



The carbohydrate-deficient transferrin as a marker of chronic alcohol consumption and assessment of its usefulness in patients with cardiovascular diseases – a pilot study

Original Study

Joanna Dominika Waś^{1*}, Magdalena Niedolisteck¹, Dorota Sokołowska¹, Joanna Wiśniewska², Tomasz Zieliński², Anna Mierzyńska³, Daniel Rabczenko⁴, Beata Orzechowska⁵, Dominika Franz⁵, Ewa Augustynowicz-Kopeć⁶, Anna Lutyńska¹

¹ National Institute of Cardiology – State Research Institute, Warsaw, Poland

² Department of Heart Failure and Transplantation, National Institute of Cardiology – State Research Institute, Warsaw, Poland

³ Department of Rehabilitation, Medical Centre of Postgraduate Education, Grucza Orthopaedic and Trauma Teaching Hospital, Otwock, Poland

⁴ Department-Center for Monitoring and Analyses of Population Health Status, National Institute of Public Health – National, Institute of Hygiene, Warsaw, Poland

⁵ Laboratory of Virology, Department of Immunology of Infectious, Hirsfeld Institute of Immunology and Experimental Therapy, Wrocław, Poland

⁶ Department of Microbiology National Reference Laboratory for Mycobacteria, Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Received 25 May, 2022, Accepted 31 March, 2023

Abstract

Introduction. Cardiovascular diseases (CVDs) are the leading cause of death in the world. The dose of 60 g of alcohol / day has been estimated to significantly increase the risk of developing high blood pressure, arrhythmias, hemorrhagic stroke, and cardiomyopathy. The aim of the study was to evaluate the diagnostic value of CDT when compared with gammaglutamyltransferase (GGT), alkaline phosphatase (ALP), N-terminal fragment brain natriuretic peptide (NT pro-BNP) and troponin T biomarkers in patients with heart failure.

Materials and Methods. The study was conducted on 59 patients with advanced heart failure with reduced ejection fraction (EF<40%). CDT serum levels were measured using CDT kit, Chromsystems Instruments & Chemicals GmbH, Germany and were expressed as a percentage of total transferrin using high-performance liquid chromatography (HPLC).

Results. Nineteen patients (n = 19) expressed a normal level of carbohydrate deficiency transferrin <1.2% of the total transferrin found in plasma. The results are obtained from 34 patients (n = 34) were within the range doubtful for the test (1.2-2.5%). In 6 Patients (n = 6) the results of more than 2.5% of CDT content were observed, which could indicate chronic alcohol consumption.

Conclusions. No statistically significant correlations between CDT and troponin T or NT pro-BNP were identified.

Keywords

carbohydrate deficient transferrin CDT% • GGT (Gamma-Glutamyl Transferase) • alcohol abuse • asialotransferrin • disialotransferrin

1. Introduction

Cardiovascular diseases (CVD), including ischemic heart disease, ischemic stroke, and peripheral arterial disease, are, next to cancer, the most common cause of death both in Poland and worldwide. WHO estimates that 16.6 million people die every year due to cardiovascular disease, and 600 million are in the so-called group of increased risk of death. In Europe, the number of deaths due to CVD is estimated at 4.3 million. Both genetic predispositions and environmental factors have an important influence on the development of cardiovascular diseases [1,2]. A risk factor for cardiovascular diseases is considered to be any measurable trait the occurrence of which in the population of healthy people is associated with a statistically significant increase in morbidity or death frequency from CVD [3–5]. The INTERHEART study, conducted in 32 countries, clearly indicated the importance of “classical” risk factors as responsible for the development of CVD, such as dyslipidemia, smoking, hypertension, diabetes, and abdominal obesity [1,6,7]. Moreover, adverse psychosocial factors, inadequate diet, excessive alcohol consumption, lack of regular physical activity, aging, and

gender may also influence general cardiovascular status. CVD biomarkers can detect pathological changes at a very early stages of the disease, and thus are suitable for use in the determination of short- and long-term risks of cardiovascular events. The intensive development of methods used in clinical biochemistry has allowed the identification of many new, promising markers which are so-called new risk factors and markers: inflammatory biomarkers (hs-CRP, fibrinogen), thrombotic biomarkers (homocysteine, Lp-associated phospholipase A2), and specific organ markers (N-terminal fragment brain natriuretic peptide, troponin T) [8–12].

The guidelines of the European Society of Cardiology (ESC) on the prevention of CVD, published in 2019, recommend especially healthy lifestyle modifications and reducing alcohol consumption to a minimum. The Sixth Joint Working Group of the European Society of Cardiology and other scientific societies on CVD prevention in clinical practice have confirmed that excessive alcohol consumption is associated with increased mortality influencing progress of CVD. Ischemic disease mortality was

* E-mail: j.was@ikard.pl



found to be higher among men (65%) who consume significant amounts of alcohol. Among these diseases, heart failure occupies a special place. Heart failure causes over 100,000 deaths annually in Europe. Heart failure is a condition in which the heart is unable to pump enough blood to cover the tissue needs due to impaired structure or function, or by increasing the filling pressure [13]. A decrease in the strength of the heart's contraction can be caused by a number of diseases that lead to heart muscle damage. One of the most common causes is ischemic heart disease, which reduces the oxygen supply to the heart muscle due to the presence of atherosclerotic plaques and thrombotic events in coronary vessels, leading to myocardial infarctions and loss of contractile functions of the left ventricle. Other causes of heart failure include hypertension, arrhythmias, myocarditis, heart valve defects, and cardiomyopathies (congenital heart defects). Cardiomyopathies are one of the more common causes of sudden cardiac death or heart failure, and in some advanced heart failure patients they are an indication for heart transplantation. Heart failure often affects young people of productive age, which significantly affects and increases the cost of treatment. Guzzo-Merello et al. [14] emphasized the importance of chronic alcohol consumption in the development of cardiomyopathy.

Numerous studies showed the adverse effects of alcohol (ethanol) consumption on the heart and circulatory system. With alcohol abuse—over (60 g alcohol/day)—an increased risk of arterial hypertension and the development of cardiac arrhythmias, hemorrhagic stroke, and cardiomyopathy are observed [11,15,16].

Paradoxically, so far scientific societies dealing with the problem of alcohol consumption have not developed uniform recommendations regarding the acceptable levels of alcohol consumption versus an increased risk of development of cardiovascular diseases [17–19].

Desialized transferrin as a marker of chronic alcoholism showed a high prognostic value and enables the identification of groups of patients who do not comply with medical recommendations, thus contributing to the optimization of treatment costs.

Alcohol markers can be a very useful screening tool among general practitioner's patients, in emergency rooms and internal medicine departments, as well as in the assessment of the impact of alcohol in the disease progression, and diagnosis or confirmation of the effectiveness of the therapy [20]. Detection of increased levels of desialized transferrin could also be valuable within the heart transplant qualification process since alcohol use disorder may also be one of the contraindications for this treatment. The goal of the study was to confirm the diagnostic value of desialized transferrin among patients with heart failure as measured by N-terminal fragment brain natriuretic peptide (NT pro-BNP), troponin T, gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) levels.

2. Materials and methods

2.1. Patients

The study was carried out on 59 patients with advanced heart failure with reduced ejection fraction (EF <40%). All patients were diagnosed in the Department of Heart Failure and Transplantology of the National Institute of Cardiology in Warsaw. Inclusion criterion for the study was the diagnosis of advanced heart failure with reduced ejection fraction (EF <40%). The exclusion criteria from the study were: (1) lack of the patient's informed consent to the examination, (2) age <18 years. All patients underwent a screening regarding alcohol consumption which was performed by psychologists of the National Institute of Cardiology. This evaluation was supplemented by validated alcohol screening tools—the Alcohol Use Disorders Identification Test (AUDIT). The control group consisted of 20 healthy people declared as non-drinkers during last 6 months in the AUDIT study. Patients from the study group were tested for markers of myocardial damage: NT pro-BNP [with reference values: 0–125 pg/ml] and troponin T [with reference values: 0–14 ng/L]. Additionally, biochemical tests on the levels of GGT and ALP were performed [with reference values of <55 U/L and 40–129 U/L, respectively]. The results of the study were correlated with the levels of desialized transferrin in a group of patients with diagnosed heart failure.

2.2. CDT analysis

A certified analytical kit dedicated for the specific determination of CDT in serum including the percentage of transferrin isoform such as asialo-, disialo-, trisialo-, tetrasialo-, and pentasialo-transferrin – CDT in serum Reagent Kit® (Chromsystems Instruments & Chemicals GmbH, Germany) was used. The reference values of CDT percentage were applied according to manufacturer recommendations: CDT <1.2% of total transferrin is normal, results between 1.2% and 2.5% should be repeated within 14–17 days from the last test, and CDT >2.5% of total transferrin is pathological.

Determination of transferrin isoform: asialo-, disialo-, trisialo-, tetrasialo-, pentasialo-transferrin was tested for with the use of the HPLC system, Spectra System (Thermo Scientific, USA), consisting of autosampler, a binary gradient pump, and PDA detector was used. CDT results were expressed as the area percent of total transferrin (sum of all peaks). Sample preparation was performed according to the manufacture instruction. A 200 µl of serum was transferred to a 1.5 ml standard Eppendorf tube, then mixed with 100 µl of reagent mix, and the sample was vortexed for 60 seconds and placed in the fridge (at 2–8°C) for 30 min. After centrifugation for 10 min at 9000 g, 200 µl supernatant was diluted with 400 µl ultrapure water (HPLC grade) and transferred to an autosampler vial. Two hundred microliters of the supernatant were injected into the HPLC system column. Universal laboratory centrifuge (5417C, Eppendorf, Hamburg,

Germany) was used for sample preparation. Data for analysis was obtained via ChromQuest software.

2.3. Statistical methods

Spearman rank correlation coefficients were used to assess correlation between CDT and other parameters. ROC curve analysis was performed to assess ability of NT pro-BNP, troponin T, ALP, and GGT parameters to detect patients, for which CDT is over 2.5 and over 1.2. The area under the ROC curve with 95% confidence interval was computed to describe the ability of analyzed parameters to detect patients with CDT over threshold values. Cut-off points maximizing sum of sensitivity and specificity were marked on ROC curve plots.

Computations were done using R 4.2.1 software (R Core Team [2022]) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (URL: <https://www.R-project.org/>), package pROC [21,22] was used to perform ROC analysis.

3. Results

Asialo-, disialo-, trisialo-, tetrasialo-, pentasialo-transferrin were eluted at retention times of 4.08, 7.10, 8.00, 10.12, and 11.20, respectively, and peaks obtained fulfilled validity criteria according to shape and separation. The specificity of the method was confirmed using serum samples randomly taken from 20 healthy patients from the control group. No interfering peaks were observed in chromatograms from patient samples and quality controls. The representative chromatograms are shown in Figure 1.

Measurements of CDT, NT pro-BNP, troponin T, ALP, and GGT were available for 59 patients.

Descriptive statistics for CDT and other analyzed parameters are presented in Table 1. Mean CDT in overall group was 1.5 (± 0.9). Six (10.2%) patients had CDT over 2.5 and 40 (6.7%) over 1.2.

Analysis of correlation between CDT and other parameters revealed significant positive, yet not very strong association between CDT and ALP and GGT (both coefficients 0.327, $p < 0.05$; Figure 2).

In the next step, ROC curve analysis was performed to assess ability of NT pro-BNP, troponin T, ALP, and GGT parameters to detect patients, for which CDT is over 2.5 and over 1.2 (Figures 3 and 4).

The area under the ROC curves along with its 95% confidence interval for each curve is presented in Table 2.

For the cut-off point 2.5, the only variable that can be considered to have predictive power was GGT; for cut-off point 1.2, the variable that can be considered to have predictive power was NT pro-BNP (as lower limit of 95% CI is over value 0.500). For GGT and CDT, the cut-off of 2.5 value of 286 was selected as the best threshold resulting in sensitivity 88.8% and specificity 83.3%. For cut-off 1.2, the best threshold for NT pro-BNP was 2662.5 resulting in sensitivity 84.2% and specificity 60.0%.

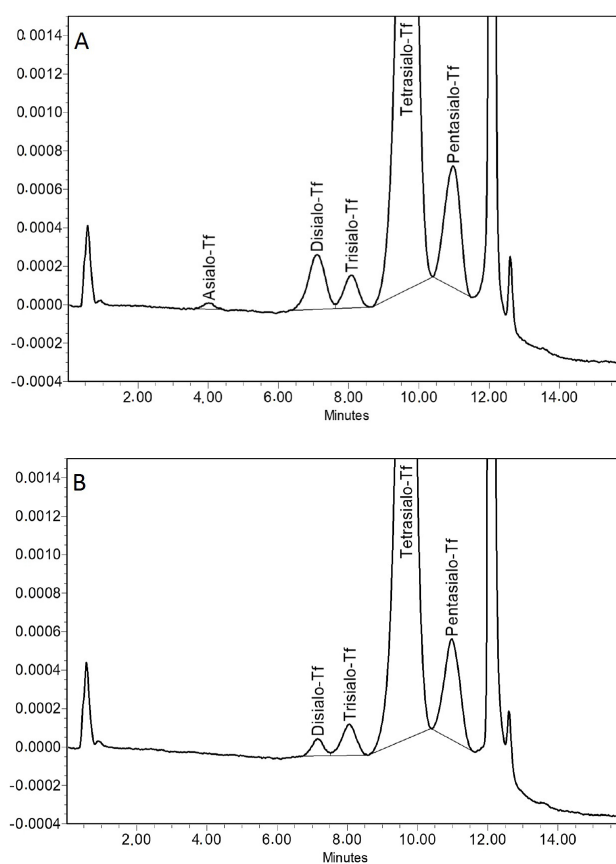


Figure 1. An representative chromatogram shows the characteristic higher peaks occurring in the case of alcohol consumption - isoform asialotransferrin and disialotransferrin - positive result CDT) - A & sample chromatogram where no asialotransferrin is present and disialotransferrin is much lower - negative CDT - B (Chromsystems, Germany)

Table 1. Descriptive statistics for analyzed parameters

	CDT	NT pro-BNP	Troponin T	ALP	GGT
N	59	59	59	59	59
Mean (SD)	1.5 (0.9)	5 123.1 (5 911.5)	159.4 (576.1)	115.6 (89.5)	172.3 (175.0)
Median	1.3	2,983.0	28.1	92.0	102.0
Range	0.4 - 5.1	180.3 - 33 576.0	4.2 - 3,694.0	25.0 - 636.0	16.0 - 782.0

Table 2. Area under the ROC curves for different parameters and CDT cut-offs

Parameter	AUC	95% C.I.
CDT cut-off 2.5		
NT pro-BNP	0.654	(0.475;0.833)
Troponin T	0.635	(0.406;0.865)
ALP	0.733	(0.444;1.000)
GGT	0.855	(0.685;1.000)
CDT cut off 1.2		
NT pro-BNP	0.716	(0.569;0.862)
Troponin T	0.445	(0.281;0.609)
ALP	0.564	(0.416;0.713)
GGT	0.565	(0.413;0.718)

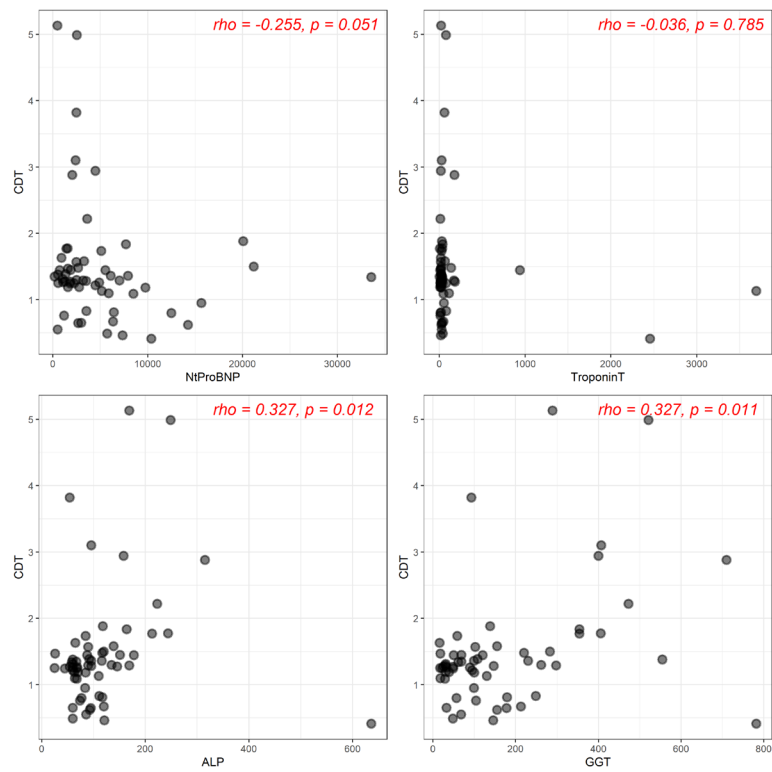


Figure 2. Analysis of correlation between CDT and NT pro-BNP, troponin T, ALP and GGT

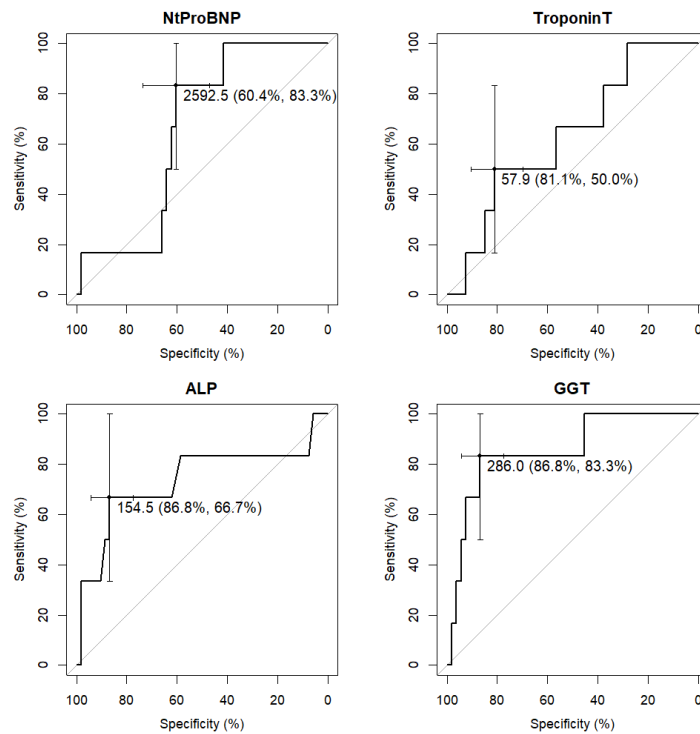


Figure 3. ROC curve analysis of CDT, NT pro-BNP, Troponin T, ALP and GGT parameters to detect patients, for which CDT is over 2.5

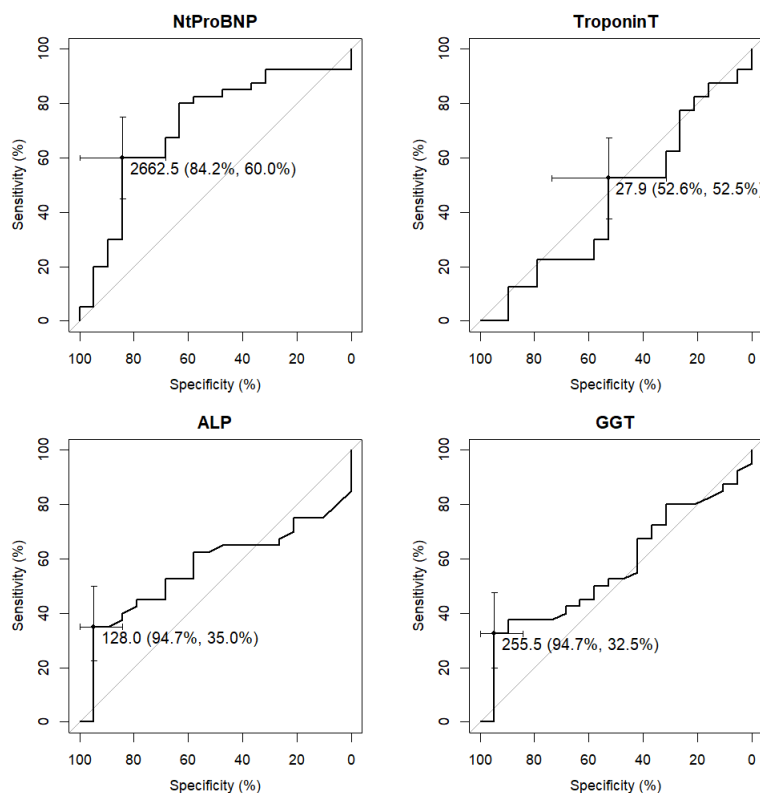


Figure 4. ROC curve analysis of CDT, NT pro-BNP, Troponin T, ALP and GGT parameters to detect patients, for which CDT is over 1.2

4. Discussion

Desialylated transferrin was found to increase significantly after a minimum weekly consumption of at least 50–80 g of alcohol per day. During the period of abstinence, values become normalized within 14–17 days, which is the mean half-life of CDT. Interestingly, consumption of even small amounts of ethanol during the period of abstinence leads to a re-increase of CDT levels in sera. The concentration of desialylated transferrin returns to physiological values after a few weeks of discontinuing the consumption of ethyl alcohol [23]. The application of specific analytical tool capable to monitor of alcohol consumption is highly expected as alcohol consumption is constantly increasing and covers as much as 80% of the population in Poland. We can, however, suspect that patients' consumption of alcohol might be lower than observed in the general population, as patients with different disorders are generally more caring about maintaining a healthy lifestyle.

It is assumed that the lack of a clear position or recommendation from medical authorities on the allowed alcohol uptake issue may be related to the not fully explained but frequently followed belief in the so-called "beneficial" effects of small amounts of alcohol, which unfortunately can lead to addiction. Nevertheless, chronic alcohol consumption

is a problem commonly recognized worldwide, not only in Poland, resulting in many cases in irreversible health damages, especially in patients under medical therapy. During continuous alcohol consumption, a substance belonging to the iron-transport group of glycoproteins named transferrins with a reduced amount of sialic acid, called a marker of chronic alcoholism, appears in the body within 7 days, thus can be potentially detected in each suspected patient. Chronic alcohol abuse causes disturbance of the transferrin biosynthesis or tissue damage resulting the presence of asialo-transferrins among more than about 3% disialo-transferrins. Due this processes desialylated transferrin has a reduced amount of sialic acid (asialo-, monosialo-, and disialo-transferrin) [24]. So, excessive consumption of alcohol contributes to an increase in the amount of low-carbohydrate isoforms of so-called desialylated transferrins, which under the physiological conditions of the body occur at a very low level. Thus, ethyl alcohol and its metabolites affect the functioning of transferrin-modifying enzymes, limit the attachment of sialic acid to transferrin molecules and lower formation of desialized forms, and disrupt the functions of hepatocyte receptors responsible for the elimination of desialylated transferrin [25].

We found a high incidence of the CDT above 2.5%, especially in a patient with high GGT. Higher than 1.3% percentage of desialylated transferrin detected in the study group is considered as the result of the so-called “gray area”, which means that these heart failure patients should be carefully monitored for possible alcohol consumption despite negative screening psychological tests. However, the scale of the health risk is greater in patients when the results coexist with other factors (e.g., GGT, ALP biomarkers).

ALP and traditional marker of chronic alcohol abuse γ – GGT are located in the bile and are related with some type of damage of biliary canaliculi. ALP has been reported as a rather novel risk marker (inflammatory mediator) for cardiovascular disease, and GGT is regarded as a sensitive indicator of hepatocyte damage. GGT or ALT increased serum activity is mainly observed as a result of the intensified biotransformation of xenobiotics, mainly ethyl alcohol [23,25,26].

Salaspuro [26], in a study comparing the value of CDT to other laboratory markers (e.g., GGT), found a high relevance of CDT. Their ROC analysis confirmed that CDT was the best biological marker, although its CDT-ROC curve did not differ statistically significantly from that of the conventional laboratory markers.

In our pilot study, 59 patients and 20 healthy volunteers were asked about the level of daily alcohol consumption, however without any statement success. Nevertheless, mean CDT in overall group was 1.5 (± 0.9). Six (10.2%) patients had CDT over 2.5, and 40 (6.7%) over 1.2. The results obtained were taken into account by clinicians regarding perspective of further general treatment after conversation with the patients asking for lowering alcohol consumption.

In our study a statistical positive correlation was found between ALP, GGT (a marker for excessive alcohol consumption) with transferrin and possible correlation of NT pro-BNP with transferrin as shown on scatter plot (Figure 2). No statistical correlation between transferrin and troponin T was found. Determination of GGT and ALP concentrations increases the diagnostic sensitivity of liver damage and thus disorders of hepatocyte integrity. Alcohol damage to the liver is associated with the toxic effects of acetaldehyde and the development of reduction stress caused by an excess of reduced form of nicotinamide dinucleotide phosphate (NADPH). The consequence is disturbances in the oxidation processes in the liver cells leading to changes in the metabolism of carbohydrates, lipids, uric acid, and porphyrins. Identification of alcohol consumers based on family and community history, as well as the use of questionnaires might be optimized when specific diagnosis of alcohol abuse is taking place consequently. Desialylated transferrin as a marker of chronic alcohol consumption of high prognostic value enables the identification of a group of non-compliant patients, thus contributing to the effectiveness and optimization of treatment.

The results of ROC analyses for NT pro-BNP and troponin T were shown no correlation between marker of alcohol abuse and specific markers of heart failure tested during the study. NT pro-BNP as a one of the predictors of higher mortality in the course of heart failure with troponin T—a protein involved in the regulation of myocardial contraction are those almost routinely determined during the diagnosis of myocardial infarction, acute coronary syndromes, and prognosis of patients with other causes of heart damage [27–29]. Patients hospitalized due to cardiovascular diseases, including heart failure, often do not report or underestimate their excessive alcohol consumption in clinical interviews. As a result of patient untruthfulness regarding alcohol abuse, disorders described above, especially when NT pro-BNP and troponin T are increased, might easily progress and result in the patient's non-compliance with medical recommendations, such as development of irreversible consequences such as heart failure and/or qualification for heart transplantation or mechanical heart support.

Unlike other alcohol markers (such as GGT or mean red blood cell volume), desialylated transferrin levels do not increase in the presence of other diseases such as nonalcoholic liver disease. The CDT methodology has so far been validated by a reference laboratory and has shown satisfactory linearity and precision [30], and low false-positive rates are observed [31,32]. In our study, we have confirmed that CDT evaluation can be very informative for clinicians, as some patients are rather afraid to make a statement about some level of alcohol abuse.

The original methodology developed in the course of the pilot study has become a reliable analytical and applicable tool for the diagnosis and monitoring of patients with alcohol problems treated at the National Institute of Cardiology. An effective lifestyle modification via lowering or excluding alcohol consumption in patients with CVD could improve the quality of life, treatment effectiveness, and reduce possible adverse complications. The determination of the content of desialylated transferrin in blood serum may provide valuable support for the overall diagnosis and accurate monitoring of excessive alcohol consumption which might lead to chronic damage to the heart muscle. So far, CDT as marker of excessive level of alcohol consumption or complete abstinence is widely accepted for CVD patients. There are still many difficulties regarding the range of objective evaluation of the acceptable consumption of alcohol in individuals with increased cardiovascular risk and patients with cardiovascular diseases. The application of an accessible method to verify patients' declarations of alcohol consumption may contribute to the development of more precise recommendations on alcohol consumption or abstinence in this group [4].

5. Conclusions

The use of a desialylated transferrin as a marker of alcohol consumption might improve the effectiveness of treatment and

also identify those who need special care regarding abuse. More large-scale studies are needed to further confirm the diagnostic utility of carbohydrate-deficient transferrin.

Abbreviations

ALP - alkaline phosphatase, **AUDIT** - Alcohol Use Disorders Identification Test, **CDT** - carbohydrate-deficient transferrin, **CVD** - Cardiovascular diseases, **ESC** - European Society of Cardiology, **GGT** - gamma-glutamyltransferase, **HPLC** - high performance liquid chromatography, **NADPH** - nicotinamide dinucleotide phosphate, **NT pro-BNP** - N-terminal fragment brain natriuretic peptide, **ROC** - Receiver Operating Characteristic, **Tf** - Transferrins,

Authors' contribution

J.W.1: Research concept and design, supervising the project, carrying out the experiments, acquisition of data, data analysis and interpretation, writing – original draft preparation, literature review, final proofreading and approval of the version for publication; M.N.: acquisition of data, data analysis and interpretation, writing – original draft preparation, literature review, final proofreading and approval of the version for publication; D.S.: acquisition of data, data analysis and interpretation, writing – original draft preparation, literature review, final proofreading and approval of the version for publication; J.W.2: acquisition of data, data analysis and interpretation, visualization; T.Z.: data analysis and interpretation, writing -review and editing; A.M.: acquisition of data, data analysis and interpretation, writing -review and editing; D.R.: acquisition of data, data analysis and interpretation, visualization, writing -review and editing; B.O.: data analysis and interpretation, writing

– original draft preparation, writing -review and editing, final proofreading and approval of the version for publication; D.F.: acquisition of data, data analysis and interpretation, visualization; E.A-K.: writing – original draft preparation, writing -review and editing A.L.: Research concept and design, supervising the project, writing – original draft preparation, writing -review and editing, final proofreading and approval of the version for publication;

ORCID

Joanna Dominika Waś <https://orcid.org/0000-0002-4904-6398>
Beata Orzechowska <https://orcid.org/0000-0002-5125-8947>
Tomasz Zieliński <https://orcid.org/0000-0001-9277-0552>
Ewa Augustynowicz-Kopeć <https://orcid.org/0000-0001-6162-8748>
Anna Lutyńska- <https://orcid.org/0000-0002-3905-5872>

Funding

The study was supported by statutory projects 3.9/I/17 from the National Institute of Cardiology – State Research Institute in Warsaw (Poland).

Conflict of interests

There is no conflict of interest.

Ethics approval

Ethics Committee approval has been granted.

The study was approved by Field Bioethics Commission at the National Institute of Cardiology- State Research Institute (KB-1633).

References

- [1] O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H, Rao-Melacini P, Zhang X, Pais P, Agapay S, et al. INTERSTROKE investigators. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. *Lancet*. 2016; 388: 761–775.
- [2] World Health Organization. 2018. Global status report on alcohol and health 2018.
- [3] Brotman DJ, Golden SH, Wittstein IS. The cardiovascular toll of stress. *Lancet*. 2007; 370: 1089–1100.
- [4] Mykletun A, Bjerkeset O, Dewey M, Prince M, Overland S, Stewart R. Anxiety, depression, and cause-specific mortality: the HUNT study. *Psychosom Med*. 2007; 69: 323–331.
- [5] Rosengren A, Tibblin G, Wilhelmsen L. Self-perceived psychological stress and incidence of coronary artery disease in middle-aged men. *J Am Cardiol*. 1991; 68: 1171–1175.
- [6] Leong DP, Smyth A, Teo KK, McKee M, Rangarajan S, Pais P, Liu L, Anand SS, Yusuf S. INTERHEART Investigators. Patterns of alcohol consumption and myocardial infarction risk: observations from 52 countries in the INTERHEART case-control study. *Circulation*. 2014; 130: 390–398.
- [7] Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S, et al. Emerging Risk Factors Collaboration/EPIC-CVD/UK Biobank Alcohol Study Group. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet*. 2018; 391: 1513–1523.
- [8] Britton A, McKee M. The relation between alcohol and cardiovascular disease in Eastern Europe: explaining the paradox. *J Epidemiol Community Health*. 2000; 54: 328–332.
- [9] Corrao G, Rubbiati L, Bagnardi V, Zamboni A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. *Addiction*. 2000; 95: 1505–1523.
- [10] Einvik G, Ekeberg Ø, Klemsdal TO, Sandvik L, Hjerkin EM. Physical distress is associated with cardiovascular events in a high risk population of elderly men. *BMC Cardiovasc Disorders*. 2009; 9: 14.

- [11] Miller PM, Anton RF. Biochemical alcohol screening in primary health care. *Addict Behav.* 2004; 29: 1427–1437.
- [12] Roest AM, Martens EJ, de Jonge P, Denollet J. Anxiety and risk of incident coronary heart disease: a meta-analysis. *J Am Coll Cardiol.* 2010; 56: 38–46.
- [13] ESC/EASD Guidelines on Diabetes, Pre-Diabetes and Cardiovascular Diseases, 2019. Available at: <https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines/Diabetes-Pre-Diabetes-and-Cardiovascular-Diseases-developed-with-the-EASD>
- [14] Guzzo-Merello G, Cobo-Marcos M, Gallego-Delgado M, Garcia-Pavia P. Alcoholic cardiomyopathy. *World J Cardiol.* 2014; 6: 771–781.
- [15] Anton RF. Carbohydrate-deficient transferrin for detection and monitoring of sustained heavy drinking. What have we learned? Where do we go from here? *Alcohol.* 2001; 25: 185–188.
- [16] Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kühn U, Maisch B, McKenna WJ, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J.* 2008; 29: 270–276.
- [17] Knott CS, Bell S, Britton A. The stability of baseline-defined categories of alcohol consumption during the adult life-course: a 28-year prospective cohort study. *Addiction.* 2018; 113: 34–43.
- [18] Rehm J, Roerecke M. Cardiovascular effects of alcohol consumption. *Trends in Cardiovasc Med.* 2017; 27: 534–538.
- [19] Roerecke M, Rehm J. Alcohol consumption, drinking patterns, and ischemic heart disease: a narrative review of meta-analyses and a systematic review and meta-analysis of the impact of heavy drinking occasions on risk for moderate drinkers. *BMC Med.* 2014; 12: 182.
- [20] Bergström JP, Helander A. Influence of alcohol use, ethnicity, age, gender, BMI and smoking on the serum transferrin glycoform pattern: Implications for use of carbohydrate-deficient transferrin (CDT) as alcohol biomarker. *Clin Chim Acta.* 2008; 388: 59–67.
- [21] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-Ch, Müller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011; 12: 77.
- [22] <http://www.biomedcentral.com/1471-2105/12/77/>
- [23] Niemelä O. Biomarkers in alcoholism. *Clin Chim Acta.* 2007; 377: 39–49.
- [24] Golka K, Sondermann R, Reich SE, Wiese A. Carbohydrate-deficient transferrin (CDT) as a biomarker in persons suspected of alcohol abuse. *Toxicol Lett.* 2004; 151: 235–241.
- [25] Rinck D, Frieling H, Freitag A, Hillemacher T, Bayerlein K, Kornhuber J, Bleich S. Combinations of carbohydrate-deficient transferrin, mean corpuscular erythrocyte volume, gamma-glutamyltransferase, homocysteine and folate increase the significance of biological markers in alcohol dependent patients. *Drug Alcohol Depend.* 2007; 89: 60–65.
- [26] Salaspuro M. Carbohydrate-deficient transferrin as compared to other markers of alcoholism: a systematic review. *Alcohol.* 1999; 19: 261–271.
- [27] Chrostek L, Cylwik B, Szmitkowski M, Korcz W. The diagnostic accuracy of carbohydrate-deficient transferrin, sialic acid and commonly used markers of alcohol abuse during abstinence. *Clin Chim Acta.* 2006; 364: 167–171.
- [28] Klatsky AL. Alcohol and cardiovascular mortality: common sense and scientific truth. *J Am Coll Cardiol.* 2010; 55: 1336–1338.
- [29] Xin X., He J., Frontini M.G., Ogden L.G., Motsamai O.I., Whelton P.K.: Effects of alcohol reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension*, 2001; 38: 1112–1117
- [30] Weykamp C, Wielders JPM, Helander A, Anton RF, Bianchi V, Jeppsson JO, Siebelder C, Whitfield JB, Schellenberg F. IFCC Working Group on Standardization of Carbohydrate-Deficient Transferrin (WG-CDT). Toward standardization of carbohydrate-deficient transferrin (CDT) measurements: III. Performance of native serum and serum spiked with disialotransferrin proves that harmonization of CDT assays is possible. *Clin Chem Lab Med.* 2013; 51: 991–996.
- [31] Bortolotti F, Sorio D, Bertaso A, Tagliaro F. Analytical and diagnostic aspects of carbohydrate deficient transferrin (CDT): A critical review over years 2007-2017. *J Pharm Biomed Anal.* 2018; 147: 2–12.
- [32] Helander A, Husa A, Jeppsson JO. Improved HPLC method for carbohydrate-deficient transferrin in serum. *Clin Chem.* 2003; 49: 1881–1890.