

The development of an objective methodology to measure medication adherence to oral thiopurines in paediatric patients with acute lymphoblastic leukaemia—an exploratory study

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Abstract

Aims To develop a method that prospectively assesses adherence rates in paediatric patients with acute lymphoblastic leukaemia (ALL) who are receiving the oral thiopurine treatment 6-mercaptopurine (6-MP).

Methods A total of 19 paediatric patients with ALL who were receiving 6-MP therapy were enrolled in this study. A new objective tool (hierarchical cluster analysis of drug metabolite concentrations) was explored as a novel approach to assess non-adherence to oral thiopurines, in combination with other objective measures (the pattern of variability in 6-thioguanine nucleotide erythrocyte concentrations and 6-thiouric acid plasma levels) and the subjective measure of self-reported adherence questionnaire.

Results Parents of five ALL patients (26.3%) reported at least one aspect of non-adherence, with the majority (80%) citing “carelessness at times about taking medication” as the primary reason for non-adherence followed by “forgetting to take the medication” (60%). Of these patients, three (15.8%) were considered non-adherent to medication according to the self-reported adherence questionnaire (scored ≥ 2). Four ALL patients (21.1%) had metabolite

profiles indicative of non-adherence (persistently low levels of metabolites and/or metabolite levels clustered variably with time). Out of these four patients, two (50%) admitted non-adherence to therapy. Overall, when both methods were combined, five patients (26.3%) were considered non-adherent to medication, with higher age representing a risk factor for non-adherence ($P < 0.05$).

Conclusions The present study explored various ways to assess adherence rates to thiopurine medication in ALL patients and highlighted the importance of combining both objective and subjective measures as a better way to assess adherence to oral thiopurines.

Keywords Thiopurines · 6-mercaptopurine · Paediatrics · Acute lymphoblastic leukaemia · Medication adherence

Introduction

Poor adherence to medication is an ever present and complex problem contributing to substantial worsening of disease control and quality of life. In addition, it gives rise to higher costs within health-care systems [1]. In a recent quantitative review of 569 studies, conducted over 50 years, non-adherence to medical treatment averaged 24.8% (with a range of 0–95.4%) demonstrating the extent of this public health issue [2]. Poor adherence to therapeutic regimens is typically higher in patients with chronic conditions [3]. Approximately one-half of patients receiving treatment for a chronic illness have problems following their prescribed regimens to the extent that they are unable to obtain optimum clinical benefit [4]. In this study non-adherence to medication in paediatric patients with acute lymphoblastic leukaemia (ALL) was investigated.

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Childhood ALL is unique among childhood cancers in that, for the majority of patients, a prolonged course of oral chemotherapy is essential to achieve long-term, disease-free survival. When ALL children reach remission, they become practically asymptomatic but remain under extended and complex treatment. Therefore, non-adherence is expected in these patients. Reports on children and adolescents with ALL have detailed non-adherence in 2–54% of cases [5–8], with higher percentages occurring in adolescents [5, 6]. The required level of adherence needed to cure ALL is unknown [7], however, it has been suggested that at least 95% of the prescribed medication should be taken by ALL patients to be considered adherent with therapy.

Several methods are reported to assess adherence to medication which can be broken down into objective direct measures (e.g. measurement of the level of drug and/or its metabolites or specific biomarkers) and subjective indirect approaches (e.g. patient questionnaires and assessment of patient's clinical response). In spite of the availability of many methods, none are entirely valid or reliable [9]. Questioning the patient (or using a questionnaire) is the most widely applicable method of measuring adherence in the clinical setting since this approach is inexpensive and easy to use. However, it can be susceptible to patient's misrepresentation and tends to result in overestimating adherence to medication [10].

Drug or metabolite levels in blood or urine provide more objective measures of adherence to medication, however, this approach can be expensive and is not always available. In addition, drug or metabolite levels may differ widely because of intra- and inter-individual differences in metabolism (due to inherited differences in the activity of enzymes and genetic polymorphisms) or due to differences in rates of absorption (e.g. when drugs are administered orally) [11]. Among the various medications used in ALL maintenance chemotherapy, the oral thiopurine medication 6-mercaptopurine (6-MP) is considered the easiest to monitor because it is taken daily and has stable intracellular metabolites. The frequency of non-adherence detected through the measurement of 6-MP and its metabolite levels in blood was 10–20% [12–14]. In a recent study, Bokemeyer et al. assessed adherence to thiopurine treatment in patients with Crohn's disease by therapeutic drug monitoring (through the quantification of relevant thiopurine metabolite levels in red blood cells) as well as by patients' self-report using a standardized questionnaire [15]. The authors found a high degree of concordance in adherence rates between the two methods (75%).

6-MP is a prodrug which requires intracellular activation into thioinosine monophosphate (TIMP) and subsequently into thioguanine nucleotides (6-TGNs), the active cytotoxic metabolites. This activation is in competition with two other metabolic pathways—oxidation of 6-MP by xanthine

oxidase into 6-thiouric acid (6-TU), an inactive metabolite, and methylation of both 6-MP and TIMP by thiopurine methyltransferase into 6-methylmercaptopurine and 6-methylmercaptopurine ribonucleotides (6-mMPNs), the latter of which are potent inhibitors of de novo purine synthesis [17].

Wide variations in the cytotoxic 6-TGN metabolites have been observed between patients prescribed similar 6-MP doses. This variability in 6-TGN concentrations can be explained, in part, by the inherited variability in TPMT activity. TPMT favours the formation of 6-mMPNs at the expense of 6-TGNs through competitive metabolic pathways [18]. Very high TPMT activity, therefore, results in the formation of low levels of 6-TGNs but high levels of 6-mMPNs and vice versa [19]. The existence of a genetic polymorphism is responsible for a large part of the inter-individual differences observed in TPMT activity and the tri-modal distribution of TPMT activity in the general Caucasian population. The inter-individual differences in patients with normal or high TPMT activity, however, are largely unknown to date. In addition, differences in TPMT activity alone are insufficient to explain the wide variation in 6-TGN concentrations within the same patient while prescribed the same dose of 6-MP. Furthermore, low concentrations of both 6-TGNs and 6-mMPNs, although possibly the result of poor absorption, are hard to explain on metabolic grounds. An alternative explanation is the possibility of non-adherence to medication [16] or sub-optimal therapeutic dosage.

The aim of the present study was to explore the value of combining various objective measures (including determination of drug and metabolite levels together with a newly applied method of hierarchical cluster analysis) and subjective methods (self-reported adherence) to assess adherence rates in ALL patients who are receiving oral thiopurine medication.

Methods

Study subjects

A total of 19 paediatric patients with ALL attending the Haematology and Oncology Outpatient Department at the Royal Belfast Hospital for Sick Children, Northern Ireland, were consecutively recruited in this study. Patients were eligible for inclusion in the study if they had been receiving 6-MP treatment for at least 1 month prior to enrolment but had not received RBC transfusion within the previous 2 months since the latter can mask the true levels of 6-MP metabolites. It was also ascertained that patients did not receive RBC transfusions prior to metabolite measurements during the study. The demographic and clinical characteristics of the participants are

shown in Table 1. Maintenance chemotherapy for ALL patients consisted of daily oral 6-MP (given as a single dose) and weekly oral methotrexate interrupted by monthly pulses of vincristine and glucocorticoid therapy. 6-MP dose was titrated to a target protocol dose of $75 \text{ mg m}^{-2} \text{ day}^{-1}$, adjusted for each child according to leucocyte count and the presence of clinically relevant infections.

Study design

Parents/guardians were asked to complete an adherence questionnaire relating to their child's 6-MP treatment at each clinic visit (Morisky self-report measure of adherence [20]). Blood samples for the determination of metabolite content (1.5 ml) were obtained from children with ALL during the course of their routine treatment and at a phase of treatment when they had an indwelling cannula for vincristine therapy. These samples ($n=64$) were taken on a maximum of five occasions, at monthly intervals, over the study period (6 months). An additional sample of blood (1 ml) from each patient, taken on one occasion only, was collected for genotyping the TPMT enzyme (200 μL of whole blood was sufficient for this purpose).

In addition to accurate information on dosing and times of sampling, the following data were collected for each patient: age, weight, height, gender, ongoing pathology (e.g. renal and/or hepatic impairment), concomitant drug therapy, clinical laboratory test results and records of any side-effects experienced.

Ethical considerations

The research was approved by the NHS Office for Research Ethics Committees in Northern Ireland (ORECNI). Chil-

dren were included in the study only after their parents or guardians had been fully informed and had signed the study consent form. In addition, verbal assent was obtained from older children (> 10 years) before enrolment in the study.

Self-reported medication adherence

Self-reported adherence (by parents) with prescribed medications was assessed using a four-item adherence questionnaire developed by Morisky et al. [20]. The original dichotomous (yes/no) version of the questionnaire was used (with slight modifications since it was administered to parents/guardians). Scores using this methodology range from 0–4, with higher scores indicative of worse adherence. Patients were considered non-adherent if they scored 2 or more.

TPMT genotype and assay of 6-MP metabolites

Genotyping of the most common TPMT variant alleles (*TPMT*3A*, *TPMT*3B* and *TPMT*3C*) was performed using validated TaqMan genotyping assays (ABI, Foster City, CA, USA) as previously described [21, 22]. RBC and plasma concentrations of 6-MP and its metabolites, 6-TGNs, 6-TU and 6-mMPNs, were measured using a reversed-phase high-performance liquid chromatography methodology that was developed earlier [23].

Non-adherence was assumed if a patient had wide variations (≥ 1.9 -fold) in their intracellular levels of 6-TGNs on different occasions while prescribed the same dose of 6-MP. The value of 1.9 was chosen as the cut-off point as suggested by Lancaster et al. [14]. Furthermore, patients were considered non-adherent to therapy if they had low concentrations of both 6-TGNs and 6-mMPNs which is hard to explain on metabolic grounds. Low concentrations of 6-TGNs and 6-mMPNs were defined according to hierarchical cluster analysis (an analysis method based on distances between points). This approach was used in order to investigate the pattern of variability in 6-TGN and 6-mMPN metabolites in the study subjects and to group these patients according to their metabolite levels. Finally, 6-MP and 6-TU levels in plasma were examined to identify patients who took a dose just prior to a clinic attendance probably to mask a missed earlier scheduled dose.

Statistical analysis

Statistical analysis was performed using SPSS computer software (version 15, SPSS, USA). Results were expressed as mean/median and range, median and inter-quartile range, or as frequencies.

Hierarchical cluster analysis was used to generate the possible number of clusters for the measured intra-

Table 1 The demographic and clinical characteristics of the study population

Parameter	ALL patients ($n=19$)
Gender (F:M)	6:13 (32%:68%)
Age (years)	10 (3–17)
Weight (kg)	33.4 (13.2–77.5)
6-MP daily dose (mg/m^2)	40.4 (5.88–76.27)
6-MP daily dose (mg/kg)	1.43 (0.16–3.41)
Metabolite concentrations	
6-TGNs ($\text{pmol}/8 \times 10^8 \text{ RBCs}$)	285.1 (92.6–1,114.2)
6-mMPNs ($\text{pmol}/8 \times 10^8 \text{ RBCs}$)	5,859.3 (248.5–28,733.8)
TPMT genotypes (heterozygotes/homozygotes)	
<i>TPMT*3A</i>	1/0
<i>TPMT*3B</i>	–
<i>TPMT*3C</i>	2/0

Values are median (range) or number

erythrocyte concentrations of 6-TGN and 6-mMPN metabolites [normalised per dose per patient surface area (SA)]. Hierarchical clustering is a standard statistical technique usually used to classify a particular data set into subsets (or clusters) so that the data in each subset share some common trait (often proximity) according to some defined distance measure. This hierarchical approach was based on Euclidean distances between the different points. Euclidean distance is the sum of the squared differences over all of the variables:

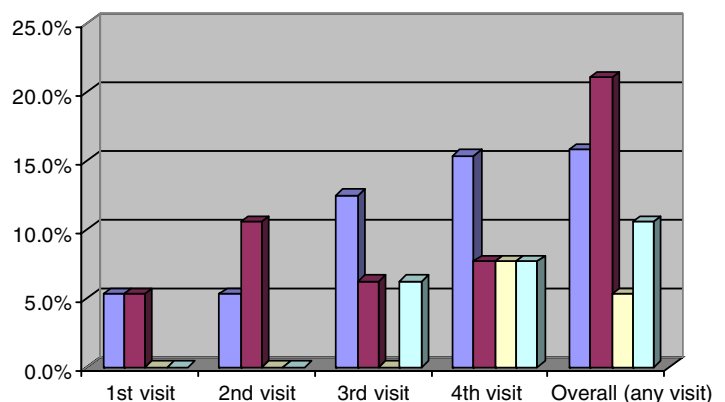
$$d_{ij} = \sqrt{\left(\sum_{k=1}^p [x_{ik} - x_{jk}]^2\right)}$$

where d_{ij} is the distance between the points i and j .

Values were standardised (divided by the standard deviation) before distances were calculated so that all values were equally important in determining these distances. This method was chosen because it is a multivariate technique that is completely numerical and could be used to establish cut-off points for screening purposes. The mathematical algorithm was the sole source for determining cluster membership for all data points.

Standard statistical methods were used to evaluate the various univariate relationships; correlations between the different metabolites were sought using the Spearman rank correlation coefficient. Association of non-adherence with categorical variables (e.g. gender) was assessed using the chi-squared (χ^2) test with one degree of freedom. Association of non-adherence with continuous variables (e.g. age) was evaluated using the Mann-Whitney U-test. Associations were tested defining non-adherent patients as those who were detected through any one of the methods developed in this study. A P -value of <0.05 (two-tailed) was used to define statistical significance.

Fig. 1 Self-reported adherence among ALL patients



■ Forget to take medication?	1 (5.3%)	1 (5.3%)	2 (12.5%)	2 (15.4%)	3 (15.8%)
■ Careless at times?	1 (5.3%)	2 (10.5%)	1 (6.3%)	1 (7.7%)	4 (21.1%)
■ Stop when feel better?	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	1 (5.3%)
■ Stop when feel worse?	0 (0%)	0 (0%)	1 (6.3%)	1 (7.7%)	2 (10.5%)

Results

Estimating adherence using Morisky self-report measure of adherence

All questions on the Morisky self-reported adherence questionnaire were answered by the patients's parents/guardians at each clinic visit. Adherence in the ALL patients, as measured by the Morisky questionnaire, is presented in Fig. 1. Three patients had only two clinic visits and another three had three clinic visits only.

An interesting result was the fact that the mean and the range of scores increased as the number of clinic visits increased (more patients reported poor adherence). Overall, five patients (26.3%) reported at least one aspect of non-adherence and three of them (15.8%) were classified as non-adherent to the prescribed medication since they had scores of 2 or more.

Developing a method to assess adherence using 6-MP metabolites

Cluster analysis of intra-erythrocyte concentrations of 6-TGNs and 6-mMPNs

For the 64 samples assayed in the ALL population, there was no statistically significant correlation between RBC 6-TGN and 6-mMPN metabolite concentrations ($r_s=0.209$, $P>0.05$). However, the relationship between the two metabolites was not random (Fig. 2). The number of children who had both metabolite values within the lower quartiles was 2.5 times higher than those having both metabolite values in the upper quartiles at least once. When cluster analysis of 6-TGN and 6-mMPN metabolite concentrations (adjusted

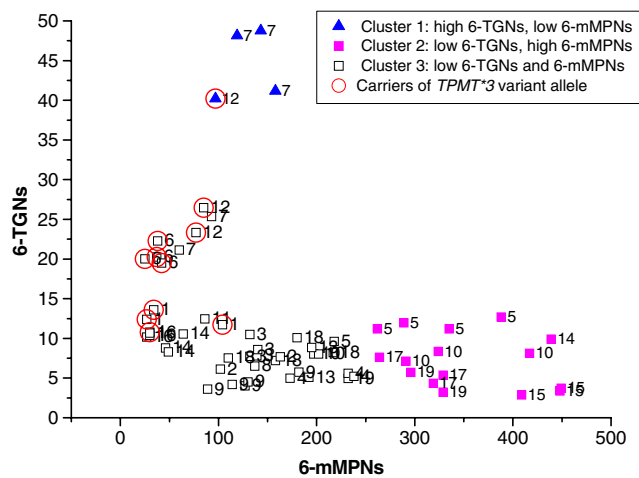


Fig. 2 Scatter plot showing the three different clusters formed after hierarchical clustering of the ALL study sample. Data for 6-TGNs and 6-mMPNs are the metabolite levels (adjusted per dose/SA). Data points related to the same patient are assigned the same number

per dose/SA) was performed, three clusters were retained as the optimal solution for the test data (Fig. 2).

Cluster 1 was characterised by a high 6-TGN level and a low 6-mMPN level, while Cluster 2 was characterised by a low 6-TGN level and a high 6-mMPN level. These two clusters exhibited the expected negative correlations between the two metabolites ($r_s = -0.637$, $P = 0.003$). Cluster 3, however, formed the bulk of the data, and it was further separated into three clusters to produce five clusters overall (Fig. 3). The newly formed cluster 1 and cluster 2 together with the previous two clusters (now called cluster 4 and cluster 5) again exhibited negative correlations between the two metabolites ($r_s = -0.417$, $P = 0.002$). In contrast, cluster 3 had very low levels of both 6-TGN and 6-mMPN metabolites which could be due to suboptimal therapy or poor adherence to therapy. Cluster 3 was, therefore, used as a cut-off point for determining non-adherence if under-dosing was excluded. The distribution of 6-TGN and 6-mMPN measurements within the various clusters formed is shown in Table 2.

Eleven data points were located in cluster 3. Eight points belonged to three patients who had all of their data points located within cluster 3. These patients were suspected to be receiving suboptimal therapy since all of their points, which were obtained at various time points during the study period, were located within the same cluster (cluster 3). Upon review of their medical records, one patient (patient no. 1) was found to be receiving less than the target dose (due to miscalculation of his SA) and, hence, received subsequent dose adjustment as necessary after the end of the study. This patient was a heterozygous carrier of *TPMT*3* variant allele. The remaining 3 of 11 data points were related to one patient (patient no. 14) whose 6-TGN

and 6-mMPN measurements were grouped in a different cluster at least once without any increase in the prescribed 6-MP dosage. Therefore, this patient was considered non-adherent to 6-MP treatment.

Non-adherence was also suspected in another three patients (patients 5, 7 and 12; 13 data points) since their intra-erythrocyte levels of 6-TGN and 6-mMPN metabolites were grouped in different clusters when measured on different occasions while prescribed the same dosage. Patient no. 12 was also a heterozygous carrier of the *TPMT*3* variant allele. All of these patients reported at least one aspect of non-adherence using the Morisky scale and two of them were classified as non-adherent to the prescribed medication since they had scores ≥ 2 .

Ratio of highest-to-lowest intra-erythrocyte concentration of 6-TGNs

Among the ALL study group, wide fluctuations in RBC concentrations of 6-TGNs (>1.9 -fold variations) were found in one patient only (5.3%; patient no. 7) which is indicative of partial or non-adherence with oral 6-MP therapy.

Plasma concentrations of 6-MP and 6-TU

In the present study, high plasma levels of either 6-MP or 6-TU (indicative of recent drug intake) were not detected in any ALL patient. This confirms that the study patients took their 6-MP medication the day before the clinic as prescribed by their doctors.

Estimating adherence using 6-MP metabolite analysis

The compounds involved in identifying non-adherence to therapy were either the drug itself (6-MP), its direct

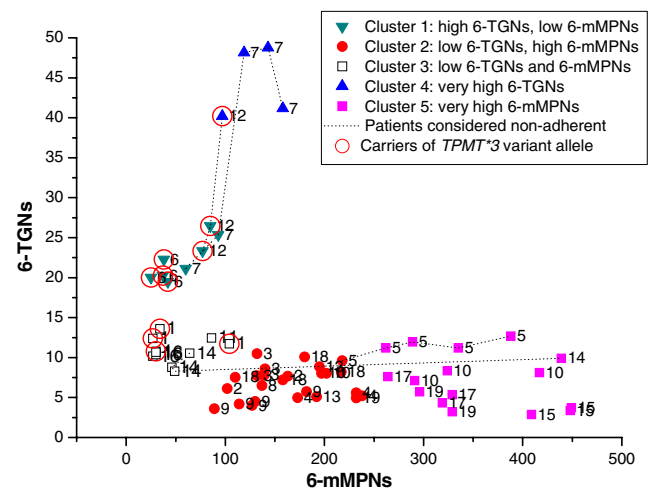


Fig. 3 The five clusters obtained in ALL patients after separating cluster 3 in the previous plot into three different clusters

Table 2 6-TGN and 6-mMPN values^a by cluster for the study sample

Cluster	Number	6-TGNs [pmol (8×10^8 RBCs) ⁻¹ mg ⁻¹ m ²]	6-mMPNs [pmol (8×10^8 RBCs) ⁻¹ mg ⁻¹ m ²]
1	8	21.7 (20.07, 24.85) [19.49–26.48]	51.32 (37.44, 82.92) [25.05–92.84]
2	25	7.21 (5.03, 8.12) [3.6–10.49]	163.33 (130.79, 199.56) [88.81–237.53]
3	11	10.66 (10.18, 12.40) [8.29–13.59]	33.71 (29.89, 64.07) [27.05–103.7]
4	4	44.67 (40.45, 48.62) [40.2–48.77]	131.17 (102.28, 154.09) [96.75–157.63]
5	16	7.36 (3.88, 10.87) [2.86–12.68]	329.07 (291.88, 414.72) [262.4–449.39]

^a Values are medians (inter-quartile ranges) [ranges of absolute values]

metabolite (6-TU) or the end metabolites that are associated with efficacy and toxicity (6-TGNs and 6-mMPNs; metabolites from two competing routes). Methods for evaluating adherence using 6-MP and its metabolites are illustrated in Table 3. Patients who are adherent with 6-MP therapy should have their RBC 6-TGN and 6-mMPN concentrations located consistently in one cluster other than cluster 3. In addition, they should have very low or unquantifiable plasma concentrations of 6-MP and 6-TU to confirm that they did not take their medication just prior to their clinic visit. Finally, their highest-to-lowest concentration ratio of both 6-TGNs and 6-TU in RBCs should be less than 1.9.

Types 1 and 2 identify failing to take the medication as one aspect of non-adherence whereas type 3 measures variations in the timing of dosage intake as another aspect of non-adherence. In the ALL population, four patients (21.1%) had type 1 non-adherence with one patient (5.3%) having type 2 as well. No patients, however, had type 3 non-adherence (taking the medication just before coming to the clinic). Overall, four patients (21.1%) had at least one aspect of non-adherence to the prescribed medication using this methodological approach.

Comparison among the different measures of adherence

Of the ALL patients who reported any form of non-adherence ($n=5$), three were identified by the method developed for estimating adherence using blood and plasma samples. Two of these five patients (patients 6 and 12) were carriers of *TPMT*3* variant allele. The last patient who had

*TPMT*3* variant allele (patient no. 1) was not considered non-adherent to therapy but was found to be receiving a sub-optimal therapeutic dose. Out of the three patients who were considered non-adherent (based on self-reported scores ≥ 2 ; 15.8%), two (10.5%) were also identified by examining their blood and plasma samples. Thus, the concordance rate between self-assessment and metabolite determination was 66.7%. This emphasises the importance of combining the results obtained from blood and plasma samples together with the self-reported adherence method to achieve more accurate estimation of non-adherence. When both methods were combined, five non-adherents (26.3%) were identified out of the 19 ALL patients studied.

Investigation of demographic factors affecting adherence to medication

The only demographic factors examined for possible association with non-adherence were gender and age. Gender was similarly distributed between adherents and non-adherents and was not related to non-adherence identified by any of the methods used in ALL patients (metabolites method, $P>0.05$; self-reported method, $P>0.05$). On the other hand, higher age was significantly associated with non-adherence ($P=0.046$) in children with ALL as reported by a score of 2 or more (self-reported method) and with non-adherence ($P=0.027$) as identified by the metabolites method. This coincides with previous studies that recognised adolescents as the worst compliers in the paediatric age range [24].

Table 3 Evaluating adherence to thiopurines using 6-MP metabolite analysis

Type of non-adherence measured	Metabolite level assessment	Adherent	Non-adherent
1 Not taking the medication sometimes or most of the time	Cluster analysis of RBC 6-TGNs and 6-mMPNs	Measured metabolites located within one cluster except cluster 3	Metabolites located within cluster 3 or within different clusters while prescribed the same 6-MP dose
2 Not taking medication regularly; skipping or missing some doses	Ratio of highest-to-lowest RBC 6-TGN concentrations	<1.9	≥ 1.9
3 Taking a dose prior to clinic attendance instead of the day before	Plasma concentrations of 6-MP and 6-TU	Low or unquantifiable plasma levels of 6-MP and 6-TU	Relatively high plasma levels of 6-MP and 6-TU

Discussion

The present study explored various ways of measuring adherence rates to oral thiopurine treatment in paediatric patients with ALL and investigated the added value of combining both objective and subjective measures to better describe non-adherence to medication in these patients.

Self-reported non-adherence was the subjective approach of adherence assessment in the present study. The majority of ALL non-adherent population as detected by this technique cited “carelessness at times about taking medication” as the primary reason for non-adherence (80%) followed by “forgetting to take the medication” (60%). This was expected since all of ALL patients took their 6-MP dose once daily. Another interesting result found in this study was the fact that as the number of clinic visits increased, more ALL patients reported non-adherence and answered more questions positively. This may indicate that patients need some time to report non-adherence, reflecting the importance of confidence and assurance that the patients should receive from the person who is administering the questionnaire. It is also possible that patients are more likely to adhere to their medication at the start of the study but lose their adherence toward the end, as reflected by a decline in 6-TGNs and 6-mMPNs in a recent study by Dilger et al. [25].

The objective measures examined in the present study included clustering patients according to their drug metabolite concentrations (hierarchical cluster analysis) and according to the extent of fluctuations in their intracellular 6-TGN concentrations. Hierarchical cluster analysis was used to identify cut-off points of 6-TGNs and 6-mMPNs that may indicate non-adherence to therapy [<14 and <104 pmol/ $(8 \times 10^8$ RBCs) $\text{mg}^{-1} \text{m}^2$ for 6-TGNs and 6-mMPNs respectively]. The idea of cluster analysis in relation to 6-MP active metabolites has been applied in only one study before [25], but it was used to identify patients at risk of suboptimal drug therapy rather than non-adherence. In addition, it was limited to adolescent subjects with ALL and, unfortunately, did not provide information on patient's genotypes or whether patients belonged consistently to one cluster or another over serial samples. The latter information was utilised in the present study to help differentiate between patients who were non-adherent to therapy and those who received suboptimal therapeutic doses. Upon the application of such method, four ALL patients (21.1%) were considered non-adherent to medication. These patients had their 6-TGNs and 6-mMPNs located within cluster 3 or within variable clusters while prescribed similar 6-MP doses. Therefore, cluster analysis, via the identification of patients with persistently low levels of metabolites or with metabolite levels clustered variably with time, could alert clinicians to non-adherent patients who would require clinical or behavioural interventions.

Monitoring wide fluctuations in RBC 6-TGN concentrations (>1.9 -fold variation), on the other hand, requires the measurement of the intracellular concentrations of 6-TGNs on more than one occasion. In the present study, one ALL patient (5.3%) had wide fluctuations indicative of non-adherence. The final objective measure examined in the present study used 6-MP and 6-TU plasma levels as adherence tracers to identify variations in the pattern of drug intake rather than not taking the medication completely or intermittently (e.g. patients taking their medicine just prior to a clinic attendance probably to mask a missed earlier scheduled dose). However, this method provides an assessment of short-term adherence only due to the short half-life of 6-MP and 6-TU.

When the objective and subjective measures were compared in the ALL population, the objective measures (via measurement of metabolite levels) were more effective in identifying higher numbers (four patients, 21.1%) of non-adherents than self-reported non-adherence with a score of 2 or more (three patients, 15.8%). However, this does not mean that the self-reported measure of adherence is not adequate for detecting non-adherence. In contrast, these results would encourage the combination of various methods as a valid way of increasing sensitivity for detecting non-adherence. Since non-adherence is potentially harmful to the outcome of ALL children, sensitivity would be more important than specificity in this case. Overall, five patients (26.3%) were considered non-adherent to their medication according to at least one method.

Age of children is an important factor affecting adherence, and it is well recognised that adolescents are the worst compliers among patients classified as children [6]. In this study, a significant association between higher age and non-adherence was found in the ALL population. Younger children are usually given the medication by their parents/guardians. On the other hand, older children and adolescents are usually in charge of their own medication, and hence, in possible denial about their illness, may choose not to take their medication with no obvious consequences at the time [16]. This could be a risk factor for non-adherence in adolescents. Adolescents, therefore, should be monitored carefully, particularly in the second year of treatment when they are asymptomatic.

A limitation of the present study is the relatively small ALL population investigated. It should be noted, however, that all patients under maintenance chemotherapy who attended the Haematology and Oncology Outpatient Department in the study site hospital were enrolled in this study which provides some confidence as to the validity and representativeness of the results.

As a conclusion, the present study explored an objective methodology to assess non-adherence to thiopurine medication and compared the results of both objective and

subjective measures. This methodology could pave the way toward a much larger study to evaluate non-adherence to thiopurine medication in more depth. The results of this study, however, demonstrated the significance of non-adherence to 6-MP therapy in ALL patients and highlighted the fact that non-adherence is also a clinically significant problem in children receiving anti-cancer therapy. This highlights the need for programmes to help patients better understand their disease and for the implementation of disease-specific interventions to improve adherence to medication.

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