Letter to the Editors

Lethal outcome of 5-fluorouracil infusion in a patient with a total DPD deficiency and a double *DPYD* and *UTG1A1* gene mutation

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5-Fluorouracil (5-FU)-based chemotherapy has been widely used for almost 50 years in the treatment of solid malignancies, especially in digestive cancers. Its main effect consists in the inhibition, via its active metabolite, of thymidylate synthase, a major enzyme involved in DNA synthesis. In normal conditions, less than 20% of the administered 5-FU is used in this anabolic pathway while more than 80% is catabolized via dihydropyrimidine dehydrogenase (DPD) [1]. This is the initial and rate-limiting enzyme in the catabolic pathway and therefore a partial or complete DPD deficiency is associated with different degrees of 5-FU toxicity whose most frequent manifestations include neutropenia, mucositis and diarrhoea [2].

The study of the polymorphism of the *DPYD* gene that encodes for DPD showed at least 39 different mutations, half of them resulting in a significantly decreased enzyme activity. The most frequently described mutation is a single nucleotide polymorphism (SNP) in intron 14, with a G→A translation: IVS14+1G>A leading to the lack of a sequence (165-bp) in the corresponding mRNA and to the synthesis of abnormal non-functional DPD [3, 4].

Screening for the presence of the IVS14+1G>A mutation in *DPYD* in a general Caucasian population showed that from 0.94% to 2.4% were heterozygotes, and from 1/18 000 to 1.2/10 000 were homozygotes [5–7]. While DPD activity is reduced in heterozygote patients, it is non-existent in homozygote patients. In such patients, 5-FU administration may generate severe toxicity. Therefore either dose-adaptation needs to be considered or non-fluoropyrimidine-based chemotherapy should be prescribed.

There have been an increasing number of articles reporting severe (including lethal) 5-FU toxic side-effects

due to DPD deficiency [7–9]. However, the relative complexity of the screening tests and their lack of availability in the majority of centres do not allow the introduction of these tests into routine clinical practice.

In our centre, given the fact that the majority of patients with metastatic colorectal cancer are likely to receive irinotecan during the course of their disease, we perform systematically a simultaneous screening for *DPYD* and *UTG1A1* (the gene responsible for metabolism of irinotecan). This appears a reasonable approach especially because it does not require an additional blood sample.

Here we report the first to our knowledge published case of lethal 5-FU toxicity related to an IVS14+1G>A homozygote mutation in *DPYD* gene associated with the TA7/7homozygote mutation in the in *UTG1A1* gene promotor.

A 75-year-old male patient presented with an abscessed sigmoid lesion with a suspected liver tumour. The lesion was diagnosed as an infiltrated, moderately differentiated adenocarcinoma and the patient underwent colonic resection according to Hartmann's procedure. The histological analysis of the surgical specimen confirmed an adenocarcinoma, classified pT4 pN0 pM1 due to the existence of two synchronous liver metastases of 20 mm of diameter each, confirmed by computed tomography.

Following the decision of the multi-disciplinary team, first line metastatic chemotherapy was proposed to the patient. A secondary hepatectomy was planned in a case of a good response. At this time, the patient was in good shape with a performance status (PS) of 1. Chemotherapy started 4 weeks after surgery and consisted of oxaliplatin (85 mg m⁻² 2 h⁻¹ infusion), calcium levofolinate (200 mg m⁻² 2 h⁻¹ infusion), and 5-FU (400 mg m⁻² 15 min⁻¹ infusion and 2400 mg m⁻² 48 h⁻¹ infusion), according to the

FOLFOX 6 protocol, which should be repeated every 2 weeks.

The second day after the first infusion, the patient developed odynophagia. Then, his general status started to deteriorate rapidly (PS = 3) with aggravation of odynophagia, appearance of stomatitis and diarrhoea. At day 7, he was admitted to the hospital with symptoms of dehydration, functional renal insufficiency and multivisceral failure. A hypothesis of a septic shock was first adopted. The patient was admitted to the Intensive Care Unit and treated with a broad-spectrum antibiotic (amikacin and imipenem), rehydration and haemodynamic support with dopamine. The following day, his general status deteriorated significantly, his renal function worsened and he developed leucopenia (WBC count = 500 mm^{-3}) and thrombocytopenia ($46\,000 \text{ mm}^{-3}$). No infectious entry could be found and all bacteriological samples were sterile. Given the clinical presentation and association with the recent 5-FU administration, the existence of DPD deficiency was suspected. Accordingly, blood samples were obtained and sent to the Department of Onco-Pharmacogenetics of Cancer Center Papin in Anger for analyses. Informed written consent for the pharmacogenetic testing was obtained from the patient's family (the patient being unconscious and unable to sign this consent by himself). Despite intensive medical care, the patient died 2 days later, at day 10 after the initial 5-FU infusion.

Heparinized blood samples (5 ml) were obtained for phenotype (UH_2/U), DNA isolation and determination of genotypes.

For the genotype determination, peripheral blood mononuclear cells were isolated by centrifugation of the blood at 3500 g for 15 min. DNA was extracted using the DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Molecular Diagnostics, Meylan, France). Each sample was controlled with respect to DNA isolation by UV-transillumination of ethidium bromide-stained gels from subsequent electrophoretic separation in 1.2% agarose. The detection of SNP was performed according to a previously published real-time pyrosequencing method [10].

The phenotypic study included the measurement of uracil (natural substrate for DPD) plasma concentration and the measurement of the dihydrouracil:uracil ratio ($\text{UH}_2:\text{U}$) in plasma. Both dihydrouracil and uracil concentrations ($\mu\text{g l}^{-1}$) were simultaneously determined in plasma by an improved liquid chromatography method, and their ratios were assessed as previously described [11]. The coefficients of variation of this technique were $< 10\%$ and the limits of determination of this method were 1.25 and 0.625 ng ml^{-1} [11].

Additionally, a search for the mutation in the promoter of the *UTG1A1* gene, coding for the enzyme responsible for metabolism of irinotecan, was also performed by pyrosequencing analysis, as described previously [12].

The genotypic study revealed that the patient presented a homozygote IVS14+1G>A mutation. The phenotypic study showed a uracil plasma concentration of $16\,480 \mu\text{g l}^{-1}$ (normal median value = $13 \mu\text{g l}^{-1}$) and dihydrouracil:uracil ratio of 0.002 (normal median ratio = 7.5) [13]. Additionally, the patient also presented a TA7/7homozygote mutation in the *UTG1A1* promotor. *UTG1A1* is responsible for the metabolism of irinotecan, frequently used in combined chemotherapy in digestive tumours.

It is noteworthy that the screening test was offered to all three of the patient's daughters and all of them showed, as suspected, a heterozygote IVS14+1G>A mutation. A few months later, breast cancer was diagnosed in one of them. Given the existence of the known mutation, a non-fluoropyrimidine-based chemotherapy was introduced preventing the risk of severe 5-FU-related toxicity.

This is, to our knowledge, the first report of a case of lethal toxicity to 5-FU in a patient presenting a double homozygote mutation in *DPYD* and *UTG1A1* genes, coding for the enzymes responsible for metabolism of 5-FU and irinotecan, respectively.

5-FU is one of the most frequently used drugs in chemotherapy, either in infusion or in oral form (UFT and capecitabine). Several toxic-related deaths and cases of severe toxicity related to DPD deficiency have been reported and numerous studies aimed to determine the predictive markers of this toxicity but so far, none of these markers has been validated for use in clinical practice. The two most reliable tests that can predict 5-FU-toxicity are genotypic study or phenotypic assessment (uracil plasma concentration and dihydrouracil:uracil plasma ratio). About 60% of patients who develop severe 5-FU-associated toxicity have a decreased DPD activity [2, 14] with heterozygote or homozygote *DPYD* gene mutations. Moreover, it appears that patients with DPD activity $< 70\%$ of that observed in the normal population might be prone to develop severe 5-FU-associated side effects [15].

Some cases of lethal toxicity have also been reported [6, 7, 9, 15–17]. To our knowledge, most of them are due to heterozygote mutations, and only two cases concern homozygote IVS14+1G>A mutations [6, 7]. One case of a double mutation in the *DPYD* gene (IVS14+1G>A) and in the *UGT1A1* gene (TA, 6/7), has been reported by Steiner *et al.* [18]. These mutations resulted in partial deficiency of DPD and of *UGT1A1* responsible for a severe gastrointestinal and haematological toxicity after combined 5-FU/ folinic acid/irinotecan treatment for a sigmoid colon adenocarcinoma, with a lethal outcome after the second drug administration. Compared with our case, however, the patient was heterozygous for both mutations.

Here we report, to our knowledge, the first published case of a double homozygote mutation in IVS14+1G>A and *UTG1A1* genes. Although detection of *UTG1A1* gene mutation, which was searched for in a systematic manner, did not have a direct consequence for this patient who did not receive irinotecan, this analysis may be important for

the patient's family, and especially for his children, who may face the necessity of irinotecan administration in the future.

The most important message from this case report is that if the patient had been tested for DPD deficiency before 5-FU perfusion, he would have probably have been still alive today. Furthermore, the screening for DPD deficiency in his daughter has probably saved her from severe side-effects of fluoropyrimidine-based chemotherapy.

Considering the wide use of 5-FU chemotherapy and the relatively high prevalence of severe 5-FU toxicity due to DPD deficiency, DPD deficiency screening in cancer patients prior to the administration of 5-FU, should be considered in clinical practice. According to our experience, based on the published and unpublished data from over 8000 patients studied in our centre, a two-step strategy combining firstly SNP detection and uracil plasma measurement, followed, in cases where metabolic deficiency was suspected, by dihydrouracil:uracil ratio determination to confirm deficiency, allowed the reliable identification of DPD deficiency and avoided 5-FU-related toxicity [13]. Before introducing this approach into clinical practice, however, large randomized clinical studies are necessary to confirm efficacy and especially to evaluate the cost-effectiveness of this approach [19, 20]

Competing interests

There are no competing interests to declare.

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