Transferrin Isoform Analysis for the Diagnosis and Management of Hazardous or Dependent Drinking

The article by Legros et al. (1) in this issue is the latest of several descriptions and evaluations of serum transferrin isoform separation by capillary electrophoresis (CE). It is of particular interest because their method is able to separate all of the major isoforms and because the sensitivity and specificity of asialo- and disialo-transferrin for assessment of alcohol use are compared against those of carbohydrate-deficient transferrin (CDT) by a commercially available anion-exchange–immunoassay method.

The diagnosis of alcohol dependence or the detection of relapse to harmful drinking in those known to be alcohol dependent poses a challenge. Patients may underestimate or conceal their drinking (2), and doctors may overlook the condition (3). As a result, laboratory tests for excessive alcohol intake have been developed, tested, and applied. These have been based on measurements of liver enzymes (particularly serum γ-glutamyl transferase); on comparatively short-term metabolic consequences of alcohol intake (blood or breath ethanol, serum or urine ethyl glucuronide, and urine 5-hydroxytryptophol/5-hydroxy-indoleacetic acid ratio); or on changes in the carbohydrate composition of serum glycoproteins (particularly transferrin).

The measurement of transferrin isoforms, the carbohydrate sidechains of which have less than the usual complement of terminal sialic acid residues, has been investigated for >20 years (4), but widespread application has been held up by labor-intensive or expensive methods and concerns about the sensitivity and specificity to be expected in clinical use. There have also been understandable concerns about the effectiveness of any laboratory test in improving outcomes in a condition that primarily affects behavior and where persistence in that behavior, despite knowledge of the resulting harm, is a defining characteristic.

The success of any test in classifying patients depends on both the performance of the analytical method and the frequency distributions of the analyte concentrations in affected and unaffected individuals. Poor technical characteristics (such as imprecision or poor specificity for the analyte) will undermine even the best of diagnostic tests, and sometimes a refinement in the methods available will lift a test to a new level of usefulness. This is the prospect of the application of CE to measurement of transferrin isoforms, as described in several recent reports (1, 5–7). Most previous work relied on either separation of transferrin isoforms by isoelectric focusing with quantification by immunofixation, staining, and densitometry or separation by anion-exchange chromatography, followed by immunoassay.

The anion-exchange methods measure a mixture of isoforms, generally including the asialo-, mono-, di-, and all or part of the trisialo transferrins, and the total of these components is reported as CDT. Such mixtures of molecular species may contain some components with good diagnostic performance and others that provide little discrimination between patient and control groups; therefore, the overall performance is worse than for more analytically specific methods. Comparison of literature reports (8) has suggested that isoelectric focusing with immunofixation gives better results than the commercial anion-exchange–immunoassay approach. Legros et al. (1) have now shown that the diagnostic performance available from measurement of asialotransferrin alone is better than that for measurement of CDT by anion exchange and immunoassay.

However, there is an important issue that cannot be overcome by technical improvements. Alcohol use is widespread, and most users are not harmed; they may even benefit in both the social and medical spheres. Therefore, the issue is not to discriminate between two separate groups but to assess whether alcohol use is above or below some "safe limit" based on epidemiologic evidence. Even with tests for the diagnosis of diseases that should be either present or absent, there is evidence that use of highly contrasting groups for assessment of sensitivity and specificity leads to overestimation of test performance (9). Because their evaluation was based on abstainers and patients in treatment for alcohol dependence, the 92% sensitivity found by Legros et al. (1) is (as they acknowledge) a best-case estimate.

Nevertheless, the data allow a prediction of the likely sensitivity of CE asialotransferrin measurement in a mixed group of drinkers. The consensus odds ratios estimated from metaanalysis of CDT studies (8) suggest that at cutoff values providing 90% specificity, the anion-exchange–immunoassay methods will have ~55% sensitivity. At a cutoff value of 3% for the Axis-Shield %CDT method, Legros et al. (1) report a specificity of 90% and sensitivity of 80%. This is consistent with an overestimation of achievable sensitivity (80% vs 55%) because of spectrum bias. A similar effect may be expected for the CE asialotransferrin results, perhaps bringing the sensitivity in clinical practice down to ~70%.

Apart from the issue of diagnostic performance, other recent work gives important insights into the nature of the transferrin isoforms. Purification of the naturally occurring isoforms from serum, combined with enzymatic treatment using neuraminidase, selective binding of lectins to carbohydrate components, or mass spectrometry, has provided further evidence of their structure. Most methods, including CE, depend on differences in charge to separate the isoforms; therefore, it is difficult to know whether the differences in transferrin isoforms in alcoholics are attributable to the absence of one or both of the carbohydrate chains or merely to loss of the terminal sialic acid residue. There is some conflict on this point, with labeling and analysis of the glycan chains suggesting loss or lack of synthesis of the entire carbohydrate chain (10). On the other hand, chromatographic separation of asialo-

transferrin from alcoholic patients showed heterogeneity in this peak (11). Mass spectrometry and lectin binding studies suggested that the heterogeneity was attributable to "a mixture of transferrins with two N-glycan chains with shortened antennae". This issue is an important one because it is possible that separation of asialotransferrins might lead to further improvements in diagnostic sensitivity for excessive drinking. Mass spectrometry, being able to distinguish isoforms of the same charge, may be particularly useful for this purpose (11, 12).

Even in healthy individuals, multiple serum transferrin isoforms coexist, and presumably this mixture serves some physiologic function. In pregnancy, an increase in the more highly sialylated forms has been reported in maternal serum (13) and in cultured placental cells (14). This may be related to changes in iron requirements and the transfer of iron to the fetus. The isoform profile is changed in several pathologic conditions not related to alcohol, particularly the carbohydrate-deficient glycoprotein syndrome (15). In both healthy persons and alcoholics or heavy drinkers, the proportions or concentrations of the less sialylated transferrin isoforms are affected by physiologic or pathologic conditions including sex; age in women, probably because of variation in hormonal effects or in iron stores; obesity; and insulin resistance (16–19). Liver disease also affects the value of CDT (8). These physiologic and pathologic effects may impact on test specificity, sensitivity, or both, and they emphasize that variation in transferrin isoforms is not just a marker created for our convenience but a consequence of dynamic metabolic processes.

With increasing emphasis on protein variation and the discovery that we have many more proteins than genes, more attention will be given to posttranslational modification of proteins and the measurement of isoforms. CE is likely to play a substantial role in such investigations and in the diagnostic tests that may result. Ideally, specific immunoassays for individual isoforms would lead to high-throughput testing, but immunoassays or combined lectin- and antibody-based techniques for individual transferrin isoforms are not currently available and present some difficulties in design. Nevertheless, methods for detection of variation in glycosylation of other proteins, such as human chorionic gonadotropin, have been developed.

Overall, the evolution of CE methods for transferrin isoforms presents an interesting example of how improved methods can make tests more specific for a defined chemical species rather than a group of similar molecules and how this may (if its promise is borne out in practice) lead to better clinical information from the laboratory.

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