A case of a life-threatening toxicity following capecitabine treatment: advocacy for dihydropyrimidine dehydrogenase deficiency screening.

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Summary

We discuss a life-threatening case of capecitabine toxicity due to the presence of a heterozygous variant on exon 14 (c.1905+1G>A, rs3918290) of the dihydropyrimidine dehydrogenase gene (*DPYD*). We advocate the need for dihydropyrimidine dehydrogenase deficit screening, which could become mandatory in Belgium, as in France, before any fluoropyrimidine administration to avoid cases of foreseeable toxicity.

Introduction

Severe fluoropyrimidine (*e. g.* 5-fluorouracil and capecitabine) toxicity is common (10-40%) and potentially life-threatening (3-5%) or lethal (0.2-0.8%) (1, 2). It is characterized by myelosuppression, mucocutaneous manifestations (alopecia, dermatitis, palmo-plantar erythrodermia, oro-pharyngeal ulcers) and gastrointestinal involvement (nausea, vomiting and intractable diarrhea). The vast majority of these adverse events are due to partial (or complete) dihydropyrimidine dehydrogenase (DPD) deficiency, which can be unmasked either by genotyping the corresponding gene (*DPYD*) (3) or phenotyping the DPD enzyme by functional tests (4). The primary objective of the screening is to detect the few completely deficient individuals (0.01-0.5% of the population), who will die after a first course of fluropyrimidine, and the more common partially deficient individuals (3-8%), who may experience life-threatening toxicity.

In France, since 2019, screening for DPD deficiency has been made mandatory before fluropyrimidine administration. In Belgium, the screening tests are also available and will probably become mandatory soon following European Medicine Agency (EMA) recommendations.

Here, we briefly discuss the different DPD deficiency screening strategies, in light of a recently observed real-world case.

Case report

A 42-year old woman was admitted to the hospital on October 21, 2019 for fever and intractable diarrhea (up to 15 stools/day) after a first cycle of capecitabine (1500mg twice daily for 14 days) prescribed for metastatic triple negative breast cancer with lymph node and bone involvement. The initial cancer was diagnosed in December 2016. As adjuvant chemotherapy she received dose-dense epirubicine/cyclophosphamide and weekly paclitaxel without any complications. Two years later she relapsed and was treated within the frame of a clinical trial aimed at testing the combination of carboplatine, placitaxel, anti-PDL-1 durvalumab, with or without anti-CD73 (adenosine-generating enzyme) oleclumab (*Synergy* trial). Due to disease progression, the patient was withdrawn from the trial and oral chemotherapy with capecitabine was started on October 7, 2019, given the lack of any other trial at that time. At Day 14 of the first cycle, she was admitted through the emergency room, for febrile neutropenia. Her ECOG was 3. Severe ulcerated, necrotic oral and genital lesions were observed. Clinical examination was otherwise normal. Grade 4 neutropenia was diagnosed (90 neutrophils/μl), together with Grade 2 anaemia (Hb: 9.2 g/dl) and Grade 1 thrombocytopenia (77,000/μl). CRP (38.5 mg/L; normal values <5 mg/L) and LDH (494 U/L; normal values < 243 U/L) levels were marginally elevated. Despite immediate treatment with piperacillin/tazobactam (4x4g/500 mg) and filgrastim (30 MU/day), patient's condition did not improve, with persistence of fever and Grade 4 neutropenia (nadir: 0/μl), thereby leading to the addition of vancomycin (1,800 mg/day) and fluconazole (200 mg/day), shift from piperacillin/tazobactam to meropenem (3x1 g) and doubling of filgrastim dose (30 MU twice daily). Parenteral nutrition was required as well as administration of RBC packs, platelets and fresh plasma. Two weeks after admission, neutrophils started to rise (89/μl) and were normalized on day 16 (2,850/μl). Oral and genital ulcers healed but hand and feet desquamation occurred. Patient left the hospital on day 17 after complete recovery.

Subsequent genotyping of the dihydropyrimidine dehydrogenase gene (*DPYD*) revealed the presence of a heterozygous variant on *DPYD* exon 14 (c.1905+1G>A, rs3918290) predisposing to toxicity of fluoropyrimidine-based chemotherapy. This led to a review of current and foregoing recommendations for DPD deficiency screening.

Discussion

This case illustrates a severe capecitabine toxicity, with an unusual 14 day-lasting period of myelosuppression, due to the presence of *DPYD* variant. Capecitabine is an orally administered fluoropyrimidine drug which is metabolized *in vivo* in 5-flurouracil (5-FU), the latter being further transformed in active cytotoxic nucleotides (5). Inactivation of 5-FU is mainly controlled by an enzyme, called dihydropyrimidine dehydrogenase (DPD). If the corresponding *DPYD* gene bears a least one loss-of-function variant, 5-FU accumulates and more active metabolites are produced. *DPYD* gene is located on chromosome 1 and contains 23 exons. More than 100 variants have been described but, so far, only four variants have been clinically associated with increased toxicity of fluoropyrimidines, mainly in Caucasians, and are recommended with a "strong evidence level" by the *Clinical Pharmacogenetics Implementation Consortium* (CPIC) for DPD deficiency screening strategies (6). Homozygous variant carriers usually do not survive after standard fluoropyrimidine exposure (1, 2), while heterozygous carriers may experience severe life-threatening adverse events. Table I summarizes the 4 most common *DPYD* variants, the estimated percentages of hetero- and homozygous variants carriers and the partial or total loss of enzymatic activity due to the variant.

The CPIC has computed a fluoropyrimidine metabolizer score based on the results on *DPYD* genotyping, which should be used to adapt the drug doses (6). Each of the two *DPYD* genes is given a score of 1 if none of the four variants are detected, a score of 0.5 if one of the two variants associated with partial loss (*vide supra*) is present and a score of 0 if one of the two variants associated with complete loss is present. The total score (in a wild-type patient) is 2. Based on the score calculated in a patient elected for treatment with fluoropyrimidines, the following dose reductions should apply : normal dose if score = 2 ; 50% dose reduction if score = 1 or 1.5 (at least for the first two doses; doses may be increased later in the absence of adverse effects according to patient tolerance); no treatment with fluoropyrimidines if score = 0 or 0.5. Of note, these recommendations apply only to a Caucasian population. The patient whose clinical case is described here was genotyped *DPYD*1/*2A* (activity score 1) and, according to the CPIC recommendations, should therefore

have received an initial dose reduced by 50% compared to the standard dose during the first cycles of treatment. It should be stressed that targeted genotyping of only 4 *DPYD* variants does not exclude the presence of other rare variants that could induce a DPD poor-metabolizer status. Full sequencing of the *DPYD* gene would allow the detection of such rare variants but the genotype/phenotype relation could still be uncertain in these cases. Moreover, the turn-around time (TAT) of the full *DPYD* sequencing should remain compatible with its use in pre-emptive DPD deficiency screening [maximum 10 days according to *Haute Autorité de Santé* (HAS) recommendations] (7).

Another approach to diagnose DPD deficiency is to measure the *in vivo* functional activity of the enzyme by determining the plasma level of uracil (U) and the dihydrouracil (UH2)/uracil ratio. Uracil is a pyrimidine base which is metabolized in dihydrouracil by DPD. A lower DPD activity will result in higher levels of plasma uracil and lower UH2/U ratios. According recent HAS recommendations, it is considered that a U plasma level <16 ng/ml is suggestive of a normal DPD activity. At the opposite, a full DPD deficient patient would have an uracil plasma level >150 ng/ml, thereby contra-indicating the use of fluropyrimidines. Values between 16 and 150 ng/ml would suggest partial DPD deficiency and should lead to fluoropyrimidines dose reduction after a “clinical-biological dialogue” (7). These uracilaemia cut-offs, proposed by HAS, still need further validation in the real-world practice. This phenotyping test was also applied to our patient with the following results: uracilaemia measured at 16.4 ng/ml, UH2 at 78,0 ng/ml and UH2/U ratio at 4.8. Although the uracilaemia value is higher than 16 ng/ml, the measured value is still quite low in relation to the genotype status of the patient (heterozygous with a complete loss of function gene) and to the clinical situation. This therefore raises the question of the relevance and validation of the currently HAS recommended cut-offs. From this point of view, it is interesting to emphasize that the value of 16 ng/ml was proposed on the basis of a paper where fluoropyrimidine toxicities were still significantly increased at uracilaemia levels between 14 and 16 ng/ml (8). Therefore a 14 ng/ml threshold for uracilaemia would certainly be more appropriate not only in view of the current case report but also in relation to the experience acquired in the determination of plasma uracil with more than 90% of the population below 14 ng/ml. Anyway, even with an uracilaemia value very close to the proposed cut-off, the

UH2/U ratio left no doubt about the presence of a DPD deficiency in our patient. Indeed, UH2/U ratios are always greater than 10 in patients with normal DPD activity. This clinical case therefore illustrates the importance of taking into account not only the value of uracilaemia but also the value of the UH2/U ratio for a correct interpretation of the results. Compared to the genotyping approach, it must be stressed that the main limitation of the phenotyping test is the very strict pre-analytical requirements. Indeed, U level rapidly increases in whole blood mainly when the sample is kept at room temperature and the maximum delay for centrifugation and plasma freezing is 90 minutes after blood collection. Furthermore, precise recommendation regarding dose reduction for patients with U values between low (14 ng/ml) and high cut-off must always be validated in prospective studies.

Conclusion

Fluoropyrimidines have been used for decades without performing tests aimed at detecting individual susceptibility towards severe adverse events. Recent unravelling of the genetic control of fluoropyrimidine metabolism lead to wide availability of screening tests. From an ethical perspective, it would be – in our view – unfair not to offer this screening to cancer patients who could therefore escape major treatment-related side effects. From an economical viewpoint, it should be stressed that the cost linked to life-threatening adverse events may well overcome the cost of systematic screening.

Key messages for clinical practice

- Fluoropyrimidine toxicity is life-threatening in 3-5% of patients and potentialy lethal in 0.2-0.8%.
- Toxicity is often related to partial or complete dihydropyrimidine dehydrogenase deficiency, which can be unmasked either by genotyping (only validated in Caucasian patients) of the corresponding gene (*DPYD*) or phenotyping of the enzyme by functional tests.
- Dose adjustments are recommended based on the results of the screening tests.
- Assessment of partial or complete dihydropyrimidine dehydrogenase deficiency may become mandatory, based on forthcoming European recommendations.

Table I : *DPYD* variants and hetero- and homozygous carrier rates in Caucasians

Variants	Percentage of heterozygous carriers	Percentage of homozygous carriers	Loss of enzymatic activity
*DPYD**2A (IVS14+1G>A,c.1905+1G>A, rs3918290)	1,5%	0,01%	Complete
*DYPD*13* (c.1679T>G,p.I560S, rs55886062)	0,2%	0,0001%	Complete
c.2846A>T (p.D949V, rs67376798)	1%	0,004%	Partial
HapB3 (3 SNPs) (c.1129-5923C>G, rs75017182, c.1236G>A, p.E412E, rs56038477 and c.483+18G>A, rs56276561	4,6%	0,06%	Partial

After INCa, HAS (7).

References

1 : Loriot M.A, Ciccolini J., Thomas F., *et al*. Dihydropyrimidine dehydrogenase deficiency screening and securing of fluoropyrimidine-based chemotherapies: Update and recommendations of the French GPCO-Unicancer and RNPGx networks. Bulletin du cancer 2018; 105: 397-407.

2 : Boisdron-Celle M., Capitain O., Faroux R., *et al*. Prevention of 5-fluorouracil-induced early severe toxicity by pretherapeutic dihydropyrimidine dehydrogenase deficiency screening: Assessment of a multiparametric approach. Semin Oncol 2017; 44: 13-23.

3 : Wigle T.J., Tsvetkova E.V., Welch S.A., *et al*. DPYD and fluorouracil-Based Chemotherapy : Mini Review and Case Report. Pharmaceutics 2019; 11: 199

4 : Gamelin E., Boisdron-Celle M., Guérin-Meyer V. *et al*. Correlation between uracil and dihydrouracil plasma ratio, fluorouracil (5-FU) pharmacokinetic parameters, and tolerance in patients with adanced colorectal cancer: a potential interst for predicting 5-FU toxicity and dertermining optimal 5-FU dosage. J Clin Oncol 1999; 17: 1105-1110.

5 : Miura K., Kinouchi M., Ishida K., *et al*. 5-FU Metabolism in Cancer and Orally-Administrable 5-FU Drugs. Cancers 2010; 2: 1717-1730.

6 : Amstutz U., Henricks L.M., Offer S.M., *et al*. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing : 2018 Update. Clinical Pharmacology & Therapeutics, November 2018.

7 : Recherche de déficit en dihydropyrimidine déshydrogenase en vue de prévenir certaines toxicités sévères survenant sous traitement comportant des fluoropyrimidines (5-fluorouracile), Recommandations et référentiels, Institut National du Cancer, Haute Autorité de Santé, France, Decembre 2018. www.has-sante.fr

8: Meulendijks D., Henricks L.M., Jacobs B.A.W., *et al*. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated Toxicity. Br J Cancer 2017; 116: 1415-1424.