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Commentary On: Ethylglucuronide and Ethyl Sulfate Assays in Clinical Trials, Interpretation 1 and Limitations: Results of a Dose Ranging Alcohol Challenge Study and Two Clinical Trials

Raymond F. Anton

Medical University of South Carolina, 67 President St., MSC 861, Charleston, South Carolina 29425, United States, T: (843)792-1226, F: (843)792-1724

Raymond F. Anton: antonr@musc.edu

In this issue, Jatlow and colleagues have provided the field with the report of a well done and timely scientific evaluation of one of the newer alcohol consumption biomarkers, urinary ethylglucuronide (EtG), and a related metabolite, ethylsulfate (EtS). These naturally occurring chemical conjugates between ethanol and glucuronide (EtG) or sulfate (EtS), while elegant in their simplicity, have caused a certain amount of discussion and controversy not only in the literature, but also in clinical practice (Center for Substance Abuse Treatment, 2012; Litten et al., 2010). EtG and EtS have been around for some time (Wurst et al., 2003), but highly structured investigations into their interpretation and utility have been few (Wurst et al., 2004, Hoiseth et. al., 2012). The concerns which have haunted both investigators and clinicians alike, and which have spilled over into forensic circles, involve cut-off levels, windows of detection of alcohol consumption, sensitivity and specificity of measurement, and the potential for use for monitoring abstinence in clinical practice and in clinical trials. In this paper, the authors have provided crucial information that increases the knowledge in most of these areas.

One consistent problem in the alcohol biomarker field is that, for the most part, discovery and clinical use have been based on investigations in either treatment-seeking patients or during clinical trials. For practical and ethical reasons, it is generally difficult to give alcohol to volunteers in a sufficiently controlled manner in order to gauge its effects on the biomarker of interest. In this current study, the investigators did their best to better understand the parameters and limitations of urine EtG/EtS by giving alcohol at various doses to volunteers without alcohol use disorders. They took a very pharmacological and pharmacokinetic approach to EtG/EtS measurement in the clinical laboratory. They went one step further by also showing how EtG could be applied in a clinical study population. Both studies provide important insights that will inform clinical practice while raising a cautionary note amongst clinical investigators.

I will begin with the take-home messages from this work, then I will return to the existing controversies and, with the help of the new data provided in this article, try to shed some light on these issues. Here are the main findings: 1) increasing amounts/doses of alcohol leads to increased urine EtG levels, 2) using different cut-offs for urinary EtG (e.g. 100 vs. 500 ng/ml) will provide different sensitivities of detection of previous alcohol use which vary by the time of the measurement after the last drink, 3) for the vast majority of people,

EtG is not likely to detect even moderate to heavy alcohol consumption more than 48 hours after the last drink, and at lower levels of consumption (1–2 drinks), no more than 24 hours after the last drink, 4) EtS (a more minor ethanol conjugation product) adds very little to EtG measurement despite the complexity and possible increased costs associated with its detection, 5) when used appropriately during clinical investigation, elevated levels of EtG will likely detect subjects who verbally under-report their alcohol intake (e.g. falsely claim abstinence).

Why are these findings important? There has been a bit of uncertainty about many of them, and while some issues remain unresolved (see below), this data does provide more clarity. For instance, there has been uncertainty about how long after low-level drinking EtG might remain positive. We now know, based on this work, that it is not very long. Clinicians should not expect urine EtG to detect one or two drinks greater than 12–24 hours after consumption, and they would also be advised to use the lower level of detection cut-off (100 ng/ml) to maximize the confidence in their detection. Drinking at higher levels (up to 6 drinks in a short period of time) will be detected over a longer time period. While there is declining confidence beyond 24 hours after the last drink, there is still some chance of detection up to 48 hours later.

Based on some concern expressed both in the literature and in “clinical lore” that exogenous alcohol products (e.g. mouthwash, hand sanitizer) can significantly elevate EtG, there has been some movement towards setting the positive threshold or cut-off at a higher level (e.g. 500 ng/ml) (Costantino et al., 2006), especially for forensic uses. Jatlow and colleagues have shown the perils of this approach. The marked loss in sensitivity probably does not justify setting a 500 ng/ml cut-off in routine clinical practice. Also, patients could, and should, be instructed (probably in writing) that certain products and behaviors could theoretically cause a false positive result. In the CAP/CLIA certified lab that I direct, we provide two interpretive comments for EtG results: one for the 100 ng/ml level (less conservative) and one for the 500 ng/ml level (more conservative). This allows the clinician to choose which is most appropriate for the particular situation (e.g. 100 ng/ml for clinical vs. 500 ng/ml for forensic uses). In addition, the true measured value of EtG (or EtS) should be supplied so the clinician can better interpret the value in relationship to the cut-off utilized. The data supplied by Jatlow and colleagues support such an approach.

Another controversial issue has been whether the additional measurement of EtS could increase the certainty of detecting recent alcohol consumption. This is based on two different concerns. The first involves the suggestion that EtS will be able to detect drinking in a more sensitive or perhaps “additive” manner to EtG. The Jatlow and colleagues data strongly suggest that this is not the case; therefore, measuring EtS does not appear to be worth the extra effort and possibly cost for the small, if any, incremental increase in drinking detection. The second concern has to do with exogenous production or destruction of EtG. Theoretically, EtG could be degraded in unrefrigerated urine over time and/or produced by bacteria working on sugar in the urine (Helander et al., 2007), but it has not been widely studied. The latter could potentially occur in diabetics, for instance, who might have high urine sugar content and, if certain yeast are present, could ferment the sugar to alcohol, which in turn could be conjugated to naturally occurring glucuronide leading to false

positive EtG results. A case has been made that EtS, which would not be produced in the same way, could provide added certainty that the EtG measurement is an accurate reflection of the patient's drinking status, and not some artifact of storage or secondary condition. While Jatlow and colleagues do not address this directly, a case can be made, especially for non-forensic situations, that if such conditions are mitigated by proper sample handling (e.g. refrigeration) and with knowledge of the patient's clinical condition (e.g. uncontrolled diabetics), then EtS measurement is likely to be superfluous.

The second aim of the Jatlow and colleagues study was to evaluate 1) how EtG would perform in a clinical trial under natural conditions and 2) how it would compare to verbally-reported drinking. Addressing the first objective, they found that about 75–85% of subjects who reported any alcohol consumption within 24 hours of urine collection showed elevated EtG (the cut-off of 100 ng/ml was the most sensitive and 500 ng/ml was the least). The rate of detection dropped to about 20–40% (depending on cut-off) between 25–48 hours post-consumption and then to below 20% detection for all cut-offs after 48 hours of consumption. This roughly paralleled what was observed during the clinical laboratory alcohol administration study and is an important clinical validation. In regards to their second aim, they found about a 10–15% discrepancy between verbally reported abstinence in the 48 hours before urine collection and urine EtG levels (at the most sensitive 100 ng/ml cut-off). In other words, a number of people who proclaimed abstinence during that period did not provide an accurate verbal report. However, it is possible, based on their clinical laboratory data (see figure 2C), that some individuals who drank very heavily (binged) greater than 48 hours prior to urine collection might still have been identified using the 100 ng/ml cut-off. Nevertheless, these results should raise a cautionary note in regards to clinical trial subjects' self-report of abstinence (see below for more discussion), an area in need of more investigation.

Although this is a very elegant and well-performed study, it left a few issues unanswered. The first one is whether high exogenous alcohol contact, as in frequent hand sanitizer use, could elevate EtG above the most sensitive threshold (Ali et al., 2013). This very controversial area was not addressed by this work. In addition, for practical reasons, the highest dose of alcohol (equivalent to about 6 drinks in a short period of time) achieved a BAC of about 140 mg%. While clinically meaningful, this peak BAC level might still be considered in the low range for alcohol dependent individuals who often achieve blood alcohol levels well in excess of 200 mg%. What remains unanswered is whether very high levels of intake would be detected at 48 or more hours after ingestion, an issue germane to treatment seeking chronic high alcohol users. Finally, whether EtG could be created or destroyed in vitro was not examined.

Nevertheless, this investigation has addressed important concerns regarding the use of urinary EtG and EtS as tests for recent alcohol consumption. Because of this work, both investigators and clinicians alike have new valid information to inform their interpretive use of this biomarker. As such, this work has set a standard for these types of investigations. It also shows that EtG, when used correctly, could find a rightful place in clinical studies and clinical trials. In clinical experiments where abstinence is needed for scientific interpretation and validity, using EtG as a confirmation of no recent alcohol use seems worthwhile. In

clinical trials where abstinence needs to be validly confirmed and documented, using EtG (probably in a random or semi-structured fashion) also seems worthy of consideration. Additional study of exogenous alcohol sources, using methods similar to the Jatlow et. al. paper, are needed to better address the appropriate EtG cutoff for forensic use, which should likely remain at 500 ng/ml until further systematic study is conducted.

Finally, funding from the National Institute on Alcohol Abuse and Alcoholism (NIAAA), which has had a focus on biomarker development, was crucial in allowing this investigation to be “done right”. This reflects well on the public health mission of NIAAA, and responds to a suggestion for support of this type of work made in a similar commentary four years ago (Anton, 2010). The current study by Jatlow and colleagues is an excellent example of applied research at its finest and should serve the field in that capacity well into the future.

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References

- Ali SS, Wilson MP, Castillo EM, Witucki P, Simmons TT, Vilke GM. Common hand sanitizer may distort readings of breathalyzer tests in the absence of acute intoxication. *Acad Emerg Med*. 2013; 20:212–215. [PubMed: 23406081]
- Anton RF. Editorial Commentary: Alcohol Biomarker Papers. *Alcohol Clin Exp Res*. 2010; 34:939–940. [PubMed: 20374210]
- Center for Substance Abuse Treatment. The role of biomarkers in the treatment of alcohol use disorders. *Substance Abuse Treatment Advisory*. 2012 Jun.11
- Costantino A, Digregorio EJ, Korn W, Spayd S, Rieders F. The effect of the use of mouthwash on ethylglucuronide concentrations in urine. *J Anal Toxicol*. 2006; 30:659–662. [PubMed: 17137525]
- Helander A, Olsson I, Dahl H. Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. *Clin Chem*. 2007; 53:1855–1857. [PubMed: 17717128]
- Høiseth G, Nordal K, Pettersen E, Mørland J. Prolonged urinary detection times of EtG and EtS in patients with decreased renal function. *Alcohol Clin Exp Res*. 2012; 36:1148–1151. [PubMed: 22316172]
- Litten RZ, Bradley AM, Moss HB. Alcohol biomarkers in applied settings: recent advances and future research opportunities. *Alcohol Clin Exp Res*. 2010; 34:955–967. [PubMed: 20374219]
- Wurst FM, Skipper GE, Weinmann W. Ethyl glucuronide--the direct ethanol metabolite on the threshold from science to routine use. *Addiction*. 2003; 98:51–61. [PubMed: 14984242]
- Wurst FM, Wiesbeck G, Metzger J, Weinmann W. On sensitivity, specificity, and the influence of various parameters on ethyl glucuronide levels in urine--results from the WHO/ISBRA study. *Alcohol Clin Exp Res*. 2004; 28:1220–1228. [PubMed: 15318121]