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Renal impairment and abnormal liver function tests in pre-therapeutic phenotype-based DPD deficiency screening using uracilemia: a comprehensive population-based study in 1138 patients

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Abstract

Background: Dihydropyrimidine dehydrogenase (DPD) deficiency screening is a pre-therapeutic standard to prevent severe fluoropyrimidine-related toxicity. Although several screening methods exist, the accuracy of their results remains debatable. In France, the uracilemia measurement is considered the standard in DPD deficiency screening. The objective of this study was to describe the hyperuracilemia (⩾16 ng/mL) rate and investigate the influence of hepatic and renal impairment in uracilemia measurements since the guidelines were implemented.

Patients and methods: Using a cohort of 1138 patients screened between 18 October 2018 and 18 October 2021, basic demographic characteristics, date of blood sampling, and potential biological confounders including liver function tests [aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), gamma-glutamyl transferase (γGT), alkaline phosphatase (ALP), and bilirubin] and estimated glomerular filtration rate (eGFR) were collected. The second same-patient uracilemia analysis was also performed. Temporal change was graphically represented while potential confounders were stratified to show linearity when suspected.

Results: Hyperuracilemia was diagnosed in 12.7% ($n = 150$) samples with 6.7%, 5.4%, 0.5%, and 0.08% between 16 and 20 ng/mL, 20 and 50 ng/mL, 50 and 150 ng/mL, and >150 ng/mL, respectively. The median uracilemia concentration was 9.4 ng/mL (range: 1.2 and 172.3 ng/mL) and the monthly hyperuracilemia rate decreased steadily from >30% to around 9%. Older age, normalized AST, γGT, ALP results, bilirubin levels, and decreased eGFR were linearly associated with higher plasma uracil concentrations (all $p < 0.001$). In the adjusted multivariate linear model, AST, eGFR, and ALP remained associated with uracilemia ($p < 0.05$). When measured twice in 39 patients, the median uracilemia rate of change was −2.5%, which subsequently changed the diagnosis in nine patients (23.1%).

Conclusions: Better respect of pre-analytical conditions may explain the steady decrease in monthly hyperuracilemia rates over the 3 years. Elevated AST, ALP levels, and reduced eGFR could induce a false increase in uracilemia and second uracilemia measurements modified the first DPD deficiency diagnosis in almost 25% of the patients.

Keywords: dihydropyrimidine dehydrogenase deficiency, false-positive reactions, fluorouracil, kidney failure, liver function tests, uracil

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Highlights

- Hyperuracilemia was diagnosed in 12.7% of 1177 consecutive samples over a 3-year period.
- Better respect for pre-analytical conditions may explain the decrease followed by stability of monthly hyperuracilemia rates.
- Renal impairment and elevated aspartate aminotransaminase and alkaline phosphatase were associated with higher plasma uracil concentration.
- When uracilemia was measured twice in 39 patients, the median rate of change was −2.5%, and changed the diagnosis of dihydropyrimidine dehydrogenase deficiency in 23.1% of patients.

Introduction

Fluoropyrimidines, including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, are the backbone for digestive, breast as well as head and neck cancer treatments in both early and advanced stages.[1–3] The most common adverse events (AEs) comprise of hematologic and digestive issues such as diarrhea, nausea, mucositis, and/or cutaneous events (hand–foot syndrome).[2,4] According to the NCI Common Terminology Criteria for Adverse Events, severe AEs (Grade 3 to 4) have been reported in 20–30% of patients receiving fluoropyrimidines and lethal toxicity has been shown to occur in less than 1% of these cases.[3–7]

Fluoropyrimidines are antimetabolite-pyrimidine analogues with a chemical structure similar to endogenous pyrimidine molecules such as uracil and thymidine. As well, these are catabolized by the same pathway.[8] Fluoropyrimidine-related toxicity is frequently attributed to the deficiency of the dihydropyrimidine dehydrogenase (DPD) enzyme, which is responsible for almost all of its catabolism.[9,10] DPD is encoded by the *DPYD* gene on chromosome 22 and is expressed mostly in the liver.[2] After administration, 5-FU is rapidly metabolized by DPD into dihydro-5-FU, which is then converted into multiples derivatives and subsequently excreted in urine (almost 80% of the received dose).[1,11]

Demographically, partial DPD deficiency is present in 3–5% of Caucasians, while complete DPD deficiency is rarer (estimated prevalence of 0.01–0.1%).[12] Several studies have shown that patients with a partial or complete DPD deficiency are at risk of severe AEs because of fluoropyrimidine accumulation.[5,11,13,14] Different methods have also been assessed to identify a DPD deficiency based on phenotyping (direct or indirect measurement of enzyme activity) or genotyping (detection of inactivating polymorphism on the *DPYD* gene).[5,8,9,12,15–18] Despite the lack of prospective validation, thresholds have been defined to identify DPD-deficient patients, based on previous studies.[15,19] Thus, an uracil concentration (uracilemia) value over 150 ng/mL indicates a complete DPD deficiency that contraindicates the use of fluoropyrimidines.[15] When ranging between 16 and 150 ng/mL, the deficit is considered partial and the initial dose of fluoropyrimidines can be reduced or re-adjusted for the second course of treatment based on patient tolerance.[20]

In France, guidelines have recommended a systematic DPD deficiency screening prior to fluoropyrimidine-based treatment by phenotyping using plasma uracil quantification since September 2018.[20,21] In April 2020, the European Medicines Agency recommended genotyping and phenotyping based on plasma uracil levels to identify patients with DPD deficiency.[22] Similarly, phenotyping based on plasma uracil levels is now also recommended in Belgium.[23]

While systematic DPD screening is designed to avoid severe toxicity in the small proportion of partial, and in particular, total DPD-deficient patients, an overestimated assessment of DPD deficient status could lead to fluoropyrimidine underexposure and thus to suboptimal anticancer treatment in a large proportion of non-DPD-deficient patients. Plasma uracil quantification is based on high-performance liquid chromatography coupled with UV or mass spectrometry detection.[24,25] Because uracil is highly unstable, its measurement requires specific equipment and pre-treatment as well as rigorous pre-analytical and transport conditions.[8,20,26] Moreover, case reports and small series studies have shown that false-positive DPD-deficient diagnoses in both dialysis and/or tumor lysis patients.[27,28] Recently, a large prospective study in the Netherlands identified potential drawbacks in the clinical use of pretreatment uracil levels to test for DPD deficiency.[29]

DPD is expressed in many tissues, especially the liver. Therefore, hepatic impairment could increase uracilemia even though this phenomenon has not been described. As a result, a better

understanding of cofactors explaining high levels of uracil concentration could be necessary.[30] The objective of this study was to describe the number of DPD-deficient patients since the implementation of the French *Haute Autorité de Santé* (HAS) guidelines and identify cofactors that influence uracilemia measurement, especially hepatic or renal impairment which could improve treatment choices and dose adaptation.

Methods

Study design and patients

A regional cross-sectional comprehensive population-based observational study was conducted in two tertiary oncology centers in France (Centre Hospitalier Universitaire de Reims and the Institut Godinot Reims UNICANCER in the Champagne region) and two secondary centers (Centre Hospitalier Auban-Moët d'Epernay, and Centre Hospitalier de Chalons-en-Champagne). We retrospectively reviewed the databases of these four centers. All patients over 18 years who had pre-treatment uracil concentration measurements between 18 October 2018 and 18 October 2021 were included whether or not they received fluoropyrimidine-based anticancer treatment. This study was reported in accordance with the STROBE statement.

Pre-analytical procedures and uracil quantification analysis methods

The time allotted to sampling, processing, storage, and transportation of blood were standardized. Without separating gel, and with the anticoagulant (ethylenediaminetetraacetic acid), the blood samples were stored on ice and centrifuged within 4 h. The plasma was subsequently stored at −80°C. The time of sampling and the time of the last meal before a blood draw were not, however, standardized. Uracil measurements were performed at the Besancon University Hospital from the beginning of the study to 7 September 2020, and afterwards at Reims University Hospital (from 7 September 2020 to the end of the study). A private laboratory (Bioxa) carried out analyses from 8 November 2019 to the end of the study for patients sampled at the Godinot Cancer Institute. From 31 August 2019, blood samples were also collected at home or in private laboratories where uracilemia measurement was centralized. In accordance with the latest French guidelines, the DPD phenotype was determined using a sensitive ultra-performance liquid chromatography-tandem mass spectrometry system. Plasmatic uracil was quantified after appropriate solid-phase extraction, chromatographic separation, and appropriate mass spectrometric detection using stable isotopes. Also, as recommended by French guidelines, a cutoff of 16 ng/mL was used to define hyperuracilemia. Partial DPD deficiency was defined as uracil concentration values between 16 and 150 ng/mL. Readings above 150 ng/mL were considered as a total deficit.

Data collection

An anonymized list of all patients who had undergone a uracil concentration measurement in these four centers was used to collect the data. Blood sampling took place in one of the four participating centers. Electronic medical records of included patients were retrospectively reviewed to collect relevant data such as basic patient characteristics [age, gender, weight, height, and body mass index (BMI)], biological data related to uracil concentration measurements [value, date and time of sampling, and where the analysis was carried out (public or private laboratory)]. In addition, liver function tests [alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST)], gamma glutamyl transferase (γGT), bilirubinemia, alkaline phosphatases (ALP), and renal creatinine clearance [estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease EPIdemiology (CKD EPI) formulae] confounders were potentially analyzed.[31] The same data were collected when a second uracil concentration measurement was performed in the same patient. Biological confounders were measured during a period up to 28 days before or 28 days after the uracil concentration measurement was taken.

To distinguish the different levels of organ dysfunction, we normalized cytolysis and cholestasis by their upper normal limit (UNL) established by the laboratory analyzing the samples before stratifying them into three groups (<2UNL, 2–5 UNL, and >5UNL). We stratified the eGFR values into classes of 30 points (>90, 90–60, 60–30, and <30 mL/mn). Renal impairment was defined as having an eGFR value below 90 mL/min.

Outcomes

The primary outcome was to assess the frequency of hyperuracilemia in a population of to-be-treated patients over 3 years using a monthly

hyperuracilemia rate. The secondary outcome was to describe the influence of cytolysis, cholestasis, and renal impairment on uracil concentration measurements using their established, normalized laboratory levels for UNL and eGFR.

Statistical analysis

Quantitative data were described using means with standard deviation or medians with interquartile range(s) (IQR), whereas qualitative data were expressed as rates. Data were compared using Student's tests, Wilcoxon tests, chi-squared tests, or Fisher's exact tests depending on the conditions of application. The Mann–Whitney test was performed for variables without a normal distribution. Monthly hyperuracilemia diagnosis rate trends were modeled (method LOESS) and visually analyzed. No additional statistical tests were performed. Continuous variables were stratified to explore linearity. When confirmed, an adjusted multivariate linear model analysis was performed after verifying the independence, homoscedasticity, and normality of the distribution of the analyzed cofactors. Residuals were plotted and visually analyzed. Aberrant data could be dropped out of the model. The threshold for significance was $p < 0.05$. All statistical analyses were performed using R version 3.6.3.

Results

Population characteristics and DPD phenotyping by measuring uracilemia

A total of 1177 samples from 1138 patients were included in the study. Median patient age was 68 years (IQR: 59.3–74.4 years), and the gender ratio was balanced. Patients with hyperuracilemia were significantly older [70.9 years (IQR: 63.8–76.6 years) *versus* 67.2 years (IQR: 58.8–74.0 years), $p < 0.001$], heavier (70.0 kg, IQR: 58.0–83.2 kg *versus* 69.0 kg, IQR: 58.0–80.0 kg, $p = 0.017$), and had higher BMI (24.6 kg/m², IQR: 21.8–28.9 kg/m² *versus* 24.1 kg/m², IQR: 20.7–27.5 kg/m², $p = 0.029$) (Table 1).

The clinical relevance of these differences however, is questionable. Hyperuracilemia was observed in 150 samples (12.7%). When over 16 ng/mL, 79 samples (52.7%) had an uracilemia reading under 20 ng/mL, 64 (42.7%) had between 20 and 50 ng/mL, 6 (4%) had between 50 and 150 ng/mL, and only one (0.7%) had over 150 ng/mL. More than half of the uracilemia tests were carried out by public biology laboratories (63.1%, and higher hyperuracilemia rates were detected among them; $p < 0.001$). The median uracil concentration was 9.4 ng/mL (IQR: 7.4–12.6 ng/mL, range: 1.2–172.3 ng/mL) with a significant difference between samples depending on where they were taken ($p < 0.001$) (Figure 1).

Monthly hyperuracilemia rate over a 3-year period

Hyperuracilemia rates increased from October 2018 to February 2019 and subsumed more than 30% of the samples (Figure 2). Thereafter, hyperuracilemia rates decreased continuously from March 2019 to reach its nadir in September 2021 (<5%). The monthly hyperuracilemia rate then stabilized at around 9%. The number of hyperuracilemia cases incidentally peaked in February 2020 at almost 35%.

The association of hyperuracilemia with liver function tests

Hyperuracilemia was more frequent in patients with elevated AST levels with a median of 38.0 UI/l, 1.0 UNL (IQR: 20.5–100.0 UI/l, and 0.6–2.2 UNL, respectively) compared to patients without hyperuracilemia ($p < 0.001$) having a median of 24.0 UI/l, 0.6 UNL (IQR: 18.0–38.0 UI/l, and 0.4–0.9 UNL, respectively). Among patients with hyperuracilemia, a significant association between renal impairment and/or hepatic cytolysis (AST increased) and/or hepatic cholestasis (ALP increased) was observed (all $p < 0.001$) (Table 1).

ALT levels were significantly different, but they remained inferior to clinically relevant differences (0.7 UNL, IQR: 0.4–1.5 UNL *versus* 0.5 UNL, IQR: 0.3–0.9 UNL, $p = 0.002$) (Table 1). Hepatic cholestasis (γGT, ALP, and bilirubin elevation) was significantly higher in patients with hyperuracilemia (all three $p < 0.001$) (Table 1). Uracilemia concentrations depending on AST levels, ALT levels, and bilirubin levels are shown in Figures 3(a), (c), and (e), respectively, and visual linear trends are illustrated in Figures 3(b), (d), and (f), respectively.

The association of hyperuracilemia with renal impairment

Renal impairment was observed in 562 patients (49.1%). Among the 150 patients with hyperuracilemia, 106 had a renal impairment (74.1%)

Table 1. Patient characteristics of the included samples.

Characteristics	Total *N* (%)	Missing *N*	Levels	Uracilemia <16 ng/mL	Uracilemia ⩾16 ng/mL	Total	*p* Value
Age (years)	1177 (100.0)	0	Median (IQR)	67.2 (58.8–74.0)	70.9 (63.8–76.6)	68.0 (59.3–74.4)	<0.001
Uracilemia (ng/mL) *N* (%)	1177 (100.0)	0	Median (IQR)	8.9 (7.1–11.2)	19.8 (17.7–23.6)	9.4 (7.4–12.6)	<0.001
			<16	1027 (100)	–	1027 (87.3)	
			Between 16 and 20	–	79 (52.7)	79 (6.7)	
			Between 20 and 50	–	64 (42.7)	64 (5.4)	
			Between 50 and 150	–	6 (4.0)	6 (0.5)	
			>150	–	1 (0.7)	1 (0.1)	
Analyzing laboratory	1169 (99.3)	8	Private	408 (39.7)	18 (12.0)	426 (36.2)	<0.001
			Public	612 (59.6)	131 (87.3)	743 (63.1)	
			(Missing)	7 (0.7)	1 (0.7)	8 (0.7)	
Weight (kg)	1171 (99.5)	6	Median (IQR)	69.0 (58.0–80.0)	70.0 (58.0–83.2)	69.4 (58.0–80.0)	0.017
BMI (mg/m²)	1166 (99.1)	11	Median (IQR)	24.1 (20.7–27.5)	24.6 (21.8–28.9)	24.2 (20.8–27.7)	0.029
eGFR (mL/mn)	1145 (97.3)	32	Median (IQR)	90.0 (80.0–96.0)	78.0 (51.5–90.0)	90.0 (77.0–96.0)	<0.001
Renal impairment (mL/min) *N* (%)	1145 (97.3)	32	⩽30	5 (0.5)	11 (7.3)	16 (1.4)	<0.001
			⩾30–60	74 (7.2)	34 (22.7)	108 (9.2)	
			⩾60–90	377 (36.7)	61 (40.7)	438 (37.2)	
			⩾90	546 (53.2)	37 (24.7)	583 (49.5)	
			(Missing)	25 (2.4)	7 (4.7)	32 (2.7)	
ASAT (IU/L)	1126 (95.7)	51	Median (IQR)	24.0 (18.0–38.0)	38.0 (20.5–100.0)	25.0 (18.0–41.0)	<0.001
Normalized AST (UNL)	1125 (95.6)	52	Median (IQR)	0.6 (0.4–0.9)	1.0 (0.6–2.2)	0.6 (0.4–1.0)	<0.001
Stratified AST *N* (%)	1125 (95.6)	52	>5UNL	15 (1.5)	12 (8.6)	27 (2.4)	<0.001
			Between 2 and 5UNL	70 (7.1)	29 (20.9)	99 (8.8)	
			<2UNL	901 (91.4)	98 (70.5)	999 (88.8)	
ALAT (IU/L)	1125 (95.6)	52	Median (IQR)	23.0 (15.2–40.0)	28.0 (17.0–63.5)	24.0 (16.0–42.0)	0.006
Normalized ALT (UNL)	1123 (95.4)	54	Median (IQR)	0.5 (0.3–0.9)	0.7 (0.4–1.5)	0.5 (0.4–1.0)	0.002
Stratified ALT *N* (%)	1123 (95.4)	54	>5UNL	23 (2.3)	4 (2.9)	27 (2.4)	0.001
			Between 2 and 5UNL	72 (7.3)	23 (16.7)	95 (8.5)	
			<2UNL	890 (90.4)	111 (80.4)	1001 (89.1)	

(Continued)

Table 1. (Continued)

Characteristics	Total *N* (%)	Missing *N*	Levels	Uracilemia <16 ng/mL	Uracilemia ⩾16 ng/mL	Total	*p* Value
GGT (IU/L)	1020 (86.7)	157	Median (IQR)	54.0 (28.0–171.5)	108.0 (36.0–419.0)	57.0 (28.0–202.0)	<0.001
Normalized GGT (UNL)	1018 (86.5)	159	Median (IQR)	1.0 (0.5–3.3)	2.3 (0.7–7.9)	1.1 (0.5–3.9)	<0.001
ALP (IU/L)	1106 (94.0)	71	Median (IQR)	99.0 (74.0–159.0)	120.0 (78.0–436.0)	100.0 (74.0–175.0)	<0.001
Normalized ALP (UNL)	1104 (93.8)	73	Median (IQR)	0.8 (0.6–1.3)	1.1 (0.7–3.4)	0.8 (0.6–1.4)	<0.001
Bilirubin (micromol/L)	1115 (94.7)	62	Median (IQR)	8.0 (6.0–12.0)	10.0 (6.0–19.0)	8.0 (6.0–13.0)	0.018
Stratified bilirubin (micromol/L) *N* (%)	1115 (94.7)	62	>100 µmol/L	34 (3.3)	13 (8.7)	47 (4.0)	0.002
			20–100	92 (9.0)	19 (12.7)	111 (9.4)	
			Less than 20	848 (82.6)	109 (72.7)	957 (81.3)	
			(Missing)	53 (5.2)	9 (6.0)	62 (5.3)	
Biochemical disturbances							
AST elevation	1125 (95.6)	52	>2N	88 (8.9)	41 (29.5)	129 (11.5)	<0.001
ALT elevation	1123 (95.4)	54	>2N	97 (9.8)	27 (19.6)	124 (11.0)	0.001
ALP elevation	1104 (93.8)	73	>2N	168 (17.4)	52 (38.0)	220 (19.9)	<0.001
GGT elevation	1018 (86.5)	159	>2N	311 (35.1)	69 (52.3)	380 (37.3)	<0.001
Renal impairment	1145 (97.3)	32	<90 ml/min	456 (45.5)	106 (74.1)	562 (49.1)	<0.001
Association of biochemical data (renal impairment and/or AST cytolysis and/or ALP cholestasis)	1090 (92.6)	87	None	405 (39.4)	17 (11.3)	422 (35.9)	<0.001
			One	435 (42.4)	69 (46.0)	504 (42.8)	
			Two	93 (9.1)	25 (16.7)	118 (10.0)	
			Three	23 (2.2)	23 (15.3)	46 (3.9)	
			(Missing)	71 (6.9)	16 (10.7)	87 (7.4)	

ALP, alkaline phosphatase; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; BMI, body mass index; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; IQR, interquartile range; IU/L, international unit per liters; mL/mn, milliliters per minute; ng/mL, nanograms per milliliter; UNL, number of times the upper normal limit.

($p < 0.001$). Median eGFR was lower in patients with hyperuracilemia (78.0 mL/min, IQR: 51.5–90.0 mL/min) ($p < 0.001$). Uracilemia concentrations depending on eGFR are shown in Figure 3(g) and (h).

Results from the multivariate analysis

Age, AST, eGFR, γGT, ALP, and bilirubin were potential confounders and were considered in the adjusted multivariate linear model (Table 2). The maximum uracil value (172.3 ng/mL) was dropped

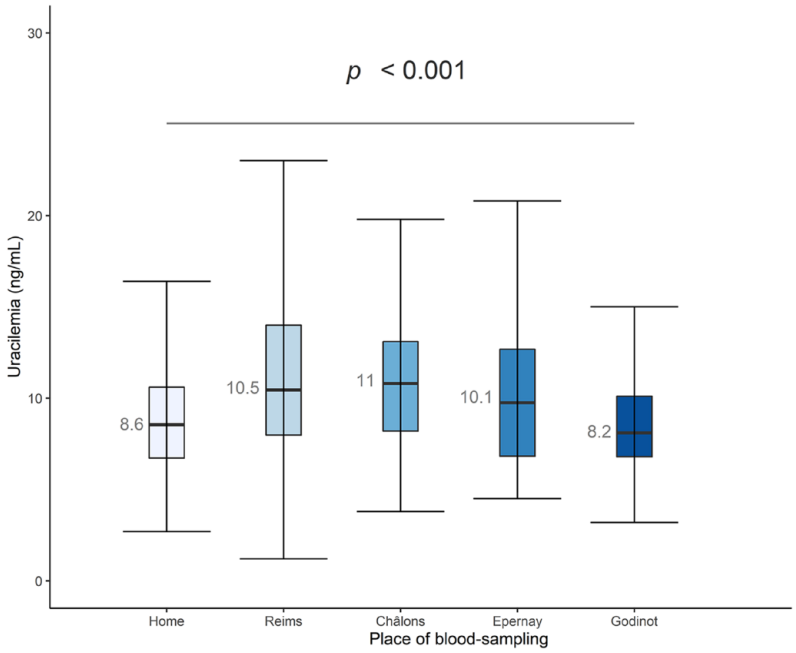

Figure 1. Differences in median uracilemia (ng/mL) and interquartile ranges according to the location of the blood drawing center in France using 1177 samples from 1138 patients.
The gray number on the left side of each box is the median at each referenced center.
ng/mL, nanograms per milliliter.

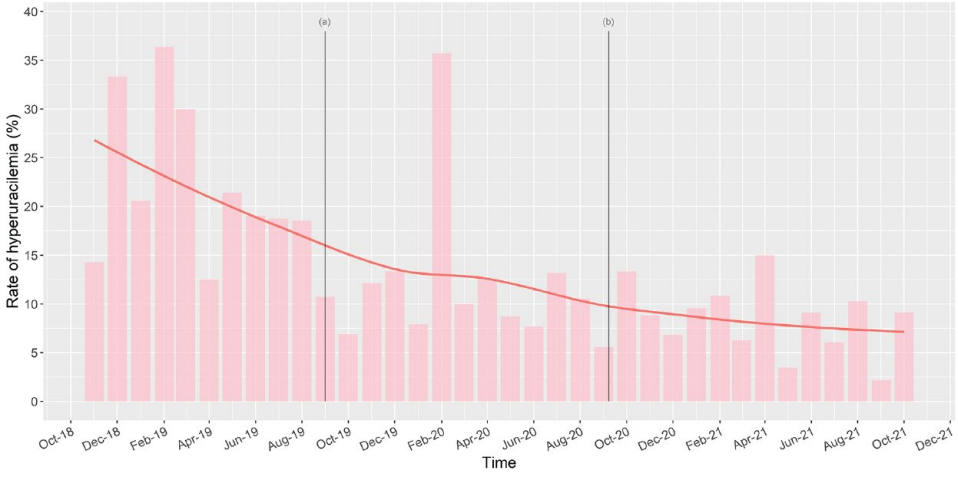

Figure 2. Monthly hyperuracilemia rate (%) between September 2018 and September 2021.
The red line represents the temporal evolution of the monthly hyperuracilemia rate (Method LOESS), (a) the first grey vertical line represents the date (31 August 2019) when uracil measurement was available and reimbursed to private biology laboratories and (b) The second gray vertical line represents the date (7 September 2020) after the uracil measurement was available to the Department of Pharmacology at Reims University Hospital.
ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; eGFR, estimated glomerular filtration rate,[30]; mL/mn, milliliters per minute; ng/mL, nanograms per milliliter; UNL, number of times upper normal limit.

out of the plotted linear model due to its aberrant value. AST (UNL), eGFR (mL/mn), and ALP (UNL) remained significantly associated with uracilemia. In contrast, age, γGT (UNL), and bilirubin did not show any significance ($p=0.16$, $p=0.39$, and $p=0.44$, respectively). For one

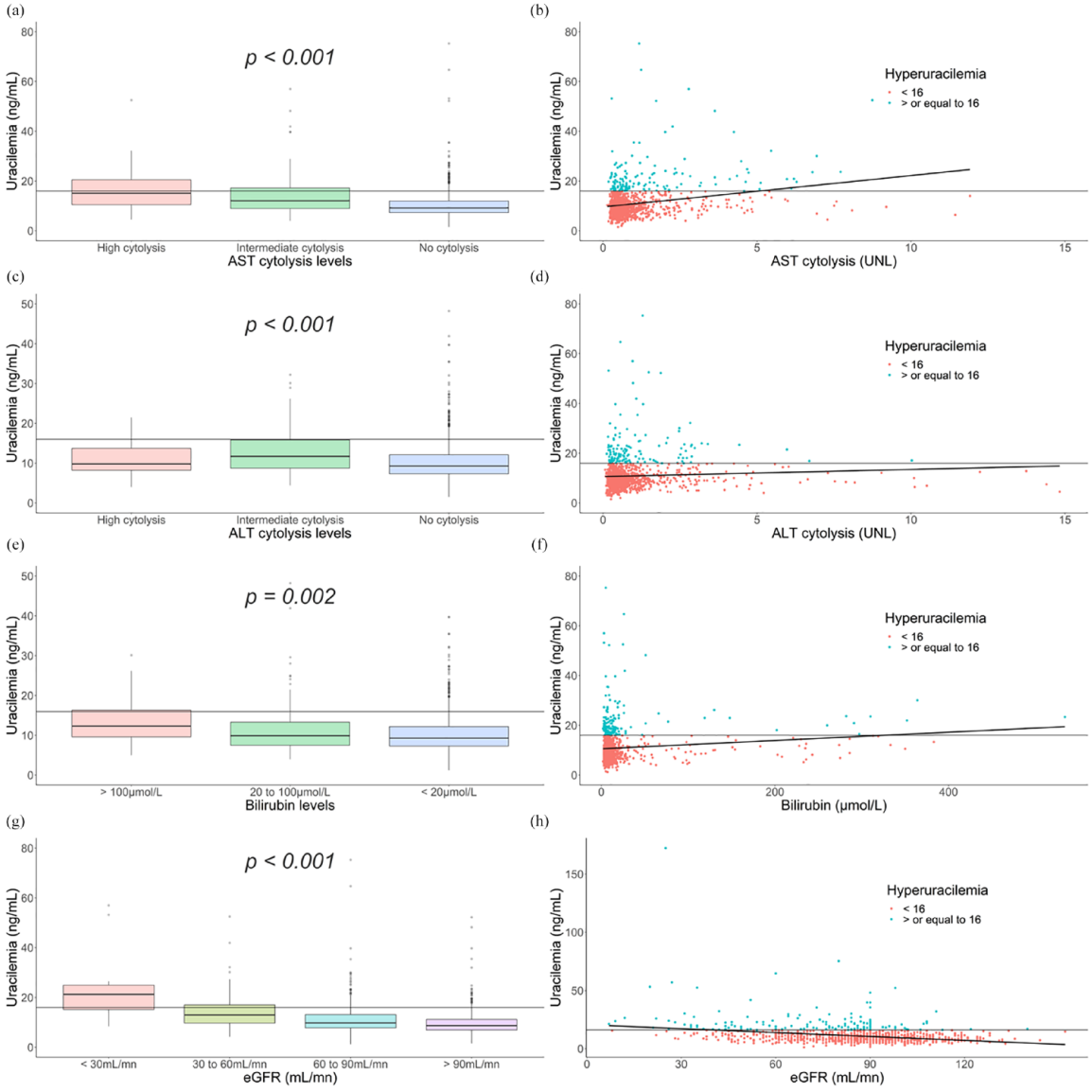

Figure 3. Uracilemia depending on stratified and normalized potentials influencing hepatic and renal biological factors: (a) Uracilemia depending on stratified AST cytolysis (box plots). (b) Uracilemia depending on normalized AST cytolysis (points). (c) Uracilemia depending on stratified ALT cytolysis (box plots). (d) Uracilemia depending on normalized ALT cytolysis (points). (e) Uracilemia depending on stratified bilirubin (box plots). (f) Uracilemia depending on normalized bilirubin (points). (g) Uracilemia depending on stratified eGFR (box plots). (h) Uracilemia depending on eGFR (points). High cytolysis was defined as >5 times the UNL; intermediate cytolysis as between 2 and 5 times the UNL and low cytolysis as <2 times the UNL. The black lines are linear models representing the correlation between *x* and *y*. The blue points represent uracilemia above 16 ng/mL, whereas the red points represent uracilemia below 16 ng/mL.
ng/mL; nanograms per milliliter; UNL, upper normal limit.

additional time, the AST (UNL) and the ALP (UNL), uracilemia might increase by 0.435 ng/mL (95% CI: 0.05–0.819) and 0.795 ng/mL (95% CI: 0.479–1.11), respectively. The influence of eGFR was also significant with an increase in uracilemia by 0.125 ng/mL (95% CI: 0.151–0.099) for each additional eGFR point.

Singular clinical situations

One patient (73-year-old woman) was classified as DPD deficient with a uracil value of 75.3 ng/ml sampled during a 5-FU infusion; however, renal and hepatic functions were normal. The only patient with uracilemia >150 ng/mL (172.3 ng/mL) was a 73-year-old woman who had acute

Table 2. Univariate and adjusted multivariate analyses using a linear model*.

Characteristics	Univariate			Multivariate*		
	Expected change in uracilemia (ng/mL) for one unit	Confidence interval (CI)	*p* Value	Expected change in uracilemia (ng/mL) for one unit	CI	*p* Value
Age (years)	0.056	0.021 to 0.091	<0.001	−0.03	−0.073 to 0.012	0.1617
AST (UNL)	0.973	0.717 to 1.23	<0.001	0.435	0.05 to 0.819	0.0268
ALT (UNL)	0.185	−0.024 to 0.393	0.0827	–	–	–
γGT (UNL)	0.171	0.117 to 0.225	<0.001	−0.039	−0.131 to 0.052	0.3948
ALP (UNL)	0.894	0.71 to 1.078	<0.001	0.795	0.479 to 1.11	<0.001
Bilirubin (micromol)	0.017	0.009 to 0.024	<0.001	−0.004	−0.013 to 0.006	0.4435
eGFR(mL/mn)	−0.091	−0.11 to −0.074	<0.001	−0.125	−0.151 to −0.099	<0.001

ALP, alkaline phosphatase; AST, aspartate aminotransaminase; eGFR, estimated glomerular filtration rate; mL/mn, milliliters per minute; ng/mL, nanograms per milliliter; UNL, number of times the upper normal limit; γGT, gamma-glutamyl transferase.
*One value (maximum uracil: 172.3 ng/mL) was dropped from the model.

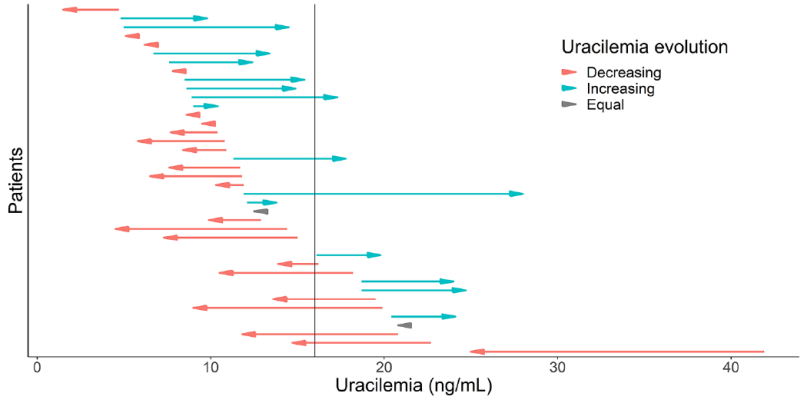

Figure 4. The change in uracilemia between the first and second blood samples for each patient who was sampled twice ($n = 39$). The second measurement was discordant with the first in nine patients (23.1%). The vertical black line represents the 16 ng/mL threshold which defines hyperuracilemia. The blue arrows represent the increasing uracilemia between the first and the second blood samples, whereas the red arrows represent the decreasing uracilemia between the first and the second blood samples. The gray arrows represent the equal uracilemia between the first and the second blood samples.

multiple organ failure and readings of eGFR (25 mL/min), AST (137 UNL), ALT (21 UNL), γGT (6 UNL), ALP (10 UNL), and bilirubin level (165 micromol/L). She did not receive any cytotoxic chemotherapy.

Second uracil concentration measurement

In all, 39 patients were subjected to a second uracilemia measurement. The median delay between the two blood samples was 26.5 days (IQR: 13–105.5). Hyperuracilemia was observed in the first sampling in nine patients (23.1%). Results of the second sampling are shown in Figure 4. Similar trends were observed between uracilemia and eGFR, AST, ALT, γGT, ALP, and bilirubin levels (see Supplemental Table 1).

A median difference of −0.2 ng/mL (IQR: −4.6 to 4.9 ng/mL) was observed between the two measurements taken from the same patient and showed a median difference rate of −2.5%. A

first diagnosis (normal or hyperuracilemia) was confirmed in 76.9% by a second blood sample. However, secondary measurements were discordant with the primary ones in the nine patients (23.1%) (see Supplemental Table 1).

Discussion

This study was the first to describe the inconsistency of hyperuracilemia rate, a progressive decrease, since the release of the HAS guidelines on the pre-treatment of DPD deficiency screening using phenotyping. We found a significant and gradual linear association between AST cytolysis, ALP elevation, renal (eGFR decrease) impairment, and hyperuracilemia. Second uracilemia measurements also did not confirm the first DPD deficiency diagnosis in 23.1% patients.

The observed decrease in hyperuracilemia rate over 3 years (from 30% to 9%) is inconsistent with the non-dynamic phenomenon of DPD deficiency, which normally should remain stable over time. In one study, among a monocentric cohort of 5886 phenotype patients over the same study period, 249 patients (6.8%) were identified with partial DPD deficiency and two patients (0.05%) with complete DPD deficiency.[18]

Pre-analytical conditions are an essential prerequisite to a reproducible and reliable uracil measurement.[25,26] Our results could be partially explained by the improvement of the pre-analytical conditions, namely a more efficient adherence to the duration of pre-analytical sample handling and temperature requirements. As de With *et al.*[29] also indicated, we observed between-center differences in uracil levels. However, these differences were less noticeable compared to the Dutch study. It may also be worth noting that we observed an unexpected peak in hyperuracilemia rates in February 2020 which remains an enigma with regard to the rest of the temporal curve. Less respected pre-analytical conditions during the COVID-19 pandemic could be one of the hypotheses to explain the unexpected peak.

Using genotyping, partial DPD deficiency has shown to be present in 3–5% of Caucasians, while in our study, we found that 12.7% of Caucasians with uracilemia had over 16 ng/mL.[12] These results suggest that the phenotyping approach might overestimate the diagnosis of DPD deficiency. However, genotyping seems less sensitive than phenotyping. In a Dutch prospective study that included 905 patients, the association between pre-treatment uracil and DPD activity in peripheral blood mononuclear cells and fluoropyrimidine-related toxicity could not be found.[29]

As reported in other literature, the influence of food intake and Circadian rhythms on uracilemia cannot be excluded.[10,26,27,32–34] In this study, both the time of the last meal and the time of sampling before blood draw were not standardized. However, no influence was observed in regard to the hour of sampling and no national or international guidelines indicate if fasting is necessary prior to uracil measurement. The number of included patients in studies concerning the Circadian rhythm and food intake remained low, which may hinder the level of proof.[10,26,27,32–34] Further prospective studies are required to assess the influence of food intake and Circadian rhythm on uracilemia measurement.

Gaible *et al.*[27] observed elevated uracilemia levels in 20 patients with end-stage renal disease before dialysis, which was improved after dialysis. They did not identify any *DPYD* variants in whole-gene sequencing for the same patients. This strengthens the hypothesis that decreased renal function may hamper uracil clearance.[27] Our large-scale observational study confirmed the influence of decreased eGFR on uracilemia indicating that renal impairment was strongly, linearly and inversely associated. Decreased uracil clearance in renal impairment cases most likely explains this phenomenon. The presence and level of renal impairment must be considered when measuring pre-therapeutic uracilemia to ensure maximum patient safety without compromising the efficacy of anticancer treatment. Thus, hyperuracilemia should be confirmed by a second sample, especially in cases of renal impairment or transitory decreases in eGFR. Another strategy would be to monitor the dihydrouracil/uracile (UH(2))/U) ratio since it is unaffected by renal function.[27]

In another study, Launay *et al.*[28] reported a patient with neuroendocrine carcinoma and liver metastasis who was classified as DPD deficient with uracil values measured successively at 321, 140, and 139 ng/mL. The patient had tumor lysis syndrome which resulted in the release of nucleic acids and their degradation products into the blood leading to increased uracil values. In our study, only one patient had uracilemia values over 150 ng/mL and multiple acute organ failures at the time of the blood sample (AST level was 137

times the UNL and eGFR was 25 mL/mn). Tumor lysis syndrome might lead to hyperuracilemia and DPD deficiency misinterpretation, although further studies are required.

When cytolysis occurs in the liver or the tumor, nucleic acids are released and might cause an overestimation of basal physiologic uracilemia. However, to our knowledge, the association between hepatic cytolysis and hyperuracilemia has never been reported. In our study, we observed a strong, linear association between ALP and AST elevation and hyperuracilemia. Nevertheless, the weak association with ALT levels suggests that cytolysis-induced hyperuracilemia is not liver specific. A second uracilemia level could be measured after hepatic improvement whenever feasible to limit AST and ALP influence on a DPD deficiency diagnosis. Moreover, lactate dehydrogenase can be used to assess tumor burden and lysis syndrome.

The multivariate-adjusted linear model did not confirm any influence of age, γGT, and bilirubin, which corresponds to our hypotheses. Cholestasis may be a side effect of liver impairment that accompanies liver cytolysis. Further descriptive studies should be carried out to better explain this phenomenon. Because ALP can originate in bones, the role of cholestasis remains unclear.

In our study, only one patient was sampled for uracil measurement while under continuous infusion of 5-FU (uracilemia 75.3 ng/mL). This case is in line with the observations of Thomas *et al.*[35] who reported 17 cases sampled for uracil measurement after fluoropyrimidine exposure. Due to their similar chemical structure, the competition between uracil and 5-FU for DPD-mediated metabolism is likely to explain these falsely positive chromatographic results.[36] Another hypothesis is that patients with significant hepatic tumor burden may induce cell apoptosis leading to higher uracil levels, whether spontaneously or through anticancer chemotherapy efficacy.

Regarding the laboratory results, we observed a difference between private and public laboratory results that could be explained by selection bias. Hospitals generally manage more advanced cancer cases in patients who more frequently have pre-existing conditions such as renal or liver dysfunction. This may also be a result of inter-laboratory variability, as observed in several previous studies.[29,37]

To our knowledge, this study was also the first to describe several patients who underwent a second uracil concentration measurement. Intra-individual test reproducibility should be considered to evaluate its accuracy. These variations are significant [median delta −2.5% (IQR: −35.1% to +30.2%)] and might reveal different DPD deficiency conclusions for the same patient (23.1%). This raises the question of the reliability of uracilemia measurement. This is a major concern, as there is a risk of potentially severe toxicity, or even suboptimal treatment depending on the uracil value. The lack of consensual guidelines on 5-FU dose adjustments in partial DPD deficiency highlights a grey area in terms of diagnosis and management. Further investigation should be proposed to better understand the implications of dose adjustments in these patients.

In terms of strengths, this study was the first to describe hyperuracilemia rates and base results on a large scale of patients, intra-individual phenotyping test reproducibility and interference with hepatic or renal dysfunction. Our findings further support previous small-sized studies and also consider the physio-pathological hypothesis.

Regarding limitations, the retrospective design did not allow us to analyze the impact of DPD deficiency screening on chemotherapy dose adjustments nor on the occurrence of severe fluoropyrimidine-induced toxicity. A recent matched retrospective study, using a propension score analysis in 198 patients, suggested that pre-therapeutic plasmatic uracil assessment, along with 5-FU dosage adjustment, may be beneficial in reducing 5-FU toxicity in real-life patients.[37] However, the retrospective design limited the sensitivity to detect toxicities below grade 3, and further prospective studies are needed. Second, biological confounders were measured during a period up to 28 days before or after the uracil concentration measurement which could have impacted the results. In future prospective studies, it would be useful to perform a standardized biochemical evaluation at the same time as uracilemia measurement, with kidney and liver tests, and screening for tumor lysis syndrome, to better analyze the association of uracilemia with other confounding factors. Third, biological data on eGFR and liver function tests may have been incomplete and the UH(2))/U ratio was not collected. In future studies, this ratio could be useful to identify any non-respected pre-analytical conditions as well as with dialysis patients.[15,27,38] Last, the limited number of patients

with discordant uracilemia assays between the first and second samples prevent us from performing precise statistical analyses.

Conclusion

In conclusion, hyperuracilemia rates consistently decreased since the screening guidelines were published in 2018. Renal impairment and tumor lysis, through APL and AST elevation, may lead to falsely positive results with artificial increase of uracilemia. When feasible, checking uracilemia levels with a second blood sample after renal and hepatic improvements may be an option to overcome these biases. With fluoropyrimidine dose adjustments in patients with partial DPD deficiency being non-consensual, further studies are required to investigate chemotherapy exposure since DPD deficiency misdiagnosis may lead to chemotherapy under-exposure or chemotherapy-induced toxicities. A large prospective study investigating the effect of phenotype-guided dosing based on pretreatment uracilemia is ongoing (clinicaltrials.gov identifier NCT04194957).

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Helsinki Declaration. Patient records were anonymized prior to analysis. A database was created in accordance with the National Commission of Liberties and Informatics reference methodology MR004 (2206749, on 13 September 2018). A non-opposition form was sent to each living patient included. As per French regulations concerning the retrospective study, no informed consent or additional ethical committee review was required.

Consent to participate

Not applicable.

Author contribution(s)

Sidonie Callon: Resources; Writing – original draft.

Mathias Brugel: Conceptualization; Formal analysis; Methodology; Resources; Supervision; Writing – review & editing.

Damien Botsen: Conceptualization; Methodology; Resources; Supervision; Writing – review & editing.

Bernard Royer: Writing – review & editing.

Florian Slimano: Resources; Supervision; Writing – review & editing.

Catherine Feliu: Writing – review & editing.

Claire Gozalo: Writing – review & editing.

Céline Konecki: Writing – review & editing.

Bruno Devie: Conceptualization.

Claire Carlier: Writing – review & editing.

Viktor Daire: Resources.

Nicolas Laurés: Resources; Writing – review & editing.

Marine Perrier: Writing – review & editing.

Zoubir Djerada: Writing – review & editing.

Olivier Bouché: Conceptualization; Methodology; Resources; Supervision; Validation; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The data presented in this study are available upon request from the corresponding author.

Supplemental material

Supplemental material for this article is available online.

References

1. Longley DB, Harkin DP and Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; 3: 330–338.
2. Vodenkova S, Buchler T, Cervena K, *et al.* 5-fluorouracil and other fluoropyrimidines in colorectal cancer: past, present and future. *Pharmacol Ther* 2020; 206: 107447.

3. Twelves C, Wong A, Nowacki MP, *et al.* Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 2005; 352: 2696–2704.
4. Meta-Analysis Group In Cancer, Lévy E, Piedbois P, *et al.* Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. Meta-analysis group in cancer. *J Clin Oncol* 1998; 16: 3537–3541.
5. Meulendijks D, Henricks LM, Sonke GS, *et al.* Clinical relevance of DPYD variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. *Lancet Oncol* 2015; 16: 1639–1650.
6. Hoff PM, Ansari R, Batist G, *et al.* Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* 2001; 19: 2282–2292.
7. Cheung WY, Renfro LA, Kerr D, *et al.* Determinants of early mortality among 37,568 patients with colon cancer who participated in 25 clinical trials from the adjuvant colon cancer endpoints database. *J Clin Oncol* 2016; 34: 1182–1189.
8. Hodroj K, Barthelemy D, Lega JC, *et al.* Issues and limitations of available biomarkers for fluoropyrimidine-based chemotherapy toxicity, a narrative review of the literature. *ESMO Open* 2021; 6: 100125.
9. Mercier C and Ciccolini J. Profiling dihydropyrimidine dehydrogenase deficiency in patients with cancer undergoing 5-fluorouracil/capecitabine therapy. *Clin Colorectal Cancer* 2006; 6: 288–296.
10. van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* 2004; 40: 939–950.
11. Diasio RB, Beavers TL and Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J Clin Invest* 1988; 81: 47–51.
12. Henricks LM, Lunenburg CATC, de Man FM, *et al.* DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* 2018; 19: 1459–1467.
13. Harris BE, Carpenter JT and Diasio RB. Severe 5-fluorouracil toxicity secondary to dihydropyrimidine dehydrogenase deficiency. A potentially more common pharmacogenetic syndrome. *Cancer* 1991; 68: 499–501.
14. Milano G and Etienne MC. Potential importance of dihydropyrimidine dehydrogenase (DPD) in cancer chemotherapy: *Pharmacogenetics* 1994; 4: 301–306.
15. Boisdron-Celle M, Remaud G, Traore S, *et al.* 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Lett* 2007; 249: 271–282.
16. Meulendijks D, Henricks LM, Jacobs BAW, *et al.* Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. *Br J Cancer* 2017; 116: 1415–1424.
17. Knikman JE, Gelderblom H, Beijnen JH, *et al.* Individualized dosing of fluoropyrimidine-based chemotherapy to prevent severe fluoropyrimidine-related toxicity: what are the options? *Clin Pharmacol Ther* 2021; 109: 591–604.
18. Pallet N, Hamdane S, Garinet S, *et al.* A comprehensive population-based study comparing the phenotype and genotype in a pretherapeutic screen of dihydropyrimidine dehydrogenase deficiency. *Br J Cancer* 2020; 123: 811–818.
19. Etienne-Grimaldi MC, Le Guellec CB, Boyer JC, *et al.* Prevention of 5-fluorouracil–induced early severe toxicity by pre-therapeutic dihydropyrimidine dehydrogenase deficiency screening: the multiparametric approach is not convincing. *Semin Oncol* 2017; 44: 159–160.
20. Loriot MA, Ciccolini J, Thomas F, *et al.* Dépistage du déficit en dihydropyrimidine déshydrogénase (DPD) et sécurisation des chimiothérapies à base de fluoropyrimidines: mise au point et recommandations nationales du GPCO-Unicancer et du RNPGx. *Bull Cancer (Paris)* 2018; 105: 397–407.
21. Haute Autorité de Santé. Des recommandations pour prévenir certaines toxicités sévères des chimiothérapies par fluoropyrimidines.https://www.has-sante.fr/jcms/c_2892234/fr/des-recommandations-pour-prevenir-certaines-toxicites-severes-des-chimiotherapies-par-fluoropyrimidines (2018, accessed 10 August 2022).
22. European Medicines Agency. EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine. https://www.ema.europa.eu/en/news/ema-recommendations-dpd-testing-prior-treatment-fluorouracil-capecitabine-tegafur-flucytosine (2020, accessed 10 August 2022).

23. Casneuf V, Borbath I, Van den Eynde M, *et al.* Joint Belgian recommendation on screening for DPD-deficiency in patients treated with 5-FU, capecitabine (and tegafur). *Acta Clin Belg* 2022; 77: 346–352.

24. Jacobs BAW, Rosing H, de Vries N, *et al.* Development and validation of a rapid and sensitive UPLC–MS/MS method for determination of uracil and dihydrouracil in human plasma. *J Pharm Biomed Anal* 2016; 126: 75–82.

25. Gamelin E, Boisdron-Celle M, Larra F, *et al.* A simple chromatographic method for the analysis of pyrimidines and their dihydrogenated metabolites. *J Liq Chromatogr Relat Technol* 1997; 20: 3155–3172.

26. Capiau S, Van Landschoot A, Reyns T, *et al.* Pre-analytical considerations for the analysis of uracil and 5,6-dihydrouracil in heparin plasma. *Clin Chem Lab Med CCLM* 2022; 60: e112–e115.

27. Gaible C, Narjoz C, Loriot MA, *et al.* Pretherapeutic screening for Dihydropyrimidine dehydrogenase deficiency in measuring uracilemia in dialysis patients leads to a high rate of falsely positive results. *Cancer Chemother Pharmacol* 2021; 88: 1049–1053.

28. Launay M, Guitton J, Balluet R, *et al.* Clinical considerations for DPD deficiency testing in advanced cancer patients: tumor lysis syndrome should be considered as a major interference. *Ann Oncol* 2022; 33: 850–852.

29. de With M, Knikman J, de Man FM, *et al.* Dihydropyrimidine dehydrogenase phenotyping using pretreatment uracil: a note of caution based on a large prospective clinical study. *Clin Pharmacol Ther* 2022; 112: 62–68.

30. Dolat M, Macaire P, Goirand F, *et al.* Association of 5-FU therapeutic drug monitoring to DPD phenotype assessment may reduce 5-FU under-exposure. *Pharmaceuticals* 2020; 13: 416.

31. Levey AS, Stevens LA, Schmid CH, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604.

32. Henricks LM, Jacobs BAW, Meulendijks D, *et al.* Food-effect study on uracil and dihydrouracil plasma levels as marker for dihydropyrimidine dehydrogenase activity in human volunteers: food effect-study on U and DHU levels. *Br J Clin Pharmacol* 2018; 84: 2761–2769.

33. Jacobs BAW, Deenen MJ, Pluim D, *et al.* Pronounced between-subject and circadian variability in thymidylate synthase and dihydropyrimidine dehydrogenase enzyme activity in human volunteers. *Br J Clin Pharmacol* 2016; 82: 706–716.

34. Jiang H, Lu J and Ji J. Circadian rhythm of dihydrouracil/uracil ratios in biological fluids: a potential biomarker for dihydropyrimidine dehydrogenase levels: the UH2/Ura ratio is a biomarker for DPD levels. *Br J Pharmacol* 2004; 141: 616–623.

35. Thomas F, Maillard M, Launay M, *et al.* Artificial increase of uracilemia during fluoropyrimidine treatment can lead to DPD deficiency misinterpretation. *Ann Oncol* 2021; 32: 810–811.

36. Terret C. Dose and time dependencies of 5-fluorouracil pharmacokinetics. *Clin Pharmacol Ther* 2000; 68: 270–279.

37. Laures N, Konecki C, Brugel M, *et al.* Impact of guidelines regarding dihydropyrimidine dehydrogenase (DPD) deficiency screening using uracil-based phenotyping on the reduction of severe side effect of 5-fluorouracil-based chemotherapy: a propension score analysis. *Pharmaceutics* 2022; 14: 2119.

38. Robin T, Saint-Marcoux F, Toinon D, *et al.* Automatic quantification of uracil and dihydrouracil in plasma. *J Chromatogr B* 2020; 1142: 122038.