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ORIGINAL RESEARCH

Impact of renal impairment on dihydropyrimidine dehydrogenase (DPD) phenotyping

B. Royer[1,2*†], M. Launay[3†], J. Ciccolini[4], L. Derain[5], F. Parant[6], F. Thomas[7] & J. Guitton[6,8,9]

[1]Laboratoire de Pharmacologie Clinique et Toxicologie, CHU Besançon, Besançon; [2]Univ. Franche-Comté, INSERM, EFS BFC, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, Besançon; [3]Pôle de Biologie-Pathologie, Hôpital Nord-CHU Saint Etienne, Saint Etienne; [4]SMARTc Unit, Centre de Recherche en Cancérologie de Marseille Inserm U1068 Aix Marseille Université and Assistance Publique Hôpitaux de Marseille, Marseille; [5]Service de Néphrologie, Dialyse, Hypertension et Exploration Fonctionnelle Rénale, Hospices Civils de Lyon, Hôpital E. Herriot, Lyon F-69003; University of Lyon 1; CNRS UMR 5305, Lyon; [6]Laboratoire de Biochimie et Toxicologie, Centre Hospitalier Lyon-Sud, Hospices Civils de Lyon, Pierre-Bénite; [7]Laboratoire de Pharmacologie, Institut Claudius Regaud, Inserm CRCT, Université de Toulouse, Toulouse Cedex 9; [8]Laboratoire de Toxicologie, ISPB, Faculté de Pharmacie, Université Lyon 1, Université de Lyon, Lyon; [9]Inserm U1052, CNRS UMR5286 Centre de Recherche en Cancérologie de Lyon, Lyon, France

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Background: The chemotherapeutic agent 5-fluorouracil (5-FU) is catabolized by dihydropyrimidine dehydrogenase (DPD), the deficiency of which may lead to severe toxicity or death. Since 2019, DPD deficiency testing, based on uracilemia, is mandatory in France and recommended in Europe before initiating fluoropyrimidine-based regimens. However, it has been recently shown that renal impairment may impact uracil concentration and thus DPD phenotyping.

Patients and methods: The impact of renal function on uracilemia and DPD phenotype was studied on 3039 samples obtained from three French centers. We also explored the influence of dialysis and measured glomerular filtration rate (mGFR) on both parameters. Finally, using patients as their own controls, we assessed as to what extent modifications in renal function impacted uracilemia and DPD phenotyping.

Results: We observed that uracilemia and DPD-deficient phenotypes increased concomitantly to the severity of renal impairment based on the estimated GFR, independently and more critically than hepatic function. This observation was confirmed with the mGFR. The risk of being classified 'DPD deficient' based on uracilemia was statistically higher in patients with renal impairment or dialyzed if uracilemia was measured before dialysis but not after. Indeed, the rate of DPD deficiency decreased from 86.4% before dialysis to 13.7% after. Moreover, for patients with transient renal impairment, the rate of DPD deficiency dropped dramatically from 83.3% to 16.7% when patients restored their renal function, especially in patients with an uracilemia close to 16 ng/ml.

Conclusions: DPD deficiency testing using uracilemia could be misleading in patients with renal impairment. When possible, uracilemia should be reassessed in case of transient renal impairment. For patients under dialysis, testing of DPD deficiency should be carried out on samples taken after dialysis. Hence, 5-FU therapeutic drug monitoring would be particularly helpful to guide dose adjustments in patients with elevated uracil and renal impairment.

Key words: dihydropyrimidine dehydrogenase—DPD, renal impairment, fluoropyrimidine, DPD phenotype, uracil, multicentric study

INTRODUCTION

Fluoropyrimidine drugs (5-fluorouracil or 5-FU and its prodrug capecitabine) are widely used in the treatment of numerous solid tumors in adults. Approximately 85% of administered 5-FU is rapidly catabolized in the liver into dihydrofluorouracil (5-FUH_2) by dihydropyrimidine dehydrogenase (DPD), leaving only a small fraction of the initial drug for an eventual transformation into cytotoxic metabolites. Fluoropyrimidine (FP) drug administration is associated with 10%-40% of severe toxicities and also 0.2%-0.8% of fatal toxicities which are frequently linked to DPD deficiency, resulting in partial or total loss of the ability to detoxify 5-FU in the liver.[1]

Since 2019, testing of DPD deficiency is mandatory in France before fluoropyrimidine-based treatments, using the determination of plasma uracil (U) as a surrogate. Indeed,

*Correspondence to: Dr Bernard Royer, Pharmacology Department, University Hospital of Besançon, rue C. Bried, F-25000 Besançon, France. Tel: +33-370-63-20-42

E-mail: broyer@chu-besancon.fr (B. Royer).

†These two authors contributed equally.

DPD is also physiologically involved in the reduction of endogenous U into dihydrouracil (UH_2); the lower the DPD activity, the higher the U levels. The European Medicine Agency (EMA) recommends screening for DPD deficiency (either by genotyping or phenotyping approaches), albeit without considering this test mandatory. In France, the threshold for a partial DPD deficiency phenotype was set at 16 ng/ml based on studies reporting the link between uracilemia and severe toxicities.[2-4]

However, various factors may modify endogenous U concentrations, such as liver function,[5-7] tumor lysis syndrome[8] or dialysis.[9,10] Indeed, Gaible et al. demonstrated that pre-therapeutic screening for DPD deficiency using uracilemia leads to a high rate of false positivity in patients under dialysis,[9] with increased U and UH_2 concentrations before compared to after dialysis. Moreover, Callon et al. described an increase in uracilemia, while the estimated glomerular filtration rate (eGFR) decreased.[6] However, to our knowledge, the impact of renal impairment on DPD phenotyping has so far not clearly been assessed.[6] Here, we evaluated in a multicentric study this impact on DPD phenotyping using a large number of patients (i) before treatment with fluoropyrimidine, (ii) under dialysis and (iii) with acute renal impairment.

PATIENTS AND METHODS

This retrospective biological observational study was approved by the institutional review board (IRBN1262022/CHUSTE). All consecutive patients (from March 2019 to December 2021) with blood samples collected for routine DPD phenotyping (using U concentrations) were anonymously included in this study among three French centers: the Hospice Civil de Lyon (HCL) (university hospital), the Institut Claudius Regaud in Toulouse (Cancer Research Center) and the University Hospital of Besançon. These analyses were carried out following the requirements of the French authorities, i.e. samples were taken before starting every new FP-based treatment. Because of the design of this study, using anonymous extraction of biological data, no clinical data (such as demographics, data on pathology, performance status or stage of disease) have been collected. Pre-analytical requirements were as follows: samples were centrifugated and frozen at $-20^{\circ}C$ within 1 h of sampling.[11,12] The eGFR, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatases (ALP), gamma glutamyl transferase (GGT) and total bilirubinemia values were collected, alongside U and UH_2 values, if their level was assessed within 2 days of sampling for DPD phenotyping. For the concomitant analysis of patients under dialysis, renal impaired and patients with a normal renal function, samples were obtained from the Nephrology and Dialysis departments of the HCL to reduce inter-center variability. The measured glomerular filtration rate (mGFR), based on iohexol clearance determination,[13] was performed with patients from the same departments.

Determination of U and UH_2 was carried out in the Pharmacology departments of the three centers. These laboratories used validated methods according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use-EMA guidelines[14] with liquid chromatography coupled either to triple quadrupole (LC-MS/MS) or high-resolution mass spectrometry (LC-MS/HRMS). Precision and accuracy values were below 10% for the three laboratories. Of note, the three laboratories participate in the same external quality controls and all have similar values for these controls, ensuring the comparability of the values.

Quantitative variables were compared using either paired *t*-test or non-parametric analysis of variance (Kruskal–Wallis test) followed by Dunn's *post hoc* test. DPD deficiency, expressed as percentage, was compared using chi-square tests. After verification of collinearity, the impact of renal (eGFR) and hepatic (AST, ALT, ALP, GGT and total bilirubinemia) functions on hyperuracilemia ($U < 16$ versus $U \geq 16$ ng/ml) was investigated using a multinomial logistic regression model. The impact of different stages of renal function on DPD deficiency was investigated using logistic regression. Pearson's correlation was tested on the relationship between mGFR and U. Differences were considered significant when $P < 0.05$.

RESULTS

Routine patients with lower eGFR have a higher frequency of displaying a DPD deficiency phenotype

Plasma U ($n = 3039$) and UH_2 ($n = 2845$) concentrations were measured in three independent centers. When classified with respect to eGFR values, a statistical increase in uracilemia was observed in patients with the lowest eGFR values (Figure 1A). This was accompanied with a higher frequency of DPD-deficient phenotypes (Figure 1B), thus suggesting that the lower the eGFR values, the higher the frequency of DPD deficiency. Such a phenomenon was not observed with UH_2, leading to a statistically higher UH_2/U ratio in patients with lower eGFR values (Figure 1C and D).

A greater probability of DPD-deficient phenotype was observed when GFR was measured in patients with mild renal impairment and from dialyzed patients before dialysis

As the estimation of renal function using the eGFR formula is less relevant for patients with very low eGFR values,[15] we then excluded any bias due to low eGFR measurements. As shown in Figure 2, a statistically significant link between low mGFR and high uracilemia was observed, as for eGFR and uracilemia. To explore whether a high frequency of the DPD-deficient phenotype is associated with different levels of renal impairment, samples from 22 dialysis patients (eGFR), 21 impaired renal function patients (mGFR) and 24 normal renal function patients (mGFR) were analyzed. To do so, the association between the probability of DPD deficiency (i.e. uracilemia above 16 ng/ml) and these clinical conditions was studied. Logistic regression analysis showed that sampling before dialysis and impaired renal function were both

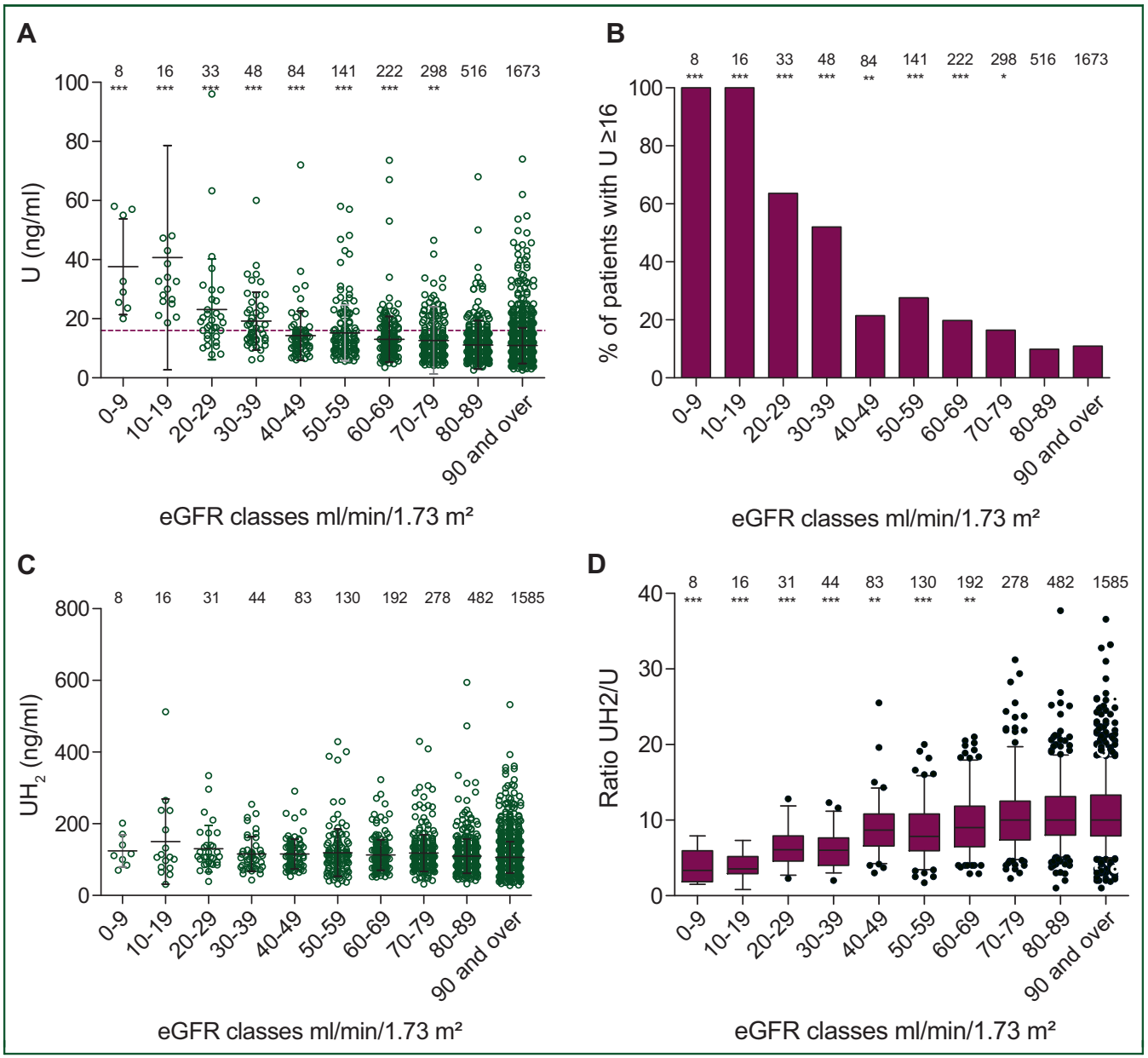

Figure 1. Distribution of uracilemia (A), percentage of samples with uracil concentrations or U ≥ 16 ng/ml (B), UH_2 (C) and UH_2/U ratio (D) with respect to eGFR classes. The red dashed line represents the DPD deficiency threshold (16 ng/ml—A). Three very high uracilemia (179, 179 and 151 ng/ml for eGFR values of 12, 72 and 85 ml/min/1.73 m^2, respectively) were discarded for a better clarity of the graph, but included for the analysis (A). The number of patients is indicated above each class. Non-parametric statistical differences (ANOVA then Dunn's tests) were analyzed versus the normal renal function class (≥ 90 ml/min/1.73 m^2). Levels of statistical differences as compared to the '90 and over' group were expressed as ***($P < 0.001$), **($P < 0.01$) and *($P < 0.05$).
ANOVA, analysis of variance; DPD, dihydropyrimidine dehydrogenase; eGFR, estimated glomerular filtration rate.

significantly associated with DPD deficiency with odds ratios of 145.7 and 25.3, respectively (Figure 3). When samples for DPD phenotyping were collected after dialysis, this statistical association was no longer observed.

Dialysis modifies DPD phenotyping

Having shown that the association between hyperuracilemia and eGFR disappears after dialysis, its impact on DPD phenotyping was then explored. Twenty-two dialyzed patients were sampled before and after dialysis. Before dialysis, uracilemia was statistically higher than that after dialysis. After dialysis, values were comparable to those of patients with normal renal function (12.2 ± 6.0 ng/ml) (Table 1). The frequency of DPD-deficient phenotypes was also statistically lower after dialysis. As UH_2 was not impacted by dialysis, the UH_2/U ratio was lower before dialysis than after dialysis (Table 1).

Renal impairment was a more important independent factor of hyperuracilemia than hepatic function

Data to carry out the analysis could be obtained from 1591 patients. According to logistic regression, eGFR, AST, ALT and ALP have independent significant impact on hyperuracilemia. The estimated coefficient of GFR was −0.02998,

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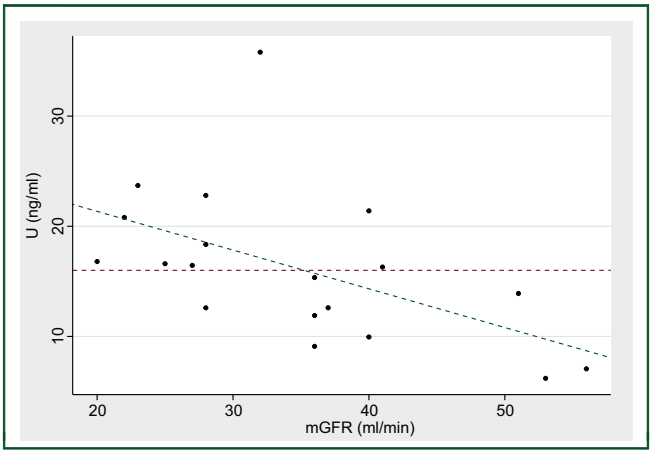

Figure 2. Uracil concentrations according to measured GFR in patients with impaired renal function. A statistical link ($P = 0.01911$) is observed between the measured GFR and uracilemia in patients with impaired renal function. The red dashed line represents the DPD deficiency threshold (16 ng/ml). The green dashed line represents the regression line. The equation used was $U = -0.3515 \times mGFR + 28.3849$.
GFR, glomerular filtration rate; mGFR, measured glomerular filtration rate (ml/min/1.73 m^2); U, uracil concentrations (ng/ml).

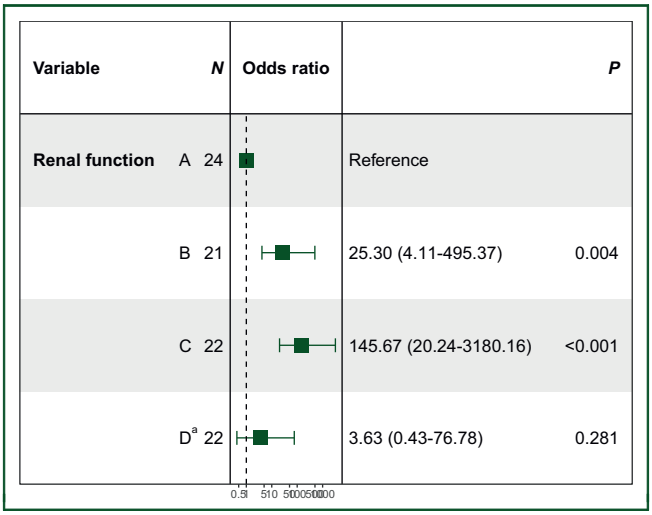

Figure 3. Association between GFR and hyperuracilemia. A logistic regression test was conducted to assess the association between the probability of uracilemia >16 ng/ml and the renal status of patients with normal renal function (A—mGFR), impaired renal function (B—mGFR) and samples taken from the same patients before dialysis (C—eGFR) or after dialysis (D—eGFR).
[a]The regression was carried out twice by considering samples taken before and after dialysis independently.
eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate.

whereas that of AST, ALT and ALP were 0.00807, −0.00587 and 0.00120, respectively. Odds ratio and 95% confidence intervals are shown in Figure 4.

Patients with transient renal impairment may have a transient modification of their DPD phenotype

The impact of renal function modifications on DPD phenotyping was assessed using patients as their own control when two samples were taken. For all patients, a minimum of 3 days elapsed between the two samplings. U was quantified in 12 patients (group B) for whom the eGFR was lower than 50 ml/min/1.73 m^2 on one occasion and higher than 50 ml/min/1.73 m^2 on the other occasion. The mean values ± SD of eGFR were 36.2 ± 10.9 and 62.5 ± 15.6 ml/min/1.73 m^2, respectively. Uracilemia was also determined twice in two control groups, in which patients constituted their own control: group A with five patients with a stable impaired renal function for two samples (eGFR < 50 ml/min/1.73 m^2, mean ± SD of eGFR were 33.6 ± 12.3 and 39.6 ± 13.0 ml/min/1.73 m^2, for the first and the second sample, respectively); and group C with 65 patients with a stable renal function for two samples (eGFR > 50 ml/min/1.73 m^2, mean ± SD of eGFR were 95.2 ± 16.5 and 96.5 ± 16.7 ml ml/min/1.73 m^2, for the first and the second sample, respectively). A statistically significant difference in uracilemia was observed between the two samples, only when there was a change in eGFR values (Figure 5A—group B). Interestingly, for these patients, when the eGFR was < 50 ml/min/1.73 m^2, uracilemia was not statistically different from that of group A (i.e. patients with stable low eGFR), whereas when the eGFR was >50 ml/min/1.73 m^2, uracilemia was not statistically different from that of group C (i.e. patients with stable higher eGFR). For patients in group B, if they were sampled when the eGFR was <50 ml/min/1.73 m^2, the frequency of DPD deficiency was 83.3%, whereas if they were sampled when the eGFR was >50 ml/min/1.73 m^2, this frequency dropped to 16.7% (Figure 5B). When the renal function was stable, uracilemia measured on the two occasions led to discordant DPD phenotypes in 12.9% (groups A and C), whereas this occurred in over 65% of the patients when the renal function was unstable (group B).

DISCUSSION

Renal impairment was recently reported to impact uracilemia.[6,10] In the present study, we also observed an increase in uracilemia as renal function decreased. However, we went further by investigating the consequences of such a uracilemia increase on the uracilemia-based DPD phenotype according to renal disease stages. Indeed, if the frequency of the DPD-deficient phenotype seemed constant when eGFR was within the normal range (≥ 90 ml/min/m^2), it gradually increased to reach 100% for the rare patients

Table 1. Paired uracil concentration, DPD deficiency, dihydrouracil concentration and dihydrouracil-to-uracil ratio in 22 dialyzed patients before and after dialysis

	Before	After
Uracil (U; ng/ml)	22.5 ± 9.1 $P < 0.0001$*	12.2 ± 6.0
Patients with DPD-deficient phenotype (%)	86.4 $P = 0.0002$*	13.7
Dihydrouracil (UH_2; ng/ml)	103.6 ± 38.2 $P = 0.1473$ (NS)	117.0 ± 41.6
UH_2/U ratio	5.1 ± 2.3 $P < 0.0001$*	10.5 ± 3.5

Uracil, dihydrouracil and UH_2/U ratio were expressed as mean ± standard deviation.
DPD deficiency was expressed as a percentage.
DPD, dihydropyrimidine dehydrogenase; NS, not statistically significant.
*Statistically significant.

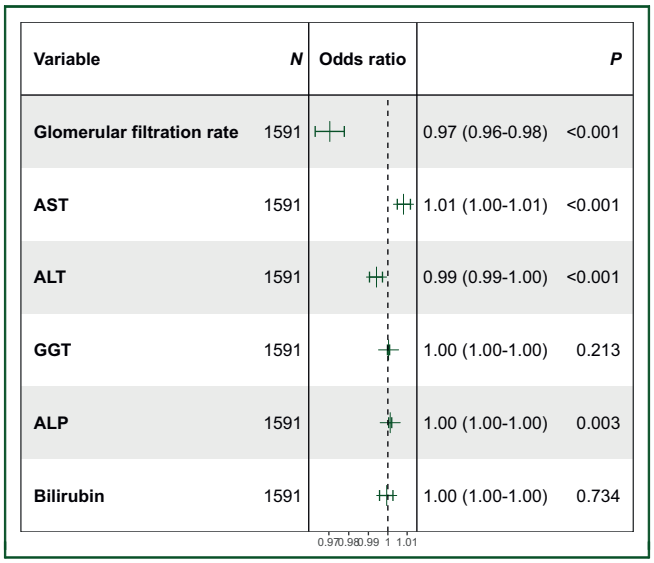

Figure 4. Impact of different biological factors on hyperuracilemia. A multinomial logistic regression was carried out to assess the impact of estimated glomerular function rate (eGFR), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatases (ALP), gamma glutamyl transferase (GGT) and total bilirubinemia as independent factors on hyperuracilemia (U $\geq$ 16 ng/ml).

with an eGFR $<$ 20 ml/min/1.73 m^2 (Figure 1B). To confirm such a phenomenon, we assessed the link between GFR and the probability of DPD deficiency phenotyping in patients with normal renal function, renal impairment and dialyzed patients. Logistic regression confirmed that there was a significant relationship between kidney status and the probability of DPD deficiency phenotype (Figure 3). Interestingly, this link disappeared after dialysis.

This raises the question of the sampling time for a dialyzed patient. Actually, it cannot be ruled out that dialysis can cause an unusually large decrease in uracilemia leading to a value below 16 ng/ml in a DPD 'true' deficient patient, leading to a false-negative diagnosis. However, we thought that it was more relevant to collect the sample after dialysis for the following reasons. Firstly, we observed a correlation between uracilemia before and after dialysis (r = 0.80—see Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101577) indicating that high uracilemia potentially remains high even after dialysis. Secondly, the uracilemia does not fall below 16 ng/ml after dialysis for all patients, as illustrated by the decrease in the frequency of patients with DPD-deficient phenotype from 86.4% to 13.7%. Moreover, both the mean values of uracilemia and the frequency of DPD deficiency after dialysis were similar to that of patients with a normal renal function. Thirdly, as described in the present work, there is a link between DPD deficiency and samples collected before dialysis, but this link disappeared when samples were collected after dialysis. Thus, taking into account all these data, we advise against collecting samples before dialysis and suggest it relevant to collect after dialysis.

We also investigated as to what extent modification of the renal function of a patient was associated with a change in the assigned DPD phenotype, as a result of an artifactual increase in uracilemia. Although the number of patients analyzed with a transient renal impairment was low, we showed that patients who had an improvement in their renal function reduced their probability of being classified as DPD deficient. We observed that >65% of the patients with a transient but significant change in their kidney function also had a discordant DPD phenotype between the evaluations, whereas the assignment of a different DPD phenotype between two occasions occurred only in 12.9% of the patients when the renal function was stable (Figure 4B).

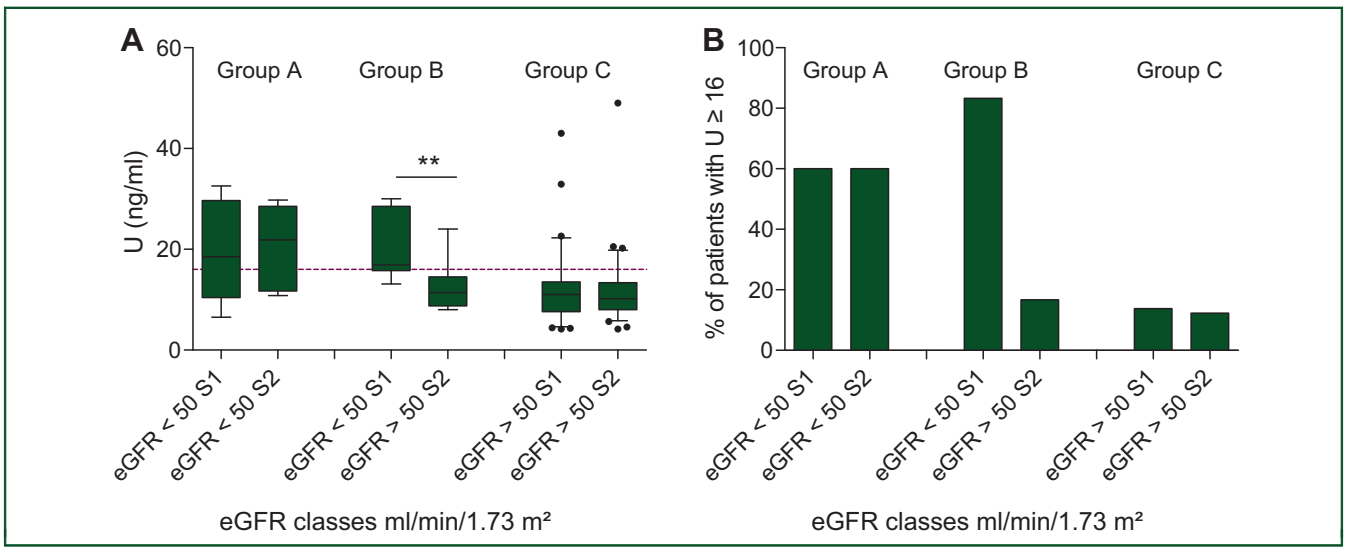

Figure 5. Uracilemia of patients for which two samples (S1 and S2) could be obtained on different days (A), and percentages of samples with U $\geq$ 16 ng/ml (B). The percentages of patients whose DPD phenotype changed between the two samples were 0%, 66.7% and 12.9% for groups A, B and C, respectively. Group A (n = 5): eGFR $<$ 50 ml/min/1.73 m^2 for both samples; group B (n = 12): patients have one sample with eGFR $<$ 50 ml/min/1.73 m^2 and one sample with eGFR $>$ 50 ml/min/1.73 m^2; and group C (n = 65): both samples have eGFR $>$ 50 ml/min/1.73 m^2. **Statistically different (P $<$ 0.01) between the two groups.
DPD, dihydropyrimidine dehydrogenase; eGFR, estimated glomerular filtration rate.

Hepatic function has been recently described as one of the factors impacting uracilemia and therefore DPD phenotype.[6,7] We thus assess the mutual impact of eGFR, AST, ALT, GGT, ALP and total bilirubinemia on hyperuracilemia (U $\geq$ 16 ng/ml). Logistic regression showed that renal function was an independent predictor variable of hyperuracilemia, the latter being less frequent with increased eGFR values. As previously described,[6,7] hepatic enzymes such as AST and ALP have been also already identified as potential predictor variables on DPD phenotyping. However, in our study renal function impact on hyperuracilemia was 4-fold higher than that of AST and 25-fold higher than that of ALP, confirming its preponderant role in hyperuracilemia.

Recent studies have highlighted that pre-therapeutic screening for DPD deficiency by measuring uracilemia in patients under dialysis leads to a higher uracilemia and a higher rate of DPD deficiency.[9,10] This is consistent with our results since 86.4% of the patients would be identified as DPD deficient before dialysis compared to 13.7% after dialysis. However, Gaible et al. suggested that clinicians preferably rely on the UH_2/U ratio to identify DPD deficiency in such patients because they observed that UH_2 was also higher before dialysis.[9] In our study, dihydrouracil was impacted neither by renal impairment nor by dialysis. This argues in favor of reconsidering the assessment of UH_2/U ratio as a surrogate marker of DPD phenotype in case of renal impairment. We assume that such a discrepancy might be due to analytical conditions leading to the absence of separation of UH_2 and an interference (see Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101577). Additional studies are currently being carried out to confirm such data. Taken together, these studies advocate for measuring the DPD phenotype through uracilemia after dialysis.

Metabolomic studies have shown that chronic kidney diseases are accompanied by a decrease in urine uracil that is significantly correlated with eGFR.[16,17] It can be thus hypothesized that the decrease in renal clearance associated with renal impairment might lead to an increase in plasma uracil concentration. However, since over 80% of the dose of 5-FU is rapidly metabolized by DPD, only a small quantity is eliminated unchanged by renal clearance.[18] This was confirmed for uracil after administration of 13C-uracil.[19] Moreover, several studies reported that the kidneys play no major role in 5-FU elimination.[20,21] Thus, renal impairment may be associated with a modification of DPD activity. For instance, an increase in endogenous compounds potentially leading to DPD inhibition could be observed in the plasma of patients with renal impairment.[20,22-24] Nevertheless, a case of dialyzed patients who tolerated standard 5-FU doses despite high levels uracilemia has been observed,[10] questioning the uracilemia-based phenotype in this situation. In the case of 'false' hyperuracilemia, as a pre-therapeutic plasma value of U $\geq$ 16 ng/ml may lead to a decrease in the 5-FU dose prescribed in the first line, there is a risk of 5-FU underexposure.[25] As previously suggested,[10] the adjustment of the 5-FU dose through therapeutic drug monitoring in this case is strongly recommended to potentially restore an adequate 5-FU dose as soon as possible.

It should be noted that several factors may lead to a U $\geq$ 16 ng/ml, including a deficiency in DPD activity or renal impairment, with or without DPD deficiency, for instance. However, we observed that a transient renal impairment was frequently associated with a discordance in DPD phenotype classification. Thus, to avoid misclassification, it seems relevant to carry out a further uracilemia-based DPD phenotype on patients collected after improvement in their renal function. Additionally, genotyping of DPD gene might give more elements allowing to appreciate the DPD status in such a situation.

Conclusion

The use of pre-therapeutic uracilemia and subsequent adaptive dosing should not be questioned as it allows a decrease in the rate of 5-FU toxicity.[26] However, DPD phenotyping using uracil concentrations might be misleading in patients with impaired renal function. If the patients are dialyzed, even if false-positive results could not be excluded, phenotyping of DPD deficiency should preferably be carried out with samples collected after dialysis. Otherwise, in case of a DPD-deficient phenotype observed in patients with impaired renal function, a 'true' DPD deficiency cannot be excluded. If possible, we suggest to collect another sample after recovery of the renal function. We also suggest the use of 5-FU therapeutic drug monitoring to allow the administration of an adequate 5-FU dose during the following lines of treatment.

FUNDING

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DISCLOSURE

The authors have declared no conflicts of interest.

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