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Thiopurine monitoring in children with inflammatory bowel disease: a systematic review

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AIMS
The aim was to systematically review the evidence on the clinical usefulness of thiopurine metabolite and white blood count (WBC) monitoring in the assessment of clinical outcomes in children with inflammatory bowel disease (IBD).

METHODS
Medline, Embase, Cochrane Central Register of controlled trials and http://www.clinicaltrials.gov were screened in adherence to the PRISMA statement by two independent reviewers for identification of eligible studies. Eligible studies were randomized controlled trials (RCTs), cohort studies and large case series of children with inflammatory bowel disease (IBD) (<18 years) who underwent monitoring of thiopurine metabolites and/or WBC.

RESULTS
Fifteen papers were identified ($n = 1026$). None of the eligible studies were RCTs. High 6-thioguanine nucleotide (6TGN) concentrations were not consistently associated with leucopenia. Leucopenia was not associated with achievement of clinical remission. A positive but not consistent correlation between 6TGN and clinical remission was reported. Haematological toxicity could not be reliably assessed with 6TGN measurements only. A number of studies supported the use of high 6-methylmercaptopurine ribonucleotides (6MMPR) as an indicator of hepatotoxicity. Low thiopurine metabolite concentration may be indicative of non-compliance.

CONCLUSION
Thiopurine metabolite testing does not safely predict clinical outcome, but may facilitate toxicity surveillance and treatment optimization in poor responders. Current evidence favours the combination of thiopurine metabolite/WBC monitoring and clinic follow-up for prompt identification of haematologic/hepatic toxicity safe dose adjustment, and treatment modification in cases of suboptimal clinical outcome or non-compliance. Well designed RCTs for the identification of robust surrogate markers of thiopurine efficacy and toxicity are required.

Introduction

Thiopurines are commonly used for maintenance of remission in children with inflammatory bowel disease (IBD) [1, 2]. Patient response to treatment exhibits inter-individual variability, thought to partially result from pharmacogenetic variation in drug metabolism [3–5]. Poor clinical outcome, toxicity or non-compliance may respond to dose

adjustment or treatment modification [6] and therefore therapeutic drug monitoring is clinically indicated [7, 8].

In paediatric clinical practice, the development of monitoring tools has not always kept pace with management protocols and evidence from paediatric studies. Children can have extensive disease at diagnosis and more severe symptoms in the first years after diagnosis in comparison with adults [9]. Most paediatric centres would use thiopurines in more than 70% of all children with IBD, at potentially higher dosages than in adults, estimated on the basis of mg kg^{-1} body weight [10, 11].

Thiopurine methyltransferase (TPMT) genotype/erythrocyte activity and thiopurine metabolite measurement have been implemented for therapeutic drug monitoring [12]. Controversy exists with regards to the applicability, predictive value and cost effectiveness of these markers [13, 14]. A review by Gisbert & Gomollon [15] reported that myelosuppression could not be explained by TPMT deficiency in adults, therefore continuous surveillance is necessary. Aberra *et al*. reviewed adult and paediatric studies [16] and observed great variability in the sensitivity and specificity of 6TGN concentrations for assessment of clinical remission. Hindorf *et al*. reported a dynamic nature of thiopurine metabolism, with children demonstrating a significantly lower median TPMT activity than adults [17].

Thiopurine dose adjustments are largely based upon white blood count monitoring (WBC) [18]. A meta-analysis of 12 studies (nine adult and three paediatric) by Higgs *et al*. investigated the risk of myelosuppression in patients with intermediate TPMT activity [19] and highlighted the importance of TPMT status in thiopurine metabolite production, but also the heterogeneity of leucopenia definitions.

Due to the lack of reviews with focus on paediatric IBD and the ongoing uncertainties regarding the clinical usefulness of thiopurine metabolite monitoring in children, we have undertaken a systematic review to evaluate the current evidence on the association between these markers and clinical outcome. The latter is defined as any observed clinical response to treatment, including adverse drug reactions such as haematological and hepatic toxicity. This manuscript provides a timely review of this topic, taking into account the differences in disease manifestation, progress, treatment response and the possibly distinct drug metabolism in children as compared with adult patients with IBD.

Thiopurine metabolism

Thiopurines are represented by mercaptopurine (MP), azathioprine and thioguanine [20]. Both MP and azathioprine are used in paediatrics; these undergo extensive intestinal and hepatic metabolism by a number of enzymes, including hypoxanthine phosphoribosyltransferase (HPRT), TPMT, xanthine oxidase (XO) and inosine monophosphate dehydrogenase (IMPDH). Various metabolites are produced. 6-thiouric acid (6TU) and 6-methylmercaptopurine (6MMR) are both inactive, while 6-methylmercaptopurine ribonucleotides (6MMPR) and 6-thioguanine nucleotides (6TGN) mainly mediate the pharmacological effect of thiopurines [21].

6MMPR is formed by TPMT and principally mediates thiopurine induced hepatotoxicity [22, 23]. A value >5700 pmol/8×10^8 red blood cells (RBC) has been associated with liver toxicity [24, 25]. Thiopurine-induced liver toxicity has been divided into three categories: hypersensitivity, idiosyncratic cholestatic reactions and nodular regenerative hyperplasia [26, 27]. Regular monitoring of 6MMPR concentrations and liver transaminases may be useful for identification of patients at risk of hepatotoxicity [28].

Production of 6TGN is catalyzed by IMPDH. Concentrations ≥235 pmol/8×10^8 RBC and ≥450 pmol/8×10^8 RBC have been associated with clinical remission and haematological toxicity respectively [28, 29]. Haematological toxicity, commonly manifesting as leucopenia, has been linked to homozygous recessive TPMT genotype and intermediate or low TPMT activity [15].

Methods

Search strategy and study selection

A literature search of the following databases was conducted: Medline from 1966 to 01/04/2013, (interfaces used were the UK National Health System health Databases and PubMed US National Library of Medicine, National Institutes of Health), Embase (1980 to 01/04/2013), Cochrane Central Register of controlled trials (CENTRAL) and the http://www.clinicaltrials.gov website.

The following search terms were used: 'thiopurine' and 'inflammatory bowel disease'. The filters used were English language, 'humans' and child 'birth to 18 years'. One hundred and twenty-nine studies were found in Pubmed (US National Library of Medicine, National Institutes of Health). An alternative Medline search was conducted electronically on 01/04/2013 via the UK National Health System health databases (http://www.evidence.nhs.uk), with the following search criteria (((((azathioprine.ti,ab) OR (exp azathioprine/)) AND (mercaptopurine.ti,ab) AND (((blood AND monitoring).ti,ab) AND (monitoring, physiologic/ OR adult/ OR drug monitoring/ OR middle aged/))) OR ((TPMT.ti,ab) AND (methyltransferases/ OR azathioprine/ OR 6-mercaptopurine/ OR immunosuppressive agents/ OR polymorphism, genetic/ OR inflammatory bowel diseases/ OR kinetics/ OR erythrocytes/)))) [4]. One hundred and eighty-one studies were retrieved. The same search strategy was repeated electronically on Embase via http://www.evidence.nhs.uk. ($n = 111$ studies). No eligible studies were found in the Cochrane database and no eligible on-going or completed clinical trials were identified from the http://www.clinicaltrials.gov website (accessed

on 01/04/2013). The database search algorithm is demonstrated in Figure S1 and is in accordance with the PRISMA checklist (Table S1) and PRISMA statement [30].

Inclusion and exclusion criteria

Randomized controlled trials (RCTs), cohort studies and case series (>five patients) of children (<18 years old) with IBD, who underwent therapeutic drug monitoring – assessment of treatment response and toxicity surveillance – with thiopurine metabolite measurement (6TGN and 6MMPR) and/or WBC, were included. Eligible studies were required to have clinical assessment tools of disease activity and/or clinical outcome, as well as biochemical evidence of adverse drug reactions or toxicity following thiopurine introduction. The thiopurines could have been administered at any time following diagnosis, by any route and at any dose. Concomitant medications were allowed. Studies published in English only were included. The exclusion criteria were the following:

1 Individual case reports and case series (≤five patients);
2 Adult studies (age ≥18 years);
3 Review articles
4 Abstracts and
5 Studies not published in English.

Data extraction methodology and data quality assessment

The list of titles and abstracts was reviewed and any irrelevant studies were removed. For the remaining papers, abstracts and full text articles were retrieved and each was assessed individually for eligibility. Reference lists of all identified studies were scrutinized for further papers of potential relevance. This process was undertaken independently by two reviewers (AK and AA) with differences resolved by discussion and consultation with the senior authors (WEM and MP). During quality assessment none of the authors was blinded to article details, such as journal, author and institution, year of publication or study findings.

Data were extracted in accordance with the methods set out in the Cochrane Handbook [31] and were subsequently recorded in data extraction forms, following iteration in the course of reviewing the first five eligible studies. Data extraction mainly focused on the pre-specified outcomes of interest in this review, following agreement by all authors. The information extracted included type of study, total number of patients and median age, mean duration of thiopurine therapy, findings on WBC and thiopurine metabolite concentrations, disease activity tools and clinical outcome definitions, interpretation of zero/negligible metabolite concentrations and effect of metabolite guided thiopurine dose adjustments. The average numerical values of thiopurine metabolites and WBC were investigated in relation to clinical outcomes, and a comparison between markers with regard to their clinical usefulness was made. Methodological quality assessment of each included study was independently performed by two authors (AK and AA), using National Institute of Clinical Excellence (NICE) defined criteria for quality assessment of case series [32], (Table S2).

Results

Fifteen studies fulfilled the eligibility criteria [4, 33–46]. 'Potentially' eligible studies which were assessed and subsequently excluded are shown in Table S3.

The eligible studies included 1026 paediatric IBD patients treated with thiopurines. None of the included studies were RCTs. Nine retrospective and six prospective case series were identified as eligible.

Nine out of fifteen studies had well defined inclusion and exclusion criteria. All studies had clearly stated aims but therapeutic drug monitoring was not necessarily the primary outcome. Study findings were stratified for patients, according to disease stage, blood test results, patient characteristics, concomitant medications and median treatment duration. Clinical outcome definitions were clearly described in all but three studies [38, 42, 45]. The characteristics and findings of the included studies are summarized in Tables S4 and S5.

Monitoring of clinical remission

Peripheral blood cell counts

Eight out of fifteen studies [4, 34, 35, 37, 39, 43, 44, 46] investigated the association between leucopenia and clinical remission. The median leucocyte count of patients in remission was $>4 \times 10^9\ l^{-1}$ in seven of these studies, and therefore leucopenia was not associated with clinical remission. Gupta *et al.* [34] observed higher remission rate in patients with leucopenia (67%) than in patients with normal leucocyte count (49%), but this difference did not reach statistical significance. Ohtsuka *et al.* [35] noted that mean WBC was overall significantly higher in active disease than in remission.

Thiopurine metabolite concentrations

Twelve out of 15 studies investigated how clinical remission related to 6TGN and 6MMPR concentrations. From these studies, five studies of 'average or above average' quality (score≥4), (Table S2) with a total number of 297 patients, reported a higher probability of remission with increasing 6TGN concentrations.

These five studies further investigated whether a specific 6TGN cut off value was associated with clinical remission. Dubinsky *et al.* [36] and Ooi *et al.* [44] concluded that a level above 235 pmol/8×10^8 RBC significantly increased the odds of clinical remission. Grossman *et al.* [39]

observed an increased trend of achieving remission if 6TGN ≥235 pmol/ 8×10^8 RBC, but this finding was not statistically significant. Pozler *et al*. [41] reported a level of 6 TGN ≥ 235 pmol/8×10^8 RBC in 10 out of 11 patients in clinical remission. Banerjee & Bishop [40] concluded that metabolite monitoring resulted in earlier dose tailoring with higher final doses, significantly improved clinical outcomes and less frequent corticosteroid treatment. In this study ($n = 101$ patients), the 6TGN concentration in just over half of the patients in clinical remission was reported to be >200 pmol/8×10^8 RBC.

Conversely, six studies of 'average or above average' quality (score ≥4, $n = 333$ patients) and one study of 'below average quality' ($n = 51$, score 3) identified non-significant differences in 6TGN levels between treatment responders and non-responders. In detail, Cuffari *et al*. [4] reported variable 6TGN concentrations in patients in remission, Cangemi *et al*. [33] showed a trend of lower 6TGN values in non-responders. However the association between a specific 6TGN target concentration and clinical remission was overall reported to be weak. Armstrong *et al*. [37] did not recommend a target 6TGN concentration for prediction of clinical remission. Ohtsuka *et al*. [35] found that 6TGN concentrations were independent of disease activity. Gupta *et al*. [34] reported higher median 6TGN concentrations in Crohn's patients in remission, but higher 6TGN did not overall increase the probability of clinical remission. Nguyen *et al*. [43] and Paerregaard *et al*. [45] similarly reported no significant 6TGN differences between patients in remission and active disease.

Monitoring of thiopurine toxicity

Haematological toxicity

Gupta *et al*. [34] and Dubinsky *et al*. [36] noted that leucopenic patients had higher 6TGN concentrations. Armstrong *et al*. [37] noted significantly higher WBC levels in patients with 6TGN <235 pmol/8×10^8 RBC and reported 6TGN values >450 pmol/8×10^8 RBC in six patients with bone marrow suppression ($P < 0.001$). Cangemi *et al*. reported one such case [33].

Conversely, Ooi *et al*. [44] reported that leucopenia could be observed with variable 6TGN concentrations. Ohtsuka *et al*. [35] and Paerregaard *et al*. [45] did not report any association between 6TGN concentrations and haematological toxicity. Pozler *et al*. [41] reported no cases of haematological toxicity. Cuffari *et al*. [4], Grossman *et al*. [39] and Cangemi *et al*. [33] did not reach any conclusions due to very few reported cases. Therefore, a weak association between WBC and thiopurine metabolite concentrations was described in three studies ($n = 126$ patients), inconclusive results were reported by four studies ($n = 120$ patients) due to very few or no reported cases, and a possible inverse correlation between 6TGN and WBC was suggested by three studies ($n = 263$ patients). The proposed median cut off 6TGN >450 pmol/8×10^8 RBC value for haematologic toxicity cannot be recommended. However it is possible that patients with relatively higher 6TGN concentrations may be at increased risk of toxicity (Table S6).

Hepatotoxicity

Five ($n = 343$ patients) out of eight studies ($n = 503$ patients) which investigated the association between 6MMPR concentrations and hepatotoxicity (elevated liver enzymes) [4, 34, 36, 39, 40], clearly supported the cut off value of 5700 pmol/8×10^8 RBC as a useful adjunct surrogate marker of hepatotoxicity (Table S6). These studies were of comparable, average or above average quality (Table S2).

Interpretation of negligible or zero metabolite concentrations

Nine out of the 15 studies investigated the significance of very low thiopurine metabolite concentrations. Eight out of the nine studies reported that low 6TGN suggested non-compliance.

Cuffari *et al*. [4] advised that low 6TGN may be due to suboptimal dose or lack of clinical response to treatment. Grossman *et al*. [39] agreed that low concentrations may correspond to low drug efficacy, whereas Gupta *et al*. [34] found that patients with low 6TGN had less chance of achieving remission, thereby suggesting that thiopurine dose adjustment or treatment change may be appropriate.

Six out of the nine studies reported low 6MMPR to be associated with lack of compliance to treatment. Cuffari *et al*. [4] reached the same conclusion and also suggested that negligible 6MMPR and measurable 6TGN concentrations may be due to TPMT deficiency.

Effect of metabolite guided dose adjustment on clinical outcome

The effect of metabolite-guided thiopurine dose adjustment on clinical outcome was systematically investigated in only four out of the 15 included studies. More rapid and safer dose escalation towards clinical remission was reported by Gupta *et al*. [34], Grossman *et al*. [39], Banerjee & Bishop [40] and Ooi [44] and colleagues.

Discussion

The large majority of included studies showed that leucopenia was not predictive or indicative of clinical remission. Nevertheless, the association between relative leucopenia ($4 \times 10^9\ l^{-1}$–$5 \times 10^9\ l^{-1}$) and clinical outcome was not investigated by any of the included studies. There is a lack of evidence for this subgroup of patients. Routine WBC monitoring is highly recommended for prompt identification of haematological toxicity.

Evidence to date demonstrates an existing but not consistent association between rising 6TGN concentrations and clinical remission; however this finding did not reach statistical significance in the majority of studies. This may be interpreted as lack of true association or lack of power due to small sample sizes. Further research is therefore required. At present no target 6TGN concentration can be safely recommended as a surrogate marker of clinical remission. The evidence on the existence of a consistent correlation between WBC and 6TGN levels is insufficient. The predictive value of 6TGN>450 pmol/8×10^8 RBC as a cut-off level for diagnosis of haematological toxicity cannot be safely established from the included studies [44]. Arguably, the relatively very few patients reported to have suffered serious haematological toxicity, manifesting as myelosuppression, does not allow safe and generalizable conclusions to be drawn. However high or rising 6TGN trends may assist clinicians in the identification of patients at increased risk of thiopurine toxicity. Current evidence favours the use of a 6MMPR concentration >5700 pmol/8×10^8 RBC as an additional surrogate marker of hepatotoxicity risk, although caution must be exercised until further high quality research in larger prospective cohorts is performed.

Metabolite monitoring is particularly recommended for assessment of non-compliance in cases of very low or negligible 6TGN concentrations. Low baseline metabolite concentrations and metabolite guided dose alterations may be implemented so as to guide safe and prompt dose escalation in patients requiring relatively higher doses to achieve clinical remission. Treatment alteration may be considered in true non-responders.

The strengths of this review include a thorough, reproducible search strategy across major electronic databases, detailed data extraction according to the Cochrane Handbook [46], and qualitative and quantitative quality assessment by independent assessors according to validated NICE criteria. As shown in Table S2, four out of 15 studies, were rated to be of 'above average' quality (scored ≥6 points out of highest score of 8), 10 studies were of average quality (score 4–5) and one study was of 'below average quality' (score ≤3). Nine out of 15 studies were of retrospective design. Five studies included ≤35 patients.

A limitation of this review is the exclusion of abstracts, conference proceedings, unpublished studies or studies published in languages other than English. A lack of RCTs, prospective case control and cohort studies is noted. Well-designed RCTs and large multicentre, longitudinal studies are clearly higher in the evidence hierarchy. Therefore their findings would carry more weight in comparison with case series.

Variability in design and methodology of the included studies and inherent bias due to non- randomization and non-blinding may have influenced study outcomes and conclusions. Sources of study heterogeneity were assessed at both methodological and outcome levels. These are choice of design (retrospective *vs*. prospective design), median duration, dose and type of thiopurine treatment, diverse follow-up arrangements, use of heterogeneous disease activity/clinical outcome assessment tools and variability in baseline patient characteristics, for example ethnic differences. Adult studies that may have included adolescent patients' data have been excluded from this review. Factors which may have played a confounding role are differences in concomitant medication, variation in timing of blood sampling and metabolite testing methods and variability in clinical practice. No missing information or selective reporting issues have been identified in any of the included studies. Disease related and genetic factors (such as ITPA genetic polymorphisms), or environmental influences, may have had an unpredictable effect on clinical outcomes. Underestimated or under-reported lack of compliance may have also affected the findings of included studies. It is important to note that we were only able to assess the quality of studies based on published data.

It was beyond the scope of this review to address how thiopurine metabolite concentrations and WBC may be influenced by age, gender, administration of concomitant medications, mean duration and type of thiopurine treatment or different dosing regimens. Such parameters may have an effect which has not been investigated but is clearly highlighted. Our strategy, however, enabled us to assess thiopurine drug monitoring in pragmatic circumstances.

Conclusion

The clinical usefulness of 6TGN concentrations of >235 pmol/8×10^8 RBC and >450 pmol/8×10^8 RBC as surrogate markers of clinical remission and drug toxicity, respectively, is controversial. A number of studies to date demonstrate an increased probability of clinical remission with increasing 6TGN concentrations. Haematological toxicity may be suspected in patients with disproportionately high 6TGN concentrations, however it may present in patients with any 6TGN concentration. The majority of included studies are supportive of the use of a 6MMPR cut-off value >5700 pmol/8×10^8 RBC as an indicator of increased hepatotoxicity risk. Low or negligible metabolite concentrations are indicative of non-compliance. The monitoring of thiopurine metabolite trend may therefore be a useful adjunct to clinical surveillance in patients with suspected toxicity and suboptimal clinical outcome. Current evidence does not justify recommendation of generalized, routine metabolite monitoring. However such practice may be advisable at the clinician's discretion.

In conclusion, the combination of WBC and metabolite testing in conjunction with clinic follow-up enhances toxicity surveillance and treatment optimization, with

facilitation of dose escalation or treatment modification in patients with poor clinical outcome. High quality evidence, however, is required on the clinical usefulness of universal, routine metabolite monitoring and on the effect of metabolite guided dose/treatment alterations in children. Emerging evidence on the impact of genetic polymorphisms in additional genes implicated in thiopurine metabolism warrants consideration of potential influences on observed clinical outcomes.

Competing Interests

Guarantors: Munir Pirmohamed and Wael El Matary

Specific author contributions

AK and WEM: study concept and design. AK and AA: acquisition, quality assessment and interpretation of data. AK drafted the manuscript. LJB peer reviewed the manuscript and offered specialist advice. WEM interpreted data and critically reviewed the manuscript as senior author. MP critically reviewed the manuscript as senior co-author.

All authors have approved the final version of the manuscript.

WEM received travel support from Janssen Ltd and served as an advisory board member for both Janssen and AbbVie.

'All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work and no other relationships or activities that could appear to have influenced the submitted work.'

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1
Database search algorithm-PRISMA 2009 Flow diagram
Table S1
PRISMA statement
Table S2
Quality assessment of case series.
Reference: National Institute of Clinical Excellence (NICE). http://www.nice.org.uk/nicemedia/pdf/Appendix_04_qualityofcase_series_form_preop.pdf
Accessed on 01/04/2013
Table S3
'Potentially' eligible studies excluded and reasons for exclusion
Table S4
Characteristics of included studies (RBC: red blood cells, WBC: white blood cells, P: prospective study, R: retrospective study, 6TGN: 6 thioguanine nucleotides, 6MMPR: 6 methylmercaptopurine ribonucleotides, ALT: alanine aminotransferase, MCV: erythrocytes mean corpuscular volume, TPMT: thiopurine-methyltransferase, c/s: corticosteroids
Table S5
Characteristics of included studies (continued)

UC: ulcerative colitis, CD: Crohn's disease, 6TGN: 6 thioguanine nucleotides, 6MMPR: 6 methylmercaptopurine ribonucleotides, TPMT: thiopurine methyltransferase HBI: Harvey–Bradshaw index, PCDAI: Paediatric Crohn's disease activity index, PUCAI: Paediatric ulcerative colitis disease activity index, SCCAI: Simple clinical colitis activity index, PGA: Physician global assessment, c/s: corticosteroids

Table S6
Reported thiopurine metabolite concentrations and WBC in clinical remission and thiopurine toxicity. RBC: red blood cells, 6TGN: 6 thioguanine nucleotide, 6MMPR: 6 methylmercaptopurine ribonucleotides