

Letter to the Editor

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Impact of hemolysis on uracilemia in the context of dihydropyrimidine dehydrogenase deficiency testing

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To the Editor,

Fluoropyrimidines, 5-fluorouracil (5-FU) and its oral prodrug capecitabine, are the most prescribed anticancer drugs, mainly administered in digestive, breast and head and neck cancers, accounting for around 2 millions of patients worldwide annually [1] and more than 75,000 patients each year in France [2]. Severe hematological and digestive toxicities (grade 3–4) occur in almost 20 % of patients. The main identified cause of fluoropyrimidine-related severe toxicities is a deficiency in the dihydropyrimidine dehydrogenase enzyme (DPD), the rate-limiting enzyme for 5-FU catabolism. DPD also metabolizes uracil (U) into dihydrouracil (UH2) making plasma U and UH2/U ratio [3] surrogate markers of DPD activity. Since 2018, French Health Authorities (Haute

Autorité de Santé and Institut National du Cancer) have recommended to screen DPD deficiency by measuring plasma uracil concentration (uracilemia) before any fluoropyrimidine administration [4] and this screening was made mandatory in France on April 2019. Strict and traceable pre-analytical handling is necessary as we recently showed that blood sample should be centrifuged within 60 min followed by immediate freezing of the plasma sample until analysis to ensure results reliability [5]. To our knowledge, the impact of sample hemolysis on uracilemia has not been reported so far, whereas it has been shown that plasma concentration of some analytes can be influenced by hemolysis [6]. The objective of this study was to determine whether hemolysis influence uracilemia and plasma UH2/U ratio and may impact their interpretation in the context of DPD deficiency screening.

Between February 2023 and May 2023, we assessed the effect of sample hemolysis on 39 patients samples addressed to two hospital laboratories for uracilemia testing. Blood samples centrifugation was performed in a mean time of 33 min (min–max: 8–100 min), with only one sample with a delay higher than 60 min. For each patient, uracilemia was measured in parallel in a non-hemolyzed plasma and in a hemolyzed plasma mechanically (shear stress) obtained by vortexing the blood sample (10–20 s) followed by either 20 or 30 suction/discharge with a 1 mL 22 G syringe depending on the degree of expected hemolysis. After blood centrifugation, the hemolysis degree was determined according to the visual scale proposed by Ingelse et al. [7], and both non-hemolyzed (H–) and hemolyzed (H+) plasmas were stored at –80 °C until their analysis (U and UH2) in the same run. The two laboratories used similar analytical methods (UPLC-MS/MS, quantification limit=2.5 ng/mL for U and UH2) validated according to European requirements (EN ISO 15189) and participate to the same External Quality Assessment Scheme (Asqualab, Paris, France) with satisfactory results.

In non-hemolyzed plasma, mean (range) U, UH2 and UH2/U were 9.4 (4.7–16.9) ng/mL, 110.8 (52.9–174.8) ng/mL

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Table 1: U, UH2 and UH2/U ratio in hemolyzed vs. non-hemolyzed plasma samples.

		Mean (±SD)	Mean (±SD)	Mean change % ^a (±SD)	
Per condition (hemolyzed H+ vs. non-hemolyzed H-)		H-	H+		p-Value ^b
All samples (n=39)	U (ng/mL)	9.4 (±3.5)	11.9 (±4.8)	+28.5 (29.2)	<0.0001
	UH2 (ng/mL)	110.8 (±33.8)	111.5 (±31.6)	+1.8 (10.6)	0.78
	UH2/U	12.6 (±4.0)	10.2 (±3.4)	-17.5 (16.9)	<0.0001
Per percentage of hemolysis		H-	H+		p-Value
0.5 % (n=13)	U (ng/mL)	9.5 (±3.7)	10.3 (±3.2)	+12.3 (14.7)	0.0181
	UH2 (ng/mL)	120.6 (±34.4)	121.9 (±33.5)	+1.7 (5.3)	0.7729
	UH2/U	14.0 (±5.6)	12.4 (±3.9)	-7.9 (13.8)	0.0134
1 % (n=14)	U (ng/mL)	10.1 (±3.8)	12.6 (±5.9)	+24.6 (27.6)	0.0005
	UH2 (ng/mL)	112.9 (±30.5)	109.6 (±28.0)	-2.3 (5.5)	0.1726
	UH2/U	11.9 (±2.5)	9.7 (±2.7)	-18.7 (15.6)	0.0002
2 % (n=8)	U (ng/mL)	8.5 (±3.4)	12.2 (±5.8)	+42.7 (23.6)	0.0156
	UH2 (ng/mL)	92.1 (±31.7)	96.5 (±29.4)	+7.0 (14.6)	0.1094
	UH2/U	11.3 (±2.4)	8.7 (±2.4)	-23.4 (15.1)	0.0078
10 % (n=2)	U (ng/mL)	7.9 (±1.8)	14.5 (±0.1)	+88.7 (44.1)	Not evaluable
	UH2 (ng/mL)	131.3 (±59.6)	118.7 (±50.9)	-8.9 (2.6)	Not evaluable
	UH2/U	16.2 (±3.9)	8.2 (±3.6)	-50.5 (10.1)	Not evaluable

^a100 × [(H+) - (H-)]/(H-). ^bWilcoxon matched pairs (H- vs. H+) signed rank test (two-tailed). Significant p-values are shown in bold.

and 12.6 (6.4–23.7), respectively. Firstly, we compared hemolysed (H+) vs. non-hemolyzed (H-) plasmas whatever the hemolysis degree. As shown in Table 1, U significantly increased and UH2/U significantly decreased in H+ plasmas (both $p < 0.0001$), whereas UH2 was not impacted. Analysis according to the degree of hemolysis clearly showed that the greater the hemolysis, the higher the uracilemia and the lower the UH2/U ratio (Spearman correlation $p = 0.0013$ and 0.0069 , respectively, Figure 1). For uracilemia, the mean percent change vs. non-hemolyzed plasma were +12.3 %, +24.6 %, +42.7 % and +88.7 % in 0.5, 1, 2 and 10 % hemolyzed plasmas, respectively (Table 1). No significant difference was observed for UH2 concentration across the different hemolysis degrees (Spearman correlation $p = 0.40$).

According to French recommendations [4], a uracilemia between 16 and 150 ng/mL is indicative of partial DPD deficiency that should lead to consider a fluoropyrimidine dose reduction, while an uracilemia above 150 ng/mL reflects a complete deficiency leading to treatment contraindication. In the present study, only 2 patients exhibited a partial deficiency (non-hemolyzed plasma), with uracilemia raising from 16.1 to 22 ng/mL, and 16.9–25.5 ng/mL in H+ samples. Importantly, for 4 patients out of the 39 (10.3 %), uracilemia changed from <16 ng/mL (no DPD deficiency) in non-hemolyzed samples to greater than 16 ng/mL (18.3, 22.3, 16.7 and 18.2 ng/mL) in hemolyzed samples, leading to misinterpretation of DPD activity. Since dihydrouracil was

not modified by hemolysis, the UH2/U ratio was not a more relevant indicator of DPD activity in case of sample hemolysis.

Hemolysis interference is a well-known issue in clinical biology that can interfere with blood tests by involving the release of the blood cell content that may lead to an overestimation of certain analytes (for instance lactate dehydrogenase or potassium) or to interference in colorimetry-based methods. For uracilemia analysis, the plasma sample undergoes a liquid–liquid extraction followed by UPLC with mass spectrometry detection, ruling out any color-based interference. It can be hypothesized that the uracilemia increase observed in hemolyzed plasma comes from a release of U from red blood cells. Since there is no DPD activity in red blood cells [8], it is expected that UH2 is not produced in such cells and therefore it explains why UH2 is not influenced by sample hemolysis.

One limitation of our study is the determination of hemolysis degree only based on visual inspection by the operator that can be prone to subjectivity. While the measurement of the hemolysis index (free hemoglobin concentration in the plasma) obtained with clinical laboratory analyzers would be more accurate, it is less available in pharmacology laboratories performing uracilemia analyses.

In conclusion, preanalytical hemolysis of blood samples causes an overestimation of uracilemia, that is dependent on

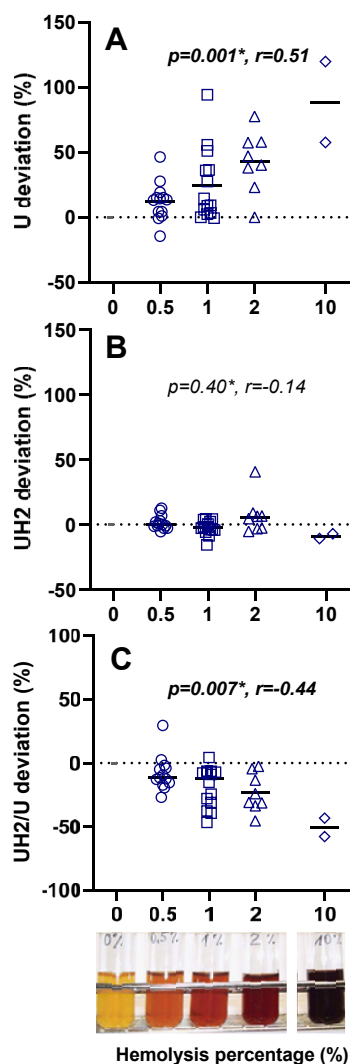


Figure 1: Individual plot of percent change as a function of hemolysis degree according to the scale proposed by Ingelse et al. [7], reproduced here with permission. *p-Values (along with r-values) correspond to the non-parametric Spearman rank correlation.

the hemolysis degree. In the case of uracilemia close to or higher than the 16 ng/mL cut-off, this overestimation may lead to misinterpretation of results and subsequent non-relevant deleterious fluoropyrimidine dose reduction in non-deficient DPD patients whereas negligible impact is expected for lower values of uracil. Therefore, we recommend that uracilemia above the threshold value of 16 ng/mL in the case of hemolyzed samples should be considered as non-interpretable and that a new blood sample properly handled (i.e., sampling, transportation and centrifugation) should be requested. In addition to the respect of the 1-h maximum delay between blood sampling and plasma freezing, careful evaluation of sample hemolysis is required in order to guarantee a correct interpretation of uracilemia results in the context of DPD deficiency screening.

Research ethics: The local Institutional Review Board deemed the study exempt from review as the samples used in this research come from patients' samples taken in the course of treatment. Patients are clearly informed on their arrival at the institute that, in the absence of any objection on their part, the samples taken may be used for research projects carried out at the institute. We made sure that patients did not object. The samples are pseudoanonymised and the confidentiality of the data is guaranteed.

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