0145-6008/03/2703-0471$03.00/0
Alcoholism: Clinical and Experimental Research

Vol. 27, No. 3
March 2003

Ethyl Glucuronide Discloses Recent Covert Alcohol Use Not Detected by Standard Testing in Forensic Psychiatric Inpatients

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Background: Considerable lives and money could be saved if one could detect early stages of lapsing/relapsing behavior in addicted persons (e.g., in safety-sensitive workplaces) and could disclose harmful drinking in social drinkers. Due to the serious public health problem of alcohol use and abuse worldwide, markers of alcohol use have been sought. Both ethyl glucuronide (EtG) and phosphatidyl ethanol (PEth) appear to have high sensitivity and specificity and a time frame of detection that may elucidate alcohol use not detected by standard testing. Our aim was to assess their potential for detecting recent covert alcohol use under controlled conditions.

Methods: Thirty-five forensic psychiatric inpatients in a closed ward who had committed a substance-related offense (§64 StGB), were followed for 12 months. The complete time spectrum of possible alcohol consumption was covered by the complementary use of breath and urinary ethanol (hours), urinary EtG (days), %carbohydrate-deficient transferrin (CDT)/PEth (weeks), and γ-glutamyltranspeptidase (GGT)/mean corpuscular volume (MCV) (weeks-months).

Results: Fourteen of the 146 urine samples examined were positive for EtG. In all EtG-positive cases, patients reported alcohol consumption of between 40 and 200 g of ethanol 12–60 hr prior to testing. Urinary and breath ethanol were positive in only one case. In the blood samples, PEth was not positive in any case and %CDT did not exceed the reference value. Isoelectric focusing showed no abnormal Tf subtypes.

Conclusions: The findings emphasize the diagnostic and therapeutic usefulness, specificity, and sensitivity of EtG as a marker of recent alcohol use. Such a test is needed in numerous settings, including alcohol and drug treatment (to detect lapse/relapse), in safety-sensitive work settings where use is dangerous or in other settings where use may be inappropriate (e.g., such as driving, workplace, pregnancy, or monitoring physicians or other professionals who are in recovery and working), or for testing other groups (such as children or those with medical problems) where alcohol use would be unhealthy or unsafe. The health, social and socioeconomic benefits arising from the future use of these markers is hard to overestimate.

Key Words: Biological Markers, Alcohol Drinking, Glucuronates, Ethyl Glucuronide, Physician Health Programs, Therapy Effectiveness, Cost Reduction.

RECENTLY THE TOPIC of physicians and addiction has been raised (Verghese, 2002) following the outbreak of bloodstream infections in an intensive care unit. This brings to discussion the question of useful tests for drug and alcohol use and monitoring individuals in safety-sensitive jobs. Alcohol and drug testing have become commonplace in the United States following the establishment of federally mandated workplace testing in the late 70s. Testing is performed for forensic, occupational, public health, and therapeutic reasons. For example, State Physician Health Programs routinely monitor recovering physicians (who make a commitment to abstinence) to document their sobriety while the physicians continue to practice medicine.

Regulatory licensing boards have generally supported the existence of these programs, as it is known that the disciplinary process is time-consuming, expensive, and associated with a high rate of suicide among disciplined licensees (Crawshaw, 1980) and, on the other hand, the success rates of these programs have been high (Shore, 1987). Thus, urine toxicology has become an important component of these health programs. Alcohol, which remains the most common drug of choice among chemically dependent health professionals (44% in Alabama), is the least amenable to detection. Furthermore, false-positive results are possible due to fermentation in the urine post

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Received for publication July 24, 2002; accepted November 14, 2002.

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DOI: 10.1097/01.ALC.0000057942.57330.E2

voiding (Saady et al., 1993). A better means of detecting alcohol lapse and relapse is needed.

Major shortcomings restrict the routine use of currently available and emerging biological state markers of alcohol consumption. These shortcomings include: (1) the time spectrum of detection they reflect (e.g., only hours for serum ethanol); (2) the amount of alcohol to be consumed before they are elevated (e.g., >1000 g of ethanol within 2 weeks for carbohydrate-deficient transferrin (CDT) (Lesch et al., 1996; Stibler et al., 1986); (3) the availability and practicability of the test (e.g., special labs being required for hydroxytryptophol/hydroxyindole acetic acid (HTOL/HIAA ratio), dolichol, acetaldehyde adducts, and so on; and (4) influences on test results by other factors including age, gender, and a variety of substances and nonalcohol-associated diseases (Gilg and Soyka, 1997; Laposata, 1999; Salaspuro, 2000). A biological state marker for disclosing recent alcohol consumption with high sensitivity and specificity with a subacute time of detection between that of the short-term markers (such as ethanol in serum and urine, methanol, and HTOL/HIAA ratio) and long-term markers (such as CDT, GGT, or MCV) is needed.

Mainly in the last decade, some nonoxidative ethanol metabolites have been studied for that purpose. Promising markers include fatty acid ethyl esters (FAEE), ethyl glucuronide (EtG), and phosphatidyl ethanol (PEth), each having a characteristic time spectrum of detection of ethanol consumption: FAEEs up to 24 hr, EtG up to 5 days, PEth up to 2 weeks (Alling et al., 1983,1984; Gunnarsson et al., 1998; Hansson et al., 1997; Varga et al., 1998,2000). Because FAEEs, although promising (Diczfalusy et al., 1999,2001; Laposata and Lange, 1986), cover a time frame that is included in the time spectrum of detection for EtG, FAEEs were not determined in this study.

EtG is a direct metabolite of ethanol that can be determined in various body fluids, tissues, and hair and is nonvolatile, water soluble, and stable upon storage (Alt et al., 2000; Jaakonmaki et al., 1967; Kamil et al., 1952; Kozu, 1973; Neubauer, 1901; Nishikawa et al., 1999; Schmitt et al., 1995,1997; Seidl et al., 2001; Wurst et al., 1995,1999a,b,2000,2002; Wurst and Metzger, 2002). The conjugation of ethanol with activated glucuronic acid in the presence of membrane-bound mitochondrial UDP glucuronyl transferase to form EtG represents 0.02–0.06% in humans (Dahl et al., 2002) and – dose dependent – about 0.5–1.5% of total ethanol elimination in rabbits (Kamil et al., 1952) and, thus, a minor detoxifying pathway. With its specific time frame of detection intermediate between short- and long-term markers and preliminary evidence of a high sensitivity and specificity, EtG is a promising marker of alcohol consumption. It can be detected in urine beginning a few hours after alcohol consumption and remains positive for up to 4 days after the complete elimination of alcohol from the body (Dahl et al., 2002; Schmitt et al., 1995,1997; Seidl et al., 2001; Wurst et al., 1995,1999a,b,2000,2002; Wurst and Metzger, 2002).

So far, in more than 2500 serum and urine samples determined by different groups, no false positives or false negatives have been reported (Dahl et al., 2002; Nishikawa et al., 1999; Schmitt et al., 1995, 1997; Seidl et al., 2001; Wurst et al., 1999a,b,2000,2002a; Wurst and Metzger, 2002b).

Recent studies have suggested and given support to the use of PEth in blood as a marker of alcohol abuse. Chronic alcoholics admitted for detoxification had mean PEth levels of 13.2 μmol/liter on the first day, detectable up to 14 days after admission (Hansson et al., 1997). Using liquid chromatography (HPLC-ELSD) and electrospray mass spectrometric detection, PEth has been detected in extracts of blood from alcoholics (Gunnarsson et al., 1998). These patients had PEth levels of 5–13 μmol/liter, detectable up to 3 weeks after the beginning of an alcohol-free period. A third study on chronic alcoholics showed mean PEth levels of 2.5 and 5.1 μmol/liter in two different groups, respectively (Varga et al., 2000). A study on healthy volunteers revealed that a single dose of ethanol (32–47 g) does not produce measurable amounts of PEth (Varga et al., 1998). However, out of twelve volunteers who consumed between 624 and 2134 g of ethanol during 3 weeks, eight persons had detectable levels of PEth (1.0–2.1 μmol/liter). A threshold of total ethanol intake yielding detectable PEth seems to be around 1000 g, with a mean daily intake of about 50 g. So far, analysis of PEth has been performed by the use of whole blood. A recent study on blood from chronic alcoholics showed that almost all PEth was found in the erythrocyte fraction (Varga et al., 2000).

The aim of the present study was to further elucidate the potential of EtG and PEth as alcohol intake markers. This study was performed in the well-controlled and defined conditions of a closed ward for forensic psychiatric inpatients who had committed a substance-related offense. Our hypothesis was that EtG should give the best information on recent alcohol intake and PEth, in addition to γ-glutamyltranspeptidase (GGT), mean corpuscular volume (MCV), and %CDT could help exclude regular consumption of greater amounts of alcohol. If EtG would turn out to be useful, this would have tremendous impact on future testing for alcohol use in various settings and lead to significant improvement in therapy effectiveness, quality of life of the patients, and socioeconomic benefit.

PATIENTS AND METHODS

The forensic psychiatric inpatients who were studied (3 female, 32 male; median age, 33 years; range, 26–56) were all sentenced according to §64 StGB (penal code), an option of German law. This law is applicable for substance-related offenses when it is deemed that the individual was incapable, because of impairment (§20/21 StGB), of recognizing the wrongfulness of the crime. During the consecutive 12-month period of the study, all patients were hospitalized on a specialized ward exclusively for these patients. Due to the inclusion criteria (start at a given date and end

Table 1. Descriptive Statistics for the Patients and Parameters Determined

Parameter	*n*	mean	median	SD	min	max
Age [years]	35	34.2	33	7.39	26	56
%CDT 1; ref. <6.5	18	1.05	0.65	1.24	0.2	4.73
%CDT 2; ref. <6.5	20	0.93	0.35	1.27	0.20	4.28
PEth 1 [nmol/L]; ref. <LOD	28	—	—	—	<LOD	<LOD
PEth 2 [nmol/L]; ref. <LOD	23	—	—	—	<LOD	<LOD
EtG [mg/L]; ref. <LOD	146	1.03	0	5.5	0	46.9
GGT 1 [U/L] ref. 6–28	27	21.1	12	20.1	6	78
GGT 2 [U/L]; ref. 6–28	23	24.1	16	24.2	7	94
ASAT 1 [U/L]; ref. <18	27	13.77	9	12.64	4	54
ASAT 2 [U/L]; ref. <18	23	13.08	9	8.74	6	33
ALAT 1 [U/L]; ref. <18	27	21.11	11	29.54	3	140
ALAT 2 [U/L]; ref. <18	23	22.47	13	26.34	5	105
MCV 1 [fl]; ref. 82–101	27	90.7	91.1	4.19	76	98
MCV 2 [fl]; ref. 82–101	25	89.3	90.0	5.54	67	95

PEth, phosphatidyl ethanol; EtG, ethyl glucuronide; 1, t1 = month 4; 2, t2 = month 11.

of study 12 months later), the patients were hospitalized between 4 days and the entire observation period. Five were alcoholics, 17 were drug dependent, and 13 had both diagnoses. Comorbid disorders were mainly personality disorders, depressive disorders, sleep disorders, and one case of schizophrenia.

A total of 146 urine samples from 35 patients were collected randomly. One to eight urine tests were performed per patient for EtG. Urine samples were all tested for specific gravity, urinary ethanol, EtG, and illicit drugs (immunologic bedside test and REMEDI, see below). Breath ethanol tests were performed randomly. In month 4 and 11 and in cases of suspicion of consumption blood samples were also drawn and %CDT, PEth, MCV, GGT, aspartate aminotransferase (ASAT), and serum alanine aminotransferase (ALAT) were determined. This resulted in 43 samples for CDT from 26 patients (including 7 samples from one patient, 2 samples from 11 each, and one sample from 14 individual patients). For PEth analysis, 42 samples from 26 patients were obtained (including four samples from 1 patient, three samples from 2 patients, two samples from 8 patients, and one sample from 16 patients). Self-reports for alcohol consumption were obtained routinely at least once a week by interview.

Methods

I. Urine testing.

All urine samples were collected under direct observation.

1) To further help exclude manipulation of the urine, specific gravity and pH were determined with Combur 9 Test (Roche Diagnostics Inc., Basel, Switzerland).
2) EtG was determined with a gas chromatography-mass spectrometry (GC/MS) method with deuterium-labeled EtG (d_5-EtG) (Medichem Inc., Stuttgart, Germany) as an internal standard as described elsewhere (Alt et al., 1997, Wurst et al., 1999a,b,2000). The limit of determination for EtG was 0.1 mg/liter. The limit of detection was 0.03 mg/liter. Reference value: <limit of determination.
3) The determination of urinary alcohol concentration was performed on a Perkin Elmer Sigma 2000/HS 100 head-space-gas chromatograph system with FI detection (Perkin Elmer Sciex, Wellesley, MA).
4) Testing for illicit drugs was initially performed using an immunologic rapid testing device (Triage 8 test; Biosite Diagnostics, San Diego, CA) and then the urine samples were tested at a laboratory of the Department of Legal Medicine using REMEDI (Global Medical Instrumentation, Inc., Albertville, MN). The urine screening for drugs was performed by the automated HPLC-System REMEDI. The active agents were identified by online comparison of UV-spectra with a spectra data bank.
5) For the determination of breath ethanol concentration, a Draeger Alcotest 7410 (Draeger Safety Inc., Durango, CO) was used.

II. Whole blood/serum testing.

1) PEth is measured in heparinized whole blood (as described elsewhere) with a high-pressure liquid chromatography (HPLC) combined with an evaporative light-scattering detector (ELSD) method (Varga et al., 2000).
2) CDT estimation was performed on duplicate serum samples using the %CDT turbidimetric immunoassay kit (Bio-Rad Laboratories, Philadelphia, PA) according to manufacturer's instructions. This method is based on micro anion exchange chromatography followed by turbidimetric measurement. Isoelectric focusing to exclude rare genetic D-variants was undertaken as previously described followed by semiquantitative evaluation by means of a scanner (Bean and James, 1994; Kuchheuser et al., 1995).

Table 2. Self-Reported Alcohol Consumption, Time of Last Drink, and Other Biomarkers Positive for Those With a Positive Urinary EtG

Patient	EtG [mg/L]	Self-reported amount of alcohol consumed	Time of last drink	Other biomarkers positive
3	0.4	40 grams	3.5 days ago	None
5	4.2	40 grams	2 days ago	None
7	4.7	60 grams	2 days ago	None
7	46.9	100 grams	12 hours ago	Breath ethanol 0.05 mg/L Urinary ethanol 0.4 g/L
12	4	50 grams	1 day ago	None
20	13.7	200 grams	2.5 days ago	None
20	7.4	150 grams	3 days ago	None
23	0.6	80 grams	2.5 days ago	None
25	43.7	100 grams	1 day ago	None
25	3.4	100 grams	3 days ago	None
29	0.3	40 grams	3 days ago	None
30	8	40 grams	2 days ago	None
30	10.1	40 grams	2 days ago	None
33	7.6	60 grams	1.5 days ago	None

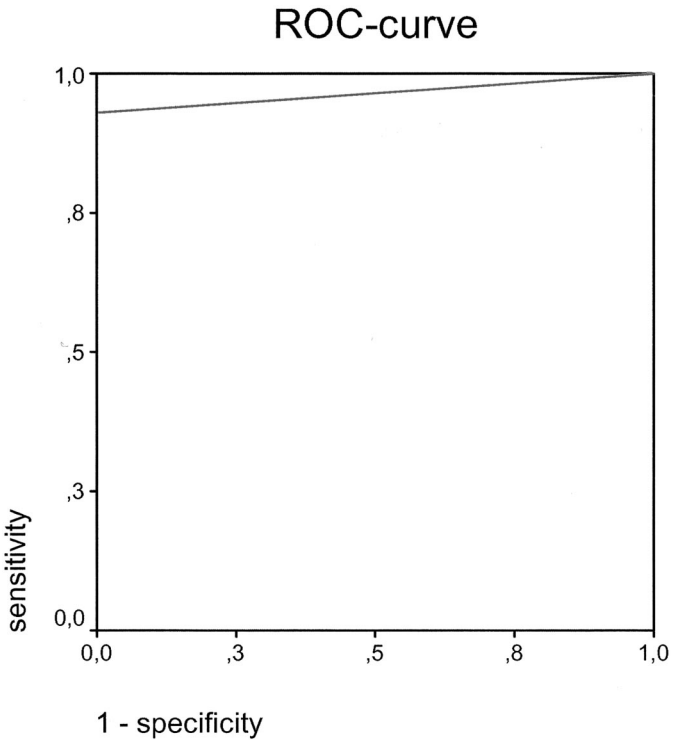

Fig. 1. ROC curve analysis for EtG as test variable and self-reported alcohol consumption (yes/no) as state variable. AUC = 0.964.

III. Statistical analysis. For statistical analysis (descriptive statistics, Spearman correlation, Wilcoxon test, ROC curve analyses), SPSS 10.07 was used (SPSS Inc., Chicago, IL).

RESULTS

Descriptive statistics for the parameters determined (*n*, mean, median, SD, minimum, maximum) are given in Table 1. During the entire study period, breath ethanol concentration was positive in only one case at 0.05 g/liter. Urinary ethanol was positive in the same individual at 0.4 mg/liter. Specific gravity of urine was, in all cases, 1.015 (reference value >1.020) or higher. One urine sample was positive for cocaine, 16 were positive for prescribed drugs such as amitriptyline, doxepin, paroxetine, promethazine, trazodone, oxymetazoline, and bisoprolol. Fourteen of the 146 urine samples were positive for EtG (46.9 mg/dL max). In all cases, patients reported alcohol consumption of between 40 and 200 g of ethanol 12–60 hr prior to testing (Table 2). None of patients, from whom the 132 EtG-negative samples were drawn self-reported alcohol consumption. ROC curve analysis for EtG as test variable and self-reported alcohol consumption (yes/no) as state variable shows an area under the curve (AUC) of 0.964 (Fig. 1).</HEAD>

No blood sample was PEth positive in any case nor did %CDT exceed the reference value (6.5). However, in four cases of those who were tested twice for %CDT, there was an increase (not exceeding the reference value) (Table 3); in four cases a decrease; and in four cases it was identical. The Tf subtypes showed no allelic D-variants. In both samples of a patient, isoelectric focusing showed rare genetic Tf-subtype C2D, which generates no higher CDT. The subtypes were: C1, 28 samples; C1–2, 10 samples; C1–3, 2 samples; C2, 1 sample; and C2D, 2 samples.

For traditional biological state markers, a good correlation was found between month 4 (t1) and month 11 (t2) (GGT: $r = 0.756$, $p < 0.001$; MCV: $r = 0.895$, $p < 0.001$). In the Wilcoxon test, no significant differences were found for these parameters and CDT at t1 and t2.

DISCUSSION

To prevent and control health and social problems related to alcohol use for the individual and society, biological state markers and marker combinations capable of monitoring alcohol consumption with a high sensitivity and specificity are needed. Such markers may help to (a) identify high risk groups; (b) evaluate current treatment programs; (c) develop more effective treatment strategies; (d) disclose recent drinking in social drinkers in inappropriate and risky situations; and (e) elucidate the role of neuropsychological impairment following alcohol intake (i.e., the hangover state).

The well-defined setting of a closed forensic psychiatric ward, in combination with an extensive marker battery, offered the unique opportunity to study the usefulness of EtG, PEth, and their combination, with traditional markers and clinical impression to monitor putative alcohol consumption in persons committed to abstinence. All patients studied are substance dependent and had committed a substance-related offense. Under German law, they were committed for a term of monitoring and treatment in lieu of jail. During later stages of their therapy, patients were allowed to leave the ward (during weekdays and on weekend passes) to begin social reintegration. Despite commitment to abstinence, it was during these periods that lapses occurred. EtG was by far the most effective in detecting

Table 3. Comparison of Different Parameters of the Four Patients With an Increase of %CDT During the Study Period

Patient	Age	Gender	%CDT1	%CDT2	GGT1 [U/L]	GGT2 [U/L]	MCV1 [fl]	MCV2 [fl]	EtG: Number of tests (positives); results [mg/L]	
7	27	m	1.4	2.5	13	12	90	87	6 (2)	4.7; 46.9
8	40	m	0.2	4.25	12	16	93	95	2 (0)	<LOD
16	30	m	0.5	0.8	9	8	92	94	8 (0)	<LOD
20	26	f	0.8	4.28	7	7	76	67	6 (2)	13.7; 7.4

LOD, limit of determination; 1, t1 = month 4; 2, t2 = month 11.

surreptitious alcohol use. In contrast, traditional markers and clinical impression gave no indication for alcohol consumption. Neither PEth nor %CDT was positive in any case. This supports the hypothesis that the patients were lapsing, not relapsing, and that relatively minor amounts of alcohol were being consumed. Theoretically the entire time spectrum of possible alcohol consumption was covered by the complementary use of ethanol (hours), EtG (days), %CDT/PEth (weeks), GGT/MCV (weeks-months). CDT and PEth are indeed similar in regarding the amount of alcohol that needs to be consumed for the marker to become positive. The current knowledge on PEth, however, indicates that sensitivity and specificity seem to be higher.

Pharmacological interaction with prescribed or illicit drugs can be excluded, as all samples positive for EtG were found to be negative for any other kind of drug. To date, including this study, no measurable EtG concentrations were observed in serum or urine of nonrelapsing patients, nondrinking drivers, or teetotalers with negative breath ethanol and/or self-reported abstinence (Nishikawa et al., 1999; Schmitt et al., 1995, 1997; Seidl et al., 2001; Wurst et al., 1999a,2000,2002; Wurst and Metzger, 2002).

Recent findings from the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence (WHO Global Status Report On Alcohol (1999); Wurst and Metzger, 2002) showed the following significant ($p < 0.001$) correlations for the Spearman rank correlation for the total sample ($n = 304$) between EtG and other variables, including: sobriety in days ($r = -0.6$); the HTOL/HIAA ratio ($r = 0.58$); ethanol level ($r = 0.433$); methanol level ($r = 0.198$); CDT ($r = 0.458$); GGT ($r = 0.428$); and total grams of ethanol consumed in the previous month ($r = 0.467, p < 0.001$). When comparing results between EtG levels (>limit of determination) and the HTOL/HIAA ratio (>15 pmol/nmol), 68.8% ($n = 119$) of those positive for EtG did not have elevated values for the HTOL/HIAA ratio. Thirty-one percent (31.2%) were positive for both parameters (Wurst and Metzger, 2002). This might reflect the longer time specturm of detection for EtG (up to 5 days) as compared to HTOL/HIAA ratio (<1 day). These results underline (in a big sample) the potential of EtG.

An algorithm as suggested by the data on ethanol, EtG, PEth, and GGT for the assessment of alcohol intake could be: (1) ethanol: recent consumption of alcohol within the last day; (2) EtG: consumption not longer ago than 5 days, detection even of small amounts like a bottle of beer; (3) PEth: 1000 g of ethanol to be consumed within 2 weeks; and (4) GGT: consumption of greater amounts over a longer time.

A test like that for EtG is needed in numerous settings, including alcohol and drug treatment (to detect lapse/relapse), in safety-sensitive work settings where use is dangerous or in other settings where use may be inappropriate (e.g., such as driving, workplace, pregnancy), or for testing other groups (such as children or those with medical problems) where alcohol use would be unhealthy or unsafe. The health, social, and socioeconomic benefit arising from the use of these markers is hard to overestimate.

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