Carbohydrate-deficient transferrin testing: a truly traceable and specific biomarker for chronic alcohol misuse

by Jean Deenmamode

The concept of testing breath, blood or urine for alcohol is familiar to the general public, and blood alcohol testing is widely used across a range of industries, from aviation to driver licensing. But is a snapshot alcohol measurement really the best approach to ensuring long-term safety in these industries, or identifying individuals at risk from complications of chronic excessive alcohol consumption?

IFCC-approved carbohydrate-deficient transferrin assay

The risks associated with driving or operating machinery directly after consuming alcohol are widely known, and the 'morning after' effects of excess alcohol intake are equally well understood. Unfortunately, the mid- to long-term effects of routinely high alcohol consumption are less widely recognized. Historically, most people have associated this with liver disease, but it is just one facet of disease; it also affects the kidneys, the brain – leading to depression and other neurological problems – fertility, and many other aspects of human physiology. Dealing with the biochemical and psychological changes associated with excess alcohol intake requires a holistic approach, looking at everything from specific biomarkers to physiology and socio-economic factors, with no one-size-fits-all solution.

Recognizing a problem

Regardless of where affected individuals interact with the health-care system (through primary care, industry-associated mandatory testing or frequent alcohol-related admissions to the emergency department), the first challenge is to establish that there is an issue. Simple blood alcohol level testing only provides a measure of short-term alcohol consumption – limited to hours, not days – making it easy to 'cheat' by simply abstaining from drinking immediately before testing. Liver function tests [including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase (ALP), albumin and total protein, among others] are commonly used to assess the health of the liver, and can provide an indication of alcohol-associated liver disease, but are obviously highly non-specific and only suggest very long-term alcohol abuse. Similarly, mean corpuscular volume (MCV) – an index of red blood cell size – has historically been used as a marker of excessive alcohol intake, and can be detected even after an extended period of abstinence, but offers poor sensitivity and can be affected by a wide range of other factors.

Gamma-glutamyl transferase (GGT) is one of the most commonly used biomarkers of chronic drinking. Excessive alcohol consumption over a period of weeks significantly raises levels of this liver enzyme, and it takes, on average, three weeks of abstinence for GGT levels to return to within reference limits. The ability of this test to detect long-term heavy drinking in the recent past has made GGT a useful tool for monitoring abstinence, but liver disease – a common problem in recovering alcoholics – can also increase GGT levels, leading to false-positive results.

Ethyl glucuronide (EtG) in urine is another potential marker, and has the advantage of non-invasive sample collection, avoiding the need for a blood draw. When combined with concomitant measurement of ethyl sulphate it provides a meaningful measure of recent alcohol consumption. However, EtG is only present in urine at detectable levels for around four to five days after heavy alcohol consumption, making it a good marker of binge drinking, but limiting its use for more chronic misuse.

Phosphatidylethanol (PEth) testing is increasingly being seen as a reliable method to provide a mid-term picture of alcohol consumption. Phosphatidylethanols are abnormal phospholipids formed in the presence of ethanol, which bind to the membrane of red blood cells. Unfortunately, there are no commercially available PEth assays at this time, making it impractical as a universally applicable biomarker.

One of the most promising biomarkers for chronic excess alcohol consumption is carbohydrate-deficient transferrin (CDT). Like GGT, this is another blood-based protein that increases in concentration with high alcohol intake. In normal serum, the majority of transferrin isoforms will have four carbohydrate moieties attached to them, forming tetrasialotransferrin. High alcohol consumption over a

period of one to two weeks leads to a greater percentage of carbohydrate-deficient disialotransferrin. For example, consumption of half a bottle of wine, two cans of beer, or 125 ml of spirits daily for a period of seven to 10 days will lead to an elevated CDT result. Furthermore, with a half-life of about two weeks, CDT is a useful 'retrospective' marker. The advantage of this over other biomarkers is that it is highly specific to chronic excessive alcohol consumption.

History of CDT testing

CDT was first proposed as a marker of chronic excess alcohol consumption in the late 1970s, by a group of Swedish researchers investigating a broad spectrum of blood- and CSF-based molecules as potential biomarkers for alcoholism. Technological advances, particularly the advent of commercially available capillary electrophoresis platforms in the early 2000s, has resolved many of the issues associated with CDT measurement, but a lack of awareness – and therefore demand – among healthcare professionals continues to be a challenge today.

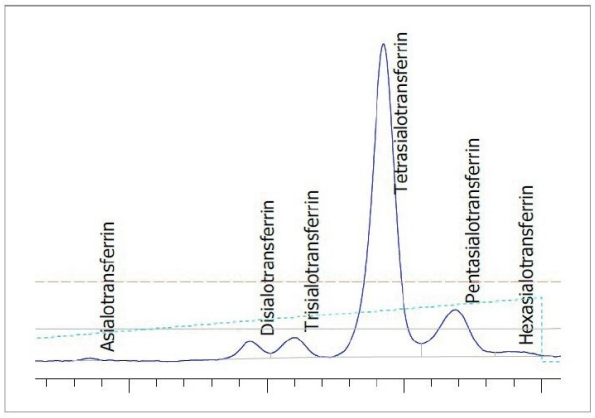
The aviation industry was one of the first sectors to take a serious interest in CDT testing, as a means of identifying alcohol misuse among commercial airline pilots, prompted by a number of high profile aviation accidents. Investment from this sector led to a number of developing commercial CDT assays. Unfortunately, the novelty of this biomarker for diagnostic applications led to a number of issues, including poor testing availability, inconsistent sample processing and a lack of uniformity between tests from different manufacturers. Although a number of airlines – as well as driving licence issuing authorities in some territories – have embraced this assay as a valuable tool, these issues prevented more widespread adoption in other areas, such as heavy industry or primary care.

Establishing a standard for consistent results

Recognizing the potential of this marker, the International Federation of Clinical Chemistry (IFCC) set about establishing an effective, traceable standard for CDT testing. This began by setting up a working group to look at the various assays on the market, and collaborate with manufacturers to create an internationally recognized standard. Close partnership between kit providers and reference labs around the world eventually led to the creation of an IFCC-approved gold standard reference method for CDT in 2017, along with the harmonization of many commercially available assays traceable to this standard. These 'CDT-IFCC' assays are now available from the majority of CDT assay manufacturers, creating a benchmark and enabling the comparison of results obtained using different technology platforms. Using this approach, any individual with CDT result of 1.8% or higher is considered 'clinically positive' across the globe, while any result over 2% is 'forensically positive' to eliminate the possibility of legal challenges.

Managing change – how to increase uptake

Four years on from the creation of the reference method, many labs are still reluctant to switch to the more rigorously controlled CDT-IFCC assays. There are a number of reasons for this, but the primary one is economics. With the airline industry still one of the predominating markets for CDT testing, swapping from a non-standardized method to one that might lead to consistently higher results – and therefore more positive results – creates a potential challenge for both labs and their airline customers. There is a perceived risk that airlines will take their business elsewhere if labs do not continue with the existing, potentially under-reporting assays. This is a major challenge for both individual labs and CDT testing as a whole as, without the more reproducible and reliable testing offered by CDT-IFCC assays, more widespread adoption will be difficult to achieve.



All possible sialotransferrin peaks identifiable by the IFCC HPLC method

Fortunately, many labs have already switched to the CDT-IFCC assays – with as driving licence issuing authorities in both Sweden and the Netherlands having approved the standardized test. In other cases, there is still a lack of understanding among those requesting testing as to the advantages of using this standardized approach. User education is the key to overcoming this, and there has been some success in countries such as France, where labs are engaging with primary care and occupational health to promote more accurate and insightful testing. This collaborative approach, bringing together CDT assay manufacturers, laboratories, clinicians, psychiatrists and local authorities, will help CDT-IFCC to gather momentum and become widely recognized as a way to tackle long-term alcohol misuse.

Opportunities in primary care – a drinking lifestyle assessment

Beyond driver licensing and aviation, there are a number of industries or regulatory sectors where reliable CDT testing has potential applications, including any industry where workers operate heavy or dangerous machinery, as well as driving or operating licence issuing authorities. However, one of the biggest markets for CDT testing should be primary care, allowing clinicians to provide individuals with a measurable biochemical assessment of their drinking habits to support a wider conversation on lifestyle. Many patients attending a GP appointment complaining of general feelings of lethargy, depression or isolation – or even mild physical symptoms of alcohol misuse – may not realize that the amount of alcohol they consume as affecting their bodies. Reliable CDT testing provides primary care workers with a valuable tool to help further the conversation on drinking lifestyles, potentially helping thousands of individuals with 'hidden' physical or mental health issues linked to chronic alcohol consumption.

Conclusion

CDT testing is proving to be the most reliable marker of chronic alcohol consumption, and the commercial availability of CDT-IFCC assays traceable to an international reference standard makes this test an ideal candidate for widespread clinical use. The challenge for proponents of this assay is now to educate a wider audience to the benefits of CDT testing to patients and the broader healthcare environment, allowing the value of this assay to society to truly be realized.

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