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PHARMACOKINETICS AND DISPOSITION

The impact of liver resection on the dihydrouracil:uracil plasma ratio in patients with colorectal liver metastases

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Abstract

Purpose The dihydrouracil (DHU):uracil (U) plasma ratio is a promising marker for identification of dihydropyrimidine dehydrogenase (DPD)-deficient patients. The objective of this study was to determine the effect of liver resection on the DHU:U plasma ratio in patients with colorectal liver metastases (CRLM).

Methods An observational study was performed in which DHU:U plasma ratios in patients with CRLM were analyzed prior to and 1 day after liver resection. In addition, the DHU:U plasma ratio was quantified in six additional patients 4–8 weeks after liver resection to explore long-term effects on the DHU:U plasma ratio. Quantification of U and DHU plasma levels was performed using a validated ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay.

Results The median (range) DHU:U plasma ratio in 15 patients prior to liver resection was 10.7 (2.6–14.4) and was significantly reduced to 5.5 (< quantification limit (LLOQ-10.5) 1 day after resection ($p = 0.0026$). This reduction was caused by a decrease in DHU plasma levels from 112.0 (79.8–153) ng/mL to 41.2 (< LLOQ-160) ng/mL 1 day after resection ($p = 0.0004$). Recovery of the DHU:U plasma ratio occurred 4–8 weeks after liver resection, which was shown by a median (range) DHU:U plasma ratio in six patients of 9.1 (6.9–14.5).

Conclusion Liver resection leads to very low DHU:U plasma ratios 1 day after liver resection, which is possibly caused by a reduction in DPD activity. Quantification of the DHU:U plasma ratios directly after liver resection could lead to false-positive identification of DPD deficiency and is therefore not advised.

Keywords Uracil · Dihydrouracil · Dihydropyrimidine dehydrogenase · Liver resection · Capecitabine · 5-Fluorouracil

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Introduction

Colorectal cancer remains one of the most commonly diagnosed cancer types worldwide [1]. Approximately 50% of the patients with advanced colorectal cancer develop liver metastases [2, 3]. For patients with resectable colorectal liver metastases (CRLM), partial liver resection is the standard of care. In addition, some patients undergo adjuvant treatment with the 5-fluorouracil (5-FU) pro-drug capecitabine in order to improve survival [4].

After oral administration, capecitabine is rapidly converted to 5-FU through a three-step enzymatic cascade. Only 1–3% of the formed 5-FU is intracellularly anabolized to metabolites that possess anti-cancer properties. Approximately 80% of the formed 5-FU is catabolized by the enzyme dihydropyrimidine dehydrogenase (DPD) to the inactive metabolite dihydro-5-FU, which is further degraded and renally excreted [5, 6]. The liver highly expresses DPD and plays an important role in the clearance of 5-FU [7].

Most commonly reported capecitabine side effects are hand–foot syndrome, diarrhoea, nausea and vomiting [8, 9]. In particular, DPD-deficient patients are at risk for developing severe and sometimes lethal toxicity [10, 11]. Upfront screening for single-nucleotide polymorphisms in the gene encoding DPD, *DPYD*, could identify patients at risk of fluoropyrimidine-induced toxicity [10, 12–14]. The sensitivity of *DPYD* genotyping approaches, however, remains rather low.

Phenotyping approaches for DPD activity might further improve the identification of patients at risk of developing fluoropyrimidine-induced severe toxicity. Most DPD phenotyping methods are based on ex vivo quantification of DPD activity in peripheral blood mononuclear cells (PBMCs) [15, 16]. Although DPD activity in PBMCs is associated with fluoropyrimidine-induced severe toxicity and clearance [17–20], this approach remains laborious and is not suitable for examination of dynamic changes in systemic DPD activity. Alternatively, determination of the ratio between dihydrouracil (DHU) and the endogenous DPD substrate uracil (U) in plasma might be used for phenotyping DPD activity.

The pre-therapeutic DHU:U plasma ratio and U plasma level showed good correlation with clearance of 5-FU [21, 22] and fluoropyrimidine-induced toxicity [23–26]. Upfront determination of the DHU:U plasma ratio is an attractive approach and less laborious than examination of DPD activity in PBMCs. Moreover, since the DHU:U plasma ratio is quantified in human plasma, it is likely that this marker is useful for detecting dynamic changes in systemic DPD activity.

There is, however, limited data on factors, such as hepatic function, which potentially play an important role in the regulation of the DHU:U plasma ratio. Identification of such factors is essential for interpretation of the DHU:U plasma ratio with respect to DPD phenotype-guided dosing. Since DPD is highly expressed in liver tissue, changes in liver tissue possibly affect the DHU:U plasma ratio. The aim of the study was to determine the effect of liver resection by quantification of the DHU:U plasma ratio in patients with CRLM prior to and 1 day after liver resection. Furthermore, we explored whether changes in DHU:U plasma ratio after liver resection were reversible and whether they were associated with capecitabine-induced toxicity.

Methods

Patient population and sample collection

The primary study objective was to determine the DHU:U plasma ratio in patients with CRLM prior to and 1 day after partial liver resection. Patients were considered eligible in case heparinized plasma for quantification of the DHU:U ratio was obtained both at the day of liver resection, prior to the surgical operation and 1 day after the resection (group A). The included patients either participated in a multicentre randomized phase III clinical trial (www.clinicaltrials.gov, study identifier: NCT00394992), in which subjects were randomized after liver resection to receive capecitabine plus oxaliplatin (CAPOX) or CAPOX plus bevacizumab (CAPOX-B) [27], or underwent partial liver resection for CRLM in the University Medical Center Utrecht as standard of care. Both studies were approved by the Medical Ethical Committee of the University Medical Center Utrecht. Written informed consent was obtained from all patients. For the patients participating in the phase III trial [27], toxicities were evaluated after every cycle of chemotherapy and assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTC-AE) version 3.0.

In addition, we were interested in long-term changes in the DHU:U plasma ratio after partial liver resection. Therefore, we also explored the effects of liver resection on the DHU:U plasma ratio in samples that were collected 4–8 weeks after resection from patients, who participated in the phase III trial [27], but for whom no plasma was available prior to and 1 day after liver resection (group B). The plasma samples were stored at − 70 °C until analysis.

Quantification of uracil and dihydrouracil plasma levels

U and DHU were quantified in plasma using an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay as described previously [28]. In short, an internal standard solution containing 1,3-U-$^{15}N_2$ and 5,6-DHU-$^{13}C_4$,$^{15}N_2$ was added to 300 μL of plasma. Protein precipitation was performed using 900 μL of methanol and acetonitrile (1:1, *v*/*v*). Samples were vortex-mixed for 10 s, shaken for 10 min and centrifuged at 14.000*g* for 10 min. The supernatants were dried under a stream of nitrogen at 40 °C and reconstituted in 100 μL of 0.1% formic acid in water. Chromatographic separation was performed on an Acquity UPLC® HSS T3 (150 × 2.1 mm ID, particle size 1.8 μm; Waters, Milford, USA) column. Mobile phases consisted of 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B) at a flow of 0.3 mL/min. The following gradient was used: 0% B from 0 to 3.0 min, 0–90% B from 3.0–3.2 min, 90% B from 3.2–3.7 min and 0% B from 3.7–5 min. A Qtrap 5500 triple quadrupole mass spectrometer (AB Sciex, Framingham, USA) was operated in the negative mode for quantification of U and in the positive mode for quantification of DHU. Validated concentration ranges for U and DHU were 1–100 and 10–1000 ng/mL, respectively.

Data analysis

Differences between DHU:U plasma ratios, and the U and DHU plasma levels, prior to and 1 day after liver resection

were assessed using the two-tailed Wilcoxon matched pair test. The two-tailed Mann–Whitney test was used for comparing DHU:U plasma ratios, and U and DHU plasma levels, 1 day after liver resection of CRLM (group A) and 4–8 weeks after liver resection (group B). The statistical analyses were performed in R (version 3.3.0) [29]. *P* values < 0.05 were considered statistically significant. Observations below the quantification limit (< LLOQ) were considered to be zero for statistical analyses. Absolute concentrations were used to calculate the DHU:U plasma ratio.

Results

Patient characteristics

Plasma samples from 21 patients who underwent partial liver resection for CRLM were available for quantification of the DHU:U ratio. From 15 patients, plasma samples were collected prior to and 1 day after resection (group A). In addition, the exploratory analysis of the DHU:U plasma ratio 4–8 weeks after resection was performed in samples from six patients (group B). Patient characteristics of both study groups are summarized in Table 1.

Table 1 Demographic and disease characteristics

Characteristic	Group A	Group B
Number of subjects	15	6
Age (years)		
Median (range)	67 (54–82)	60 (40–73)
Gender		
Male	9	5
Female	6	1
Location of primary tumour		
Caecum	1	–
Colon	6	4
Rectosigmoid	–	1
Rectum	8	1
Clinical stage		
T3 N0	7	2
T3 N1	6	1
T3 N2	2	1
T4 N0	–	1
T4 N2	–	1
Number of CRLM		
Median (range)	1 (1–6)	1 (1–1)
Radical resection		
R0	14	6
R1	1	–

CRLM colorectal liver metastases

Reduction in DHU:U plasma 1 day after liver resection

The median (range) DHU:U plasma ratio prior to liver resection was 10.7 (2.6–14.4). The DHU:U plasma ratio was significantly reduced to 5.5 (< LLOQ-10.5) 1 day after resection ($p = 0.0026$; Fig. 1a). U plasma levels prior to and 1 day after resection were 11.0 (8.6–36.9) ng/mL and 11.1 (3.9–17.1) ng/mL ($p = 0.3232$; Fig. 1b), respectively. In all patients except for two, the DHU plasma level was decreased 1 day after liver resection. The median (range) DHU plasma level prior to resection was 112.0 (79.8–153) ng/mL and was 41.2 (< LLOQ-160) ng/mL 1 day after resection ($p = 0.0004$; Fig. 1c). In four patients, the DHU plasma levels were < LLOQ 1 day after liver resection.

The DHU:U plasma ratio 4–8 weeks after liver resection

The median (range) time interval of plasma collection in group B was 46.5 (29–55) days after liver resection. The median (range) DHU:U plasma ratio in this group was 9.1 (6.9–14.5), which was significantly higher compared to DHU:U plasma ratios 1 day after resection ($p = 0.0135$; Fig. 2a). As shown in Fig. 2b, U plasma levels were not statistically different in the samples that were collected 1 day after liver resection or 4–8 weeks after. Contrarily, the median (range) DHU plasma level was 106.5 (88.1–120.0) ng/mL 4–8 weeks after resection and was significantly higher than the DHU levels 1 day after resection ($p = 0.003$; Fig. 2c).

Tolerability of capecitabine

Treatment characteristics of four patients in group A were available for exploratory analysis of treatment toxicity (Supplementary Table 1). Capecitabine was administered on days 1–14 of every 21-day cycle with a dose of 1000 mg/m^2 bi-daily. Oxaliplatin 130 mg/m^2 and bevacizumab 7.5 mg/kg, for patients who underwent treatment with CAPOX–B, were administered on day 1 of each cycle. In case of the four patients, adjuvant chemotherapy was started 51–57 days after liver resection.

The DHU:U plasma ratio of patient 1, a 73-year-old male, was increased from 5.9 to 8.2 1 day after liver resection. He received three cycles of CAPOX–B and poorly tolerated the chemotherapy. The patient was admitted to the hospital for capecitabine-induced diarrhoea (grade 3). He also experienced a severe infection (grade 4) and multiple grade 1–2 toxicities. Furthermore, the patient required treatment delay after two cycles.

Patient 2, a 72-year-old male, and patient 3, a 66-year-old female, both had undetectable DHU plasma levels 1 day after liver resection. Both patients received eight cycles of adjuvant chemotherapy. Nonetheless, both patients required a

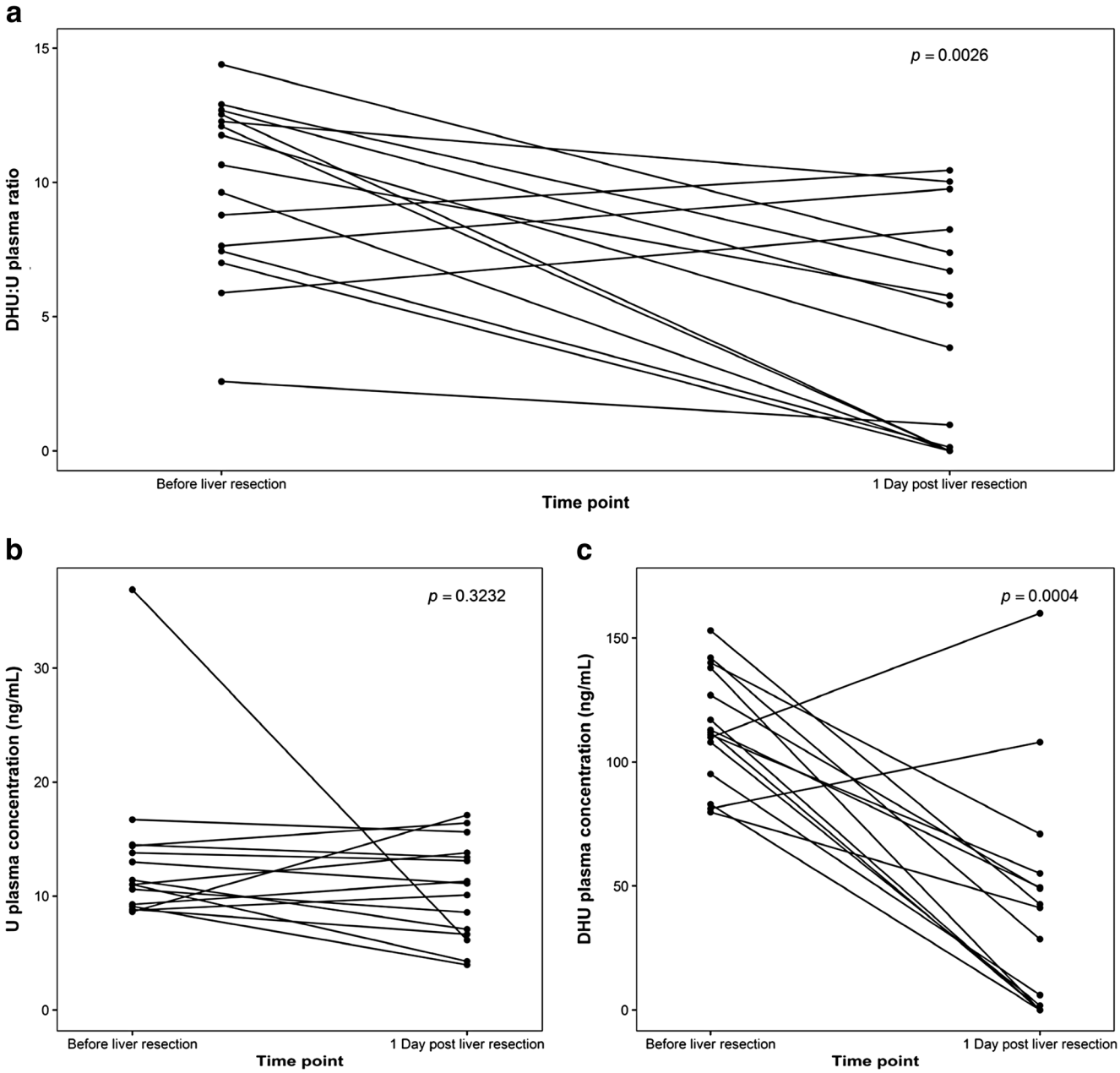

Fig. 1 The dihydrouracil:uracil plasma ratio (**a**), uracil (**b**) and dihydrouracil (**c**) plasma levels of 15 patients prior to and 1 day after liver resection for colorectal liver metastases. DHU dihydrouracil, U uracil

capecitabine dose reduction after cycle 4 and cycle 3, respectively, and a treatment delay. Patient 2 suffered from severe nausea (grade 3) and multiple grade 1–2 toxicities. Patient 3 experienced severe hyperglycemia (grade 3) and several grade 1–2 toxicities.

Patient 4, a 61-year-old female, was treated with two cycles of CAPOX. The DHU:U plasma ratio was relatively low 1 day after liver resection. She experienced severe vomiting (grade 3) and dehydration (grade 3), for which she was hospitalized. In addition, she suffered from multiple grade 1–2 toxicities. After two cycles, it was decided to switch from CAPOX to folinic acid, fluorouracil and oxaliplatin (FOLFOX). During the FOLFOX treatment, she also experienced grade 1–3 toxicities.

Discussion

The results of this study clearly show that the DHU:U plasma ratio is decreased 1 day after liver resection. The reduction in the DHU:U plasma ratio is the result of ~ 50% decrease in the DHU plasma level. Our results also show that the decrease in

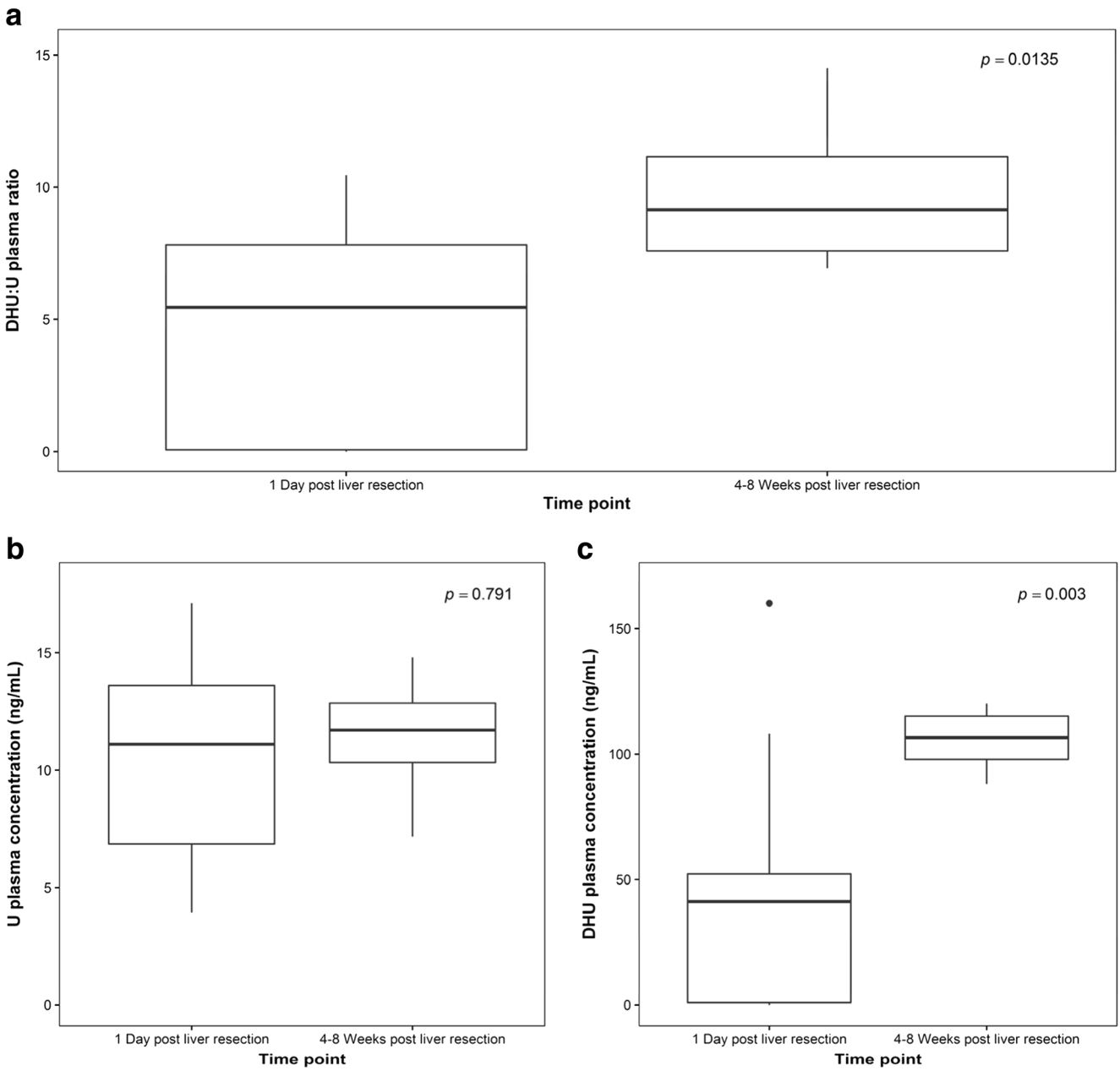

Fig. 2 The dihydrouracil:uracil plasma ratio (**a**), uracil (**b**) and dihydrouracil (**c**) plasma levels 1 day ($n = 15$ patients) and 4–8 weeks ($n = 6$ patients) after liver resection in patients with colorectal liver metastases. DHU dihydrouracil, U uracil

DHU:U plasma ratio is reversible, since the DHU:U plasma ratios 4–8 weeks after liver resection were in line with values prior to liver resection and comparable to DHU:U ratios in healthy volunteers [30]. This is, to our knowledge, the first study to report dynamic changes in the DHU:U plasma ratio after liver resection in humans.

The effect of unresected CRLM on a DPD phenotype marker was recently studied by Van Staveren et al. [31]. In their study, the DPD phenotype was assessed by uracil pharmacokinetics after administration of an oral dose of uracil. They found unaltered uracil pharmacokinetics in patients with CRLM [31]. In our study, patients with CRLM showed DHU:U plasma ratios prior to liver resection that were comparable to DHU:U plasma ratios of healthy volunteers (reference mean (± s.d.) DHU:U plasma ratio: 10.6 ± 2.4) [30]. Based on these findings, it seems that the DPD phenotype markers are unaltered in patients with unresected CRLM.

The recovery of the DHU:U plasma ratio after 4–8 weeks could be related to liver regeneration. Liver regeneration is a complex physiological process that immediately starts after liver resection and discontinues when the liver reaches its original volume. A study with human patients demonstrated

that liver function recovers within 30 days after partial liver resection [32]. Preclinical experiments in rats showed that it only takes 5–7 days until the liver reaches its original volume [33]. More specifically, a study of liver resection in rats demonstrated that DPD activity recovers 4 days after liver resection [34]. One day after liver resection, however, DPD activity in rat liver was reduced by 45% [34]. In vitro experiments further illustrated that uracil metabolism in regenerating rat liver was only 25% compared to normal rat liver [35]. So it seems, during the first phase after liver resection, hepatic DPD activity can be reduced, but rapidly recovers thereafter. Results of our study demonstrate that DPD activity is reduced directly after liver resection and that DPD activity recovers within 4–8 weeks after resection, which is in line with the data on human liver regeneration and DPD activity in rat liver. The exact mechanism behind the recovery in DPD activity remains unclear and warrants further research.

Based on our cases series of four patients, it seems unlikely that the DHU:U plasma ratio 1 day after liver resection gives an appropriate representation of the DPD phenotype during adjuvant chemotherapy. The DHU:U plasma ratio in patient 1 was increased 1 day after liver resection, while it was highly reduced in patients 2, 3 and 4. Adjuvant chemotherapy was started 51–57 days after liver resection. All four patients poorly tolerated CAPOX–B and mainly suffered from severe diarrhoea, nausea and vomiting. These toxicities can be related to capecitabine, but could also be caused or provoked by oxaliplatin and bevacizumab. Larger studies are needed to examine the relationship between DHU:U plasma ratio after liver resection and capecitabine-induced toxicity. Since capecitabine-induced toxicity is associated with genetic mutations in *DPYD* [10, 11], the predictive value of the DHU:U plasma ratio should also be tested in combination with the *DPYD* genomic status.

The primary aim of our study was to quantify changes in DHU:U plasma ratio after liver resection. Although the number of patients was relatively small, the results of the study clearly demonstrated a reduction in DHU:U plasma ratio after liver resection. A limitation of the study is that we cannot rule out that the reduction in DHU:U plasma ratio is the effect of reduced systemic DPD activity. Dynamic changes in other enzymes, such as dihydropyrimidinase, which is important for the degradation of DHU and which is also expressed in liver tissue, might also affect DHU levels. However, the question remains whether this would be of clinical relevance since dihydropyrimidinase deficiency has thus far not been associated with capecitabine-induced toxicity. Furthermore, other studies are warranted to determine whether changes in DHU:U plasma ratio are caused specifically by liver resection or by non-specific surgical effect(s), such as the use of anaesthesia. In addition, the association between changes in DHU:U plasma ratio and possible covariates, such as gender, age and the extent of liver resection requires additional research.

Besides liver resection, we previously also found that circadian rhythmicity is a factor that influences the DHU:U plasma ratio [30]. More research is needed to study the role of other factors, such as exposure to light, intake of food and physical activity that contribute to the DHU:U plasma ratio. This research is warranted for the validation of the DHU:U plasma ratio in order to allow clinical implementation of this DPD phenotype marker.

In conclusion, liver resection leads to very low DHU:U plasma ratios 1 day after liver resection. Quantification of the DHU:U plasma ratios directly after liver resection might lead to false-positive identification of DPD deficiency. Therefore, DPD phenotype-guided fluoropyrimidine dosing should not be based on DHU:U plasma ratios in samples that are collected directly after liver resection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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