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COMMENT

Growing potential and remaining uncertainties in assessing prenatal alcohol exposure in dry blood spots

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Recent prevalence estimates of Fetal Alcohol Spectrum Disorders (FASD), with 1.1–5% of school-age children being affected, places FASD among the most common developmental disorders.[1] National surveys report that more than a quarter of pregnant women use alcohol during pregnancy; however, self-report is likely to severely underestimate true prevalence due to social desirability bias. Social desirability bias and fear of legal consequences, as well as limited tools in routine prenatal care to systematically assess quantity and frequency of alcohol use, all contribute to under-reporting and limit opportunities for early intervention and harm reduction of teratogenic effects to the fetus. While alcohol screening during pregnancy presents multiple ethical/legal questions, including but not limited to autonomy, selection of patients for screening (universal vs. targeted), beneficence, confidentiality, potential criminalization, and availability of referral and treatment options, accuracy of the assessment is crucial in making a decision of whether or not to incorporate it in clinical practice.

Direct and indirect ethanol metabolites are routinely used to assess alcohol use, both in pregnant women and newborns. To date, no ethanol biomarker has been shown to be 100% accurate in detecting alcohol exposure, and the accuracy dramatically drops with decreasing quantity and frequency of alcohol use. An "ideal biomarker" would have high sensitivity and specificity, can detect low levels and intermittent patterns of alcohol consumption, have a long detectability window, and can differentiate various levels of alcohol consumption and a temporal window of exposure. Breathalyzer and urinalysis of ethanol concentration, while highly specific, can only identify recent alcohol use. The longer-term "traditional" ethanol biomarkers, such as a Food and Drug Administration-approved carbohydrate-deficient transferrin, can detect only heavy chronic alcohol use. In newborns, fatty acid ethyl esters measured in meconium remain the most established and widely used test to assess prenatal alcohol exposure (PAE); however, delayed sample collection and potential contamination with postnatally produced stool induce false-positive results.[2] A direct ethanol metabolite—phosphatidylethanol (PEth), which can be measured in liquid blood and dry media, such as dried blood spots (DBS), received a lot of attention recently due to its higher accuracy and ease of collection, storage, and transportation.[3]

In this issue of *Pediatric Research*, Umer et al.[4] present results of testing for PAE by measuring PEth in 1729 newborn residual DBS cards in West Virginia. Information on a limited number of maternal and infant factors was obtained from the Project WATCH, which collects birth data on all infants from West Virginia birthing facilities. Results of PEth analysis in DBS cards were matched with available data in the Project WATCH (94% of records were matched). The study found that 8.1% of DBS cards had PEth values above the limit of quantitation (8 ng/ml) and 1.7% had PEth >20 ng/ml. The prevalence ranged from 2.27% to 17.11% across sub-state regions. Significant correlates of a positive PEth test included tobacco use during pregnancy, preterm delivery, birth weight, and breastfeeding intent and practice at hospital discharge. The study also compared the results of PEth analysis in DBS cards with previous estimates obtained by the PRAMS (Pregnancy Risk Assessment Monitoring System; self-reported telephone survey) and earlier PEth testing in umbilical cord tissue specimens in West Virginia. In this commentary, we discuss the primary strengths and significance of Umer et al.'s[4] findings, as well as potential limitations and interpretation of the results in a broader context of accurate identification of PAE.

One of the important contributions of the Umer's study is a large sample size of residual DBS cards, randomly selected from the West Virginia screening repository, which was matched with key perinatal variables of interest collected through another statewide program. However, it appears that examination of correlates of PAE included only bivariate analyses. Thus, given the strong established correlation between prenatal tobacco and alcohol use, it is unclear how much other factors independently contributed. Additionally, accuracy of some maternal behaviors known to be prone to information bias (e.g., tobacco use, breastfeeding intention), obtained from the statewide surveillance data, is uncertain. Prevalence estimates presented in the study by Umer et al., while alarming, are lower than estimates obtained from the Texas repository of residual DBS cards where 8.4% of samples had PEth >20 ng/ml.[5] Two previous smaller studies reported 4%[6] to 15.5%[3] prevalence at PEth ≥8 ng/ml cutoff. Given that samples from West Virginia were analyzed on average 6–8 months after collection, and some potentially had longer time between collection and analysis, the true prevalence estimates might be higher, thus more comparable with the Texas data. Previous reports indicate 13.5%[7] to 31.2%[6] degradation of the initial concentration after 9 months of storage at room temperature. With the range of positive PEth values reported between the LOD and 346 ng/ml and a mean of 20.7 ng/ml (median was not reported), it would be interesting to see the differences in patient characteristics by PEth concentration quartiles. Attempts have been made to approximate quantity of

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alcohol consumption based on PEth value in liquid blood; however, differentiation between moderate and heavy drinking was challenging,[8] and the utility of PEth to quantify lower levels of consumption is not well characterized.

Despite growing literature supporting higher utility of PEth in DBS over other biomarkers and biological matrices, there are a number of uncertainties that need to be addressed before wider use. First, it is unclear whether total PEth concentration or specific PEth homologs (16:0/18:1, 16:0/18:2, 16:0/20:4) need to be included in the diagnostic test. Some variability in the field with respect to PEth-DBS sensitivity and specificity might be explained by the differences in laboratory approach. Recent preclinical studies indicate that the homolog distribution profiles are tissue specific.[9] Another challenge is the lack of clearly established and validated cutoff concentration to constitute a "positive test". The authors acknowledge this challenge to the field of alcohol research. To date, a wide range of cutoff concentrations was used across the studies (in pregnant and non-pregnant populations) from the limit of detection to as high as 274 ng/ml. The tradeoff between the cutoff concentration and sensitivity (lower cutoff increases sensitivity) and specificity (higher cutoff increases specificity) of a test deserves careful consideration. As a direct ethanol metabolite, PEth theoretically cannot be formed unless alcohol was consumed. Post-collection synthesis of PEth in dry media was not demonstrated.[10] However, recent reports indicate that the prevalence of a positive PEth is higher in infant DBS cards compared to PEth measures in maternal samples.[11]

There are three potential explanations for such discrepancies: (1) sensitivity of PEth in liquid samples is lower than in DBS cards; (2) potential false positives in DBS specimens introduced either by perinatal confounders or differences in laboratory analyses between liquid and dry media; (3) the metabolism of PEth in pregnant women and fetus/newborn is different, which affect PEth concentrations in maternal and fetal/newborn samples. The latter hypothesis is particularly intriguing and deserves further examination. Animal models might help to address differences in alcohol metabolism. Human studies with objective quantification of alcohol exposure (via wearable electronics and other novel assessment methods) will also help to further determine sensitivity and specificity of PEth in pregnant women and newborn children.

In summary, while results of the study by Umer et al. need to be interpreted in light of uncertainties of PEth-DBS sensitivity, specificity, and a clinically relevant cutoff concentration, they add to the growing literature with a consistent message that PAE is much more prevalent when previously thought. Recent estimates of FASD at 1.1–5% of school-aged children highlight the needs for accurate, early, and readily available tests to confirm or rule out PAE to improved early identification and treatment of FASD.[1] Assessment of PEth in residual DBS cards offers a promise of not only testing at birth, but potentially accessing the banked specimens from the state repository for diagnostic purposes in suspected FASD cases. Complex legal and ethical issues, including autonomy and beneficence, are not trivial and require careful examination from multi-stakeholder perspective. It is unlikely that either a single self-report measure or ethanol biomarker will be sufficient to accurately determine PAE, thus future research should examine the most promising assessment batteries to identify different patterns of alcohol consumption.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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