
Dietmar Stöckl

Inst. für Standardisierung und Dokumentation im Med. Lab. e.V.
Johannes Weyer Strasse 1
D-40225 Düsseldorf, Germany

Using Relation Between Urinary Cannabinoid and Creatinine Excretion to Improve Monitoring of Abuser Adherence to Abstinence

To the Editor:

Testing urine for drugs of abuse is now widespread. The performance of such testing was improved by the establishment of US federal guidelines (1). Although these guidelines were mainly intended for employee testing in the US, they have received acceptance also in the context of treatment of drug abusers. Screening for cannabinoids in urine is usually done qualitatively with immunological methods, with a preset specific cutoff limit (commonly 100 µg/L). Positive results are verified with gas chromatography-mass spectrometry (GC-MS), with a cutoff limit of 15 µg/L, and the final result is given as “positive” or “negative.”

One problem when monitoring cannabis abusers is the difficulty in differentiating between new intake and previous abuse, because of the slow elimination of cannabinoids and their metabolites (2). Quantitative measurements of cannabinoids in urine may give additional information and aid in the interpretation of results. Many factors may affect the amount of cannabinoids in the urine, including amount of drug taken, time elapsed since consumption, metabolic rate and extent, fluid intake, and the effects of biological variability on each of these factors. In this context, creatinine excretion may be used to monitor and compensate for the degree of dilution of the urine (3). The use of such measurements has been suggested (1, 3, 4), but their clinical usefulness has not been validated.

We decided to evaluate the use of quantitative measurements of cannabinoids in urine. We used a fluorescence polarization immunoassay (FPIA; Abbott Diagnostics, Chicago, IL) that we had evaluated previously (5). We confirmed the agreement of the FPIA method (x) with GC-MS (y) in the range of the calibrators for FPIA, i.e., 25 and 150 µg/L: y = -4.3021 + 1.0411x (r² = 0.984, Sₓᵧ = 13.1 µg/L, n = 52). Creatinine in urine was measured with a method based on the Jaffé reaction.

We assayed samples from 16 former heroin addicts (13 men, 3 women) from the Stockholm County Methadone Program, who had been in treatment for between 1 month and 3 years. The study was approved by the local Ethics Committee. Patients were included in the study after confirmed positive findings of cannabinoids by GC-MS, whether they had a previous history of cannabis abuse or not. FPIA results were included in the data analysis when values of 25–150 µg/L were found, and the patients were monitored prospectively until two consecutive negative (<25 µg/L) results were obtained. The mean (±SD) time to reach undetectable ‘cannabinoid” (THC-COOH equivalents) excretion was 27 ± 18 days (range 10–69 days). For each sample the ratio of cannabinoids (FPIA results expressed as µg/L) to creatinine (expressed as mmol/L) was calculated. The first measurable ratio was 19.5 ± 14.6 (range 7.4–56.1), and the last measurable ratio was 2.5 ± 1.2 (range 1.0–5.3) µg/mmol creatinine, significantly lower (P < 0.001). The apparent elimination rate, expressed as “half-life” of the ratio, was 12.8 ± 7.9 days (range 3–30 days).

The correlation coefficients for cannabinoid and creatinine excretions for each patient were statistically significant (P < 0.05) in all patients, but varied among individuals. Peak concentrations of cannabinoids >100 µg/L, with subsequent declines in the cannabinoid/creatinine ratios, were observed in five patients (illustrated for one patient in Fig. 1). Those patients displayed no clinical signs of relapse, and the ratios were all in steady decrease. Three patients were clinically suspected of relapses during treatment; all three had lower correlation coefficients for the excretion ratios: r² = 0.31, 0.31, and 0.60 (range for other patients, 0.64–0.99).

These data indicate steadily declining excretions of cannabinoids in abstaining patients, when normalized by creatinine measurements. Thus, parallel and quantitative measurements of creatinine may prove valuable not only for the detection of diluted urine samples, with a higher risk of false-negative results in testing for drugs of abuse as we previously have reported (4), but also for improved detection of relapses of cannabis abuse.

The additional cost for quantification of creatinine constituted 10% of the total cost for the analyses (cannabinoids and creatinine), overheads not included. This cost has been found acceptable by our customers as compared with other solutions, e.g., retesting with higher cutoff or prolonged intervals between tests, because it raises the efficacy of the analyses and the treatment programs.

We conclude that measurement of cannabinoids excretion in relation to creatinine excretion yields more de-

Fig. 1. Some of the measurements from a typical abstinent patient.

On day 20 the urinary cannabinoid concentration was again >100 µg/L, but there was no clinical indication of relapse. As shown, the concentration of creatinine was also increased in the same sample, and the ratio of cannabinoids to creatinine showed a steady decline. This ratio thus revealed a false-positive result related to a lesser dilution in the day 20 sample.
pendable data when monitoring patients' adherence to abstinence.

References

Pierre Lefoli
Olof Beck
Paul Hjemdahl
Dept. of Clin. Pharmacol.
Karolinska Hospital
S-104 01 Stockholm

Stefan Borg
Alcohol and Drug Dependence Unit,
Dept. of Psychiatry
St. Gorana Hospital, Karolinska Inst.
P.O. Box 125 57
S-102 29 Stockholm
Sweden

Author for correspondence.

Inhibition of the Polymerase Chain Reaction by Mucolytic Agents

To the Editor:

In clinical microbiology, the development of the polymerase chain reaction (PCR) has enabled the direct testing of patients' specimens for the pathogen of interest. However, for maximum sensitivity, it is sometimes necessary that the organism be concentrated into a smaller volume before direct assay by PCR, or that relatively large sample volumes be used in a PCR reaction. Similarly, if specimens are to be directly tested by PCR, steps must be taken to ensure that the organisms are uniformly distributed throughout the sample before a small aliquot is removed for analysis. In this regard, sputum specimens can pose a significant problem: They may be ex-

jected to PCR analysis. DTT at a final concentration of 0.1 g/L (0.65 mmol/L) had little or no effect on the yield of amplified product (Fig. 1, lane 5), but increasing the concentration to 0.15 g/L (0.97 mmol/L) and 0.2 g/L (1.3 mmol/L) resulted in greatly diminished yields (lanes 6 and 7), much the same as seen with NALC. Treatment of sputum with DTT at a concentration of 1 g/L (6.5 mmol/L), as has been done in some studies (3), will result in a final concentration of DTT in the PCR reaction of 0.2 g/L (1.3 mmol/L) if 10 µL of the sputum specimen is added directly to a 50-µL reaction mixture. As shown above, this concentration of DTT has the potential to be strongly inhibitory to the subsequent amplification reaction.

We conclude that the presence of NALC or DTT at high concentrations is either directly inhibitory to the Taq DNA polymerase itself or to some other component of the amplification reaction. We have not examined Taq DNA polymerases from other manufacturers or other thermostable DNA polymerases for sensitivity to NALC or DTT. Nevertheless, our results suggest that caution may be in order if sputum specimens known to have been treated with mucolytic agents

Fig. 1. Effect of NALC and DTT on the PCR amplification of M. pneumoniae DNA.

The PCR reaction was carried out with oligonucleotide primers PL-178 and PL-331 (4) as described in the text; 35 cycles of amplification were performed, with the thermal-cycling conditions previously described (4). We electrophoresed 15 µL of the final PCR products through a 1.4% agarose gel and stained them with ethidium bromide. The arrow indicates the position of the 153-bp amplified product specific for M. pneumoniae. Lane 1, control; no NALC or DTT was added to the PCR reaction. Lanes 2-4. NALC was present in the PCR reaction mix at final concentrations of 0.5, 0.75, and 1.0 g/L, respectively. Lanes 5-7. DTT was present in the PCR reaction mix at final concentrations of 0.1, 0.15, and 0.2 g/L, respectively.

CLINICAL CHEMISTRY, Vol. 40, No. 1, 1994 171