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Further characterization of a ^{13}C-dextromethorphan breath test for CYP2D6 phenotyping in breast cancer patients on tamoxifen therapy

Frans Opdam, Anil Modak, Hans Gelderblom, Henk-Jan Guchelaar

J Breath Res 2015 Apr 20;9(2):026003

ABSTRACT

In a previous study, we found that the CYP2D6 phenotype determined by ^{13}C-dextromethorphan breath test (DM-BT) might be used to predict tamoxifen treatment outcome in breast cancer patients in the adjuvant setting. However, large variation in the Delta-over-baseline (DOB) values was observed in the extensive metabolizer predicted phenotype group based on single point measures. In the present work we aimed to analyze the variability of phenotype results and determine reproducibility to further characterize the clinical utility of DM-BT by introducing multiple breath sampling instead of single breath sampling and by administration of a fixed dose of ^{13}C-DM. A total of 6 extensive metabolizers (EMs) patients by predicted phenotype with early breast cancer using tamoxifen in the adjuvant setting were phenotyped for CYP2D6 by the DM-BT on three separate days using a fixed 50 mg dose of ^{13}C-DM to ascertain reproducibility. Breath samples were collected on t = 0, 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 min. The ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath samples collected at baseline and at different time points post-ingestion of ^{13}C-DM were measured using infrared spectrometry. DOB values were calculated from baseline and postdose $^{13}CO_2/^{12}CO_2$ ratios, reflecting the CYP2D6 phenotype. Variation in area under the curve ($AUCDOB_{0-4h}$), was determined and compared to single breath sampling parameters. Intra- and interday analytical variation of DOB_{50} results were 9.9 and 11.2% respectively. Time to maximum DOB value ranged from 30–120 min with the median time of 54.4 min. Mean *inter*individual variation in $AUC_{DOB0-4h}$ was 22% compared to 3.3–15.5% for *intra*individual *inter*day variation. DOB_{50} values with a fixed dose of 50 mg ^{13}C-DM were 10.3–75.6% higher compared to predicted DOB_{50} values when using a 0.5 mg kg^{-1} dose, suggesting higher metabolic rate with higher dosing in CYP2D6 EMs. The findings suggest that the analytical variation in DM-BT results is sufficiently low. Although multiple breath samples theoretically might predict endoxifen serum levels better than single-point breath test, for practical purposes as a point of care diagnostic test, a single breath sample collection might be preferable. Using a higher fixed dose of the substrate probe ^{13}C-DM should be considered in future validation studies.

INTRODUCTION

Stable isotope-labeled substrates can be used to provide rapid in vivo phenotype assessment of drug metabolizing enzymes. A few rapid, non-invasive, phenotype breath tests have been researched using [^{13}C]-pantoprazole [1-6], [^{13}C]-dextromethorphan [7-9], [^{13}C]-uracil [10-11], [^{13}C]-L-DOPA [12], [^{13}C]-Phenylalanine [13] and [^{13}C]-methacetin [14] for assessing CYP2C19, CYP2D6, Dihydropyrimidine Dehydrogenase (DPD), aromatic L-amino acid decarboxylase (AADC), phenylalanine hydroxylase (PAH) and CYP1A2 enzyme activity respectively. These rapid, non-invasive, point of care, phenotype breath tests using stable isotope labeled substrates to generate the biomarker $^{13}CO_2$ in breath are viable and superior alternatives to the routinely used clinical phenotype tests measuring metabolites of drugs in blood which are time consuming and expensive. Besides the phenotype breath tests are vastly superior to genotype tests since they encompass both genetic and non-genetic (age, diet, environment, liver disease, drug drug interactions) factors for variability in enzyme activity.

Tamoxifen is commonly used for the treatment of adjuvant breast cancer. Only one third of women with localized hormone receptor positive breast cancer benefit from adjuvant tamoxifen. This variable response to tamoxifen treatment might be at least partially explained by interindividual variability in tamoxifen biotransformation to active metabolites, of which endoxifen is the most critical in determining efficacy. CYP2D6 mediated metabolism is considered the main route of activation of tamoxifen into active endoxifen along with CYP3A4. In a recent study, endoxifen serum levels of >5.97 ng/mL have been associated with lower breast cancer recurrence rate [15]. However, variability in endoxifen levels is only explained for 23% by *CYP2D6* genotype [16] suggesting that other extraneous factors might also play an important role in variability of endoxifen levels.

CYP2D6 phenotype includes both *CYP2D6* genotype and interacting environmental factors or co-medications. Therefore, determination of CYP2D6 phenotype by a noninvasive single-point ^{13}C-dextromethorphan breath test (DM-BT) [17] might be of clinical use in prediction of serum endoxifen levels and thus tamoxifen treatment outcome. We have shown that this test is useful in distinguishing between patients with suggested therapeutic serum endoxifen levels and those with suggested subtherapeutic levels [8]. However, variability in endoxifen levels was explained for only 48.7% by CYP2D6 phenotype by single-point DM-BT. This might be explained by the fact that other enzymes than CYP2D6, such as CYP3A, might be involved in tamoxifen metabolism. However, even a large interpatient variability in DM-BT results in the subgroup of patients with

EM *CYP2D6* genotype was observed, which could not be explained by co-medication (such as CYP2D6 inhibitors), since the usage was not allowed in the study. It is therefore hypothesized that variability in DM-BT results might partially be due to technical aspects of the DM-BT and therefore, further characterization of test performance is warranted. Several factors might contribute to the large interpatient variability in DM-BT results, including variability in dextromethorphan (the test probe) disposition and moderate test performance due to single-time-point breath sampling. This study focussed on the further characterization of the existing single-point DM- BT by introducing multiple breath sampling over time using a fixed dose of the probe substrate ^{13}C-DM versus the weight based dose in the previous study. We hypothesized that CYP2D6 phenotype by using DM-BT based on collection of multiple breath samples might help explain the variability in endoxifen levels compared to CYP2D6 phenotype using DM-BT based on single point breath sample.

METHODS

Study design

This pharmacokinetic study was conducted in the Leiden University Medical Center, approved by the institutional review board of the study site and all patients provided written informed consent. A total of 6 patients were recruited from the multicenter, prospective CYPTAM study (NTR1509). In this substudy, *CYP2D6* genotype and CYP2D6 phenotype by DM-BT were determined. Women 18 years and older with early stage estrogen receptor positive breast cancer who were on adjuvant tamoxifen therapy at a daily dose of 20 mg for at least two months, were eligible. Exclusion criteria for this study were a poor metabolizer (PM) CYP2D6 predicted phenotype by means of the genotype, since DM-BT results were near zero, patients unwilling to fast overnight, to abstain from alcohol for at least 24 hours prior to the DM-BT, with known allergy to DM, with grade ≥3 liver or renal impairment, hypercapnia, concomitant use of MAO-inhibitors or medication slowing gastrointestinal motility. Patients underwent CYP2D6 phenotyping by DM-BT on 3 separate days, with a minimum of 24 hours in between. Patient characteristics and concomitant use of medication were recorded on each visit.

Analysis of *CYP2D6* genotype and translation to predicted phenotype

Blood samples were collected to determine *CYP2D6* genotype by the Amplichip array (Amplichip, Roche). *CYP2D6* genotypes were translated to predicted phenotypes (extensive, intermediate

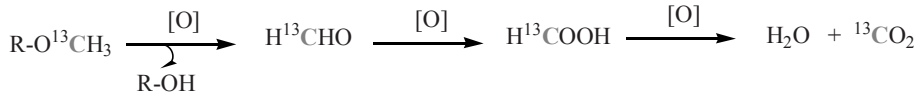
or poor metabolizer). By definition, the CYP2D6 intermediate metabolizer phenotype predicted by genotype consisted of patients homozygous for a decreased activity allele (e.g. *41/*41) or heterozygous for an absent activity allele (e.g. *1/*4 and *41/*4). A patient could only be classified to a certain CYP2D6 phenotype if genotyping was successfully done for the *CYP2D6* alleles with a reported frequency in Caucasians of more than 5%.

Determination of serum levels of tamoxifen and metabolites

Ten mL of blood was collected on the first visit, centrifuged and stored at -20°C for serum analysis of tamoxifen and metabolites. Blood samples were taken up to 12 hours before the next dosage of tamoxifen (trough levels). Serum levels of tamoxifen and its metabolites 4-hydroxytamofen, N-desmethyltamoxifen and endoxifen were determined by liquid chromotography, coupled with tandem-mass-spectrometry (LC-MS) [18].

DM-BT

Isotopically labeled CO_2 breath test have been investigated as minimally invasive procedures for estimating drug biotransformation activity [19]. CYP2D6 mediated *O*-demethylation of ^{13}C-DM leads to dextrorphan and the released methyl group is involved in the formation of $^{13}CO_2$ that is released in expired breath over time [17]:



The concentration of $^{13}CO_2$ and $^{12}CO_2$ in the exhaled breath samples was measured by infrared spectrometry using a POCOne® spectrophotometer (Otsuka Electronics Co. Ltd., Osaka, Japan). Enrichment of $^{13}CO_2$ in expired air as a consequence of ^{13}C-DM is expressed as the increase in the $^{13}CO_2/^{12}CO_2$ ratio relative to predose baseline sample and is termed delta-over baseline (DOB), reflecting CYP2D6 activity:

> DOB= 1000x[($^{13}CO_2/^{12}CO_2$) Postdose - [($^{13}CO_2/^{12}CO_2$) Baseline]/R_{PDB} where DOB is expressed in units of Δ per mil (‰), and R_{PDB}=0.012373 is $^{13}C/^{12}C$ in PDB (international standard Pee Dee Belemnite).

Clinical trial material grade ^{13}C-DM (API) was synthesized by Cambridge Isotope Laboratories (Andover, Massachusetts, U.S.A.) as a powder meeting USP standards. Production of the drug substance meets Good Manufacturing Practice (GMP) guidelines. The oral liquid formulation (2.08

mg/mL) was manufactured under GMP conditions in the GMP facility of Confab Laboratories Inc. in Montreal, Canada. Just before administration of fixed dose of 50 mg of ^{13}C-DM formulation, patients received a solution of water and 2 tablets of 1000 mg anhydrous citric acid, 344 mg potassium bicarbonate, and 1050 mg sodium bicarbonate (AlkaSeltzer Gold [ASG]) for optimization of breath test results [17].

Patients underwent CYP2D6 phenotyping by DM-BT on three separate days, with of minimum of 24 hours in between. Patients were fasting for at least 8 h and had abstained from alcohol for at least 24 hours before the DM-BT.

Prior to the administration of Alka Seltzer solution and ^{13}C-DM, patients exhaled in a 1.3 L breath bag (baseline breath sample). Breath samples were subsequently taken at 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 minutes postdose (Figure 5.1). Patients were clinically monitored during the test and adverse events were recorded.

DOB values from single-point breath sampling (at t=50 min) were compared to calculated Areas under the Curve (AUCs (*DOBxh*)) using the trapezoidal rule from multiple breath sampling for correlation with serum endoxifen levels Pharmacokinetic profiles consisted of ten breath samples collected over 4 hours (0 (baseline) and 10, 20, 30, 40, 50, 60, 90, 120, 180 and 240 minutes postdose).

Trapezoidal $AUC_{0–4h}$ (full AUC) was calculated from all measured DOB-timepoints (T=0–4h) using trapezoidal rule. Additionally time to which maximum DOB value (T_{DOBmax}) was reached was calculated.

Intraday analytical variation of DM-BT was determined in triplicate by analysis of three single breath samples on one single day ten times, and expressed as variation coefficient (VC=standard deviation (SD)/mean).

Interday analytical variation of DM-BT expressed as VC was determined by repetition of breath sample analysis of one single breath sample on day 0, 1, 2, 3, 4, 5, 10, 15, 20, and 30.

Results from DM-BT identified the CYP2D6 phenotype of participating subjects. From each patient and on each day, $AUC_{DOB0–4h}$ was calculated using the trapezoidal method. Intrapatient variation in T_{DOBmax}, CYP2D6 phenotype expressed as VC of $AUC_{DOB0–4h}$, were calculated for each patient (n=3 days).

Figure 5.1 Breath samples (300 cc) were obtained at baseline (T=0) and after 30, 40, 50, 60, 90, 120, 180 and 240 minutes after administration of 50 mg ^{13}C-dextromethorphan ^{13}C-DM).
BS, Breath sample.

RESULTS

Analytical variation

Mean intraday analytical variation of DOB50 for three breath samples was 9.9% as shown in Table 5.1a. Interday analytical VC was 11.7% (Table 5.1b). There was no trend in decreasing DOB50 values over time, excluding the possibility of adsorption of ^{13}C to the aluminum wall of the breath bag, consistent with the finding by Mattison et al. [20] who had shown that the $^{13}CO_2$ content in breath collection bags stored at room temperature is stable up to 210 days.

Intrapatient interday variability in CYP2D6 phenotype

Intrapatient interday variability in CYP2D6 phenotype determined by DM-BT was studied in respect to variation in time by which maximum DOB value was reached (T_{DOBmax} min) and the $AUC_{DOB0–4h}$(‰*x*h) (Table 5.2). Except for the large variation of T_{DOBmax} in patient 5 (60–120 minutes), TDOBmax was in the range of 30 to 60 minutes in the other patients. In patient 5 the T_{max} was delayed which might be due to slower gastrointestinal absorption of ^{13}C-DM and transfer to the liver. Figure 5.2 shows the average DOB breath curves of all 6 patients.

Figure 5.3 shows the correlation of DOB_{50} with AUCs $DOB_{0–4\ h}$ (‰*x*h) and AUCs $DOB_{0–2\ h}$ (‰*x*h) for all 6 patients on three different days. Although there is a large (22%) interpatient variation in AUCs, the intrapatient interday variation in AUCs is considerable low (VC 3.3–15.5%), stressing the unique and consistent ^{13}C-DM drug disposition in each patient. The DOB_{50} correlates better

Table 5.1a Intraday analytical variation in ^{13}C-DM-BT DOB_{50} results to determine CYP2D6 phenotype of three (A, B, C) different breath samples,repeatedly measured on one single day for ten times

Breath sample	A	B	C
No. of repeated analyses	10	10	10
Mean DOB_{50} (‰)	2.57	5.61	1.68
Min. DOB_{50} (‰)	2.3	4.8	1.4
Max. DOB_{50} (‰)	2.9	6.7	1.9
SD DOB_{50} (‰)	0.183	0.644	0.18738
VC	0.0712	0.1147	0.1115
Mean VC	0.0991		

Abbreviations: No., number; ^{13}C-DM-BT, ^{13}C-dextromethorphan breath test; DOB_{50}, delta-over-baseline on t = 50 min; Min.,minimum; Max., maximum; SD, standard deviation; VC, variation coefficient.

with AUCs $DOB_{0-2\,h}$ (‰*x*h) than AUCs DOB_{0-4h} (‰*x*h) since it more closely measures the first pass metabolism of the ^{13}C-DM following ingestion.

Table 5.1b Interday analytical variation in DM-BT DOB_{50} results of one single breath sample to determine CYP2D6 phenotype in a single measurement on 10 separate days

Day	0	1	2	3	4	5	10	15	20	30
DOB_{50} (‰)	1.7	2.1	2.1	2.1	1.9	2.5	2.2	2.5	2.4	2.2
Mean DOB_{50} (‰)	2.17									
Min. DOB_{50} (‰)	1.7									
Max. DOB_{50} (‰)	2.5									
SD DOB_{50} (‰)	0.254									
VC	0.1171									

Abbreviations: No., number; ^{13}C-DM-BT, ^{13}C-dextromethorphan breath test; DOB_{50}, delta-over-baseline on t = 50 min; Min., Minimum;Max., maximum; SD, standard deviation; VC, variation coefficient.

Table 5.2 Interday intrapatient variation in CYP2D6 phenotype parameters determined by ^{13}C-dextromethorphan breath test in 6 patients on 3 separate days

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patent 6
Genotype	*1/*2	*1/*1	*1/*1XN	*1/*1	*1/*1	*1XN/*4
Predicted phenotype	EM	EM	EM	EM	EM	EM
T_{DOBmax} (min)						
Day 1	60	60	40	40	120	50, 60
Day 2	50	50	60	50	90	40
Day 3	50	40	50	30	60	40
DOB_{50} (‰)						
Day 1	4.5	4.3	6.0	8.7	3.7	5.0
Day 2	5.3	7.4	5.8	6.7	3.0	3.5
Day 3	4.0	5.0	6.8	6.3	4.9	4.3
Avg	4.6	5.6	6.2	7.2	3.9	4.3
SD	0.7	1.6	0.5	1.3	1.0	1.0
VC	0.15	0.286	0.048	0.18	0.256	0.233
AUC (‰ × hour)						
Day 1	8.8	10.6	14.7	15.9	10.3	9.4
Day 2	9.1	11.5	12.0	12.4	11.1	8.5
Day 3	8.5	9.2	16.4	14.3	11.6	9.4
Avg	8.8	10.4	14.4	14.4	11.0	9.1
SD	0.3	1.2	2.2	1.8	0.7	0.5
VC	0.034	0.115	0.153	0.125	0.064	0.055
Endoxifen C_{trough} (mg/L)	10.5	18.9	29.5	13.5	11.8	8.1

Abbreviations: T_{DOBmax} (min), time to maximal DOB; DOB, Delta-over baseline; SD, standard deviation; VC, varation coefficient; AUC, Area under the Curve; Avg, average.

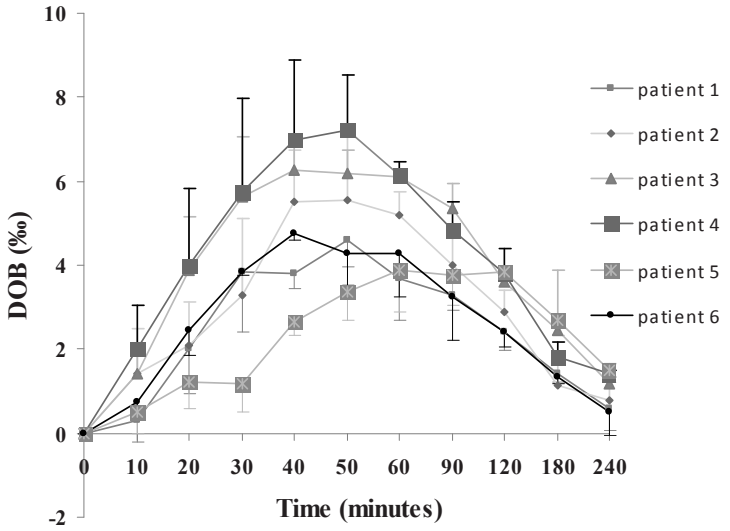


Figure 5.2 DOB (‰, average (point) and SD (lines)) versus time curves for 6 different patients undergoing CYP2D6 phenotype test by DM-BT on 3 separate days.
Abbreviations: DOB, Delta-over=Baseline; DM-BT, ^{13}C-dextromethorphan breath test; SD, standard deviation.

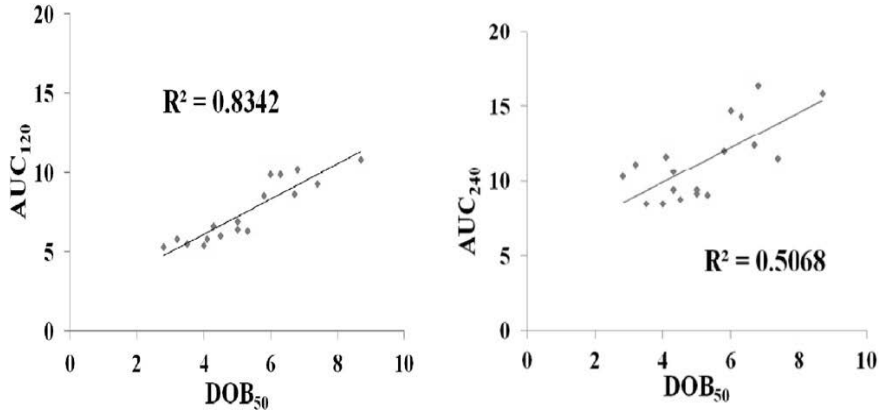


Figure 5.3 Correlation between CYP2D6 phenotype parameters DOB_{50} (‰) and $AUC_{DOB0–120min}$ (‰.h) and $AUC_{DOB0–240min}$ (‰.h) determined with DM-BT.
Correlation between DOB_{50} with $AUC_{0–120min}$ (left) is better than with $AUC_{0–240min}$ (right), but it should be stated that patients numbers are low. Abbreviations: AUC, Area under the Curve; DOB, Delta-over = Baseline; DM-BT, ^{13}C–dextromethorphan breath test.

Effect of probe dose on CYP2D6 activity

In our previous study [8], we observed a non-linearity between gene activity score and DOB_{50} in CYP2D6 IMs and EMs, with flattening of the curve in EMs, suggesting that CYP2D6 enzyme

Table 5.3 DM-BT results in 6 patients with CYP2D6 EM predicted phenotype determined with 0.5 a weight-based (previous study [8]) probe dose versus a fixed probe dose of 50 mg ^{13}C-DM

Previous study with 0.5 mg kg^{-1} ^{13}C-DM dose				Dose response	Present study with fixed 50 mg ^{13}C-DM dose		
Patient	Weight kg	^{13}C-DM dose (mg)	DOB_{50} ‰	Predicted DOB for 50 mg	^{13}C-DM dose (mg)	Avg DOB n=3	((Predicted/True DOB_{50}) – 1))*100%
1	101	50.5	3.6	3.6	50	4.6	+ 27.8
2	61	30.5	2.6	4.3	50	5.6	+ 30.2
3	69	3.5	4.2	5.9	50	6.8	+ 15.3
4	64	32	2.6	4.1	50	7.2	+ 75.6
5	78	39	1.9	2.4	50	3.4	+ 41.7
6	90	45	3.5	3.9	50	4.3	+ 10.3

Abbreviations: ^{13}C-DM-BT, ^{13}C-dextromethorphan breath test; CYP2D6, Cytochrome P450 2D6; EM, extensive metabolizer; ^{13}C-DM, ^{13}C-dextromethorphan.

activity was saturable in IMs and limited by ^{13}C-DM dose in EMs. In the context of this potential "ceiling" effect of DOB_{50} values, results of DM-BT with the fixed ^{13}C-DM dose of 50 mg were compared to DM-BT results from the weight based dose of 0.5 mg/kg used in our previously performed study (Table 5.3). DOB_{50} values in this fixed dose study were 10.3–75.6% higher compared to predicted DOB_{50} values (by linear extrapolation of data from the weight-adjusted dose study to a 50 mg fixed dose of ^{13}C-DM, suggesting higher metabolic rate with higher dosing.

DISCUSSION

This is the first pharmacokinetic study which investigated the analytical and patient related variability in clinical CYP2D6 phenotype by DM-BT. DM-BT was introduced as a rapid phenotyping assay for CYP2D6 activity, using single point determination of $^{13}CO_2$ enrichment by infrared spectrometry (delta-over-baseline (DOB) value) in expired air breath samples [17]. In our previous study [8], we have shown that in 65 breast cancer patients, assessment of the CYP2D6 phenotype by DM-BT might discriminate patients with suggested subtherapeutic (<5.97 ng/mL) and therapeutic (≥5.97 ng/mL) endoxifen levels [15]. However, in the large subset of patients with EM predicted phenotype, we observed a large interpatient variation in the CYP2D6 phenotype determined by DM-BT, which could not be explained by use of CYP2D6 inhibiting medication.

DM-BT measures a metabolic response to a dose of the administered ^{13}C-enriched DM substrate. $^{13}CO_2$ is the biomarker, which is analyzed in breath samples.

*Intra*patient *inter*day variation of the CYP2D6 phenotype was assessed for various DM-BT parameters. Except for the large variation of T_{DOBmax} in patient 5 (60–120 minutes), T_{DOBmax} was in the range of 30 to 60 minutes in the other patients, which is consistent with the expected time of peak plasma concentration (T_{max}) for dextrorphan [21]. Although all patients were fasting (for a minimum of 8 h), sober (for a minimum of 24h) and abstained from medication which might slow GI motility (for a minimum of 12h), differences in T_{DOBmax} over days could be attributable to differences in GI motility, for which recently a circadian rhythm in the fasted state has been discovered [22]. Although an *intra*patient *inter*day variation in C_{DOBmax} was observed, *intra*patient *inter*day variation in AUCs was considerable low (VC 3.3 to 15.5%), stressing the unique and consistent metabolism of the substrate in each patient. To date, no circadian rhythm in CYP2D6 first-pass metabolism has been observed. In contrast, *inter*patient difference in AUCs were 22% higher (Table 5.2), consistent with the high variability of DM-BT DOB_{50} results we observed in patients with CYP2D6 EM predicted phenotype in our previous study [8] and stressing the heterogeneity of CYP2D6 phenotype among genetically identical subjects.

DOB_{50} values in this fixed 50 mg DM dose study were higher compared to predicted DOB_{50} values (by linear extrapolation of data from the previous [8] weight-adjusted dose study to a 50 mg fictive dose of ^{13}C-DM (Table 5.3), suggesting higher metabolic rate with higher dosing.

This finding is consistent with the fact that CYP2D6 mediated DM metabolism follows first-order pharmacokinetics. This data confirms our hypothesis that EM patients with higher CYP2D6 enzyme activity may not have received adequate dextromethorphan-^{13}C to truly reflect their enzyme activity. A mean C_{max} of 14 uM dextrorphan (DXO) was observed [23] after administration of 60 mg DM, far below the DXO Michaelis constant Km (which is the substrate concentration at which the reaction rate is half of the maximum metabolic speed (V_{max})) of 1220 uM [24]. Therefore, with the 50 mg DM dose in our study, CYP2D6 was expected not to be saturated in EMs and the nonlinearity between genotype and phenotype we observed between subjects with IM and EM predicted phenotype in our previous study could not be explained by enzyme saturation. We have shown that DM-BT was able to predict suggested therapeutic endoxifen levels of ≥5.97 ng/mL with a positive predictive value of 100% and a negative predictive value of 90% [8]. However, we observed a large interindividual variation in endoxifen levels in the EM group of patients. Patient numbers in this study are however too low to correlate CYP2D6 phenotype with endoxifen levels in respect to single or multiple breath sampling. Further studies should investigate whether limited multiple breath sampling would be able to improve prediction of endoxifen levels compared to single breath sampling in all three genotype subgroups (PM, IM, EMs). With an already 100% PPV

and 90% NPV with single breath sampling, further improvement by introducing a time consuming limited multiple breath sampling might not improve clinical decision making.

In summary, with our study, we further characterized the DM-BT in respect to analytical variation, an alternative fixed dose regimen and the introduction of multiple breath sampling. Our findings suggest that the analytical variation in DM-BT results is sufficiently low. With a fixed dose of 50 mg ^{13}C-DM compared to using a 0.5 mg/kg dose higher DOB were observed, suggesting higher metabolic rate with higher dosing in CYP2D6 EMs. The optimal dosing of the ^{13}C-DM probe should be part of further research. Due to the low number of patients in the study no conclusions can be drawn regarding the potential superior correlation between CYP2D6 phenotype and endoxifen serum levels by introducing limited breath sampling compared to a slngle breath test. However, from practical considerations, a single breath test might be preferable and adequate. Further research would be necessary to determine if limited multiple breath sampling will influence the decision to choose between tamoxifen (for patients with predicted normal therapeutic endoxifen level) and an aromatase inhibitor (for patients with predicted subtherapeutic endoxifen level). Our results do not support any change to the (weight-based) dose regimen and single-point sampling strategy of the DM-BT at this time.

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