Evolution of carbohydrate-deficient transferrin testing: Technologies, diagnostic performance, and benefits

Accurate and sensitive biomarkers that can objectively reflect whether a person is engaging in sustained heavy drinking are invaluable because often medical problems can be explained or exacerbated by abusive drinking.

By Pamela Bean, PhD

Medical attention and treatment for abusive drinking usually occurs only after more serious problems develop such as difficulties at work, impulsive social behavior, severe depression, and impaired driving. Considering these circumstances, there is no doubt that the proper detection of chronic and harmful alcohol consumption via biological tests can contribute significantly to diagnosis, early intervention, and successful treatment of alcohol abuse and dependence.

Currently, physicians and other caregivers are limited to very brief interviews to assess alcohol use and misuse in their clinics. Over 85 types of scientifically validated questionnaires are available to inquire about all kinds of factors: pressures to drink, beliefs about drinking, personal coping skills, family history, and health habits. The main advantage of questionnaires is that they cost very little to administer and there are many to choose from. Their main weaknesses relate to their dependence on the recall and honesty of the patient and the doctor's reluctance to conduct them due to lack of time in their busy schedules. Even more, many health professionals agree that an objective evaluation done by a laboratory test is preferable than interview because conclusions from a questionnaire are often less compelling.

Biomarkers to detect alcohol use and abuse have been classified into two main groups: direct and indirect.[1] Direct biomarkers are those that measure alcohol itself or a product of alcohol metabolism. Indirect biomarkers are those that measure the effects of alcohol in the body.

The best example of a direct biomarker is the measurement of blood or urine alcohol. Blood alcohol concentrations are an excellent measure of alcohol use and abuse because they reflect very accurately the amount of alcohol present in the body. However, both these indicators clear away within a matter of hours after alcohol consumption and this short window of detection is too narrow for reliably indicating if abuse is occurring.

The best-known example of an indirect biomarker is the liver enzyme gamma glutamyl transferase (GGT) whose elevations in any given person reflect liver damage. The main challenge with GGT is its poor specificity. In other words, GGT becomes elevated with many conditions unrelated to alcohol abuse including age and body mass index.[2] This lack of specificity has limited GGT's reliability as a tool to detect heavy drinking in the medical community.

Another indirect biomarker of sustained heavy drinking is carbohydrate-deficient transferrin (CDT) – a specific isoform of the human protein transferrin. Transferrin is synthesized in the liver as an 80-kDa polypeptide chain containing 679 amino acids and two complex carbohydrates (Figure 1). Chronic alcohol consumption of 60 grams (5 beers, 4 glasses of wine, 3 standard drinks) per day for at least one week induces the liver to produce molecules of transferrin which are missing one or two of its carbohydrate chains and thus the name carbohydrate-deficient transferrin.[3] The two main mechanisms for the accumulation of CDT in serum have been attributed to acetaldehyde-mediated inhibition of the protein glycosylation processes in the Golgi apparatus and/or increased trimming of normal transferrin molecules in the serum.[4]

CDT was discovered in Europe in the late seventies and several CDT assays have been developed around the world during the last 20 years. All these assays have two steps: the first step separates CDT from the normal transferrin molecules and the second step quantifies CDT. For example, the first CDT kit was developed in Europe and consisted of an ion exchange chromatography procedure which used mini-columns to separate the CDT isoforms from normal transferrin.[5] Nephelometry was then used to quantify the CDT molecules that eluted from the mini-columns. The second CDT assay was an isoelectric focusing procedure considered the gold standard in the mid-nineties, which separated CDT by gel electrophoresis and quantified the CDT bands by western blotting and laser densitometry.[6] Other CDT test developments through the years include the following technologies: high performance liquid chromatography, capillary electrophoresis, and mass spectrometry.

In general, mainstream laboratory tests proceed through several stages of development prior to their acceptance by pathology labs and the medical community. First, the test must be accurate in that it must expose the patient to minimal

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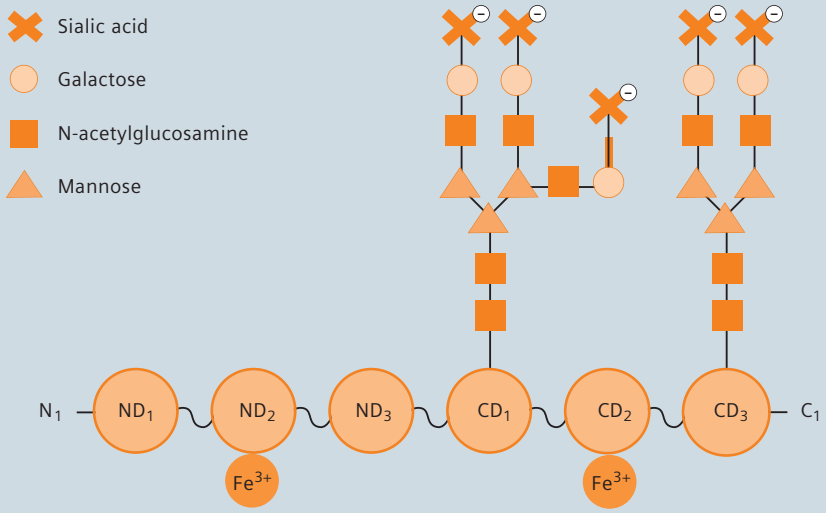

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risk in terms of false positives and false negatives. Second, the test must be affordable in terms of technician involvement, equipment, and reagents. Third, the test must be user-friendly, which means it must be performed quickly with a minimum of training by a laboratory technician in almost any laboratory. Last but not least, the test must be automated and adapted to run in widespread instruments for maximum efficiency and accessibility.

For the most part, previous CDT tests lacked one or more of the features mentioned above; they are specialized tests requiring skilled technicians to run them, they use expensive equipment or costly reagents, and the turnaround time has been less than adequate. These limitations have made them unsuitable for routine use. More recently, Siemens developed the first direct nephelometric immunoassay which uses monoclonal antibodies against CDT.[7] This is the first direct %CDT test to reach the market and it is the only fully automated %CDT test available worldwide. It is very likely that these new features will change the scope of CDT testing, making its use more popular and widespread.

CDT assay performance

Several hundred publications in the last decade have helped our understanding of the diagnostic performance of CDT in several populations. The main difference between CDT and the previous, more traditional, markers of alcohol abuse is diagnostic accuracy. Indeed, CDT's major asset is its high specificity. This means that a CDT positive result is almost always indicative of sustained heavy drinking. For instance, CDT is not affected by any of the following diseases; hypertension, asthma, bronchitis, angina pectoris, diabetes mellitus, adiposity, lipid metabolism disorder, depression, and disorders of the digestive tract.[8] Studies have shown that there are only a few non-alcohol related conditions that will render false positives for CDT: genetic variants of transferrin, the carbohydrate-deficient glycoprotein syndromes, and severe chronic viral hepatitis.[9, 10]

Genetic variants are molecules of transferrin that can cause not only false positives but also false negatives when running previous CDT tests. For example, when running the Sebia Capillarys CDT test, the main tetrasialo-transferrin isoform is taken as a reference and it constitutes the central peak; B-variants show the same central peak plus additional peaks towards the anode and D-variants show additional peaks towards the cathode. As seen in Figure 2, B-type variants produce false negatives by Sebia's capillary electrophoresis method and D-variants produce false positives. Similarly, the micro-column CDT method also produces false positives for transferrin variants of the D type as recently reported.[7] Surprisingly, the Siemens Healthcare Diagnostics method appears not to be affected by either the B or the D phenotypes most likely because the monoclonal antibodies do not react with transferrin genetic variants. This is a strong competitive advantage for the newly automated CDT test.

The sensitivity of the CDT test, measured as its ability to detect harmful alcohol consumption when it truly exists, depends on several parameters: amount of alcohol ingested, extent of drinking behavior, time of sample collection after cessation of drinking, age, and gender.[11] Previous CDT tests have been shown to perform best in middle-age Caucasian males with

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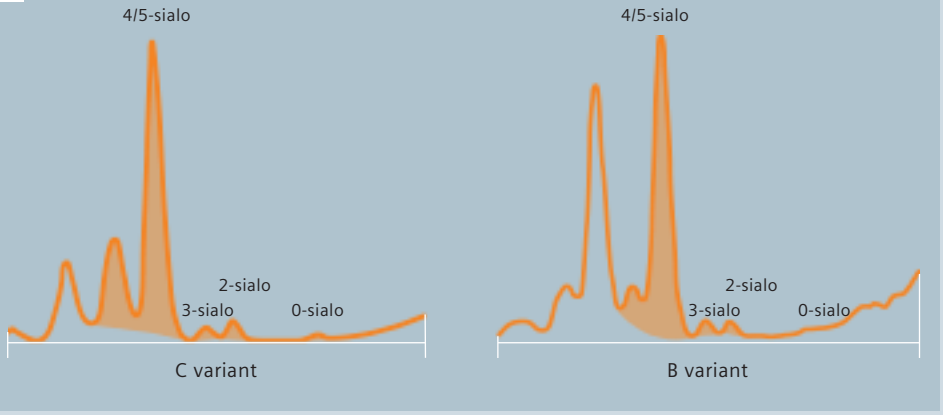

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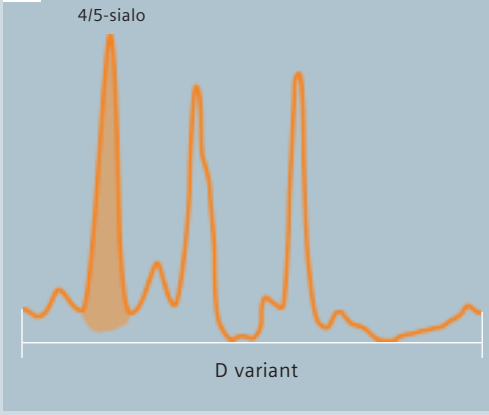
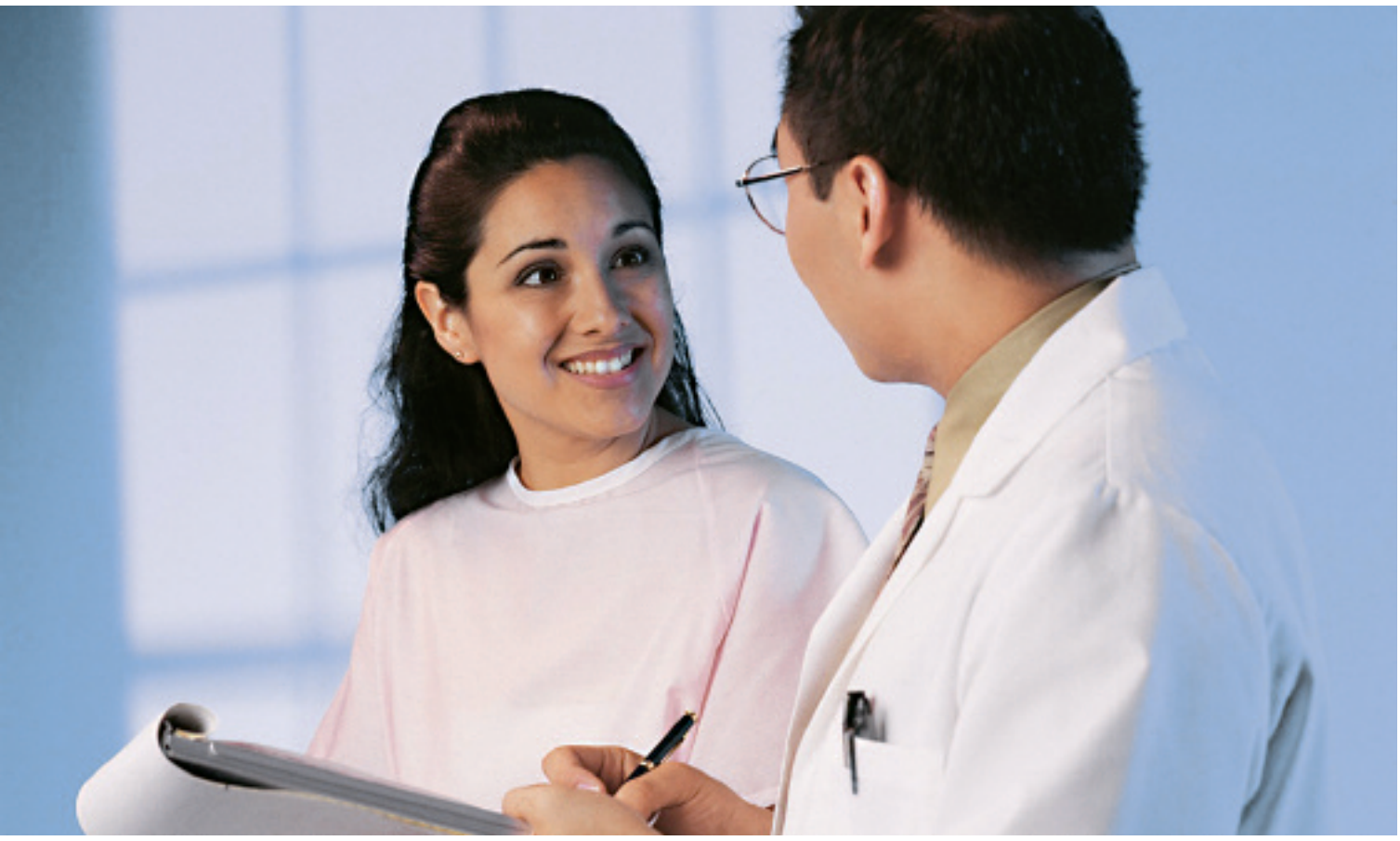


2A **Chromatograms for transferrin types C and B:** Both these sample tested positive (3%) by the CDT N-Latex method, identifying both subjects as heavy drinkers. However, when these samples were tested by the Capillarys method the sample in the left, obtained form a subject carrying the most common C variant of transferrin, tested CDT positive but the sample in the right, obtained from a subject carrying the B variant of transferrin, tested CDT negative. Capillary electrophoresis shows negative results for B variant samples because CDT represents a low percentage of the total transferrin present in these subjects.
The B variant is represented by the much higher peak at the left of the shaded area, compared to C variant. (4/5 sialo represents molecules of transferrin with 4 and 5 sialic acids.)

2B **Chromatogram for transferrin D variant:** this sample tested negative (1.94%) by the N Latex CDT method and positive (7.2%) by the BioRad microcolumn CDT kit. When tested by the Capillarys method, the presence of additional peaks towards the cathode helped to identify this individual as a carrier of a genetic variant of the D type. The Sebia instrument does not calculate a value for genetic variants.

Acknowledgements: The Sebia CDT test was run under the direction of Dr. Cristian Saez during his tenure as Assistant Laboratory Director at Heritage Laboratories (Olathe, KS) and the N-Latex test was run under the direction of Dr. Ronald Haas during his tenure as Laboratory Director at the Clinical Chemistry Section Laboratory in Marshfield Clinic (Marshfield, WI).



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Group	n =	1.0%	2.5%	Median	97.5%	99.0%
Male	255	1.19%	1.27%	1.77%	2.36%	2.47%
Female	306	1.20%	1.37%	1.76%	2.35%	2.44%
Total	561	1.19%	1.29%	1.76%	2.35%	2.47%
Adolescent	141	1.38%	1.45%	1.91%	2.40%	2.47%

3 **Distribution of CDT values produced by the N Latex CDT assay**[7]

a less than optimal diagnostic performance in females.[12] Another competitive advantage of the direct CDT test is that it shows optimal diagnostic performance in adolescents and adults, both males and females (Figure 3).

Benefits of the use of CDT

One of the main uses of CDT in the US refers to the practice of reflex testing in the insurance industry.[13] Reflex testing is effective in detecting alcohol abusers because it is only done when there is already a significant suspicion pointing to possible heavy drinking. The initial screening tests perceived by insurers as the most helpful in identifying potential abusive drinking is GGT.

Insurers also look for alcohol-related driving convictions as reported on the motor vehicle report. It is estimated that two-thirds of US and Canadian life insurance companies use the CDT test by reflexing from elevated GGT or other factors suspicious for alcohol abuse. Rather than screening, CDT is used by insurers to confirm a suspicion of abuse.

Another application of CDT testing that is gaining popularity in the US is its use as a tool to monitor abstinence and relapses in repeat intoxicated drivers. Third- and fourth-repeat offenders in Waukesha County (Wisconsin) are monitored with biomarkers during the entire duration of their Drivers Safety Plan (DSP).

When a biomarker is used to identify drinking in any given person over time, the result of the test can be compared against the previous biomarker levels in the same subject.[14] In the Waukesha pilot, subjects are asked to provide a sample of blood for biomarker testing during their assessment and then again every three months. This type of comparison is often used during longitudinal testing to assist a person in recovery (Figure 4). If at any time during the 12 months follow-up period the biomarker result indicates a relapse to heavy drinking, represented by a 25 percent increase in biomarker value, then the subject is monitored more often and the treatment support is intensified until the biomarker value goes down to non-drinking levels.

Moving forward

More than 300 studies in the last decade describe the use of CDT to identify sustained, harmful alcohol consumption of 60 grams of alcohol per day for 7 to 10 consecutive days. Astute practitioners are now beginning to understand that alcohol abuse has many definitions. The role of the laboratory is to promote the use of this test and to assist in results interpretation with the intent of guiding the medical professional towards early diagnosis and treatment of heavy drinking.

It is very possible that as a result of automation and availability, this new CDT test will become more widely available in the US. Educational efforts to raise awareness into the best applications of the CDT test are paving the way to help those in need of better treatment by providing stronger support to manage their condition.

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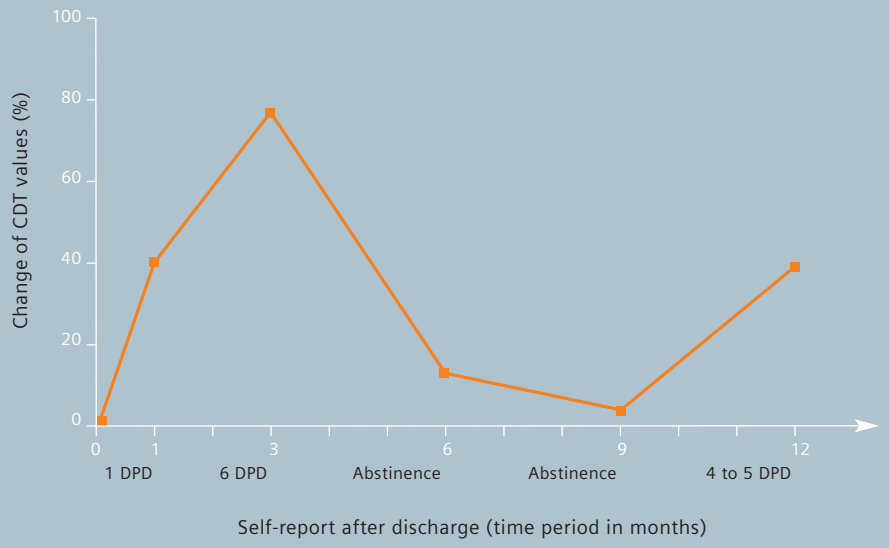

4 Monitoring of alcohol consumption: CDT values and self-report

An adult female was monitored with the CDT test after discharge of 57 days in residential treatment for alcohol dependency. Follow-up monitoring was done after one month and then regularly every three months.

At each time period, the client was asked to go to the laboratory to draw a blood sample for CDT testing and a phone interview was conducted to obtain information on her alcohol consumption for the previous 15 days before sampling.

Alcohol consumption is reported as standard drinks per day (DPD). CDT measures are reported as percent change from discharge, which represents her abstinent baseline.

This example serves as "proof of concept" for the use of the CDT test to monitor abstinence and relapses: every time the subject reported alcohol use, the CDT values went up and when the subject reported abstinence, the CDT values went down. Specifically, one and three months after discharge the patient reported consumption of 1 and 6 DPD, respectively and the %CDT values went up. She then reported abstinence at six and nine months post-discharge and the %CDT values went down. Close to the end of the monitoring period she again reported consumption of 4 to 5 drinks daily which resulted in an increase in the %CDT value at 12 months.

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