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Simple Urine Test to Evaluate Adherence to Oral 5-ASA in Teenagers With Ulcerative Colitis: Proof of Concept

*Alie Dijkstra, †Daan J. Touw, and *Patrick F. van Rheenen

ABSTRACT

Objectives: 5-Aminosalicylic acid (5-ASA) is an important maintenance drug for patients with ulcerative colitis. A proportion of the ingested dose is excreted in the urine. Measuring 5-ASA and its metabolites in urine requires mass spectrometry, which is not widely available for this purpose. Urinary 5-ASA can be measured by colorimetry using the serum salicylic acid assay and is a surrogate marker of recent 5-ASA ingestion. We evaluated whether measuring 5-ASA in first morning voids or in random spot urine samples correctly identifies teenagers with poor adherence to oral 5-ASA.

Methods: Teenagers who were prescribed a current regimen including >40 mg·kg^{-1}·day^{-1} of 5-ASA were invited to collect their spot urine with various time lapses since their last presumed 5-ASA ingestion. Classification of adherence was based on a composite method that included a patient-reported adherence scale and 6-thioguanine levels in erythrocytes.

Results: Teenagers who were classified as ''good adherers'' had 66 of 69 (96%; 95% confidence interval 87%–99%) spot urine samples with detectable 5-ASA levels. ''Poor adherers'' had 30 of 45 (67%; 95% confidence interval 52%–79%) spot urine samples with *un*detectable 5-ASA levels. The ''good adherers'' with false-negative urine tests were on a once daily dosing regimen and had collected a spot urine sample shortly before the next dosage. Their first morning voids had detectable 5-ASA levels.

Conclusions: Undetectable 5-ASA levels in the first morning void confirms short-term nonadherence to oral 5-ASA.

Key Words: 5-aminosalicylic acid, medication adherence, mesalamine, ulcerative colitis, urine

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What Is Known

- Adherence to commonly prescribed oral 5-aminosalicylic acid for ulcerative colitis is challenging for patients.
- Not being able to identify those not following their prescribed medications could lead to a premature switch of medication.

What Is New

- We used a commercially available colorimetric urine test to distinguish medication failure from failure to take medication.
- First morning voids are more reliable than spot urine samples to assess recent ingestion of 5-aminosalicylic acid.
- Evaluate first morning voids for 5-aminosalicylic acid to protect the patient from a premature step up to biologic therapies.

5-Aminosalicylic acid (5-ASA) is the medical treatment of choice for induction and maintenance of remission in ulcerative colitis (UC) (1–3). Nonadherence is an important cause of unresponsiveness to 5-ASA, but few practical tools exist to accurately and routinely detect it. Failure to identify those not following their prescribed medications could lead to premature treatment escalations and expensive biologic therapies.

The therapeutic effect of 5-ASA occurs topically at the site of the inflamed colonic mucosa. A multitude of 5-ASA preparations have been formulated to minimize systemic absorption at the small bowel level and maximize drug availability in the colon. Although the main part of the ingested dose of 5-ASA leaves the body with the stool, a proportion of the drug is excreted in the urine.

Gas chromatography-mass spectrometry is the most accurate, sensitive, and reliable method of urine drug testing, but the test is time-consuming, requires a high level of expertise to perform, and is costly. A commercially available colorimetric test (Architect Salicylate test, Abbott Diagnostics, Hoofddorp, The Netherlands), which is normally used to measure serum salicylate levels, can also be used to detect 5-ASA and its metabolites in urine (off-label use) (4,5). This test is relatively cheap and widely available in clinical laboratories. There is excellent correlation between urine levels of 5-ASA and readout signal of the test with $R^2 = 0.999$ and a crossreactivity of 63%. This result indicates that the concentration of urinary 5-ASA levels as measured by the salicylic acid test can be used as an indicator for recent 5-ASA ingestion, provided that the patient is not using any other salicylate-containing drugs, such as aspirin.

In the present study we examined whether measuring salicylate levels in first morning voids or in spot urine samples correctly identifies teenagers with poor adherence. Second, we aim to give recommendations for the use of this test in clinical practice.

MATERIALS AND METHODS

We used a prospective case-control design. We recruited teenagers with a diagnosis of UC according to the European Society

of Pediatric Gastroenterology Hepatology and Nutrition criteria (6) and who were treated at the pediatric inflammatory bowel disease (IBD) clinic in the University Medical Center in Groningen (UMCG). The UMCG is a tertiary care hospital and provides health services to pediatric patients with IBD living in the Northern region of the Netherlands. Teenagers who were prescribed a current regimen including >40 mg·kg^{-1}·day^{-1} of 5-ASA (ie, therapeutic range) were eligible to participate. Cases were teenagers with UC who were classified as ''poor adherers,'' and controls were teenagers with presumed ''good adherence.''

In the absence of a perfect reference standard for adherence we used a composite method (7) to classify the degree of adherence (Table 1). All study participants completed the Morisky Medication Adherence Scale (MMAS-8), an 8-item self-report instrument to measure day-to-day adherence (8). MMAS-8 consists of 7 questions with a dichotomous scale and 1 featuring a 5-point Likert scale. The total score ranges from 0 to 8, higher scores representing higher adherence. For the purpose of the present study fewer than 6 points is interpreted as poor adherence; and 6 to 8 points as good adherence. The MMAS-8 is a validated questionnaire and is widely used in patients with chronic conditions including IBD (8,9). Next, for participants on thiopurines we checked the electronic health database for recently measured levels of active metabolites (6-thioguanine [6-TGN]). A validated and routinely performed analytical procedure was used for determination of 6-TGN levels (10). With this method 6-TGN levels >600 pmol/8 × 10^8 red blood cells are a proxy for adequate oral ingestion of thiopurine medication (11).

Patients and parents or legal guardians were preinformed of the study before the clinic visit. We explained the purpose of the new urine test, and advised that the treating physician would not be aware of the results, to increase recruitment to the study. When individuals agreed to participate, written and verbal instructions on how to collect urine samples were given. Urine samples were frozen at −80°C for subsequent analysis in batches. Cases and controls were analyzed together and laboratory personnel were unable to distinguish among cases and controls. Urine 5-ASA levels were measured using reagents and instrumentation from Abbott Diagnostics (Off label use of the Salicylate measured on the Architect c8000 platform, Abbott Diagnostics). The lower limit of quantitation was 20 mg/L. A test result >20 mg/L was considered as positive for the presence of 5-ASA.

Dichotomous variables were compared between groups by χ^2 test or Fisher exact test where appropriate. Continuous variables were compared using t tests if normally distributed, or Mann-Whitney U test if non-normal.

The Medical Ethics Review Committee of the UMCG approved the study (file number METc 2015/350). The data were collected and recorded by the investigators in such a manner that subjects could not be identified, neither directly nor through identifiers linked to the subjects. The legal guardians from all participants, and the participants themselves gave informed consent for participation.

RESULTS

Between January 2015 and March 2016, we collected a total of 114 spot urine samples for salicylate measurements. Eight teenagers, who were classified as ''poor adherers,'' collected a total of 45 urine samples, of which 7 samples were first morning voids. Seventeen ''good adherers'' collected a total of 69 urine samples, and 12 of these were first morning voids.

Table 2 shows that the majority of participants were on a combination therapy of 5-ASA and thiopurine immunomodulation. Two thirds of the participants were recommended to take 5-ASA once daily, and none were taking remission induction medication at the time of urine collection.

Figure 1 shows that ''good adherers'' had 66 of 69 (96%; 95% confidence interval 87%–99%) spot urine samples with detectable 5-ASA levels. ''Poor adherers'' had 30 of 45 (67%; 95% confidence interval 52%–79%) spot urine samples with *un*detectable 5-ASA levels. The ''good adherers'' with false-negative urine tests were on a once daily dosing regimen and had collected a spot urine sample shortly before the next dosage. Their first morning voids had detectable 5-ASA levels. All 12 first morning voids of ''good adherers'' contained 5-ASA, including 3 samples of those with negative random spot urine samples. Four of 7 first morning voids of ''poor adherers'' had undetectable 5-ASA levels.

DISCUSSION

In this case-control study we included 25 teenagers with previously diagnosed UC. We used a paired design in which study participants first collected several urine samples for salicylate testing, and then completed the MMAS-8 questionnaire. Classification of drug adherence was based ultimately on a composite method combining MMAS-8 result and, when available, 6-TGN levels in erythrocytes. In the absence of a single reference standard providing adequate classification of adherence, this composite method was as close as we could get to define adherence in our study population (7). We found that undetectable 5-ASA levels in the first morning void correctly identifies short-term nonadherence to oral 5-ASA.

Implications of Key Findings

Screening patients for short-term nonadherence by measuring 5-ASA in their first morning void is of value only if it results in

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TABLE 1. Composite method to classify the degree of adherence

Composite score	Interpretation
−1	Probably poor adherer
0	Possibly poor adherer
1	Possibly good adherer
2	Probably good adherer

Degree of adherence can be calculated from the following formula.
Composite score = Morisky Medication Adherence Scale (MMAS-8) + 6-thioguanine (6-TGN).
MMAS-8-scores are coded 0 for <6 points or 1 for ≥6 points.
6-TGN results are coded −1 for inadequate levels, 0 for not tested, or 1 for adequate levels.

TABLE 2. Patient characteristics at the time of urine collection

Characteristics	Good adherers (n = 17)	Poor adherers (n = 8)
Age in years, median (IQR)	14.2 (12.8–15.9)	15.5 (13.5–16.4)
Male sex, n (%)	11 (65%)	3 (38%)
Years since diagnosis, median (IQR)	2.0 (1.1–4.6)	3.2 (1.3–6.2)
Daily 5-ASA dosage in mg/kg, mean (SD)	55 (13)	53 (12)
5-ASA dosing frequency, n (%)		
Once a day	9 (53%)	6 (75%)
Twice a day	4 (24%)	2 (25%)
Three times a day	4 (24%)	0
Thiopurine use, n (%)	15 (88%)	7 (88%)

5ASA = 5-aminosalicylic acid; IQR = interquartile range; SD = standard deviation.
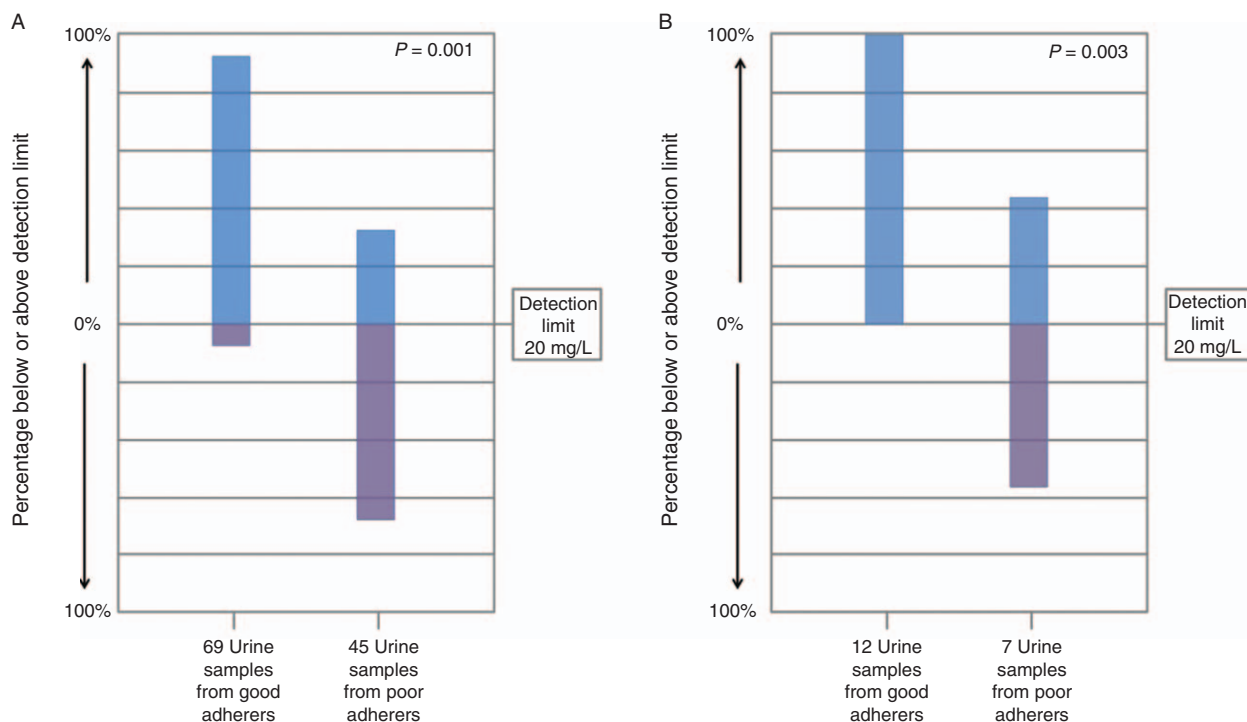
FIGURE 1. Distribution of urinary 5-aminosalicylic acid (5-ASA) levels above or below the detection limit according to classification of adherence. We show the urinary 5-ASA levels as measured by the salicylic acid test in (A) the total number of urine samples (n = 114) and (B) the first morning urine samples (n = 19).

improved outcomes for patients. For this reason we infer the effect of urinary 5-ASA measurement on patient outcome in the event of a disease flare. Key questions are whether the numbers of false negatives (adherent patients being counseled for nonadherence) and false positives (nonadherent patients being treated with 5-ASA dose escalation, whereas stimulating regular ingestion should suffice) are acceptable when urine 5-ASA testing is introduced as screening strategy. In the ''new'' strategy teenagers with a disease flare and undetectable salicylate levels in their first morning void require counseling for adherence instead of recommending dose escalation. Table 3 shows the implications of the testing scenario.

Comparison With Other Studies

An American research group recently recommended random urine salicylate testing to identify adult patients with UC with short-term nonadherence, and advised to consider levels >150 mg/L as indicator for 5-ASA ingestion in the prior week (5). We used a 10-fold more sensitive assay (lower limit of quantitation of 20 mg/L) and noticed that many urine 5-ASA levels in adherent teenagers

were much lower than 150 mg/L. Use of an insensitive assay would classify immeasurable urinary 5-ASA levels as coming from non-adherent patients and could lead to unjust adherence counseling sessions. The majority of teenagers in our study cohort were on a once-daily regimen of 5-ASA, and false-negative tests exclusively occurred in this dosing regimen.

We found that measuring salicylate in first morning void is most reliable, because this urine is more concentrated than any other time of the day (see Table 4, for background information on urine drug testing). The three 5-ASA products employed in the present study (Mezavant, Salofalk, and Pentasa) were not developed for once daily dosing, but so far have not shown differences in efficacy between once-daily and multiple-daily dosing (12).

Methodological Limitations

As it is not uncommon for respondents to give socially accepted answers to MMAS-8 questions, we used the level of active metabolites of thiopurine medication (6-TGN) measured within 6 months from urine collection to make a composite

TABLE 3. Impact of 5-aminosalicylic acid testing in first morning urine on patient outcome

Test result	Number per 100 patients	Presumed influence on patient outcome
True positive	63	Benefit from 5-ASA dose escalation or switch to different remission induction therapy
True negative	21	Benefit from counseling to improve 5-ASA adherence
False positive	16	Detriment from 5-ASA dose escalation, whereas regular ingestion should suffice
False negative	0	Detriment from unjust adherence counseling session
Sensitivity	100%	
Specificity	57%	

In this scenario we assume that urinary 5-ASA is measured for increased disease activity.
5-ASA = 5-aminosalicylic acid.

TABLE 4. Recommendations for use of the urinary 5-ASA measurement in clinical practice

Advantage	5-ASA and metabolites found in urine are stable
	Analysis is simple because of absence of proteins and cellular material in urine
	Commercial reagents and analytical systems are wide available
Disadvantage	Drinking a lot will reduce the concentration of 5-ASA in the urine and can produce lower than expected levels (requesting a first morning void will help to produce a more concentrated sample)
Why	To monitor a patient's adherence to 5-ASA therapy
	To identify the need for further counseling
	To help provide better medical therapy
Who	Patients with presumed medication failure
	Patients with an imminent disease flare
How	The optimum sample volume is 30 mL or more
Interpretation	Different patients have different rates of 5-ASA absorption and metabolism, so expect variability of test results between patients on the same dosages
	There is also absorptive and metabolic variability within a patient

reference standard that is more likely to correctly classify adherence than MMAS-8 alone. This interval, however, may be too long for the adherence status to remain stable. One may argue that the composite reference standard was not sufficiently accurate for answering our research question (or, put differently, that the urine 5-ASA test is actually better than the composite reference standard). This possibility represents a fundamental flaw in the test accuracy study design, because the urine 5-ASA test can never be deemed to perform better than the composite reference standard, and its value may therefore be underestimated.

As an alternative to the classical diagnostic accuracy paradigm the concept of clinical test validation is recommended (7). The present study design did not allow us to perform a meaningful comparison of urine salicylate levels in first morning urine with the need to start remission induction therapy in the 6 months that follow. We aim to evaluate the predictive validity of the urine salicylate test in a future study.

Ethical Considerations

During this research project patients were advised that their physician would not be aware of the urine results, to reduce the possibility that nonadherers declined participation. When urine 5-ASA screening is introduced in clinical practice, health providers should not intentionally deceive their patients into the purpose of the test, given the requirement for informed consent. The justification of the deception (to avoid nonadherers resuming medication only shortly before the urine collection) does not outweigh the objections (duty to be truthful, respect for patient autonomy and the ''right to know'') (13). Where it is clearly unethical to use deception to steer a patient toward urine testing, nudging offers an ethically legitimate opportunity to steer people's choices in directions that will improve their own outcome (14). We advise to explain the purpose of urine 5-ASA testing to the patient at the introduction of 5-ASA therapy.

CONCLUSIONS

We consider measuring 5-ASA levels in first morning voids a useful tool for identifying teenagers with short-term nonadherence. Performing this test in patients with an imminent disease flare may reduce needless treatment escalations and expensive biologic therapies, in which counseling for nonadherence would suffice.

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