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Advancing Alcohol Biomarkers Research

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Abstract

Biomarkers to detect past alcohol use and identify alcohol-related diseases have long been pursued as important tools for research into alcohol use disorders as well as for clinical and treatment applications and other settings. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) sponsored a workshop titled “Workshop on Biomarkers for Alcohol-Induced Disorders” in June 2008. The intent of this workshop was to review and discuss recent progress in the development and implementation of biomarkers for alcohol use and alcohol-related disorders with a goal to formulate a set of recommendations to use to stimulate and advance research progress in this critical area of alcoholism research. Presentations at this workshop reviewed the current status of alcohol biomarkers, providing a summary of the history of biomarkers and the major goals of alcohol biomarker research. Moreover, presentations provided a comprehensive overview of the current status of several well-recognized biomarkers of alcohol use, a summary of recent studies to characterize novel biomarkers and their validation, along with perspectives and experiences from other NIH institutes and from other federal agencies and industry, related to regulatory issues. Following these presentations, a panel discussion focused on a set of issues presented by the organizers of this workshop. These discussion points addressed: (i) issues related to strategies to be adopted to stimulate biomarker discovery and application, (ii) the relevance of animal studies in biomarker development and the status of biomarkers in basic science studies, and (iii) issues related to the opportunities for clinical and commercial applications. This article summarizes these perspectives and highlights topics that constituted the basis for recommendations to enhance alcohol biomarker research.

Biomarkers have long been pursued as tools for the detection of alcohol use, alcohol-related diseases and for prognostic identification of alcohol risk factors. Currently, the armamentarium of existing biomarkers is largely aimed at providing information related to recent alcohol use or past at-risk drinking behavior. However, questions of biomarker specificity and sensitivity remain and the relationship between past alcohol use and biomarker activity is generally only poorly quantifiable (see the reviews and commentaries by Litten et al., Jatlow and O’Malley, and Freeman and Vrana in this issue). As a result, the

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majority of traditional and new alcohol biomarkers still appears to serve as more qualitative indicators of alcohol use. Some of these qualitative indicators distinguish between longer-term alcohol use, such as γ-glutamyl transferase (GGT), carbohydrate-deficient transferrin (CDT), mean corpuscular volume (MCV), which require several weeks or months of sustained alcohol consumption to be significantly elevated (Anton et al., 2002; Litten et al., 2010, Sharpe, 2001), and short-term alcohol use (e.g., 5-hydroxytryptophol, ethyl glucuronide), measurable during the few days following an acute exposure (Jatlow and O'Malley, 2010; Litten et al., 2010). In addition, the metabolic activities that give rise to a wide variety of biomarkers may be subject to individual differences related to age, gender, racial or ethnic backgrounds, and genetic make-up. Therefore, improved validation of the information provided by individual biomarkers and their susceptibility to individual variation is essential. In this context, the preliminary report presented by Vrana, Freeman and coworkers at the NIAAA workshop (Vrana et al., 2008) showing a unique plasma proteomics profile in a carefully monitored ethanol-fed nonhuman primate population maintained by Dr. Kathleen Grant, Oregon, Health & Science University, is currently the most detailed attempt to bring the biomarker field to a higher level of quantification (see Freeman et al., 2006, for background on this experimental model). However, it remains unclear whether this approach and others in experimental animal models will be able to provide a more specific characterization of the impact of different patterns of alcohol use observed in human populations.

This issue of "quantifiability" of biomarkers is particularly relevant in the context of the specific kind of information that is being sought from the biomarker analysis. Issues of biomarker sensitivity and specificity vary depending on the needs for the biomarker. In some situations, evidence of recent alcohol use is relevant, even at a moderate level, e.g., in monitoring compliance or relapse in remedial programs. Sometimes, any use, remote or acute, may be important to document, i.e., in fetal alcohol syndrome (FAS) studies of prenatal alcohol exposure. At other times, it is essential to distinguish between at-risk drinking and moderate drinking histories. An improved qualification of such factors and their impact on the biomarker profile is urgently needed.

Cost effectiveness is a critical consideration in the use of biomarkers and may tip the balance between biomarker sensitivity and specificity. For instance, for prenatal alcohol exposure, a biomarker with high sensitivity but lower specificity could be used as an initial screening tool for mother, infant, or both, with a more specific (and probably more expensive) second-stage diagnostic tool being employed for ultimate identification of exposed persons.

A mechanistic basis for the functionality of individual biomarkers is for the most part poorly characterized. Thus, the field would benefit from more in-depth analyses of the factors that determine in a quantifiable manner the turnover and maintenance of specific biomarkers. Again, improved analysis of the time- and dose-dependence of the relationship to drinking history in a quantitative manner is essential for this purpose. Animal studies that allow for experimental intervention to test relevant mechanistic hypotheses are generally more appropriate to answer such questions. However, it is essential to extend mechanistic insights from animal studies to human populations. Mechanistic studies are obviously much more

difficult to conduct in a human population. Nevertheless, it will be important to encourage and facilitate investigations that build on carefully screened and characterized human alcohol users with the goal to address and analyze specific mechanistic questions. Some current studies may offer potential in this direction, if they build on such a well-characterized study population. For example, Rubin and colleagues, at Thomas Jefferson University, are undertaking proteomic-based investigations to identify a set of reliable diagnostic and prognostic protein biomarkers of alcoholic cardiomyopathy (ACM) in humans (Anni et al., 2007; Yohannes et al., 2009). Their studies first attempt to identify a set of early disease ACM-protein biomarkers in serum and heart tissue from rats maintained on an ethanol-containing diet. These studies will ultimately translate into human patients by characterizing protein expression profiles in muscle biopsies and serum of human alcoholics with clinical ACM and myopathy when compared to asymptomatic alcoholics and nonalcoholic controls. Work by E.B. Rimm and colleagues is focused on identifying groups of biomarkers associated with alcohol and other comorbidity risk factors such as diabetes, hypertension, and coronary heart disease (Beulens et al., 2008; Wong et al., 2007).

Biomarkers that are indicative of alcohol-related disease are generally less well characterized and may not be specific for an alcohol-dependent disease state. For instance, it is to be expected that biomarkers for alcoholic liver disease are not by themselves unique and would be difficult to distinguish from biomarkers used to identify liver diseases occurring in the absence of alcohol exposure. Therefore, combinations of general liver disease biomarkers with biomarkers specific for alcohol exposure (i.e., consumption) may be more informative in identifying alcoholic liver disease. A similar strategy may be applied to other tissues and diseases of interest in the context of alcohol use. However, it remains unclear whether there are unique features that distinguish clinical features of alcohol-related disorders from related diseases that are not associated with alcohol use. Strategies aimed at identifying and selecting biomarker candidates that can distinguish between nonalcoholic and alcoholic disease states may lead to the development of unique panels of disease biomarkers for diagnosis. Indeed, Dr. C. Wu and colleagues, University of Colorado-Denver, are engaged in studies using a quantitative proteomics approach to identify distinct sets of protein biomarkers for alcohol and nonalcoholic liver disease (5R01AA016171 Quantitative Proteomic Analysis of Alcoholic Fatty Liver Biogenesis; see Sikela et al., 2006; Kline and Wu, 2009 for a discussion of relevant methods).

These general considerations and others that were presented and discussed at the NIAAA workshop provided the backdrop to the discussions, the gist of which is presented below.

STRATEGIES TO STIMULATE BIOMARKER DISCOVERY AND APPLICATION

The discussion considered several short-term strategies that could be adopted to pursue different goals. First, there is a fairly extensive panel of alcohol use biomarkers that have in many respects complementary characteristics (e.g., see Table 3 provided in Litten et al., this issue). In addition, other recent studies (Alatalo et al., 2009) have made significant attempts to investigate the characteristics of a combination of different biomarkers in human populations that were carefully selected using the time-line follow back procedure of

establishing recent patterns of alcohol use. However, these studies also identified potential problems with some of the more traditional biomarkers that could not effectively distinguish patients with a history of moderate alcohol use and those with more high-risk drinking behavior. What remains poorly defined is whether this inability stems from a problem characterizing the drinking of patient populations or short-comings of the biomarkers being investigated, i.e., that relatively moderate ethanol use already can trigger significant biomarker responses in the absence of conditions that could lead to disease. Some of the more novel biomarkers of alcohol exposure, such as ethyl glucuronide, fatty acid ethyl esters (FAEE), mono-amino oxidase-B (MAO-B), or acetaldehyde adducts need more rigorous testing in various patient populations and situations to evaluate what exactly can be added by the addition of these new biomarkers to the traditional panel of markers currently available for routine use, in terms of time and dose dependence of detected alcohol use and biomarker specificity/sensitivity relative to cost effectiveness. A detailed discussion of the issues surrounding the use of ethyl glucuronide is presented by Jatlow and O'Malley in this volume.

Second, there is a need for a more mechanistic approach to biomarker investigations. This can take the form of carefully controlled animal studies, such as those being carried out by the group of Vrana, Grant, and coworkers (Freeman et al., 2006) but with more attention paid to a mechanistic focus for study design and functional outcomes measured. Similarly, studies designed for use with rodent models of alcohol consumption may also be useful in providing increased flexibility to gain essential mechanistic insights. Ultimately, human studies will be required, to develop increased understanding of the individual factors that contribute to the biomarker response and therefore determine some of the reasons for the tremendous variability in the strength of the response. A carefully characterized patient population would be critical for the success of such studies. Ultimately, a better understanding of the mechanisms by which biomarker levels or activities are controlled will provide essential insight into the metabolic, genetic, or other factors that can cause variability in their outcomes.

As mentioned previously, another intermediate strategy to expand the use of biomarkers of exposure is to combine them with biomarkers of disease detection. In this context, it may not be possible (or necessary) to discover novel biomarkers of alcohol-dependent diseases. There are numerous biomarkers of liver injury and disease that can be used, which, when combined with biomarkers of ethanol exposure could be evaluated for their suitability to distinguish alcoholic liver disease from other forms of liver disease. Also, other tests are available to help physicians diagnose viral-, autoimmune-, or obesity-induced hepatitis versus alcoholic hepatitis. However, significant questions would need to be resolved related to the impact of more moderate alcohol consumption or a more distant history of alcohol use as risk factors for the liver disease in question. At present, it remains an unresolved issue whether there are unique characteristics that can be used to distinguish alcoholic liver disease from nonalcoholic liver disease. Similar questions remain for other alcohol-related disorders.

Third, in the long term, the goal should be to develop and validate a "suite" of biomarkers, possibly combinations of such markers, that can be used to set standards for use to achieve

the different goals that were identified during the course of the workshop: markers that identify recent alcohol use (e.g., exemplified by the metabolite marker, ethyl glucuronide), when compared to markers that identify more chronic high-risk drinking, in addition to markers of prominent alcohol-related disease conditions that may need early detection.

Furthermore, long-term strategies should also address the use of biomarkers for prognostic evaluation of risk for alcohol-related disorders, e.g., focusing on efforts to find biomarkers for patient and risk group classification as well as treatment response and prognosis. Funding mechanisms that promote collaborative projects between bench and clinical scientists would be needed to stimulate a real "bench-to-bed-side" approach to this problem. Ethical considerations, e.g., related to patient confidentiality, will need careful scrutiny when considering a prognostic biomarkers approach.

THE PLACE OF ANIMAL STUDIES IN BIOMARKER DEVELOPMENT

As evident in many published studies and discussed in several of the workshop presentations on past and ongoing efforts at biomarker characterization, animal studies can contribute much to the discovery and characterization of biomarkers of alcohol use. Validation and mechanistic studies in controlled, laboratory animal studies are without a doubt critical for bringing the biomarker field to a higher level. Mechanistic studies can be much more informative in a system that allows for experimental observation, such as what is offered by animal experimentation. One could argue, however, that lack of progress in the biomarker field may be due at least in part to insufficient or poorly conducted animal model studies. Different animal models are appropriate, with the nonhuman primate model developed by Dr. Kathleen Grant (Freeman et al., 2006) providing an excellent example. Mouse studies are also useful to gain access to the battery of knock-out and transgenic mice that are available or that can be generated to improve the experimental flexibility and mechanistic investigation and validation of biomarkers. This approach is illustrated in the studies of Dr. A. Fornace and colleagues, Georgetown University, using various knockout mice models to identify alcoholic liver disease–specific biomarkers (Fornace and Gonzalez, 2008). Sheep studies are relevant to modeling of FAS, as gestational timing of brain development and size of the fetus resemble that of humans. An example of this approach is demonstrated in the work of Dr. Timothy Cudd and colleagues of Texas A&M University, using timed pregnant sheep (Ramadoss et al., 2008).

Of course, the main concern with animal models is the question of whether the results garnered from animal studies will translate into the human diseases of alcoholism. Clearly, this is not always the case because of species differences in metabolism, immunology, genetics, etc. Even in the best experimental animal models, comparisons to the alcohol-exposed human are limited. In this context, it is possible that improved metabolic modeling studies can make significant contributions, as exemplified by the flux-balance, systems biology, and bibliomic studies advocated by Palsson and coworkers (e.g., Duarte et al., 2007). However, in their current state, these approaches do not consider important kinetic determinants of metabolic parameters and therefore cannot account adequately for differences between human conditions and animal models of alcoholic diseases. Future refinement of such "in silico" models that take account of different characteristics related to

enzyme expression levels and kinetic properties may have significant potential to improve biomarker discovery and validation.

Finally, it is important to recognize that the clinical or research questions “pushing” the research enterprise must be clearly defined. Only after a clear, testable research hypothesis is articulated can the most appropriate animal model be selected or developed to assess validity of the biomarker in question. The formation of networks between clinical scientists and animal experimentalists with long-standing expertise in the field should be promoted to stimulate such collaborations among the wider university and industry-wide communities.

A related matter concerns the overall interest or enthusiasm that basic scientists may have for biomarker-focused research enterprises. Undoubtedly, the overviews of the current status of biomarkers for alcohol use provided during the course of the workshop raised the question of the underlying mechanisms that are being affected by ethanol treatment and that are detected by the different biomarkers. To the extent that the biomarker analysis is mechanistically informative, this may inform and enhance basic science research. However, a research study that specifically targets the mechanisms underlying biomarkers related to alcohol use may not be viewed as high priority by a study section in the current research climate and a different format, such as a Small Business Innovation Research (SBIR) grant may be more appropriate. Another way basic science research may benefit from the use of biomarkers would be through validation of new and/or existing animal models, e.g., to verify/validate a novel model of alcoholic hepatitis, cardiomyopathy, pancreatitis, or fetal alcohol syndrome compared to other established or older models of these diseases. Also, biomarkers might be helpful when trying to verify the efficacy of new therapeutic or behavioral interventions.

OPPORTUNITIES FOR CLINICAL AND COMMERCIAL APPLICATIONS

Clinicians have different priorities for biomarker research than basic scientists. The discussions during the NIAAA workshop focused in large part on the question of what would be required to generate a serious interest in biomarkers on the part of the pharmaceutical industry and the use an effective biomarker would find in the clinical and treatment community. It was obvious that the major requirements related to cost and ease of use must include portability, robustness, and simplicity. Again, it may be that this kind of goal is more readily reached through SBIR grant mechanisms. Some of the current projects supported by this type of funding mechanism offer opportunities in that direction with research focused on monoclonal antibody–mediated biomarker discovery for alcoholic liver disease (Lohocla Research Corporation, Aurora, CO) and transcriptomic fingerprints as biomarkers for chronic alcohol abuse (Genome Exploration, Inc., Memphis, TN).

In general, the pursuit of alcohol biomarkers might also benefit from collaborations with other existing biomarker initiatives that have established infrastructure, resources, and expertise and that can be mobilized to move alcohol biomarker research forward in a timely fashion. In addition, there may be utility in considering a central core lab or facility supported by federal funding to centralize and standardize biomarker analyses for several reasons, including cost effectiveness, standardization, as well as for method development.

The addition of a more ambitious program of biomarker characterization using prognostic or high-throughput technologies may become of interest in the longer term. In this case, issues of privacy and cost reimbursement obviously must be considered and will become prominent. Currently, the evidence is only suggestive that this will provide clinically relevant information.

SUMMARY

As a result of these discussions, several recommendations could be made to enhance research into alcohol biomarkers and stimulate their application in different settings. Where possible, involvement of industry in this research could be enhanced via the SBIR mechanism.

First, there is an urgent need for the development of well-characterized biomarkers providing strong reasons to support biomarker discovery with a focus on better quantitative characterization of time and dose dependence and the impact of age, gender, racial/ethnic, and genetic differences.

Exploration of the relationships between different biomarkers/different matrices and their utility in combination, particularly in the context of the detection of alcohol-related disease conditions deserve further study. In particular, further exploration of the potential of prognostic biomarkers can identify at-risk individuals or personalized treatment potential based on early characterization.

Continued pursuit of mechanistic studies is needed to focus on biomarker formation and variability through use of well-established, characterized experimental animal models of alcoholic diseases with complementary human studies on well-characterized patient populations.

It may be of interest to promote the establishment of a central core laboratory to perform alcohol biomarker analyses for a broad variety of clinical and translational studies. Such a core laboratory should provide optimal opportunities to interact with biomarker core facilities at other institutes.

It will be through these and similar initiatives that the broader alcohol research community will begin to capitalize on the use of biomarkers for quantifying alcohol use and for diagnosis and prognosis for alcohol-related diseases and pathologies.

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