Aliment Pharmacol Ther 2004; 20: 593–599. doi: 10.1111/j.1365-2036.2004.02124.x

Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease

J. WINTER*, A. WALKER†, D. SHAPIRO‡, D. GAFFNEY§, R. J. SPOONER‡ & P. R. MILLS*
Gastroenterology Unit, Gartnavel General Hospital, Glasgow; †Robertson Centre for Biostatistics, University of Glasgow, Glasgow; ‡Departments of Clinical Biochemistry, Gartnavel General Hospital, Glasgow and §Royal Infirmary, Glasgow, Scotland

Accepted for publication 19 June 2004

SUMMARY

Background: Azathioprine is a useful agent in the management of inflammatory bowel disease. Its use is limited by its side-effect profile. Marrow toxicity occurs in approximately 3.2% of patients and is known to be associated with diminished thiopurine methyltransferase enzyme activity resulting from genetic polymorphisms.
Aim: To evaluate the cost-effectiveness of screening for thiopurine methyltransferase gene polymorphisms prior to initiation of azathioprine therapy.
Methods: Analysis of the literature was undertaken to calculate the expected frequency of leucopenia and its relationship with thiopurine methyltransferase polymorphisms in a model of theoretical inflammatory bowel disease patients. Decision analysis was then applied to assess the cost of a pre-treatment genotyping strategy, taking account of direct costs and cost per life-year saved.
Results: In 1000 inflammatory bowel disease patients treated with azathioprine, 32 will develop myelosuppression and one will die because of this. Of those who develop myelosuppression during azathioprine therapy, 32% are attributable to lower thiopurine methyltransferase activity. Pre-treatment genotyping costs £347 per life-year saved for a 30 year old and £817 per life-year saved for a 60 year old. This compares favourably with other health care technologies.
Conclusion: The use of pre-treatment screening for thiopurine methyltransferase polymorphisms in inflammatory bowel disease patients commencing azathioprine therapy represents good value for money.

INTRODUCTION

Azathioprine is an extremely useful second-line agent in the management of inflammatory bowel disease (IBD).[1, 2] Unfortunately, it has a significant side-effect profile. Adverse reactions occur in 15% of patients and include gastrointestinal, hepatic and bone marrow toxicity. Marrow toxicity leading to leucopenia is usually an early event and occurs in approximately 3% of patients. It is unpredictable and can result in death. Current practice is to monitor the blood count regularly to detect marrow toxicity at a stage early enough to stop therapy and avoid life-threatening sepsis. This is not infallible, as profound leucopenia can develop suddenly and unpredictably in between blood tests.

There is a recognized association between myelosuppression and diminished activity of thiopurine methyltransferase (TPMT), an enzyme involved in the hepatic metabolism of azathioprine. TPMT activity can be predicted by genotype analysis,[3] with approximately 11% of the population carrying one mutant allele (the commonest being 2, 3A or 3C) for the enzyme which is

Correspondence to: Dr J. Winter, Division of Cardiovascular and Medical Sciences, Gardiner Institute, Western Infirmary, Glasgow G11 6NT, UK. E-mail: jackwwinter@btopenworld.com

associated with reduced activity. One in 300 patients are homozygous for these mutations and have very low activity, entailing a high risk of early, profound leucopenia.[4]

Although low TPMT activity is associated with an increased risk of early myelosuppression, it is not the sole mechanism. In a study of 41 patients who developed this complication,[5] 29% of cases of leucopenia were caused by a known TPMT polymorphism. In two other studies detailing the influence of TPMT status on single centre patient populations, polymorphisms accounted for 50% of cases of leucopenia.[6, 7]

Hepatic TPMT activity can be indirectly assessed either by measuring red blood cell enzyme activity (phenotype) or be predicted by measuring white blood cell TPMT genotype. Either assay can be used, but the latter is more stable and not influenced by drug interactions[8–10] or blood transfusion.

Pre-treatment screening for TPMT genotype was found to be cost-effective in a Canadian study of patients with rheumatological diseases.[11] However, these findings cannot be extrapolated to patients with IBD in the United Kingdom. It is recognized that approximately 9% of patients with rheumatoid arthritis experience leucopenia when treated with thiopurine drugs,[11–13] approximately three times as frequent as in IBD. Currently gastroenterologists have concerns about myelotoxicity when commencing patients on azathioprine and have medico-legal concerns about whether they should be using the molecular biological tools now available to allow them to screen for susceptibility. There are no guidelines available to advise on this issue.

The aim of our study is to evaluate the cost-effectiveness of screening for TPMT polymorphisms prior to initiation of azathioprine therapy for patients with IBD.

MATERIALS AND METHODS

Literature review and risk profiles

Frequency of leucopenia in patients receiving thiopurine drugs. Medline was searched from 1989 to identify case series reporting frequency of severe leucopenia in adults with IBD treated with azathioprine or its metabolic product mercaptopurine. Relevant referenced studies were also examined. Seven studies are reported (Table 1), with an overall frequency of leucopenia of 3.2%, and, in one series, two deaths out of 739 patients (0.29%). These are all retrospective studies.

Table 1. Frequency of leucopenia in adult IBD patients treated with thiopurine drugs

Author	Number of patients	Number with leucopenia requiring withdrawal of drug (%)	Definition of leucopenia	Deaths
Ansari[6]	106	2 (1.9)	N < 2.0	0
Schwab[7]	93	4 (4.3)	WCC < 3.0	0
Connell[14]	739	28 (3.8)	WCC < 3.0	2
Fraser[15]	622	21 (3.4)	WCC < 3.0	0
Present[16] (MP)	396	8 (2)	WCC < 2.5	0
Bouhnik[17]	157	3 (1.9)	WCC < 3.0	0
Qasim[18]	110	5 (5.5)	Not specified	0
Total	2223	71 (3.2)		2

WCC, total white cell count; N, neutrophil count; IBD, inflammatory bowel disease; MP, mercaptopurine.

Association of leucopenia with TPMT deficiency. Only in two of the seven case series was TPMT status investigated, with 50% of cases of leucopenia associated with TPMT deficiency in each: one of the two patients in the Ansari[6] study had intermediate red cell TPMT enzyme activity and two of four patients in the Schwab[7] study had TPMT genotypic variants – one was a homozygote and one a heterozygote.

A retrospective study by Colombel *et al.*[5] examined the TPMT genotype in a cohort of 41 patients who had experienced myelosuppression while receiving azathioprine therapy (38 leucopenia, three thrombocytopenia). Eleven (29%) of the 38 patients with leucopenia had a mutant TPMT polymorphism. Thirty-one of these patients had azathioprine discontinued and seven had their dose reduced.

A further study by Gearry *et al.*[19] examined the TPMT genotype of 56 patients with IBD who had suffered a range of adverse effects from azathioprine requiring cessation of therapy. Although there was no overall statistically significant association between abnormal TPMT genotype and cessation of therapy, there did appear to be an association for leucopenia. Four patients suffered leucopenia, two of whom had abnormal genotypes (one heterozygote and one homozygote). Genotype analysis was not performed on the other two patients, making the association rate at least 50%.

Case model

A model was established based on a theoretical population of 1000 patients receiving azathioprine

therapy for IBD (Figure 1) and undergoing standard haematological monitoring as is current practice.

Using the data from Table 1, we would expect that 32 patients per 1000 would experience leucopenia when treated with azathioprine. It is difficult to be sure of the precise risk of leucopenia associated with TPMT polymorphisms. However, to provide a reasonable estimate, we combined the results of the Colombel, Ansari and Schwab[5–7] studies and applied a 95% confidence interval (CI) to cover the potential range. Overall, this gave an association rate of 32% (95% CI: 20–47).

Cost estimates

Decision analysis was applied to our case model to ascertain if prospectively genotyping IBD patients who require azathioprine would be a worthwhile allocation of resources. The net cost of a genotyping strategy was calculated by accounting for the gross cost of the test and the cost saving from reduction in morbidity and mortality. This provided an estimate of 'cost per life-year saved', allowing comparison with other health care technologies which have been incorporated into clinical practice.

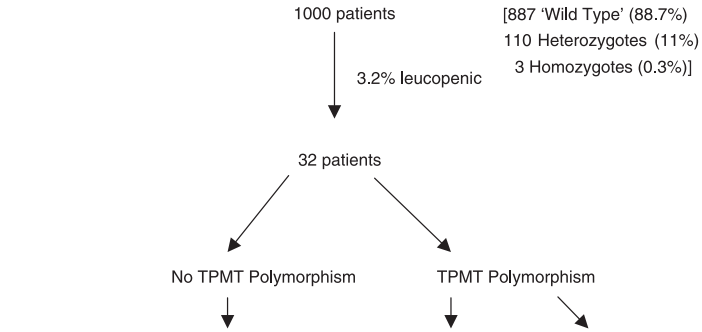
RESULTS

Risk profiles for TPMT polymorphisms

Homozygotes (three patients) will almost always develop leucopenia regardless of the strength of association for all TPMT mutations. Twenty-nine further patients will develop leucopenia, and the relative frequency for heterozygotes and 'wild types' will depend on the overall association between TPMT mutation and leucopenia. With a TPMT association rate of 32% (10 patients), then 22 patients (95% CI: 17–26) with leucopenia will be wild type (Figure 1). Three of the remaining 10 patients (95% CI: 6–15) are homozygotes, leaving seven heterozygotes (95% CI: 3–12).

Using this model, we can calculate the absolute and relative risks of leucopenia from azathioprine for each TPMT genotype. These are depicted in Figure 1 and Table 2. The absolute risk of leucopenia is 100% in homozygotes, 6.4% in heterozygotes and 2.5% in the wild-type population.

If we are to assume that homozygotes would not receive azathioprine, and that heterozygotes would receive a reduced dose and therefore avoid myelotoxicity, then the frequency of leucopenia could be reduced



Association strength	'Wild type'	Heterozygotes	Homozygotes	Total
32%	22 patients *	7 patients	3 patients	*n* = 32
	(2.5% 'wild type')	(6.4% heterozygotes)	(100% homozygotes)	
Limits of 95% confidence interval				
20%	26 patients	3 patients	3 patients	*n* = 32
	(2.9% 'wild type')	(2.7% heterozygotes)	(100% homozygotes)	
47%	17 patients	12 patients	3 patients	*n* = 32
	(1.9% 'wild type')	(11% heterozygotes)	(100% homozygotes)	

Figure 1. Case model demonstrating risk of leucopenia in 1000 theoretical IBD patients. Association of leucopenia with thiopurine methyltransferase (TPMT) deficiency is 32% (95% CI: 20–47)

* 32 patients suffer leucopenia. If TPMT polymorphisms account for 32% of leucopenia, then 68% of patients with leucopenia will be 'wild type' (22 patients). Of the remaining 10 patients, 3 will be homozygotes as there is a 100% association between homozygosity and leucopenia. The remaining 7 patients are therefore heterozygotes. The same calculations can be applied to different association strengths as shown above.

Table 2. Risk profiles for leucopenia and relationship with thiopurine methyltransferase (TPMT) genotype

Genotype	Absolute risk	Relative risk
Any	32/1000 = 3.2%	1
Wild type	22/887 = 2.5%	0.78
(95% confidence interval)	(1.9–2.9)	(0.59–0.9)
Heterozygote	7/110 = 6.4%	2
(95% confidence interval)	(2.7–11)	(0.84–3.52)
Homozygote	3/3 = 100%	31

by 32%. This would result in a reduction in the frequency of leucopenia from 32 to 22 per 1000. Therefore, the number needed to screen (NNS) to avoid one adverse event is 100 (1000/10), with a 95% CI of 67–167.

Validity as a screening test

The validity of TPMT genotype as a screening test is depicted in Table 3. Overall sensitivity and positive predictive value are low, largely reflecting the low incidence and risk of myelosuppression in this population. Indeed, there is a crossing of the confidence limits for the absolute risks attached to heterozygotes and 'wild types' indicating that we cannot say with certainty that heterozygotes have an increased risk. The negative predictive value of 97% is high but unfortunately not clinically useful as all the patients still have to be monitored intensively.

However, the absolute risk for homozygotes is so high and the consequences of missing them so potentially devastating that their detection as the sole purpose of screening would be worthwhile if found to be cost-effective.

Cost estimates of genotyping

Gross cost of the test. The cost of testing for TPMT polymorphisms by polymerase chain reaction is approximately £30 per patient. Therefore, to test 1000 patients costs £30 000.

Table 3. Validity of TPMT genotype as a screening test for leucopenia risk. Population frequency 3.2%. Association rate 32%.

	Disease present (significant myelosuppression)		
TPMT variant	Yes	No	Total
Yes	10	103	113
No	22	865	887
	32	968	1000

Sensitivity = 31%; specificity = 89%; positive predictive value = 9%; negative predictive value = 97%.

Costs saved. Savings have to be examined on two levels: the cost of morbidity [reflecting the cost to the National Health Service (NHS) of treating an adverse event] and the cost of mortality (representing the cost to society of losing a life). It must be borne in mind that many IBD patients treated with azathioprine are young and in employment.

Cost of morbidity. It is assumed that two-thirds of patients suffering significant leucopenia could be managed as an out-patient, requiring two extra medical clinic visits at £115 per attendance. The remaining one-third would require hospital admission because of infective complications. On average, they would spend 10 days in hospital and require barrier nursing, intravenous antibiotics, and possibly blood transfusion or granulocyte colony-stimulating factor. We equated this with the cost of a 10-day stay in a haematology ward at £402/day. Our costs were obtained from the Information and Statistics Division of the Common Services Agency in Scotland.[20]

Cost of mortality. This is expressed as 'cost per life-year saved', which allows comparison with other interventions offered by the NHS. We propose as a conservative estimate that we would avoid one death per 1000 patients if those with absent TPMT activity were identified by screening and either avoided azathioprine or received a much lower dose.

Current statistics[21] (from 2001) detail life expectancy in Scotland as follows: at age 30 it is 44.8 for males, 49.6 for females; at age 60 it is 18.4 for males, 22 for females. Therefore, avoiding a death at 30 will gain 47 life-years and avoiding a death at 60 will gain 20 life-years.

Calculation. If the frequency of myelosuppression is 3.2% and in 32% of patients this is associated with TPMT then pre-treatment screening would avoid 10

cases of leucopenia per 1000 patients when compared with standard haematological monitoring. Seven patients would have been managed as an out-patient (requiring two extra visits to a medical clinic at a cost of £115 per visit) costing £1610. Three patients would have required in-patient treatment (needing 10 days in a haematology ward at a cost of £402/day) costing £12 060. Therefore, the net cost of avoiding morbidity by screening 1000 patients is £16 330 (£30 000–1610–12 060) or £1633 per patient.

If we were to avoid one death by screening, then for a 30 year old, we save 47 life-years, costing £347 per life-year saved. For a 60 year old, we save 20 life-years, costing £817 per life-year saved. If we were to avoid two deaths, then the cost per life-year saved would halve.

Applying our earlier 95% CI for association rate, the cost per life-year saved for a 30 year old lies between £162 and £448, and for a 60 year old lies between £380 and £1052.

When discounted at 1.5% (the rate recommended by HM Treasury for the discounting of future benefits in public sector programmes), the cost per life-year saved rises to £487 (95% CI: 227–627) for a 30 year old and £951 (95% CI: 443–1225) for a 60 year old.

DISCUSSION

Comparison with other interventions

The cost of screening patients with IBD by TPMT genotype prior to commencing thiopurine drugs compares favourably with other health care technologies. For example, using a statin for secondary prevention in coronary heart disease costs £5100 per life-year gained,[22] using pegylated interferon with ribavirin for 48 weeks in chronic hepatitis C instead of a standard non-pegylated combination costs £4000–11000 per quality adjusted life-year (QALY)[23] and anti-tumour necrotic factor (TNF)-α antibody therapy for severe Crohn's disease costs £27 500/QALY.[24]

The above figures for TPMT screening represent a reasonably conservative estimate of the benefit gained. This cost would come down even further if the death rate was higher or if the association of myelosuppression with TPMT was >32%. Although the total combined mortality from the current studies lies just under 0.1%, this could well be an underestimate given the retrospective nature of these investigations. Screening for TPMT genotype prior to commencing therapy with azathioprine therefore represents good value for money. This is an assay which is readily established in a regional laboratory and can allow rapid reporting of results.

We do not believe that there will be any increase in treatment failure by adopting a screening programme. By reducing the dose of azathioprine administered to heterozygotes and avoiding azathioprine in homozygotes the number of cases of significant leucopenia will be reduced. Although the majority of heterozygotes will not experience leucopenia at standard doses, there is evidence to suggest that low TPMT activity appears to be predictive of a favourable response in patients treated with low doses of azathioprine.[25] Indeed, it may even be possible to safely treat homozygotes with very low doses as has been demonstrated by some case reports.[26]

Service provision

Currently, few clinicians routinely test for TPMT status and, at time of writing, the authors are aware of only two centres in the UK which offer a diagnostic service. Testing for red blood cell TPMT enzyme activity can be an expensive laboratory service to initiate, but genotype testing is equally helpful in identifying individuals at risk and can be more simply set up in regional centres which already test for genetic diseases such as haemochromatosis. Increased availability and use of testing will reduce the time taken for the results to be made available to the clinician.

Interpretation of the available data

Our information comes from retrospective data. This has two implications. First, the predictive value of TPMT polymorphisms in determining patients susceptible to leucopenia may have been exaggerated as it is based on data from small retrospective studies, which may overemphasize the risk. In any prospective study, patients would either not be treated or receive a lower dose of azathioprine and hence the value in preventing leucopenia would be difficult to judge. Secondly, retrospective series often underestimate morbidity and mortality and the true risk of death may in fact be higher than the 1 per 1000 we have used for our calculations. Indeed, it is the small but tangible risk of iatrogenic death which makes screening cost-effective.

It must always be remembered that the majority of cases of leucopenia are not TPMT-related and therefore

genotype screening can never be viewed as a substitute for the current practice of regular monitoring of the white blood cell count.

Potential weaknesses

Our calculations make the assumption that identifying TPMT-deficient patients at risk of myelosuppression will reduce mortality from thiopurine-related leucopenia. However, this may not be the case, as illustrated by the two arguments below.

We have assumed that there is an equal risk of death from leucopenia regardless of whether or not it is attributable to TPMT deficiency. It is unclear if the remaining 68% cases of myelosuppression unrelated to TPMT may be due to deficiencies in enzymes which have yet to be identified or may be of viral origin. It could be argued that risk of death from myelosuppression of unknown source (e.g. viral infection) may be greater than that from TPMT deficiency, where leucopenia is usually self-limiting once the drug has been discontinued. Therefore, screening might identify potentially leucopenic patients with a better overall prognosis.

It is also possible that leucopenia secondary to TPMT deficiency may occur earlier in treatment than unrelated causes of myelosuppression and thus be more likely to be readily detected by haematological screening. In this event, screening for TPMT deficiency may be identifying patients who would have been detected by careful haematological monitoring in normal circumstances and therefore possibly not contributing significantly to alteration in prognosis.

Both of the above arguments are plausible. However, in the absence of any sound confirmatory evidence in the literature it would be difficult to currently justify failing to screen for TPMT deficiencies on this basis, especially given the degree of cost-effectiveness illustrated.

CONCLUSION

Screening for TPMT genotype is an acceptable and cost-effective intervention in the management of patients with IBD requiring therapy with azathioprine. Most centres should offer a diagnostic service so that clinicians can utilize this test. However, screening should be viewed as an adjunct to standard haematological monitoring and not as a replacement.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Alex McConnachie from the Robertson Institute of Biostatistics, University of Glasgow, for his assistance with the statistical analysis.

REFERENCES

1 Pearson DC, May GR, Fick G, Sutherland AR. Azathioprine for maintenance of remission in Crohn's disease (Cochrane Review). In: *The Cochrane Library*, Issue 3, 2004. Chichester, UK: John Wiley and Sons, Ltd.
2 Sandborn W, Sutherland L, Pearson D, May G, Modigliani R, Prantera C. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease (Cochrane Review). In: *The Cochrane Library*, Issue 3, 2004. Chichester, UK: John Wiley and Sons, Ltd.
3 Yates CR, Krynetski EY, Loennechen T, *et al.* Molecular diagnosis of thiopurine-s-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997; 126: 608–14.
4 Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 1980; 32: 651–62.
5 Colombel JF, Ferrari N, Debuysere H, *et al.* Genotypic analysis of thiopurine s-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. Gastroenterology 2000; 118: 1025–30.
6 Ansari A, Hassan C, Duley J, *et al.* Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment Pharmacol Ther 2002; 16: 1743–50.
7 Schwab M, Schaffler E, Marx C, *et al.* Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine s-methyltransferase polymorphism. Pharmacogenetics 2002; 12: 429–36.
8 Szumlanski C, Weinshilboum RM. Sulfsalazine inhibition of thiopurine methyltransferase: possible mechanism for interaction with 6-mercaptopurine and azathioprine. Br J Clin Pharmacol 1995; 39: 456–9.
9 Lewis LD, Benin A, Szumlanski CL, *et al.* Olsalazine and 6-mercaptopurine-related bone marrow suppression: a possible drug-drug interaction. Clin Pharmacol Ther 1997; 62: 464–75.
10 Lennard L. Clinical implications of thiopurine methyltransferase – optimisation of drug dosage and potential drug interactions. Ther Drug Monit 1998; 20: 527–31.
11 Marra C, Esdaile J, Aslam HA. Practical pharmacogenetics: the cost effectiveness of screening for thiopurine s-methyltransferase polymorphisms in patients with rheumatological conditions treated with azathioprine. J Rheumatol 2002; 29: 2507–12.
12 Black AJ, McLeod HL, Capell H, *et al.* Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. Ann Intern Med 1998; 129: 716–8.
13 Suarez-Alamazor ME, Spooner C, Belseck E. Azathioprine for treating rheumatoid arthritis (Cochrane Review). In: *The*

Cochrane Library, Issue 3, 2004. Chichester, UK: John Wiley and Sons, Ltd.
14 Connell WR, Kamm MA, Ritchie JK, Lennard-Jones JE. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. Gut 1993; 34: 1081–5.
15 Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut 2002; 50: 485–9.
16 Present DH, Meltzer SJ, Krumholtz MP, Wolke A, Korelitz BI. 6-Mercaptopurine in the management of inflammatory bowel disease: short and long term toxicity. Ann Intern Med 1989; 111: 641–9.
17 Bouhnik Y, Lemann M, Mary JY, *et al.* Long term follow up of patients with Crohn's disease treated with azathioprine or 6-mercaptopurine. Lancet 1996; 347: 215–9.
18 Qasim A, Seery J, Buckley M. TPMT in the treatment of inflammatory bowel disease with azathioprine (letter). Gut 2003; 52: 767–74.
19 Gearry RB, Barclay ML, Burt MJ, *et al.* Thiopurine-S-methyltransferase genotype does not predict adverse reactions to thiopurine drugs in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2003; 18: 395–400.
20 Scottish Health Service Costs. Year Ending 31st March 2003. The Costs Book 2003. Information and Statistics Division of the Common Services Agency, http://www.isdscotland.org/isd/info3.jsp. Last accessed 28 July 2004.
21 Office for National Statistics Autumn 2003. Expectation of Life (in years) at Birth and Selected Age, 1981 Onwards: Population Trends 114. http://www.census2001.gov.uk/STATBASE/Expodata/Spreadsheets/D8625.csv. Last accessed 28 July 2004.
22 Pickin DM, McCabe CJ, Ramsay LE, *et al.* Cost effectiveness of HMG CoA reductase inhibitor (Statin) treatment relates to the risk of coronary heart disease and the cost of drug treatment. Heart 1999; 82: 325–33.
23 National Institute for Clinical Excellence. Interferon-alfa (Pegylated and Non-pegylated) and Ribavirin for the Treatment of Chronic Hepatitis C. Technology Appraisal Number 75. January 2004, http://www.nice.org.uk. Last accessed 28 July 2004.
24 National Institute for Clinical Excellence. Full Guidance for the Use of Infliximab in Crohn's Disease. Technology Appraisal Number 40. May 2002, http://www.nice.org.uk.
25 Campbell S, Kingstone K, Ghosh S. Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low dose azathioprine. Aliment Pharmacol Ther 2002; 16: 389–98.
26 Kaskas BA, Louis E, Hindorf U, *et al.* Safe treatment of thiopurine s-methyltransferase deficient Crohn's disease patients with azathioprine. Gut 2003; 52: 140–2.