DETECTION AND ASSESSMENT

Urinary Ethyl Glucuronide and Ethyl Sulfate Testing for Detection of Recent Drinking in an Outpatient Treatment Program for Alcohol and Drug Dependence

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INTRODUCTION

In many parts of the world, a high and rising frequency of excessive alcohol use, abuse and dependence makes a major contribution to social, economic and health problems for the individual and for society. Alcohol use disorders are common in many somatic and psychiatric health-care services (Neumann and Spies, 2003) and are associated with increased risks for medical complications and a worse treatment outcome (Spies and Rommelspacher, 1999; Tonnesen, 1999). Efforts aiming to reduce the harm caused by alcohol include early detection of those at risk by interview or use of questionnaires and instruments such as the Alcohol Use Disorders Identification Test (AUDIT) and timeline follow-back (TLFB). However, because many people tend not to report their alcohol intake correctly, but rather deny drinking or at least underreport the true amount (Helander et al., 1999; Midanik, 1989), obtaining reliable information about a person’s drinking habits is often a difficult task. For that reason, there is a frequent failure to identify alcohol-related disorders.

Laboratory tests that are directly or indirectly related to the amount and/or period of alcohol ingestion, and/or severity of the disorder, represent an objective way to identify persons with risky drinking habits. Such alcohol biomarkers are also useful to confirm abstinence or detect relapse while undergoing outpatient treatment (Allen et al., 2001; Helander, 2003). Biomarkers mainly targeting heavy long-term drinking are carbohydrate-deficient transferrin (Helander et al., 2010a; Jeppsson et al., 2007) and phosphatidylethanol (Helander and Zheng, 2009; Varga et al., 1998), while liver enzymes such as γ-glutamyltransferase are used to indicate associated organ damage (Niemelä and Alatalo, 2010). To confirm or rule out recent alcohol consumption, the standard way is to check for the presence of ethanol in blood, urine or breath (Jones, 1990). A general problem with ethanol testing is that the substance is cleared fairly rapidly from the body, and this method is therefore limited to detect only very recent (within ~12 h) ingestion of alcohol (Bendtsen et al., 1998; Helander et al., 1996).

The conjugated ethanol metabolites ethyl glucuronide (EtG; Schmitt et al., 1997) and ethyl sulfate (EtS; Helander and Beck, 2004) are other measures used to confirm or rule out recent drinking. Although EtG and EtS account for only <0.1% of the ingested ethanol dose (Dahl et al., 2002), they remain detectable in urine for several hours up to some days longer than ethanol, the time-lag largely depending on the amount consumed (Helander et al., 2009; Hoiseth et al., 2008). Even a very small ethanol dose can be detected in this way (Stephanson et al., 2002). A positive finding of EtG and/or EtS is thus indicative of recent drinking, also when this is denied and the ethanol itself cannot be detected.

The interest in biomarkers focusing on recent alcohol consumption has grown rapidly, following a large number of clinical and forensic application studies and case reports where such tests have been indicated to play an important role (Erim et al., 2007; Helander, 1998; Helander et al., 1992, 1999, 2009; Johnson et al., 2005; Kip et al., 2008; Kroke et al., 2001; Neumann et al., 2008; Pichini et al., 2009; Schmitt et al., 1997; Spies and Rommelspacher, 1999; Voltaire et al., 1992; Wurst et al., 2003, 2008). The present study aimed to evaluate the extra information obtained about recent drinking, when introducing urinary EtG and EtS testing into routine practice in outpatient treatment programs for alcohol and drug dependence.

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MATERIALS AND METHODS

Patients and study protocol

The study involved alcohol- and drug-dependent patients participating in routine outpatient treatment programs at the Maria Addiction Centre (Stockholm, Sweden); either in a 12-step program for alcohol-dependent subjects, or a 1–3 months open treatment program for former heroin addicts, or in methadone maintenance therapy. In connection with a regular visit at the unit, which included being questioned about and leaving supervised urine samples for testing of alcohol and illicit drug use, patients were invited to also complete an anonymous single-question form about recent drinking (‘Did you drink any alcohol in the past 3 days—Yes/No?’). They were informed about the aim of the evaluation and that self-reports, and laboratory data were immediately de-identified and only connected by a code with no possibility to track the individual. The information was only given on one occasion within each treatment program. Participation in the evaluation was voluntary and there were no exclusion criteria. The information and the administration of self-reports and urine samples were made by a staff member who was not involved in the treatment programs, and the routine treatment staff did not have access to the results of the study. The study was conducted in accordance with the Declaration of Helsinki.

A total of 24 patients (21 men and 3 women, aged 21–52 years) agreed to participate in the evaluation. They were allocated to three subgroups depending on the type of outpatient treatment program; Group A included 8 individuals (7 men and 1 woman) undergoing treatment for alcohol dependence; Group D included 10 individuals (9 men and 1 woman) being treated for drug dependence; and Group M included 6 individuals (5 men and 1 woman) undergoing methadone maintenance therapy.

Urinary EtG and EtS analysis

The urine samples were stored frozen at −20°C until analyzed for EtG and EtS, using a routine electrospray ionization liquid chromatography-mass spectrometry (LC-MS) method, using selected ion monitoring in the negative ion mode (Helander and Beck, 2004). The ions monitored were m/z 221 for EtG, m/z 226 for EtG-d₅ (penta-deuterated internal standard), m/z 125 for EtS and m/z 130 for EtS-d₅ (internal standard). The concentrations of EtG and EtS were calculated from the peak-area ratio to the corresponding internal standard, by reference to calibration curves. The reporting limits were 0.5 mg/l for EtG and 0.1 mg/l for EtS (Helander et al., 2009, 2010b).

RESULTS

Each of the 24 patients provided 4–14 (mean 8.9, median 9) urine samples over a 2–8-week (mean 5.4, median 5) period, the total number of samples being 214 (Table 1). In 211 (98.6%) of the 214 cases, the anonymous self-report form about recent alcohol use was successfully completed. Depending on local routines for twice-weekly return visits to the outpatient unit, sampling mostly occurred on Mondays (40.7% of cases) and Fridays (43.5%).

Altogether 55 (25.7%) of the 214 urine samples collected from 12 (50%) of the 24 patients tested positive at least once for EtG (concentration range = 0.5–434 mg/l, mean = 47.1, median = 13.6) and/or EtS (range = 0.1–87 mg/l, mean = 10.8, median = 2.6). In the majority of cases (N = 40; 72.7%), both metabolites were present at levels above the corresponding reporting limits. Another six (10.9%) samples tested positive for EtS, while EtG was detected at levels below the reporting limit (i.e. <0.5 mg/l). One patient (D4; Table 1) provided nine urine samples all of which were clearly positive for EtS (range = 2.4–12.0 mg/l, mean = 5.8, median = 3.3) but with no detectable (<0.05 mg/l) EtG. These results were confirmed by LC-MS/MS analysis (Helander et al., 2010b).

In 45 (21.3%) of the 211 self-reports collected from 11 (45.8%) patients, ingestion of alcoholic beverages was admitted in the 3-day period prior to urine sampling. In 184 (87.2%) of the 211 complete cases (i.e. both self-report and urinary data were available), the information provided by self-report agreed with the corresponding EtG/EtS results (i.e. either true positives or true negatives; Table 1). In 18 (8.5%) cases, however, the urine tests indicated recent drinking, while this was not admitted by self-report. In another nine (4.3%) cases, recent drinking was reported but the corresponding urine tests turned out negative. In the three cases where self-report data of recent drinking was missing, one urine sample tested positive and two negative for EtG/EtS.

There were significantly more positive self-reports of recent drinking when sampling occurred on Mondays (32.6%) compared with on Fridays (14.3%; P = 0.0068, χ² test). There was also a higher frequency of positive EtG/EtS findings in urine specimens collected on Mondays (32.2%) compared with on Fridays (21.5%), but this difference was not statistically significant (P = 0.146).

There were marked differences in alcohol consumption between the three patient subgroups (Table 1). The frequency of drinking (Fig. 1) was significantly higher in the drug-dependent group compared with the alcohol-dependent group, both according to self-reports (34.3 vs. 3.1% cases; P < 0.0001) and urinary EtG/EtS results (40.8 vs. 10.9%; P = 0.0001). The frequency of drinking was also significantly higher in the drug-dependent group compared with the methadone maintenance therapy group, according to urinary EtG/EtS (12.8%; P = 0.0013) but not to self-reports (17.8%; P = 0.0672). Furthermore, there was a significant difference in the frequency of self-reported drinking between the alcohol-dependent and methadone maintenance therapy groups (P = 0.0225) but not according to urinary EtG/EtS (P = 0.993). Only 2 out of 10 (20%) patients in the drug-dependent group abstained completely from drinking according to both measures, compared with 5 of 8 (62.5%) in the alcohol-dependent group and 3 of 6 (50%) in the methadone maintenance therapy group.

DISCUSSION

Due to the rather short detection time for ethanol after each alcohol intake, biomarkers with a longer detection window for recent drinking, such as the urinary ratio of 5-hydroxytryptophol to 5-hydroxyindoleacetic acid (Beck and Helander, 2003), and EtG and EtS have been introduced as
more sensitive measures, of which EtG/EtS is the currently most used (Palmer, 2009). A positive laboratory result taken as proof of relapse into alcohol and/or drug abuse might lead to discharge from treatment. Several studies have provided data on the urinary detection times for EtG and EtS following intake of different alcohol doses, which represents important information in clinical practice (Helander et al., 2009). It should be considered that even consumption of a very small ethanol dose (<10 g), and use of ethanol-based products such as mouthwash (Costantino et al., 2006) and hand sanitizers (Rohrig et al., 2006) may result in a detectable EtG level in urine (Helander and Beck, 2005; Stephanson et al., 2002; Wurst et al., 2006). Furthermore, incorrect storage of infected specimens implies risks for false-positive and false-negative EtG results (Dahl et al., 2002; Goll et al., 2002; Helander and Dahl, 2005; Helander et al., 2007), but this is seemingly a minor problem with EtS. Related to this, the cutoff used to distinguish between a positive and a negative result is always a critical issue, although the risk for such problems was indicated to be low at the reporting limits for EtG and EtS applied in this study (Helander et al., 2009, 2010b).

This study aimed to estimate the value of urinary EtG and EtS testing when introduced as a routine indicator for recent drinking during outpatient treatment for alcohol and drug dependence. In an effort to obtain reliable comparison data, the patients were asked to provide anonymous self-reports of recent drinking. They were informed that participation was voluntary, that all results were immediately de-identified and not traceable to the individual, and that declining to attend would not influence their treatment in any way.

Table 1. Frequency of recent alcohol consumption according to anonymous self-reports and urinary EtG and EtS measurement in outpatient treatment programs for alcohol and drug dependence

<table>
<thead>
<tr>
<th>Group and ID No.</th>
<th>Sex (M/F)</th>
<th>Data collection (weeks)</th>
<th>Samples (N)</th>
<th>Self-reported drinkingb/urinary EtG and EtS resultsc</th>
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aOutpatients undergoing treatment programs for primarily (A) alcohol dependence or (D) drug dependence, or (M) opiate addicts participating in methadone maintenance therapy.

bAny intake of alcoholic beverages in the past 3 days—Yes/No?.

cThe reporting limits were 0.5 mg/l for EtG and 0.1 mg/l for EtS.

dThese urine samples were only positive for EtS.

Fig. 1. Indications of recent drinking based on anonymous self-report (any drinking in the past 3 days?) or a positive urinary EtG and/or EtS test in outpatient treatment programs for alcohol and drug dependence. ‘Overall agreement’ is the total frequency of concordant results (i.e. the sum of results being positive for alcohol by both measures or negative by both measures). The reporting limits were 0.5 mg/l for EtG and 0.1 mg/l for EtS.
is probably the reason for the very good outcome in the evaluation, with 99% of all self-report forms being completed successfully. In 21% of the cases, alcohol consumption in the 3-day period prior to sampling was admitted, and these results were in good agreement with the urinary EtG/EtS results (range for overall agreement = 83.5–89.1%). If the self-reports had not been anonymous, these figures would probably have been considerably lower (Helander et al., 1999; Midanik, 1989), given that alcohol use was not permitted while participating in the treatment programs. In this connection, it should be noted that a positive finding of alcohol use could have immediate consequences for the patients undergoing treatment for alcohol dependence, whereas the other two programs were mainly concerned with relapse into illicit drug use. It was therefore interesting to note that the frequency of drinking was markedly lower among patients undergoing treatment for alcohol dependence compared with the other groups. These results may suggest that patients in the treatment program focusing primarily on alcohol-related problems were more motivated to avoid drinking.

The EtG and EtS results were given in absolute amounts (mg/l) and not normalized to the creatinine content. Creatinine measurement is a common way to check for unphysiological urine dilution and possible sample adulteration in drug testing. However, this strategy would not have influenced test sensitivity (i.e., number of samples positive for recent drinking) in this study, because samples with an EtG/EtS concentration below the detection limit of the method would still be negative irrespective of the creatinine concentration. In this respect, it should be noted that urinary EtG values are highly variable both within and between subjects after taking the same dose of alcohol, even after correcting for creatinine (Sarkola et al., 2003).

In conclusion, alcohol represents a common problem among patients dependent on illicit drugs, and heavy drinking is associated with an increased risk for relapse into drug use and discharge from treatment (Anglin et al., 1989; Stenbacka et al., 2007). Although based on a small sample size, the results of this study suggested that biomonitoring of alcohol use by urinary EtG and EtS testing is a valuable objective tool for detection of recent drinking during outpatient treatment.

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**REFERENCES**


