When helping the minority of patients may hurt the majority: The case for DPD phenotyping and 5-fluorouracil therapeutic drug monitoring

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Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosures: The authors declare no conflict of interest regarding this study.

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Word count: 1317 words

Dear Editor,

5-fluorouracil (5-FU) is still the backbone in some cancer treatments particularly in digestive cancers. The drug is associated with potential severe adverse events leading in some cases in substantial morbidity and mortality. As the drug is essentially catabolized through an enzymatic process mediated by dihydropyrimidine deshydrogenase (DPD), DPD deficiency is thought to be the main mechanism leading to 5-FU accumulation and toxicity [1]. Identifying patients with DPD deficiency and notably patients with complete deficiency, is then a relevant approach to limit the risk of severe adverse event. Recently, the European Agency for Medicine has therefore recommended DPD deficiency screening prior a treatment with fluorouracil or one of these derivatives [2].

The rate for complete DPD deficiency in Caucasian population is estimated to up to 0.5% to 1%, while up to 9% of patients may have some kind of partial enzymatic deficiency [2]. Thus, considerable effort should be undertaken to recognize these patients before any initiation of 5-FU treatments. Genotyping approach allows identifying patients carriers of one the four most common allelic variants associated with a loss or a decrease of function of DPD. Although frequencies of the variants of interest are low, these pharmacogenetics tests could be useful in some cases to explain an enzymatic deficiency. However, on a large cohort, genotyping was shown to be poorly predictive of DPD deficiency [3]. Actually, the phenotyping approach appears more appropriate to screen DPD deficiency. Because DPD catabolized uracil into its metabolite, dihydro-uracil, measuring uracil plasma level before any treatment initiation is the recommended phenotyping method aiming at preventing the drug toxicity at the first treatment cycle. In France, a concentration threshold of 16 ng/mL of uracil in plasma is proposed to categorize patients with potential partial DPD deficiency while a concentration above 150 ng/mL prohibits any 5-FU treatment. For patients with uracil plasma level between 16 and 150 ng/mL, caution is required and the treatment dosage may be decreased to prevent drug toxicity [4,5]. This situation can be frequent since a recent study reported that 15 % of the patient of its cohort (n= 526) had uracil concentration within this range [6]. As a result, an important part of patients treated with 5-FU receives a reduced drug dosage.

However, it is known that there is a wide inter-individual variability of 5-FU exposure and that in digestive cancer, most of patients treated with 5-FU are underexposed to the drug even when treated with the standard regimen [7]. At usual dosage, around 50% of patients displays low drug exposure, when targeting a 5-FU area under the curve of drug concentrations (AUC) exposure of 20-30 ng.h/mL [7]. Consequently, the risk for low 5-FU exposure might be further increased due to the DPD phenotyping. It is of utmost importance since 5-FU exposure has been related to overall response rate in a randomized multicenter study conducted in metastatic colorectal cancer patients [8].

The aim of the present study was to assess the relationship between DPD phenotype and the 5-FU exposure during the first treatment cycle.

We included in the analysis patients treated for digestive or head and neck cancers in Rennes University Hospital and the Eugène Marquis Anticancer Center, with a regimen including continuous infusion of 5FU. Data were collected from April 2019 to June 2020 based on the routine follow-up of patients. The study was approved by the local ethical committee (authorization n° 20.98). DPD phenotyping was performed by measuring uracil concentration in plasma (uracilemia) before the chemotherapy according to national recommendations [4,5]. Uracilemia was measured using a validated high performance liquid chromatography tandem mass spectrometry method in the Rennes University Hospital laboratory. At the first 5-FU cycle (C1), concentration of 5-FU in plasma was determined in a sample collected during the infusion (between 18h after the start of the infusion and 4h before the end) in order to measure 5-FU at steady state according to Adjei *et al.* [9]. Because 5-FU is very unstable *ex-vivo* in the blood sample (due to the remaining DPD metabolism), a DPD inhibitor was added in the sample immediately after blood withdrawal. Then, 5-FU was quantified by an immunoassay [10]. Genotype of *DPYD* (the gene encoding the DPD) was available when it was requested by clinicians for patients who consented to this analysis. The following demographic and therapeutic parameters were collected routinely and prospectively: age, sex, weight, chemotherapy regimen, dose of 5-FU continuous infusion at C1.

A total of 42 patients were enrolled in the study. Their characteristics are reported in Table 1. No linear correlation was found between uracil concentration and 5-FU clearance or 5-FU exposure at C1 (Figure 1). Furthermore, sixty-six percent of patients had 5-FU AUC below the therapeutic threshold of 20-30 ng.h/mL at C1. The rate of underexposed patients according to DPD status revealed that 5-FU underexposure trended to be more frequent in patient with partial DPD deficiency than in patients without DPD deficiency (75% vs 57% in uracilemia $\geq$ 16 ng/mL and < 16 ng/mL respectively. p=0.28, F-test) (Figure 2). Besides, according to the recommendations, all patients with pre-therapeutic uracilemia $\geq$ 16 ng/mL received a decreased dose of 5-FU at C1 (of at least -20%) compared to the protocol-specified dose. In patients without DPD deficiency, only 13% received a decreased dose at C1.

Systematic screening of DPD deficiency before any 5-FU (or other fluoropyrimidine) based treatment is crucial to prevent rare but early and highly severe 5-FU related toxicities. In France, this screening is now mandatory by the phenotyping approach based on uracilemia. Indeed, several studies have shown that uracilemia was a surrogate marker of 5-FU early toxicity [5]. The range of uracil concentrations associated with partial DPD deficiency is wild (16-150 ng/mL) and to date, no algorithm is available to choose the appropriate starting 5-FU dose based on uracilemia. Thus, it is challenging for clinicians and laboratory scientists to accurately determine the magnitude of the dose decrease to apply at C1. Usually, an empirical reduction of 20 to 30 % of the standard dose is applied for patients with moderate increase of uracilemia (16-40 ng/mL). In the present work we reported that this strategy tended to worsen the proportion of patients underexposed to 5-FU especially when uracilemia was slightly high as the majority of DPD deficient patients from our cohort. Despite differences were not statistically significant, likely due to the small sample size of our cohort, we wanted to alert readers on the potential risk of 5-FU underexposure meaning that more than never patients would benefit of 5-FU plasma concentration measurement as early as C1. The recent work from Chamorey *et al.* supports this statement, since this team evidenced that patients with high DPD activity (so likely low 5-FU plasma exposure) had poorer overall survival, progression free survival and response rate [11]. We admit that a limit of our work is the lack of data regarding patient toxicity

at C1. Guidelines clearly state that if a dose reduction was applied at C1, the dose should be increased at the next cycle when the first cycle was well tolerated, in order to maximise the treatment efficacy [5]. However, it can be challenging and take several cycles to find the patient's optimal 5-FU dose in this context. It appears much more efficient to monitor 5-FU concentration in plasma at C1 and at the next cycle if needed, since a validated dosage algorithm is available to safely choose dose the increase leading to 5-FU therapeutic exposure [10]. Thus, therapeutic drug monitoring of 5-FU is a time saving approach, easy to implement in clinical practice and strongly recommended to optimized and individualized 5-FU dosages [7]. Hence, to our point of view, the required DPD phenotyping approach aiming at preventing toxicities in patients with deep DPD deficiency should be systematically associated with 5-FU exposure evaluation using therapeutic drug monitoring, otherwise it could be detrimental for the treatment efficacy in a significant proportion of patients who had no toxicity following the first cycle of 5-FU based chemotherapy.

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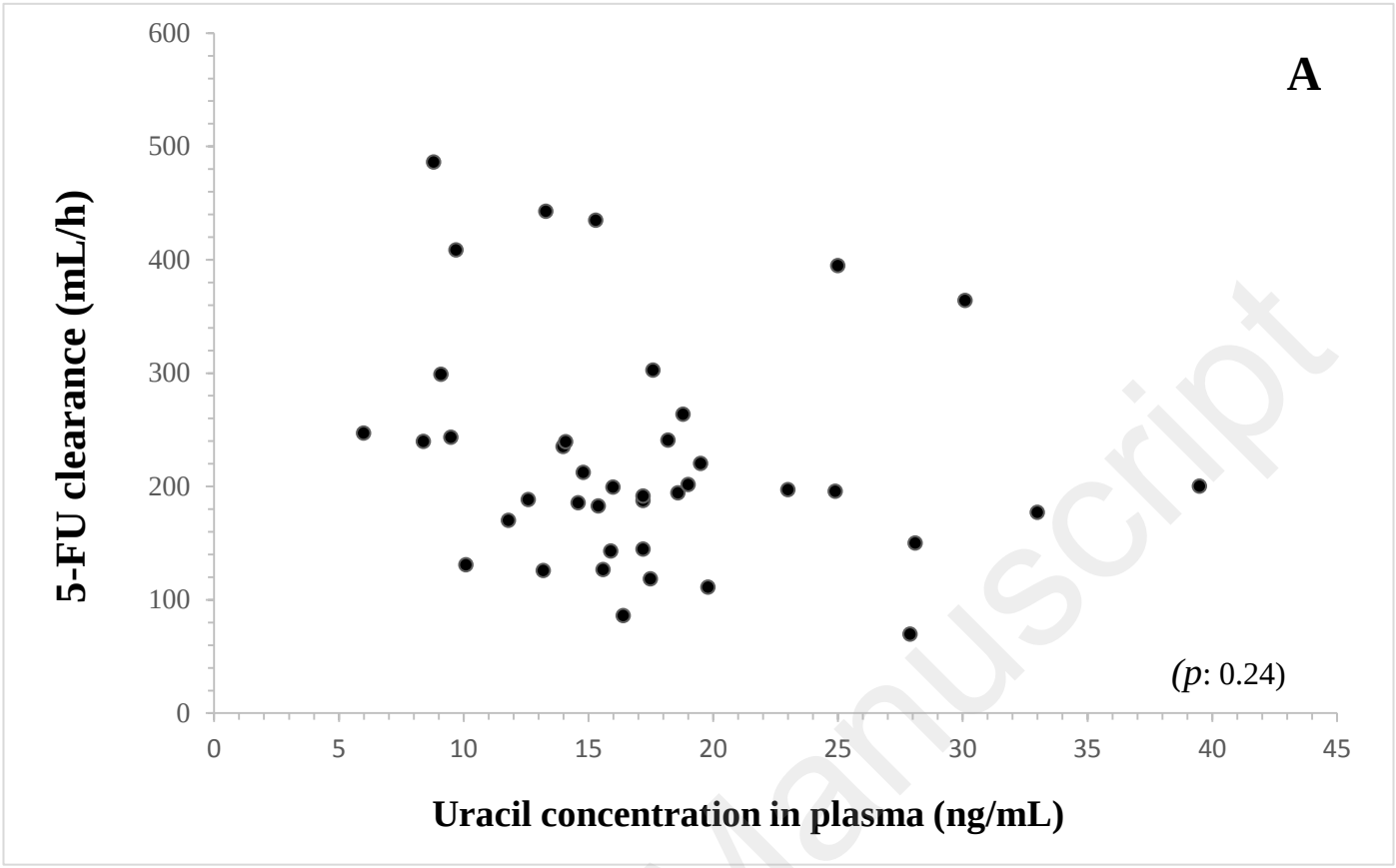
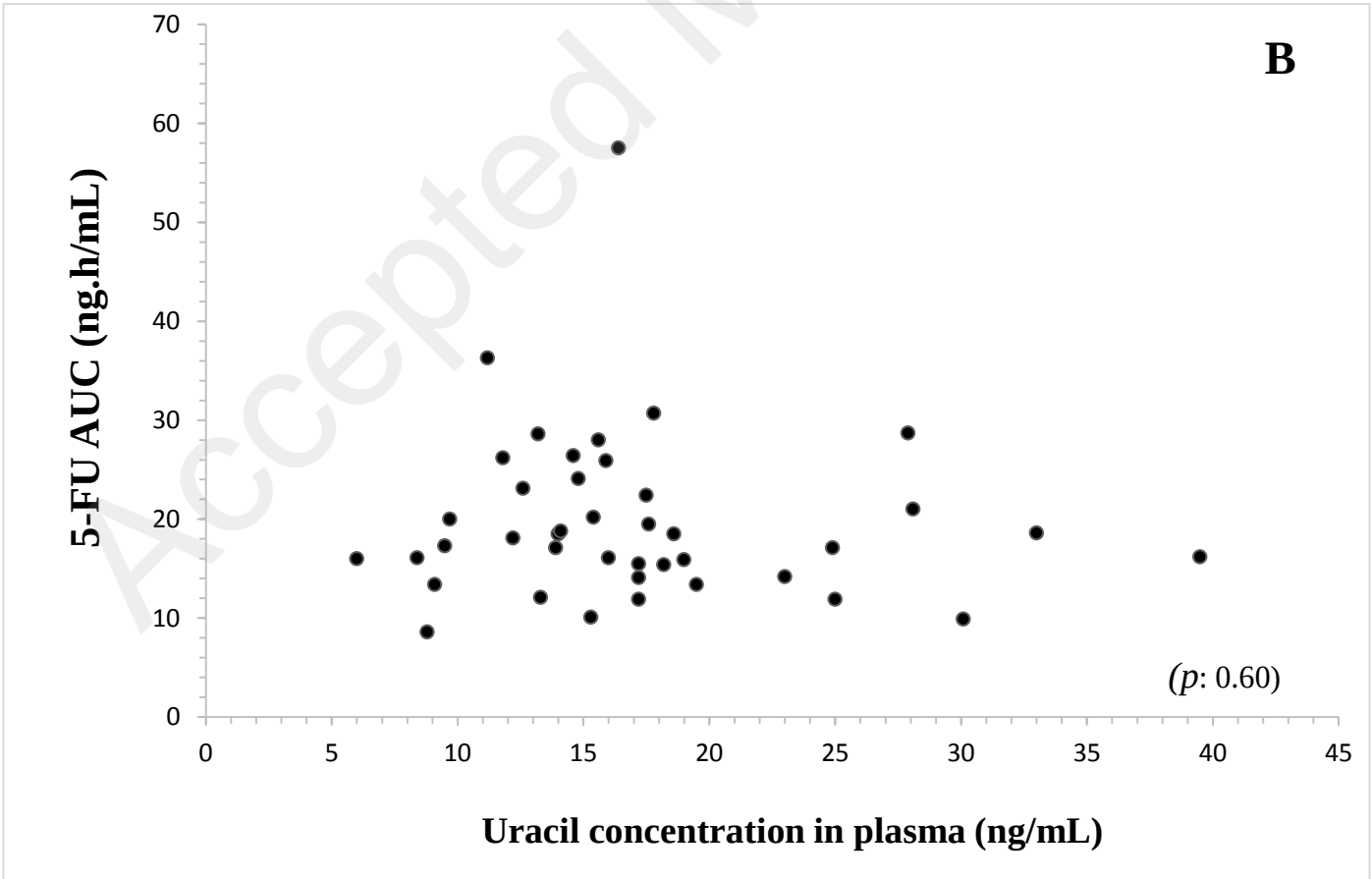
Figures legends:

Figure 1: Relationship between uracilemia and 5-FU clearance (A) or exposure (B) at first cycle. *p*: p-value pearson correlation test. Clearance was calculated as follow : Dose 5FU continuous infusion (mg) / AUC 5-FU (mg.h/mL).
5-FU: 5-fluorouracil; AUC: area under the curve of drug concentrations

Figure 2: Percentage of patients with 5-FU AUC values below, within or above the therapeutic range at first cycle depending on the DPD activity status
5-FU: 5-fluorouracil; AUC: area under the curve of drug concentration; DPD: dihydropyrimidine deshydrogenase





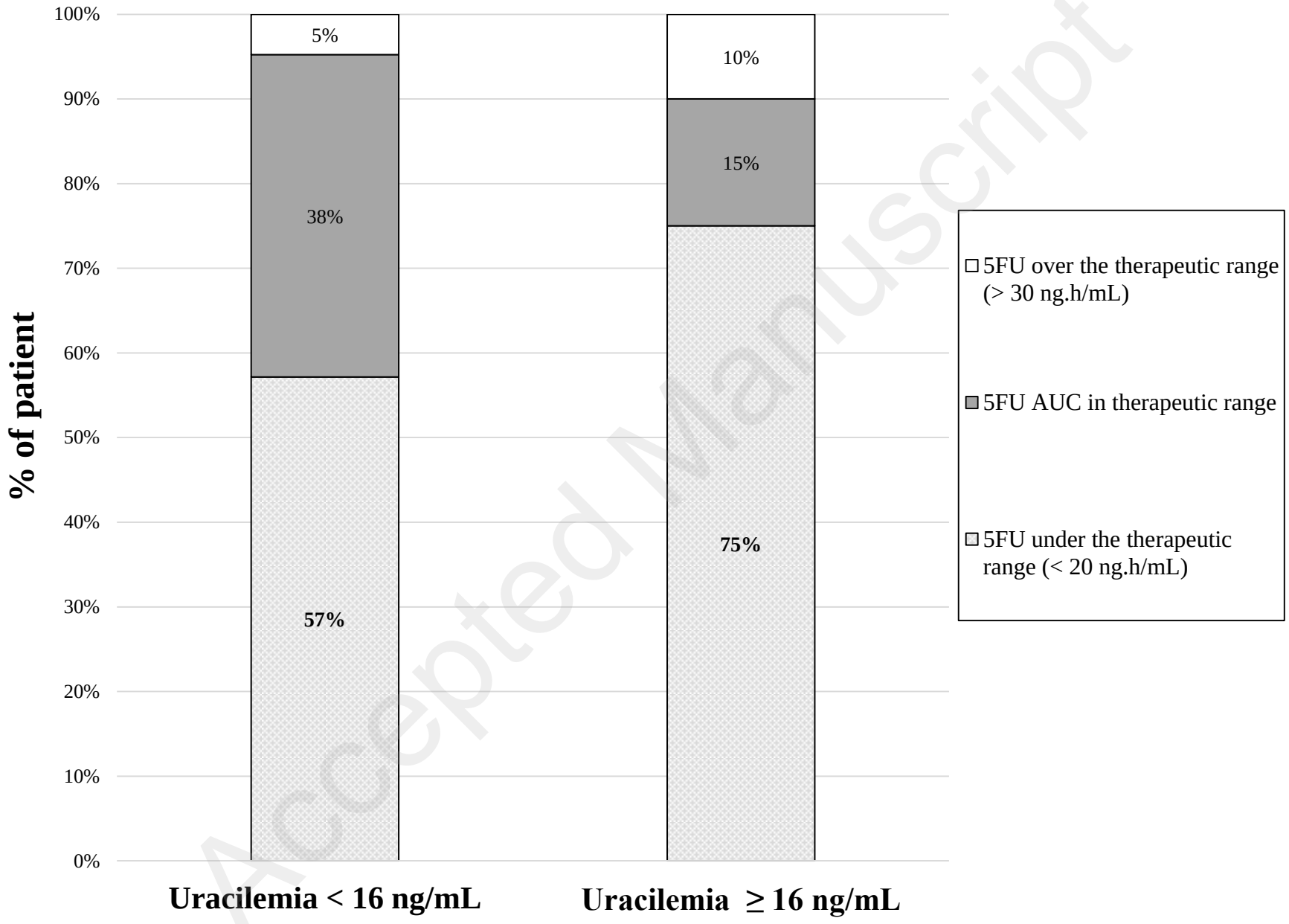


Table 1: Patients characteristics (n=42)

Age (years) (mean±SD)		66 ± 9.6
Sex		
	M (n)	31
	W (n)	11
Weight (kg) (mean±SD)		68.3 ± 14.5
Indications		
	Digestive cancer (n)	39
	Head and neck cancer (n)	3
5-FU protocol (n)		
	carboplalin-5FU	3
	FOLFIRI	1
	FOLFIRINOX	10
	FOLFOX	26
	LV5FU2	1
	DCF	1
***DPYD* Genotype**[a]		
	No variant (n)	18
	hapB3, c.1129-5923C>G (heterozygous) (n)	2
5-FU dose at C1 (mean (CV))	mg/m^2	2094 (22%)
	mg	3768 mg (24%)
5-FU exposure (AUC in ng.h/mL) (mean (CV))		19.3 (43%)
Uracile		
	Mean (ng/mL)	17.5
	Min-max (ng/mL)	6-39.5
	No DPD deficiency (%) (Uracilemia <16ng/mL)	51
	DPD deficiency (%) (Uracilemia ≥16ng/mL)	49

5-FU : 5 fluorouracile ; C1: first cycle; FOLFIRI (leucovorin + 5FU + irinotecan); FOLFIRINOX (leucovorin + 5FU + irinotecan + oxaliplatin); FOLFOX (leucovorin + 5FU + oxaliplatin); LV5FU2 (leucovorin + 5FU) ; DCF (docetaxel + cisplatin + 5FU) ; M: men ; W: women ; AUC: area under the curve ; SD: standard deviation ; CV: coefficient of variation; DPYD: gene encoding dihydropyrimidine deshydrogenase

[a]The four variants of *DPYD* included in the analysis were : rs55886062 (*13, no function allele), rs3918290(*2, no function allele), rs67376798 (decrease function allele), rs75017182 (HapB3, decrease function allele)