5
General Discussion
GENERAL DISCUSSION

In this thesis knowledge of environmental and genetic drug interactions, advances in bioanalytical technology and economic evaluations were integrated to explore the value of precision drug therapy in depression. These aspects were all combined in the design of a pragmatic randomized controlled trial which is currently being conducted among old age depressed patients.

5.1. ENVIRONMENTAL AND GENETIC INFLUENCES ON PSYCHOTROPIC DRUG EXPOSURE

In the first chapter of this thesis we aimed to illustrate environmental and genetic influences on drug exposure. An observation of a different phenotype than predicted by genotype is referred to as phenoconversion (1). In this light Chapter 2.1 can be considered as a typical example of phenoconversion induced by environmental factors like smoking. In the described case report, a patient was treated with clozapine which is metabolized by cytochrome P450 1A2 (CYP1A2). CYP1A2 is known to be induced by smoking (2). As such, genetically extensive metabolizers who smoke, frequently display phenoconversion towards an ultra-rapid metabolizer phenotype. Not only co-medication, but also changes in behavior like switching to e-cigarettes can have an effect on enzyme induction which can result in a different phenotype which was demonstrated in the case-report. This could lead to substantial changes in drug exposure within the same patient over time.

To further study the phenomenon of phenoconversion, we were interested if aging could be considered as an environmental factor to provoke phenoconversion towards a reduced metabolism of CYP2D6. In Chapter 2.2. an old aged population (>60 years) was studied and surprisingly no support for this hypothesis was found with regard to the use of and exposure to venlafaxine. On the contrary, phenoconversion was directed towards an increased metabolism and it was found that for venlafaxine men had a higher risk on this type of phenoconversion. This could be related to the fact that venlafaxine is partly metabolized by other enzymes than CYP2D6, such as CYP3A4 which could compensate for a loss in CYP2D6 function (3). As for nortriptyline, which is more dependent on CYP2D6 than venlafaxine, we found some phenotypes with less metabolic capacity than would have been expected based on genotype, among intermediate metabolizers. However, due to the post-hoc nature of this study, we cannot rule out that other mutations than the CYP2D6 *3 and *4 alleles, like the *5, *6, *10, *41, etc. which were not addressed in this study could have played a role in these patients. These other, “untested mutations” could also explain the decreased metabolism observed in some of the patients.
The fact that phenoconversion occurred, does not imply genotyping is misleading or of less value, but results should be interpreted by trained professionals. These professionals should incorporate co-medication and environmental factors like gender and smoking in their dosing recommendations. To achieve precision drug therapy, it is important that young clinicians who are now trained with knowledge of a growing amount of genetic variations, continue to look at the patient and talk about behavioral changes like compliance, smoking, caffeine intake, grapefruit juice, etc (4). These tasks might become complicated and decision support, which could be incorporated in electronic prescription software and updated based on current knowledge, might be used to facilitate precision drug therapy in practice. To monitor and study the influence all mentioned variables, therapeutic drug monitoring (TDM) remains of undoubted importance.

5.2. NEW SAMPLING STRATEGIES FOR THERAPEUTIC DRUG MONITORING OF TRICYCLIC ANTIDEPRESSANTS AND VENLAFAXINE

To facilitate TDM, a Dried Blood Spot (DBS) method was developed for six common tricyclic antidepressants, venlafaxine and desmethylvenlafaxine (ODV). The same extraction method was used for all compounds and the methods were validated according to FDA and EMA guidelines (5, 6). As is required by these guidelines, accuracy and precision, selectivity, matrix effects, carry over, recovery, linearity, and stability were assessed and the outcomes are described in Chapter 3.1 and 3.2. In addition, we studied some DBS-specific characteristics like spotting volume, punch position and different hematocrit concentrations (7). For the clinical interpretation of DBS concentrations, a comparison between plasma and DBS concentrations was made. We found all guidelines requirements were met, except for the selectivity of the method for desmethylclomipramine (DCMP) which was a little too low as well as the precision for clomipramine (CMP). We choose for a pragmatic solution and increased the lower limit of quantification (LLOQ) for both compounds towards 40 instead of 20 µg/L. Since the lower limit of the therapeutic range for the sum of CMP and DCMP is 200 µg/L we felt a LLOQ of 40 µg/L was still sufficient for TDM purposes. With respect to DBS specific characteristics, like variation in hematocrit, we found a trend towards a negative bias at a lower hematocrit for all compounds as is reported in many studies for various compounds (8-11). Although the results did not indicate major bias at physiological hematocrit concentrations, the bias observed for amitriptyline, desmethylclomipramine, venlafaxine, and desmethylvenlafaxine indicated the need for further examination. Spotting volume and punch position did not introduce any substantial bias. In theory, drugs can be distributed equally over plasma and whole blood and if so, DBS concentrations mirror plasma concentrations.
However, for most compounds this distribution is not a 1:1 distribution and in line with this we found that DBS concentrations of the antidepressants differed from plasma concentrations. Therefore a clinical validation study in which we compared paired patients samples of DBS and plasma concentrations was performed which is described in Chapter 3.3. Not only the relationship between these two matrixes, but also the influence of different hematocrit concentrations on this relationship was assessed. To measure the hematocrit in patient samples, we used potassium as a derivative. A large set of patient samples was analyzed and we applied a Passing and Bablok regression to test the comparability of the DBS and plasma method (12, 13). We found significant proportional differences between the DBS and plasma concentrations which was in line with our findings in the analytical validation. To correct for the proportional difference, the slope of the regression lines can be used as a correction factor (14, 15). We did not find a quantifiable hematocrit effect, except for ATP in the patient samples. Especially for venlafaxine and desmethylvenlafaxine these findings were unexpected. The most simple explanation for this difference was the preparation procedure of the different hematocrit concentrations in the first validation studies. This procedure was recently found to give lower hematocrit concentrations than anticipated by calculations based on the different fractions of plasma and red blood cells (16). As a result, our first study could have overestimated the influence of hematocrit. Besides this simple explanation, it might be that more complicated unknown influences are at play, which compensate the negative hematocrit bias. Such factors could be related to the recovery, which can be influenced by hematocrit (17). As we did not study these influences, strict coherence towards our sample preparation and analysis procedure is required. For example, the use of a 4 mm punch instead of the validated 6 mm punch could give, hypothetically, a major influence on the assays accuracy, because it might be more sensitive to variations in hematocrit.

The field of DBS analysis has been a field of research for more than a decade, however, more and more issues which could introduce bias are being identified (17-19). As a consequence, validation of a DBS method becomes a comprehensive task and it is unlikely all laboratories will be equipped for this task in the future. On the other hand, one could argue that it is not necessary to know all the variations which are ongoing in a certain assay as long as the outcome is of sufficient quality. To facilitate this quality, we studied an additional validation step in Chapter 3.3. For this step, a new set of patient samples was used and we applied the correction factor to calculate plasma concentrations of nortriptyline based on DBS concentration. Biases between the calculated and measured plasma concentrations were calculated for individual data points. For the individual biases we calculated a limit of acceptance based on a Monte Carlo simulation. In this simulation we compared in silico results of 10 000 method comparisons to calculate a 95% predicting interval for acceptance. This principle is illustrated in figure 1. Note that
the regression line of example one is closer to the line of unity than in example 2, but the error of the individual points of example 1 is unacceptable whereas the error of the individual points of example 2 is acceptable. If the error of individual points is taken into account, one can be confident that possible “unknown” bias in the DBS assay will not result in unacceptable bioanalytical performance according to the FDA and EMA requirements.

Current quality of TDM is maintained by external quality control programs in which laboratories participate. Although the Dutch authority (i.e. Dutch Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology, KKGT) which is conducting quality control programs for TDM is taking steps towards development of such programs for DBS methods, DBS specific programs are not yet in place (21). Until the field of DBS analysis is also covered by such programs, comprehensive analytical and clinical validation studies of DBS methods as we illustrated in Chapter 3 remain of utmost importance.

**Example 1 does not meet additional criterium**

**Example 2 does meet additional criterium**

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**Figure 1.** Demonstration of additional validation criteria.
5.3. EVALUATING EFFECTS AND COSTS ASSOCIATED WITH THE APPLICATION OF GENETIC TESTING TO IMPROVE PHARMACOTHERAPY

In Chapter 4.1 an update on a systematic review concerning cost-effectiveness of pharmacogenetic and pharmacogenomic screening (PGx) tests was given. In order to enable integration of outcomes with data from previous reviews, the same search strategy was used as in the previous reviews (22, 23). We found that the number of studies was doubled from 2010 till 2014. Furthermore, the quality was improved with regard to more comprehensive sensitivity analyses and reporting of study limitations. This is likely due to the availability and better adherence to guidelines on the conduct of cost-effectiveness analyses such as the CHEERS statement which was published in 2013 (24). When the added papers were studied in more detail, it appeared that most of the reported incremental cost-effectiveness ratios (ICERs) were not giving specific information about the cost-effectiveness of the PGx test itself, but rather gave an analysis of a comparison of (drug) therapies including genetic tests. As such, the ICER was mainly dependent on the costs of the comparator drug and gives no information about the added value or costs of the PGx tests (i.e. the intrinsic value of the PGx test). To facilitate a clear as possible comparison, a distinction between studies that gave information about the intrinsic value of the PGx tests and studies that did not was made. In this way, more insight was given into the comparability of the studies. Furthermore, general and PGx specific recommendations for improvement of economic evaluations of PGx tests were formulated. As the amount of PGx tests will likely increase in the future, our recommendations together with those of others could be used for the formulation of guidelines for cost-effectiveness analysis of PGx tests (25). Most studies which were included in the review involved genetic testing in the field of oncolytic and antithrombotic treatment. In the field of psychotropic medicine, two studies were found which evaluated a screening for a serotonin transporter (5-HTTLPR) to select either citalopram or bupropion for treatment of major depressive disorder. In the first study, the ICER for genotype guided selection of antidepressant was found not cost-effective at a $50,000 per quality adjusted life year (QALY) cut-off (26). In the second study, 5-HTTLPR screening was only costs-effective (ICER<3 times the gross domestic product per capita) for high income countries (27). No studies assessing cost-effectiveness for screening for CYP enzymes in combination with psychotropic agents were found. Nevertheless, initiatives are undertaken in the Netherlands to perform genotyping for CYP2D6 and CYP2C19 enzymes, when antidepressants are used (28). To either support or discourage such screening initiatives, a cost-effectiveness analysis was performed in chapter 4.2 to assess cost-effectiveness of a routine screening for CYP2D6. We identified nortriptyline as the best study example, because of the evidence which is available concerning the dose-
effect relationship and evidence for a major influence of genetic variation in CYP2D6 activity on plasma concentrations of nortriptyline (29).

A decision tree model was constructed to assess the cost-effectiveness of genotyping for CYP2D6 at the start of nortriptyline treatment. An old aged (> 60 years) depressed inpatient population was chosen. This patient population was chosen, because of a higher burden of adverse drug reactions and a possible reduction in hospitalization duration which could result in more health gains and lower health care costs (30, 31). We found at current costs (i.e. €190 per test) of genotyping, genotyping was not a cost-effective (i.e. €50 000 per QALY) strategy to follow, unless the price for genotyping was reduced toward €40 per test. On the other hand, if genotyping costs would be less than €35 per test, genotyping was found to be a dominant treatment option (i.e. cost savings and better health outcomes). The analysis contained limitations, which should be considered when interpreting the results. The most important limitation is the assumption that genotyping can prevent sub- or supra-therapeutic dosing. Although many studies have confirmed sub- or supra-therapeutic dosing occurs in deviating genotypes (i.e. poor, intermediate, or ultra-rapid metabolizers) (32-36), it has not been shown, that genotyping could prevent such dosing. In addition, under naturalistic conditions, in which TDM guided flexible dosing is applied, plasma concentrations might be regulated to such an extent that variations in plasma concentrations between genotype groups are minimalized. This is supported by the findings of Hodgson et al. who found no relation between genotype and adverse drug reactions after eight weeks of nortriptyline pharmacotherapy in patients of different ages (i.e. 19-72 years) under naturalistic conditions (37). They did not find a significant relation between adverse drug reactions and nortriptyline plasma concentrations. Therefore, if there are beneficial effects of genotyping, one might expect that they occur at the very start of treatment when no TDM results are available yet. Moreover, they should be studied in a naturalistic setting in which TDM based dose adaptations are applied. In the model, effects were incorporated in a very short time span, i.e. the first twelve weeks of pharmacotherapy and in addition to TDM to give the most realistic simulation of effects.

To establish effects, which were assumed in the pharmacoeconomic model, of genotyping for CYP2D6 under naturalistic conditions, genotyping should ideally be studied in a randomized controlled trial (RCT). The design of such a trial is described in Chapter 4.3. Two CYP2D6 substrates (i.e. nortriptyline and venlafaxine) were selected for this study. In the Cyp SCreening Elderly trial (CYSCE), genotype information is given to the treating psychiatrist within 10-15 days of treatment. The patients are followed until six till eight weeks of treatment and various outcomes like adverse drug reactions (including severity), quality of life, and blood plasma concentrations are measured. Plasma concentrations are measured by DBS sampling. Unfortunately, mental care faced extensive reorganization in the period 2012-2014 and as a result inclusion of patients in
a trial, in which timing is of major importance, was taking more efforts than anticipated. Nevertheless, by the time this thesis is written, one third of the anticipated patients completed the CYSCE trial. Since sufficient power to detect any differences in outcomes is of utmost importance, it was decided to extent the inclusion period by one year. With the CYSCE trial, questions about the capability of genotyping to optimize treatment with the selected antidepressants under naturalistic conditions can be answered by a RCT in the near future.

5.4. FUTURE PERSPECTIVE

DBS sampling for monitoring plasma concentrations of antidepressants is not only a patient friendly alternative for venous sampling, but it could also serve to centralize drug analysis. As a result, logistics of (scientific) studies can be optimized. This was demonstrated by the application of the described DBS methods for TDM of nortriptyline and venlafaxine in the CYSCE trial. In the trial samples from ten different locations are collected by even more health care workers (i.e. psychiatrists, medical doctors, nurses). All samples are analyzed and reported in a uniform way and in this way heterogeneity between centers can be reduced. By the time this thesis is written >175 DBS samples were collected for TDM of nortriptyline or venlafaxine in the CYSCE Trial. Although not all DBS samples which were collected were of perfect quality (i.e. repeatedly only one out of four spots on a card was suitable for analyses), > 98% could be analyzed which can be considered as a successful application of DBS sampling. The timing of DBS sampling was found quite challenging, like for example sampling of nortriptyline which should be performed ~12 hours after nortriptyline intake. This might not be a specific problem for DBS sampling as this is also a requirement for venous sampling. Nevertheless, attention towards this aspect when implementing a DBS method is important. In addition, control measures to assure the right timing of the sample should be in place. When taking these considerations into account, DBS sampling could facilitate patients to obtain a TDM sample by themselves and at home, which would likely reduce TDM costs and patients discomfort. In addition to TDM, genetic information will likely become part of psychotropic pharmacotherapy. In the CYSCE trial most health care professionals were positive about genetic testing and wanted to apply pharmacogenetic information for optimization of pharmacotherapy of their patients. Whether the CYSCE trial will show an added value of CYP2D6 screening is still unknown. However, there is a willingness and interest from the field as well as patients to start implementing genetic knowledge. This is supported by the findings from a Canadian group who found 80% of physicians thought pharmacogenetics will be applied in the future of mental health care (38). Nevertheless, communication about when and how genetic information should be given
and in what kind of format might be different between different settings and hospitals. Local (clinical) pharmacies could start to facilitate these aspects, together with mental health care institutions within their regions. In some Dutch regions around Rotterdam and Assen, such initiatives are already undertaken. Note that a (clinical) pharmacist does not have to perform the genetic testing him-/herself, however (s)he could be the responsible party to give sufficient education, logistic support and interpretation of pharmacogenetic information based on the national pharmacists guidelines (39, 40). Such efforts, can be the key towards cost-effectiveness of precision drug therapy with regard to psychotropic treatment. As a result, in the hopefully near future, precision drug therapy with psychotropic agents will become care as usual.
5.5. REFERENCES


