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Screening for dihydropyrimidine dehydrogenase deficiency to prevent severe 5-fluorouracil and capecitabine-associated toxicity

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5-Fluorouracil (5FU) and capecitabine are the cornerstones of all currently applied regimens for the treatment of patients with cancers of the gastrointestinal tract, breast, head and neck. Dihydropyrimidine dehydrogenase (DPD) plays a pivotal role in the metabolism of 5FU and as such, a deficiency of DPD has been recognised as an important risk factor, predisposing patients to develop severe 5FU-associated toxicity. In this manuscript, we discuss a wide range of methods that have been established to assess the genetic and functional status of DPD. Genotyping of *DPYD* is used to identify DPD deficient patients. However, its suitability for pre-treatment testing is under debate, not least due to conflicting genotype-phenotype relations in mutation carriers and relatively low positive predictive values. In addition to genetic screening, a number of phenotype-based methods have been introduced which appear to be well suited for clinical laboratories and which are an attractive option for monitoring of the DPD status. These phenotype-based screening approaches to detect DPD-deficient patients warrant further clinical validation.

Key-words: 5-fluorouracil, dihydropyrimidine dehydrogenase, DPYD

5-fluorouracil (5FU) and its oral prodrug capecitabine (Xeloda®) are two of the most frequently prescribed chemotherapeutic drugs for the adjuvant and palliative treatment of patients with cancers of the gastrointestinal tract, breast, head and neck (1, 2). For colorectal cancer, the addition of oxaliplatin to continuous infusion of 5FU and folinic acid (LV) has improved the 5 years disease-free survival and 6 years overall survival for stage III colon cancer only (3). Despite the demonstrated efficacy of adding irinotecan, bevacizumab or cetuximab to 5FU-based regimens in the treatment of patients with metastatic colorectal cancer, no improvement in survival outcomes has been observed in the adjuvant setting (4, 5). Thus, although 5FU has been introduced into clinical practice 50 years ago, it has remained the cornerstone and the most important component of all currently applied regimens.

However, therapeutic success is often limited by frequently occurring acute drug-adverse events. An analysis involving 974 patients with colorectal cancer treated with 5FU/leucovorin, according to the Mayo Clinic regimen, showed that grade III or IV neutropenia, stomatitis and diarrhea occurred in 26%, 14% and 13% of the patients, respectively (2). These severe toxicities often result in interruption of the chemotherapy and therefore an increased risk of disease progression. Regarding the high number of patients receiving 5FU-based therapies per year and the deleterious effects that are exerted by severe toxicities on their quality of life and disease cure, it is of major clinical interest to reduce the incidence of 5FU-related adverse events. In this respect, it has been shown that a dihydropyrimidine dehydrogenase (DPD) deficiency is a major determinant of severe 5FU-associated toxicity. DPD is the initial and rate-limiting enzyme in the degradation of the pyrimidine bases thymine and uracil but also of 5FU. Since more than 80% of the administered 5FU is catabolized by DPD, patients with a complete or partial DPD deficiency have a strongly reduced capacity to degrade 5FU and therefore, an increased likelihood of suffering from severe multivisceral toxicity, which may result in death (6, 7). To date, various strategies have been proposed to screen patients for a DPD deficiency and in this manuscript we describe the implications of using genotyping or phenotyping procedures.

Genotype-based screening procedures to identify DPD-deficient patients

Population studies have shown that the prevalence of a partial DPD deficiency in the general population is at least 3-5% (8, 9). To date, many mutations and polymorphisms have been described in the gene encoding DPD (*DPYD*) with the c.1905+1G>A mutation as the most commonly detected (52%) mutation in patients with a DPD deficiency (7, 10). In addition, there is a relatively high frequency of this mutation in the populations from Northern Europe, with a frequency of 1-1.8% in the German and Dutch population, respectively (7). Analysis of the prevalence of the various mutations in *DPYD*, in cancer patients experiencing severe toxicity, showed that the splice-site mutation c.1905+1G>A and the c.2846A>T (p.D949V) mutation are most commonly involved (7, 9, 11, 12). However, the prevalence of the c.1905+1G>A mutation in patients suffering from severe 5FU-associated toxicity varied considerably, ranging from 0-28% (13).

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Although ample evidence has been provided that carriers of the c.1905+1G>A have a strongly increased risk of developing toxicity, not all patients heterozygous for the c.1905+1G>A mutation develop severe toxicity after the administration of 5FU (9, 11). This phenomenon most likely reflects the fact that some heterozygous carriers of the c.1905+1G>A mutation possess low normal DPD activity (14, 15). The application of a genotype-based dose reduction strategy would result in undertreatment of such patients (16). In addition, the percentage of patients with severe toxicity correctly identified through screening for c.1905+1G>A mutation is low (table 1) (9, 11, 17, 18). The sensitivity of the genotype test is, however, increased when additional mutations are included (table 1).

A significant drawback of the genotyping approach is the fact that a significant number of patients with a reduced DPD activity, do not possess mutations in the coding part of *DPYD* (19-21). The observation that a *DPYD* haplotype not containing any nonsynonymous or splice-site mutations was associated with 5FU toxicity, suggested the presence of additional genetic variations in the noncoding region of *DPYD* (22). Subsequently, we showed that a deep intronic mutation (c.1129-5923C>G) affected pre-mRNA splicing and this mutation was significantly enriched in patients with severe 5FU-associated toxicity (23). In addition, we have shown that genomic deletions affecting *DPYD* occur in 7% of pediatric patients with a complete DPD deficiency and provide a molecular basis for cancer patients with a phenotypically established DPD deficiency (24). Thus, these observations demonstrate that screening for coding mutations alone cannot unambiguously identify all patients at risk.

Phenotype-based screening procedures to identify DPD-deficient patients

The advantage of phenotype-based procedures over the genotyping assay is that all genetic variations resulting in either a systemically altered DPD activity or altered 5FU metabolism will be detected with these approaches. Various phenotyping procedures have been proposed to screen patients for a DPD deficiency, including: 1) measurement of the uracil/dihydrouracil ratio; 2) the assessment of the DPD activity in peripheral blood mononuclear cells; 3) an oral loading test using (stable-isotope labeled) uracil, and 4) the application of a test dose of 5FU (6, 21, 25-30). Importantly, a recent clinical study suggested a clinical benefit for DPD deficient patients when the DPD phenotypic status is determined prior to treatment and subsequent dose-tailoring of 5FU is achieved (30).

Compelling results have shown that patients with a partial or complete DPD deficiency have a reduced capacity to degrade 5FU and are at risk of developing severe 5FU-associated toxicity (6, 31-34). We showed that pharmacokinetic 5FU profiling of patients treated with a dose of 5FU of 300 mg/m^2, using a single 5FU concentration at 60 min, may be useful for identification of DPD deficient patients in order to reduce severe toxicity (6). In addition, it is worthwhile to note that toxicity was observed in only 2 out of 30 patients heterozygous for the c.1905+1G>A mutation after the administration of the single dose of 5FU (6). The possibility of inflicting toxicity is prevented in case uracil, instead of 5FU, is administered to patients (29). It has been shown that the pharmacokinetics of uracil and dihydrouracil in patients with DPD deficiency differed significantly as compared to patients with normal DPD activity (29).

Although the results of loading tests to measure the *in vivo* capacity of DPD and the other enzymes of pyrimidine degradation pathway are promising, they do warrant further clinical validation. Accordingly, the measurement of the DPD activity in peripheral blood mononuclear cells remains the golden standard. The DPD activity in controls (9.9 ± 2.8 nmol/mg/h) and obligate heterozygotes (4.8 ± 1.7 nmol/mg/h) follows a normal or Gaussian distribution, with the mean DPD activity in individuals heterozygous for a pathological mutation in *DPYD* being 48% of that observed in controls (14). The fact that individuals heterozygous for a mutation in *DPYD* can have a (low) normal DPD activity might explain the observation that for patients heterozygous for the c.1905+1G>A mutation, still a wide variation in fluoropyrimidine tolerability has been observed (16). The importance of a DPD deficiency in the etiology of unexpected severe 5FU toxicity has been demonstrated by the fact that in 39-61% of the cases, a decreased DPD activity could be detected in peripheral blood mononuclear cells (14, 19, 35, 36). Interestingly, the mean DPD activity in peripheral

Table 1. Accuracy of classification of patients at risk of developing toxicity through analysis of a single or multiple SNPs in the DPD gene

Study	Treatment	Patients	SNPs	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Schwab et al (11)	5FU monotherapy	683	c.1905+1G>A	5.5	99	46	85
Deenen et al. (17)	[1]CAIRO2	567	c.1905+1G>A	1	100	100	0.15
			c.2846A>T	1	99	88	15
Morel et al. (9)	5FU-containing therapy	487	c.1905+1G>A, c.2846A>T, 1679T>G	31	98	62	94
Loganayagam et al. (18)	5FU-containing therapy	430	c.1905+1G>A	3	100	>99	78
			c.1601G>A,1905+1G>A, c.2846A>T, 1679T>G	23	100	>99	80

[1]CAIRO2: capecitabine, oxaliplatin, bevacizumab ± cetuximab.
Sens: sensitivity (percentage of patients with severe toxicity (≥ grade 3) correctly identified). Spec: specificity (percentage of patients with no toxicity (grade ≤ 2) correctly identified). PPV: positive predictive value. NPV: negative predictive value.

blood mononuclear cells proved to be increased in patients experiencing grade I/II neutropenia when compared to patients without neutropenia and those suffering from grade III/IV neutropenia (37). Thus, patients with a high-normal DPD activity proved to be at risk of developing mild toxicity upon treatment with 5FU-leucovorin, suggesting an important role of DPD in the etiology of toxicity associated with catabolites of 5FU.

Conclusion

DPD plays a pivotal role in the metabolism of 5FU and as such, a deficiency of DPD has been recognised as an important risk factor, predisposing patients to develop severe 5FU-associated toxicity. Considering the common use of 5FU in the treatment of cancer patients, pre-treatment screening for patients at risk is warranted. To this end, a wide range of methods has been established to assess the genetic and functional status of DPD. As specific sequence variations in the *DPYD* gene have been clearly associated with impaired breakdown of 5FU followed by severe toxicities, genotyping of *DPYD* is used to identify DPD deficient patients. However, its suitability to identify patients at risk is subject to debate, not least due to conflicting genotype-phenotype relations in mutation carriers and relatively low positive predictive values. In addition to genetic screening, a number of phenotype-based methods have now been introduced which appear to be well suited for clinical laboratories and which are an attractive option for (pretreatment) monitoring of the DPD status. Therefore, we feel that the phenotype-based screening approaches to detect DPD-deficient patients warrant further clinical validation.

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Samenvatting

van Kuilenburg ABP, Ferdinandusse S, Wanders RJA. Screening op dihydropyrimidine dehydrogenase deficiëntie om ernstige 5-fluorouracil en capecitabine-geassocieerde toxiciteit te voorkomen. Ned Tijdschr Klin Chem Labgeneesk. 2013; 38: 202-205.

5-Fluorouracil (5FU) en capecitabine zijn de meest gebruikte chemotherapeutica bij de behandeling van patiënten met colorectaal en borstkanker. Dihydropyrimidine dehydrogenase (DPD) vervult een belangrijke rol bij de afbraak van 5FU en patiënten met een DPD deficiëntie hebben een sterk verhoogd risico op het ontwikkelen van ernstige (letale) toxiciteit na toediening van een op 5FU gebaseerde chemotherapie. In dit artikel behandelen we een aantal mogelijkheden om patiënten te testen op een DPD deficiëntie waaronder genotypering en fenotypering. Het screenen op mutaties in het DPD gen kan DPD deficiënte patiënten identificeren maar er zijn nog onduidelijke genotype-fenotype relaties en een relatief lage voorspellende waarde m.b.t het ontwikkelen van toxiciteit. Naast genotypering zijn er nu diverse fenotypische methodes beschikbaar die geschikt zijn om DPD deficiënte patiënten te kunnen identificeren. Het grote voordeel van fenotypering is dat alle genotypische veranderingen die resulteren in een verlaagde DPD activiteit in principe kunnen worden opgespoord. Verdere klinische validatie van deze fenotypische testen is dan ook aan te bevelen.

Trefwoorden: 5-fluorouracil; dihydropyrimidine dehydrogenase; DPYD