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The following discussion was presented as part of the American Academy of Insurance Medicine Medical Management and Procedures Committee meeting on October 6, 1991, at Marina Del Rey, California.

A discussion of Osborn Laboratories' experience with Tumor Markers by Warren Kleinsasser, MD, Senior Vice President and Medical Director:

In the assessment of a tumor marker, a major obstacle has been the fact that a number of tumor markers can be produced by malignant tissues. Essentially, tumor markers fall into three categories: 1) Host Injury Response Substances which are immune responses to altered antigens originating from a tumor with subsequent antigen-antibody complex. An example of these markers are Tumor Associated Antigen (TAA) and beta-2 microglobulin. 2) Oncofetal Antigens normally found in injured tissue undergoing regeneration, or in the newborn and during pregnancy. They include alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigens (CA) 15.3, 19.9, and 125, and tissue polypeptide antigen (TPA). 3) Markers of Differentiation which are produced by specific types of tissue, thus helping to identify specific tissues which may be affected by a malignancy. These include prostate specific antigen (PSA), prostate acid phosphatases, (PAP) beta human chorionic gonadotropin (Beta HCG), neurone-specific enolase (NSE), and squamous cell carcinoma antigen (SCC). These tumor markers are very helpful in identifying specific tissues that may be affected by a malignancy.

As stated earlier, many of the tumor markers occur in benign conditions and such conditions must be excluded in the evaluation of an adverse laboratory result. For example, CA 125 may be elevated with pregnancy and endometriosis, while AFP and CA 19.9 may be found to be elevated in hepatic or gastrointestinal illness. Beta-2 microglobulin can be elevated on Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis, disorders related to immune suppression, or infectious diseases. Therefore, the sensitivity and specificity of these markers are not adequate for use as a screen. But combined with a general tumor marker in selected populations, specificity is increased enough to provide a significant diagnostic test.

Dr. Donald Morton, discoverer of TAA, has determined that TAA is apparently a unique tumor marker, and has developed an immunologic test based upon the antigen/antibody reaction. It is not a genetic test. TAA detects a variety of solid tumors in organs including the bladder, prostate, colon, breast, lungs, etc. It has not been shown, however, to be of much help in Hodgkin's disease, or the leukemias.

In pursuing its research, Osborn Laboratories currently uses the TAA marker screen in conjunction with a secondary panel of recognized tumor markers as described earlier. Cases are considered to be positive only when a secondary panel marker is abnormal in addition to a positive TAA. The test is not commercially available, but it is being marketed with the cooperation of a major insurer. Since October 1990, approximately 25,000 tests were performed. The insurance company's first year cancer claims experience has fallen from 41 in 1989 and 38 in 1990, to 18 through September 10, 1991. None of the first year cancer claims were tested for TAA, thus there have not been, as of that date, any cancer claims on TAA tested business. The early data is encouraging, and the TAA research will continue.

The second type of test is to assay for the presence of the drug (alcohol) or its metabolite. A model for this approach is cocaine. When we want to know if a person abuses cocaine, we don't look for the appearance of end-organ liver damage or changes in the electrocardiograms. The assay for the principle metabolite of cocaine (benzoylcegonine) identifies the cocaine abuser. It is quite clear from this example that direct markers are preferred over indirect or secondary markers.

Acetaldehyde is the first product in the intermediate metabolism of ethanol. It has been suggested as one of the contributory factors for the development of liver disease in some alcoholics. As a metabolic product of alcohol, its measurement provides a method to detect recent
alcohol use. Transferrin is a glycoprotein that is synthesized principally in the liver and is involved in iron homeostasis. In the alcoholic, there is an increased accumulation of a desialated form of transferrin, commonly referred to as the pI 5.7 isoform. The accumulation of this glycoprotein is most probably the result of a failure to complete the terminal sialylation of transferrin resulting in the accumulation of carbohydrate deficient transferrin (CDT).

There are an infinite number of patterns for alcohol use and abuse. While acetaldehyde will tell of recent use, carbohydrate deficient transferrin occurs in the chronic user. By coupling these two markers, one can identify the recent and long term user.

These markers, at CRL, will be used in the following manner:

**Algorithm I.** The two new markers, acetaldehyde-protein and carbohydrate deficient transferrin may be used as a reflex test for applicants with abnormal liver enzyme findings. It can identify those 20-40% of patients with elevations of liver enzymes having benign or insignificant findings unrelated to alcohol abuse. For the applicant with liver enzyme elevations and normal reflex test there will be an opportunity to offer an insurance contract.

**Algorithm II.** Acetaldehyde-protein will be added as a general marker to the serum panel. If the sample is reactive, the report will indicate alcohol use. Alone, it should not be interpreted as an indication of alcohol abuse. If the aldehyde marker is elevated, the isoforms of transferrin will be assayed and reported as normal or abnormal. All samples with abnormal liver enzyme levels would be assayed for CDT (DST).

In summary, DST is an exciting new test for alcohol abuse, confirmed by numerous investigators world-wide. The positive and negative predictive values are markedly improved and the adequate stability of the sample have made it the best alcohol marker currently available.

GIB Laboratories was represented by John Boffa, Vice President, Technical Services, who spoke on Markers for Alcohol Abuse:

No single parameter has had sufficient specificity and sensitivity, by itself, to determine alcohol abuse. The difficulty exists partly because diverse conditions and etiological factors can effect the liver, serum enzymes and other constituents, and partly because abnormalities in any given parameter are not universally present in all populations of drinkers.

The most currently used enzyme parameter in the blood profile for the detection of alcohol abusers is the biochemical markers: GGTP, SGOT, SGPT, and alkaline phosphatase. The individual sensitivity of these tests reported by the Mayo Clinic and Rochester Methodist Hospital Alcoholism and Drug Dependence Unit are as follow: GGTP (63%), SGOT (48%), SGPT (56%), and Alk Phos (16%). Combinations such as SGOT and GGTP, SGOT and MCV, GGTP and MCV, or SGOT, GGTP, and MCV may optimize sensitivity and specificity to approach 100%.

Another clinical laboratory parameter which can be correlated with alcohol abuse is carbohydrate deficient transferrin (CDT). The mechanism whereby transferrin is produced is unknown. The electrophoretic ability of transferrin seems to be altered by conditions in which sialic acid residues on the carbohydrate terminals of the glycoprotein side chains of the transferrin molecule are altered. As the amounts of both sialic acids and carbohydrate depletion are uncertain, the term carbohydrate deficient transferrin is preferable to desialyed transferrin (DST).

False positive results are rarely found, whereas most subjects consuming an average of 80g of alcohol or more a day for at least three days show a positive result irrespective of the nature or severity of the liver damage. Abnormal CDT can remain detectable in the serum for several days after withdrawal of alcohol which suggests that the test is superior to random blood alcohol determinations.

The percentage of both true positive and true negative results with CDT are reported to be higher than for any other test. The Royal Hallamshire Hospital of Sheffield, England, reported sensitivity and specificity for CDT to be 90.5% and 98.8% respectively. Whereas the sensitivity and specificity for MCV and GGTP were reported to be 65%, 92.6% and 90.5%, 41% respectively.

The criteria for an acceptable diagnostic test for the insurance industry are that it should be inexpensive and easy to perform, that it measure the condition it is designed to measure, and finally, the test should have high diagnostic validity. Currently, CDT detection is expensive and the methodology is labor intensive for the laboratory to perform.

GIB Laboratories has been collaborating with a diagnostic manufacturing company in the research and development of a new technology to detect CDT which is less labor intensive and less expensive to perform. When fully developed, in the near future, the CDT alcohol marker test will be offered by GIB Laboratories as a supplemental test to be performed whenever elevated liver enzymes are reported on the blood profile.

The Senior Vice President and Chief Pathologist of the Home Office Reference Laboratory, Carl W. Ludvigsen, Jr., MD, PhD, JD, FACP, FCLM, presented a Preliminary Summary of β-Hexosaminidase and Hemoglobin Associated Acetaldehyde (HAA) Data:

The studies presented are in the process of being repeated and all of the data contained in the first studies have not been fully evaluated.

Approximately 70% of the population imbibes alcohol. Six to ten percent of alcohol consumers can be classified as alcoholic. It is estimated that 70% of alcoholics are employed and would presumably be in the 2.9% to 4.5% insurance buying public. Therefore, alcohol specific markers should reach a total "hit" rate of roughly 2.9% to 4.9%.

Various studies have shown sensitivities and specificities of GGT detection of alcoholism is approximately 50% (+/-20% to 25%). It suggests that GGT misses the alcoholics approximately
half the time, and half the time when GGT is elevated it is due to a cause other than alcohol. Therefore, a research program was begun to search for a screening test that would pick up alcohol abuse that was missed by GGT and to develop a confirmatory test for the alternative screening test, beta hexosaminidase, and GGT to improve specificity.

In preliminary studies, it appears that the sensitivity for β-hexosaminidase and HAA seem to have no variation due to age of the gender of the individual. Both β-hexosaminidase and HAA appear to be stable at room temperature at least for two weeks and perhaps much longer. Therefore, β-hexosaminidase appears to fulfill the role as an alternative screening test.

Hemoglobin acetaldehyde at a cutoff of 12.5 mmol (10.5 mmol)* has a sensitivity of 75% (95%)* and a specificity of 99%. The half life of β-hexosaminidase appears to be 7 to 10 days and the half life of HAA appears to be approximately three weeks. It appears that HAA, as well as β-hexosaminidase and GGT, takes approximately three weeks of heavy drinking, 80 grams per day of 100% ethanol, to detect significant elevations. Preliminary total HAA positive "hit" rates are approximately 3.8% in a low risk insurance buying population fitting into the assumed targeted population of 2.9% to 4.9%.

Carbohydrate deficient transferrin (CDT), also referred to as Desialylated Transferrin (DST), appears to be increasingly unstable with time of storage. At 72 hours, the results are potentially false positives. This is seen with either the isolectric focusing or the micro-anionic exchange methods for determination of CDT. Further studies on sample stabilization are being conducted.

Further studies of alcoholics, social drinkers, teetotalers and the insurance buying population continue to be conducted.


Nancy J. Haley, PhD, Director of MetLife Laboratories, reported on biological markers aimed at evaluating alcohol use. The data were take from presentations of her research group at the Research Society on Alcoholism and from other investigators also in search of such markers. Her collaborators in this area are John P. Richie, Jr., PhD, and Barbara Vogt, MS, of the American Health Foundation where Dr. Haley heads a research team evaluating lifestyle-related biomarkers:

Moderate to high consumption of alcohol has been associated with increased risk for the development of certain cancers and other diseases which might impact on excess morbidity and early mortality. A major need in the assessment of risk for the development of such diseases is the identification of alcohol abuse. To this end, the development of biological markers of alcohol use should provide an early detection system for the evaluation of risk for underwriting and medical management purposes.

Acetaldehyde is a chemically reactive compound which can form Schiff bases with amino-terminal and lysl-side chain amino groups of proteins. Adducts of acetaldehyde have been chemically synthesized and characterized for their association with hemoglobin and other serum proteins. Therefore, an acetaldehyde-hemoglobin (AcH-Hb) adduct should be useful in the determination of alcohol abuse due to the long half-life of hemoglobin and the possibility that a simple and inexpensive test could be produced through immunological methods.

The objective was to develop an immunological assay for AcH-Hb and utilize this test in specific populations of alcoholics and persons at risk for certain cancers. Additionally, it was hoped to explore the questionable association of breast cancer with alcohol consumption. It was recognized at the start that competing sources of acetaldehyde (metabolism and tobacco smoke) could provide low background levels of this compound. Both polyclonal and monoclonal antibodies were generated against reduced AcH-Hb and tested in competitive ELISAs. All antibodies were reactive to synthesized AcH-Hb and a linear response was obtained over a wide and expected dynamic range. However, the assay system did not elicit a response when tested with the blood of alcoholics, self-reported heavy drinkers, or the blood of alcohol-fed mice.

Pharmacokinetics analysis of the in vivo AcH-Hb adduct by HPLC methods confirmed that the t 1/2 (biological half-life) of this adduct was much shorter than that of the red blood cell, thus suggesting that an unstable adduct might be formed in vitro. It is possible that low rate of formation and the instability of the naturally occurring adduct limits its detection in plasma and accounts for the high variability seen in previous studies. It has now been confirmed by other researchers that these adducts can dissociate or are not formed in significant amounts in vivo due to the rapid metabolism of acetaldehyde.

Dr. Charles Lieber and colleagues have suggested that carbohydrate deficient transferrin (CDT) might be a more stable marker for alcohol use. Since immunological tests can be developed for this compound, making measurement possible in large populations, and the ratio of CDT to total transferrin should be a stable value, this test might help to fill the need for a biological marker of alcohol abuse in insurance populations as well as a test for recidivism in recovering alcoholics. Once large numbers of tests are completed and the results evaluated, the association of this marker with alcohol-related diseases and selected cancers can be studied with greater precision than has been possible in the past.

Warren Kleinsasser, MD, Senior Vice President and Medical Director, presented Osborn Laboratories' research and development of "Laboratory Underwriting of Alcohol Abuse":

Primary liver enzymes used to evaluate alcohol abuse have been alkaline phosphatase, AST (SGOT), ALT (SGPT) and GGT. Unfortunately, these enzymes are adversely affected by many substances in addition to alcohol. Some of the offenders are acetaminophen (Tylenol), aspirin, numerous antibiotics, Ibuprofen (Advil, Motrin), other non-steroidal anti-inflammatory drugs, isotretinoin and tretinoin (Retin-A) niacin, oral contraceptives, thiazides, vitamins A and B.
It has become evident also that GGT values are not as stable as previously believed.

Acetaldehyde-protein binding was first considered to be an ideal candidate. Published investigations have shown that hemoglobin associated aldehyde and serum protein associated aldehyde, like GGT, are only 50% to 65% sensitive.

Various lysosomal enzymes including n-acetyl-beta-d-glucosaminidase (NAG) and beta-hexosaminidase (Beta-Hex) in the urine have been found to be unstable and affected by several clinical conditions.

More recently, investigation of desialylated transferrin (also known as desialo-transferrin, DST, carbohydrate deficient transferrin, CDT), has demonstrated it to be a useful, sensitive test for detection of the chronic heavy alcohol user. Each transferrin molecule contains four to five sialic acids. In a chronically insulted liver from heavy alcohol use, there is a loss of one or more of the sialic acid molecules resulting in the formation of desialo-transferrin (DST). DST half-life is 7-17 days, and therefore, values remain elevated for 17 to 30 days after the cessation of heavy drinking.

Osborn Laboratories, through its research, has developed a unique methodology for assaying DST which is significantly less expensive and has set to rest the question of DST instability. The medical literature and Osborn Laboratories' experience have shown DST sensitivity of 81% to 100% and specificity of 97% to 99%. This translates into a positive predictive value of 71% to 90% and a negative predictive value of 98.3% to 99.9%.

The next presenter was Mark E. Williamson, MD, representing PRL Laboratory Services. The Molecular Biology/Cytogenetic division of PRL Laboratory Services, under the direction of Don Lightfoot, PhD, Marilyn Lloyd, PhD, and Norman Vigfusson, PhD, continue to monitor the applications of molecular genetics testing for alcoholism. Dr. Williamson was kind enough to present a summary of the developments in this field to the Committee:

The medical literature and lay press have recently reported a genetic marker for type II alcoholism. The proposed genetic marker is a variant of the D2 Dopamine receptor found on the membranes of brain cells. The gene for this type of Dopamine receptor is located on the Q arm of chromosome 11, in the 22-23 band region, and is called the A-1 allele. A DNA test has been developed to detect the A-1 allele. The test is non specific, however, since only 65% of severe alcoholics test positively for the A-1 allele. Furthermore, the A-1 allele may be found in 15% of nonalcoholics, as well as in Tourett's syndrome and several other behavioral disorders. In summary, it is not felt that the test has any current utility in the assessment of insurance applicants for familial alcoholism. The development of the test, however, is an important first step in the understanding of the genetic basis for familial alcoholism.

The Medical Management and Procedures Committee is aware that the terminology "Alcohol Marker" should be avoided in the insurance industry. Dr. Hefti reported that a similar issue arose in Europe when the insurers identified a series of parameters, as the history, variety of laboratory tests, etc. as "Alcohol Markers." To satisfy marketing considerations, communications with the applicant, agent or the attending physician, the terms "Indicators of Alcohol Consumption" was finally adopted and became generally accepted.

The Committee prefers the term "Ethanol Indicator." There is a need for education of these new tests, which should be assumed by the laboratories. A first step would be for the laboratories to uniformly adopt the recommended terminology of "Ethanol Indicator" in all their future communications.