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Tumour Necrosis Factor- α and Its Relationships to the Biochemical Factors Potentially Involved in Atherosclerotic Processes in Alcohol Dependent Male Patients after Alcohol Withdrawal*

Stężenie czynnika martwicy guza typu α oraz jego związek z poziomem czynników biochemicznych, potencjalnie związanych z rozwojem miażdżycy w okresie abstynencji u mężczyzn uzależnionych od alkoholu

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Abstract

Objectives. Cytokines play an important role in pathogenesis of atherosclerosis. The aim of this study was to estimate the level of TNF- α and its relationships to the levels of some other factors potentially involved in atherosclerosis progression in alcoholics during early abstinence period.

Material and Methods. We studied 47 alcohol dependent male patients who stopped alcohol misuse period not later than two weeks before the study beginning. In all at the study start and after 4 weeks abstinence we determined the levels of: tumour necrosis factor alpha (TNF- α), plasma lipids, platelets count and their mean volume (MPV) and total antioxidative status (TAS). These examination we repeated in 27 subjects who turned-up at 6-month visit, among their only 17 remained abstinent.

Results. In studied alcoholics more or less 50% had determinable (> 0.05 pg/ml) TNF-alpha level at all three visits. This cytokine concentration decreased with time of abstinence duration. Alcoholics with determinable TNF- α level ($n = 26$) at the beginning of the study, in comparison to "TNF-negative" alcoholics had greater plasma lipids concentration and greater platelets volume at the study start, and after 4 weeks and 6 months abstinence they had greater collagen induced platelets aggregation and lower total antioxidative plasma state.

Conclusions. 1. In studied alcoholics TNF- α plasma concentration was the highest after alcohol withdrawal and decreased within abstinence period. 2. In TNF-positive alcoholics the unfavourable changes in cardiovascular event risk factors during following weeks of abstinence were more expressed, what suggests usefulness of TNF- α determination as a marker of unfavourable changes in atherosclerosis risk factors during anti-relapse therapy (Adv Clin Exp Med 2005, 14, 3, 511–521).

Key words: tumour necrosis factor alpha, atherosclerosis – risk factor, alcohol dependence, abstinence, lipids.

Streszczenie

Cel pracy. Cytokiny odgrywają istotną rolę w patogenezie miażdżycy zarówno bezpośrednio (aktywacja zapalenia), jak i pośrednio przez wpływ na tzw. „klasyczne” czynniki ryzyka. Celem badania była ocena zmian stężenia czynnika martwicy guza (TNF- α) oraz ich związek z poziomem lipidowych, hemostatycznych czynników ryzyka miażdżycy oraz parametrów równowagi antyoksydacyjnej u mężczyzn z zespołem zależności alkoholowej podczas 6-miesięcznej abstynencji.

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Materiał i metody. Zbadano 47 mężczyzn z zespołem zależności alkoholowej (z.z.a.), którzy zakończyli okres nadużywania alkoholu nie wcześniej niż 14 dni przed włączeniem do badania. U każdego na początku badania i po 4 tygodniach abstynencji oznaczono stężenie TNF- α , podstawowych frakcji lipidów osocza, liczbę płytek krwi i ich średnią objętość (MPV) oraz całkowity stan antyoksydacyjny osocza (TAS). Badania te powtórzono u 27 pacjentów, którzy zgłosili się na wizytę kontrolną po 6 miesiącach obserwacji, wśród nich tylko 17 utrzymało w tym czasie abstynencję.

Wyniki. Spośród badanych pacjentów z z.z.a. około połowa miała „oznaczalne” ($> 0,05$ pg/ml) stężenie TNF- α na każdej z wizyt. Średnie stężenie cytokiny zmniejszało się istotnie z czasem trwania abstynencji. Mężczyźni z oznaczalnym stężeniem TNF- α ($n = 26$) na początku badania, w porównaniu z pacjentami „TNF-ujemnymi”, mieli większe stężenie lipidów osocza i większe MPV po włączeniu do badania, a po 4 tygodniach i 6 miesiącach abstynencji stwierdzono bardziej nasiloną agregację płytek krwi pod wpływem kolagenu i mniejszą wartość TAS.

Wnioski. 1. U mężczyzn z z.z.a. średnie stężenie TNF- α było największe w ciągu 14 dni po odstawieniu alkoholu i zmniejszało się z czasem trwania abstynencji. 2. Pacjenci z „oznaczalnym” stężeniem TNF- α na początku abstynencji mieli bardziej nasilone niekorzystne zmiany w zakresie czynników ryzyka miażdżycy w kolejnych tygodniach abstynencji alkoholowej. Sugeruje to oznaczanie TNF- α jako markera proaterogennych zmian podczas terapii przeciw nawrotowi picia (*Adv Clin Exp Med* 2005, 14, 3, 511–521).

Słowa kluczowe: czynnik martwicy guza, miażdżycy – czynniki ryzyka, alkohol, abstynencja alkoholowa, lipidy.

Heavy drinkers and teetotallers have the greater cardiovascular diseases prevalence than regular, moderate (1–2 standard drinks per day) alcohol drinkers, arise from J-shaped relationships between coronary artery disease (CAD) epidemiology and quantity of alcohol drinking [1]. It is known that regular drinking more than 3 standard drinks per day or binge drinking [2] may favour atherogenesis via hypertensive effect, secondary hypertriglyceridemia, increase of LDL cholesterol concentration and its oxidation or acetylation (such lipids modification enhances their uptake by macrophages, leading to “foam cells” generation) [1, 3]. On the other hand, cardiovascular events risk may increase also after alcohol withdrawal, what is suggested by left arm of mentioned J-shaped curve and by lack of favourable effect of moderate alcohol drinking, producing HDL cholesterol concentration increase, anti-platelets effect, decrease of PAI-1 activity, improvement of endothelial function [4, 5], anti-inflammatory effect [6] and antioxidative effect of non-ethanolic components (flavonoids) of alcoholic beverages [1]. Above mentioned mechanisms of potential harmful effect of alcohol abuse, binge drinking and alcohol withdrawal may be important factors responsible for high CAD prevalence in people alcohol misused. In Poland this problem concerns at least several millions of people, alcohol dependent or harmfully drinking. It is similarly in the Europe and in the world.

The recent studies results confirmed the unchallenged role of traditional atherosclerosis risk factors as total cholesterol, hypertension, diabetes and smoking [7]. However, raised level of acute phase proteins found in patients with acute cardiovascular events [8] and current theory of atherosclerosis pathogenesis gave the base to recognize some new important pathogenic factors among markers of systemic inflammation, such as C-reactive protein determined using high sensitive me-

thod (hsCRP) [9, 10] or cytokines [11]. It is known, that heavy drinking may favour increase of cytokines level, especially tumour necrosis factor alpha (TNF- α) [12], because of intestinal barrier injury and its increased permeability for bacterial endotoxins [13]. TNF- α is a proinflammatory cytokine produced by monocytes, macrophages and T cells. It can affect proliferation, differentiation and function of virtually every cell type. The biological effects of TNF- α include stimulation of the acute phase response, cytotoxicity, cachexia, shock, interleukins 1 and 6 production, expression of adhesion molecules, procoagulant activity and fibroblast proliferation [14–16]. Potentially, these mechanisms of TNF- α acting may accelerate inflammatory processes in vascular wall and stimulate atherosclerosis progression in alcoholics. However, in this context alcohol drinking cessation should have an anti-atherogenic effect, what is not comparable with the J-shaped relationships between quantity of alcohol drinking and coronary artery prevalence [1, 2, 4]. Such obscurities gave the premise to undertake this study. Its aim was to estimate the level of TNF- α and its relationships to the levels of some other factors potentially involved in atherosclerosis progression in alcoholics during early abstinence period. In our knowledge this is the first work, in which inflammatory mechanisms of proatherogenic effect of alcohol withdrawal and not alcohol drinking in heavy drinkers was investigated.

Material and Methods

The investigation was done in 47 alcohol dependent male patients, diagnosed according to ICD-10 (International Classification of Diseases Tenth Revision) criteria, hospitalized and treated with training to cope with alcohol craving in Addiction Treatment Unit, Department of Psychiatry, The Ludwik Rydygier Medical University in Byd-

goszcz (Poland) in 1999 and 2000. The inclusion criteria to the patients group were: male sex, age between 30–50 years, ICD-10 criteria of alcohol dependence performance, motivation of abstinence keeping and end of misuse period not longer than 14 days before the study start. The exclusion criteria were: acute and chronic inflammatory processes symptoms (may affect cytokine and free radicals production), the presence of the other diseases, which could have an influence on the lipids metabolism (for example liver failure, nephrotic syndrome) or blood coagulation, psychotic or demential disorders, addiction to other substances than alcohol (except smoking), and any drugs taking.

For first eight weeks patients have been hospitalized in Addiction Treatment Unit. Next they were studied as outpatients. For the observation period studied persons had not taken any drugs. The diet for all of them was hypolipemic, according to European Atherosclerosis Society (EAS) recommendations [17]. Energy consumption was in average 1800 kcal per day, but in patients with body mass index (BMI) above 25 kg/m² the reduced diet (to 20 kcal/kg body mass) was recommended. A daily calories consumed consisted in a third of cereal products, in a quarter of vegetables, in a fifth of fruits, in 15% of milk products and in last part of meat, fish or leguminous plants. In this way daily cholesterol consumption was lower than 300 mg and daily fat-energy consumption was lower than 30% (saturated fatty acids below 10%, monounsaturated fatty acids 10–15% and polyunsaturated fatty acids 7–10% of energy). In patients with BMI above 25 kg/m² and in patients with hypertriglyceridemia (TGL > 150 mg/dl) no sugar consumption was recommended. Standard meal was given in the morning, after blood sampling. It consisted of two 50 g pieces of rye bread, butter (0.5 g per kilogram of body mass) and one cup of black coffee.

In studied subjects blood samples for biochemical determinations were taken after 14 hours of fasting, at the study start and after 4 weeks of abstinence period. TNF- α concentration was assayed in Immunological Laboratory by ELISA method, using the set manufactured by ENDOGEN. Sensitivity of this method allows to determine TNF- α level above 0.05 pg/ml. According to this value at the study start (on the basis of the first estimation) we divided studied subjects in groups with determinable (TNF-positive) and undeterminable (TNF-negative) plasma level of this cytokine. Demographic and clinical data of studied alcoholics in relation to TNF- α plasma presence are showed in Table 1.

Moreover, using routine laboratory methods, we determined concentration of fasting total cho-

lesterol (TC), HDL cholesterol (HDL) and triglycerides (TGL). LDL cholesterol (LDL) concentration was calculated using Friedewald pattern, only for patients with triglycerides level below 400 mg/dl. We determined also platelets count, mean platelets volume (MPV), and collagen induced platelets aggregation (using resistance method and aggregometer 530-VS manufactured by Chrono-log), prothrombin time expressed as INR, and fibrinogen (FBG) concentration. Moreover we estimated the levels of following antioxidative balance blood parameters: total antioxidant plasma status (TAS), using colometric method and reagents manufactured by RANDOX, as well as aldehyde products of lipids peroxidation (mainly malonyldialdehyde and 4-hydroxyenal; MDA + 4HNE), using colometric method and “Bioxytech LPO-586TM” set manufactured by Oxis International.

In the end we performed glucose tolerance test, and determined peripheral blood morphology, creatinine and thyreotropin (TSH) concentration, as well as biochemical markers of alcohol abuse level (activity of: γ -glutamyltransferase (GTP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), mean corpuscular volume (MCV)). Because the aminotransferases activities decreases within abstinence period, which was not longer than 6 months, we did not make a liver biopsy [18, 19].

Abstinence keeping was controlled during eight weeks long hospitalisation on the basis of physical examination as well as alcohol presence in exhaled air and the above mentioned biochemical markers of alcohol abuse. After discharge from the Addiction Treatment Unit alcohol drinking was diagnosed on the basis of medical history, the level of biochemical markers of alcohol abuse (determined during control visits), objective familial interview, and medical documentation analysis (from outpatients clinic).

All subjects gave their informed consent to participate in this study, which was approved by the Local Ethics Committee of The Ludwik Rydygier Medical University in Bydgoszcz. The investigation was in compliance with the Declaration of Helsinki for medical research.

The results were presented as the median \pm 95% CI (confidence interval), as mean \pm standard deviation or as percentage (%) of quantity. Normal distribution of variables using Kolmogorow-Smirnow test was assessed. Statistical significance of differences was determined using, respectively, Mann-Whitney test, unpaired Student's *t*-test, χ^2 test, Fisher exact test and two-factorial ANOVA with two- and three repetitions and Tukey post hoc test in statistical software *STATISTICA PL 5.0* for Windows.

Table 1. Demographic and clinical features of studied alcohol dependent patients divided in relation to presence of determinable TNF- α level at the study start**Tabela 1.** Demograficzne i kliniczne dane pacjentów z zespołem zależności alkoholowej w zależności od oznaczalnego lub nieoznaczalnego stężenia TNF- α na początku badania

Feature (Wskaźnik)	TNF(+) (n = 26)	TNF(-) (n = 21)	p
Age – years (Wiek – lata)	41.8 \pm 8.0	41.1 \pm 8.0	0.99
Age of alcohol dependence onset – years (Wiek początku uzależnienia – lata)	23.5 \pm 6.4	21.1 \pm 7.7	0.23
Length of alcohol dependence – years (Czas trwania zespołu zależności alkoholowej – lata)	17.6 \pm 7.7	17.5 \pm 8.0	0.97
Number of drinking days during 90 days before the study start (Liczba dni picia alkoholu podczas 90 dni przed rozpoczęciem badania)	57.2 \pm 24.6	48.3 \pm 23.9	0.22
Number of standard drinks drunk during 90 days before the study start (Liczba standardowych porcji alkoholu wypitych w ciągu 90 dni przed rozpoczęciem badania)	1092 \pm 707	855 \pm 595	0.23
Number of standard drinks drunk during 30 days before the study start (Liczba standardowych porcji alkoholu wypitych w ciągu 30 dni przed rozpoczęciem badania)	287.5 \pm 169	252.8 \pm 177.6	0.5
Mean daily nicotine dose – mg/day (Średnia dzienna dawka nikotyny – mg/dobę)	25.8 \pm 11.8	27.2 \pm 18.0	0.80
Mean daily tar dose – mg/day (Średnia dzienna dawka substancji smolistych – mg/dobę)	24.9 \pm 7.7	27.6 \pm 8.4	0.9
Systolic blood pressure – mm Hg (Skurczowe ciśnienie tętnicze krwi – mm Hg)	113.9 \pm 15.4	115.0 \pm 13.4	0.8
Diastolic blood pressure – mmHg (Rozkurczowe ciśnienie tętnicze krwi – mm Hg)	76.3 \pm 7.3	73.8 \pm 10.4	0.35
BMI – kg/m ² (Wskaźnik masy ciała – kg/m ²)	25.1 \pm 2.8	24.4 \pm 3.4	0.43
WHR (Wskaźnik talia/biodro)	0.97 \pm 0.05	0.95 \pm 0.06	0.20

TNF(+) – alcoholics with determinable (> 0.05 pg/ml) TNF- α plasma concentration, TNF(-) – alcoholics with undeterminable (< 0.05 pg/ml) TNF- α plasma concentration, BMI – body mass index, WHR – waist hip ratio.

TNF(+) – pacjenci z „oznaczalnym” (> 0.05 pg/ml) stężeniem TNF- α w osoczu, TNF(-) – pacjenci z nieoznaczalnym (< 0.05 pg/ml) stężeniem TNF- α w osoczu, BMI – wskaźnik masy ciała, WHR – wskaźnik talia/biodro.

Results

At the study start, within 14 days after alcohol drinking cessation, determinable TNF- α plasma level was observed in 26 (55%) alcoholics, after following four weeks long abstinence period in 23 (49%). At six month visit turned-up 26 (55%) subjects, but only 17 (36%) was abstinent during half of year observation, but the others failed to remain abstinent. Among abstainers after six months long observation determinable TNF- α level was found in 8 (49%). So, percentage of abstinent alcoholics with determinable TNF- α level (TNF-positive) did not change during observation period. However, among TNF- α positive alcoholics we found significant decrease in TNF- α level after four weeks abstinence (median \pm 95% CI; 1.2 \pm 0.84–2.8 vs.

0.5 \pm 0.35–1.2 pg/ml; $p = 0.023$). After six months TNF- α level in this subjects subgroup decreased as well, but without ($p = 0.10$) statistical significance (0.3 \pm -0.1–1.5 pg/ml) (Fig. 1). Alcoholics with determinable TNF- α plasma level at the beginning of the study were a resemblance to patients without TNF- α plasma presence in relation to the demographic and clinical data, indirectly showing insulin resistance (BMI, WHR, glucose loading test) too (Tab. 1, Tab. 2a).

The results of epidemiological investigations show, that heavy drinking increase risk of cardiovascular events. However, we found (Tab. 2a) that their probability was higher in patients with TNF-positive alcoholics, because at the study beginning they had greater TC, LDL and fibrinogen concentrations than alcoholics, in whom plasma

TNF- α was not detected. Moreover, at the study start they also had higher TGL level. TNF-positive alcoholics, in comparison to the second patients group, had also borderline shorter prothrombin time expressed as lower INR value (Tab. 2a). After four weeks long abstinence period they had greater collagen induced platelets aggregation, still higher triglycerides concentration as well as borderline lower total plasma antioxidative status (Tab. 2b). The significant main effect of TNF- α positivity on changes in TC ($F = 8.6$; $p < 0.005$) and TGL ($F = 8.18$; $p = 0.01$) concentrations, as well as in collagen induced platelets aggregation ($F = 6.55$; $p < 0.015$) and INR value ($F = 5.53$; $p < 0.024$) during first four weeks observation was confirmed using ANOVA method with two repetitions.

Similar sense of the observations we made in twenty six alcoholics, who turned up to the third visit after six months of observation. Nine of them failed to remain abstinent till this period. Among alcoholics remained abstinent at six-month visit ($n = 17$) at the study start TNF- α concentration was detected in eight males. In comparison to subjects with undetectable level of this cytokine ($n = 9$), they had significantly greater collagen induced platelets aggregation and lower total antioxidative plasma status, as well as borderline higher TGL concentration and lower MDA + 4HNE level (Tab. 2b). Moreover, using two-factorial ANOVA method with three repetitions, we found the significant main effect of TNF-positivity on fasting TGL concentration changes during six months of abstinence ($F = 5.68$, $p = 0.026$), as well as significant effect of interaction between plasma presence of this cytokine and time of abstinence duration

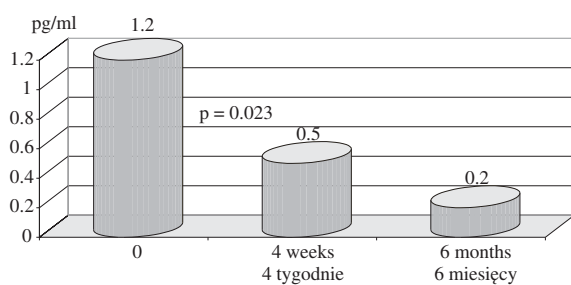


Fig. 1. The median and 95% CI of TNF- α concentration in alcohol dependent patients at the study start, after four weeks and six month of abstinence period (values enumerated only for subjects with determinable TNF- α level and abstinent during the whole investigation period)

Ryc. 1. Średnie i 95% przedziały ufności stężenia TNF- α u pacjentów z zespołem zależności alkoholowej na początku badania, po 4 tygodniach i 6 miesiącach abstynencji (wartości wyliczono tylko dla badanych z „oznaczalnym” stężeniem TNF- α oraz zachowujących abstynencję podczas całego okresu badania)

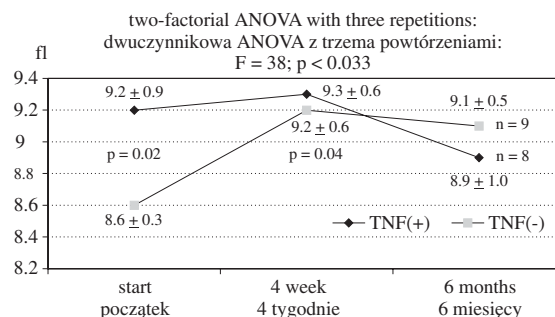


Fig. 2. Changes in mean platelets volume (MPV) values in respective determinations during abstinence period in relation to presence of TNF- α in determinable level at the study start ($n = 17$)

Ryc. 2. Zmiany średniej objętości płytek krwi w poszczególnych oznaczeniach podczas okresu abstynencji w zależności od stężenia TNF- α (oznaczalne, nieoznaczalne) na początku badania ($n = 17$)

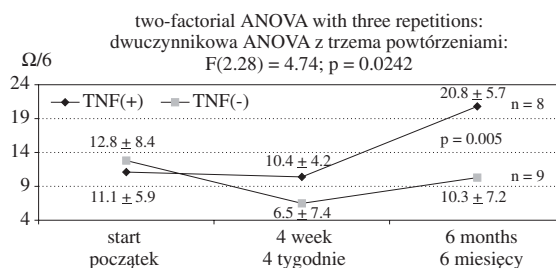


Fig. 3. Changes in collagen induced platelet aggregation in respective determinations during abstinence period in relation to the presence of TNF- α in determinable level at the study start ($n = 17$)

Ryc. 3. Zmiany zdolności agregacji płytek krwi pod wpływem kolagenu w zależności od stwierdzenia lub nie wykrywalnego TNF- α podczas okresu abstynencji ($n = 17$)

on changes in MPV value (Fig. 2) and collagen induced platelets aggregation (Fig. 3). The abstinence keeping status at six month visit have no influence on the median of TNF- α plasma concentration at six month visit (median \pm 95% CI, abstinent; $n = 17$ vs. relapsed; $n = 9$; $0 \pm -0.08-0.4$ vs. $0.6 \pm -0.1-1.68$ pg/ml; $p = 0.020$ in U Mann-Whitney test). Lack of significant differences in TNF- α concentration between alcoholics, who at the six month visit remained abstinent, and who failed to remain abstinent during whole observation period we found also among subjects with TNF- α determinable level at the study beginning ($n = 3$ vs. 5), respectively $0 \pm -0.77-1.2$ vs. $0.6 \pm -0.38-2.34$ pg/ml; $p = 0.46$ in U Mann-Whitney test). However, at six month visit alcoholics, who remained abstinent, in comparison to relapsed subjects, had significantly lower HDL concentration (44.6 ± 11.8 vs. 62.2 ± 28.9 mg/dl, $p = 0.03$), and greater MPV (9.3 ± 0.7 vs. 8.5 ± 0.8 ; $p = 0.01$)

and INR (0.97 ± 0.09 vs. 0.88 ± 0.08 ; $p = 0.014$) values. We did not find relationships between abstinence status at the six month visit and TNF- α positivity at the study beginning as well as any relationships between kind of alcohol previously drunk and changes in studied factors levels.

Discussion

The choice of TNF- α in our study, as inflammatory processes mediator, was based on its known role in pathogenesis of heart disease [20] and alcohol abuse complications, especially alcoholic liver disease [21]. Our observations suggest, that alcohol drinking or early abstinence period

stimulates TNF- α production (Fig. 1). References data are ambiguous. The results of some reports showed the increase of TNF- α production caused by alcohol drinking [21, 22, 23], independently to liver status [12], but other authors observed its decrease in the same circumstances [24, 25]. The dysregulated cytokine pathway (IL-10, IL-12, and IFN- γ), associated with the symptoms of hangovers, reported also Kim [26], but in this work TNF- α level was similar before and thirteen hours after drinking beverages with concentration of 1.5 g of alcohol per kg of body mass.

The proatherogenic role of TNF- α consist in stimulation of surface adhesive molecule (ICAM-1, VCAM-1), selectin E and local C-reactive protein (CRP) expression [27], what may favour atherosclerosis.

Table 2a. Values of biochemical parameters in studied alcoholics at the study start in relation to TNF- α plasma presence at the beginning of the study ($n = 47$)

Tabela 2a. Wartości wskaźników biochemicznych u pacjentów na początku badania w zależności od obecności TNF- α w osoczu ($n = 47$)

Parameter (Wskaźnik)	TNF(+) ($n = 26$)	TNF(-) ($n = 21$)	p
GTP – U/l	78.7 ± 46.1	54.7 ± 28.5	0.042
AST – U/l	23.4 ± 8.4	32.4 ± 7.2	0.35
ALT – U/l	33.1 ± 19.5	32.1 ± 41.4	0.92
Glucose fastly – mg/dl (Glukoza na czczo – mg/dl)	85.4 ± 12.4	92.5 ± 16.6	0.12
Glucose 2 h after 75 g of glucose orally (Glukoza 2 godz. po podaniu 75 g glukozy)	113.6 ± 35.8	128.6 ± 26.4	0.13
Total cholesterol – mg/dl (Cholesterol całkowity – mg/dl)	245.9 ± 47.9	193 ± 31.0	0.001
HDL cholesterol – mg/dl	49.4 ± 15.4	52.5 ± 11.4	0.45
LDL cholesterol – mg/dl	154.9 ± 36.5	115.6 ± 29.4	0.001
Triglycerides – mg/dl (Trójglicerydy – mg/dl)	207.8 ± 114.8	126.5 ± 56.4	0.005
Platelets count – G/l (Liczba płytek krwi – G/l)	234.3 ± 93.0	228.9 ± 66.1	0.31
MPV – fl	8.8 ± 0.9	8.6 ± 0.7	0.47
Collagen induced platelet aggregation – $\Omega/6$ min (Agregacja płytek krwi indukowana kalogenem – $\Omega/6$ min)	15.0 ± 8.2	11.6 ± 7.2	0.17
INR	0.92 ± 0.07	0.96 ± 0.08	<u>0.051</u>
Fibrinogen – g/l (Fibrynogen – g/l)	3.7 ± 1.1	4.1 ± 0.96	0.21
MDA + 4HNE – $\mu\text{mol/l}$	2.3 ± 1.5	2.4 ± 1.3	0.90
TAS – mmol/l	1.2 ± 0.3	1.1 ± 0.3	0.69
Systolic blood pressure – mm Hg (Skurczowe ciśnienie tętnicze krwi – mm Hg)	114.0 ± 15.4	115.0 ± 13.4	0.81
Diastolic blood pressure – mm Hg (Rozkurczowe ciśnienie tętnicze krwi – mm Hg)	76.3 ± 7.3	73.8 ± 10.4	0.35

Table 2b. Values of biochemical parameters in studied alcoholics after four weeks (n = 47) and after six month (n = 17) abstinence period in relation to TNF- α plasma presence at the study start (n = 47)**Tabela 2b.** Wartości wskaźników biochemicznych u pacjentów z zespołem zależności alkoholowej po 4 tygodniach (n = 47) i po 6 miesiącach (n = 17) abstynencji w zależności od obecności TNF- α w osoczu na początku badania (n = 47)

Parameter (Wskaźnik)	After 4 weeks (Po 4 tygodniach)			After 6 months (Po 6 miesiącach)		
	TNF(+) n = 26	TNF(-) n = 21	p	TNF(+) n = 8	TNF(-) n = 9	p
GTP – U/l	41.7 \pm 40.8	23.2 \pm 16.7	<u>0.064</u>	43.7 \pm 35.2	36.7 \pm 47.2	0.7
AST – U/l	21.4 \pm 7.2	21.8 \pm 7.4	0.10	23.5 \pm 14.0	25.6 \pm 15.6	0.73
ALT – U/l	30.7 \pm 17.4	22.8 \pm 13.1	0.10	32.6 \pm 24.7	21.6 \pm 14.1	0.21
Total cholesterol – mg/dl (Cholesterol całkowity – mg/dl)	226.8 \pm 61.6	210.7 \pm 37.1	0.31	233.0 \pm 60.0	220.5 \pm 47.2	0.58
HDL cholesterol – mg/dl	41.6 \pm 13.9	44.1 \pm 13.6	0.55	47.3 \pm 17.0	56.9 \pm 24.5	0.27
LDL cholesterol – mg/dl	155.1 \pm 39.8	141.2 \pm 35.1	0.23	150.8 \pm 61.6	143.0 \pm 51.8	0.74
Triglycerides – mg/dl (Trójglicerydy – mg/dl)	189.7 \pm 80.8	132.5 \pm 67.5	0.02	173.8 \pm 88.5	110.7 \pm 48.8	<u>0.084</u>
Platelets count – G/l (Liczba płytek krwi – G/l)	234.3 \pm 49.2*	224.6 \pm 59.5	0.6	213.9 \pm 52.0	200.1 \pm 67.6	0.58
MPV – fl	9.1 \pm 0.5	9.1 \pm 0.9	0.94	8.9 \pm 1.0	9.1 \pm 0.5	0.50
Collagen induced platelet aggregation – Ω /6 min (Agregacja płytek krwi indukowana kalogenem – Ω /6 min)	14.0 \pm 9.1	7.9 \pm 6.3**	0.02	20.8 \pm 5.7	10.3 \pm 7.2	0.005
INR	0.95 \pm 0.07	0.94 \pm 0.09	0.75	0.96 \pm 0.07	0.92 \pm 0.13	0.31
Fibrinogen – g/l (Fibrynogen – g/l)	3.4 \pm 0.8*	3.6 \pm 0.5**	0.36	3.1 \pm 0.5	3.0 \pm 0.5	0.54
MDA+4HNE – μ mol/l	1.8 \pm 1.1	2.1 \pm 1.0	0.25	1.2 \pm 0.5	1.70.9	<u>0.08</u>
TAS – mmol/l	1.1 \pm 0.3	1.2 \pm 0.2	<u>0.08</u>	1.1 \pm 0.2	1.3 \pm 0.1	0.02

TNF(+) – alcoholics with determinable (> 0.05 pg/ml) TNF- α plasma concentration, TNF(-) – alcoholics with undeterminable (< 0.05 pg/ml) TNF- α plasma concentration, GTP – γ -glutamyltransferase, AST – asparagines aminotransferase, ALT – alanine aminotransferase, MPV – mean platelets volume, INR – international normalized ratio – prothrombin index, MDA + 4HNE – malonylodialdehyde and 4-hydroxyenal aldehyde products of lipids peroxidation, TAS – total antioxidative plasma status. Significance of difference between respective alcoholics group (paired Student *t*-test): * – $p < 0.05$; ** – $p < 0.01$.

TNF(+) – pacjenci z oznaczalnym ($> 0,05$ pg/ml) stężeniem TNF- α na początku badania, TNF(-) – pacjenci z nieoznaczalnym ($< 0,05$ pg/ml) stężeniem TNF- α na początku badania, GTP – aktywność γ -glutamylotransferazy, AST – aktywność aminotransferazy asparaginianowej, ALT – aktywność aminotransferazy alaninowej, MPV – średnia objętość płytki krwi, INR – znormalizowany wskaźnik aktywności protrombiny, MDA + 4HNE – stężenie malonylodialdehydu i 4-hydroksyenu alu (aldehadowych produktów peroksydacji lipidów), TAS – całkowity stan antyoksydacyjny osocza. Istotność różnic między poszczególnymi grupami badanych (test *t*-Studenta dla zmiennych zależnych): * $p < 0,05$; ** $p < 0,01$.

lerosis progression, via inflammatory cells accumulation, extracellular matrix-degradation and plaque destabilisation [28–30]. These molecular mechanisms of inflammatory factors involvement in atherosclerosis pathogenesis have confirmation in observational studies showing increased CRP and proinflammatory cytokines in patients with acute coronary syndromes [9, 10]. On the other hand, TNF- α -induced adhesion of monocytes to endothelial cells was abolished after alcohol con-

sumption, especially after red wine [6]. This effect was explained by the down-regulation of adhesion molecules on the monocyte surface. All these mechanisms explained potential role of cytokines in atherosclerosis pathogenesis in alcoholics and in cardioprotection in moderate drinkers.

However, the results of our study showed, that TNF- α may potentially accelerate atherosclerosis progression in heavy drinking not only as proximate cause, especially during early abstinence period

(Fig. 1) via above mentioned mechanisms, but also indirectly, via unfavourable effect on the changes in the levels of plasma lipids, procoagulative and prooxidative factors observed at four weeks and six months visit (Tab. 2a, 2b, ANOVA, Fig. 2–4). In our previous study we found, that in the early abstinence period occur such changes in plasma lipids level, which may cause potentially proatherogenic effect [5]. Whereas, the present study results showed, that these changes in the first four weeks of observation period were presented only in TNF-positive patients (Tab. 2a). Moreover, in TNF-positive subjects who remain abstinent within whole observation period, at the beginning of the study we found greater MPV value (Fig. 2), suggesting increased platelets renewal, probably secondary to platelets consumption in thrombotic processes [31]. In following weeks of abstinence period we also observed increase of collagen induced platelets aggregation-ability (Fig. 3) and lack of total antioxidative plasma status (TAS) level increase, which appeared in patients with undeterminable TNF- α level at the study start (Tab. 2a, 2b). These observations, in the context of similar level of potentially confounding factors (Tab. 1), suggested, that plasma level of TNF- α above 0.05 pg/ml in early abstinence period may be a marker of unfavourable changes in factors potentially affecting cardiovascular risk (lipids, platelets, antioxidative balance). So, TNF-positive alcoholics would be more endanger to potentially harmful effect of abstinence period, what is also suggested by the left arm of above mentioned J-shaped curve [1, 32].

Influence of TNF-positivity on proatherogenic changes in plasma lipids level observed in our study (Tab. 2a) were comparable with previous investigation. The results of Feingold et al. study, performed in Syrian hamsters, showed, that the acute phase response induces a multitude of changes in lipoprotein metabolism including hypertriglyceridemia, enrichment of LDL by triglycerides, and decrease of HDL levels accompanied by changes in HDL composition, such as increase in free cholesterol and triglycerides and a decrease in esterified cholesterol [33]. There were also reported striking positive correlations between TNF- α and plasma triglycerides, VLDL triglycerides and VLDL cholesterol concentrations in patients with systemic lupus erythematosus [34] and with coronary artery disease [28]. Proatherogenic sense of changes in plasma lipids level observed in our study among TNF-positive subjects arose from well documented key-role of plasma lipids arteriosclerosis pathophysiology and CAD prevalence [7, 35–37].

TNF- α influence on MPV value, platelets aggregation-ability and prothrombin time value (Tab. 2b, Fig. 2, 3) observed in our study imply

usefulness of this cytokine determination as a predictor of disturbances in haemostasis after alcohol misuse period and show the second, haemostatic mechanism of indirect TNF- α proatherogenic effect. The last suggestion resulted from observational study, which show, that among other haemostatic factors, thrombocytosis, greater MPV value, spontaneous and ADP-induced platelets aggregation as well as increased fibrinogen and longer prothrombin time (lower INR value) may predispose to acute coronary syndromes [31, 38, 39]. However, greater collagen induced platelets aggregation after four weeks and six months of abstinence period in TNF-positive than TNF-negative alcoholics (Tab. 2b, Fig. 3), might issue in our study not only from cytokine acting [40], but also from triglycerides effect [41].

Alcohol abuse stimulates free radicals production and reduces antioxidative defence [42–45]. Logically effect of alcohol withdrawal seems to be improvement in antioxidative balance after alcohol withdrawal. However, in our study this effect only in TNF-negative patients was found (Tab. 2b). In TNF-positive alcoholics we observed lack of TAS level increase and lower TAS level in TNF-positive than TNF-negative subjects at six month of abstinence. This observation may imply third potential mechanism of proatherogenic, indirect TNF- α acting in alcoholics after alcohol withdrawal [46]. In references we did not find information concerning TAS, however they displayed different cytokines effect on the antioxidative enzymatic defence. TNF- α induced depletion of glutathione (GSH) [47]. The expression of extracellular superoxide dismutase (EC-SOD) was up-regulated by interferon-gamma (IFN- γ) and interleukin 4 (IL-4) and was down-regulated by TNF- α . The manganese containing superoxide dismutase (Mn-SOD) activity was strongly up-regulated by TNF- α and IL-1- α and moderately by IFN- γ . The CuZn-SOD activity of the smooth muscle cells was not significantly influenced by any of the cytokines [48].

Our study have some limitations, arising mainly from small subjects number, especially at six month visit, lack of liver biopsy and CDT determination as a alcohol abuse marker. Nevertheless, its results suggest, that TNF- α determination in patients treated against alcohol drinking relapse may be helpful in identification of alcoholics more menaced potential harmful metabolic changes occurring after alcohol withdrawal. These patients would need more intensified prophylactic procedures (diet, exercises, smoking discontinue, pharmacology) in order to prevent cardiovascular event, but this problem need a further study. It seems, that it might interesting be to estimate an effect of probiotics, which have no adverse effects

and exert the hepatoprotective, anti-cytokine, hypolipemic and anticoagulative action [49, 50].

In conclusions, our observations showed, that 1. Determinable TNF- α plasma concentration was found in about half of studied alcoholics. 2. The TNF- α plasma concentration in TNF-positive sub-

jects was the highest after alcohol withdrawal and decreased significantly after four weeks abstinence period. 3. In TNF-positive alcoholics the unfavourable changes in cardiovascular event risk factors during following weeks of abstinence were more expressed.

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