LETTER TO THE EDITOR

Plasma Uracil as a DPD Phenotyping Test: Pre-Analytical Handling Matters!

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To the Editor:

We read with interest the article by de With *et al.*[1] reporting the results of a prospective multicenter study where pretreatment uracil blood samples were collected and handled in 17 Dutch hospitals but analyzed in the reference hospital. Significant differences in plasma uracil concentrations were observed between samples from the reference hospital and those from eight other hospitals, six of which showing significant higher concentrations. Unfortunately, the number of patients concerned with this pitfall is not given. The authors suggested that this variability could be explained by deviations in the pre-analytical rules. Indeed, the existence of pre-analytical instructions (blood stored on ice and centrifuged within 30 minutes, as mentioned in the Methods section) does not guarantee their application. Many studies by our group[2] and others[3,4] clearly demonstrated that extended blood storage at room temperature before centrifugation significantly increases uracilemia as a result of uridine conversion into uracil by uridine phosphorylase. The authors analyzed all uracil data irrespective

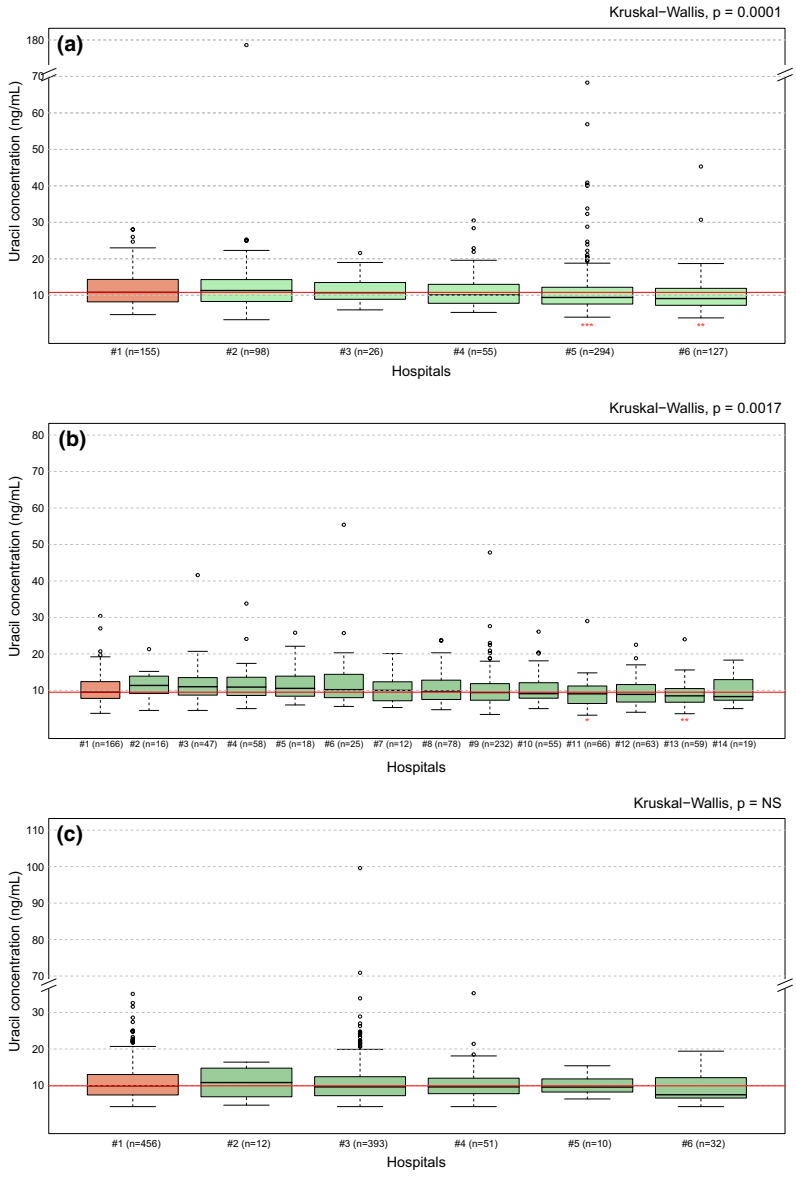

Figure 1 Pretreatment plasma uracil measured in 3 French academic laboratories (lab (**a**) 755 patients, lab (**b**) 914 patients, lab (**c**) 954 patients). The first orange boxes (#1) correspond to plasma uracil concentrations obtained from blood of patients collected and handled in the reference hospital that performed the analysis. Green boxes show uracil concentrations of patients from other centers that provided frozen plasma to the reference hospital for uracil analysis. All samples fulfilled French pre-analytical recommendations. Within each laboratory, between-centers differences were tested using Kruskal-Wallis test, and when significant each center was compared to the reference hospital (#1) using Mann–Whitney test. The red line corresponds to the median uracil concentration in each reference hospital. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

of the sample origin and concluded on a lack of correlation between uracilemia and toxicity. We feel that inclusion of such flawed uracilemia is highly questionable and undermines the reliability of this conclusion. We suggest the authors to repeat the analyses after excluding all samples who do not meet pre-analytical requirements or alternatively after excluding the six centers with significantly higher uracilemia relative to the reference hospital. Furthermore, the authors do not discuss the fact that excluding *DPYD* variant carriers from toxicity analyses does not allow to determine the performance of phenotyping, by minimizing the relationship between DPD phenotype and toxicity.

In 2019, French authorities have made mandatory uracilemia testing before fluoropyrimidine chemotherapy,[5] along with pre-analytical recommendations (maximum blood storage 1 hour and 30 minutes at room temperature or 4 hours at +4°C) to ensure uracilemia reliability. **Figure 1a–c** shows an example of real-life uracilemia measured in three French academic laboratories (2,623 patients in total), each receiving frozen plasma samples from different hospitals. The respect of pre-analytical recommendations was checked for each sample upon reception by verifying that sampling and centrifugation times as well as blood storage temperature were correctly documented and compliant with French recommendations. The three laboratories use analytical methods (ultraperformance liquid chromatography-tandem mass spectrometry) validated according to European requirements (EN ISO 15189) and participate to an External Quality Assessment Scheme (Asqualab, Paris, France) with satisfactory results. The extent of between-centers variability is much lower than that observed in De With's paper. No hospital shows significantly higher uracilemia compared with the reference hospital, thus limiting the risk of DPD status misclassification and showing the feasibility of large-scale implementation of uracilemia testing.

In conclusion, strict adherence to pre-analytical requirements is mandatory to validate uracilemia for fluoropyrimidine dose adaptations. In their current form, the De With results do not provide useful information regarding the performance of uracilemia for predicting severe toxicity.

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CONFLICT OF INTEREST
The authors declared no competing interests for this work.

1. de With, M. *et al*. Dihydropyrimidine dehydrogenase phenotyping using pretreatment uracil: a note of caution based on a large prospective clinical study. *Clin. Pharmacol. Ther.* **112**, 62–68 (2022).
2. Maillard, M. *et al*. Quantitative impact of pre-analytical process on plasma uracil when testing for dihydropyrimidine dehydrogenase deficiency. *Br. J. Clin. Pharmacol.* (2022). https://doi.org/10.1111/bcp.15536. [e-pub ahead of print].
3. Jacobs, B.A.W. *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.* **126**, 75–82 (2016).
4. Capiau, S., Van Landschoot, A., Reyns, T. & Stepman, H. Pre-analytical considerations for the analysis of uracil and 5,6-dihydrouracil in heparin plasma. *Clin. Chem. Lab. Med.* **60**, e112–e115 (2022).
5. Recommandations et référentiels, INCa, HAS, décembre 2018. Recherche d''un déficit en dihydropyrimidine deshydrogénase visant à prévenir certaines toxicités sévères associées aux traitements par fluoropyrimidines. <https://www.has-sante.fr/portail/upload/docs/application/pdf/2018-12/recherche_dun_deficit_en_dihydropyrimidine_deshydrogenase_visant_a_prevenir_certaines_toxicites_severes_associees_aux_traite.pdf>.

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