Should Screening for DPD Deficiency Be Mandatory before 5-FU Exposure?

Toxicity from chemotherapy is a substantial clinical problem, with a major impact on patient comfort and safety. The spectrum of toxicity observed after systemic chemotherapy is wide and the severity highly variable. A broad spectrum of agents target rapidly dividing cells, resulting in diarrhoea, neutropenia, mucositis and alopecia. In exceptional circumstances, these toxicities lead to fatal outcomes, for example from neutropenic sepsis. Other drugs also bring relatively unique toxicities such as skin rash after gefitinib or capcitabine-associated palmar-plantar desquamation. Patients require additional care and therapy to ameliorate toxicities, translating to higher healthcare cost.

The fluoropyrimidines are antimetabolites that cause a deficiency in thymidine by inhibiting thymidylate synthase (TS). This results in failure of DNA synthesis leading to cell death. The prototypical drug is 5-fluorouracil (5-FU) which was developed in 1957 when it was observed that tumor cells were more efficient in using uracil for DNA synthesis than normal cells. 5-FU is metabolized intracellularly to 5-FUTP, and then to its active form 5-FdUMP. This has a greater affinity for TS than uracil forming a stable ternary complex with TS, and 5’10’ methylenetetrahydrofolate [1]. This results in inhibition of TS activity that is essential for production of the DNA precursor thymidine. Errorneous incorporation of 5-FUTP into RNA also interferes with RNA translation [2]. 5-FU is widely used in adjuvant therapy and palliative treatment of colorectal, gastric, pancreatic, head and neck, and cervical cancers. Dihydropyrimidine dehydrogenase (DPD) is the enzyme responsible for catabolism of 5-FU and is responsible for >85% of 5-FU elimination [3]. Deficiency of DPD due to polymorphisms of the DPD gene is a potential cause of such toxicity [4, 5]. DPD deficiency due to a genetic defect gives rise to severe 5-FU toxicity from reduced catabolism [6]. This pharmacogenetic ‘DPD syndrome’ manifests typically as severe or fatal diarrhea and mucositis/stomatitis and neutropenia/myelosupression. These side-effects are observed with the first or second dose of 5-FU. Hepatitis, encephalopathy, and acute cardiac ischemia are uncommon manifestations (table 1). This toxicity has been reported with bolus or continuous infusion 5-FU, and oral 5-FU pro-drugs [8]. There are now more than 30 described mutations in the gene encoding DPD (DPYD) [7]. It is unknown which of these mutations are of clinical consequence. Common polymorphisms have been described that are non-functional [9]. Also, there is no known clinical phenotype that prospectively predicts for someone who has DPD deficiency.

The most compelling reason to introduce routine DPD testing is to avoid severe toxicity in the many patients who receive 5-FU therapy. There are currently two ways to screen for DPD deficiency – measurement of DPD catalytic activity and genotyping for the IVS 14+1G → A (DPD*2A), the most commonly observed polymorphism associated with toxicity [10]. Measuring DPD levels is difficult. The tissue that has the greatest DPD activity is liver [11]. Liver biopsy, however, is associated with significant morbidity and mortality, making this approach of dubious clinical utility. Peripheral blood lymphocyte levels (PBL) of DPD have been correlated with liver tissue activity and can be used as surrogate tissues [12]. Unfortunately the sample methods are tedious and PBL levels of DPD have inconsistent correlations with 5-FU toxicity [13–15]. DPD activity also has a circadian rhythm with associated changes in 5-FU concentrations of up to 5-fold [16]. Time of sampling is hence important. Long assay time, requirement for radioisotopes, and high-performance liquid chromatography or mass spectrometry further limit the utility of this test to specialized centers only.

Routine screening for DPD*2A is specific for the mutation, but interpretation of this test is fraught with debate given the available published data. The prevalence of DPD*2A in polymorphisms in the population at risk is low (around 1%) [17].
Therefore screening 100 patients would yield only one patient who carries the mutation. From separate studies of DPD*2A in patients who had at least grade 3 toxicity from 5-FU treatment, two additional observations can be made [17, 18]: One is that the frequency of DPD*2A in patients with severe toxicity was highly variable. The DPD*2A polymorphism was present in only 15/41 patients with at least grade 3 or 4 toxicity in two studies [17, 18]. Therefore DPD deficiency attributable to this splice-site polymorphism [17, 18] and other known DPD polymorphisms do not fully explain 5-FU toxicity [19]. The excess variance can be attributed to several causes. Rare polymorphisms with corresponding low levels of DPD can cause severe toxicity [20]. Functional genetic polymorphisms in downstream enzymes such dihydropyrimidinase with normal wild-type DPD [21] are also reported to cause severe toxicity. DPD deficiency has also been described in patients with no known sequence variation in the coding region of the DPD gene [24]. Sequencing the entire gene for polymorphic variants is therefore not useful unless they can be associated with decrease in DPD activity. The second observation is that the specificity of DPD*2A in predicting low DPD activity was studied in a subset of patients with known grade III/IV toxicity, and not in a population of cancer patients at risk from 5-FU therapy. This gives us no information on the true predictive power of this test. In summary, the prevalence of DPD*2A in the population is low, the sensitivity of DPD*2A to detect decreased DPD activity is unknown, and testing for DPD*2A will only detect a small and variable proportion of patients who could suffer severe toxicity. For these reasons, its value as a tool for population-wide pharmacogenetic screening is diminished. Routine screening using currently available tests cannot be advocated for patients who need 5-FU therapy. What then should be done? Simple tests of DPD activity are needed. A novel 2-13C uracil breath test is being developed [24]. 2-13C uracil is ingested orally, and metabolized by the DPD pathway to 13CO2 that is exhaled by the lungs. The ratio of 13CO2 to 12CO2 in exhaled breath is then quantitated using an infrared spectrophotometer. Currently it requires patient cooperation for 3 hours to collect exhaled air at several time-points by blowing into a balloon. This test uses the same equipment to analyze the results as is widely available for diagnosis of Helicobacter pylori infection of the gastrointestinal tract. There may be difficulty implementing it in ill cancer patients, or institutions with limited access to such equipment. However, it is able to differentiate between patients with severe, partial and no DPD deficiency, and may represent a breath of fresh air in testing of DPD activity. A prospectively conducted screening trial of DPD*2A with concurrent measurement of DPD activity in a population of cancer patients treated with 5-FU is required. This will establish definitively the clinical utility (sensitivity and specificity) of this pharmacogenetic test in predicting both DPD activity and 5-FU toxicity in a population at risk. 

**Table 1. Features of 5-FU toxicity**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Associated with DPD deficiency</th>
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<tbody>
<tr>
<td>Mucositis</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>+</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>+</td>
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<tr>
<td>Palmar-planter syndrome</td>
<td>–</td>
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<tr>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>Ecephalopathy</td>
<td>+</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>–</td>
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<tr>
<td>Cardiac ischemia</td>
<td>–</td>
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</table>

Lazar and colleagues have done an excellent summary of the potential hazards of 5-FU-related toxicity in their case report [22]. Most interestingly, mucositis, diarrhea or neutropenia were not pre-eminent in this patient. These are by far more common manifestations of 5-FU toxicity, including those associated with DPD deficiency. Most papers that have described toxicity from DPD deficiency relate early-onset diarrhea, stomatitis, and myelosuppression. These serve as harbingers for severe 5-FU toxicity. This case also illustrates the difficulty of establishing firm genotype/phenotype correlations in the absence of a reliable test. DPD activity levels were not available. Sequencing the gene in their patient revealed two common missense mutations and combinations of polymorphisms that have been previously described [19]. The combination of G1601A and IVS13 G40A is a possible cause of the purported toxicity. Each of these polymorphisms is associated with a broad range of DPD activity, and alone may not be associated with a reduced DPD phenotype. However, the presence of both could account for reduced DPD activity [19]. The non-conserved amino acid change induced by G1601A is Ser534Asn and protein conformational analysis suggests that this could affect enzymatic function [23].

References

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