Avoiding azathioprine toxicity due to TPMT mutations in renal transplant recipients

David Power - Austin Health
COMMENTS

30. Pursuant to section 67(3) of the Coroners Act 2008, I make the following comments connected with the death:

1. At present, there is no Australian guideline regarding TPMT testing, and the decision to perform routine genotyping or enzymatic analysis prior to prescription of thiopurine\textsuperscript{24} medication is determined by individual clinician or health care service.

2. Access to testing should not be considered a significant barrier since genotyping and TPMT functional assays are now readily available, and the cost of such testing is not high.

3. A significant barrier that may remain is the lack of knowledge amongst prescribers regarding access to testing and the implementation of specific recommendations at a clinical level, across the different subspecialty groups that are largely responsible for prescribing these medications
Coroner’s recommendation

RECOMMENDATION

31. Pursuant to section 72(2) of the Coroners Act 2008 (Vic), I make the following recommendation connected with this death:

1. That TPMT genotyping for the common alleles should be mandatory for patients prior to the commencement of thiopurine containing medications.
TGA and SCV response to the Victorian Coroner’s Findings

“The Coroner’s recommendation is under consideration. The TGA will review the available evidence for the appropriate place for TPMT testing prior to the use of thiopurine medications in the Australian context, including seeking the advice of appropriate external experts. The timeframe for a decision following this review is estimated to be twelve months. The relevant contact person responsible for the…..”

“However, it is the considered view of SCV that mandatory testing of thiopurine methyltransferase (TMPT) genotyping ahead of thiopurine administration is not justified.

About 11% of the population have reduced thiopurine methyltransferase (TPMT) activity and 0.3% of the population had a true deficiency of TPMT. There are 28 allele variants that may be tested for, but of these it is likely that only a few are clinically relevant. Of course, the cost of testing increases with every allele screened.

Our concerns are that the current evidence does not support routine use of genetic testing in preference to simple routine monitoring of blood counts. This alone would detect the problem in an individual without the expense of testing 99.7% of the treated population unnecessarily.

For these reasons, the Coroner's recommendation is under consideration by Safer Care Victoria. We are aware …..”
Interstate case: MinterEllison Report

• It was for the Crohn’s colitis that the deceased sought medical management at [Redacted] in early February 2015.

• The deceased was initially treated with corticosteroids (steroids), however concerns soon arose about the possible harmful effects of the steroids on the deceased’s mental health. The deceased was taken off the steroids and commenced on the immunosuppressant drug 6-Mercaptopurine (6-MP) to help control inflammation of the bowel.

• On 10 February 2015, the deceased was discharged from [Redacted].

• The deceased continued to experience pain and on 1 March 2015, he collapsed at his father’s home. He was transferred by ambulance to [Redacted] and admitted to [Redacted] Intensive Care Unit (ICU), where the 6-MP was withdrawn following a full blood count test.

• On 5 March 2015, the deceased died from septic shock and marrow aplasia.

• TPMT testing was not routine practice, but it was ordered and returned on 19/2/15.
Interstate coroner’s recommendations (abbreviated)

• That __ put in place its own internal robust systems for tracking lists of patients in iCM for whom tests have been ordered and received.
• That the Department of Health consider whether an operational directive or instruction is required to support governance within public hospitals regarding the implementation of systems for tracking test results, particularly where patients have been discharged.
Thioguanine metabolism

AZA → 6-MP → 6-TIMP → 6-TXMP → 6-TGN (active metabolites)

6-thiouric acid (inactive) → 6-methyl TIMP → 6-TGTP

Reduced de novo purine synthesis
Rac1 blockade: apoptosis
Incorporation into DNA and inhibition of replication

6-methyl MP (inactive)
6-thiouric acid (inactive)
6-TIMPA 6-MP
6-TXMP
6-TGMP (inactive)
6-TGN (active metabolites)
HGPRT
IMPDH
TPMT
XO
TPMT functional testing
Advantages

- Should detect ALL variants
  - However, at least one false negative resulting in neutropenia has been described in IBD
- Medicare rebated

Disadvantages

- Slow return – likely to be batched
- May involve use of radioisotopes
- Falsely high following recent blood transfusion
- Falsely low with salicylates, XO inhibitors
- Some racial groups do not have easily discernable peaks

Azathioprine metabolite testing
Measurement of metabolites: 6-MMP and TGN

6-methyl MP (inactive)

6-Thiouric acid (inactive)

6-TIMPA 6-MP

6-methyl TIMP

6-TXMP (active metabolites)

6-TGMP (inactive)

AZA → 6-MP → 6-TIMP → 6-TXMP

XO → 6-thiouric acid (inactive) → 6-methyl TIMP → 6-TXMP

6-TGN (active metabolites)

Reduction of de novo purine synthesis

Rac1 blockade: apoptosis

Incorporation into DNA and inhibition of replication
Assay of 6-TGN and 6-MMP: summary

- Can be measured in red cells by HPLC
- Measure 4-6 weeks after commencement or dose change
- Reflect compliance and metabolic status
  - Data comes from studies of IBD
- High ratio of 6-MMP to 6-TGN may predict hepatotoxicity and poor response to therapy
  - Preferential MMP metabolizers – can respond to dose reduction and allopurinol or BD dosing
- Now Medicare rebatable

TPMT genetic testing
<table>
<thead>
<tr>
<th>Homozygous wild-type (&quot;normal&quot;) 90%</th>
<th>High enzyme activity. Found in ~86–97% of patients.</th>
<th>Two or more functional TPMT alleles</th>
<th>*1/*1</th>
<th>Start with normal starting dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous 10%</td>
<td>Intermediate enzyme activity. Found in ~3–14% of patients.</td>
<td>One functional TPMT allele plus one nonfunctional TPMT allele</td>
<td>*1/*2 &lt;br&gt; *1/*3A &lt;br&gt; *1/*3B &lt;br&gt; *1/*3C &lt;br&gt; *1/*4</td>
<td>If disease treatment normally starts at the “full dose”, consider starting at 30–70% of target dose (e.g., 1–1.5 mg/kg/d), and titrate based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment.</td>
</tr>
<tr>
<td>Homozygous or compound heterozygous variant 0.3%</td>
<td>Low or deficient enzyme activity. Found in <del>1 in 178 to 1</del>3736 patients.</td>
<td>Two nonfunctional TPMT alleles</td>
<td>*3A/*3A &lt;br&gt; *2/*3A &lt;br&gt; *3C/*3A &lt;br&gt; *3C/*4 &lt;br&gt; *3C/*2 &lt;br&gt; *3A/*4</td>
<td>Consider alternative agents. If using azathioprine start with drastically reduced doses. Azathioprine is the likely cause of myelosuppression.</td>
</tr>
</tbody>
</table>

Effect of mutations

• A systematic review of TPMT testing in adults and children with chronic inflammatory diseases reported that, compared with a wild-type TPMT genotype, those who were TPMT heterozygotes or TPMT deficient were at an increased risk of developing leucopenia (odds ratios 4.29, 95% CI 2.67, 6.89 and 20.84, 95% CI 3.42, 126.89, respectively).

What is testing for?

- To detect those carrying the rare homozygous or compound heterozygous mutations that will lead to profound leucopenia
- To dose azathioprine more accurately in those with heterozygous mutations.
Enzyme activity or mutation detection?

- Close correspondence between the two assays for absence of activity (98.4% in normals). Less for intermediate activity (86%)

- Enzymatic assays have problems
  - Confounders are recent blood transfusion and some drugs, such as allopurinol and salicylates
  - Can involve use of isotopes
  - Probably batched and slow reporting
  - The phenotype assay will miss 11% of TPMT deficient individuals who are misclassified as TPMT intermediate activity

- Genotyping will miss rare variants
  - Risk estimated at 1 in 7416 for a heterozygote to have an undetected rare variant

- Pooled estimates of sensitivity suggest that genotype testing has higher sensitivity than phenotype testing as long as both TPMT*2 and TPMT*3 are tested

- Genotype assays report in 3-5 days

- Both tests Medicare rebatable

Who should be tested?

• The US Federal Drug Administration recommends but does not mandate TPMT testing prior to the initiation of thiopurine therapy.

• Current British Society of Gastroenterology guidelines do not specifically recommend testing, although a role is suggested in predicting early events rather than long term control. (Lennard L. Pharmacogenomics and Pharmacoepigenetics. April 2014; 77: 704-714)

• Current Australian Gastroenterology Guidelines for IBD do not mention TPMT testing but discuss 6-TGN and 6-MMP monitoring.

Is it worth it?

- “The utility of pretesting for TPMT status before initiating thiopurine treatment remains in question, because insufficient evidence demonstrates that this strategy is effective to reduce harm or is superior to the established clinical standard of hematologic monitoring.” (Booth RA, et al. Ann Intern Med. 2011;154(12):814-823)

- “Unless treated with very low thiopurine doses the TPMT deficient patient will experience profound myelosuppression when treated with thiopurine drugs. It is cost-effective to routinely perform pre-treatment TPMT testing to identify these individuals alone.” (Lennard L. Pharmacogenomics and Pharmacoepigenetics. April 2014; 77: 704-714)
Conclusion

• Coroner has recommended mandatory testing of TPMT genotype prior to thiopurine use
  • This recommendation currently under review by the TGA
• Only a few groups mandate this prior to therapy, although it is widely recommended
• Available in Australia and Medicare rebatable for both functional and genetic assays
• Genetic assays probably superior but may miss a small number of compound heterozygotes who have minimal TPMT activity
• Assay of azathioprine metabolites now commonly used in IBD. Role in renal transplantation unclear.
My recommendations for renal transplant recipients

- TPMT genotype prior to prescription of azathioprine
- No testing prior to a plan to use the drug
- No current plans to tailor initial dose to genotype/phenotype
  - Planned use is to avoid dosing those with no TPMT activity
  - But should we?
- No plans to measure 6-TGN/6-MMP
  - But should we?