er geen verschil in klinische fenotype gevonden tussen deze patiënten en patiënten met andere mutaties die de ernstige infantiele nefropathische vorm geven (17). Sedoheptulose is ook verhoogd in bloedspots, al tijdens de neonatale periode (18). Deze bevinding kan zorgen voor een snelle (pre)-symptomatische detectie van cystinose patiënten homozygoot voor de 57-kb deletie, waardoor een behandeling vroeg kan worden gestart.

Functie PPP

De belangrijkste functie van het oxidatieve gedeelte van de PPP is de productie van NADPH uit $NADP^+$ en daarmee de cytosolische NADPH concentratie te behouden. NADPH is belangrijk bij de verdediging tegen oxidatieve stress veroorzaakt door reactieve zuurstofradicalen. De PPP is sterk verbonden met de glycolyse door de intermediairen glyceraldehyde-3P en fructose-6P. Het is bekend dat de PPP activiteit omhoog gaat bij oxidatieve stress in zoogdiercellen. Het exacte mechanisme hiervan was onduidelijk. Onlangs is aangetoond in gistcellen met een verhoogde resistentie voor oxidatieve stress geproduceerd door het thiol-reducerende reagens diamide dat het onderliggende mechanisme gebaseerd is op een omleiding van de metabole flux van de glycolyse naar de PPP, waarbij de redox status van het cytosolisch NADP(H) wordt verhoogd (19). Verder is aangetoond dat de PPP een metabole redox sensor is en transcriptie reguleert tijdens de reactie op antioxidanten (20).

Toekomstig onderzoek

Het toekomstige onderzoek zal gericht zijn op het verkrijgen van meer kennis over de pathofysiologie in de twee aangeboren defecten (TALDO en RPI deficiëntie), met hopelijk een werkzame behandeling als gevolg. Tevens willen we graag de nog niet volledig gekarakteriseerd enzymen die betrokken zijn bij de vorming van de polyolen verder beschrijven. Onze technieken voor het meten van suiker-P, polyolen en C7-suikers zullen verder gebruikt worden in de diagnostiek van bekende en misschien nieuwe defecten in de PPP en onderzoek naar de functie van de PPP o.a. tijdens oxidatieve stress.

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Forever an alcoholic?
The Dutch approach in using CDT as alcohol biomarker in forensic medicine

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In the Netherlands the CBR (Central Office Driving ability) is the Dutch statutory body responsible for the administering of driving tests including withdrawal and regranting of the driving licences. The CBR Driver Assessment Division is responsible for the 'disqualification procedures' for example in case of drunk driving. Roughly ten thousand driver assessments are yearly initiated in the Netherlands, involving a medical examination by specialized psychiatrists. From medical, ethical and social perspectives both high sensitivity and specificity of the laboratory tests used, are needed for correct judgement of excessive alcohol drinking. Measuring of the alcohol biomarker Carbohydrate Deficient Transferrin (CDT) has become the keystone laboratory parameter used by psychiatrists in the clinical evaluation of drunk drivers for chronic alcohol abuse and for assessment of abstaining. In comparison with previously used laboratory parameters as MCV and γGT, CDT scores the highest on diagnostic accuracy. We focus both on analytical aspects as testing, development, and standardization of CDT methods, as well as development of guidelines for a proper use of CDT in Dutch and International (IFCC) settings.

Measuring CDT, method testing and development

Transferrin is the major iron transport protein, consisting of a single polypeptide chain of 679 amino acids, two iron binding sites and two N-linked complex oligosaccharide chains. Over 30 different polypeptide chain variants are known. The most common variant in Caucasians is the homozygous C1 variant. Heterozygous transferrin BC and CD variants are present in approximately 1% of the Caucasian population. The oligosaccharide chains have a large variety of microheterogeneity; the glycan chains can be di-, tri- and tetra-antennary, and each antennary is normally terminated by a sialic acid residue. The total number of sialic acid residues gives its name to the glycoform. Different transferrin glycoforms occur in serum (figure 1). Prolonged alcohol abuse causes a disialotransferrin (DiST) increase together with an increased ratio DiST/total transferrin; only in heavy alcohol abuse, asialotransferrin is present (figure 1). In the early days CDT indicated the sum of asialotransferrin (exclusively present in heavy drinking) and disialotransferrin as a fraction of total transferrin. Nowadays the ratio DiST/total transferrin is defined as official measure.

The first commercially available CDT method from Pharmacia (CDTect) and its successor method from Axis Shield (expressing CDT as a fraction of total transferrin) included a small ion exchange chromatography procedure for separating the disialofraction from the other constituents. This method was very sensitive for genetic variants and other microheterogeneities as demonstrated among others by our group. Combined with the co-existence of several methods (all with their own characteristics and cut-offs), this resulted in the initiation of a Dutch Advisory Board on alcohol markers by CBR (nowadays NVKC WG on CDT).

Early work from our laboratory was on method correlation, demonstrating that the Analis CE method was highly correlated to HPLC (1). We participated in the multicenter validation of N-Latex CDT (Dade Behring/Siemens), an automated, particle-enhanced, homogeneous immunonephelometric assay for directly determining CDT without the need of a sample work-up (2). We demonstrated that the N-Latex method is apparently not disturbed by the presence of genetic variants. Recently we provided a major contribution to the multicenter validation study of the Sebia CE method (3). In this publication we used the new concept of measurement uncertainty as required by ISO 15189.

In order to provide evidence in court that the early methods were error prone, we developed transferrin genotyping by sequence analysis. We demonstrated that an occasional occurring unidentified compound in between the disialo- and trisialotransferrin peak in the chromatogram was not a genetic variant, as previ-

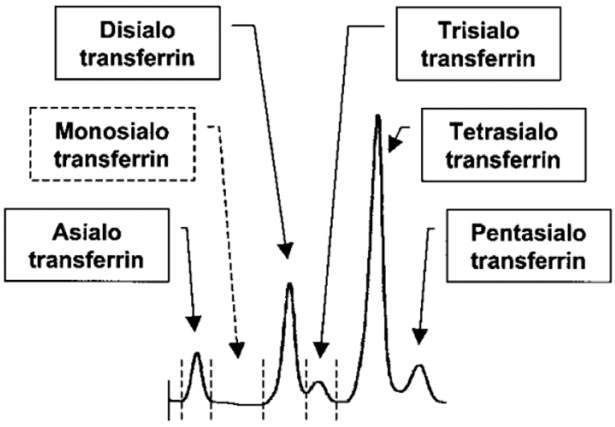

Figure 1. Distribution of transferrin isoforms in a serum sample from an alcoholic with HPLC analysis (5).

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ously assumed in literature, but that this di-tribridging phenomenon was associated with liver cirrhosis (4).
Our laboratory as an IFCC reference lab is considered as a national referral lab for confirmation of disputed CDT results in court, by using the HPLC method of Helander et al. (5). We published the existence of a new C2 genetic variant (6), other new variants discovered in our laboratory are waiting to be published.

International method standardisation and reference laboratory activities

In the last years, several methods for CDT quantification were introduced. Each technique provides results that may differ significantly to each other. Yet, manufacturers, physicians and patients need comparable results. This prompted the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to form a working group (WG-CDT) to achieve the standardization of CDT measurement. The standardization work aims to define and validate the analyte, select a reference method, work out definition and production of reference materials and propose reference intervals based on the reference procedure. In addition, the WG will provide recommendations on clinical applications for CDT.
In its first paper (7), the WG proposed that disialotransferrin should be the primary target molecule for CDT measurement and the single analyte on which CDT standardization is based. Furthermore, it proposed HPLC as the reference method and to express the results of in relative amount. In a second publication (8) the candidate reference method demonstrated reproducible results within a network of reference laboratories. Candidate reference material was found to be commutable and stable upon storage. Publications about additional validation of the proposed reference HPLC method (5) and further standardisation and correlation activities are in progress. Introducing a set of native serum calibrators will allow standardisation of all CDT methods on the market.

The Dutch approach for CDT measurement

Since the nineties, psychiatrists in the Netherlands have been using several methods for evaluating chronic alcohol abuse among drivers, being physical examination, anamnesis, and the measurement of CDT, MCV, γGT, Hb, and AST/ALT. However, the interpretation of the laboratory data combined with the anamnesis and test results is rather difficult. Discussions in court over the interpretation of the medical report between CBR doctors and lawyers on one side, and the driver and lawyer on the other side, were very intense, especially in the first years when the Pharmacia and Axis methods where used and no guidelines from either NVKC or NVvP (Dutch psychiatrists organisation) were available. This is illustrated by a very critical journal article “Voor altijd alcoholist” (Forever an alcoholic) describing cases where people once involved in drunk driving were incorrectly held to be drinking year after year based on false high CDT results. Additional laboratory tests (HPLC and genotyping when needed) performed in our lab proved the presence of a genetic variant or other interferences giving erroneous high results. Discussions about erroneous results even led to a special hearing by a committee of the Dutch parliament in June 2008.
Work from the Dutch Working group on CDT resulted in November 2008 in a NVKC guideline for CDT analysis in the clinical laboratories and a list of methods suitable for this purpose (9). In this NVKC guideline, analytical and biological variation was taken into account by presenting the concept of a critical difference. This critical difference expresses the uncertainty in the result, required for the judgement of proven existence of excessive alcohol intake. Recently, the IFCC confirmed that they support the Dutch approach of taken into account the measurement uncertainty in the individual CDT results. Furthermore, the NVKC guideline defines specific requirements for the analytical methods approved, and for the laboratories performing the analysis of CDT in forensic medicine.
To explain the characteristics of the different alcohol biomarkers available (including CDT and ethylglucuronide) we wrote a review for general practitioners, psychiatrist and other health workers (10).
In a combined taskforce of all parties involved, the NVvP published in 2011 a guideline for psychiatrists about evaluating and reporting alcohol abuse in driving license affairs. This guideline (11) includes an extensive chapter on the diagnostic accuracy, the use, and diagnostic pitfalls of laboratory tests. The Dutch psychiatrists followed the technical proposals of the NVKC regarding CDT analyses. Over the last three years the number of disputed elevated CDT results and the need for confirmation in our lab has decreased over 80%. However, the occasional erratic interpretation of traditional alcohol biomarkers MCV, γGT, AST/ALT lingers on.

Concluding

The proper use of CDT in driver licence affairs over the last years is much improved after 2008, both by the development of the new methods HPLC, CE and N-Latex and by the introduction of the NVKC and NVvP guidelines. Studies and publications from our lab and from both the Dutch and the IFCC Working Group on CDT have contributed to this progress.

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