DPD-deficiency and capecitabine-related toxicity: clinical relevance of measuring DPD-activity

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Abstract

Background. Capecitabine (Xeloda ®) is a widely used oral chemotherapeutic drug, which potentially could cause severe toxicity. This might be due to a deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD). Therefore it could be useful to screen every patient for DPD-deficiency before start of chemotherapy. The aim of this study was to determine the clinical relevance of DPD-deficiency in patients treated with capecitabine, by determining a cutoff level to predict toxicity and by defining the consequences concerning interventions of treatment.

Method. We included adult patients treated with capecitabine from October 2011 until March 2014 in two hospitals in the Netherlands. Two equal groups were identified, consisting of patients of whom the DPD-activity was determined or not. From all patients, data concerning capecitabine-related toxicity, interventions and basic demographic data were collected from the digital records.

Results. A total of 164 patients were analysed, of whom in 77 (47%) cases the DPD-activity was determined. DPD-deficiency was seen in 12 (16%) patients. The best cut-off value with a sensitivity of 63% and a specificity of 61% was a DPD-activity of 9,65 nmol/mg/h. There was no significant difference in mean DPD-activity between the different grades of toxicity. Independent of DPD-measuring, reducing the dose of capecitabine decreased the grade of toxicity.

Conclusion. No cut-off value to define DPD-deficiency based on the degree of DPD-activity and the grade of toxicity could be identified. Furthermore, measuring the DPD-activity played a minor role concerning interventions after the occurrence of toxicity. This study points out the doubt if determining the DPD-activity is a genuinely added parameter to predict toxicity in patients undergoing chemotherapy with capecitabine. Further studies are warranted to clarify the importance of the measurement of DPD-activity.
Samenvatting

Achtergrond. Capecitabine (Xeloda®) is een veel gebruikt oraal chemotherapeuticum waarbij in sommige gevallen ernstige toxiciteit kan ontstaan. Dit is mogelijk te wijten aan een tekort van het enzym dihydropyrimidine dehydrogenase (DPD). Het is mogelijk nuttig om elke patient, voorafgaand aan de start van de chemotherapie, te screenen op DPD-deficiëntie, maar er bestaan nog veel onduidelijkheden. Het doel van deze studie was om bij patienten die behandeld worden met capecitabine, te bepalen wat de klinische relevantie van DPD-deficiëntie is. Dit door een afkrapwaarde te vinden om toxiciteit te voorspellen en door vast te leggen wat de consequenties van therapeutische interventies zijn.

Methoden. In de periode oktober 2011 tot en met maart 2014 hebben we alle volwassen patienten die werden behandeld met capecitabine in twee ziekenhuizen in Nederland geïncludeerd. Er waren twee gelijke groepen, respectievelijk bij wie de DPD-activiteit bepaald was en bij wie niet. Van alle patienten werden gegevens verzameld uit het elektronisch patiëntendossier omtrent capecitabine-gerelateerde toxiciteit, interventies en demografische gegevens.

Resultaten. In totaal werden 164 patienten geanalyseerd, waarvan bij 77 (47%) de DPD-activiteit was bepaald. DPD-deficiëntie werd gezien bij 12 (16%) patienten. De beste afkapwaarde was bij een DPD-activiteit van 9,65 nmol/mg/h, met een sensitiviteit van 63% en een specificiteit van 61%. Er werd geen significant verschil gezien in de gemiddelde DPD-activiteit bij verschillen graden van toxiciteit. Onafhankelijk van het meten van de DPD-activiteit, zorgde een dosisreductie van capecitabine voor een verlaging van de toxiciteitsgraad.

Conclusie. We konden geen bruikbare afkapwaarde vinden om DPD-deficiëntie te definieren, gebaseerd op de DPD-activiteit en de toxiciteitsgraad. Bovendien is er slechts een kleine rol weggelegd voor de DPD-activiteit als het gaat om interventies na het ontstaan van toxiciteit. Deze studie brengt de twijfel naar voren of het vaststellen van de DPD-activiteit echt een toegevoegde waarde is om toxiciteit te kunnen voorspellen.
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Introduction

5-Fluorouracil (5-FU) was first synthesized in 1957 and is one of the most commonly used chemotherapeutic drugs for either the curative or palliative treatment of in particular tumors of the gastrointestinal tract, breast, head and neck [1]. Belonging to the fluoropyrimidines, 5-FU interferers with Desoxyribo Nucleic Acid (DNA) synthesis which eventually leads to cell death [1,2]. Given the unpredictable pharmacokinetics of oral administered 5-FU, the primary route of the chemotherapeutic drug has traditionally been intravenous. However, to avoid the disadvantages of intravenous therapy, including major burden for patients, high costs and the possibility of infection of the infusion entrance site, since 1967 oral pro-drugs of 5-FU have been developed [3, 4]. Capecitabine (Xeloda®) is one of these pro-drugs. It is rapidly and nearly completely absorbed through the intestines and metabolized in the liver to the active 5-FU by three activation steps. The last enzyme necessary for this activation, the thymidine phosphorylase, is significantly more active in tumor tissue than in normal tissue, which makes capecitabine tumor-selective [5,6,7]. Because of the advantages of oral administration and an equal effect compared to intravenous 5-FU, capecitabine is a preferable option for the treatment of different tumors [8]. It can be used as monotherapy or combined therapy. Capecitabine is generally well tolerated. The most common therapy-related adverse events are myelosuppression, mucositis, hand-foot syndrome, nausea, vomiting, diarrhea and fatigue, of which the hand-foot syndrome is seen significantly more in patients using capecitabine comparing to intravenous 5-FU [3,8]. Approximately 5-10% of the patients develop severe toxicity, sometimes even life-threatening [9,10]. The cause of these serious adverse events is due to a deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD) [11, 12, 13]. This enzyme is seen in most tissues, including tumors, and is highly active in the liver and in peripheral lymphocytes [14]. DPD is responsible for the irreversible converting of more than 85% of the active 5-FU to the inactive metabolite 5,6-dihydro-5-fluorouracil, which will eventually be converted to the fluoro-β-alanine and will be excreted in the urine [15, 16, 17] [figure 1].

![Metabolism of 5-FU and role of DPD as the rate-limiting enzyme.](image)

Without this conversion an excessive availability of the active metabolites will arise, leading to severe toxicity in about 80% of the patients with a DPD-deficiency within a few days until weeks after the start of the therapy [18]. The treatment will have to be stopped or interrupted,
and as a result there could be long hospitalizations associated with high costs. [19] The DPD-activity in the general population varies considerably. Research shows that 3-5% of the general population has a partial DPD-deficiency and a complete deficiency is seen in about 0.5% of the general population [20, 21, 22]. There is no significant difference in DPD-activity related to gender or age, but some studies have shown significant variability among different ethnic groups [20, 23, 24, 25].

A number of screening methods have been developed to detect DPD-deficiency, either by genotyping or phenotyping. The last years, more than 40 mutations associated with DPD-deficiency were reported in the literature, of which c.1905+1G>A is the most common mutation (52%) [26]. However, there is barely a correlation between the patients with this mutation and the development of severe toxicity [22, 27,28]. Besides, a significant number of patients with reduced DPD-activity did not show a mutation in the coding region of the gene [29,30]. The sensitivity of genotyping as a screening method was 5.5% for only the c.1905+1G>A mutation and 31% for this mutation in combination with two other mutations most frequently associated with toxicity [27]. This demonstrates that genotyping can only predict the risk of developing severe toxicity in a small percentage of patients [31].

There are different phenotype-based procedures to determine DPD-deficiency. Testing can occur either directly or indirectly. Directly determining the enzymatic activity can be through ex-vivo DPD assays whereas the indirect way is based on the monitoring of endogenous or exogenous substrates of DPD as surrogate markers [32].

Directly testing can be done by the measurement of the DPD-activity in peripheral blood mononuclear cells (PBMC’s). Already in 1989, this was the first method proposed to assess overall DPD functionality [33]. As said before, DPD is markedly expressed in the liver, but also in peripheral lymphocytes or fibroblasts. The DPD-activity in the liver is strongly correlated with the activity in the PBMC’s [34]. As these mononuclear cells are easier to access than liver cells, it makes them preferable for testing DPD-activity. The method of measuring the DPD-activity in PBMC’s consists of incubating isolated lymphocytes with radioactive labeled 5-FU or thymine and measuring the resulting rate of catabolite formation by high-performance liquid chromatography (HPLC) [35,36]. These isolated PBMC subfractions of blood cell components can be variable, what influences the precision [46]. The isolation of the PBMC’s is relatively easy and all required materials are commercially available, however this method is labour intensive and therefore expensive [37]. Literature suggests a sensitivity of 60%, though this estimate is based on little data [45]. Nonetheless, measuring the DPD-activity in peripheral blood mononuclear cells remains the preferred method to diagnose DPD-deficiency.

The first method of indirectly testing is the oral uracil-loading test dose, using uracil, a natural substrate for DPD, as a marker to detect possible DPD-deficiency. Uracil is administrated orally followed by multiple blood sampling to measure the amount of uracil and its metabolite dihydouracil (DHU) in plasma. However, plasma uracil showed poor correlation with DPD-activity or 5-FU clearance and the sensitivity and specificity of this test are not yet determined [24, 38]. Secondly, there is an uracil breath test, whereby after ingestion of an aqueous solution of uracil the reduced uracil catabolism in patients with DPD-deficiency results in decreased exhaled CO2 levels. This test is rapid and non-invasive and has been extensively validated. Disadvantages are the limited availability of the expensive aqueous solution of uracil and the resources to analyse which are not commonly available [37].

Thirdly, there is the method of analyzing endogenous uracil and/or DHU levels or their ratio in plasma or urine. An impaired breakdown of uracil when the DPD-activity is low, gives increased levels of endogenous uracil and decreased levels of DHU in plasma and urine [39]. Research showed contradictive correlations between the DHU/U ratio and 5-FU half-life, clearance and plasma levels, indicating that this test of analyzing the U/DHU ratio may not
always reflect correctly 5-FU levels [40]. Another method is the 5-FU therapeutic drug monitoring, whereby the plasma concentration of 5-FU and its metabolite dihydro fluorouracil (DHFU) will be determined. Those plasma concentrations can be determined using HPLC, liquid chromatography - mass spectrometry (LC-MS)/MS or immunoassay, whereby all have been validated [39, 41]. A disadvantage of this test is the possible toxicity in patients who are severe DPD-deficient, caused by the given test dose of 5-FU.

In general, genotyping is easier and less invasive than phenotyping, but it is accompanied by a low sensitivity and specificity [37]. Phenotyping seems to have a stronger correlation with the development of 5-FU-toxicity, but the sensitivity and specificity need to be improved [37]. A high specificity is important, because a low specificity might lead to undertreatment of the cancer. With respect to the cost-effectiveness, there are barely data about the different screening methods.

Although research shows that it might be useful to screen every patient for DPD-deficiency before they start their 5-fluorouracil-based chemotherapy, there is still no consensus on the cutoff level to define DPD-deficiency. Some studies define a DPD-deficiency whenever the activity is below the 95th percentile, other studies use the 70th percentile as cutoff point, whereby almost 14% of the general population is at risk of 5-FU-induced toxicity [34, 42, 43]. This was based on previous experiences, but only with a small amount of patients and no correlation with the grade of toxicity was made [21, 34, 44].

Another problem is that even when a DPD-deficiency is established, it is not clear which consequences will follow.

This raises the question: can there be found a cutoff level of DPD-activity to define DPD-deficiency? Also, is there a correlation between the DPD-activity and the grade of toxicity? Thirdly, what are the results of the determination of a DPD-deficiency in regard to clinical interventions and dosage capecitabine?

The aim of this study is to determine the clinical relevance of DPD-deficiency in patients treated with capecitabine.
Patients and methods

This retrospective study took place in two hospitals, in the Kennemer Gasthuis, a top-clinical hospital in Haarlem and in the Spaarne Ziekenhuis, a top-clinical hospital in Hoofddorp, both in the Netherlands. No medical ethics approval was needed for this study.

Study population

The patient population contained adult patients with mainly a tumor of breast, gastric or colon. Included patients were those who were treated with capecitabine monotherapy or combined therapy and those who were treated with the same therapy and from who the DPD-activity was determined.

Patients excluded were those who were on drugs known to interfere with DPD activity such as raltitrexed or other urine derivatives such as antiviral sorivudine.

Data collection

First, there was a selection of patients treated in the Kennemer Gasthuis and in the Spaarne Ziekenhuis, from who the DPD-activity was determined by the laboratory in the Amsterdam Medical Centre, Amsterdam in the Netherlands, a university hospital, between October 2011 until March 2014. The method used was the measurement of the DPD-activity in peripheral blood mononuclear cells (PBMC’s). From these selected patients, data concerning capecitabine-related toxicity and basic demographic data such as age, sex and location of the tumor were collected from the digital records. Secondly, there was a selection of an equal group of patients treated in the Kennemer Gasthuis, but without the measurement of DPD-activity. From these selected patients, the same data was collected from the digital records.

Endpoints

The primary endpoint was the cut-off point to define DPD-deficiency based on the degree of DPD-activity and the grade of toxicity. Toxicity was scored by using the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, which consists of standardized definitions for adverse events for patients receiving cancer treatment graded as mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4) or death (grade 5). In our study, grade 1 was considered as no toxicity, because no action was needed after this grade of toxicity. DPD-deficiency was based on the values used in our hospital, whereby the normal values of DPD-activity are defined between 5.9 and 14.0 nmol/mg/h. Secondary endpoints were the consequences of toxicity, such as duration of hospitalization measured by searching in digital records for the date of admission and the date of discharge, the clinical interventions after the occurrence of toxicity, such as stopping the capecitabine or dose-reduction and from the group who DPD-activity was determined, the clinical interventions after measuring the DPD-activity, such as continuing the therapy as before, dose-reduction, changing from therapy or restarting capecitabine.

Statistical analysis

Data are stored in a web-based database (Research Manager, version 3.7.0.2, the Netherlands) and exported to SPSS version 22. Patient characteristics are reported descriptively. Possible correlation between patient characteristics and the occurrence of toxicity was demonstrated by means of univariate and multivariate analysis, using Chi-square or logistic regression. A P-value < 0.05 was considered statistically significant. To determine whether the DPD-activity is normally distributed we used the Kolmogorov-Smirnov test. The analysis of variance (ANOVA) was used for the difference in mean DPD-activity between the different grades of toxicity. If the groups were too small we used the Kruskal Wallis test.
Receiver operating characteristic (ROC) curves were used to correlate DPD-activity and the grade of toxicity to find a cut-off level. They were made for toxicity $\geq$ grade 2 as well for toxicity $\geq$ grade 3.
Results

Patient characteristics

A total of 164 patients were included in this study. Table 1 shows baseline characteristics for all 164 patients. Most of the patients had colon carcinoma (74%). Metastases were seen in 93% of the patients, either lymph node metastases, distant metastases or both. There was an equal distribution between curative or palliative treatment, respectively 49% and 51%.

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- male</td>
<td>87 (53%)</td>
<td></td>
</tr>
<tr>
<td>- female</td>
<td>77 (47%)</td>
<td></td>
</tr>
<tr>
<td>Age in years</td>
<td>64.18 ± 10.40</td>
<td></td>
</tr>
<tr>
<td>Body surface area in m²</td>
<td>1.89 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Type of malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Colon carcinoma</td>
<td>122 (74%)</td>
<td></td>
</tr>
<tr>
<td>- Breast cancer</td>
<td>19 (12%)</td>
<td></td>
</tr>
<tr>
<td>- Gastric carcinoma</td>
<td>8 (5%)</td>
<td></td>
</tr>
<tr>
<td>- Pancreatic cancer</td>
<td>6 (4%)</td>
<td></td>
</tr>
<tr>
<td>- Ovarian cancer</td>
<td>4 (2%)</td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>5 (3%)</td>
<td></td>
</tr>
<tr>
<td>Metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>9 (6%)</td>
<td></td>
</tr>
<tr>
<td>- Lymph node metastases</td>
<td>65 (39%)</td>
<td></td>
</tr>
<tr>
<td>- Distant metastases</td>
<td>51 (31%)</td>
<td></td>
</tr>
<tr>
<td>- Both</td>
<td>39 (24%)</td>
<td></td>
</tr>
<tr>
<td>Indication of treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Curative</td>
<td>81 (49%)</td>
<td></td>
</tr>
<tr>
<td>- neo-adjuvant</td>
<td>10 (12%)</td>
<td></td>
</tr>
<tr>
<td>- adjuvant</td>
<td>71 (88%)</td>
<td></td>
</tr>
<tr>
<td>- Palliative</td>
<td>83 (51%)</td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Monotherapy capecitabine</td>
<td>53 (32%)</td>
<td></td>
</tr>
<tr>
<td>- Combination therapy capecitabine +</td>
<td>111 (68%)</td>
<td></td>
</tr>
<tr>
<td>- oxaliplatin</td>
<td>74 (67%)</td>
<td></td>
</tr>
<tr>
<td>- oxaliplatin + bevacuzimab</td>
<td>18 (16%)</td>
<td></td>
</tr>
<tr>
<td>- other combinations</td>
<td>19 (17%)</td>
<td></td>
</tr>
</tbody>
</table>
Distribution of DPD-activity and cut-off value

Out of 164 patients included this study, DPD-activity was measured in 77 cases (47%). The values for DPD-activity were normally distributed (p-value Kolmogorov-Smirnov = 0.200) with a mean of 9.19 nmol/mg/h and a SD of 3.46 [figure 1].

**Figure 1: DPD-activity**

![Histogram of DPD-activity](image)

* normal range: 5.9 – 14.0 nmol/mg/h

DPD-deficiency was seen in 12 (16%) patients. Based on the ROC-curves, we found a DPD-activity of 9.65 nmol/mg/h as the best cut-off value with a sensitivity of 63% and a specificity of 61% for grade ≥ 2 and a sensitivity of 63% and a specificity of 49% for grade ≥ 3 [figure 2].

**Figure 2:**

- ROC-curve DPD-activity and toxicity ≥ grade 2
- ROC-curve DPD-activity and toxicity ≥ grade 3
Toxicity

In total, 106 (65%) out of 164 patients experienced toxicity ≥ grade 2. Table 2 shows the frequency of types of toxicity and the grade of toxicity. The most common types of toxicity are diarrhea (60%), nausea (52%) and anorexia (43%).

Table 2: Toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Diarrhea</td>
<td>64 (60%)</td>
</tr>
<tr>
<td>- Nausea</td>
<td>55 (52%)</td>
</tr>
<tr>
<td>- Anorexia</td>
<td>46 (43%)</td>
</tr>
<tr>
<td>- Vomiting</td>
<td>43 (41%)</td>
</tr>
<tr>
<td>- Stomach ache</td>
<td>29 (27%)</td>
</tr>
<tr>
<td>- Fatigue</td>
<td>29 (27%)</td>
</tr>
<tr>
<td>- Hand-foot syndrome</td>
<td>29 (27%)</td>
</tr>
<tr>
<td>- Leukopenia</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>- Anemia</td>
<td>19 (18%)</td>
</tr>
<tr>
<td>- Thrombocytopenia</td>
<td>15 (14%)</td>
</tr>
<tr>
<td>- Infection</td>
<td>15 (14%)</td>
</tr>
<tr>
<td>- Mucositis</td>
<td>12 (11%)</td>
</tr>
<tr>
<td>- Dizziness</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>- Rash</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>- Head ache</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>- Dyspnoea</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>- Muscle cramps</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>- Coronary spasms</td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

The time until the occurrence of toxicity, measured from the start of therapy until reported signs of toxicity, has a median of 38 days (range 2 to 231). Out of 106 patients who experienced toxicity, 48 (45%) were admitted to the hospital. The median length of hospitalization was 6 days (range 1 to 31).

There were no significant differences found in the occurrence of toxicity between monotherapy and combination therapy (p-value = 0.191) or in frequency of types of toxicity between monotherapy and combination therapy (p-values vary from 0.05 to 0.936). Also no significancy was found in grade of toxicity between these groups (p-value = 0.157).
**Influence of DPD-activity on toxicity**

Out of the 106 patients who experienced toxicity, in 59 (56%) patients the DPD-activity was determined, whereby 11 (19%) patients were DPD-deficient. A significant difference was seen between whether or not the measurement of the DPD-activity and the occurrence of toxicity (p-value < 0.01). With respect to the grade of toxicity, a significant higher grade was determined in the group where DPD-activity was measured as compared to the other group (p-value < 0.01).

Considering the group of whom DPD-activity was measured compared to the group of patients without this measurement, a significant shorter time until the occurrence of toxicity (p-value = 0.03) was found. Furthermore significant more patients were admitted to the hospital because of toxicity (p-value < 0.01) and a significant longer hospital length of stay (p-value = 0.03) was seen in the group of whom the DPD-activity was determined.

With respect to the DPD-activity in different grades of toxicity, there was no significant difference in mean DPD-activity between the different grades (p-value = 0.520) [figure 3].

**Figure 3: distribution of DPD-activity in different grades of toxicity**

Eighteen (23%) of the 77 patients of whom the DPD-activity was measured, did not experience toxicity. The DPD-activity in this group varied from 4.8 to 14.2 nmol/mg/h. One patient (6%) was DPD-deficient. From those 18 patients, 4 (22%) received a dose reduction before starting the therapy, not because of the DPD-activity, but for other reasons such as renal dysfunction. This was compared to 12 (21%) patients with a dose reduction before starting the therapy in the group who did experience toxicity.
Interventions after the occurrence of toxicity

The intervention following the occurrence of toxicity in 106 patients consisted of continuing the therapy in the same dose (2%), continuing the therapy with a reduced dose (24%) or discontinuing the therapy (74%). The mean reduced dose was 73% of the normal start dose. Independently of the amount of dose reduction, we found a significant decrease of toxicity to grade one or lower (p-value < 0.01). After this decrease of toxicity, 8 (32%) patients showed again an increase of toxicity, which started after a mean of 2 courses after the intervention. This increase was also independent of the amount of dose reduction.

Considering the patients who discontinued treatment as primary intervention, in 33 cases (42%) the therapy was restarted later (without considering DPD-activity) with a median of 11 days (range 1 to 34) between stopping and restarting the treatment. The dose at the restart was in 14 (42%) of the 33 patients the same as the starting dose. Nineteen (58%) patients had a restart with a mean dose of 68% of the starting dose. A significant decrease of toxicity to grade 1 or lower was seen in the group with dose reduction (p-value = 0.024). After a mean of 3 cycles of chemotherapy after the intervention, an increase of toxicity in 46% was observed, independently of either receiving the same dose or a reduced dose (p-value = 0.112).

Interventions concerning DPD-activity

In 30 (39%) of 77 patients of whom the DPD-activity was measured, this was done before the start of the therapy. In 2 (7%) of these patients the start dose was reduced because of a decreased DPD-activity. Table 3 shows the interventions after measuring the DPD-activity. No action was needed in 43 (56%) patients. After discontinuing the treatment for reason of toxicity, in 13 (17%) cases the treatment was restarted in a reduced dose after measuring the DPD-activity. Five (38%) patients were actually DPD-deficient. Other interventions consist of increase in dose to the same dose level or even above or discontinuing of cancer treatment.

Table 3: interventions after measuring DPD-activity

<table>
<thead>
<tr>
<th>Intervention</th>
<th>N (%)</th>
<th>DPD-deficient (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose reduction before start therapy</td>
<td>2 (3%)</td>
<td>1</td>
</tr>
<tr>
<td>No action needed</td>
<td>43 (56%)</td>
<td>2</td>
</tr>
<tr>
<td>Reduce dose</td>
<td>2 (3%)</td>
<td>2</td>
</tr>
<tr>
<td>Restart therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- same dose</td>
<td>14 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>- in reduced dose</td>
<td>- 1 (7%)</td>
<td>5</td>
</tr>
<tr>
<td>- 13 (93%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start different treatment modeling</td>
<td>8 (10%)</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>8 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>12</td>
</tr>
</tbody>
</table>

A significant decrease of toxicity to grade 1 or lower was seen in the group who restarted the therapy after measuring the DPD-activity (p-value < 0.01). In this group, after a mean of 3 cycles of chemotherapy following intervention, an increase of toxicity was seen in 83%. Six of 8 patients who started a different therapy, showed a decrease in toxicity to grade 1 or lower. From those patients, an increase of toxicity in 50% was observed after a mean of 5 cycles of chemotherapy.
Discussion

In our study, we were not able to detect an optimal cut-off point which could be of use in daily practice to predict toxicity. We found low sensitivity and specificity scores. Besides, we found no correlation between the DPD-activity and the grade of toxicity. One third of the DPD-measurements were done before start of treatment. This shows that the measurement of DPD-deficiency is not yet completely incorporated in the care for patients treated with capecitabine. With respect to the interventions after the occurrence of toxicity, a dose reduction resulted in a significant decrease of the grade of toxicity. However, one-third showed subsequently an increase of toxicity after a few cycles of reduced dose chemotherapy. Importantly, these results were independent of the measurement of DPD-activity.

DPD-activity and cut-off value

In our study, the DPD-activity was normally distributed. There were 12 (16%) of the 77 patients DPD-deficient. The primary endpoint was to find a cut-off point to predict toxicity. We found a low sensitivity and specificity, which makes it impossible to define DPD-deficiency based on the degree of DPD-activity and the grade of toxicity. In the literature, DPD-activity was also normally distributed [21]. Earlier research showed that 3-5% of the general population has a partial DPD-deficiency and a complete deficiency is seen in about 0.5% of the general population [20,21,22]. The relatively high percentage of DPD-deficient patients in our study can be explained by the retrospective character of the study and the selective group of patients of whom the DPD-activity was measured, causing an overestimation of the number of patients with DPD-deficiency. Literature shows a clear relationship between low DPD-activity and the occurrence of toxicity, but no ROC-curves were made [21,34,44]. This shows that correlation with the grade of toxicity could not be observed. Cut-off values in literature are based on the mean DPD-activity and their standard deviation [21,23]. The fact that a cut-off point could not be found, could be explained by the selective group of patients, a low amount of patients and the retrospective design. It is important to find a cut-off value which predicts the grade of toxicity, as toxicity can have serious consequences, even leading to death [47,48].

Toxicity and influence of DPD-activity

The occurrence of toxicity was seen in about two-thirds of the patients treated with capecitabine. Of these patients, almost the half were admitted to the hospital. This shows serious consequences of toxicity and is associated with high costs. A significant difference was found between the measurement of DPD-activity and the occurrence of toxicity. This was expected, because frequently the DPD-activity was determined after the occurrence of toxicity. As expected a significant higher toxicity grade was found in the group where DPD-activity was measured. The higher grade of toxicity in this group might also explain the significant shorter time until occurrence of toxicity, significant more hospital admissions caused by toxicity, and a significant longer hospital length of stay. Notable fact is, that there was no significant difference in mean DPD-activity between the different grades of toxicity. Known from the literature, the grade of toxicity should be inversely proportional with the level of DPD-activity [21,34,42,43,44]. The group who did not show toxicity was remarkable, with the same mean DPD-activity as the groups who experienced toxicity. This group included 18 patients from who one was DPD-deficient and one was at the cut-off value. Comparing to earlier research we had only one patient with DPD-deficiency who did not experience toxicity, due to the small amount of patients we
included [43,44]. Important are the consequences it could have for patients, as abstaining from the chemotherapy they need while it was not necessary.

**Interventions**

Dose reduction after the occurrence of toxicity, resulted in a decrease in toxicity to grade 1 or lower in all patients. However, one-third showed again an increase of toxicity after a few cycles, also independent of the amount dose reduction. To conclude, dose reduction after the occurrence of toxicity, independent of the measurement of DPD-activity, is successful. However, the amount of dose reduction should be established in further research. With respect to the patients who restarted the treatment after discontinuing, significant decrease in toxicity compared to patients with the same dose was seen. However, reducing the dose could have consequences for the effectiveness of the treatment [29,42].

This study shows that changing the treatment after the occurrence of toxicity was independent of the DPD-activity. Literature suggests measuring the DPD-activity as decisive for the treatment with capecitabine [29,45].

**Strengths and limitations of the study**

The strength of this study is that our results are a good reflection of daily practice. We included all patients from two hospitals from who the DPD-activity was determined in the last two and a half year and we detected every step that was taken before or after measuring the DPD-activity. Subsequently we can conclude that, in those years, DPD-activity played a minor role in making decisions about interventions. The limitations are the retrospective design of this study, the small amount of patients and thereby the selective group of patients included. Most of the time, DPD-activity was measured after occurrence of severe toxicity. Another weakness concerns the limitations of data collection from digital records.

**Considerations for practice**

As shown in the literature, it might be useful to screen every patient for DPD-deficiency before starting the chemotherapy with capecitabine [38,45]. With respect to our study, it is important to consider if screening would be an added value. First, predicting capecitabine-related toxicity depending on the DPD-activity, which could not be confirmed in this study, should be proved. Secondly, even when the DPD-activity was measured before the therapy started or after the occurrence of toxicity, the majority of the interventions such as dose reduction, were done independently of the DPD-activity. Another important consideration is the possibility patients are abstained from their chemotherapy while there could be no toxicity, even with a low DPD-activity. Besides, the measurement of DPD-activity takes time, so the consideration to wait until the result before starting the chemotherapy should be made, even when the interventions may not depend on it.

**Considerations for further research**

Further research should prove if determining DPD-activity to predict toxicity in patients who undergoing chemotherapy with capecitabine is a genuinely added value. They should focus on finding a cut-off value to predict toxicity by correlate DPD-activity and the grade of toxicity, using a large amount of patients from who the DPD-activity was measured before start of the
therapy. To assess the effectiveness of the intervention based on DPD-activity, they need to determine the number needed to harm.

Conclusion

Concerning our data with patients undergoing chemotherapy with capecitabine, we were not able to find a cut-off value to define DPD-deficiency based on the degree of DPD-activity and the grade of toxicity. Besides, measuring the DPD-activity is still no routine in daily practice. Even when the DPD-activity is determined, usually the interventions concerning the therapy depend on toxicity rather than on the DPD-activity.
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