7. Al-Daghri NM, Al-Attas OS, Bindahman LS, Saleem TH, Alokail MS, Alkharfy KM et al. Soluble CD163 is associated with body mass index and blood pressure in hypertensive obese Saudi patients. Eur J Clin Invest. 2012;42:1221-6.
8. Parkner T, Sørensen LP, Nielsen AR, Fischer CP, Bibby BM, Nielsen S et al. Soluble CD163: a biomarker linking macrophages and insulin resistance. Diabetologia. 2012;55:1856-62.
9. Soenen S, Bonomi AG, Lemmens SG, Scholte J, Thijssen MA, van Berkum F et al. Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance? Physiol Behav. 2012;107:374-80.
10. Fjeldborg K, Christiansen T, Bennetzen M, Møller HJ, Pedersen SB, Richelsen B. The macrophage-specific serum marker, soluble CD163, is increased in obesity and reduced after dietary-induced weight loss. Obesity. 2013;21:2437-43.
11. Møller HJ, Grønbaek H, Schiødt FV, Holland-Fischer P, Schilsky M, Munoz S, Hassanein T. Soluble CD163 from activated macrophages predicts mortality in acute liver failure. J of Hepatology 2007;47:671-6.
12. Kováčiková M, Sengenes C, Kováčová Z, Šiklová-Vítková M, Klimčáková E, Polák J et al. Dietary intervention-induced weight loss decreases macrophage content in adipose tissue of obese women. Int J Obes. 2011;35:91-98.

Samenvatting

Hillebrand JJG, van Berkum FNR, Mulder AHL. Geen relatie tussen sCD163 concentraties en insuline resistance in calorisch beperkte vrouwen met overgewicht. Ned Tijdschr Klin Chem Geneesk. 2014;39:182-185

sCD163 wordt sinds een aantal jaren genoemd als mogelijke marker van ontstoken vetweefsel en de ontwikkeling van type 2 diabetes mellitus (T2DM). Wij bestudeerden concentraties van sCD163 in vrouwen met overgewicht voorafgaand en na afloop van een periode van calorische beperking. Onze hypothese was dat calorische beperking zou leiden tot veranderingen in concentraties van sCD163 welke geassocieerd zouden zijn met veranderingen in BMI, vetmassa en insuline resistentie (HOMA-IR). antropometrisch (BMI, vetmassa) en metabole parameters (gevast glucose, insuline, HOMA-IR, sCD163) werden gemeten in 40 vrouwen met overgewicht voorafgaand aan en drie maanden na het volgen van het dieet (33EN%). BMI, vetmassa en sCD163 concentratie daalden als gevolg van calorische beperking en de HOMA-IR verbeterde. sCD163 en veranderingen in sCD163 concentraties waren echter niet geassocieerd met (veranderingen in) BMI, vetmassa en HOMA-IR. Wij concluderen op basis van deze data dat er geen bewijs is voor het concept dat sCD163 een directe marker is voor vroege T2DM.

Trefwoorden: obesitas, ontsteking, macrofagen, diabetes mellitus

Ned Tijdschr Klin Chem Labgeneesk 2014; 39: 185-188

Biomarkers of excessive alcohol intake in alcohol addicts with a normal nutritional status

E.E. KOK[1], J.P.M. WIELDERS[2], P.C.M. PASKER-de JONG[3], H. DEFOURNY[4], S.J.A. RONDE[5] and A. van de WIEL[1]

Introduction: **Biomarkers of excessive alcohol intake are generally measured for diagnosis and follow-up of alcohol dependency. However the micronutrient status might influence the biomarker results, especially for MCV. We studied the relationship of commonly used biomarkers with the alcohol intake in the absence of a (micro)nutrient deficiency.**
Methods: **Blood samples were taken from 70 patients (39 males, 31 females) at the start of the clinical period of an alcohol abstinence program. Vitamin B12, folic acid and albumin were used as markers for the nutritional state; γ-GT, ASAT, ALAT, MCV as well as CDT were measured as biomarkers for alcohol use. According to their self reported alcohol intake of the last month (MATE interview) patients were divided into three groups with normal, high (men 4-6 units/d, women 3-4 units/d) and excessive intake.**
Results: **No significant increase in MCV was found in the three groups. Only in excessive users of alcohol, a significant increase in the liver parameters was observed both in men and women. CDT showed a significant increase with alcohol intake especially in men. Deficiencies in albumin, vitamin B12 and folic acid were hardly present and levels of CDT were not related to the levels of the nutritional parameters.**
Conclusions: **In individuals with a normal nutritional status, CDT is the biochemical parameter most related to the reported intensity of drinking for men. ASAT, ALAT and γGT reflect liver toxicity rather than alcohol abuse and MCV increase is probably more related to vitamin and nutritional status than to excessive drinking.**

Keywords: alcohol biomarkers, CDT, MCV, nutrition, vitamin B12, folic acid

Chronic excessive alcohol intake has been associated with a number of hematological and biochemical changes in the blood (1). These changes include an increase in the mean cellular volume (MCV) of erythrocytes and a raise of the enzymes aspartate aminotransferase (ASAT), alanin aminotransferase (ALAT) and γ-glutamyl transpeptidase (γGT). Two decades ago carbohydrate deficient transferrin (CDT)

Dept of Internal Medicine[1], Meander Academy[2] and Dept of Clinical Chemistry[3], Meander Medical Center, Amersfoort; SolutionS Addiction Center[4], Voorthuizen

E-mail: jpm.wielders@meandermc.nl

was introduced as a new and more reliable marker for chronic alcohol abuse compared to the conventional ones (2).

Hematological and biochemical changes may result either from a direct toxic effect of alcohol or its metabolites on hematopoietic tissue and the liver or result from secondary nutritional deficiencies (1). Since these conditions are present in many people with alcohol dependence, the contribution of each of these conditions separately is often unclear.

The SolutionS addiction treatment center is a recognized mental healthcare institution for individuals with an addiction problem such as alcohol dependency. The center works with the 12-step Minnesota model including a clinical phase. Before going in to treatment the majority of the patients are still working, socially adapted and not malnourished. When admitted to the clinic an extensive intake is performed including the Measurements in the Addictions for Triage and Evaluation (MATE) interview, physical examination and a panel of laboratory tests. This approach allows to analyze relationships between reliable self-reported alcohol intake and the conventional and new biomarkers for chronic alcohol abuse in a population of individuals with a normal nutritional status. Significant changes of the biomarker in the absence of (micro)nutrient deficiency are then more indicative for a direct effect of alcohol or its metabolites.

Patients and methods

All patients were admitted to the SolutionS Center for the treatment of their addiction (in majority alcohol addiction) in the period July 2011 – December 2011. At the start of the treatment an extensive history and current status was scored using the MATE interview(3). This interview includes the drinking pattern of the individual during the last years as well as during the last month and has been shown to give a reliable estimate of drinking behavior and intake.

A physical examination was performed as well as a panel of blood tests. The latter included measurement of hemoglobin (Hb), hematocrit (Ht), MCV, leucocytes, platelets, γ-glutamyl transpeptidase (γGT), alanin aminotransferase (ALAT), aspartate aminotransferase (ASAT), carbohydrate deficient transferrin (CDT), folic acid, vitamin B12 and albumin. All tests were performed on routine laboratory equipment (Sysmex hematology HST analyzers and Beckman DxC 880i clinical chemistry analyzers); CDT was performed using the Siemens N-Latex CDT assay.

Nutritional status judgment was based on physical examination as well as on the biomarkers albumin, folic acid and vitamin B12 (Beckman DxC analyzers).

Statistical analyses were carried out using IBM SPSS version 19. Alcohol use was trichotomised according to expected effects, with cut off points differing for men and women. Men were considered normal drinkers if they drank up to three standard units (approximately 30 g ethanol) per day, women up to 20 g ethanol per day, which is regarded as maximum for safe drinking. Men who drank 4 to 6 units of alcoholic beverages per day and women with 3 or 4 units were considered high users. This upper level corresponds with the general accepted 60 g/day for men and 40 g/day for women above which continued alcohol use leads to clinical damage. Excessive intake reflected the use of more than 4 units (women) or 6 units (men) of alcohol per day. The correlation of drinking pattern and biomarkers, γGT, ASAT, ALAT, MCV and CDT is analyzed by ANOVA separately for women and men.

Ethics

All individuals gave informed consent for the blood tests as well as for this study. The study protocol was approved by the medical ethical commission of Meander Medical Center.

Results

The study included 70 individuals, 39 men (55.7%) and 31 women (44.3%), with a mean age of 42.2 year (SD 11.8, median 40.0). The majority of them are still working, socially active and in normal feeding condition. Table 1 shows the number of patients in each group according to alcohol intake as well as

Table 1 Alcohol intake and nutritional status of our study group.

	Normal (n=12)	High (n=13)	Excessive (n=45)
Alcoholic units per day *mean (range)*			
Men	1.50 (0.0 – 3.0)	4.86 (4.0 – 6.0)	22.54 (7.0 – 73.0)
Women	1.75 (1.0 – 2.0)	2.83 (2.0 – 3.0)	12.52 (5.0 – 22.0)
Folic Acid nmol/l *mean (sd)*	16.50 (9.2)	11.44 (3.0)	17.83 (8.7)
Low	1	0	0
Normal	10	12	35
High	1	0	8
Vitamin B12 pmol/l *mean (sd)*	263.25 (93.9)	240.58 (39.7)	321.69 (172.5)
Low	0	0	3
Normal	12	12	37
High	0	0	2
Albumin g/l *mean (sd)*	42.25 (3.2)	40.31 (4.0)	40.53 (17.3)
Low	0	0	4
Normal	12	13	41

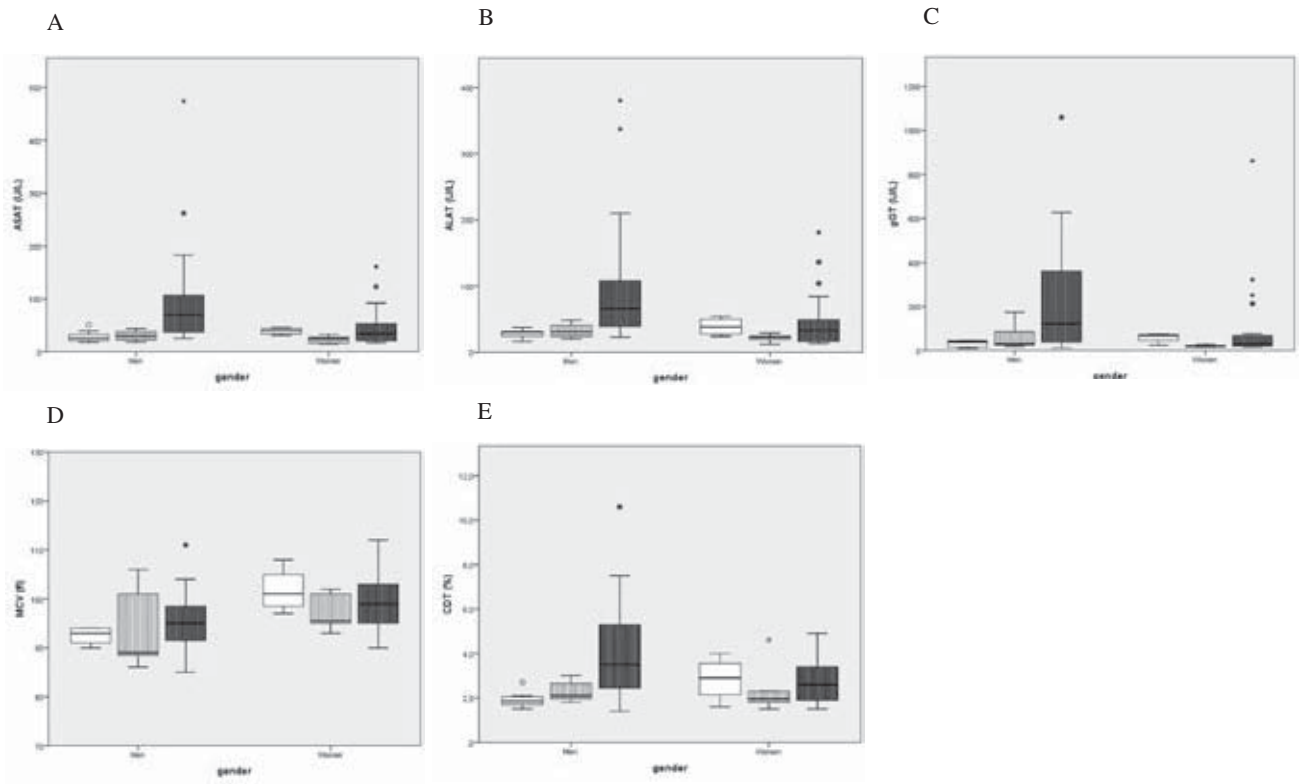

Figure 1 (A-E). Boxplots showing the distribution of the results obtained for the different biomarkers indicated and the three subgroups of alcohol drinking (from left to rhe right: normal, high, excessive) and split up in men and women. The bottom and top of the box are the borders of the first and third quartiles, the band inside the box is the median.

levels of folic acid, vitamin B12 and albumin in each group. Among men, 8 had a normal alcohol intake, 7 had high intake and 24 had excessive intake. Among women, these numbers were 4, 6 and 21, respectively. A low level of folic acid was only observed in one patient while 3 and 4 patients showed a decreased level of vitamin B12 and albumin respectively. In case of excessive drinking the daily intake in men was significantly higher in men than in women (22.5 vs.12.5 units).

Figure 1a – 1c shows the the results of the alcohol bio markers ASAT, ALAT, γGT, for the three subgroups of men and women in Box plots. Only in the groups with excessive intake, a statistically significant increase in the liver parameters was observed in men but not in women. Figure 1d shows the relationship between alcohol intake and the hematological parameter MCV. No significant differences were found between the groups characterized by the level of their alcohol intake and no significant differences were seen between men and women although the women tended to have somewhat higher levels. An elevated MCV (ref. range 80-102 fl) was observed in 12 of the 70 individuals (17%), eight of them being women. Two of these individuals had subnormal vitamin B12 levels (114 and 127 pmol/l respectively; ref. range 130-700 pmol/l). Figure 1e shows the relationship between alcohol intake and CDT. This marker showed a significant increase with alcohol intake for men.

Calculation of the Pearson Correlation between the biomarkers CDT, γGT or MCV and the individual alcohol intake yielded the following results 0.40, 0.39 and 0.18, respectively.

Discussion

In the present study of individuals with alcohol dependency but normal nutritional status CDT correlated best with the amount of alcohol consumed. The conventional liver markers ASAT,ALAT and γGT were only increased significantly in the tertile with the highest alcohol consumption indicative for a direct toxic effect of alcohol or one of its metabolites on the liver. This effect was clearly observed in men and less marked in women, but the women consumed less alcohol in all three categories. The hematological alcohol marker MCV was only increased in 17% of the studied population with no significant differences between the tertiles.

Measurement of biochemical markers has become a routine in the diagnosis, work-up and follow up of individuals with an alcohol problem. This is not restricted to clinical practice but these tests are also applied in addiction care settings and in governmental medical examinations concerning driving license withdrawing and regranting (4). In the case of alcohol abuse biochemical abnormalities will be determined partly by the toxic effect of alcohol but also by co-morbidity including nutritional status. The population of alcohol addicts following the SolutionS Center program differs from other alcoholic populations since the majority of them are still working, socially active and in normal feeding condition. Furthermore,

this setting and the use of the MATE interview, gives a good estimate of their drinking pattern, allowing us to study the relationship between the intensity of drinking and biochemical markers without interference of co-morbidity and nutritional deficiencies.

Our data show that of all biochemical parameters studied, levels of CDT are reflecting the intensity of drinking most adequately for men. Liver enzymes ASAT and ALAT were only increased in the most heavily drinking group indicating the passing of a threshold for liver toxicity. They do not discriminate, like CDT, the lighter drinkers.

Both the box plots as well as the Pearson Correlation demonstrate that MCV is not correlated to alcohol intake in our group of well-nourished alcohol abusers. MCV was increased only in a small part of the population and not discriminative for the amount of alcohol consumed. MCV is believed to be a marker for long term alcohol abuse but its response is multifactorial. Because MCV is highly depending on the availability of folic acid and vitamin B12 in the body, this parameter probably reflects nutritional status better than intensity of alcohol consumption in alcoholics. Erythroblasts require folate and vitamin B12 for proliferation during their differentiation. A deficiency of these vitamins will inhibit purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis resulting in a macrocytic anemia (5). In addition smoking may cause a significant increase of MCV(6). We noticed overall a slightly higher MCV in women in our population, possibly related to the use of oral contraceptives (6).

CDT, especially measured by modern techniques, has become the most reliable marker for the intensity of drinking and the monitoring of continuation of alcohol abuse (7). In our study the association between drinking intensity and CDT level was obvious in the males but not in the females. This may be due to the small sample size in our study with unequal distribution over the three groups, a less reliable history of alcohol abuse in the women, a better abstinence for women in the week before entering the clinic or a gender difference in CDT response to alcohol intake that has been noted before in some but not all studies (7). Some years ago, even using a less specific CDT method, it was already concluded that CDT is the best parameter for confirming alcohol abuse, concerning sensitivity and specificity (8). MCV should not be used for confirmation of alcohol abuse in driver's license affairs (4).

We conclude that in individuals with a normal nutritional status CDT is the biochemical parameter most accurately related to the intensity of drinking. ASAT and ALAT reflect liver toxicity rather than alcohol abuse. MCV is more related to vitamin and nutritional status than to excessive drinking.

References

1. Von dem Borne AE. The effect of alcohol on blood: hematological consequences of alcoholism. Ned Tijdschr Geneeskd. 1979;21:1264-9.
2. Hanuksela ML Biochemical markers of alcoholism Clin Chem Lab Med. 2007;45(8):953–61.
3. Schippers GM, Broekman TG, Buchholz A, Koeter MWJ, van den Brink W. Measurements in the Addictions for Triage and Evaluation (MATE): an instrument based on the World Health Organization family of international classifications. Addiction. 2010;105:862-71.
4. Guideline Dutch Psychiatrists "Diagnostiek van stoornissen in het gebruik van alcohol in het kader van CBR-keuringen." De Tijdstroom Utrecht 2011.
5. Koury MJ, Ponka P. New insights into erythropoiesis: The Roles of folate, vitamin B12, and iron. Annu Rev Nutr. 2004;24:105-31.
6. Chalmers DM, Levi AJ, Chanarin I, North WR, Meade TW. Mean cell volume in a working population: the effects of age, smoking, alcohol and oral contraception. Br J Haematol. 1979;43(4):631-6.
7. Bortolotti F, De Paoli G, Tagliaro F. Carbohydrate-deficient transferrin (CDT) as a marker of alcohol abuse. J Chromatogr B. 2006;841: 96-109.
8. Van Pelt J. Koolhydraat-deficiënt transferrine: een nieuwe biochemische marker voor chronisch overmatig alcoholgebruik. Ned Tijdschr Geneeskd.1997;141:773-7.

Samenvatting

Kok EE, Wielders JPM, Pasker-de Jong PCM, Defourny H, Ronde SJA, van de Wiel A. Biomarkers voor excessief alcoholgebruik in alcohol verslaafden met een normale voedingsstatus. Ned Tijdschr Klin Chem Labgeneesk 2014;39:185-188

Inleiding: Wij onderzochten de relatie tussen gebruikelijke alcoholbiomarkers en alcoholinname in afwezigheid van (micro)nutriënt deficiëntie. Met name MCV zou gevoelig kunnen zijn voor de voedingstatus.

Methoden: Bloed monsters werden afgenomen bij 70 patiënten (39 mannen, 31 vrouwen) direct na opname voor een ontwenningskuur. Als markers voor de voedingstatus werden vitamine B12, folaat en albumine gekozen, als markers voor alcohol gebruik γ-GT, ASAT, ALAT, MCV en CDT. Op basis van een gestandaardiseerd intake gesprek werden patiënten in 3 groepen ingedeeld met normale, hoge (man 4-6 eenheden/dag, vrouw 3-4 eenheden/dag) en excessieve inname.

Resultaten: Geen significante MCV stijging werd gevonden in deze drie groepen. Alleen bij excessief gebruik werd een significante toename van de lever parameters gezien. CDT correleerde met alcohol inname, vooral bij mannen. (Micro) nutriënten tekorten werden nauwelijks gezien.

Conclusie: Bij personen met een normale voedingstatus is CDT het best gecorreleerd aan de zelfgerapporteerde alcoholinname bij mannen. ASAT, ALAT en γGT zijn vooral gecorreleerd aan lever toxiciteit. MCV toename wordt waarschijnlijk meer bepaald door de vitamine status dan de alcohol inname.

Trefwoorden: alcoholmarkers, CDT, MCV, voeding, vitamine B12, folaat