Erythrocyte thiopurine methyl transferase assessment prior to azathioprine use in the UK

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Received 4 January 2002 and in revised form 21 March 2002

Summary

Background: Individuals with low activity of a key metabolic enzyme, thiopurine methyl transferase (TPMT), are more susceptible to azathioprine-induced myelosuppression.

Aim: To determine the pattern of use of TPMT activity estimation, with respect to azathioprine use, by medical practitioners in the UK.

Design: Retrospective analysis of assay use.

Methods: We analysed all test results (n = 3291), and patient and practitioner details, from inception of TPMT assay in 1990 to the end of December 2000, held at the Purine Research Laboratory, Guy’s Hospital, London. Patient details were anonymized. Repeat analyses and requests from outside the UK were excluded.

Results: The male:female ratio was approximately equal and the mean age was 46.6 (range 0.5–97) years. Thirteen different medical specialities requested assays; Dermatology and Gastroenterology were the most frequent users, together accounting for 86% of requests. The numbers of centres requesting the assay varied widely both within and between different specialities. Some 80% of individuals had normal TPMT activity, 9% enzymic activity above normal, and 10% low activity. Fifteen had no detectable enzymic activity: 0.45% (1:220) of the study population.

Discussion: This incidence of undetectable enzyme activity is significantly higher than the previously reported level of 1:300 derived from smaller studies, and makes the economics of screening more attractive.

Introduction

Azathioprine has been used extensively for immunosuppression in autoimmune disease and organ transplantation for nearly 40 years.¹ The exact mode of action of azathioprine at the cellular level remains unclear, but the drug is rapidly converted in vivo to 6-mercaptopurine, its active metabolite, before being further metabolized by three competitive enzymic routes illustrated in Figure 1. Metabolism by hypoxanthine guanine phosphoribosyl transferase (HPRT) results in the formation of thiopurine nucleotide analogues, which are thought to be responsible for the drug’s cytotoxic activity through their incorporation into DNA.² Alternative oxidation by xanthine oxidase exhibits little inter-individual genetic variability,³ but azathioprine toxicity can result if xanthine oxidase is inhibited by the concurrent administration of allopurinol.

In contrast, inactivation of azathioprine via methylation by thiopurine methyl transferase...
(TPMT) shows wide inter-individual variation in activity determined by a common genetic polymorphism. About 11% of the population have low TPMT activity and are vulnerable to overdosage and myelosuppression with conventional doses of azathioprine. Of greater concern, 1:300 of the population have very low or undetectable TPMT levels and are susceptible to profound acute intolerance to thiopurine medications including azathioprine, 6-mercaptopurine and thioguanine, resulting in early drug-induced myelosuppression. The increased cytotoxicity observed when TPMT is very low or absent results from reduced inactivation of azathioprine and a consequent dose-related (Type A) toxicity if a standard dose is administered. Regular monitoring of the full blood count (FBC) is a poor indicator of the risk of toxicity, as the bone-marrow reserve leads to delay before haematological parameters are affected. By the time this occurs, the marrow reserve is exhausted, and toxic 6-thioguanine nucleotides reach levels 100–1000 times greater than those normally seen in most patients treated with azathioprine. Red-blood-cell TPMT reflects the enzyme activity in other tissues and cells. Thus by assessing erythrocyte TPMT status prior to commencing azathioprine, toxicity can be anticipated and dosing regimen adjusted accordingly. Additionally, sub-optimal doses of azathioprine in those with very high TPMT activity can also be avoided.

Although the polymorphism was initially described in 1980, it did not appear in a mainstream medical journal until 1992. Since then, awareness of the significance of variability in TPMT activity when prescribing azathioprine has been patchy across specialities. Currently, only one centre in the UK offers TPMT assay as a service to clinicians caring for adults, and children without haematological malignancies, using a modified version of the radio-gold assay described by Chocair et al., which itself is a modified version of the method first described by Weinshilboun. The normal ranges used are based on previously established ranges, and the lower limit of the test’s sensitivity is approximately 1 nmol/h/ml red blood cells. We have collated this centre’s results from the inception of the assay in 1990 up to the end of December 2000.

Methods

The data for this study were held in the Purine Research Laboratory at Guy’s Hospital, London. As the study represents a retrospective analysis of usage of an assay, ethical permission was not sought. Data on all patient samples for whom TPMT estimations had been performed from the inception of the assay to the end of 2000 were entered into a computer database. The initiating hospital request form and the subsequent laboratory report were used to obtain anonymized data that included the age, date of birth, TPMT activity status, whether the patient was taking azathioprine at the time the sample was taken, and the hospital, consultant and speciality requesting the test. Repeat analyses were
excluded; requests from outside the UK (12 in total), and from veterinary surgeries (5) were also excluded. TPMT levels appear to be stable as long as red blood cells remain intact (John Duley, unpublished data), and thus haemolysed and frozen samples were not analysed. The absolute numbers of requests for TPMT estimation each quarter were recorded and represented graphically (Figure 2). The TPMT activity was assayed in red-blood-cell lysates by the method described in detail by Chocair et al. Briefly, the method depends upon the transfer of $^{14}$C-radiolabelled methyl from the donor molecule S-adenosyl-methionine to 6-mercaptopurine. The radioactive product, $^{14}$C-methyl-mercaptopurine, is quantified using a beta-counter. Haemoglobin content of each assay was assayed and converted to equivalent ml red cells, resulting in a unit of activity of nmol/h/ml RBC. The ranges for activity were: <3 units: TPMT deficiency; 3–8: carrier of TPMT deficiency; 8–14.5: normal TPMT; >14.5: high TPMT. The Purine Research Laboratory is the only laboratory, to our knowledge, that reports an upper limit to the normal range, above which it declares the result as ‘high’, recognizing that this small proportion of patients may be at an increased risk of nonResponsiveness to thiopurines. This is based on 10 years’ experience by the Laboratory and a retrospective study of 108 gastroenterology patients, which showed significant and linear fall-off of responsiveness with each unit of activity above 14 (Ansari et al., unpublished). The lower limit of detection was approximately 1 unit of activity. Variation within runs was consistently about 5%. Variation between runs was approximately 10% but depended also on the time of storage of red cells. For assay purposes, aliquots of washed red blood cell pellets were stored at −70 °C. Over a period of 1 month’s storage, the activity of controls consistently fell by about 10%. Thus, new controls were prepared monthly. In our experience, activity of TPMT did not fall significantly in whole blood for at least 1 week, provided that (i) the anticoagulant used was EDTA; and (ii) blood was kept at room temperature (not refrigerated). This ensures minimum disruption of red cells, and maximum stability of TPMT (and other purine-metabolizing enzymes).

Results
The results for 3291 individuals were entered into the database. It was possible to identify the sex in 3182 (96%); 1540 (48%) were males and 1642 (52%) females. The mean age was 46.6 years (range 0.5–97 years, median 46 years, upper quartile 65, lower quartile 30). Over the 10-year study period, there was an increase in the number of requests for TPMT activity estimation, particularly towards the end of the study period, with >1000 requests in the year 2000 (Figure 2). The speciality initiating the request was identifiable in 2756 (84%) results, and the breakdown of number of requests and number of requesting centres is illustrated in Table 1. Dermatology and Gastroenterology accounted for the majority of requests. Some 80% of individuals had TPMT activity within the normal range, 9% had activity above the normal range, and 10% had low activity. We identified 15 individuals who had undetectable enzymic activity. This represents 0.45% of the patient sample tested, (about 1 : 220 individuals). Of 3291 requests, 2086 (63%) stated whether or not the sample had been taken prior to starting azathioprine. Of these, 84% of specimens were sent prior to starting azathioprine, whilst 16% were already on the drug. As TPMT is considered by some to be an inducible enzyme, we analysed the results from those who were stated to be definitely not taking azathioprine at the time the sample was

![Figure 2. Number of TPMT assay requests.](image)

### Table 1  Specialities initiating requests for TPMT analysis 1990–2000

<table>
<thead>
<tr>
<th>Speciality</th>
<th>Requesting centres</th>
<th>Requests (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatology</td>
<td>68</td>
<td>1479 (54)</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>28</td>
<td>879 (32)</td>
</tr>
<tr>
<td>Oral Medicine</td>
<td>4</td>
<td>178 (6)</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>6</td>
<td>154 (6)</td>
</tr>
<tr>
<td>Rheumatology</td>
<td>7</td>
<td>24 (1)</td>
</tr>
<tr>
<td>Haematology/Oncology</td>
<td>7</td>
<td>16 (0.6)</td>
</tr>
<tr>
<td>Other specialities ($n=7$)*</td>
<td>19</td>
<td>25 (0.9)</td>
</tr>
</tbody>
</table>

*Other specialities requesting TPMT assay: Neurology, Paediatrics, Renal Medicine, Cardiology, Respiratory Medicine, Ear, Nose & Throat, General Practice. TPMT, thiopurine methyl transferase.
taken. In keeping with the larger cohort, 80% of these 1747 individuals had a normal TPMT activity, 9% high activity, 10% low activity, and 0.45% undetectable TPMT activity.

Discussion

Full blood count monitoring is a poor indicator of the risk of early azathioprine induced cytotoxicity in susceptible individuals with low TPMT activity, as high levels of cytotoxic metabolites may develop before change is detected in the peripheral blood.7 The clinical correlation between acute myelotoxicity after azathioprine administration and a very low TPMT activity has been documented in patients with a number of different conditions.2,7,13 An inverse relationship has additionally been reported between high TPMT activity and clinical efficacy on standard doses of azathioprine.11,14 Thus accurate estimation of TPMT activity (performed prior to starting azathioprine, as the enzyme may be inducible) has three main potential benefits. Firstly, it allows identification of those individuals with very low or undetectable enzyme activity that are at risk of potentially fatal acute myelotoxicity. Secondly, patients with detectable but low TPMT activity can be identified and treated with an appropriately smaller dose of azathioprine, thereby reducing the risk of sub-acute myelotoxicity. Thirdly, the 9% of subjects in the high-end TPMT activity range can be identified and treated with a more aggressive dosing regimen in order to obtain a therapeutic response. Knowledge of an individual’s TPMT status does not obviate the need for routine haematological monitoring during azathioprine therapy, which should be carried out as recommended on the azathioprine drug data sheet, but prior assessment of TPMT status provides a more confident basis for deciding whether to prescribe azathioprine and at what dosage. However, there are no prospective studies stating specific doses, different to those recommended by the manufacturers, for commencement of azathioprine treatment, and none that demonstrate improved safety and efficacy for azathioprine following TPMT assessment.

The findings of the population distribution of TPMT activity in this study broadly correlate with those of the largest studies to date which have examined 298,303 randomly selected Caucasian blood donors. As this study cohort is bigger by a magnitude of 10, the rate of TPMT deficiency of 1:220 individuals may be more reliable than the previously quoted rate of 1:300. However, our subjects were not healthy volunteers, but individuals with a wide range of medical conditions, and selection bias may therefore apply. Further, they were not restricted to specific ethnic groups (about 4% of the UK population is non-Caucasian, and studies have suggested that the incidence of mutant TPMT alleles is higher in Afro-Caribbean17 and lower in Chinese18 populations, compared to Caucasians). As such our population may be more representative of other populations likely to be prescribed azathioprine. The observation that there was the same proportion of deficient patients in the samples taken prior to azathioprine treatment as in those taken during treatment, suggests that the post-treatment group was not biased by knowledge of myelotoxicity with azathioprine.

Azathioprine is a drug used by many medical specialities. A survey of azathioprine usage in a large teaching hospital in South Wales (University Hospital of Wales, Cardiff: 968 in-patient beds, serving a population of 500,000) over a 4-year period, showed a mean of 1096 prescriptions for azathioprine each year. The relative proportions of azathioprine usage attributable to specialities were as follows: gastroenterology 19%, dermatology 13%, paediatrics 12%, renal medicine 9%, neurology 6%, rheumatology 3%, and cardiology 3%. These proportions are somewhat different to the proportions of medical specialities that requested TPMT analysis on a national basis, and suggest that there may be discrepancies between specialities in their pre-azathioprine monitoring of TPMT (Table 1).

The direct cost of hospital treatment in the UK of an episode of azathioprine-related myelotoxicity was estimated at £3200 in 1997.19 A more recent cost analysis from Canada, where each test costs CAN$100, suggests that TPMT screening is cost-neutral when one looks only at the prevention of myelosuppression in TPMT-deficient individuals, using a population incidence of 1:300 TPMT deficiency, but becomes cost-beneficial should it prevent myelosuppression of those with low TPMT activity.20 If the incidence is higher, as suggested by this series, then the economics for screening become more attractive. TPMT genotyping may be an alternative method to predict enzyme deficiency. However there are at least seven mutant alleles, which effectively renders genotyping cost-ineffective, compared to the direct assay of TPMT activity. Additionally, not all the genotypes that result in undetectable enzyme activity have been identified, so genotyping is not yet totally predictive, and it does not provide information on the functional activity of an individual’s enzyme.21

In the absence of drug interactions with xanthine oxidase inhibitors, TPMT deficiency is the most important and commonest cause of myelotoxicity to have been identified for azathioprine. However, other mechanisms for myelotoxicity with
azathioprine have been reported,\textsuperscript{4,11} and normal or high TPMT status does not completely exclude the possibility of myelotoxicity. The large and increasing literature on azathioprine has belatedly led to an appreciation of the relevance of TPMT activity to safe and effective prescribing with azathioprine. The observation that myelotoxicity may still occur in patients with normal or high TPMT activity emphasizes the need for clinicians to continue to use FBC to monitor for later-onset myelotoxicity.

In conclusion, the common genetic polymorphism in the TPMT enzyme can lead to contrasting clinical outcomes when prescribing the same dose of azathioprine: the inter-individual variability in drug response ranges from under-dosage for some patients to death from myelosuppression in others. The enzyme assay has emerged as a valuable tool to assist clinicians in prescribing azathioprine, and pre-treatment assessment is considered by some to be essential.\textsuperscript{22} Our study suggests the incidence of complete TPMT deficiency to be higher than previously thought. This analysis of usage of the TPMT assay shows that this test is not currently uniformly performed in the UK prior to commencing treatment with azathioprine. While debate about several new drugs has focused on high cost and low efficacy,\textsuperscript{23} developments in understanding the metabolic fate of azathioprine, an old established drug, appear to have assisted clinicians with efficacy and safety of prescribing and extended its lifetime of use beyond half a century. Prospective studies are now needed to underpin the intuitive enthusiasm that clinicians have demonstrated for the use of the TPMT assay as an aid to prescribing.

Acknowledgements

SAH received travel and subsistence funding from the Royal Gwent Hospital Dermatology Research Fund. No other funding was received for this study.

References


