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Dihydropyrimidine Dehydrogenase Deficiency: To Screen or Not to Screen?

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Abstract

5-fluorouracil (5-FU) and its prodrug capecitabine are frequently prescribed in oncology. While usually well tolerated, toxicity can be severe, and even life-threatening. A dihydropyrimidine dehydrogenase (DPD) deficiency can cause severe toxicity. Current testing for DPD deficiency does not meet the criteria for a routine screening test prior to 5-FU therapy. A case study of a fatality secondary to capecitabine toxicity is reviewed and literature is examined regarding general screening for DPD deficiency.

CASE STUDY

SJ is a 69-year-old female with recurrent breast cancer. She was originally diagnosed with clinical stage IIIB, human epidermal growth factor receptor 2 (HER2)-positive infiltrating ductal carcinoma of the breast. She receives neoadjuvant chemotherapy with docetaxel, pertuzumab, and trastuzumab for four cycles. Following the fourth cycle of treatment, she is admitted to the hospital with mental status changes and is found to have anti–N-methyl-D-aspartate (NMDA) receptor encephalitis thought to be a paraneoplastic syndrome secondary to her breast cancer.

SJ has a bilateral mastectomy while hospitalized. Pathologic staging is ypT1a, ypN0, and M0. Adjuvant therapy includes 5-fluorouracil, epirubicin, and docetaxel for 3 cycles and trastuzumab for a total of 1 year. Approximately 18 months following adjuvant therapy, she has localized recurrence in the chest wall and receives radiation and trastuzumab. Progression of local disease prompts a biopsy, and pathology shows metastatic breast cancer, favoring lobular, that is estrogen receptor (ER) positive, progesterone receptor (PR) negative, and HER2 positive. She is prescribed anastrozole.

In the metastatic setting, SJ also receives paclitaxel, ado-trastuzumab, and with progression, receives a drug in clinical trial with capecitabine vs. placebo with capecitabine. On day 8, she begins having mucositis. On day 11 of cycle 1, she calls the nurse triage line and is given instructions for the treatment of mucositis. Treatment is held. She presents to the cancer center on cycle 1, day 15, with grade 2 mucositis

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and grade 1 diarrhea (that began on day 10). She has some hypotension and hypokalemia, and for clinical dehydration, she is given fluids and potassium. She is unable to take her regular medications, including cardiac medications. *C. diff* and stool cultures are negative. With little response from fluids, she is admitted to the hospital with a diagnosis of atrial fibrillation, dehydration, and hypotension.

This is complicated by acute renal failure and severe hyperchloremic acidosis. On hospital day 5, SJ requires intubation due to decompensation. On hospital day 6, thoracentesis is performed, with 1,500 cc of fluid removed. She has complications of septic shock with severe metabolic acidosis and respiratory failure. On hospital day 7, the lead investigator for the clinical trial is consulted about the study drug. The lead investigator notes that this drug was not linked to mucositis or toxic colitis in previous test subjects. A *DPYD* gene mutation test is ordered on hospital day 7. SJ dies on hospital day 8 and an autopsy is not performed. Results from the *DPYD* gene mutation test are received after SJ's death. The test is negative for the IVS14+1G>A mutation in the *DPYD* gene.

Following SJ's death, it was requested that the trial be unblinded for SJ. The request was denied as SJ was deceased. Because SJ was on a clinical trial, the institutional review board (IRB) became involved and requested more information. The IRB also requested that oncology providers begin *DPYD* screening on all patients who plan to receive a 5-FU therapy.

5-fluorouracil (5-FU) and its prodrug capecitabine are frequently prescribed for curative and palliative treatment of cancers of the gastrointestinal tract, breast, and head and neck. In stage 3 colon cancer, adjuvant 5-FU therapy increases 5-year survival from 51% to 64% (Meyerhardt & Mayer, 2005). Capecitabine is used frequently in the first line in metastatic breast cancer. In most cases, these are considered fairly tolerable drugs, but toxicity can be severe and even life-threatening.

According to a 2003 review, approximately 275,000 patients receive 5-FU–based regimens in the world every year (Longley, Harkin, & Johnston, 2003). Of these, approximately 20% develop serious, including life-threatening, adverse reactions (André et al., 2003). The mortality rate from these adverse events is estimated to be 0.5%, stemming from polyvisceral reactions, including hematologic, mucosal, cutaneous, and digestive effects (Diasio & Johnson, 1999).

More than 80% of 5-FU is catabolized by dihydropyrimidine dehydrogenase (DPD), and patients with a deficiency in this enzyme risk severe toxicity. DPD enzymatic activity displays a wide range across the human population, with 5% demonstrating reduced activity and 0.2% to 0.3% with complete deficiency (Seck et al., 2005). There are multiple methods for evaluating DPD status, but none have been accepted as definitive or recommended as the standard of care (Van Cutsem et al., 2016).

DPD deficiency is linked to a genetic polymorphism in the *DPYD* gene, and over 30 variant DPD alleles have been identified (Wei, McLeod, McMurrough, Gonzalez, & Fernandez-Salguero, 1996). More than half of those likely have deleterious effects on 5-FU metabolism (Wei et al., 1996). There are several methods to detect DPD deficiency, including genotyping and the assessment of DPD activity in peripheral blood mononuclear cell (PBMC). Screening for genotype alone has not been successful for a number of reasons. For one, the functional consequences of the mutations are not always known. In addition, low DPD activity is seen in a number of patients who have no identifiable mutations in the coding region of the *DPYD* gene (van Kuilenburg et al., 2000).

SCREENING FOR DPD DEFICIENCY

A multiparametric approach, including screening for genetic mutations and phenotypic changes using a reduced dihydrouracil to uracil ratio (UH2/U) as a marker was proposed in 2017 by Boisdron-Celle and colleagues (2017). The toxicity in the prescreened group (grade 3 or higher) was 10.8%, compared with 17.5% in the standard-treatment group. The absolute risk reduction was 69/398 – 79/719 or 0.1734 – 0.1099 = 0.0635. This gives a number needed to screen (NNS) of 15; i.e.,

15 people would have to be screened to prevent one grade 3 or higher adverse event. With regard to grade 4 toxicities alone, the absolute risk was (4.2–1.2)/100 or 0.03. The NNS to prevent one grade 4 event would therefore be 33. This study was nonrandomized and felt to be strongly biased, as statistical analysis of the cohorts showed they were noncomparable at baseline (Etiennne-Grimaldi et al., 2017).

Normally, plasma concentration is dependent upon dose and rate of infusion (Schilsky, 1998). Following a bolus dose of intravenous 5-FU, normally the half-life is 8 to 14 minutes. When the dose increases, the plasma clearance decreases. When given by continuous infusion, the clearance of 5-FU is faster than when given by bolus, thus allowing higher cumulative doses of 5-FU to be given safely (Schilsky, 1998).

Bocci and colleagues (2006) examined the pharmacokinetics of 5-FU and the major metabolite 5-FDHU (5-fluoro-5,6-dihydrouracil) vis a vis DPD activity in PBMCs. 188 gastrointestinal cancer patients were given a test dose of 5-FU at 250 mg/m^2 2 weeks before starting the planned 5-FU treatment of 370 mg/m^2 plus L-folinic acid at 100 mg/m^2 for 5 days every 4 weeks. Drug levels were examined by high-performance liquid chromatography (HPLC), and toxicities were graded according to World Health Organization criteria. Of 188 patients, 3 (1.6%) had marked alterations of 5-FU/5-FDHU pharmacokinetics (i.e., 5-FU half-life [$t_{1/2\beta}$] > 5 hours, 5-FU total body clearance [CL_{TB}] < 1 L × h^{-1} × m^{-2}, and 5-FDHU time to reach maximum plasma concentration [t_{max}] ≥ 45 minutes); they were excluded from 5-FU treatments and treated with irinotecan, which was well tolerated. Unfortunately, this method has not been validated in a controlled fashion in the literature (Bocci et al., 2006).

The measurement of DPD activity in PBMCs remains the gold standard in DPD deficiency screening (van Kuilenburg et al., 2006); however, as noted, this measurement is problematic. The advantage of this approach is that genotype no longer matters; only the ultimate outcome of the genetic programming is evaluated (deficient activity or no). However, most often these tests are done in specialty labs and are not currently available for commercial use. Commercially, the DPD deficiency gene mutation analysis is available. This polymerase chain reaction test detects the IVS14+1G>A mutation in asymptomatic carriers, which accounts for approximately 50% of DPD deficiency alleles. Individuals with one copy of the IVS14+1G>A mutation are predicted to have significant side effects when treated with standard doses of 5-FU, and caution should be taken when treating with any pyrimidine-based therapy.

This test does not detect other variations or mutations in the DPD gene, which may impair 5-FU or pyrimidine-based therapy metabolism and detoxification, nor does it examine other genetic or nongenetic modifiers of DPD metabolism. This is a significant limitation since evidence suggests that deep intron mutations may affect the splicing of *DPYD* pre-mRNA, for instance (van Kuilenburg et al., 1999). A recent case report described a patient who developed severe 5-FU toxicity after undergoing treatment for metastatic pancreatic cancer and was found to be heterozygous for three different polymorphisms of the *DPYD* gene, identified only after sequencing the entire *DPYD* gene (Mukherji, Massih, Tfayli, Kanso, & Faraj, 2019). The margins of commercial testing limit the use of this for general screening of persons preparing to undergo treatment with fluorouracil-based therapy.

CASE STUDY DISCUSSION

The DNA testing indicated that SJ was negative for the IVS14+1G>A mutation in the *DPYD* gene. This mutation accounts for approximately 50% of *DPYD* deficiency alleles. However, this negative result does not rule out the presence of rare *DPYD* mutations that are not detected by this assay. Therefore, the possibility of DPD deficiency and a severe adverse reaction to treatment with pyrimidine-based chemotherapeutic agents (e.g., 5-FU and capecitabine) cannot be ruled out. Since genetic variation and other problems can affect the accuracy of the direct mutation testing, these results should always be interpreted in light of clinical and familial data. Therefore, available testing of SJ as prescreening would have been of no benefit.

The oncology and palliative care team reviewed the literature on routine *DPYD* testing for patients undergoing 5-FU therapy. No national guidelines

regarding routine screening for DPD deficiency have been published. Even if a validated protocol regarding screening for DPD deficiency existed, to quote van Kuilenburg (2006), "No clear guidelines have been formulated as to the application of alternative therapies in the event that a patient is diagnosed with a deficiency of DPD." Also, there are no data to suggest that, when a deficiency is found, it is of benefit to select the appropriate initial dose of a fluoropyrimidine. Interestingly, in this case, SJ had received 3 cycles of 5-FU–based therapy adjuvantly, although tolerance was not noted.

Cost is a significant factor if one were to consider screening all patients. The DPD deficiency gene mutation analysis test is approximately $450. Of course, this will not predict outcomes in patients who have toxicities unrelated to DPD deficiency.

The definition of a screening test requires that a proposed screen (Obuchowski, Graham, Baker, & Powell, 2001):

1. Have a high level of sensitivity (the IVS14 screen is less than 50%)
2. Screen for a treatable illness (debatable; see following section)
3. Be inexpensive (again, debatable)
4. Screen for a prevalent condition (0.2%–5%)

Therefore, the DPD activity and *DPYD* genotype testing do not meet the criteria for a proper screening test. The decision of the oncology team was to not test everyone receiving one of these drugs but to provide the option to patients for testing after appropriate counseling and documentation.

This case study also illustrates that oncology professionals must be alert for early signs of toxicity secondary to 5-FU therapy. Early signs of grade 3 or 4 mucositis and diarrhea in patients on a 5-FU therapy would suggest that the patient should be examined in the clinic with appropriate workup. Other indications of toxicity could include severe vomiting, bleeding, severe hand-foot syndrome, chest pain, and neurologic symptoms. Grading of toxicities is vital to determining the treatment and plan. It is recommended that a triage protocol be developed for those patients who have signs of early toxicity. Patients must be educated to report early onset symptoms.

Health-care providers, including those outside of oncology, should be aware of uridine triacetate indicated for fluorouracil or capecitabine toxicity to reduce or prevent further toxicity. This should be given within 96 hours of the last dose of chemotherapy. The drug is specially ordered and 10-g po doses are given every 6 hours for 20 doses. It should be noted that this drug is indicated for fluorouracil or capecitabine toxicity; the patient does not need to have a *DPYD* mutation to receive this drug. In this case, uridine triacetate was not considered, nor would it have been given within 96 hours of the last dose of chemotherapy.

ALTERNATIVE THERAPY

In terms of testing, an interesting approach may be to consider UFT (tegafur/uracil) in patients with demonstrated partial deficiency of *DPYD*. Cubero, Cruz, Santi, Silva, and del Giglio (2012) studied five colorectal cancer patients who presented with acute toxicity (grades 3 and 4) after being given the first cycle of chemotherapy using 5-FU. The *DPYD* deficiency was confirmed by gene sequencing. After a full recovery from all side effects, the regimen was changed to UFT (300 mg/m^2/day) associated with leucovorin (90 mg/day) for 21 days, with an empirical dose reduction of at least 10% in the first cycle. There were no episodes of grade 3 or 4 toxicity in the UFT-treated group. Double-blind placebo-controlled trials would likely be unethical, so this and other similar studies may be the best evidence available (Cubero et al., 2012). Unfortunately, UFT is not available in the United States.

EFFICACY AND SAFETY OF DOSE REDUCTIONS

Henricks and colleagues (2019) discussed the effectiveness and safety of *DPYD**2A genotype-based dose reductions to improve patient safety. The study involved a cohort of 40 prospectively identified heterozygous *DPYD**2A carriers treated with approximately a 50% reduced fluoropyrimidine dose. A matched-pair analysis was performed, with each *DPYD**2A carrier matched with a *DPYD**2A wild-type patient. Overall survival and progression-free survival were compared between these groups. Severe grade 3 and higher treatment-related toxicity was compared to a cohort of 1,606 wild-type patients treated with the full dose and a cohort of historical controls derived from literature. A matched control could be found for 37 out of 40 *DPYD**2A carriers.

The study concluded that reduced doses compared to full doses did not affect overall survival (median 27 months vs. 24 months, p = .47) nor progression-free survival (median 14 months vs. 10 months, p = .54). Patient safety was improved in those who received reduced doses. The risk of toxicity in *DPYD**2A carriers treated with the reduced dose was 18% compared to 23% for wild-type patients. This risk was significantly lower than the risk of 77% in *DPYD**2A carriers treated with the full dose (Henricks et al., 2019).

CONCLUSIONS

From an ethical standpoint, autonomy implies informed consent, and this requires that patients be aware of the risks, benefits, and alternatives to treatment. Patients must be informed of the risk of DPD deficiency and potential toxicities of 5-FU and its analogues. Until firm guidelines are established, it is acceptable to offer screening as long as patients understand that this is not standard of care, may mitigate some (but not all) risk, will increase cost, and may prevent treatment with a proven effective regimen in some patients who might otherwise tolerate therapy. A cost-benefit analysis using the various tools available would also be helpful. Even if hospitalization could be prevented in one out of 200 patients, the cost of screening ($90,000 at $450/test) would likely be less than the cost of any prolonged hospitalization. This could be done locally with retrospective data from the cancer center.

In this case, the oncology team developed a protocol for patients receiving 5-FU or capecitabine. Every patient will be offered DPD testing after appropriate counseling on the benefits, risks, and limitations of testing. Documentation of this must be completed before the drugs can be ordered.

Further research is required to establish a firm guideline that is proven to prevent morbidity and mortality among patients receiving 5-FU or its analogues. That this has not yet been accomplished on a national level speaks to the difficulty of screening individuals who might be at risk for adverse events. Regardless, the oncology advanced practitioner and other oncology professionals must be cognizant of the potential lethality of even the most commonly utilized agents.

Disclosure

The author has no conflicts of interest to disclose.

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