabolizer), one (intermediate metabolizer), two (extensive metabolizer) or more than two (ultrarapid metabolizer) functional CYP2D6 alleles (Lohmann et al. 2001).

3.6 Pharmacokinetic analysis

3.6.1 Pharmacokinetic models

Pharmacokinetic analysis describes the absorption, distribution and elimination of a drug in the body. This can be done by applying compartment models and estimating pharmacokinetic parameters that characterise the pharmacokinetic behaviour of a drug. Two different models were applied in this thesis.

3.6.1.1 One-compartment model

The one-compartment model was applied for mixed-effects modeling of mirtazapine. It describes a first-order absorption into a central compartment, characterised by the volume of distribution, and a first-order elimination phase that is either characterised by the elimination rate constant or the clearance (Figure 3).

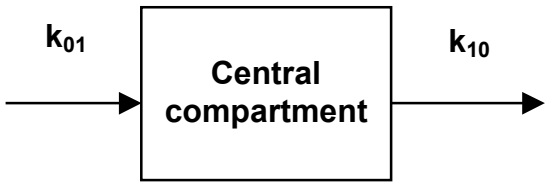


Figure 3. Schematic presentation of a one-compartment model

$$C_t = \frac{k_{01} \cdot F \cdot D}{V_d \cdot (k_{01} - k_{10})} * \left(e^{-k_{10} \cdot t} - e^{-k_{01} \cdot t}\right) \qquad \textit{Equation 1}$$

$$CL = V_d \cdot k_{10} \qquad \textit{Equation 2}$$

C_t *Plasma concentration at time t*

k_{01} *Absorption rate constant*

k_{10} *Elimination rate constant*

V_d *Volume of distribution*

D *Dose*

F *Bioavailability*

CL *Clearance*

3.6.1.2 Two-compartment model

The two-compartment model was used to characterize the pharmacokinetics of lithium. It has the same characteristics as the one-compartment-model, except that there is a peripheral compartment in equilibrium with the central compartment (Figure 4). The intercompartmental distribution follows first-order kinetics.

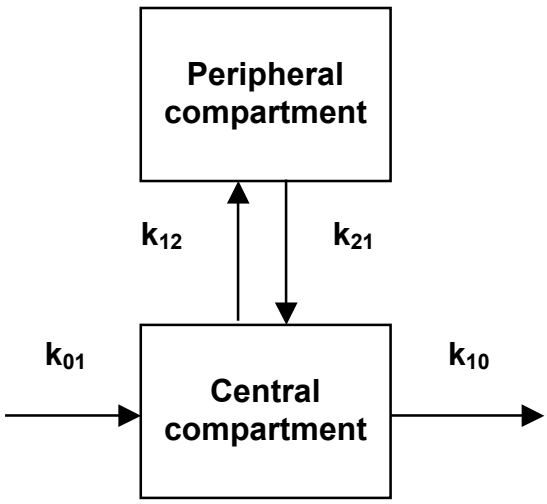


Figure 4. Schematic presentation of a two-compartment model

$$C_t = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} - C \cdot e^{-k_{01} \cdot t}$$ *Equation 3*

where $$A = \frac{D \cdot k_{01} \cdot (k_{21} - \alpha)}{V_C \cdot (\beta - \alpha) \cdot (k_{01} - \alpha)}$$

$$B = \frac{D \cdot k_{01} \cdot (k_{21} - \beta)}{V_C \cdot (\alpha - \beta) \cdot (k_{01} - \beta)}$$

$$C = A + B$$

$$\alpha = 0.5 \cdot \left(k_{12} + k_{21} + k_{10} + \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4 \cdot k_{21} \cdot k_{10}} \right)$$

$$\beta = 0.5 \cdot \left(k_{12} + k_{21} + k_{10} - \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4 \cdot k_{21} \cdot k_{10}} \right)$$

k_{12}, k_{21} *Distribution rate constants*

α, β *Hybrid rate constants*

V_c *Central volume of distribution*

3.6.2 Population pharmacokinetic analysis

Population pharmacokinetic analysis was performed with the software WinNonMix™, version 2.0.1, Pharsight Corporation, Mountain View, California. This program iteratively estimates the fixed effects representing the population mean pharmacokinetic parameters and random effects representing the inter- and intraindividual variability of these parameters in one step by fitting a population model to the data. This is different from the classical pharmacokinetic approach, where the data of each patient are analysed separately. The estimation of the fixed effects is based on generalized least squares assuming a known covariance matrix, and random effects are then estimated by maximising the restricted likelihood. Therefore, the nonlinear model function is linearised by conditional first-order Taylor expansion (FOCE) and an objective function is minimised that is proportional to twice the negative restricted log-likelihood. Individual pharmacokinetic parameters are then obtained by post-hoc Bayesian curve fitting (Pharsight Corporation 1999).

To assess the factors influencing the pharmacokinetics of mirtazapine, a model for covariate effects was built in three steps (Mandema et al. 1992): (1) Building a basic

model without covariates; (2) building the final model including all relevant covariates; and (3) assessing the appropriateness of the model.

3.6.2.1 Basic model building

The mixed effects model consists of a structural, a statistical, and a covariate model. Building the basic model means choosing the appropriate structural and statistical model.

Concerning the structural model, a one- and a two-compartment model with first-order absorption were tested. The two-compartment model was tested, because it was used previously to describe mirtazapine pharmacokinetics (Voortman and Paanakker 1995). The one-compartment model was tested to keep the model as simple as possible since only trough levels were available and this fact might prevent estimation of the distribution parameters. It was tried to estimate the pharmacokinetic parameters by the WinNonMix™ software or to fix them according to previously published data to stabilise the estimation process.

The statistical model accounts for interindividual and residual variability. Variability is usually assumed to follow normal distribution with a mean of zero. The interindividual variability (η) is described as the individual's deviation from the population mean (Θ_p) of a kinetic parameter. As individual pharmacokinetic parameters (Θ_i) are usually log-normally distributed, the interindividual variability was included in exponential form in the model:

$$\Theta_i = \Theta_p \cdot e^{\eta} \qquad \textit{Equation 4}$$

The residual variability accounts for the precision of the analysis of plasma concentrations, variations in time of drug intake or blood withdrawal and other system-related variations.

The residual error (ε) can be included in the model as a constant parameter (additive error, equation 5), a constant proportion (multiplicative error, equation 6) or a combination of both (equation 7). Each possibility was tested for the basic model.

$$\varepsilon = \sigma^2 \cdot 1$$ *Equation 5*

$$\varepsilon = \sigma^2 \cdot \left|\hat{C}\right|^a$$ *Equation 6*

$$\varepsilon = \sigma^2 \cdot \left(\alpha + \left|\hat{C}\right|^b\right)$$ *Equation 7*

σ^2 *Variance of the residual error*

a, b *Constants*

$\hat{C}$ *Predicted plasma concentration*

A model was judged reliable when (1) convergence was achived within the estimation process, (2) when the 95% confidence interval of the estimated parameter's standard error did not include zero, (3) when no covariance of the random effects was noted, and (4) when the Akaike information criterion (AIC) decreased after inclusion of an additional parameter.

3.6.2.2 Final model building

Covariates that influence the pharmacokinetic parameters of a drug can be introduced in the mixed-effects specification by additional fixed effects (Θ_1, Θ_2,…, Θ_n) representing a shift parameter or a multiplier associated with the specific covariate.

Initial screening included diagnostic plots of possible covariates versus the individual's η's that were estimated from the basic model and stepwise multiple linear regression analysis of this parameter, performed with SPSS®, version 10.0, SPSS inc., Chicago, Illinois. These covariates were then included in a stepwise forward method into the mixed-effects specification. A parameter was judged relevant if (1) convergence was achieved within the estimation process (2), if the 95% confidence interval of the estimated parameters' standard error did not include zero, (3) if the difference between the groups of categoric covariates was estimated at least 10%, (4) if the interindividual

variability was reduced, and (5) if there was a drop in the objective function value between two nested models of at least 3.84. The latter corresponded to $p < 0.05$ in the log-likelihood ratio test, assuming that the difference in the objective function values was χ^2-distributed (NONMEM Project Group 1994).

3.6.2.3 Model check

To assess the goodness-of-fit of the final model plots of observed trough plasma concentrations versus predicted plasma concentrations were examined. They should show high correlation. The weighted residuals of predicted plasma concentrations were plotted against the predicted concentrations and against time; they should be randomly distributed around zero. Histograms of individuals' η's should be normally distributed with a mean of zero.

3.6.3 Bayesian curve fitting

Bayesian curve fitting was applied to estimate individual plasma concentrations, taking into account the mean and the distribution of pharmacokinetic parameters within a standard population and measured individual plasma concentrations at the same time. The pharmacokinetic model is fitted to the data by iteration and minimising a target objective function (Φ):

$$\Phi = \sum_{i=1}^{i=N} \frac{\left(C_i - \hat{C}_i\right)^2}{\hat{\sigma}_i^2} + \sum_{j=1}^{j=M} \frac{\left(\overline{P}_j - \hat{P}_j\right)^2}{\hat{\sigma}_j^2} + \sum_{k=1}^{k=L} \frac{\left(\log\left(\overline{P}_k\right) - \log\left(\overline{P}_k\right)\right)^2}{\sigma_k^2}$$

Equation 8

C_i *I^{th} measured concentration*

$\hat{C}_i$ *I^{th} predicted concentration*

$\overline{P}_j$ *Population mean of the jth normally distributed pharmacokinetic parameter*

$\hat{P}_j$ *J^{th} predicted normally distributed parameter*

$\overline{P}_k$ *Population mean of the kth log-normally distributed parameter*

$\hat{P}_k$	*K^{th} predicted log-normally distributed pharmacokinetic parameter*
$\hat{\sigma}_i$	*Standard deviation of the ith predicted concentration*
$\hat{\sigma}_j$	*Standard deviation of the jth predicted normally distributed pharmacokinetic parameter*
$\hat{\sigma}_k$	*Standard deviation of the kth log-normally distributed pharmacokinetic parameter*
N	*Number of measured concentrations*
M	*Number of normally distributed pharmacokinetic parameters*
K	*Number of log-normally distributed pharmacokinetic parameters*

3.6.3.1 Establishment of a service for lithium dose individualisation by Bayesian curve fitting

The computer-assisted dose individualisation was established by extending the Abottbase pharmacokinetic software®, version 1.10, Abbott GmbH Diagnostika, Delkenheim, Germany.

The lithium specification was based on a literature search and included population pharmacokinetic parameters of a two-compartment model as well as covariates influencing the pharmacokinetics of lithium. For further analysis, co-medication with ACE-inhibitors, calcium antagonists and β receptorantagonists were combined in one covariate called “antihypertensives”. The covariate “diuretics” included thiazides and loop diuretics and the covariate “nonsteroidal antiinflammatory drugs” (NSAID) summarised co-medication with diclofenac and indometacine. “Obesity” included patients with a body mass index (BMI) of 30 or higher. The BMI is calculated by dividing the body weight in kilograms by the squared height in metres. Patients over 70 years of age were considered “elderly”. The creatinine clearance was calculated according to Cockcroft and Gault (1976). The lithium specification is detailed in Table 7.

The method was validated with serum concentrations from routinely monitored inpatients that were retrospectively evaluated. One to eight serum concentrations per

Table 7. Lithium specification in the Abottbase pharmacokinetic software®

Parameter	Population mean	Reference
Clearance (CL) [L / hr / 70kg]	1.5 (Range: 3.47-27.75)	Taright et al. 1994
Variability of CL [%]	38%	Taright et al. 1994
Central volume of distribution (V_c) [L / 70kg]	10.55 (Range: 3.47-27.75)	Taright et al. 1994
Variability of V_c [%]	51%	Taright et al. 1994
Distribution rate constants [hr^{-1}]		Taright et al. 1994
k_{12}	0.49 (Range: 0-1.95)	
k_{21}	1.11 (Range: 0.03-2.5)	
Absorption rate constant [hr^{-1}]	0.29	Taright et al. 1994
Bioavailability [%]		
Quilonum retard®	0.85	Manufacturers' information
Hypnorex retard®	0.95	
Correction factors for:		
Antihypertensives	CL x 0.75	Sihm et al. 2000 Krusell et al. 1997 Sproule et al. 2000
Diuretics	CL x 0.5	Sproule et al. 2000 Sihm et al. 2000
Nonsteroidal anti-inflammatory drugs	CL x 0.8	Turck et al. 2000 Reimann & Fröhlich 1981
Creatinine clearance	CL = 0.235 x CL_{crea}	Pepin et al. 1980
Obesity	CL x 1.47 V_c x 0.64	Reiss et al. 1994
Old age	CL x 0.4 V_c x 0.77	Sproule et al. 2000

patient were available for Bayesian curve fitting using the extended Abbottbase pharmacokinetic software®. Covariates were included if they reduced the residual sum of squares of the fit. To evaluate the predictive performance of the model, the accuracy and precision to predict the last observation of every patient was compared with three a-priori methods for dose individualisation (Pepin et al. 1980; Jermain et al. 1991; Yukawa et al. 1993).

3.6.3.2 Predictive performance

The predictive performance of the methods for lithium dose individualisation was assessed by calculating the mean prediction error (MPE) and the mean squared error

(MSE) representing accuracy and precision (Sheiner and Beal 1981). The closer the calculated error to zero the better the predictive performance. The corresponding equations are:

$$MPE = \frac{1}{n}\sum_{i=1}^{n}(\hat{C}_i - C_i)$$ *Equation 9*

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(\hat{C}_i - C_i)^2$$ *Equation 10*

C_i *ith measured concentration*

$\hat{C}_i$ *ith predicted concentration*

n *Number of concentrations*

3.6.3.3 Method of Pepin and colleagues (1980)

Pepin and colleagues assumed the pharmacokinetics of lithium to follow a one-compartment model and the lithium clearance to amount 23.5% of the creatinine clearance. Consequently, they defined the trough serum concentration to equal

$$CP = \frac{D \cdot e^{-k_{10} \cdot \tau}}{V_d \cdot \left(1 - e^{-k_{10} \cdot \tau}\right)}$$ *Equation 11*

where $k_{10} = \frac{\ln 2}{t_{1/2}}$ and $V_d = \frac{CL}{k_{10}}$

where $t_{1/2} = \frac{22}{1 - 0.95 \cdot \left(\frac{1 - CL_{Crea}}{100}\right)}$ and $CL = CL_{Crea} \cdot 0.235$

where $CL_{Crea}^{male} = \frac{(140 - age) \cdot IBW}{72 \cdot S_{Crea}}$ and $CL_{Crea}^{female} = 0.85 \cdot CL_{Crea}^{male}$

where $IBW_{male} = \frac{50 + 2.3 \cdot (height - 152.4)}{2.54}$

and $IBW_{female} = \frac{45.5 + 2.3 \cdot (height - 152.4)}{2.54}$

CP *Predicted trough serum concentration (mmol/L)*

τ *Dosing interval (h)*

k_{10} *Elimination rate constant (h^{-1})*

V_d *Volume of distribution (L)*

D *Dose (mmol)*

$t_{1/2}$ *Half-life (h)*

CL_{Crea} *Creatinine clearance (mL/min)*

CL *Clearance of lithium (mL/min)*

S_{Crea} *Serum creatinine concentration(mg/dL)*

IBW *Ideal body weight (kg)*

3.6.3.4 Method of Yukawa and colleagues (1993)

Yukawa and colleagues developed a population pharmacokinetic model by mixed effects modeling by retrospectivly analysing 303 serum concenrtations of 90 patients from routine serum concentration monitoring. They applieda one-compartment model to the data and found serum creatinine, total body weight and age above or below 50 years to predict the lithium clearance.

$$CP = \frac{D}{CL}$$ *Equation 12*

where $$CL_{age<50} = 31.6 + \frac{0.225 \cdot weight - 7.79}{S_{Crea}}$$

and $$CL_{age\geq 50} = 31.6 + \frac{0.225 \cdot weight - 7.79}{S_{Crea} - 0.634 \cdot (age - 50)}$$

CP *Predicted serum trough concentration (mmol/L)*

D *Dose (mmol/day)*

CL *Clearance of lithium (L/day)*

S_{Crea} *Serum creatinine concentration (mg/dL)*

3.6.3.5 Method of Jermain and colleagues (1991)

Similar to Yukawa and colleagues, Jermain and colleagues developed a population pharmacokinetic model for lithium by mixed effects modeling by analysing the data of 79 inpatients from routine drug monitoring. They used a one-compartment model and found the creatinine clearance (calculated according to Cockcroft and Gault (1976)) and the lean body weight to be significant covariates on lithium clearance.

$$CP = \frac{D}{CL}$$ *Equation 13*

where $$CL = (0.0093 \cdot LBW) + (0.0885 \cdot CL_{Crea})$$

where $$CL_{Crea}^{male} = \frac{(140 - age) \cdot weight}{72 \cdot S_{Crea}} \qquad CL_{Crea}^{female} = 0.85 \cdot CL_{Crea}^{male}$$

and $$LBW_{male} = 1.1 \cdot weight - 128 \cdot \left(\frac{weight}{height}\right)^2$$

and $$LBW_{female} = 1.07 \cdot weight - 148 \cdot \left(\frac{weight}{height}\right)^2$$

CP *Predicted serum trough concentration (mmol/L)*

D *Dose (mmol/day)*

CL_{Crea} *Creatinine clearance (L/h)*

CL *Clearance of lithium (L/h)*

S_{Crea} *Serum creatinine concentration (mg/dL)*

LBW *Lean body weight (kg)*

3.7 Statistical analysis

3.7.1 Descriptive statistics and hypothesis testing

The characteristics of a sample were described by the arithmetric mean and the relative standard deviation for continuous and normally distributed data as well as by the median and range.

The one-sample Kolmogorov-Smirnov test was applied to check wether the distribution of a sample was in accordance with a normal distribution. A $p < 0.05$ indicated a significant difference between the two distributions.

χ^2-testing was used to check wether the frequencies of categories within two populations were significantly different from each other ($p < 0.05$).

3.7.2 Correlation and regression

Correlation analysis was carried out to check the extent to which two variables were related to another. The Spearman's correlation coefficient (r_S) was used because in this thesis ordinal data or data that were not normally distributed were analysed. The correlation coefficient can be between –1 and 1: 0, no correlation, –1 and 1, complete negative and positive correlation, respectively.

Regression analysis was applied to describe a functional relationship between a dependent variable and several covariates.

Stepwise multiple linear regression was used for continuous dependent variables and continuous, ordinal or dichotomous covariates. Covariates were introduced in the linear model in a stepwise forward manner checking the significance of an improved fit after inclusion and exclusion of each covariate. The goodness-of-fit was assessed by the coefficient of determination (r^2) that is the closer to 1, the better the model approximates linearity.

The functional relationship is described as:

$$y = \alpha + b_1 \cdot x_1 + b_2 \cdot x_2 + \ldots + b_n \cdot x_n$$ *Equation 14*

y *Dependent variable*

a *Intercept*

$x_{1,2,\ldots,n}$ *Covariates*

$b_{1,2,\ldots,n}$ *Regression coefficients*

If the calculated r^2 and $b_{1,2,\ldots,n}$ differ significantly ($p < 0.05$) from 0 by the statistical test parameters of F or T, a covariate is included in the linear model. The underlying statistical procedures are analysis of variance (ANOVA) or Student's t-test.

Logistic regression was carried out on dichotomous dependent variables. Covariates were introduced in a stepwise forward manner. In this procedure, the probability of one of the two events was calculated by:

$$p = \frac{1}{1 + e^{-z}}$$ *Equation 14*

where $$z = \alpha + b_1 \cdot x_1 + b_2 \cdot x_2 + \ldots + b_n \cdot x_n$$

z *Dependent dichotomous variable*

p *Probability*

a *Intercept*

$x_{1,2,\ldots,n}$ *Covariates*

$b_{1,2,\ldots,n}$ *Regression coefficients*

A variable is included in the logistic regression model if the computed $b_{1,2,\ldots,n}$ differ significantly from 0 ($p < 0.05$) by χ^2-testing according to Wald. The Odds Ratio represents the dependent variable's relative risk for one of two events compared to the other event in predefined populations. It is calculated by:

$$OR = \frac{f_{11} \cdot f_{22}}{f_{12} \cdot f_{21}}$$ *Equation 15*

f_{11} *Number of individuals with event 1 in group 1*

f_{12} *Number of individuals with event 1 in group 2*

f_{21} *Number of individuals with event 2 in group 1*

f_{22} *Number of individuals with event 2 in group 2*

3.7.3 Receiver operating curve

The receiver operating curve was used to describe the ability to classify patients into responders or nonresponders with respect to antidepressant treatment by their mean trough plasma concentration.

The specifity is plotted versus 1 minus the sensivity of this prediction. A parameter used to predict and event should be as specific and precise as possible (Bühl and Zöfel 2002). Thus, the predicted coordinates of the curve were used to define a threshold concentration with the best sensitivity and specifity by plotting the difference between specifity and 1 minus sensitivity against the mean trough plasma concentration.

3.7.4 Goodness-of-fit

Goodness-of-fit parameters are commonly based on the minimisation of the sum of least squares (ss).

$$SS = \sum \varepsilon_i = \sum \left(C_i - \hat{C}_i \right)^2 \qquad \textit{Equation 16}$$

The Akaike information criterion (AIC) evaluates the goodness-of-fit by taking into account the number of parameters in the model (Yamaoka et al. 1978). It is calculated according to the following formula:

$$AIC = N \cdot \ln(ss) + 2 \cdot P \qquad \textit{Equation 17}$$

AIC *Akaike information criterion*
N *Number of plasma concentrations*
ss *Sum of least squares*
P *Number of model parameters*

4 Results

4.1 Impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome

4.1.1 Patient characteristics

The entire dataset for analysis of the impact of CYP2C9, CYP2C19 and CYP2D6 on treatment outcome consisted of 875 observations of 136 patients. Twenty-five patients were recruited in Mainz and received sertraline, venlafaxine or paroxetine. One patient was treated in Gabersee and received doxepin followed by venlafaxine. In addition, 110 patients were recruited in Bonn and treated with citalopram, mirtazapine, amitriptyline, sertraline, doxepin, fluvoxamine, clomipramine or paroxetine. From these data, patients were selected according to predefined criteria (see section 3.1.1.1) to evaluate the relationship between genotype, trough plasma concentration, response, and side effects. The frequencies of antidepressants within the three subsets is summarised in Table 8.

Table 8. Frequencies of antidepressants in the analysis of the relationship between genotype and treatment outcome *

	All observations	Subset for concentration evaluation	Subset for response evaluation	Subset for side effect evaluation
Amitriptyline	9 / 44	3 / 29	4 / 27	6 / 6
Citalopram	50 / 333	47 / 295	41 / 301	50 / 50
Clomipramine	2 / 2	0 / 0	0 / 0	0 / 0
Doxepin	4 / 18	4 / 17	3 / 17	4 / 4
Fluvoxamine	1 / 5	1 / 5	0 / 0	1 / 1
Mirtazapine	43 / 249	43 / 238	33 / 215	41 / 41
Paroxetine	5 / 25	6 / 22	3 / 18	4 / 4
Sertraline	14 / 90	14 / 79	9 / 82	14 / 14
Venlafaxine	17 / 109	15 / 75	12 / 91	16 / 16

**Number of patients per number of observations*

Table 9. Patient characteristics for evaluation of the impact of genotypes on trough plasma concentrations

Age [years] **		49 (14)
Gender *	Female	78
	Male	58
Height [cm] **		170 (9)
Patients' mean weight [kg] **		76.3 (17.5)
Deviation of mean dose-corrected trough plasma concentration from the substance-specific median [%] **		17.1 (80.8)
Patients' mean number of substrates of: ** #	CYP1A2	0.10 (0.27)
	CYP2C9	0.20 (0.44)
	CYP2C19	0.16 (0.36)
	CYP2D6	0.27 (0.45)
	CYP3A4	0.68 (0.72)
Patients' mean number of inhibitors of: ** #	CYP2C9	0.03 (0.16)
	CYP2C19	0.07 (0.26)
	CYP2D6	0.08 (0.23)
	CYP3A4	0.68 (0.72)
Patients' mean number of inducers of: ** #	CYP1A2	0.09 (0.01)
	CYP2C9	0.03 (0.16)
	CYP2C19	0.03 (0.16)
	CYP3A4	0.05 (0.20)
Patients' mean consumption of: ** #	Coffee +	0.92 (0.46)
	Cigarettes +	0.74 (0.90)
	Alcohol +	0.18 (0.37)

**expressed as numbers of subjects within the specific category*
***expressed as mean (standard deviation)*
#calculated over the patients' entire observation period
+for definition refer to section 3.1.1.1

The patients for evaluation of the impact of genotypes on plasma concentrations were selected according to the criteria detailed in section 3.1.1.1. The analysis included 760 trough plasma concentrations of 136 patients (Table 9).

Thirty-two observations were excluded since plasma concentrations or doses were missing or steady-state was not reached. Eighty-three observations were excluded because they were carried out on a second antidepressant that was administered to some patients within the course of the study.

Patients for evaluation of the impact of genotypes on treatment response were selected according to the criteria defined in section 3.1.1.1. These data consisted of 112 patients (Table 10).

Table 10. Patient characteristics for evaluation of the impact of genotypes on treatment response

Age [years] **		50 (14)
Gender *	Female	62
	Male	50
CYP2C9 genotype *	PM	3
	IM	32
	EM	76
CYP2C19 genotype *	PM	5
	IM	26
	EM	81
CYP2D6 genotype *	PM	5
	IM	38
	EM	66
	UM	3
Subtype of depression *	Unipolar	101
	Bipolar	9
	Dysthymia	1
	Brief recurrent	1
Mean duration of observation [weeks] **		6.7 (2.4)
HAMD total score at first rating **		23.9 (6.0)
Relative reduction of HAMD score from the first to the last observation [%] **		24.5 (38.8)
Patients' mean number of: ** #	Benzodiazepines	0.43 (0.44)
	Hypnotics	0.17 (0.33)
	Antipsychotics	0.14 (0.29)
	Mood stabilisers	0.14 (0.35)
	Other antidepressants	0.18 (0.30)

**expressed as numbers of subjects within the specific category*
***expressed as mean (standard deviation)*
#calculated over the patients' entire observation period
PM = poor metabolizers, IM = intermediate metabolizers, EM = extensive metabolizers, UM = ultrarapid metabolizers

The evaluation of the impact of genotypes on side effects was carried out on relevant side effects reported at all patients' first observation after the start of antidepressive treatment (for definition refer to 3.1.1.1). Co-medication was stratified according to its clinical effects to control for interferences (Table 11).

As the majority of patients were treated with antidepressants possessing pronounced serotonergic activity, a further subanalysis on serotonergic side effects was carried out among 85 patients treated with SSRIs or venlafaxine. Therefore, the sum of the following UKU items was evaluated at the patient's first observation: concentration

Table 11. Co-medication at the first observation from patients evaluated for the impact of genotypes on side effects

	Median	Range
Number of co-medicated drugs		
causing sedation	1	0 – 4
causing serotonergic effects	0	0 – 2
causing anticholinergic effects	0	0 – 2
causing agitation	0	0 – 2
influencing blood pressure	1	0 – 5
influencing the gastro-intestinal system	0	0 – 2

difficulties, confusion, failing memory, inner unrest, tremor, sweating, diarrhoea, and nausea. These items were selected according to Sternbach (1991).

4.1.2 Genotype distributions

Genotyping detected the funtional wildtype alleles CYP2C9*1, CYP2C19*1, CYP2D6*1 and *2, the main defective alleles CYP2C9*2 and *3, CYP2C19*2, CYP2D6*3, *4, *5, *6, *9 and CYP2D6 gene duplication. χ^2-testing for CYP2C9 and CYP2C19 showed no

Table 12. *A*llele frequencies among psychiatric patients and control groups

CYP2C9 allele	Psychiatric patients (n=270)		Nonpsychiatric patients (n=1122) (Taube et al. 2000)	
	n	%	n	%
*1	223	82	944	84
*2	26	9.6	119	11
*3	21	7.7	59	5
CYP2C19 allele	**Psychiatric patients (n=272)**		**Healthy volunteers (n=280) (Xie et al. 1999)**	
*1	229	84.2	238	85
*2	43	15.8	42	15
CYP2D6 allele	**Psychiatric patients (n=272)**		**Healthy volunteers (n=390) (Griese et al. 1998)**	
*1 or *2	203	74.6	285	73.0
*3	6	2.2	4	1.0
*4	41	15.1	76	19.5
*5	8	2.9	17	4.3
*6	3	1.1	5	1.3
*7	0	0	1	0.3
*8	0	0	1	0.3
*9	7	2.6	0	0
*16	0	0	1	0.3
Duplication	4	1.5	6	3.1

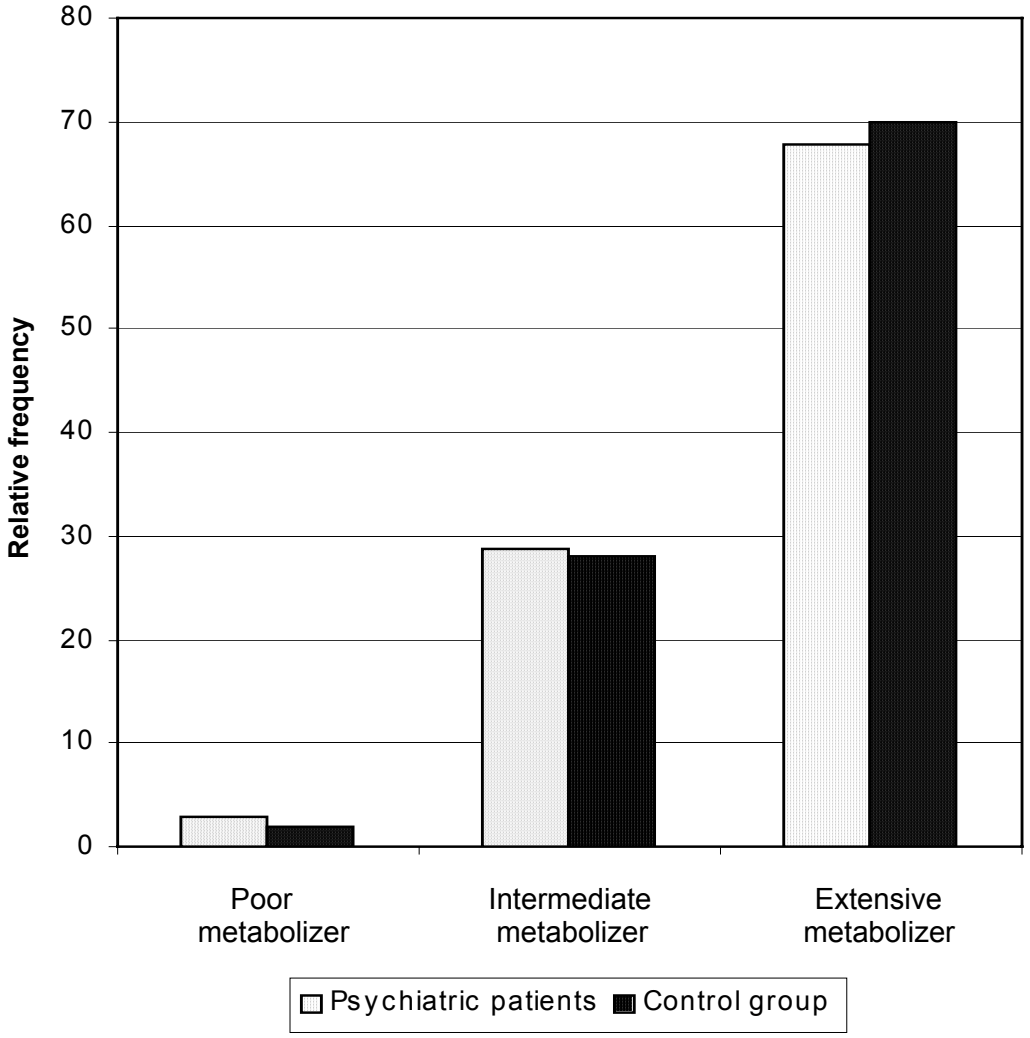


Figure 5. Comparison of the CYP2C9 genotype distribution of our psychiatric patients with the control group described by Taube et al. (2000)

significant difference with respect to allele frequencies (CYP2C9: χ^2=3.578, df=2, p=0.167 and CYP2C19: χ^2=0.140, df=1, p=0.709) when compared to other Caucasian control groups reported in the literature (Taube et al. 2000, Xie et al. 1999). Combining the rare alleles CYP2D6*7, *8, *9 and *16 in one group, there was a significant difference in the allele fequencies between our patients and 195 healthy volunteers investigated by Griese et al. (1998) (χ^2=19.578, df=6, p=0.003) (Table 12).

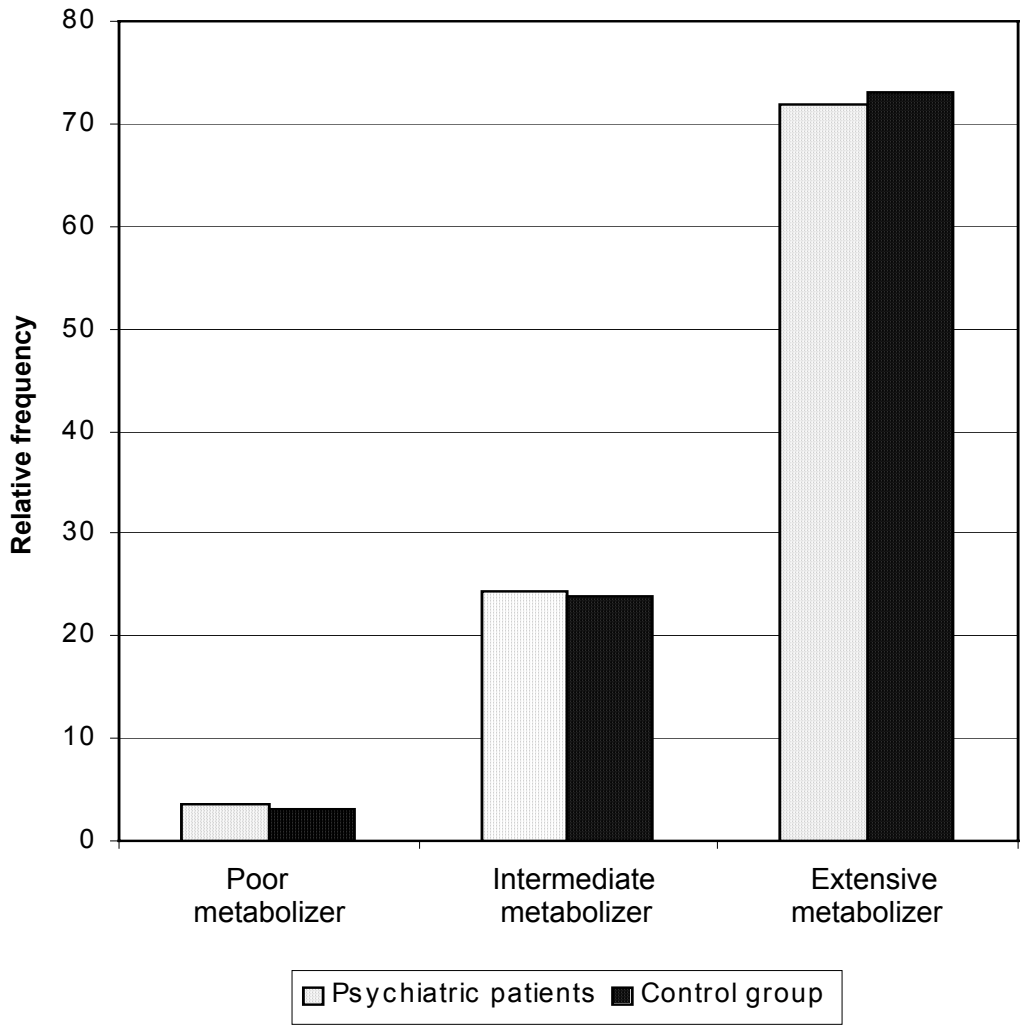


Figure 6. Comparison of the CYP2C19 genotype distribution of our psychiatric patients with the control group described by Xie et al. (1999)

According to the functionality of the detected CYP2D6 alleles the CYP2D6*1, *2 and *9 alleles were combined as functional wildtype alleles and CYP2D6*3, *4, *5, *6, *7, *8 as defective alleles without enzyme activity. There was a significant difference in the CYP2D6 genotype distribution between healthy volunteers and patients (χ^2=7.836, df=3, p=0.05): poor metabolizers of CYP2D6 were underrepresented among the patients (Figure 7).

Similar to the allele distributions there was no significant difference in the number of carriers of none, one or two functional CYP2C9 or CYP2C19 alleles (CYP2C9:

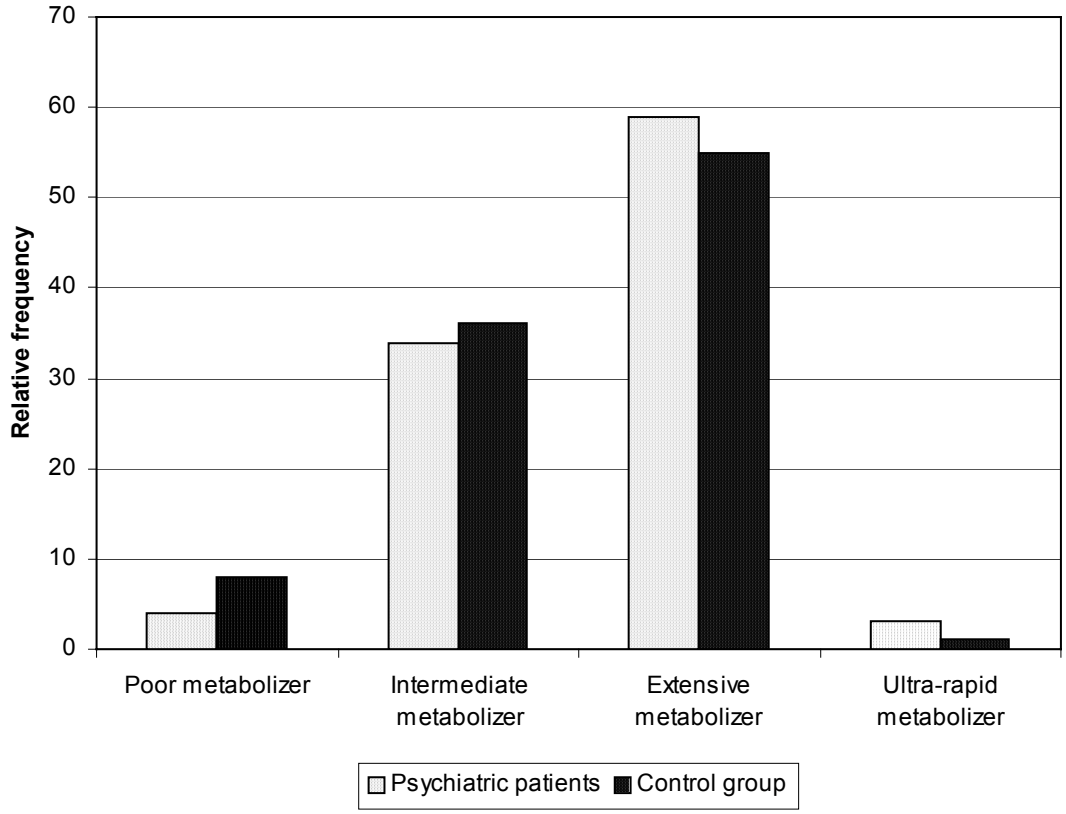


Figure 7. Comparison of the CYP2C19 genotype distribution of our psychiatric patients with the control group described by Griese et al. (1998)

χ^2=1.617, df=2, p=0.445; CYP2C19: χ^2=0.331, df=2, p=0.847) (Figure 5 and Figure 6). Thus, the CYP2C9 and CYP2C19 genotype distributions were in line with previous findings.

4.1.3 Relationship between genotype and trough plasma concentrations

Stepwise multiple regression analysis was carried out on the relative deviation of patients' mean dose-corrected trough plasma concentrations from the substance-specific median. Covariates were the influence of CYP2C9, CYP2C19 and CYP2D6 genotypes as well as gender, age, height, mean weight, coffee, alcohol and cigarette consumption, study centre, mean number of substrates, inhibitors or inducers of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The final model included CYP2D6 poor metabolizers (regression coefficient b = 108.985, T = 3.576, p < 0.001),

co-medication with CYP2D6 inhibitors (b = 102.774, T = 3.751, p < 0.001), CYP2C19 extensive metabolizers (b = -40.260, T = -2.877, p = 0.005) and smoking (b = -15.278, T = -2.160, p = 0.033). Inclusion of these covariates explained 23.1% of the dependent variable's variability (df=4 ; F=9.614; p<0.001), the corrected R^2 was 0.207. Thus, the relative deviation of mean dose-corrected plasma concentrations from the substance-specific median was significantly higher in CYP2D6 poor metabolizers or patients with co-medication inhibiting CYP2D6, and was significantly lower in CYP2C19 extensive metabolizers and smokers.

4.1.4 Relationship between genotype and treatment response

According to the HAMD-based response criterium, 48 patients (42.9%) were defined as responders and 37 patients (33%) were responders based on the CGI. These two response criteria were significantly correlated (r_S=0.73, p<0.01); 32 patients (28.6%) being responders according to both criteria. Besides the genotypes of CYP2C9, CYP2C19 and CYP2D6, logistic regression analysis tested for the following covariates that putatively influenced treatment response: Gender, age, diagnosis, antidepressant, study centre, HAMD score at first observation, length of observation, relative deviation of mean dose-corrected plasma concentrations from the substance-specific median and mean number of co-medication with benzodiazepines, hypnotics, antipsychotics, mood stabilisers or other antidepressants.

When the HAMD-defined response was evaluated by logistic regression analysis, the HAMD score at first observation had a significant impact on treatment response at the last observation (χ^2=6.854, df=1, p=0.009, Odds Ratio=1.094 (95% confidence interval 1.023-1.171). Neither the CYP2C9 genotype, nor CYP2C19 or CYP2D6 genotypes had an impact on the HAMD-defined response (χ^2=0.504, df=1, p=0.478; χ^2=0.645, df=1, p=0.422 and χ^2=0.131, df=1, p=0.717, respectively).

Evaluating the CGI-defined response, the mean number of co-medicated antidepressants and benzodiazepines were found to be significant covariates for the

prediction of response (χ^2=4.116, df=1, p=0.042, Odds Ratio=0.148 (95% confidence interval 0.023-0.937) and χ^2=4.195, df=1, p=0.041, Odd's Ratio=0.362 (95% confidence interval 0.137-0.957) respectively). No influence was seen for the CYP2C9 genotype (χ^2=0.552, df=1, p=0.457), the CYP2C19 genotype (χ^2=0.000, df=1, p=0.991) or the CYP2D6 genotype (χ^2=0.172, df=1, p=0.678).

An additional test was carried out to evaluate, if trough plasma concentrations below the limits currently used in routine drug monitoring, were associated with treatment response. These thresholds were 30 ng/mL for citalopram or mirtazapine, 20 ng/mL for sertraline, 40 ng/mL for paroxetine, 50 ng/mL for the sum of doxepin and desmethyldoxepin, 80 ng/mL for the sum of amitriptyline and nortriptyline and 195 ng/mL for the sum of venlafaxine and norvenlafaxine as outlined by the Consensus Group on therapeutic drug monitoring of the Arbeitsgemeinschaft für Neuropsychologie und Psychopharmakologie (AGNP) (personal communication). Response was not significantly different in patients with mean trough plasma concentrations above or below these thresholds when defined according to the HAMD (χ^2=0.001, df=1, p=0.982), but a trend was detected when response was defined according to the CGI (χ^2=3.018, df=1, p=0.082).

4.1.5 Relationship between genotype and side effects

Relevant side effects at first observation were experienced by 52 of the 136 patients. The influence of the CYP2C9, CYP2C19 and CYP2D6 genotypes on the occurrence of relevant side effects in the entire subset was tested by logistic regression analysis (CYP2C9: χ^2=1.156, df=1, p=0.283; CYP2C19: χ^2=0.847, df=1, p=0.357; CYP2D6: χ^2=0.283, df=1, p=0.595). Other covariates were: Gender, age, study centre, relative deviation of the dose-corrected plasma concentration to the substance-specific median and co-medication affecting blood pressure or the gastro-intestinal system, causing sedation, agitation, anticholinergic or serotonergic side effects. No covariate influenced the occurrence of relevant side effects.

Patients treated with SSRIs or venlafaxine (n=85) were then analysed separately for a relationship between genotypes and the severity of serotonergic side effects. No impact of genotypes was found but the severity of serotonergic side effects was linked to sedative co-medication (b = 0.703, T = 1.999, p = 0.049). The rating of the severity of serotonergic side effects turned out to be different among the study centres (b = -0.419, T = -3.974, p < 0.001). These findings were obtained by stepwise multiple linear regression analysis (corrected R^2 = 0.247) including the same covariates as for logistic regression analysis.

4.1.6 Plasma concentrations and clinical outcome of poor metabolizers and ultrarapid metabolizers

Clinical data of poor metabolizers of CYP2C9, CYP2C19 and CYP2D6 and ultrarapid metabolizers of CYP2D6 are given in Table 13. All poor metabolizers of CYP2C9 were inconspicious with respect to trough plasma concentrations or side effects. Two poor metabolizers of CYP2C19 showed dose-corrected plasma concentrations that were more than twice higher than the substance-specific median: one patient received 100-225 mg amitriptyline in combination with propranolol, digoxine, valproate and nifedipine or amlodipine; the other patient received 75 mg sertraline in combination with folic acid. The mean dose-corrected plasma concentration of one of the CYP2D6 poor metabolizers was 678% higher than the substance-specific median. This patient took 150 mg venlafaxine in combination with mirtazapine and zopiclon or later on with risperidone and pipamperone. Although both, risperidone and pipamperone, are also substrates of CYP2D6, trough plasma concentrations before and after initialisation of these drugs were equally high. Initially, five out of the six CYP2D6 poor metabolizers experienced relevant side effects.

Table 13. Treatment response and side effects of poor metabolizers and ultrarapid metabolizers *

	Drug	Deviation from median dose-corrected trough plasma concentrations	Relative drop of HAMD from first to last observation	Absolute drop of CGI1 from first to last observation	CGI2 at last observation	Relevant side effects at first observation
CYP2C9	Citalopram	-25%	-79%	0	4	No
PM	Venlafaxine	0%	61%	0	3	Yes
	Mirtazapine	-41%	n.a.	n.a.	n.a.	No
	Mirtazapine	-39%	22%	1	4	Yes
CYP2C19	Sertraline	188%	48%	1	4	No
PM	Amitriptyline	133%	50%	2	3	No
	Citalopram	64%	5%	1	3	Yes
	Sertraline	36%	0%	0	4	Yes
	Paroxetine	88%	-67%	0	4	No
CYP2D6	Doxepin	-12%	59%	2	3	Yes
PM	Citalopram	70%	75%	4	2	Yes
	Mirtazapine	28%	24%	2	3	No
	Citalopram	39%	-35%	0	4	Yes
	Sertraline	-67%	n.a.	n.a.	n.a.	Yes
	Venlafaxine	674%	-5%	0	4	Yes
CYP2D6	Amitriptyline	6%	n.a.	n.a.	n.a.	No
UM	Sertraline	-52%	65%	2	3	No
	Venlafaxine	-36%	83%	1	2	Yes
	Mirtazapine	4%	42%	2	3	Yes

n.a. not analysed for response **PM= poor metabolizers, IM= intermediate metabolizers, EM= extensive metabolizers, UM= ultrarapid metabolizers*

4.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine

4.2.1 Patient characteristics

Sixty-five patients were recruited who received mirtazapine. From these patients, 327 trough plasma concentrations were analysed. The length of observation among these patients was determined by either the limit of ten weeks that was set by the study

Table 14. Characteristics of patients treated with mirtazapine

	Entire data		**Subgroup for response evaluation**	
Patients *	65		45	
Gender *	Male	28	Male	21
	Female	37	Female	24
Age [years] **	49.2 (13.4)		49.7 (12.9)	
$HAMD_0$ *	23.7 (6.0)		24.4 (6.09)	
$HAMD_{end}$ *	17.5 (8.7)		17.9 (9.0)	
Observation duration [days] **	28 (18)		38 (16	
ICD-10 diagnosis at the end of study *	F 3	35	F 3	
	Moderate		Moderate	23
	Severe	22	Severe	19
	F 4	5	F 4	3
	Other	3		
Observations *	327		247	
Daily dose [mg] **	37.3 (8.6)		37.6 (8.7)	
Plasma concentration [ng/mL] **	31.7 (16.3)		32.9 (15.8)	
UKU_{global} at first observation **	1.03 (0.75)		0.86 (0.70)	
$UKU_{sedation}$ at first observation **	0.94 (0.94)		1.02 (0.90)	
$UKU_{increased\ sleep}$ at first observation **	0.30 (0.64)		0.34 (0.73)	
$UKU_{weight\ gain}$ at first observation **	0.44 (0.81)		0.24 (0.62)	
Psychiatric co-medication per observation *	Benzodiazepines	1 126	Benzodiazepines	94
	Hypnotics	47	Hypnotics	46
	Mood Stabilisers	24	Mood Stabilisers	0
	Antipsychotics	19	Antipsychotics	13
	Antidepressants	28	Antidepressants	0

** expressed as number within a specific category*

***expressed as mean (standard deviation)*

protocol (7.7%), the discharge from hospital due to the patient's recovery (58.5%), change of antidepressive medication (30.8%) because of partial response or nonresponse or due to mirtazapine-induced eczema (one case). All data were analysed for the relationship between mirtazapine trough plasma concentration and side effects (Table 14). In addition, the side effects of 32 observations that were carried out within the initial seven days of mirtazapine treatment were analysed separately. Subgroups were defined for evaluation of the plasma concentration-response relationship and for analysing factors influencing mirtazapine pharmacokinetics.

For evaluation of the relationship between mirtazapine trough plasma concentrations on treatment response, 45 patients were selected according to the criteria stated in section 3.1.1.2 (Table 14).

Patients for population pharmacokinetic analysis were selected according to the criteria defined in section 3.1.1.2. Out of the 327 trough plasma concentrations, seven were excluded because of missing dosing schedule, noncompliance (as stated by the patient or the hospital staff), or interference in the analytical assay. From this database we selected 260 observations with constant co-medication for population pharmacokinetic analysis. A subset of 49 patients, which included 213 observations gave written informed consent for genetic analysis. We determined the genotypes of CYP2C9, CYP2C19 and CYP2D6 of these patients (Table 15).

4.2.2 Therapeutic range

The relationship between mirtazapine dose and trough plasma concentration was assessed by computing Spearman coefficient of correlation because dose and plasma concentration were not normally distributed. A weak correlation between dose and trough plasma concentration was found ($r_S = 0.365$, $p < 0.01$). There was marked variability of the plasma concentrations ranging from 6-29 ng/mL, 0-73 ng/mL, 0-98 ng/mL and 14-76 ng/mL, when doses of 15, 30, 45 and 60 mg/day, respectively were administered (Figure 8).

Table 15. Characteristics of patients selected for population pharmacokinetic analysis of mirtazapine

		Entire data with constant co-medication	Data of genotyped patients
Age [years] **		49.6 (13.5)	48.7 (13.2)
Mean weight per patient [kg] **		77.7 (18)	76.8 (17)
Height [m] **		1.70 (0.09)	1.70 (0.09)
Gender *	Female	37	27
	Male	28	22
CYP2C9 genotype *	PM		2
	IM		15
	EM		31
CYP2C19 genotype *	PM		0
	IM		10
	EM		39
CYP2D6 genotype *	PM		1
	IM		18
	EM		29
	UM		1
Alcohol consumption *	No	56	44
	Yes	9	5
Coffee consumption *	No	11	9
	Yes	54	40
Smoking *	No	39	25
	Yes	26	24
Mean blood pressure per patient [mm HG] **	Systolic	119 (12)	119 (13)
	Diastolic	76 (8)	75 (8)
Mean puls per patient [per minute] **		78 (8)	79 (6)
Mean AST activity per patient [U/L] **		10.7 (3.8)	10 (2.9)
Mean ALT activity per patient [U/L] **		16.0 (9.6)	13.8 (7.0)
Mean γ-GT activity per patient [U/L] **		18.7 (38.4)	13.3 (8.9)
Mean serum creatinine concentration per patient [mg/dL] **		0.9 (0.1)	0.9 (0.2)

**expressed as numbers of subjects within a specific category*

***mean (standard deviation)of all observations of a patient during investigation*

PM = poor metabolizers; IM = intermediate metabolizers EM = extensive metabolizers;

UM = ultrarapid metabolizers

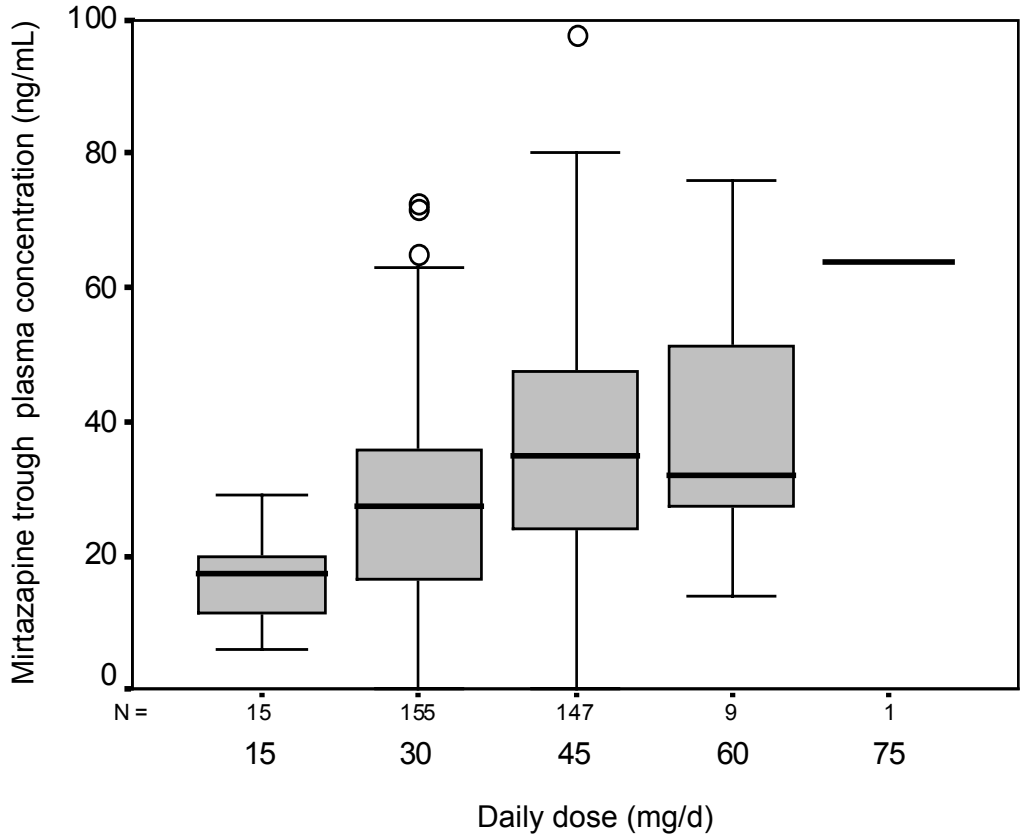


Figure 8. Boxplot of the relationship of mirtazapine daily dose to trough plasma concentrations (the median is indicated as black line, the quartiles are represented by shaded boxes and the range is expressed by error bars).

Mean mirtazapine trough plasma concentrations of responders and nonresponders were analysed for a minimum threshold concentration by a receiver operating curve (Figure 9). The best distinction between true and false positive responders was found at a threshold concentration of 30 ng/mL. For confirmation, the proportions of responders and nonresponders were compared between groups at mean trough plasma concentrations below or above 30 ng/mL and the difference was found to be significant (χ^2 = 6.017, df = 1, p = 0.014) (Table 16). Logistic regression analysis detected a small but significant impact of the individuals' mean trough plasma

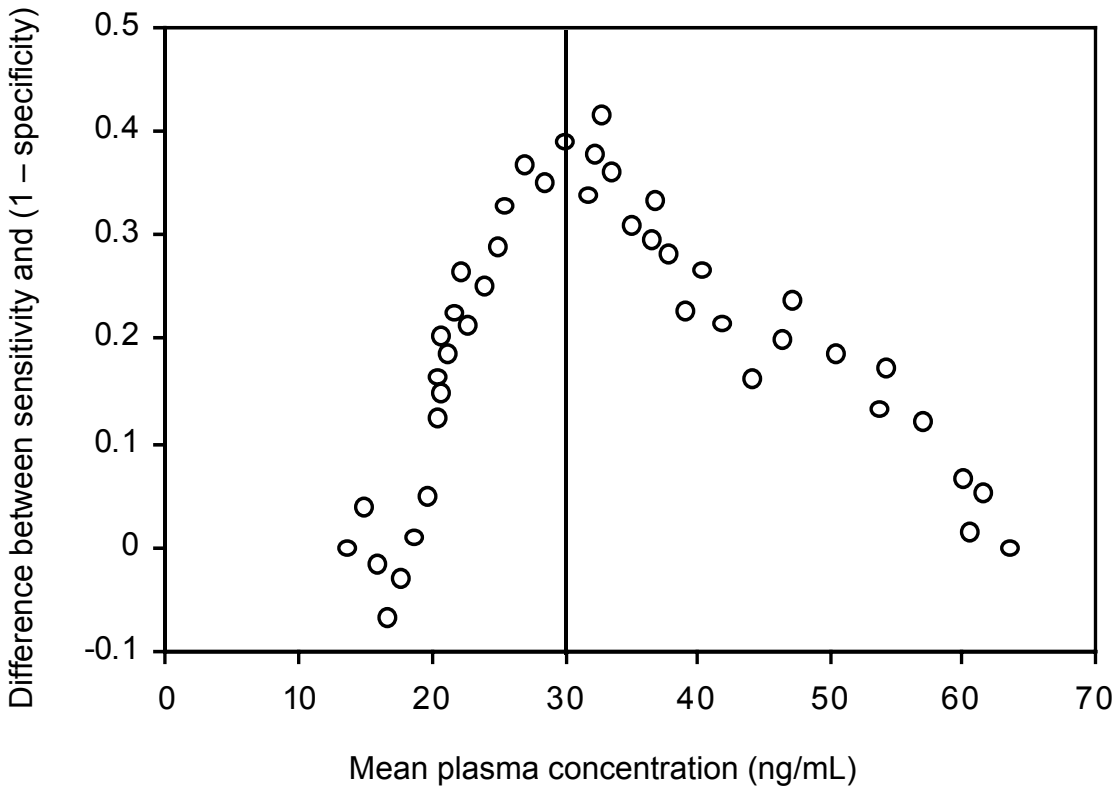


Figure 9. Receiver operating curve of response to mirtazapine treatment. The difference between sensitivity and 1-specificity is plotted against individuals' mean trough plasma concentrations.

concentration on the reponse to mirtazapine (χ^2 = 4.371, df = 1, p = 0.031, Odds Ratio = 1.054 (95% confidence interval: 1.005 – 1.106)), whereas no influence was found with respect to gender, age, duration of treatment or the HAMD score at first observation.

We tested the influence of mirtazapine trough plasma concentrations on the magnitude of the most frequently experienced side effects such as sedation, increased duration of sleep, weight gain and on the global assessment of the patient's performance. Nonparametric correlation analysis of all observations yielded no significant correlation between trough plasma concentration and the degree of sedation, weight gain or

impairment of the patient's performance. There was also no relationship between the number of additional sedative co-medication (benzodiazepines, antipsychotics, other sedative antidepressants, hypnotics or mood stabilisers) and the UKU item sedation or increased duration of sleep.

When only observations during the first week of mirtazapine treatment were included in the analysis (n = 32) a weak negative correlation was detected between serum mirtazapine concentration and sedation (r_S = - 0.321, p = 0.044) and increased duration of sleep (r_S =- 0.369, p = 0.019), while a weak positive correlation was seen between increased duration of sleep and co-medication with antipsychotics (r_S = 0.333, p = 0.036) as well as with other sedative antidepressants (r_S = 0.372, p = 0.018). We found no correlation between mirtazapine trough plasma concentration and weight gain.

Table 16. χ^2-Table for evaluation of a minimum threshold concentration for response to mirtazapine

Total	Nonresponder	Responder	Total
Mean trough plasma concentration < 30 ng/mL	17	5	22
Mean trough plasma concentration > 30 ng/mL	9	14	23
Total	26	19	45

To control the influence of co-medication on sedation, increased duration of sleep, weight gain or patient's performance within this subgroup a logistic regression analysis was performed. The relationship between the occurence of increased duration of sleep and trough plasma concentration was confirmed ($\chi 2$ = 4.479, df = 1, p = 0.034, Odd's Ratio = 0.925 (95% confidence interval: 0.861 – 0.994)); neither benzodiazepines nor antipsychotics, mood stabilisers or other antidepressants were relevant covariates on the absence or presence of the respective side effect.

4.2.3 Population pharmacokinetic analysis

The following covariates were carried forward for population pharmacokinetic analysis of mirtazapine: height, weight, age, gender, males up to 48 years, smoking, alcohol and coffee consumption, the CYP2C9, CYP2C19 and CYP2D6 genotype as well as co-medication with substrates of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The number of patients that received inhibitors or inducers of CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 were too small for further analysis. Serum creatinine, concentrations, liver enzyme activity, blood pressure and pulse were within a normal range and were therefore not further evaluated. Among patients taking mirtazapine, there was one patient with two defective alleles and another showing gene duplication of CYP2D6. As both patients did not present with conspicious plasma concentrations, the poor metabolizer was combined with the intermediate metabolizers and the ultrarapid metabolizer's data were included within the group of extensive metabolizers. For the same reason, the data of two poor metabolizers of CYP2C9 were also grouped with the intermediate metabolizers. No poor metabolizer of CYP2C19 was detected within these patients. The distributions of functional alleles did not differ significantly from other groups referring to the CYP2D6 genotype (Griese et al. 1998) (χ^2=2.664, df=3, p=0.446) or the CYP2C9 genotype (Taube et al. 2000) (χ^2=2.282, df=2, p=0.319). Comparing the frequencies of intermediate and extensive CYP2C19 metabolizers reported previously (Xie et al. 1999) with the present data, no significant difference was found (χ^2=0.551, df=1, p=0.458).

The basic model was developed with a structural one-compartment model and first-order absorption. As the data consisted of trough levels, the population mean was estimated only for clearance ($\Theta_{CL/F}$). The population mean of the absorption rate constant (k_{01}) was fixed to 1.5 hr^{-1} as estimated from the nomograph of Franke and Ritschel (1976) by the elimination rate constant and the time to reach plasma peak

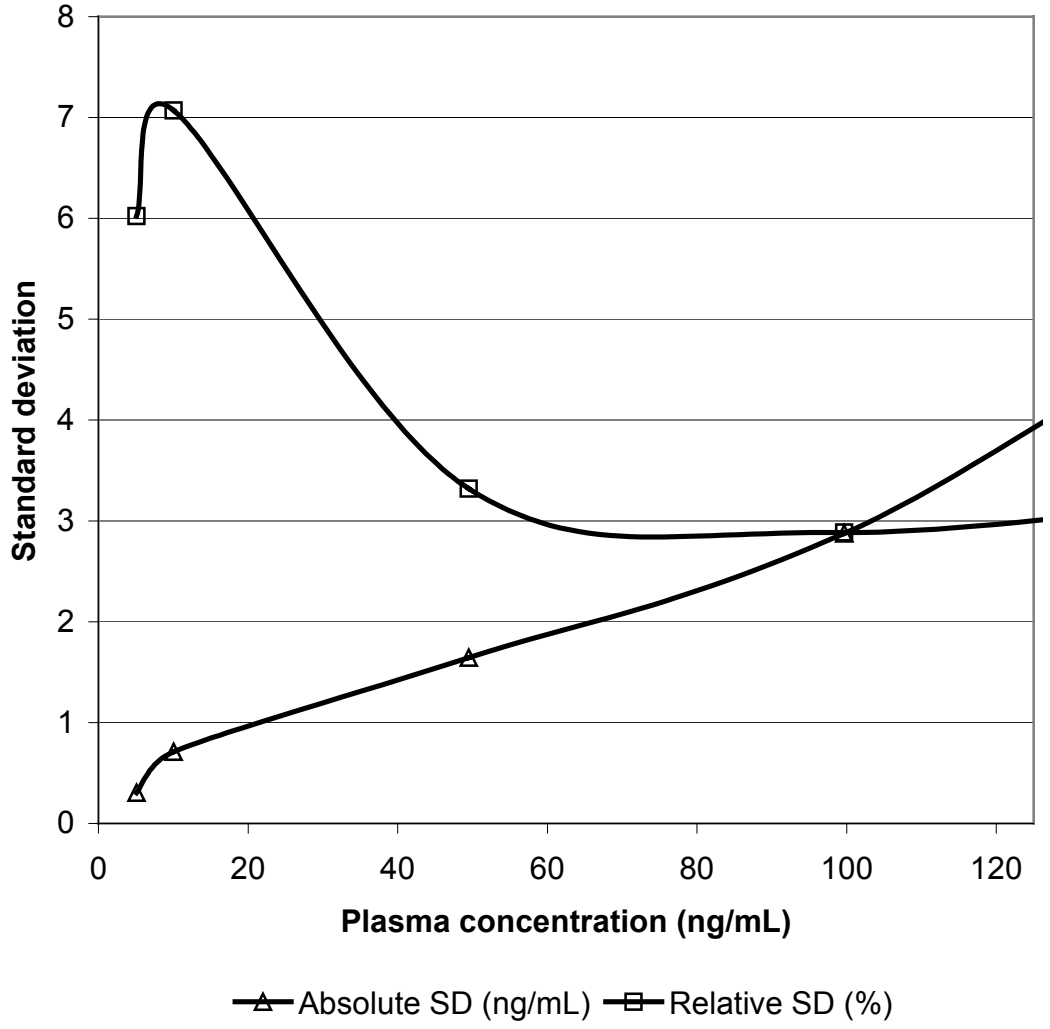


Figure 10. Precision of the HPLC method for quantification of mirtazapine plasma concentrations

concentrations reported in the literature (Timmer et al. 2000). The volume of distribution (V_d/F) was fixed to 678 L as calculated according to Voortman and Paanakker (1995). Interindividual variability of clearance $\eta_{CL/F}$ was assumed to obey normal distribution with a mean of zero. Pharmacokinetic parameters are usually log-normally distributed, thus η_{CL} was included exponentially in the model:

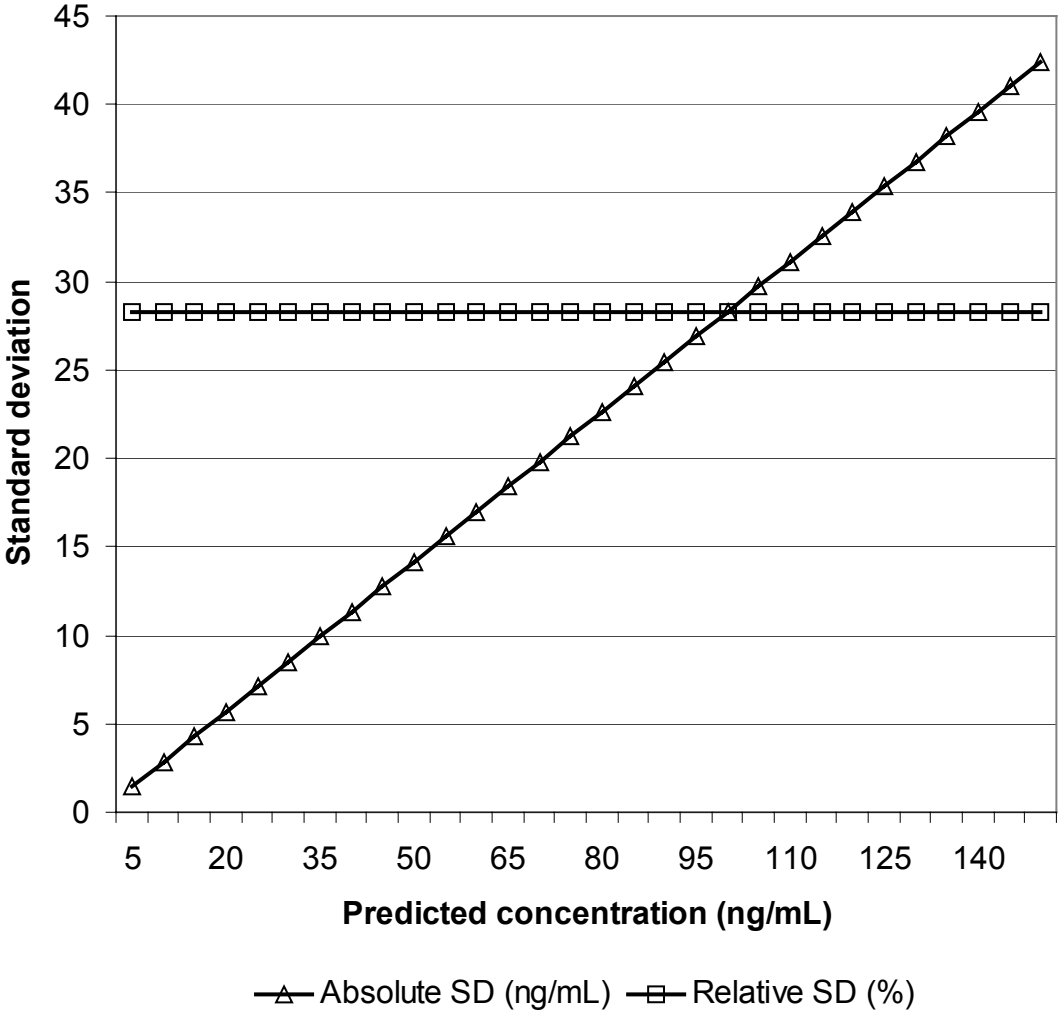


Figure 11. Residual variability estimated by mixed-effects modeling of mirtazapine

$$CL = CL/F \cdot e^{\eta_{CL/F}}$$ *Equation 18*

A multiplicative residual error model was applied:

$$\varepsilon = \sigma^2 \cdot \left|\hat{C}\right|^2$$ *Equation 19*

The latter described best the amount and distribution of the residual error, that included the analytical assay error pattern (Frahnert et al. 2003) (Figure 10) as well as

deviations from the study protocol and model misspecifications. The estimated σ^2 of 0.08 resulted in a relative standard deviation of 28.3% (Figure 11).

During the model building process, diagnostic plots and stepwise multiple linear regression analysis of η_{CL} detected the CYP2D6 genotype, smoking and co-medication with substrates of CYP3A4 as putative covariates. Then each covariate was included in the clearance mixed-effects specification in a stepwise forward manner to model interindividual variability (Table 17).

As can be seen in table 17, the final model included the CYP2D6 genotype. This was the only covariate causing a significant drop in the objective function value and reducing the interindividual variability of clearance (CL/F) from 37.4% to 32.9%. The typical value for clearance (CL/F) in intermediate metabolizers was estimated to be 26.4% lower than in extensive metabolizers.

We found no other covariate that significantly increased the goodness-of-fit. The inclusion of smoking reduced the interindividual variability of clearance to 34.3% while estimating an 30% increase in the clearance (CL/F) of smokers, but the drop in the objective function value did not reach significance. Likewise, co-medication with substrates of CYP3A4 reduced the clearance (CL/F) by 20% but failed to cause a significant drop in the objective function value.

Goodness-of-fit plots of the final model show observed trough plasma concentrations versus plasma concentrations estimated from population and individual pharmacokinetic parameters and weighted residuals (Figures 12 and 13). The histogram of individuals' CL/F approximates log-normal distribution (Figure 14). A comparison of the distributions of individual's $\eta_{CL/F}$ showed that by inclusion of the covariate CYP2D6, the bimodal distribution of the basic model was transformed into a normal distribution in the final model (Figure 15).

Table 17. Covariate model building for detection of factors influencing the clearance of mirtazapine

Clearance specification	Covariate specification	OFV	$\Theta_{CL/F}$		Θ_1 Θ_2		$\eta_{CL/F}$		σ^2	
			Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
$\Theta_{CL/F} \bullet e^{\eta}$ *		**1170.53**	**1170.99**	**96.82**			**0.101**	**0.030**	**0.080**	**0.008**
$\Theta_{CL/F}$ $e^{\eta} \bullet (1+ \Theta_1 \bullet$ (CYP2D6-2)) **	**IM=1, EM=2**	**1163.80**	**1319.20**	**172.89**	**0.264**	**0.101**	**0.081**	**0.028**	**0.079**	**0.008**
$\Theta_{CL/F} \bullet e^{\eta}$ 1+ $\Theta_1 \bullet$ (CYP2D6-2)) • (1+ Θ_2 • smoking)	IM=1, EM=2 0=no, 1=yes	1163.35	1175.92	136.98	0.231 0.230	0.080 0.132	0.073	0.020	0.079	0.011
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1 \bullet$ (CYP2D6-2)) • (1+ Θ_2 • substrate CYP3A4)	IM=1, EM=2 0=no, 1=yes	1162.24	1460.05	234.20	0.274 -0.208	0.010 0.113	0.069	0.015	0.079	0.005
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1$*(CYP2D6-2)) • (1+ Θ_2 • adult males)	IM=1, EM=2 0=no, 1=yes	1166.09	1284.73	169.05	0.276 -0.164	0.086 0.141	0.079	0.026	0.079	0.008
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1 \bullet$ smoking)	0=no, 1=yes	1168.08	1042.24	77.79	0.276	0.138	0.087	0.018	0.080	0.011
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1 \bullet$ substrate CYP3A4)	0=no, 1=yes	1170.66	1282.93	157.00	-0.198	0.101	0.093	0.030	0.080	0.006
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1 \bullet$ alcohol)	0=no, 1=yes	1171.22	1191.39	105.38	-0.081	0.028	0.101	0.031	0.078	0.008
$\Theta_{CL/F} \bullet e^{\eta} \bullet (1+ \Theta_1 \bullet$ substrate CYP2C19)	0=no, 1=yes	1172.30	1220.59	123.94	-0.177	0.104	0.098	0.032	0.080	0.007

OFV: Minimum objective function value; $\Theta_{CL/F}$: Clearance scaled by bioavailability; Θ_1, Θ_2: Shift parameters for clearance; η: interindividual variability of clearance; σ^2: Residual error; SE: Standard error; CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4: Cytochrome P450 isoenzymes 1A2, 2C9, 2C19, 2D6, 3A4
** Basic model, **Final model*

Table 17. Covariate model building for detection of factors influencing the clearance of mirtazapine (continued)

Clearance specification	Covariate specification	OFV	$\Theta_{CL/F}$		Θ_1		$\eta_{CL/F}$		σ^2	
			Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ gender)	♂=0, ♀=1	1173.06	1244.92	134.64	-0.116	0.142	0.100	0.026	0.079	0.009
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ adult males)	0=no, 1=yes	1173.93	1145.43	115.74	0.109	0.159	0.102	0.032	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ substrate CYP1A2)	0=no, 1=yes	1174.07	1174.87	101.67	-0.051	0.169	0.102	0.031	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ substrate CYP2C9)	0=no, 1=yes	1174.36	1175.70	109.74	-0.028	0.158	0.104	0.032	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ substrate CYP2D6)	0=no, 1=yes	1174.44	1164.47	101.82	0.047	0.147	0.103	0.031	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ (CYP2C19-2))	IM=1, EM=2	1174.74	1172.37	110.21	0.006	0.143	0.104	0.030	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ coffee)	0=no, 1=yes	1176.53	1194.97	212.50	-0.022	0.169	0.104	0.035	0.080	0.009
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ (age-48))	age [y]	1181.29	1173.02	96.95	-0.003	0.004	0.103	0.032	0.080	0.008
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ *(height-170))	height [cm]				Failure to estimate					
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ (CYP2C9))	IM=1, EM=2				Failure to estimate					
$\Theta_{CL/F} \cdot e^{\eta} \cdot (1+ \Theta_1 \cdot$ (weight-78))	weight [kg]				Failure to estimate					

OFV: Minimum objective function value; $\Theta_{CL/F}$: Clearance scaled by bioavailability; Θ_1, Θ_2: Shift parameters for clearance; η: interindividual variability of clearance; σ^2: Residual error; SE: Standard error; CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4: Cytochrome P450 isoenzymes 1A2, 2C9, 2C19, 2D6, 3A4
** Basic model, **Final model*

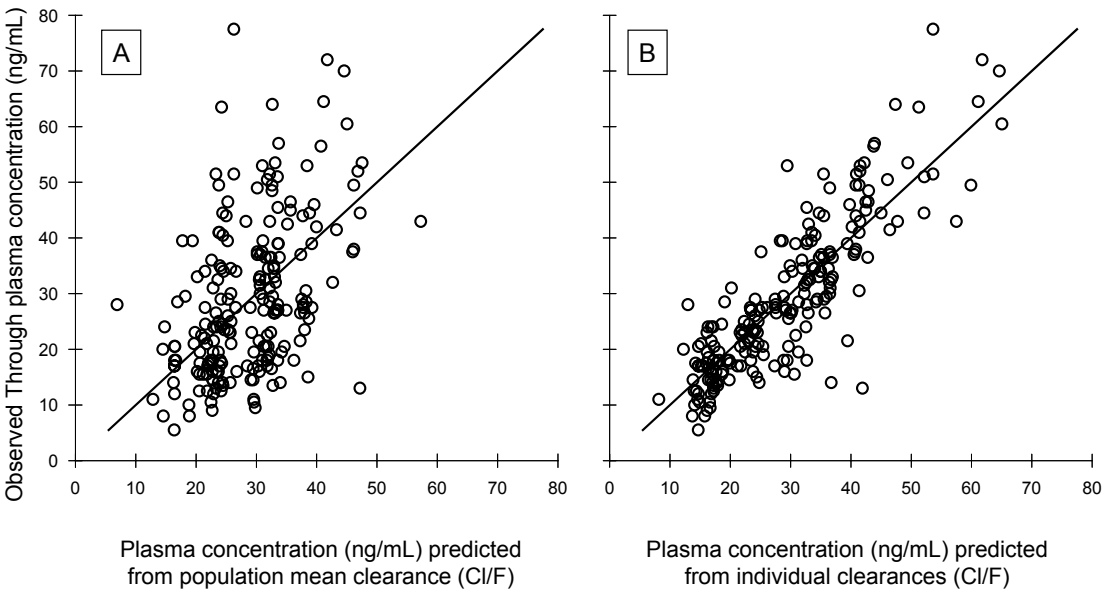


Figure 12. Observed versus predicted mirtazapine trough plasma concentrations (A) predicted from the population clearance (B) predicted from the individual estimated clearances

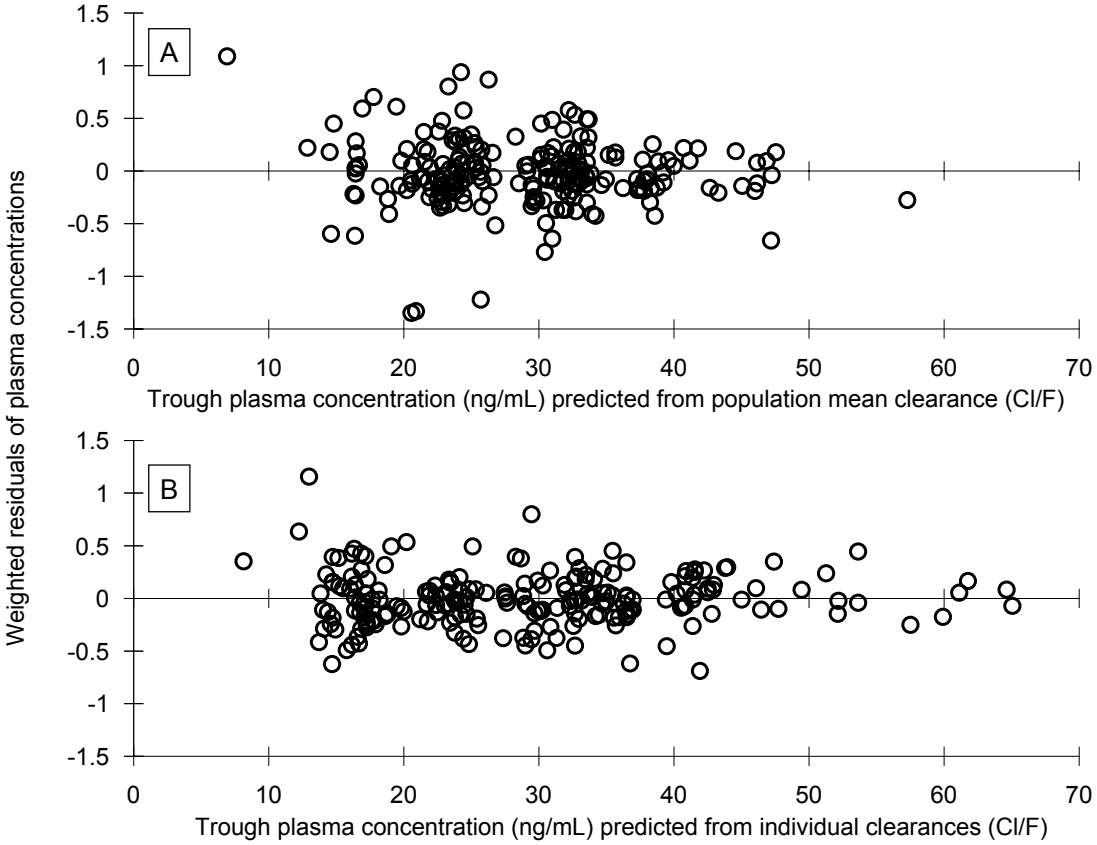


Figure 13. Weighted residuals of mirtazapine trough plasma concentrations. (A) predicted from the population clearance (B) predicted from the estimated individual clearances

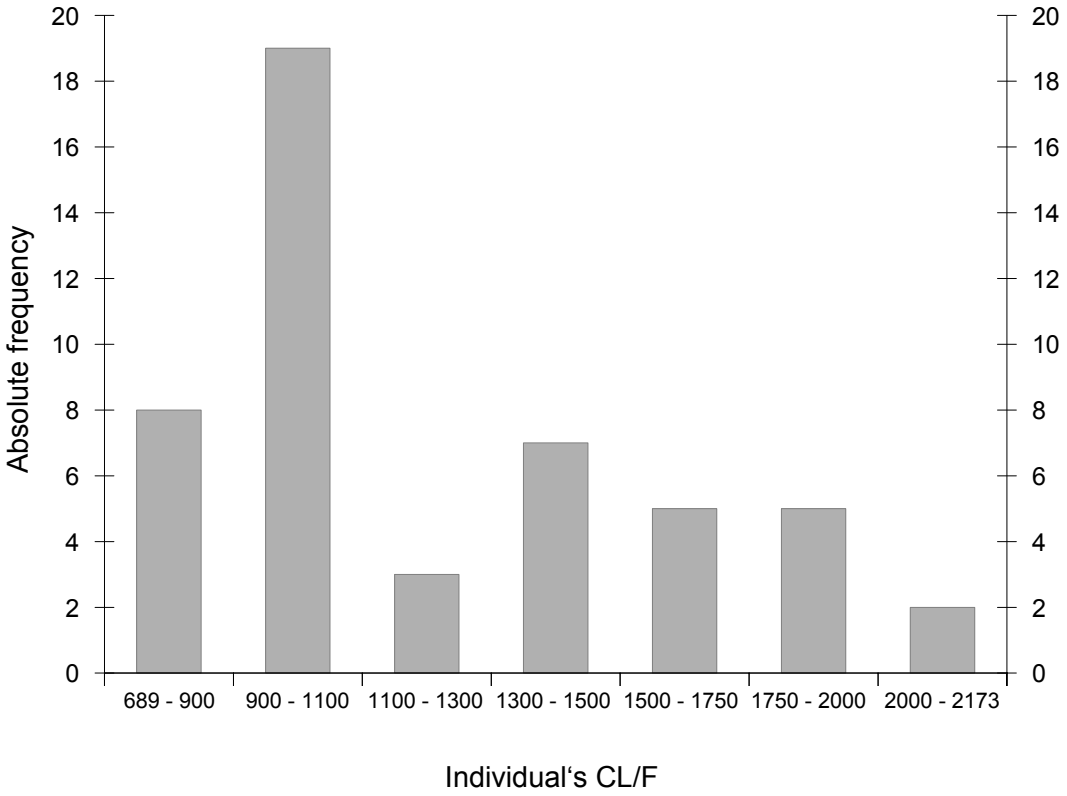


Figure 14. Distribution of individual predicted clearances (scaled by bioavailability) estimated by the final model

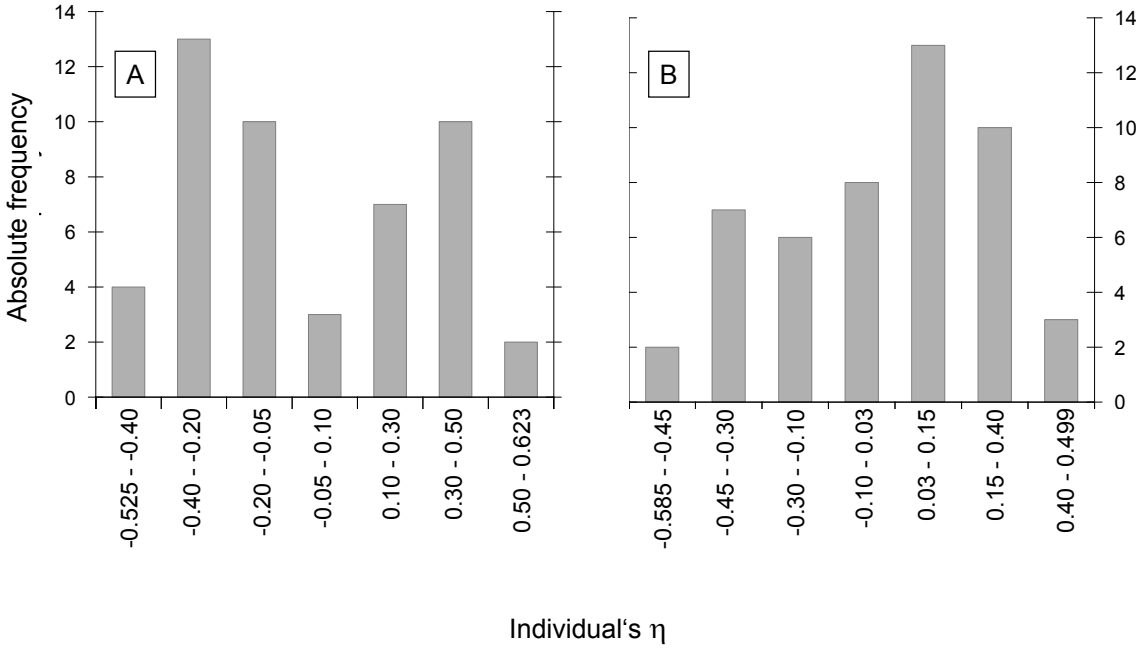


Figure 15. Comparison of the distribution of individual's variability ($\eta_{CL/F}$) from the population mean of clearance (scaled by bioavailability) estimated by (A) the basic model and (B) the final model

4.3 Computer-assisted dose individualisation of lithium

4.3.1 Patients characteristics

The data for validation of the Bayesian curve fitting method consisted of 228 lithium serum concentrations from routine monitoring of 56 inpatients (Table 18).

Patients' charts were screened for covariates with possible influence on the pharmacokinetics of lithium and revealed co-medication with ACE inhibitors (n=6), calcium antagonists (n= 3), β receptorantagonists (n=8), thiazid diuretics (n=4), loop diuretics (n=1), diclofenac (n=1), and indomet acine (n=1).

4.3.2 Validation of the method

Trough plasma concentrations of each of the 56 patients were fitted with the help of the extended Abbottbase pharmacokinetic software®. The best fit was determined by stepwise inclusion of each covariate that was noted in the patients' chart. The

Table 18. Patient characteristics for validation of the method for lithium dose individualisation

Age [years] *	46.7 ± 13.7 (21 – 81)
Height [cm] *	170 ± 8 (150–190)
Weight [kg] *	74.6 ± 12.4 (55–119)
Serum creatinine [mg/dL] *	0.95 ± 0.15 (0.7–1.3)
Number of:	
Females	32
Males	24
Elderly	5
Obesity	10
Sweating or exsiccosis	2
Co-medication with:	
Diuretics	5
Antihypertensives	14
NSAID	2

**expressed as as mean ± standard deviation (range)*
NSAID: Nonsteroidal antiinflammatory drugs

Table 19. Pharmacokinetic parameters estimated for 56 patients with the extended Abbottbase pharmacokinetic software®

	Mean (Standard deviation)	Range
Sum of least squares	1.65 (1.45)	0.003-7.86
Clearance [L/h]	1.11 (0.25)	0.46-1.77
Central volume of distribution [L]	19.78 (8.26)	8.52-61.6
Half-life [h]	13.2 (6.01)	6.29-36.6

covariates specified in the software were also used to simulate other clinical situations where similar clinical effects were observed. A covariate was included in the patient's model if it decreased the sum of least squares of the model fit. The estimated individual pharmacokinetic parameters obtained by the best fits of each patient are summarized in Table 19.

The patients' last observed plasma concentration was then carried forward to compare the predictive performance of the established method with three other methods described in the literature (Pepin et al. 1980, Jermain et al. 1991, Yukawa et al. 1993; Table 20). For that purpose, the individual pharmacokinetic parameters were estimated by Bayesian curve fitting, that included population pharmacokinetic parameters and all individual serum concentrations except for the last one. This concentration was then predicted by the individual pharmacokinetic parameters and compared to the observed concentration.

Table 20. Predictive performance of the computer-assisted method for lithium dose individualisation compared to standard methods

	Mean squared error	Mean prediction error (95% Confidence interval)
Present method	0.019	-0.023 (-0.014-0.053)
Pepin et al. 1980	0.087	0.089 (0.013-0.160)
Jermain et al. 1991	0.161	0.297 (0.075-0.246)
Yukawa et al. 1993	0.042	-0.055 (-0.007-0.091)

Out of the 56 patients 21 were best fitted by inclusion of a covariate. However, the specified covariates did not always improve the curve fit. Best fits of the fourteen patients taking antihypertensives included this covariate in six individuals; four out of the five patients taking diuretics were better fitted with the covariate “diuretics”; one of the two patients taking nonsteroidal antiinflammatory drugs was better fitted including this covariate. The pharmacokinetics of one out of ten obese patients was better described by the covariate “obesity” and one of the five patients older than 70 years was better fitted with the help of the covariate “old age”. The inclusion of a linear relationship between creatinine clearance and lithium clearance led to a better fit for only one patient who had a serum creatinine concentration of 0.8 mg/dL.

On the other hand, the pharmacokinetics of seven patients was better described

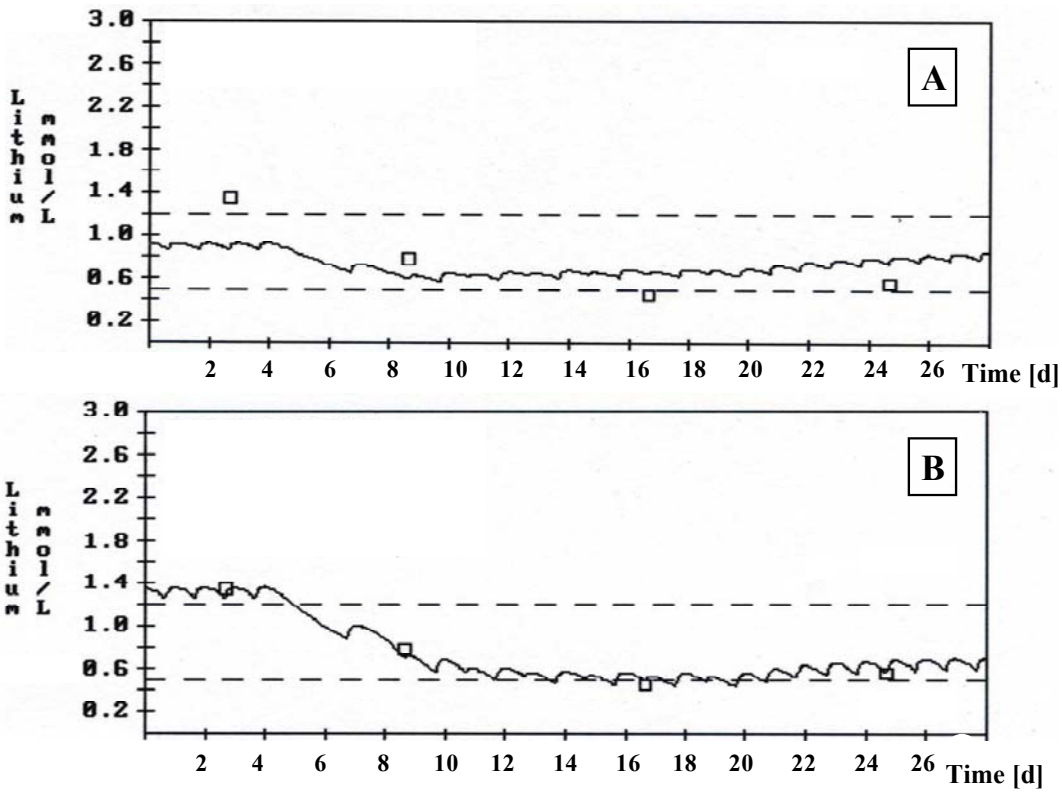


*Figure 16. Concentration-time-curve of a patient presenting with intensive sweating obtained by Bayesian curve fitting taking into account the observed serum concentrations (□): **A** without, **B** with modeling dehydratation at the first serum concentration measurement*

including covariate specifications in comparable clinical situations: including the covariate “old age” led to a better fit for three patients who did not drink enough the change in clearance by intensive sweating or the combined effect of two antihypertensives was better reflected by the covariate “diuretics” (Figure 16). A change of diaphoretic co-medication was better explained by the inclusion of the covariate “nonsteroidal antiinflammatory drugs”.

5 Discussion

In this thesis 136 patients were evaluated for an impact of CYP2C9, CYP2C19 and CYP2D6 polymorphisms on trough plasma concentrations, side effects and treatment response. These data mainly consisted of second generation antidepressants (93%) and demonstrated that changes in CYP2D6 and CYP2C19 enzyme activity and smoking significantly altered antidepressants' trough plasma concentrations. The effects on plasma concentrations were of minor clinical relevance when the entire data were anaylsed but examination of poor and ultrarapid metabolizers indicated that trough plasma concentration measurement may be of relevance for substrates of CYP2D6 in cases of nonresponse or severe side effects.

Providing a rationale for therapeutic drug monitoring of mirtazapine, a minimum threshold trough plasma concentration of 30 ng/mL may be used to optimise the dose of mirtazapine nonresponders. Dose adaption of mirtazapine may also be considered in patients with decreased CYP2D6 activity.

The computer-assisted service for dose individualisation of lithium that was established in this thesis showed good precision and accuracy and was flexible with regard to dosing schedules, times of blood withdrawal and covariates influencing the pharmacokinetics of lithium.

5.1 Impact of CYP2C9, CYP2C19 and CYP2D6 genotypes on treatment outcome

The evaluation of the impact of CYP2C9, CYP2C19 and CYP2D6 on treatment outcome showed that (1) poor metabolizers of CYP2D6 and patients taking inhibitors of CYP2D6 had significantly higher mean dose-corrected trough plasma concentrations compared to the substance-specific median; (2) poor metabolizers of CYP2D6 were underrepresented (n=6) compared to the control group of healthy volunteers and (3) five of them experienced side effects that were judged relevant; (4) extensive metabolizers of CYP2C19 and smokers showed low mean dose-corrected plasma

concentrations compared to the substance-specific median; (5) mean trough plasma concentrations above or below the lower limit of a predefined minimum threshold concentration did not predict patients' treatment outcome; (6) the HAMD score at first observation was associated with the HAMD-defined response at the end of the study period; (7) the CGI-defined lack of response was seen in patients that received a second antidepressant or benzodiazepines in the course of the study; (8) there was no general predictor for the occurrence of relevant side effects, but the administration of sedative co-medication was associated with the severity of serotonergic side effects.

An overview of the current literature about the contribution of the cytochrome P450 isoenzymes to the metabolism of the antidepressants evaluated in this thesis indicates that the three cytochrome P450 isoenzymes studied contribute to a different extent to the metabolism of the different drugs. Hydroxylation of amitriptyline, doxepin and clomipramine is predominantly catalysed by CYP2D6 while CYP2C19, CYP2D6, CYP2C9 and other isoenzymes are involved in demethylation. The impact of CYP2D6 in the metabolism of amitriptyline and clomipramine is demonstrated in numerous studies whereas only one study showed significant differences in steady-state amitriptyline and clomipramine plasma concentrations between poor metabolizers and extensive metabolizers of CYP2C19. The relevance of CYP2C9 remains unclear and so far limited information is available of the influence of CYP isoenzymes for doxepin (Kirchheiner et al. 2001). Citalopram is metabolized to desmethylcitalopram by CYP2C19 and CYP3A4 and to didesmethycitalopram by CYP2D6. CYP2C19 is relevant for enantioselective demethylation of the active enatiomere S-citalopram (Hiemke and Härtter 2000). An association was found between citalopram demethylation and the mephenytoin metabolism, a probe drug for CYP2C19 as well as between the demethylation of desmethylcitalopram and the sparteine metabolism, a probe drug for CYP2D6 (Sindrup et al. 1993). Fluvoxamine metabolism is mediated by CYP1A2 and CYP2D6 (Chiba and Kobayashi 2000). A high and a low affinity metabolic pathway were detected for paroxetine, the high affinity process being saturable and

Table 21. Contribution of CYP isoenzymes to the metabolism of the antidepressants – overview of current literature*

	CYP2C9	CYP2C19	CYP2D6
Amitriptyline	+	++	++
Citalopram	-	++	+
Clomipramine	-	++	++
Doxepin	-	++	++
Fluvoxamine	-	+	+
Mirtazapine	-	-	+
Paroxetine	-	-	++
Sertraline	+	+	+
Venlafaxine	-	-	++

++ involved, + little involved or - not involved in the metabolism of the antidepressant
**Chiba and Kobayashi 2000; Dahl et al. 1997; Delbressine et al. 1998; Hiemke and Härtter 2000, Kirchheiner et al. 2001; Sindrup et al. 1992; Sindrup et al. 1993*

mediated by CYP2D6 (Sindrup et al. 1992). Multiple isoenzymes are involved in the metabolism of sertraline in vivo, including CYP2D6, CYP3A4, CYP2C9 and CYP2C19, thus it is unlikely that changes in the metabolic capacitiy of a single isoenzyme significantly alters sertraline plasma concentrations (Kirchheiner et al. 2001). Mirtazapine is hydroxylated by CYP2D6 and CYP1A2 to 40% whereas 25% are N-oxidated by CYP3A4 or excreted as glucuronides (Dahl et al. 1997, Delbressine et al. 1998). O-demethylation by CYP2D6 is the main metabolic pathway of venlafaxine and was found to be associated with elevated plasma concentrations and arrythmia in poor metabolizers. CYP3A4, CYP2C9 and CYP2C19 are involved in N-demethylation of venlafaxine in vitro (Kirchheiner et al. 2001). Table 21 summarises the overview of current literature given in the preceeding paragraph.

The patients evaluated in this thesis were mainly treated with second generation antidepressants. Unlike the tricyclic antidepressants the different cytochrome P450 isoenzymes contribute to a varying extent to the metabolism of these drugs. Thus, the impact of CYP2D6 and CYP2C19 polymorphism on the treatment with new antidepressants can not easily be generalised. The most pronounced effect of CYP2D6

was expected for tricyclic antidepressants, venlafaxine and paroxetine whose main metabolic pathways are catalysed by this isoenzyme. Tricyclic antidepressants and citalopram were expected to be altered by CYP2C19. In addition, sertraline and amitriptyline might have been altered by CYP2C9. Taking into account the frequencies of antidepressants in the present study these data included 23% observations that should be markedly influenced by CYP2D6 activity, 45% of the data should also depend on CYP2C19 capacity and 15% may be altered by changes in the CYP2C9 function.

Evaluating these data, the impact of CYP2D6 activity was shown by elevated trough plasma concentrations when inhibitors of this isoenzyme were co-administered or when patients were poor metabolizers of CYP2D6. Although the analysis included only six poor metabolizers of CYP2D6, their trough plasma concentrations were significantly higher than the substance-specific median when analysing the entire data. This effect was even persistent when the poor metabolizer with the highest venlafaxine plasma concentration was excluded to check wether the significant result was only due to this outlier. Of note, trough plasma concentrations of venlafaxine were considerably increased in the poor metabolizer and decreased in the ultrarapid metabolizer of CYP2D6. Out of the six poor metabolizers of CYP2D6, five experienced relevant side effects at their first observation. These patients received doxepin, citalopram, sertraline and venlafaxine. Although until now only the association between high venlafaxine plasma concentrations and side effects was previously described, the incidence of relevant side effects in tended to be higher in poor metabolizers of CYP2D6 than in poor metabolizers of CYP2C19 or CYP2C9.

In contrast to CYP2C9 or CYP2C19, the distribution of functional CYP2D6 alleles within our psychiatric patients differed significantly from a group of healthy Caucasian volunteers described by Griese and colleagues (1998). The number of poor metabolizers in psychiatric patients was smaller than expected. It is unlikely that this difference was caused by analytical errors because our method for CYP2D6

genotyping is well established and has proven to be reliable in another sample before (Lohmann et al. 2001). It has also been shown that the detection of the CYP2D6 alleles *1, *2, *3, *4, *5, *6 and the gene duplication is sufficient to accurately predict enzyme activity (Sachse et al. 1998). The poor metabolizer's underrepresentation may be due to the fact that CYP2D6 poor metabolizers may be less willing to take antidepressant medication because of drug-related problems experienced in the past that were due to the drug's elevated plasma concentrations. Indeed it has been demonstrated that the occurrence of side effects is associated with high plasma concentrations of venlafaxine and of old drugs such as tricyclic antidepressants (Preskorn 1993, Lessard et al. 1999). Another study evaluating tricyclic antidepressants already indicated a genotype-dependent increase of side effects in patients treated with substrates of CYP2D6 (Chou et al. 2000). Moreover, drug-related problems caused a higher rate of treatment discontinuation with greater risk of noncompliance (Montgomery and Kaser 1998).

The impact of CYP2C19 was less pronounced than of CYP2D6. The analysis of our patients showed lower trough plasma concentrations in extensive metabolizers of CYP2C19 than in intermediate and poor metabolizers of this isoenzyme. Citalopram was the most frequently prescribed drug (37%) among the patients included in this analysis. It has been demonstrated by Sindrup and colleagues (1993) that the clearance of citalopram is twice as high in extensive metabolizers as in poor metabolizers of CYP2C19. However the results obtained with respect to CYP2C19 were less clear than for CYP2D6. Our study included five poor metabolizers of CYP2C19 and two of them received amitriptyline or citalopram that were metabolized by this isoenzyme to a significant extent. The mean trough plasma concentration of the poor metabolizer receiving citalopram were 64% higher than the substance-specific median. The elevated amitriptyline trough plasma concentration of the other poor metabolizer of CYP2C19 may also be the result of multiple pharmacokinetic drug interactions: propranolol, a substrate of CYP2D6, and calcium channel blockers, that are substrates of CYP3A4, concur in the same metabolic pathways as amitriptyline;

sodium valproate may further raise amitriptyline plasma concentrations by enzyme inhibition (Bazire 2000). Another CYP2C19 poor metabolizer showed pronounced deviation from the sertraline-specific median, but trough plasma concentrations of sertraline are low compared to other antidepressants as they ranged from 20 to 50 nl/mL, thus small changes in plasma concentrations resulted in high deviations from the population median. Only two CYP2C19 poor metabolizers experienced relevant side effects but they did not present with conspicious plasma concentrations.

Neither the CYP2D6 genotype nor the CYP2C19 genotype were found to influence the occurrence of relevant side effects at treatment initialisation in our general logistic regression analysis. This may be due to the fact that 81% of the patients studied received second generation antidepressants with a lower risk of severe side effects at high plasma concentrations than the tricyclic antidepressants. These findings may indicate that the influence of genotypes on plasma concentrations of new antidepressants is of minor relevance in a naturalistic clinical setting were multiple factors influence plasma concentrations as well as treatment outcome. The severity of serotonergic side effects of patients taking SSRIs or venlafaxine were rated differently among the study centres. This centre effect may have been a further confounding factor. Analysing these patients receiving serotonergic medication, the only association was seen between the severity of side effects and sedative co-medication. This finding probably reflects symptomatic treatment with benzodiazepines or antipsychotics to control serotonergic side effects. This effect is also described in a Dutch study on add-on medication of benzodiazepines in elderly patients (van Dijk et al. 2002).

Plasma concentration monitoring is an accepted tool to avoid nonresponse and severe side effects of tricyclic antidepressants; therapeutic ranges of these substances are well established (American Psychiatric Association Task Force on the Use of Laboratory Tests in Psychiatry 1985). The advantage of therapeutic drug monitoring of new antidepressants still remains to be elucidated. If the activity of metabolising enzymes affects plasma concentration, it should also influence clinical outcome.

However, in our analysis, trough plasma concentrations above the currently recommended minimal threshold did not predict a better treatment response for these patients. Consequently, no relationship between genotype and treatment response was seen. There might be several reasons that, taken together, are responsible for the lacking impact of CYP2D6 and CYP2C19 polymorphisms on response: firstly, patients were mainly taking SSRIs or mirtazapine. Compared to TCAs or venlafaxine these drugs owe a small dosing range and a flat dose response-relationship. Thus, the frequency of subtherapeutic doses is probably low. Secondly, the impact of the isoeenzymes' activity varies between the drugs but because of the small sample size, no subgroup analysis was carried out of patients taking antidepressants metabolized to a significant extent by CYP2D6 (amitriptyline, doxepin, venlafaxine, paroxetine) or CYP2C19 (amitriptyline, doxepin, citalopram). Thirdly, there were only four patients showing duplication of the CYP2D6 gene and at chance to show ultrarapid metabolism. Three of these patients were taking the CYP2D6 substrates amitriptyline, mirtazapine and venlafaxine but only venlafaxine's dose-corrected plasma concentrations were 36% lower than the substance-specific median. The fourth patient showed dose-corrected sertraline plasma concentration 52% lower than the median. But as sertraline's metabolism appears to be mediated by CYP3A4 it was unlikely to be affected by changes in the activity of CYP2D6 (Hiemke and Härtter 2000). Fourthly, within this natural clinical study design, a number of other factors such as additional psychotherapy or medication were likely to influence treatment outcome. When the response criteria were defined as 40% or more reduction in the HAMD score, the HAMD score at the first rating had a significant impact on treatment response. The finding that antidepressants were more effective in patients with severe depression is in accordance with Khan and colleagues (2002) who performed a meta analysis among 45 clinical trials recorded in the database of the Food and Drug Administration. When response was defined according to the CGI, the physicians' treatment strategy was

reflected: nonresponders were more likely to receive co-medication with a second antidepressant or a benzodiazepine.

5.2 Therapeutic range and population pharmacokinetic analysis of mirtazapine

The evaluation of a concentration-response relationship demonstrated marked variability of mirtazapine trough plasma concentrations, a small but significant relation of mirtazapine trough plasma concentration to treatment response and a trend of increased sedation and sleep at low mirtazapine trough plasma concentrations.

Mirtazapine was prescribed in daily doses of 15, 30, 45 or 60 mg/day resulting in plasma concentrations ranging from 0-98 ng/mL, which is in close agreement with previous findings (Timmer et al. 1995). However, there was low correlation between dose and trough plasma concentration with marked variability within the dose levels. The reasons for this variability were studied later on by population pharmacokinetic analysis.

The analysis of a minimum effective plasma concentration resulted in a threshold of 30 ng/mL. The effect of trough plasma concentrations on the reduction of the HAMD score were small (Odd s Ratio = 1.056) but significant. Within this setting, treatment response might have been not only influenced by the mean trough mirtazapine plasma concentration but also by other factors. Therefore an additional logistic regression analysis was carried out that included age, gender, severity of illness, length of observation or Hamilton score at the first observation. However, these covariates did not significantly influence the reduction of the HAMD score from the first to the last observation.

The response rate within our study was 42% which is lower than in pivotal clinical trials (Fawcett and Barkin 1998). There are different explanations for this finding. Firstly, the definition of response as 40% or more reduction in the HAMD-score might have been too rigid considering the fact that mirtazapine causes a mean drop in the HAMD-score of 25% in the first week and this time span was not taken into account in our study.

Secondly, no restriction was made in the study protocol that would have probable influence on the patient's prognosis, such as severity of illness, number of depressive episodes in the past or co-morbidity.

No naturalistic study was carried out so far that deteced a dose- or concentration-response relationship for mirtazapine. One study that showed marked variability in the response rates of different investigators failed to show a difference between patients treated with daily doses of 5, 10, 20 or 40 mg (Organon, personal communication). There is only one double-blind study comparing the effectiveness of mirtazapine to imipramine in severely depressed patients. In this study, where dosing was adjusted for a target blood concentration range of 50-100 ng/mL for mirtazapine and 200-300 ng/mL for imipramine, impramine was more effective than mirtazapine (Bruijn et al. 1996). The mean daily dose of mirtazapine was 76 mg and thus markedly higher than in pivotal clinical trials. The patients in this study had experienced several depressive episodes in the past or were severly ill, thus it remains unclear if the reason for low performance of mirtazapine resides in treatment resistance or if high doses and thus high plasma concentrations might provide evidence for a curvilinear concentration-response relationship.

However, in the present study extremly low or high plasma concentrations were underrepresented; this made it more difficult to detect minimum effective trough plasma concentrations or side effects related to high plasma concentrations. As physicians were free to choose initial doses and to adjust dosing schedules at about 4 weeks of treatment, nonresponders were more likely to receive high doses, thus no evaluation of maximum effective concentrations was carried out.

Our analysis of the relationship between mirtazapine trough plasma concentrations and side effects focussed on side effects at low plasma concentrations and included a logistic regression analysis to control for possible covariates. The doses applied to the patients in our study were proved safe and effective in earlier clinical trials for marketing application (Fawcett and Barkin 1998), therefore the finding of good

tolerability was as expected. Mirtazapine side effects were assumed to be more prominent at initialisation of antidepressant medication than under chronic treatment. A relationship of trough plasma concentrations to side effects was therefore only found for the first week of treatment. The trend to increased sedative effect associated with increased duration of sleep at low plasma concentrations is in accordance with the findings of Kasper and colleagues who noted increased sedation at low doses of mirtazapine (Kasper et al. 1997). The reason for this may be mirtazapine's higher affinity to histamine-H_1 receptors than to serotonergic receptors; thus histamine-H_1 receptor blockade is more prominent at low plasma concentrations and is counterbalanced by serotonergic effects at higher plasma concentrations. Apart from sedation or increased duration of sleep, no significant relationship was detectable between trough plasma concentration, other UKU items (global assessment of the patient's performance, sedation, weight gain), and co-medication.

The population pharmacokinetic analysis of patients treated with mirtazapine demonstrated a significant difference in the mirtazapine clearance between extensive and intermediate metabolizers of CYP2D6.

CYP2D6 and CYP1A2 catalyse the formation of 8-hydroxymirtazapine, the main metabolic pathway that contributes to 40% of mirtazapine's metabolism in vivo (Delbressine et al. 1998). In vitro experiments have shown a 65% contribution of CYP2D6 to this pathway decreasing to 20% at a high mirtazapine concentration, while the contribution of CYP1A2 increases from 30% to 50% (Störmer et al. 2000). CYP2D6 phenotyping with sparteine detected a five-fold lower clearance in intermediate metabolizers compared to extensive metabolizers, associated with a novel mutation in the flanking region of the CYP2D6 gene (Raimundo et al. 2000). Thus, our finding of a 28% reduction in the mirtazapine clearance in intermediate metabolizers further supports the relevance of CYP2D6 in psychiatric clinical routine.

No conspicious plasma concentration deviations were detected in the one poor metabolizer of these data neither has any report of high mirtazapine plasma

concentrations in poor metabolizers of CYP2D6 been published so far. One study comparing seven extensive metabolizers with seven poor metabolizers of debrisoquine found a 78% higher AUC of S-(+)-mirtazapine in poor metabolizers while the pharmacokinetics of the R-(-)-enantiomer remained unchanged; however, when analysing the racemate, no difference in mirtazapine pharmacokinetics was noted between poor and extensive metabolizers (Dahl et al. 1997). This finding is explained by enantioselective metabolism of mirtazapine: the S-(+)-enantiomer is predominantly hydroxylated via CYP2D6 and CYP1A2 and the R-(-)-enantiomer preferably undergoes N-oxidation and entero-hepatic recirculation as N^+-glucuronide. The absence of a difference between poor and extensive metabolizers may be related to the decreasing relevance of CYP2D6 in favour of CYP1A2 with increasing mirtazapine exposure, suggesting that with low CYP2D6 activity other metabolic pathways become more relevant.

One alternative metabolic pathway is 8-hydroxylation by CYP1A2. There are no polymorphisms with relevance to the metabolic function known for CYP1A2 but it is altered by several extrinsic factors. A case report has shown a three- to four-fold increase of mirtazapine serum concentrations after addition of fluvoxamine, a potent inhibitor of CYP1A2 (Anttila et al. 2001). As smoking is a known inducer of CYP1A2, it may also play a role in the clearance of mirtazapine. During model building a 30% increase of mirtazapine's clearance was estimated reducing interindividual variability from 41.4% to 37.8%, although the model fit did not significantly improved compared to the basic model. This may be due to the residual variation of trough plasma concentrations within this naturalistic clinical study, that was estimated to be 27%, thus the analysis of a large sample size might have clarified the impact of smoking.

The other alternative metabolic pathway is N-oxidation via CYP3A4. Mirtazapine plasma concentrations are found to decrease by 60% within the first weeks of co-administration of carbamazepine, an inducer of CYP3A4 (Timmer et al. 2000). Only few of the patients evaluated in this thesis received carbamazepine, some of them just

initialising this co-medication, thus preventing to evaluate the long-term effect of this important covariate. However, a 20% lower clearance in patients co-medicated with substrates of CYP3A4 was noted together with a decrease in the interindividual variability of clearance. The effect was even more pronounced when this covariate was introduced in the final model but did not significantly improve the goodness-of-fit. Similar to smoking, this effect might be masked by the variability caused by the typical clinical setting.

In our patients, neither influence of gender nor of age was detected on the clearance of mirtazapine. Previous findings reported a 50% higher AUC in adult males than in females or elderly (Timmer et al. 1996). The authors attributed this difference to altered clearance or volume of distribution between males and females or in the elderly. An effect on clearance was not supported by our clinical data.

The study presented in this thesis was carried out under clinical conditions, thus the pharmacokinetics of mirtazapine was affected by multiple other factors such as reduced adherence to the dosing schedules or co-medication. Therefore the residual variability was considerable and model restrictions such as allowing no variability of the volume of distribution, bioavailability or absorption rate constant led to a further increase of this variability. In spite of these confounding factors, the effect of the CYP2D6 genotype was consistent during the entire process of model building suggesting this covariate to be pronounced even in this clinical setting. This effect may become relevant in the management of nonresponse associated with low plasma concentrations of extensive metabolizers.

5.3 Dose individualisation of lithium

Bayesian curve fitting with the extended Abbottbase pharmacokinetic software® allowed to generate individual serum concentration-time curves for every patient. The individual pharmacokinetic parameters estimated by this software were in accordance with the literature (Ritschel, 1992).

The accuracy to predict serum concentrations was good applying the extended Abbottbase pharmacokinetic software® or the method of Yukawa and colleagues (1993) as the prediction error was close to zero and the 95% confidence interval included 0. The method described by Pepin and colleagues (1980) tended to overestimate serum concentrations but the accuracy was still in line with those of commonly applied methods (Browne et al. 1988). This trend for overestimation was not present in other evaluations including 34 (Yukawa et al. 1993) or 20 patients (Browne et al. 1988) but in these evaluations the method of Pepin was found to be associated with high random variability. Validating our Bayesian method, dose individualisation according to Jermain and colleagues (1991) performed worse than other methods described in literature (Browne et al. 1988), the results of the present evaluation being in comparable to previous findings (Yukawa et al. 1993, Taright et al. 1994). Like the method of Yukawa et al. (1993), the method of Jermain et al. (1991) was established by nonlinear mixed-effects modeling but without evaluating the predictive performance as a final model check.

The evaluations of Yukawa et al. (1993) and Taright et al. (1994) are the only studies published so far that used advanced population pharmacokinetic analysis to establish a dose individualisation method for lithium; the method of Taright et al. (1994) being based on nonparametric maximum likelihood estimation while the Yukawa method applied mixed effects modeling (Yukawa et al. 1993, Taright et al. 1994). Including the method established in this thesis that was based on the results of Taright and colleagues (1994), all validated methods that included the results of population pharmacokinetic analyses performed better than most of the standard methods (Table 22). In contrast, the predictive performance was comparable to the test dose methods of Perry and colleagues (1986) or a nonlinear regression method (Williams et al. 1989) when Bayesian forecasting was based on standard pharmacokinetic data instead of advanced population pharmacokinetics (Table 22).

Table 22. Accuracy of methods to predict lithium serum concentrations – comparison with current literature

	Our Bayesian method (n=60) *	Browne et al. 1988 (n=20) +	Yukawa et al. 1993 (n=34) *	Williams et al. 1989 (n=21) *	Taright et al. 1994 (n=35) *
Pepin method	0.089 (0.013-0.160)	0.095 (-0.14-0.27)	0.01 (-0.039-0.051)		
Yukawa method	-0.055 (-0.007-0.091)		-0.02 (-0.053-0.022)		-0.04
Jermain method	0.297 (0.075-0.246)		-0.02 (-0.218-0.343)		-0.44
Other a priori methods		-0.155 (-0.27-0.08)	-0.02 (-0.062-0.013)		
Nomo-graph		0.17 (0.00-0.38)			
Nonlinear regression				-0.034 (-0.125-0.053)	
Test dose of Perry		-0.06 (-0.19-0.01)		-0.015 (-0.075-0.035)	
Other Bayesian methods**	-0.023 (-0.014-0.053)			-0.042 (-0.109-0.049)	-0.01

**expressed as mean prediction error (95% confidence interval)*
+expressed as median prediction error (95% confidence interval)
*** based on data obtained by standard pharmacokinetic analysis*

When comparing the 95% confidence interval of the prediction error of the four methods evaluated in this thesis, the prediction of our Bayesian method was the most precise followed by the method of Yukawa, Pepin and Jermain. The Bayesian method was the only one that also took individual serum concentrations or co-medication into account thus leading to better predictions. Both, our Bayesian method and the method according to Yukawa were among the most accurate when the 95% confidence intervals were compared to other findings (Table 22). The 95% confidence interval of accuracy of the Pepin method varies among the authors but Yukawa and colleagues (1994) also found the Jermain method to be less accurate. Table 22 compares the

accuracy of different techniques of dose individualisation evaluated in this thesis with the accuracies stated by Browne et al. (1988), Yukawa et al (1993), Williams et al. (1989) and Taright et al (1994).

The inclusion of predefined covariates did not always improve the Bayesian curve fit of the extended Abbottbase pharmacokinetic software® but depended on the covariate and the clinical situation of the patients. The covariates “antihypertensives”, “diuretics” and “NSAID” described different degrees of reduction of the lithium clearance and performed well as far as no other covariate was present. In the presence of more than one factor influencing the pharmacokinetics of lithium, the appropriate covariate specification that best described their combined effect had to be chosen. For example when several drugs reducing the clearance of lithium were combined, as was the case for three patients, an additive effect was noted and either the covariate “diuretics” or a combination of the covariates “diuretics” and “antihypertensives” led to the best fit. The clinical situation of three other patients could be better understood by empiric inclusion of one of these covariates that mimiked for example extensive sweating or a change in diaphoretic medication.

The covariate “old age” was useful to describe data of four patients that were thin and probably did not drink enough. Physiological changes in the elderly are commonly related to the body composition, with increasing body fat and decreasing total body water (Sproule et al. 2000). Thus the physiological state of a dehydrated patient may be comparable to that of the elderly. The covariate definition was based on a study that was carried out in the Seventies and the physiological status of old people at that time may have been worse than that of the five old patients evaluated in this thesis (Lehmann and Merten 1974).

The inclusion of the covariate “obesity” did not explain pharmacokinetic variability of obese patients or any other clinical situation. One reason may be that the volume of distribution was calculated based on ideal body weight and therefore all patients were

automatically standardised by the Abbottbase pharmacokinetic software® with respect to weight and age.

Including a linear relationship between lithium clearance and creatinine clearance calculated according to Cockcroft and Gault did not improve the model fit for most of the patients although all authors so far found renal function to be a crucial factor in the elimination of lithium. None of the patients included for validation of this method presented with a reduced glomerular filtration rate as serum creatinine concentrations ranged from 0.7 to 1.3 mg/dL. As the inclusion of this covariate should become especially relevant in renal impairment, its usefulness could not be adequately assessed in our patient group.

Relying on the present data, the predefined covariates “diuretics”, “antihypertensives”, “NSAID” and “old age” may be of use in clinical routine and should be introduced after close examination of the patient’s state, especially when there are more factors possibly influencing lithium pharmacokinetics. Even other clinical situations may then be adequately described, as was shown for seven patients where the best fit was achieved by off-label use of one of these covariates.

6 Conclusion

These studies demonstrate that administering new generation antidepressants, the CYP2D6 genotype still significantly influences the plasma concentration. We observed a minor influence of the CYP2C19 genotype and no relevance of the CYP2C9 genotype. The changes in plasma concentrations due to CYP2D6 polymorphism are most pronounced when venlafaxine is administered. The good tolerability of the new generation antidepressants may be the reason for the minor impact of genotypes on the occurrence of side effects. According to these results, genotyping by itself is no predictor for treatment response in a clinical setting, where the treatment outcome is influenced by multiple factors such as severity of illness, co-medication or additional treatment. Nevertheless, plasma concentration measurements may be useful to assess the patients' actual metabolic capacity when treated with substrates of CYP2D6 and presenting with drug intolerance or nonresponse.

This thesis also provides a basis for the therapeutic drug monitoring of mirtazapine. A positive correlation was confirmed between mirtazapine dose and trough plasma concentration but marked variability of plasma concentrations was seen. This variablility is partly determined by the CYP2D6 genotype. Other covariates that may also influence the pharmacokinetics of mirtazapine are co-medication affecting CYP3A4 activity or smoking. A minimum effective threshold concentration of 30 ng/mL for clinical routine is postulated providing a basis for therapeutic drug monitoring in nonresponders to mirtazapine treatment. Sedative effects such as increased duration of sleep may be more pronounced at low plasma concentrations whereas weight gain is not related to plasma concentration. To which extent high plasma concentrations may affect treatment outcome and side effects remains to be studied.

In contrast to antidepressants, therapeutic drug monitoring is mandatory when lithium is administered. Several methods for dose individualisation of lithium are presented in the literature but they all necessitate prior knowledge of covariates and are based on

defined dosing schedules or times of blood withdrawal. It was demonstrated that dose individualisation of lithium is more flexible and thus more convenient for clinical routine when the Bayesian approach is used. The method presented in this thesis allows individual dose recommendations, independent of the dosing schedule or the time of blood withdrawal. Its predictive performance proved to be good when compared to standard a priori methods. Including covariates is not mandatory but may lead to a better understanding of the individual clinical situation.

7 Summary

Depression is the most frequent mental disorder occuring in 17% of the European population and its impact is assumed to grow in the next decades. Optimal treatment necessitates long-term administration of antidepressants in unipolar depressive disorder or mood stabilisers in bipolar disorder. Among mood stabilisers, lithium is the standard of care and therapeutic drug monitoring of lithium is mandatory to control wide interindividual variability in serum concentrations and to prevent severe intoxication. Several methods for dose individualisation of lithium are proposed in literature. Most of them require the adherence to defined dosing intervals, doses or serum concentration measurements and are therefore rarely applied in clinical routine. In this thesis a computer-assisted service for dose individualisation was established that applies population pharmacokinetic and individual data to predict and simulate serum concentrations by Bayesian curve fitting. This method is flexible with regard to dosing schedules and times of blood withdrawal and allows the inclusion of covariates. The predictive performance was compared to standard a priori methods for dose individualisation and showed good precision and accuracy.

Antidepressants are first choice treatment of unipolar depression. They are equally effective but vary in their tolerability and side effect profile. Tricyclic and tetracyclic antidepressants possess a narrow therapeutic range, large interindividual variability and are therefore prone to the occurence of sub- or supratherapeutic plasma concentrations when standard doses are administered. Interindividual variability is due to drug interactions, renal or hepatic impairment, and genetic polymorphism of drug metabolising cytochrome P450 isoenzymes 2D6 or 2C19. For these drugs therapeutic drug monitoring is recommended to avoid nonresponse or toxic effects. Second generation antidepressants are also characterized by a large interindividual variability in plasma concentrations but most of them proved good tolerability, a flat dose-

response relationship and less severe side effects. Therapeutic ranges are not well established and so far the usefulness of therapeutic drug monitoring is unclear.

A multicentre naturalistic study was conducted to evaluate the relevance of therapeutic drug monitoring of amitriptyline, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, imipramine, mirtazapine, nortriptyline, paroxetine, sertraline and venlafaxine. In this study inpatients' severity of depression, side effects and trough plasma concentrations were recorded weekly. One hundred and thirty-six patients were recruited that also gave informed consent for an evaluation of the genetic factors underlying the response to antidepressive treatment. These data consisted to 93% of second generation antidepressants and were evaluated for an impact of CYP2C9, CYP2C19 and CYP2D6 polymorphisms on trough plasma concentrations, side effects and treatment response. It was demonstrated that CYP2D6 plays a significant role in the treatment with second generation antidepressants. The CYP2D6 genotype influenced dose-corrected trough plasma concentrations as they were significantly higher in the six poor metabolizers compared to the substance-specific median and five of them experienced side effects that were judged to be relevant. Plasma concentrations of patients taking inhibitors of CYP2D6 were higher than the substance-specific median. Poor metabolizers of CYP2D6 were significantly underrepresented among psychiatric inpatients treated with psychotropic drugs compared to a control group of healthy volunteers, maybe because they were more susceptible to severe side effects and therefore refused to take antidepressants.

The analysis also confirmed an impact of the CYP2C19 genotype or smoking on dose corrected trough plasma concentrations that were low in extensive metabolizers and smokers when compared to the substance-specific median. CYP2C9 polymorphism did not significantly influence patients' trough plasma concentrations.

The effects on trough plasma concentrations were of minor clinical relevance as mean plasma concentrations above or below the lower limit of currently accepted minimum threshold concentrations did not predict patients' treatment outcome. Accordingly, no

association was seen between CYP2D6, CYP2C19 or CYP2C9 genotypes and response or the ocurrence of side effects at the beginning of antidepressive therapy. Some treatment strategies of the clinical routine turned out to be significant as nonresponders were more likely to receive a second antidepressant or benzodiazepines and the severity of serotonergic side effects was associated with sedative co-medication. Although the effect of genotypes was not distinct enough to be detectable for all antidepressants in the presence of multiple influencing factors of clinical routine, prominent plasma concentrations were seen for two patients that were poor or ultra rapid metabolizers of CYP2D6 and who received venlafaxine. Thus, plasma concentration measurements may be of relevance for substrates of CYP2D6 in cases of nonresponse or severe side effects. As the present investigation showed that the influence of genotypes on trough plasma concentration may be masked by multiple factors occuring in clinical routine, the determination of the actual enzyme activity by phenotyping or therapeutic drug monitoring may be of more clinical relevance than the determination of the patient's genotype alone.

Providing a basis for therapeutic drug monitoring of mirtazapine, patients that received this antidepressant were analysed separately for a concentration-effect relationship and to determine factors influencing its pharmacokinetics. Best distinction between responders and nonresponders to mirtazapine was achieved at a mean trough plasma concentration of 30 ng/mL. Sedation was more pronounced at low plasma concentrations, probably due to prominent histamine-H_1 receptor blockade; for weight gain no association was shown. Population pharmacokinetic analysis was applied to detect factors influencing the pharmacokinetics of mirtazapine. The final model included the genotype of CYP2D6 as the only covariate significantly improving the model fit. In this analysis patients were divided in intermediate and extensive metabolizers, where one poor metabolizer entered the intermediate metabolizer group and one ultra rapid metabolizer joined the extensive metabolizers. Co-medication with substrates of CYP3A4 or smoking may be other influencing factors that require further

investigation. These findings indicate that therapeutic drug monitoring of mirtazapine may be beneficial in nonresponders. Dose adaption may be considered in patients with decreased CYP2D6 activity.

8 References

Aithal GP, Day CP, Kesteven PJ, Daly AK (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 353: 717–719

Amdisen (1975) Monitoring of lithium treatment through determination of lithium concentration. Dan Med Bull 22: 277-291

Amsterdam JD, Fawcett J, Quitkin FM, Reimherr FW, Rosenbaum JF, Michelson D, Hornig-Rohan M, Beasley CM (1997) Fluoxetine and norfluoxetine plasma concentrations in major depression: a multicenter study. Am J Psychiatry 154: 963-969

Anttila SAK, Rasanen I, Leinonen EVJ (2001) Fluvoxamine augmentation increases serum mirtazapine concentrations three- to fourfold. Ann Pharmacother 35: 1221-1223

APA Task Force on the Use of Laboratory Tests in Psychiatry (1985) Tricyclic antidepressants--blood level measurements and clinical outcome: an APA Task Force report. Am J Psychiatry 142:155-162

Aynacioglu AS, Brockmöller J, Bauer S, Sachse C, Guzelbey P, Ongen Z, Nacak M, Roots I (1999) Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol 48: 409–415

Bazire (2000) Psychotropic drug directory 2000. Quay Books Division, Marc Allen Publishing Ltd, Jesses Farm, Snow Hill, Dinton, United Kingdom

Beneke M, Rasmus W (1992) "Clinical Global Impressions" (ECDEU): some critical comments. Pharmacopsychiatry 25: 171-176.

Benkert O, Hippius H (2000) Kompendium der psychiatrischen Pharmakotherapie. 2nd edition. Springer Verlag, Berlin Heidelberg, Germany

Bergmann TK, Bathum L, Brøsen K (2001) Duplication of CYP2D6 predicts high clearance of desipramine but high clearance does not predict duplication of CYP2D6. Eur J Clin Pharmacol 57: 123-127

Bertilsson L, Aberg-Wistedt A, Gustafsson LL, Nordin C (1985) Extremely rapid hydroxylation of debrisoquine. A case report with implication for treatment with nortriptyline and other tricyclic antidepressants. Ther Drug Monit 7: 478–480

Bjerkenstedt L, Flyckt L, Overo KF, Lingjaerde O (1985) Relationship between clinical effects, serum drug concentration and serotonin uptake inhibition in depressed patients treated with citalopram. A double-blind comparison of three dose levels. Eur J Clin Pharmacol 28: 553-557

Bluhm RE, Wilkinson GR, Shelton R, Branch RA (1993) Genetically determined drug-metabolizing activity and desipramine-associated cardiotoxicity: a case report. Clin Pharmacol Ther 53: 89-95

Bremner JD (1995) A double-blind comparison of Org 3770, amitriptyline, and placebo in major depression. J Clin Psychiatr 56: 519-525.

Brockmöller J, Rost KL, Gross D, Schenkel A, Roots I (1995) Phenotyping of CYP2C19 with enantiospecific HPLC-quantification of R- and S-mephenytoin and comparison with the intron4/exon5 G-->A-splice site mutation. Pharmacogenetics 5: 80-88

Broly F, Marez D, Sabbagh N, Legrand M, Millecamps S, Lo Guidice JM, Boone P, Meyer UA (1995) An efficient strategy for detection of known and new mutations of the CYP2D6 gene using single strand conformation polymorphism analysis. Pharmacogenetics 5: 373-384

Browne JL, Patel RA, Hoffman CS, Hussey BK (1988) Comparison of pharmacokinetic procedures for dosing lithium based on analysis of prediction error. Ther Drug Monit 22: 227-232

Bruijn JA, Moleman P, Mulder PGH, van den Broek WW, van Hulst AM, van der Mast RC, van de Wetering BJM (1996) A double-blind, fixed blood-level study comparing mirtazapine with imipramine in depressed in-patients. Psychopharmacology 127: 231-237

Bühl A, Zöfel P (2002) SPSS 11. Einführung in die moderne Datenanalyse unter Windows. Pearson Education Deutschland GmbH München, Germany

Bundesärztekammer (2002) Richtlinie der Bundesärztekammer zur Qualitätssicherung quantitativer laboratoriumsmedizinischer Untersuchungen. Deutsches Ärzteblatt 98: A 2747-2759 und 99: A 1187

Burke MJ, Preskorn SH (1999) Therapeutic drug monitoring of antidepressants. Cost implications and relevance to clinical practice. Clin Pharmacokinet 37: 147-165

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

Chiba K, Kobayashi K (2000) Antidepressants. In: Levy RH Thummel KE, Trager WF, Hansten PH, Eichelbaum M. Metabolic Drug Interactions. Lippincott Wiliams & Wilkins, Philadelphia, 233-243

Chou WH, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, Pho M, Xiao V, Ryder TB, Liu WW, Teiling C, Wedlund PJ (2000) Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. J Clin Psychopharmacol 20: 246-251

Collegium Internationale Psychiatriae Scalarum (CIPS) (1981) Internationale Skalen für die Psychiatrie. Beltz Test GmbH, Weinheim, Germany.

Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16: 31-41

Cooper TB, Simpson GM. Lithium dosing guide (1982) Am J Psychiatry 139: 969

Coutts RT, Urichuk LJ (1999) Polymorphic cytochromes P450 and drugs used in psychiatry. Cell Mol Neurobiol 19: 325-354

Dahl ML, Bertilsson L, Nordin C (1996) Steady-state plasma levels of nortriptyline and its 10-hydroxy metabolite: relationship to the CYP2D6 genotype. Psychopharmacology 123: 315-319

Dahl ML, Voortman G, Alm C, Elwin CE, Delbressine L, Vos R, Bogaards JJP, Bertilson L (1997) In vitro and in vivo studies on the disposition of mirtazapine in humans. Clin Drug Invest 13: 37-46

Daly AK, Brockmöller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, Huang JD, Idle JR, Ingelmann-Sundberg M, Ishizaki T, Jacqz-Aigrain E, Meyer UA, Nebert DW, Stehen VM, Wolf CR, Zanger UM (1996) Nomenclature for human CYP2D6 alleles. Pharmacogenetics 6: 193-201

Danish University Antidepressant Group (DUAG) (1999) Clomipramine dose-effect study in patients with depression: Clinical end points and pharmacokinetics. Clin Pharmacol Ther 66: 152-165

Delbressine LPC, Moonen MEG, Kaspersen FM, Wagenaars GN, Jacobs PL, Timmer CJ, Paanakker JE, van Hal HJM, Voortman G (1998) Pharmacokinetics and biotransformation of mirtazapine in healthy volunteers. Clin Drug Invest; 15: 45-55

De Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J Biol Chem 269: 15419-15422

Deutsche Gesellschaft für Psychiatrie, Psychotherapie und Nervenheikunde (DGPPN) (2001) Praxisleitlinien in Psychiatrie und Psychotherapie, Band 5: Behandlungsleitlinie Affektive Erkrankungen, Steinkopff Verlag, Darmstadt, Germany

Ebert D (2001) Psychiatrie systematisch. 4th edition, UNI-MED Verlag AG, Bremen, Germany

Eichelbaum M, Gross AS (1990) The genetic polymorphism of debrisoquine/sparteine metabolism – clinical aspects. Pharmacol Ther 46: 377–394

Fawcett J, Barkin RL (1998) Review of the results from clinical studies on the efficacy, safety and tolerability of mirtazapine for the treatment of patients with major depression. J Aff Disorders 51: 267-285

Food and Drug Administration (FDA) (1999) Guidance for industry. Population pharmacokinetics. U.S. Food and Drug Administration, Department of Health and Human Services, Rockville MD: http://www.fda.gov/cder/guidance/index.htm

Frackiewicz EJ, Sramek JJ, Cutler NR (2000) Gender differences in depression and antidepressant pharmacokinetics and adverse events. Ann Pharmacother 34: 80-88

Frahnert C, Rao ML, Grasmäder K (2003) Determination of eighteen antidepressants, four antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. J Chromat B 794: 35-47

Franke EK, Ritschel WA (1976) Quick estimation of the absorption rate constant for clinical purposes using a nomograph. Drug Intel Clin Pharm 10: 77-82

Furuta T, Shirai N, Xiao F, Ohashi K, Ishizaki T (2001) Effect of high-dose lansoprazole on intragastric pH in subjects who are homozygous extensive metabolizers of cytochrome P4502C19. Clin Pharmacol Ther 70: 484-492

Gaillot J, Steimer JL, Mallet AJ, Thebault JJ, Bieder A (1979) A priori lithium dosage regimen using population characteristics of pharmacokinetic parameters. J Pharmacokinet Biopharm 7: 579-628.

Gex-Fabry M, Balant-Gorgia AE, Balant LP (1995) Potential of concentration monitoring data for a short half-life drug: analysis of pharmacokinetic variability for moclobemide. Ther Drug Monit 17: 39-46

Griese EU, Zanger UM, Brudermanns U, Gaedigk A, Mikus G, Morike K, Stuven T, Eichelbaum M (1998) Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. Pharmacogenetics 8: 15–26

Goodnick PJ (1994) Pharmacokinetic optimization of therapy with newer antidepressants. Clin Pharmacokinet 27: 307-330

Härtter S, Hiemke C (1992) Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum. J Chromatogr Biomed Appl 587: 273-282

Härtter S, Wetzel H, Hiemke C (1992) Automated determination of fluvoxamine in human plasma by column switching high-performance liquid chromatography. Clin Chem 38: 2082-2086

Härtter S, Hermes B, Szegedi A, Hiemke C (1994) Automated determination of paroxetine and ist main metbolite by column switching and on-line high-performance liquid chromatography. Ther Drug Monit 16: 400-406

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: 199-200

Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiat 23: 56-62

Hiemke C, Weigmann H, Härtter S, Mann K (2000) Therapeutisches Drug Monitoring von Psychopharmaka. Neurotransmitter 11, 2000: 52-55

Hiemke C, Härtter S (2000) Pharmacokinetics of selective serotonin reuptake inhibitors Pharmacol Ther 85: 11-28

Hildebrand M (2003) Klinische Prüfung von Arzneimitteln. In: Jaehde U, Radziwill R, Mühlebach S, Schunack W. Lehrbuch der Klinischen Pharmazie (2nd edition). Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany: 129-140

Isacsson G, Holmgren P, Druid H, Bergman U (1997) The utilization of antidepressants--a key issue in the prevention of suicide: an analysis of 5281 suicides in Sweden during the period 1992-1994. Acta Psychiatr Scand 96: 94-100

Jaehde U (2003) Klinische Pharmakokinetik. In: Jaehde U, Radziwill R, Mühlebach S, Schunack W. Lehrbuch der Klinischen Pharmazie (2nd edition). Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany: 67-85

Jerling M, Merlé Y, Mentré F, Mallet A (1994) Population pharmacokinetics of nortriptyline during monotherapy and during concomitant treatment with drugs that inhibit CYP2D6 – an evaluation with the nonparametric maximum likelihood method. Br J Clin Pharmacol 38: 453-462

Jermain DM, Crimson LM, Martin III ES (1991) Population pharmacokinetics of lithium. Clin Pharm 10: 376-381

Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjoqvist F, Ingelman-Sundberg M (1993) Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Proc Natl Acad Sci 90: 11825–11829

Kasper S, Dotsch M, Kick H, Vieira A, Möller HJ (1993) Plasma concentrations of fluvoxamine and maprotiline in major depression: implications on therapeutic efficacy and side effects. Eur Neuropsychopharmacol 3: 13-21

Kasper S, Praschak-Rieder N, Tauscher J, Wolf R (1997) A risk-benefit assessment of mirtazapine in the treatment of depression. Drug Safety 17: 251-264

Kidd RS, Straughn AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT (1999) Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. Pharmacogenetics 9: 71-80

Kirchheiner J, Brøsen K, Dahl ML, Gram LF, Kasper S, Roots I, Sjoqvist F, Spina E, Brockmöller J (2001) CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. Acta Psychiatr Scand 104: 173-192

Khan A, Leventhal RM, Khan SR, Brown WA (2002) Severity of depression and response to antidepressants and placebo: an analysis of the food and drug administration database. J Clin Psychopharmacol 22: 40-45

Krusell LR, Jespersen LT, Christensen CK, Thomsen K, Pedersen OL (1997) Proximal tubular function in essential hypertensives on beta-blocker therapy with atenolol. Blood Press 6: 166-70

Kvist EE, Al-Shurbaji A, Dahl ML, Nordin C, Alvan G, Stahle L (2001) Quantitative pharmacogenetics of nortriptyline: a novel approach. Clin Pharmacokinet 40: 869-877

Lecrubier Y (2001) The burden of depression and anxiety in general medicine. J Clin Psychiatry 62, Suppl 8: 4-9

Lehmann K, Merten K (1974) Die Elimination von Lithium in Abhängigkeit vom Lebensalter bei Gesunden und Niereninsuffizienten. Int J Clin Pharmacol 10: 292-298

Leiri I, Kubota T, Urae A, Kimura M, Wada Y, Mamiya K, Yoshioka S, Irie S, Amamoto T, Nakamura K, Nakano S, Higuchi S (1996) Pharmacokinetics of omeprazole (a substrate of CYP2C19) and comparison with two mutant alleles, CYP2C19m1 in exon 5 and CYP2C19m2 in exon 4, in Japanese subjects. Clin Pharmacol Ther 59: 647–653

Lessard E, Yessine MA, Hamelin BA, O'Hara G, LeBlanc J, Turgeon J (1999) Influence of CYP2D6 activity on the disposition and cardiovascular toxicity of the antidepressant agent venlafaxine in humans. Pharmacogenetics 9: 435-443

Leucht S, Steimer W, Kreuz S, Abraham D, Orsulak PJ, Kissling W (2001) Doxepin plasma concentrations: Is there really a therapeutic range? J Clin Psychopharmacol 21: 432-439

Lewin HA, Stewart-Haynes JA (1992) A simple method for DNA extraction from leukocytes for use in PCR. Biotechniques 13: 522-524

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 334 Suppl: 1-100

Lohmann PL, Rao ML, Ludwig M, Griese EU, Zanger UM, Morike K, Maier W, Bagli M (2001) Influence of CYP2D6 genotype and medication on the sparteine metabolic ratio of psychiatric patients. Eur J Clin Pharmacol 57: 289-295

Løvlie R, Daly AK, Molven A, Idle JR, Steen VM (1996) Ultrarapid metabolizers of debrisoquine: characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene. FEBS Lett 392: 30-34

Lundmark J, Bengtsson F, Nordin C, Reis M, Wålinder J (2000) Therapeutic drug monitoring of selective serotonin reuptake inhibitors influences clinical dosing strategies and reduces drug costs in depressed elderly patients. Acta Psych Scand 101: 354-359

Mandema JW, Verotta D, Sheiner LB (1992) Building population pharmacokinetic--pharmacodynamic models. I. Models for covariate effects. J Pharmacokinet Biopharm. 20: 511-528

Meyer UA (2000) Pharmacogenetics and adverse drug reactions. Lancet 356: 1667–1671

Meyer-Barner M, Meinecke I, Schreeb KH, Gleiter CH (2002) Pharmacokinetics of doxepin and desmethyldoxepin: an evaluation with the population approach. Eur J Clin Pharmacol 58: 253-257

Meyers JA, Sanchez D, Elwell LP, Falkow S (1976) Simple agarose gel electrophoretic method for the identification and characterization of plasmid desoxyribonucleic acid. J Bacteriol 127: 1529-1537

Michell PB (2000) Therapeutic drug monitoring of psychotropic medications. Br J Clin Pharmacol 49: 303-312

Möller HJ (2000a) Are all antidepressants the same? J Clin Psychiatry 61, Suppl 6: 24-28

Möller HJ (2000b) (Ed.) Therapie psychiatrischer Erkrankungen. Thieme Verlag Stuttgart, Germany

Montgomery SA, Kasper S (1998) Side effects, dropouts from treatment and cost consequences. Int Clin Psychopharmacol 13, Suppl 2: S1-5

Müller MJ, Dragicevic A, Fric M, Gaertner I, Grasmäder K, Härtter S, Hermann E, Kuss HJ, Laux G, Oehl W, Rao ML, Rollmann N, Weigmann H, Weber-Labonte M, Hiemke C (2003) Therapeutic drug monitoring of tricyclic antidepressants: How does it work under clinical conditions? Pharmacopsychiatry 36: 98-104

National Institute of Mental Health (1976) CGI. Clinical Global Impressions. In: Guy, W. (Ed.): ECDEU Assessment Manual for Psychopharmacology. Rev. Ed. Rockville, Maryland: 217-222

Ninomiya H, Mamiya K, Matsuo S, Ieiri I, Higuchi S, Tashiro N (2000) Genetic polymorphism of the CYP2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. Ther Drug Monit 22: 230-232

NONMEM Project Group. Boeckmann AJ, Sheiner LB. Beal SL (1994) NONMEM Users Guide Part V. University of California, San Fransisco, USA.

Pepin SM, Baker DE, Nance KS (1980) Lithium dosage calculation from age, sex, height, weight and serum creatinine. 15th Annual Midyear Clinical Meeting, American Society of Hospital Pharmacists, San Francisco, 9 Dezember 1980.

Perry PJ, Alexander B, Prince RA, Dunner F (1986) The utility of a single-point dosing protocol for predicting steady-state lithium levels. Br J Psychiatry 148: 401-405

Pharsight Corporation (1999) WinNonMix™ Reference Guide. Pharsight Corporation, Mountain View, California.

Preskorn SH (1989) Tricyclic antidepressants: The whys and hows of therapeutic drug monitoring. J Clin Psychiatry 50, Suppl 7: 34-42

Preskorn SH (1993) Pharmacokinetics of antidepressants: Why and how they are relevant to treatment. J Clin Psychiatry 54, Suppl 9: 14-34

Preskorn SH (1997) Clinical relevant pharmacology of selective serotonin reuptake inhibitors. An overview with emphasis on pharmacokinetics and effects on oxidative drug metabolism. Clin Pharmacokinet 32, Suppl 1: 1-21

Raimundo S, Fischer J, Eichelbaum M, Griese EU, Schwab M, Zanger UM (2000) Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. Pharmacogenetics 10: 577-81

Rao ML, Bagli M, Papassotiropoulos A, Sobanski A, Deister A (1999) Zur klinischen Relevanz von Paroxetin-Serumkonzentrationen. Psychopharmakotherapie 6: 158-160

Rasmussen BB, Brøsen 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: 143-154

Reimann IW, Frölich JC (1981) Effects of diclofenac on lithium kinetics. Clin Pharmacol Ther 30: 348-352

Reiss RA, Haas CE, Karki SD, Gumbiner B, Welle SL, Carson SW (1994) Lithium pharmacokinetics in the obese. Clin Pharmacol Ther 55: 392-398

Ritschel WA (1992) Handbook of basic pharmacokinetics, 4th edition. Drug Intelligence Publications Inc., Hamilton, Illinois

Sachverständigenrat für die Konzertierte Aktion im Gesundheitswesen (2002). Bericht über Über-, Unter- und Fehlversorgung im Gesundheitswesen. Band III.3: Ausgewählte Erkrankungen: Rückenleiden, Krebserkrankungen und depressive Störungen. http://www.bundestag.de, Bundestags-Drucksache 14-6871: 193-254

Sachse C, Brockmöller J, Hildebrand M, Müller K, Roots I (1998) Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. Pharmacogenetics 8: 181-185

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Ehrlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA-polymerase. Science 239: 487-491

Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 9: 503-512

Sheiner LB, Rosenberg B, Marathe VV (1977) Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biopharm 5: 445-479

Sihm I, Thygesen K, Krusell LR, Lederballe O (2000) Long-term renal and cardiovascular effects of antihypertensive treatment regimens based upon isradipine, perindopril and thiazide. Blood Press 9: 346-354

Sindrup SH, Brøsen K, Gram LF (1992) The relationship between paroxetine and the sparteine oxidation polymorphism. Clin Pharmacol Ther 51: 278-287

Sindrup SH, Brøsen K, Hansen GJ, Aaes-Jørgensen T, Overø KF, Gram LF (1993). Pharmacokinetics of citalopram in relation to the sparteine and the mephenytoin oxidation polymorphisms. Ther Drug Monit 15: 11-17

Spina E, Gitto C, Avenoso A, Campo GM, Caputi AP, Perucca E (1997) Relationship between plasma desipramine levels, CYP2D6 phenotype and clinical response to desipramine: a prospective study. Eur J Clin Pharmacol 51: 395-398

Sproule BA, Hardy BG, Shulman KI (2000) Differential pharmacokinetics of lithium in elderly patients. Drugs Aging;16: 165-177

Steen VM, Andreassen OA, Daly AK, Tefre T, Borresen AL, Idle JR, Gulbrandsen AK (1995) Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. Pharmacogenetics 5: 215-223

Sternbach H (1991) The serotonin syndrome. Am J Psychiatry 148: 705-13

Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF, Rettie AE (1997) Genetic association between sensitivity to warfarin and expression of CYP2C9*3. Pharmacogenetics 7: 361-367

Störmer E, von Moltke LL, Shader RI, Greenblatt DJ (2000) Metabolism of the antidepressant mirtazapine in vitro: contribution of cytochromes P-450 1A2, 2D6 and 3A4. Drug Metab Dispos 28: 1168-1175

Tarright N, Mentré F, Mallet A, Jouvent R (1994) Nonparametric estimation of population characteristics of the kinetics of lithium from observational data and experimental data: individualisation of chronic dosing regimen using a Bayesian approach. Ther Drug Monit 16: 258-269

Taube J, Halsall D, Baglin T (2000) Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. Blood 96: 1816-1819

Timmer CJ, Lohmann AAM, Mink CPA (1995) Pharmacokinetic dose-proportionality study at steady-state of mirtazapine from Remeron ® tablets. Hum Psychopharmacol 10 Suppl: 97-106

Timmer CJ, Paanakker JE, van Hal HJM (1996) Pharmacokinetics of mirtazapine from orally administered tablets: Influence of gender, age and treatment regimen. Hum Psychopharmacol 11: 497-509

Timmer CJ, Sitsen JM, Delbressine LP (2000) Clinical pharmacokinetics of mirtazapine. Clin Pharmacokinet 38: 461-474

Turck D, Heinzel G, Luik G (2000) Steady-state pharmacokinetics of lithium in healthy volunteers receiving concomitant meloxicam. Br J Clin Pharmacol 50: 197-204

Ulrich S, Läuter J (2002) Comprehensive survey of the relationship between serum concentration and therapeutic effect of amitriptyline in depression. Clin Pharmacokinet 41: 853-876

Van der Weide J, Steijns LS, van Weelden MJ, de Haan K (2001) The effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. Pharmacogenetics 11: 287–291

Van Dijk KN, de Vries CS, ter Huurne K, van den Berg PB, Brouwer JR, de Jong-van den Berg LT (2002) Concomitant prescribing of benzodiazepines during antidepressant therapy in the elderly. J Clin Epidemiol 55: 1049-1053

Van Harten J (1993) Clinical pharmacokinetics of selective serotonin reuptake inhibitors. Clin Pharmacokinet 24: 203-220

Veefkind AH, Haffmans PMJ, Hoenkamp E (2000) Venlafaxine serum levels and CYP2D6 genotype. Ther Drug Monit 22: 202-208

Voortman G, Paanakker JE (1995) Bioavailability of mirtazapine from Remeron® tablets after single and multiple oral dosing. Human Pschopharmacol 10 Suppl: 83-96

Wen L (2001) Two-step cycle sequencing improves base ambiguities and signal dropouts in DNA sequencing reactions using energy-transfer-based fluorescent dye terminators. Mol Biotechnol 17: 135-142

Williams PJ, Browne JL, Patel RA (1989) Bayesian forecasting of serum lithium concentrations. Comparison with traditional methods. Clin Pharmacokinet 17: 45-52

World Health Organization (WHO) (1992) International Statistical Classification of Diseases and Related Health Problems, 1989 Revision, Geneva, 1992

World Health Organization (WHO) (1996) The global burden of desease. In: Murray CJL, Lopez AD (editors). The Global burden of disease: A comprehensive assessment of mortality

and disability from deseases, injuries and risk factors 1990 and projected. Cambridge, Mass: Harvard School of Public Health

Wormhoudt LW, Commandeur JN, Vermeulen NP (1999) Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit Rev Toxicol 29: 59-124

Xie HG, Stein CM, Wilkinson GR, Flockhart DA, Wood AJ (1999) Allelic, genotypic and phenotypic distributions of S-mephenytoin 4'-hydroxylase (CYP2C19) in healthy Caucasian populations of European descent throughout the world. Pharmacogenetics 9: 539–549

Yamaoka K, Nakagawa T, Uno T (1978) Application of the Akaike information criterion AIC in the evaluation of linear pharmacokinetics. J Pharmacokin Biopharm 6: 165-175

Yukawa E, Nomiyama N, Higuchi S, Aoyama T (1993) Lithium population pharmacokinetics from routine clinical data: role of patient characteristics for estimating dosing regimens. Ther Drug Monit 15: 75-82

Zetin M, Garber G, Antonio MD (1983) Prediction of lithium dose: a mathematical alternative to the test dose method. J Clin Psychiatry 44: 144-145

9 Publications originating from this thesis

Rao ML, Hiemke C, **Grasmäder K**, Baumann P (2001) Olanzapin: Pharmakologie, Pharmakokinetik und Therapeutisches Drug Monitoring. Fortschritte der Neurologie Psychiatrie 69: 510-517

Lohmann PL, **Grasmäder K**, Rao ML, Hiemke C (2002) Pharmakokinetik und Pharmakogenetik in der Psychiatrie. Psycho 28: 534-538

Lennecke K, Lengeling S, Hagel K, **Grasmäder K**, Liekweg A (2002) Therapie-Profile für die Kitteltasche. Wissenschaftliche Verlagsgesellschaft Stuttgart, Germany

Dragicevic A, Fric M, Geiger K, **Grasmäder K**, Härtter S, Kuss HJ, Laux G, Lohmann PL, Mletzko W, Müller MJ, Rao ML, Schanz B, Scherer A, Weber-Labonte M, Hiemke C (2003) Therapeutisches Drug Monitoring von Antidepressiva Psycho 29: 40-43

Grasmäder K, Jaehde U, Lohmann PL, Rao ML (2003) Optimierung des TDM von Lithiumsalzen durch computergestützte Dosisindividualisierung. Psychopharmakotherapie 10: 15-18

Lohmann PL, Frahnert C, **Grasmäder K**, Hiemke C, Laux G, Rao ML (2003) Klinische Relevanz des CYP2D6-Polymorphismus für die Therapie mit Antidepressiva und Neuroleptika. Psychopharmakotherapie 10: 35-38

Grasmäder K, Lohmann PL, Kuss HJ, Laux G, Hiemke C, Rao ML (2003) Therapeutisches Drug-Monitoring von Antidepressiva – therapeutischer und gesundheitsökonomischer Nutzen. Medizinische Monatsschrift für Pharmazeuten 26: 162-164

Müller MJ, Dragicevic A, Fric M, Gaertner I, **Grasmäder K**, Härtter S, Hermann E, Kuss HJ, Laux G, Oehl W, Rao ML, Rollmann N, Weigmann H, Weber-Labonte M, Hiemke C (2003) Therapeutic drug monitoring of tricyclic antidepressants: how does it work under clinical conditions? Pharmacopsychiatry 36: 98-104

Frahnert C, Rao ML, **Grasmäder K** (2003) Determination of eighteen antidepressants, four antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. Journal of Chromatography B 794: 35-47

Grasmäder K, Verwohlt PL, Kühn KU, Frahnert C, Hiemke C, Dragicevic A, Zobel A, Von Widdern O, Maier W, Rao ML (2003) Relationship between mirtazapine dose, plasma concentration, response and side effects. Submitted for publication

Grasmäder K, Verwohlt PL, Rietschel M, Dragicevic A, Hiemke C, Freymann N, Zobel A, Maier W, Rao ML (2003) Impact of polymorphisms of cytochrome P-450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects in a naturalistic clinical setting. Submitted for publication

Grasmäder K, Verwohlt PL, Kühn KU, Dragicevic A, Hiemke C, Maier W, Jaehde U, Rao ML (2003) Population pharmacokinetic analysis of mirtazapine. Submitted for publication

Abstracts

Grasmäder K, Bagli M, Lohmann PL, Kühn K-U, Rao ML (2000) Socio-economic relevance of therapeutic antidepressant drug monitoring. Poster at the 4th EURON Ph.D.-students day in Maastricht, September 14, 2000

Grasmäder K, Jaehde U, Lohmann PL, Rao ML (2002) Application of Population Pharmacokinetics to Optimise Therapeutic Drug Monitoring of Lithium by Bayesian Curve Fitting. Abstract of an oral presentation at the 5th Workshop of the AGNP working group on therapeutic drug monitoring in Magdeburg, July 4-5, 2002, published in Psychopharmacology 35: III-IV

Grasmäder K, Lohmann PL, Kuss HJ, Laux G, Oehl W, Hiemke C, Rao ML (2002) Impact of therapeutic drug monitoring on antidepressive therapy and costs. Oral presentation at the annual meeting of the DPhG in Berlin, October 9-12, 2002

10 Acknowledgements

I owe my gratidute to many people that were involved in this work and without whom this thesis would not have been possible.

First of all, I thank Prof. Dr. Marie Luise Rao for giving me the subject of this thesis and the facilities to accomplish this work under her profound supervision and care on which I could always rely.

I would like to thank Prof. Dr. Ulrich Jaehde for being my supervisor in the faculty of pharmacy, especially for giving me advice and facilities in all aspects of pharmacokinetics.

My very special thanks go to Dr. Petra Luise Verwohlt. She always had a helping hand during the conduct of the study and encouraged and consoled me whenever it was necessary. I also thank her for being a good friend and for sharing a pharmaceutical point of view in a medical world.

This work would not have been possible without my colleagues in my psychiatric home, the Neurochemisches Labor. Especially I like to thank Christine Frahnert, who was responsible for plasma concentration measurements, Sandra Schmitz, the expert in genotyping and Dr. Heike Kölsch for her helpful advice and critical comments.

I thank all colleagues from the “Kompetenznetz Depression” for the good ambiance we had when working together on the subproject 3.8. The co-operation with Prof. Dr. Christoph Hiemke and Dr. Aleksandra Dragicevic of the Department of Psychiatry of the University of Mainz was crucial not only to assure the quality of the data gathered, but to discuss all aspects of the study of the Kompetenznetz and for administration and coordination of all research activities that were involved. A specials thank goes to PD Dr. Sebastian Härtter for his spontaneous help to accomplish the analytical part of this thesis.

I also thank Prof. Dr. Wolfgang Maier, director of the Department of Psychiatry, who allowed to accomplish this work in his clinic. Because of the initiative of him and Prof. Dr. Marcella Rietschel it was possible to evaluate the pharmacogenetic aspects of this work. I thank the colleagues from the genetical division of the Department of Psychiatry for their good co-operation and entertainment when I looked out for my patients out in the Augenklinik – Heiner, thanks for making me laugh every time I came in for a little talk or a cup of coffee.

I thank Dr. Metin Bagli for sharing his passion in pharmacokinetics with me and for his suggestions when looking through this thesis.

A special thanks goes to Ann Snyder and her friend Greg for their spontaneous help in correcting my thesis when I called them at the Oktoberfest. I hope you also enjoyed the Rouladen mit Klößen.

I owe special thanks to Katrin Barkow for her crash courses in statistics and Dirk Oliver Granath for his coffee and IT support.

No clinical study can be conducted without the assistance of the medical and nursing staff at the hospital wards. I thank Prof. Dr. Reinhard Heun and Prof. Dr. Peter Falkai for allowing me to attend their visits and Dr. Kai-Uwe Kühn for his advice in psychopharmacological research. Special thanks go to the colleagues at the hospital wards Schneider and Bonhoeffer for their good co-operation allthough their time was always pessing. Allthough they are too much to mention I would especially thank Dr. Nikolaus Freymann, Dr. Lisa Schönrock, Dr. Michael Abele, Dr. Katharina Friedrichs, Dr. Frank Jessen, Dr. Kathrin Götz, Dr. Olrik von Widdern and Dr. Claas Wittig.

I also thank the colleagues at the Institute of Clinical Pharmacy of the University of Bonn who always welcomed me as one of them.

The population pharmacokinetic working group that was supervised by Prof. Jaehde and Prof. Süverkrüp was crucial for the evaluation of mirtazapine pharmacokinetics. I like to thank Markus Opitz for his company in exploring the population pharmacokinetic universe in numerous WinNonMix Peasure Sessions and Susanne Quellmann for the hot discussions – and hot Salsa nights – we had together.

I owe my deep gratidute to all patients that allowed me to accompany them during their stay in hospital and gave insight in very personal aspects of their lifes. I learned a lot from you and I hope I was able to give it back to you by listening to you and giving you some pharmacological advice.

This work would never have been begun without my family who always believed in me and gave me the courage and confidence to follow my way:
Vatter, Mutter, Oma Erna, Oma Liese, Opa Helmut, Opa Karl, Schwester, Petra - die an letzter Stelle Genannten sind immer die Wichtigsten. Ohne Euch wäre diese Arbeit nie entstanden und mir fehlen die Worte, um zu beschreiben, wie wichtig Eure Unterstützung und Liebe für mich war und ist. DANKE! Ich hab Euch lieb!

Appendix 1

Hamilton Depression Rating Scale

Therapeutisches Drug Monitoring bei Antidepressiva Patientennummer:

Datum:

Hamilton Depression Scale (17 Items)

1. Depressive Stimmung
(Gefühl der Traurigkeit, Hoffnungslosigkeit, Hilflosigkeit, Wertlosigkeit)
0 Keine
1 Nur auf Befragen geäußert
2 Vom Patienten spontan geäußert
3 Aus dem Verhalten zu erkennen (z.B. Gesichtsausdruck, Körperhaltung, Stimme, Neigung zum Weinen)
4 Patient drückt fast ausschliesslich diese Gefühlszustände in seiner verbalen und nicht verbalen Kommunikation

2. Schuldgefühle
0 Keine
1 Selbstvorwürfe, glaubt Mitmenschen enttäuscht zu haben
2 Schuldgefühle oder Grübeln über frühere Fehler und "Sünden"
3 Jetzige Krankheit wird als Strafe gewertet, Versündigungswahn
4 Anklagende oder bedrohende akustische oder optische Halluzinationen

3. Suizid
0 Keiner
1 Lebensüberdruß
2 Todeswunsch, denkt an den eigenen Tod
3 Suizidgedanken oder entsprechendes Verhalten
4 Suizidversuche (jeder ernste Versuch = 4)

4. Einschlafstörung
0 Keine
1 Gelegentliche Einschlafstörung (mehr als 1/2 Stunde)
2 Regelmäßige Einschlafstörung

5. Durchschlafstörung
0 Keine
1 Patient klagt über unruhigen oder gestörten Schlaf
2 Nächtliches Aufwachen bzw. Aufstehen (falls nicht nur zur Harn- oder Stuhlentleerung)

6. Schlafstörungen am Morgen
0 Keine
1 Vorzeitiges Erwachen, aber nochmaliges Einschlafen
2 Vorzeitiges Erwachen ohne nochmaliges Einschlafen

7. Arbeit und sonstige Tätigkeiten
0 Keine Beeiträchtigung
1 Hält sich für leistungsunfähig, erschöpft oder schlapp bei seinen Tätigkeiten (Arbeit oder Hobbies) oder fühlt sich entsprechend
2 Verlust des Interesses an seinen Tätigkeiten (Arbeit oder Hobbies), muss sich dazu zwingen. Sagt das selbst oder lässt es durch Lustlosigkeit, Entscheidungslosigkeit und sprunghafte Entschluss-Änderungen erkennen.
3 Wendet weniger Zeit für seine Tätigkeit auf oder leistet weniger. Bei stationärer Behandlung ist Ziffer 3 einzutragen, wenn der Patient weniger als 3 Stunden an Tätigkeiten (ausgenommen Hausarbeit auf Station) teilnimmt.
4 Hat wegen der jetztigen Krankheit mit der Arbeit aufgehört. Bei stationärer Behandlung ist Ziffer 4 anzukreuzen, falls der Patient an keinen Tätigkeiten teilnimmt, mit Ausnahme der Hausarbeit oder wenn er diese nur unter Mithilfe leisten kann.

Summe Seite 1:

Therapeutisches Drug Monitoring bei Antidepressiva Patientennummer:

Datum:

8. Depressive Hemmung
(Verlangsamung von Denken und Sprache; Konzentrationsschwäche , reduzierte Motorik)
0 Sprache und Denken normal
1 Geringe Verlangsamung bei der Exploration
2 Deutliche Verlangsamung bei der Exploration
3 Exploration schwierig
4 Ausgeprägter Stupor

9. Erregung
0 Keine
1 Zappeligkeit
2 Spielen mit den Fingern, Haaren usw.
3 Hin- und herlaufen, nicht still sitzen können
4 Händeringen, Nägelbeißen, Haareraufen, Lippenbeißen usw.

10. Angst - psychisch
0 Keine Schwierigkeit
1 Subjektive Spannung und Reizbarkeit
2 Sorgt sich um Nichtigkeiten
3 Besorgte Grundhaltung, die sich im Gesichtsausdruck und in der Sprechweise äußert
4 Ängste werden spontan vorgebracht

11. Angst - somatisch: Körperliche Begleiterscheinungen der Angst wie Gastrointestinale (Mundtrockenheit, Winde, Verdauungsstörungen, Durchfall, Krämpfe, Aufstoßen) - Kardiovasculäre (Herzklopfen, Kopfschmerzen) - Respiratorische (Hyperventilation, Seufzen) - Pollakisurie -Schwitzen
0 Keine
1 Geringe
2 Mäßige
3 Starke
4 Extreme (Patient ist handlungsunfähig)

12. Körperliche Symptome - gastrointestinale
0 Keine
1 Appetitmangel, ißt aber ohne Zuspruch, Schweregefühle im Abdomen
2 Muß zum Essen angehalten werden. Verlangt oder benötigt Abführmittel oder andere Magen-Darmpräparate

13. Körperliche Symptome - allgemein
0 Keine
1 Schweregefühl in Gliedern, Rücken oder Kopf. Rücken-, Kopf- oder Muskelschmerzen. Verlust der Tatkraft, Erschöpfbarkeit
2 Bei jeder deutlichen Ausprägung eines Symptoms 2 eintragen

14. Genitalsymptome wie etwa: Libidoverlust, Menstruationsstörungen etc.
0 Keine
1 Geringe
2 Starke

Summe Seite 2:

Therapeutisches Drug Monitoring bei Antidepressiva Patientennummer:

Datum:

15. Hypochondrie
0 Keine
1 Verstärkte Selbstbeobachtung (auf den Körper bezogen)
2 Ganz in Anspruch genommen durch Sorgen um die eigene Gesundheit
3 Zahlreiche Klagen, verlangt Hilfe etc.
4 Hypochondrische Wahnvorstellungen

16. Gewichtsverlust (entweder a oder b eintragen)
a. Aus Anamnese
0 Kein Gewichtsverlust
1 Gewichtsverlust wahrscheinlich in Zusammenhang mit jetziger Krankheit
2 Sicherer Gewichtsverlust laut Patient

b. Nach wöchentlichem Wiegen in der Klinik oder Praxis, wenn Gewichtsverlust
0 weniger als 0,5 kg/Woche
1 mehr als 0,5 kg/Woche
2 mehr als 1 kg/Woche

17. Krankheitseinsicht
0 Patient erkennt, daß er depressiv und krank ist
1 Räumt Krankheit ein, führt sie aber auf schlechte Ernährung, Klima, Überarbeitung, Virus, Ruhebedürfnis etc. zurück
2 Leugnet Krankheit ab.

Summe Seite 3:
+
Summe Seite 1:
+
Summe Seite 2:

Gesamtsumme :

Appendix 2

UKU side effect rating scale

Therapeutisches Drug Monitoring von Antidepressiva Patientennummer:

Datum:

Nebenwirkungs-Rating-Skala (UKU)

Bitte fragen Sie den Patienten nach jedem nachstehend aufgeführten Item und beurteilen Sie jedes nach Schweregrad und Kausalität.

Schweregrad (Grad)	Kausalität (Kaus.)
0= nicht vorhanden	0= unwahrscheinlich
1= leicht	1= möglich
2= mittel	2= wahrscheinlich
3= stark	9= nicht zutreffend
9= nicht beurteilbar	

Grad	Kaus.		Grad	Kaus.	
☐	☐	Konzentrationsstörungen	☐	☐	Verstopfung
☐	☐	Schläfrigkeit/Sedierung	☐	☐	Miktionsstörungen
☐	☐	nachlassendes Gedächtnis	☐	☐	Polyurie/Polydipsie
☐	☐	Spannung/innere Unruhe	☐	☐	Orthostatischer Schwindel
☐	☐	Erhöhte Schlafzeit	☐	☐	Herzklopfen/Tachykardie
☐	☐	Verringerte Schlafzeit	☐	☐	Erhöhte Schwitzneigung
☐	☐	Erhöhte Traumaktivität	☐	☐	Gewichtszunahme
☐	☐	Emotionale Indifferenz	☐	☐	Gewichtsabnahme
☐	☐	Konfusion/Bewusstseinstrübung	☐	☐	Verminderte Libido
☐	☐	Tremor	☐	☐	Orgasmus-Dysfunktion
☐	☐	Akkommodationsstörungen	☐	☐	Kopfschmerzen
☐	☐	Vermehrter Speichelfluss	☐	☐	Libidosteigerung
☐	☐	Verminderter Speichelfluss/ Mundtrockenheit			**bei Männern:**
			☐	☐	fehlende Erektion
☐	☐	Übelkeit/Erbrechen	☐	☐	Ejaculatio praecox
☐	☐	Diarrhoe	☐	☐	Ejaculatio retardata

Gesamteinschätzung:
Bitte beurteilen Sie nun den Einfluss der Nebenwirkungen auf das tägliche Verhalten des Patienten.
0 = keine Nebenwirkungen
1 = leichte Nebenwirkungen, die das Verhalten des Patienten NICHT beeinflussen.
2 = Nebenwirkungen, die das Verhalten des Patienten MÄSSIG beeinflussen
3 = Nebenwirkungen, die das Verhalten des Patienten DEUTLICH beeinflussen

➡ ☐

Konsequenzen:
Bitte beschreiben Sie die Konsequenzen, die sich aus den geschilderten Nebenwirkungen ergeben:
0 = Keine Massnahmen erforderlich
1 = Häufigere Untersuchung des Patienten, aber keine Reduzierung der Dosis; evtl. gelegentliche, medikamentöse Behandlung der Nebenwirkungen.
2 = Reduzierung der Dosis und/oder kontinuierliche medikamentöse Behandlung der Nebenwirkungen
3 = Absetzen des Medikaments oder Wechsel zu einem anderen Präparat

➡ ☐

Appendix 3

Clinical Global Impression Item 3

Therapeutisches Drug Monitoring von Antidepressiva Patientennummer:

Datum:

Clinical Global Impressions (CGI)

Wirksamkeits-Index

Bitte beurteilen Sie nun das **Verhältnis** von therapeutischem Effekt und auftretenden Nebenwirkungen **nur** auf Grundlage des **Medikamenten-Effekts**. Der therapeutische Effekt ist in den Zeilen links beschrieben, die *Nebenwirkungen* sind in den *Spalten rechts* aufgeführt. Suchen Sie die passende Kombination und kreuzen Sie den zutreffenden zweistelligen Code an.

Beispiel: Der Therapieerfolg wird als "mässig" beurteilt, und zu den Nebenwirkungen wird festgestellt, dass sie den Patienten nicht wesentlich beeinträchtigen.

Es ist 3/2 anzukreuzen.

	Nebenwirkungen:			
Therapeutischer Effekt:	*keine*	*Beeinträchtigen den Patienten nicht wesentlich*	*Beeinträchtigen den Patienten wesentlich*	*Überwiegen den therapeutischen Effekt*
Sehr gut - umfassende Besserung; vollständige o. fast vollständige Remission aller Symptome	2/1	2/2	2/3	2/4
Mässig - deutliche Besserung. Teilweise Remission der Symptome	3/1	3/2	3/3	3/4
Gering - leichte Besserung; eine weitere Behandlung des Patienten ist dennoch nötig	4/1	4/2	4/3	4/4
Unverändert oder schlechter	5/1	5/2	5/3	5/4
Nicht beurteilbar 1/1				

Appendix 4

Request form for plasma concentration measurement

(Including Clinical Global Impression Item 1 and 2)

Universitätsklinikum Bonn
Klinik und Poliklinik für Psychiatrie und Psychotherapie
Direktor: Prof. Dr. W. Maier
Neurochemisches Labor
Leiterin: Prof. Dr. M.L. Rao
Sigmund-Freud-Str. 25
53105 Bonn

Tel. 0228/ 287-6308/5737/6125
Fax: 0228/ 287-6383

Station Telefon Datum

Pat.-Nr.: **Studientag:**

Anwendungsbeobachtung: Therapeutisches Drug Monitoring von Antidepressiva

Bitte beachten: Blutentnahme unter Steady-State Bedingungen mind. 12 h und höchstens 24 h nach Medikamenteneinnahme, morgens nüchtern in 10 ml - EDTA Röhrchen.Bei Verdacht auf Substanzabusus oder Intoxikation zu jeder Tageszeit.

Antidepressivum:	Dosis in mg morgens	mittags	abends	nachts	Tage konst. Dosis	Bedarfsmedikation der letzten 3 Tage
						-1:
Andere Dauermedikation						
						-2:
						-3:

Zeitpunkt der letzten Medikamenteneinnahme: _____ Zeitpunkt Blutabnahme: _____

Anordnender Arzt: _____ Unterschrift Arzt: _____

Klinische Diagnose (ICD-10): _____

Gewicht:____ Körpergröße:____

Schweregrad der Krankheit (CGI)
- ☐ nicht beurteilbar (1)
- ☐ Pat. ist überhaupt nicht krank (2)
- ☐ Pat. ist ein Grenzfall psychiatrischer Erkrankung (3)
- ☐ Pat. ist nur leicht krank (4)
- ☐ Pat. ist mässig krank (5)
- ☐ Pat. ist deutlich krank (6)
- ☐ Pat. ist schwer krank (7)
- ☐ Pat. gehört zu den extrem schwer Kranken (8)

Beurteilung der Zustandsänderung
- ☐ nicht beurteilbar (1)
- ☐ sehr viel besser (2)
- ☐ viel besser (3)
- ☐ etwas besser (4)
- ☐ unverändert (5)
- ☐ etwas schlechter (6)
- ☐ viel schlechter (7)
- ☐ sehr viel schlechter (8)

Nebenwirkungen
- ☐ keine
- ☐ leicht
- ☐ mittel
- ☐ schwer

Zusammenhang mit Medikation: wahrscheinlich O möglich O unwahrscheinlich O

Relevante Begleiterkrankungen:
- ☐ Diabetes
- ☐ Niere: Kreatinin _____mg/dl
- ☐ Schilddrüsenerkrankung
- ☐ Leber: GOT____ GPT____ yGT____U/l
- ☐ EKG pathol./kardial
- ☐ sonstige Krankheiten
- ☐ andere:_____

Alkohol ☐ nein ☐ __ Glas Bier (0,3l) ☐ __Glas Wein (0,2l ml) ☐ __Glas Schnaps (2 cl)
Rauchen ☐ nein ☐ <10 Zig. ☐ >10 Zig.
Kaffee ☐ nein ☐ Anzahl der Tassen ____

RR: _____
Puls: _____

Plasmaspiegel

Trizyklische Antidepressiva (TZA)
- ☐ Amitriptylin (100-220 ng/ml)
- ☐ Nortriptylin (70-170 ng/ml)
- ☐ Clomipramin (175-450 ng/ml)
- ☐ Imipramin (175-350 ng/ml)
- ☐ Desipramin (100-300 ng/ml)
- ☐ Doxepin (50-150 ng/ml

SSRI:
- ☐ Citalopram (30-130) ng/ml
- ☐ Fluvoxamin (20-300 ng/ml)
- ☐ Paroxetin (40-120 ng/ml)
- ☐ Sertralin (20-120 ng/ml)

Neuroleptika: _____ **NU:** ( )
Benzodiazepine: _____ ng/ml ( )
Lithium: _____ mmol/L (0,5-1,2)

Beurteilung:

☐ **Patient ohne TDM** ☐ nach klinischem Bild weiterbehandeln ☐ **Achtung**, zu hoher Antidepressivaspiegel

☐ **Patient mit TDM** _____ng/ml _____ng/ml (Metabolit _____ng/ml (Summe)

Blutspiegel: ☐ ausreichend ☐ zu niedrig ☐ zu hoch ☐ im therapeutischen Bereich
Empfehlung für Dosis: ☐ beibehalten ☐ erhöhen auf _____mg/Tag ☐ reduzieren auf _____mg/Tag

Datum: **Unterschrift Labor:**