Anne Winther-Larsen*, Anne Tranberg Madsen, Peter H. Nissen, Elke Hoffmann-Lücke and Eva Greibe

Short-term biological variation of plasma uracil in a Caucasian healthy population

https://doi.org/10.1515/cclm-2022-1167
Received November 16, 2022; accepted February 22, 2023; published online March 2, 2023

Abstract

Objectives: Plasma uracil is a new biomarker to assess the activity of dihydropyrimidine dehydrogenase before cancer treatment with fluoropyrimidine drugs. Knowledge on the biological variation of plasma uracil is important to assess the applicability of plasma uracil as a biomarker of drug tolerance and efficacy.

Methods: A total of 33 apparently healthy individuals were submitted to sequential blood draws for three days. On the second day, blood draws were performed every third hour for 12 h. Plasma uracil was quantified by LC-MS/MS. The within-subject (CV_I) and between-subject (CV_G) biological variation estimates were calculated using linear mixed-effects models.

Results: The overall median value of plasma uracil was 10.6 ng/mL (range 5.6–23.1 ng/mL). The CV_I and CV_G were 13.5 and 22.1%, respectively. Plasma uracil remained stable during the day, and there was no day-to-day variation observed. No differences in biological variation components were found between sex and no correlation to age was found. Four samples were calculated to be required to estimate the homeostatic set-point ±15% with 95% confidence.

Conclusions: Plasma uracil is subject to tight homeostatic regulation without semidiurnal and day-to-day variation, however between-subject variation exists. This emphasizes plasma uracil as a well-suited biomarker for evaluation of dihydropyrimidine dehydrogenase activity, but four samples are required to establish the homeostatic set-point in a patient.

Keywords: 5-fluorouracil; biological variation; dihydropyrimidine dehydrogenase (DPD) deficiency; uracil.

Introduction

Chemotherapeutic drugs belonging to the group of fluoropyrimidines, such as 5-fluorouracil (5-FU), capecitabine, and tegafur, are frequently used for treatment of solid tumors. The drugs are administered orally or intravenously and catabolized by the enzyme dihydropyrimidine dehydrogenase (DPD), encoded by the *DPYD* gene, to inactive metabolites. Reduced activity of this enzyme poses a risk of severe toxicity, and 10–50% of patients treated with a standard 5-FU dose experience toxicities that potentially can be life-threatening [1–3].

For this reason, the European Medicines Agency (EMA) (April 2020) has recommended that patients should be tested for DPD deficiency before starting cancer treatment with 5-FU, capecitabine, or tegafur, either by *DPYD* genotyping or by phenotyping based on pretreatment plasma uracil concentrations [4]. EMA proposed cut-off values for uracilemia of 150 ng/mL (complete deficiency) and 16 ng/mL (partial deficiency) and suggested that plasma uracil concentrations above these cutoff values require reductions in 5-FU standard dose of 100 and 50%, respectively [4].

As the patient will receive reduced doses of fluoropyrimidines for treatment of their cancer, it is paramount to understand all analytical and biological aspects of plasma uracil as a biomarker for DPD activity. Although research has been devoted to the correlations between DPD deficiency, 5-FU toxicity, and plasma uracil concentrations [5–9], only a few studies have investigated the natural dynamics of plasma uracil [10, 11]. In order to base clinical decisions on plasma uracil concentrations, it is essential to understand the magnitude of between-subject and within-subject variation.

The present study was designed to evaluate the magnitude of biological variation of plasma uracil in healthy

***Corresponding author: Anne Winther-Larsen**, MD, PhD, Associated Professor, Department of Clinical Biochemistry, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Aarhus, Denmark; and Department of Clinical Medicine, Health, Aarhus University, Aarhus, Denmark, Phone: +0045 7844 0000, E-mail: anlarsen@rm.dk. https://orcid.org/0000-0002-2763-595X

Anne Tranberg Madsen, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, USA. https://orcid.org/0000-0001-9146-5943

Peter H. Nissen, Elke Hoffmann-Lücke and Eva Greibe, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; and Department of Clinical Medicine, Health, Aarhus University, Aarhus, Denmark. https://orcid.org/0000-0002-8201-1306 (P.H. Nissen)

individuals and, based on this, calculate the number of samples required to estimate the homeostatic set-point in an individual. This will help to elucidate how to interpret plasma uracil concentrations and ensure the correct dosing of fluoropyrimidine drugs for cancer treatment in the future.

Materials and methods

Subjects

From June 2018 to October 2018, 33 apparently healthy individuals were included in the study at Department of Clinical Biochemistry, Aarhus University Hospital, Denmark. The study inclusion and exclusion criteria have been thoroughly described previously [12]. In brief, subjects were eligible for inclusion if they were older than 18 years and did not suffer from any acute or chronic infection or inflammation, did not received any medication, were not pregnant or did not smoke. Furthermore, night-shift work or crossing of time-zones in the week before sampling lead to exclusion.

Subject were included despite uracil concentrations above 16 ng/mL as partial DPD deficiency is, to our knowledge, not associated with a specific disease but may be the underlying cause of toxicity against fluoropyrimidines. Therefore, we believe that these individuals are as healthy as others and should be included in the biological variation calculation.

The study was performed in accordance with the Helsinki Declaration and approved by the Central Denmark Region Committees on Health Research Ethics [1–10]. Written informed consent was provided by all subjects before inclusion.

Study design

On three consecutive days, each subject had a blood drawn at 9 AM. On the second day, blood sampling was also conducted at the following time points: 12 PM, 3 PM, 6 PM, and 9 PM. Subjects were included in the study even though they did not participate in all blood drawings as long as they participated in the three 9 AM blood drawings. Subjects were not allowed to drink alcohol or perform hard physical activity during the study period. Additionally, subjects had to refrain from food and any kind of physical activity an hour prior to each blood sampling.

The study was designed bearing the checklists for biological variation studies in mind [13–15].

Blood sample collection and processing

Handling of blood samples have previously been described in detail [12]. In short, whole blood was collected in 10 mL EDTA tubes (BD Vacutainer) and set to rest 30 min before centrifugation at RT for 10 min at 1800 *g*. The supernatant plasma fractions were carefully transferred into new tubes and subjected to a second centrifugation for 10 min at 13,000 *g*. Hereafter, the plasma was stored at −80 °C until further analysis. All sampling and processing of the blood samples were performed by the same four experienced technicians.

Laboratory analysis

Plasma uracil was measured using an ISO 15189 accredited in-house LC-MS/MS method at Aarhus University Hospital, Aarhus, Denmark in Spring 2022. The liquid chromatography was carried out on the Agilent 1,290 Infinity Series system (Agilent, Denmark) and mass spectrometric detection was performed on the Agilent 6,490 Triple Quad mass spectrometer (Agilent, Denmark), which was equipped with an electrospray ionization source. Analytical separation was performed on a Kinetex C18 HPLC column (3.0 × 150 mm, 2.6 µm) (Phenomenex, Denmark) at a temperature of 30 °C controlled by a column heater.

All samples were thawed at RT for 30–60 min before analysis. Protein precipitation was performed with ice-cold acetonitrile (VWR, Denmark) containing deuterium-marked internal standard ([^{13}C,^{15}N$_2$]-Uracil; AlsaChim, France). After vortexing and centrifugation (5 min, 16,162 *g*), the supernatant was transferred to a SPE Strata-X PRO plate (Phenomenex, Denmark) and centrifuged (10 min, 1,479 *g*, 4 °C). The filtrate was then evaporated and reconstituted in 0.1% formic acid in LCMS grade water (both VWR, Denmark). 10 µL of this solution was injected onto the column and analyzed in negative mode. Calibration curves were based on in-house made standard solutions of uracil (Toronto Research Chemicals, Canada).

Each sample was analyzed with duplicate injection in two analytical runs by a trained laboratory technician blinded to data. The intermediate precision of three plasma controls with mean ± SD concentrations of 9.02 ± 1.05 ng/mL (n=78 measures), 30.4 ± 2.3 ng/mL (n=79 measures), and 216.1 ± 12.6 ng/mL (n=81 measures) analyzed from February 2022 to April 2022 was 11.7%, 7.3%, and 5.8%, respectively. In addition, the laboratory participated in external quality schemes organized by Asqualab, France and have met the performance targets set by the organizers. All samples from the same subject were analyzed in the same run.

Statistical analysis

Outlier analyses on the analytical and within-subject level were performed using the Cochran's C test, while the Dixon-Reed criterion was used for detection of between-subject outliers [15, 16]. The data distribution was assessed by visual evaluation and by the Shapiro-Wilk test. As data followed a ln-normal distribution, a ln-transformation was performed, and data were presented as medians and ranges. The steady state of the population was assessed by using a linear regression of the median value for each blood drawing vs. the blood drawing number. The Brown-Forsythe test was used on the ln-transformed data to evaluate the variance homogeneities of within-subject variability.

The analytical variation (CV_A) was estimated from duplicates of every sample according to Fraser et al. [16]. The within-subject variation (CV_I) and between-subject variation (CV_G) were assessed using linear mixed effects models with day and sample as fixed effects and subjects as a random effect. For calculation of the CV_A, CV_I and CV_G, samples from all time points were included. The 95% confidence intervals (CIs) were calculated according to Roraas et al. [17] for all the biological variation estimates. The number of samples required to estimate an individual's homeostatic set-point (n) was calculated using the following equation proposed by Fraser et al. [16]: $n=(z \cdot \sqrt{(CV_I^2 + CV_A^2)}/D)^2$, where z is the z-score and D is the desired percentage closeness to the homeostatic set point. For the pair-wise comparisons of day-to-day and semidiurnal median values, the Bonferroni correction was used to correct for multiple comparisons.

Thus, a p-value below 0.017 (0.05/3 comparisons) was considered significant for pair-wise comparison of day-to-day values and 0.005 (0.05/10 comparisons) for pair-wise comparison of semidiurnal values.

Statistical calculations were performed in STATA 14 (StataCorp) and GraphPad Prism 9.3.1 (GraphPad Software).

Results

Description of subjects

In total, 33 subjects were included in the study of whom 23 (70%) were women. The median age of all subjects was 39 years (range 22–66 years). From the 33 individuals, a total of 184 blood samples were collected: all seven blood samples were collected in 14 subjects, five blood samples were collected in 13 subjects (all but the 6 and 9 PM blood draws), four blood samples were collected in one subject (all samples except the 3 PM, 6 PM, and 9 PM blood draws) and three blood samples were collected in five subjects (the three 9 AM blood draws) (Figure 1). An outlier was detected on the analytical level and was thus excluded from further analysis. No outliers on the within-subject and between-subject levels were detected. All participants were in steady state, and no heterogeneity of variance was detected.

Biological variation components

The median value of uracil in all subjects was 10.6 ng/mL (range 5.6–23.1 ng/mL) (Table 1). The median values and ranges of uracil in each subject are illustrated in Figure 1. The overall CV_I was 13.5% (95% CI 10.8–16.1%) and the overall CV_G was 22.1% (95% CI 15.8–28.4%). The CV_A was 7.2% (95% CI 6.2–8.1%) and nearly below the desirable analytical performance of $CV_A \leq 0.5\ CV_I$ [16]. The number of samples calculated to be required to estimate the homeostatic set-point ±10%, ±15%, and ±20% with 80%, 85%, 90%, and 95% confidence, respectively, are depicted in Figure 2. We find that four independent measurements are required to provide a trustworthy estimation of the plasma uracil

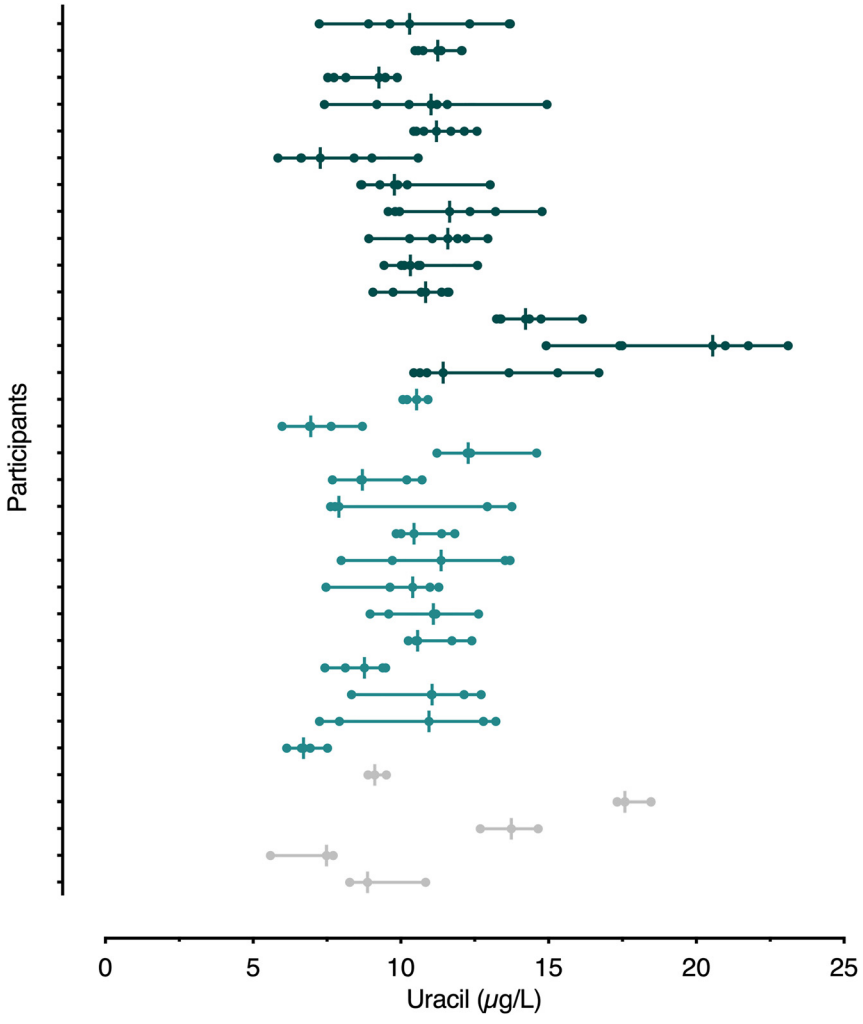

Figure 1: Plasma uracil levels in apparently healthy subjects (n=33). Subjects are coloured according to the number of samples contributed by the individual: dark green, 7 samples; light green, 5–6 samples; grey, 3–4 samples. The coloured vertical lines indicate the median values and the horizontal lines shows the ranges. The coloured spots illustrate the mean value of duplicates for each measurement.

Table 1: Components of biological variation.

	Uracil
Number of subjects	33
Number of samples[a]	183
Median, pg/mL	10.6 (10.3–11.0)
Range, pg/mL	5.6–23.1
CV_I, %	13.5 (10.8–16.1)
CV_G, %	22.1 (15.8–28.4)
CV_A, %	7.2 (6.2–8.1)
Samples required, n[b]	4

Values in parentheses are the 95% confidence intervals. [a]Number of samples analyzed after exclusion of outliers; [b]n, required to estimate homeostatic set point within 15% with 95% confidence. CV_I, within-subject coefficient of variation; CV_G, between-subject coefficient of variation; CV_A, analytical coefficient of variation.

concentration if the absolute relative deviation between the observed mean of serial samples and the homeostatic set-point can be no greater than ±15% with 95% confidence. Moreover, we find that as many as nine independent measurements are needed if a deviation of only ±10% with 95% confidence is acceptable (Figure 2).

No difference in CV_A, CV_I, and CV_G were found between sexes and no correlation with age was found for the variation components (data not shown).

Day-to-day and semidiurnal variance

The day-to-day variance components were achieved by using the 9 AM measurements on each of the three days. There was no significant difference in plasma uracil levels found between the days (p=0.64, Figure 3).

For calculation of the semidiurnal variance components, all the measurements on day 2 were included (9 AM, 12 PM, 3 PM, 6 PM, 9 PM). Again, no significant difference in plasma uracil levels was observed during the day (p=0.27, Figure 4). The median uracil level at each time point and the day-to-day and semidiurnal variance components are depicted in Table 2.

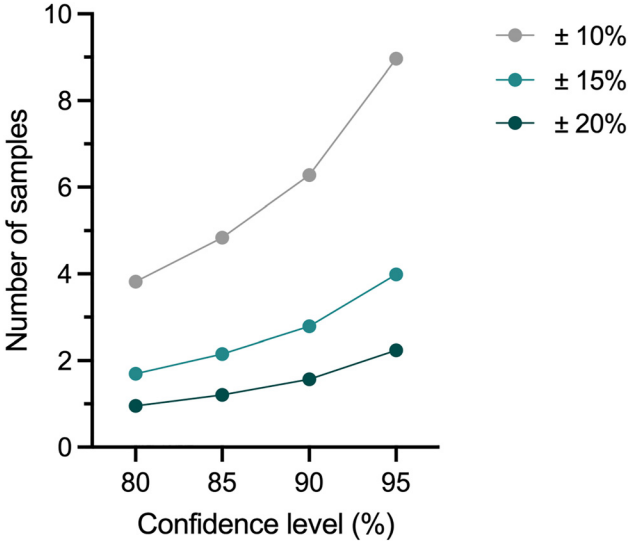

Figure 2: The number of samples required to estimate the homeostatic set-point. Three different desired percentage closeness to the homeostatic set-point are shown for four different confidence levels.

Discussion

Uracil is the endogenous substrate of the DPD enzyme, and plasma uracil concentrations are used for evaluation of DPD activity prior to cancer treatment with fluoropyrimidine drugs such as 5-FU. It has been proposed that blood sampling should be performed between 8 and 10 AM after overnight fasting as uracil display diurnal variation and is affected by food intake [10, 11, 18]. Here, we present new data on the magnitude of biological variation of plasma uracil in a cohort of healthy individuals. We found plasma uracil to remain stable during the day with no systematic changes in the concentration from day-to-day. Considerable variation between subjects was observed; however, this was not related to sex or age. Finally, we report on calculations showing that at least four samples are required to estimate the homeostatic set-point for plasma uracil in an individual. To the best of our knowledge, we are the first to present insights on this need for multiple sampling to establish a trustworthy measurement of plasma uracil.

In our cohort of 33 healthy individuals, we found a median plasma uracil of 10.6 ng/mL which is in agreement with the finding in other studies [18–20]. Furthermore, no effect of sex and age on the endogenous uracil levels was observed which supports the findings by De With et al. [20]. Importantly, we found no fluctuations in plasma uracil during the day which suggests that sampling can be carried out at all times. Our results support the findings of no diurnal variation of plasma uracil in recent studies [18, 19], however disagree with others who found plasma uracil to vary considerably during the day [11, 21]. Henricks et al. [18] have shown that plasma uracil concentrations are higher in fasting state than in fed state, which may explain observations on diurnal variation. In our study, the participants had to abstain from food for at least an hour prior to each blood sampling. For this reason, we expect any possible influence from food intake to be minor. Also, we did not observe any variation in the day-to-day concentration of

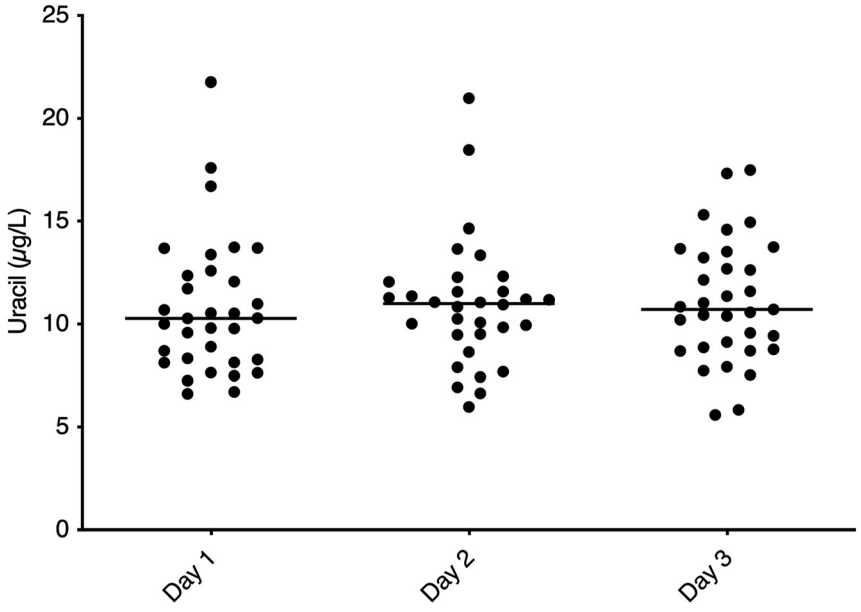

Figure 3: Day-to-day levels of plasma uracil. Individual results (spots) are depicted. The horizontal lines shows the median values.

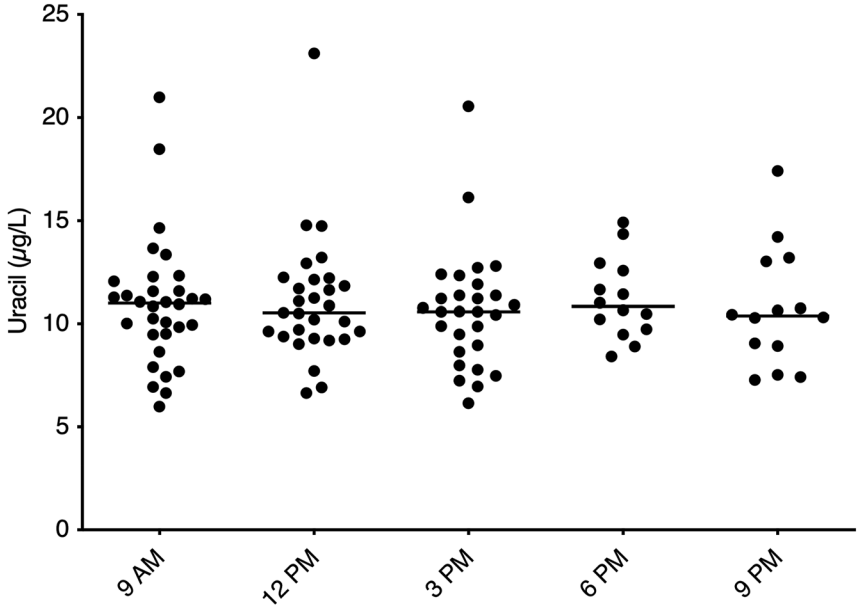

Figure 4: Within-day levels of plasma uracil from the second day. Individual results (spots) are depicted. The horizontal lines shows the median values.

plasma uracil, and our data overall indicates that the exact day and time point during the daytime for blood sampling is of less importance when measuring plasma uracil.

We are the first to present calculations on the number of samples required to estimate the homeostatic set-point for plasma uracil in an individual. We found that at least four independent measurements are necessary to provide a trustworthy estimation of the plasma uracil concentration ±15% with 95% confidence and as many as nine measurements if the deviation has to be no greater than ±10% with 95% confidence. This finding is highly important since it is current practice to use only a single measurement of plasma uracil for evaluation of DPD activity before initiation with 5-FU treatment which, build on our calculations, only will estimate the homeostatic set-point of plasma uracil ±30% with 95% confidence (data not shown). By basing the evaluation of DPD activity on only a single measurement, there is a high risk of incorrect interpretation of the plasma uracil concentration giving rise to a wrongful 5-FU cancer treatment. For two subjects, all (3/3 samples)/all but one (6/7 samples) of their plasma uracil measurements were above the clinical cut-off value of 16 ng/mL that may indicate partial DPD deficiency, and should, in accordance with guidelines [4], result in a 50%

Table 2: Day-to-day and semidiurnal components of biological variation.

	Uracil
Day-to-day	
Number of subjects	33
Day 1, pg/mL	10.3 (8.7–11.7)
Day 2, pg/mL	11.0 (9.8–11.6)
Day 3, pg/mL	10.7 (9.4–12.6)
CV_I, %	9.9 (6.2–13.6)
CV_G, %	25.6 (19.7–31.4)
Semidiurnal	
Number of subjects	29
12 PM, pg/mL	10.5 (9.6–11.8)
03 PM, pg/mL	10.6 (9.5–11.4)
06 PM, pg/mL	10.8 (9.5–13.0)
09 PM, pg/mL	10.4 (7.5–13.2)
CV_I, %	11.3 (8.6–14.1)
CV_G, %	22.1 (15.3–28.8)

Day-to-day and semidiurnal uracil levels are presented as medians. Values in parentheses are the 95% confidence intervals. CV_I, within-subject coefficient of variation; CV_G, between-subject coefficient of variation.

reduction in the 5-FU standard dose for cancer treatment. This possible reduction in DPD activity in the present cases could have been supported by the *DPYD* genotype, however these data were not available for this study. Two of the other participants showed one plasma uracil measurement above the 16 ng/mL cut-off. However, as their remaining measurements were below the 16 ng/mL cut-off, impaired DPD activity seems unlikely, and the observations may just be the result of intra-individual variation in these subjects, pre-analytical and/or analytical variation. Nevertheless, this underscores the importance of estimating the homeostatic set-point in an individual by use of at least four plasma uracil measurements before deciding on the 5-FU treatment dose. Unfortunately, as time is of the essence when cancer treatment is to be initiated, it may not be practical and possible to perform multiple samplings beforehand. For this reason, we recommend that DPD activity is always assessed by both phenotyping (plasma uracil) and *DPYD* genotyping as neither of these approaches can stand alone when it comes to providing the complete picture on a patient's DPD activity needed to decide the best 5-FU cancer treatment strategy. In a recent nationwide study, including plasma uracil measurements and genotyping of four clinically relevant *DPYD* variants, it was observed that 4.8% of 2070 patients with normal genotype, had plasma uracil levels above 16 ng/mL, similar to our findings of individuals with plasma uracil levels above the cut-off [22]. This discrepancy may be due to *DPYD* variants not targeted by the current genotyping methods, or to biological variation not attributable to the *DPYD* gene, and may imply the need for further investigation among other uracil modulating variants to further understand the biological variation of plasma uracil.

In our study, we used a relatively homogenous group of healthy individuals and fixed test conditions. Our results may therefore not directly be extrapolated to the real-world setting of cancer patients, where higher inter-individual variation in the uracil metabolism may occur. Studies of cancer patients in a clinical setting are needed to provide further recommendations. The inclusion of subjects in this study was based on clearly defined inclusion and exclusion criteria, which was assessed based on self-reported information from the subjects. Yet, no clinical tests were performed to verify the subjects' health status and, therefore, we cannot be entirely certain that all subjects are actually healthy. Lastly, blood samples included in this study had all been frozen for four years, despite no long-term stability data at −80 °C above 6 months exists [23]. However, all samples from the same individual were analyzed at the same time and thus had been frozen for the same duration of time.

In conclusion, we show that plasma uracil is subjected to tight homeostatic regulation without semidiurnal and day-to-day variation, however between-subject variation exists. This emphasizes plasma uracil as a well-suited biomarker for evaluation of DPD activity. We find no evidence that sampling needs to be carried out at a certain time of day, but we find that at least four plasma uracil measurements are required to establish the homeostatic set-point in a patient. For this reason, we recommend that *DPYD* genotyping always is used as a supplement to phenotyping by plasma uracil concentration to ensure the correct dosing of fluoropyrimidine drugs for cancer treatment.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflicts of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Central Denmark Region Committees on Biomedical Research Ethics [1–10].

References

1. Leichman CG, Fleming TR, Muggia FM, Tangen CM, Ardalan B, Doroshow JH, et al. Phase II study of fluorouracil and its modulation in advanced colorectal cancer: a southwest oncology group study. J Clin Oncol 1995;13:1303–11.
2. Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA). Assessment report: fluorouracil and fluorouracil related substances (capecitabine, tegafur and flucytosine) containing medicinal products; 2020. Available from: https://www.ema.europa.eu/en/documents/referral/fluorouracil-fluorouracil-related-substances-article-31-referral-assessment-report_en.pdf.
3. INCa and HAS. Screening for dihydropyrimidine dehydrogenase deficiency to decrease the risk of severe toxicities related to fluoropyrimidines (5-fluorouracil or capecitabine); 2018. Available from: www.has-sante.fr/jcms/c_2891090/en/screening-for-dihydropyrimidine-dehydrogenase-deficiency-to-decrease-the-risk-of-severe-toxicities-related-to-fluoropyrimidines-5-fluorouracil-or-capecitabine-inahta-brief.
4. European Medicines Agency (EMA). EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine; 2020. Available from: https://www.ema.europa.eu/en/documents/referral/fluorouracil-fluorouracil-related-substances-article-31-referral-ema-recommendations-dpd-testing_en.pdf.
5. Meulendijks D, Jacobs BA, Aliev A, Pluim D, van Werkhoven E, Deenen MJ, et al. Increased risk of severe fluoropyrimidine-associated toxicity in patients carrying a G to C substitution in the first 28-bp tandem repeat of the thymidylate synthase 2R allele. Int J Cancer 2016;138:245–53.
6. Fleming RA, Milano G, Thyss A, Etienne MC, Renée N, Schneider M, et al. Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 1992;52:2899–902.
7. Milano G, Etienne MC, Pierrefite V, Barberi-Heyob M, Deporte-Fety R, Renée N. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. Br J Cancer 1999;79:627–30.
8. Zhou ZW, Wang GQ, Wan DS, Lu ZH, Chen YB, Li S, et al. The dihydrouracil/uracil ratios in plasma and toxicities of 5-fluorouracil-based adjuvant chemotherapy in colorectal cancer patients. Chemotherapy 2007;53:127–31.
9. Gamelin E, Boisdron-Celle M, Guérin-Meyer V, Delva R, Lortholary A, Genevieve F, et al. Correlation between uracil and dihydrouracil plasma ratio, fluorouracil (5-FU) pharmacokinetic parameters, and tolerance in patients with advanced colorectal cancer: a potential interest for predicting 5-FU toxicity and determining optimal 5-FU dosage. J Clin Oncol 1999;17:1105.
10. Jiang H, Lu J, Ji J. Circadian rhythm of dihydrouracil/uracil ratios in biological fluids: a potential biomarker for dihydropyrimidine dehydrogenase levels. Br J Pharmacol 2004;141:616–23.
11. Jacobs BA, Deenen MJ, Pluim D, van Hasselt JG, Krähenbühl MD, van Geel RM, et al. Pronounced between-subject and circadian variability in thymidylate synthase and dihydropyrimidine dehydrogenase enzyme activity in human volunteers. Br J Clin Pharmacol 2016;82:706–16.
12. Madsen AT, Hojbjerg JA, Sorensen BS, Winther-Larsen A. Day-to-day and within-day biological variation of cell-free DNA. EBioMedicine 2019;49:284–90.
13. Aarsand AK, Røraas T, Fernandez-Calle P, Ricos C, Díaz-Garzón J, Jonker N, et al. The biological variation data critical appraisal checklist: a standard for evaluating studies on biological variation. Clin Chem 2018;64:501–14.
14. Bartlett WA, Braga F, Carobene A, Coşkun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. Clin Chem Lab Med 2015;53:879–85.
15. Braga F, Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: an update. Crit Rev Clin Lab Sci 2016;53:313–25.
16. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
17. Røraas T, Støve B, Petersen PH, Sandberg S. Biological variation: evaluation of methods for constructing confidence intervals for estimates of within-person biological variation for different distributions of the within-person effect. Clin Chim Acta 2017;468:166–73.
18. Henricks LM, Jacobs BAW, Meulendijks D, Pluim D, van den Broek D, de Vries N, et al. Food-effect study on uracil and dihydrouracil plasma levels as marker for dihydropyrimidine dehydrogenase activity in human volunteers. Br J Clin Pharmacol 2018;84:2761–9.
19. Capiau S, Van Landschoot A, Reyns T, Stepman H, de With M, Knikman J, et al. Pre-analytical considerations for the analysis of uracil and 5,6-dihydrouracil in heparin plasma dihydropyrimidine dehydrogenase phenotyping using pretreatment uracil: a note of caution based on a large prospective clinical study. Clin Chem Lab Med 2022;60:e112–e5.
20. de With M, Knikman J, de Man FM, Lunenburg C, Henricks LM, van Kuilenburg ABP, et al. Dihydropyrimidine dehydrogenase phenotyping using pretreatment uracil: a note of caution based on a large prospective clinical study. Clin Pharmacol Ther 2022;112:62–8.
21. Remaud G, Boisdron-Celle M, Hameline C, Morel A, Gamelin E. An accurate dihydrouracil/uracil determination using improved high performance liquid chromatography method for preventing fluoropyrimidines-related toxicity in clinical practice. J Chromatogr B Anal Technol Biomed Life Sci 2005;823:98–107.
22. Paulsen NH, Qvortrup C, Vojdeman FJ, Plomgaard P, Andersen SE, Ramlov A, et al. Dihydropyrimidine dehydrogenase (DPD) genotype and phenotype among Danish cancer patients: prevalence and correlation between DPYD-genotype variants and P-uracil concentrations. Acta Oncol 2022;61:1400–5.
23. Chavani O, Jensen BP, Strother RM, Florkowski CM, George PM. Development, validation and application of a novel liquid chromatography tandem mass spectrometry assay measuring uracil, 5,6-dihydrouracil, 5-fluorouracil, 5,6-dihydro-5-fluorouracil, α-fluoro-β-ureidopropionic acid and α-fluoro-β-alanine in human plasma. J Pharm Biomed Anal 2017;142:125–35.