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## Increased Blood Coagulation in Alcohol Dependent Male Patients During Six-Month Abstinence Period\*

### Aktywacja krzepnięcia krwi u pacjentów uzależnionych od alkoholu w czasie abstynencji trwającej sześć miesięcy

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#### Abstract

**Background.** Haemostatic factors play an important role in pathogenesis of cardiovascular events and their activity may be affected by alcohol drinking and withdrawal.

**Objectives.** The aim of this study was to determine changes in platelets and plasma coagulation factors over a 6-month abstinence period.

**Material and Methods.** Forty-seven alcohol-dependent male patients, who were abstained less than 14 days, and 18 non-alcoholic males, who did not drink alcohol for the last month, were studied. In all patients at the beginning of the study and after four weeks of abstinence period platelets count and mean platelet volume (MPV) as well as the levels of thrombin–antithrombin complexes (TAT-complexes), antithrombin (AT), fibrinogen (fbg), activated partial thromboplastin time (aPTT) and prothrombin time expressed as international rationalized ratio (INR) were determined. In 27 alcoholics, who came for the visit after 6 months, all these determinations were repeated.

**Results.** Alcohol dependent patients during first two and after next four weeks of abstinence had higher TAT-complexes and fbg concentrations than control group. After four weeks of abstinence period decrease in fibrinogen, and AT levels as well as increase in MPV and INR values were observed. Patients, who failed to remain abstinent for whole observation period ( $n = 9$ ), in comparison to abstinent patients, had significantly lower INR and MPV values and three times higher TAT-complexes concentration.

**Conclusions.** In alcohol dependent male patients during early abstinence period the authors found increase of platelets and plasma blood coagulation activation, expressed by greater TAT-complexes concentration as well as enlargement of MPV value showing increased platelets renewal. Further study are needed to estimate importance of these haemostatic changes for cardiovascular events risk in this patients group (*Adv Clin Exp Med* 2005, 14, 2, 323–331).

**Key words:** thrombin–antithrombin complexes, coagulation, platelets, atherosclerosis risk factor, alcohol dependence, abstinence.

#### Streszczenie

**Wprowadzenie.** Układ krzepnięcia odgrywa istotną rolę w patogenezie incydentu sercowo-naczyniowego. Nadużywanie alkoholu, jak również jego odstawienie mogą zmieniać aktywność układu hemostazy.

**Cel pracy.** Ocena hemostazy płytkowej i osocznego układu krzepnięcia krwi u mężczyzn uzależnionych od alkoholu w czasie abstynencji trwającej sześć miesięcy.

**Materiał i metody.** Badanie przeprowadzono na grupie 47 uzależnionych mężczyzn, którzy nie pili alkoholu dłużej niż 14 dni, oraz na 18 niepijących 30 dni przed badaniem. U każdego z badanych na początku obserwacji i po

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czterech tygodniach abstynencji oznaczono liczbę płytek krwi, ich średnią objętość (MPV) oraz poziom kompleksów trombina–antytrombina (kompleksy TAT), antytrombiny (AT), fibrinogenu (fbg), czas kaolinowo-kefalinowy (aPTT) i czas protrombinowy wyrażony jako wskaźnik INR. U 27 mężczyzn uzależnionych od alkoholu, którzy zgłosili się na wizytę po 6 miesiącach, każde z badań zostało powtórzone.

**Wyniki.** We wczesnym okresie abstynencji, w ciągu pierwszych 14 dni po odstawieniu alkoholu oraz po kolejnych 4 tygodniach mężczyźni uzależnieni od alkoholu mieli istotnie większe stężenie kompleksów TAT i fibrinogenu niż w grupie kontrolnej. Po 4 tygodniach abstynencji obserwowano znamienne zmniejszenie stężenia fbg i aktywności AT oraz wzrost wartości MPV i INR. Pacjenci, którzy nie utrzymali sześciomiesięcznej abstynencji ( $n = 9$ ) w porównaniu do mężczyzn, którzy w tym okresie nie pili alkoholu ( $n=18$ ), podczas wizyty kończącej półroczną obserwację mieli znamienne niższe wartości MPV i INR oraz trzy razy większe stężenie kompleksów TAT.

**Wnioski.** U mężczyzn uzależnionych od alkoholu we wczesnym okresie abstynencji alkoholowej obserwowano wzrost liczby płytek krwi oraz cechy aktywacji krzepnięcia krwi, wyrażonej przez podwyższone stężenie kompleksów TAT oraz większą średnią objętość płytek krwi, sugerującą ich przyspieszoną odnowę. Dalszych badań wymaga ocena klinicznego znaczenia stwierdzonej aktywacji układu krzepnięcia w kształtowaniu ryzyka wystąpienia ostrego incydentu sercowo-naczyniowego w tej grupie chorych (*Adv Clin Exp Med* 2005, 14, 2, 323–331).

**Słowa kluczowe:** kompleksy trombina–antytrombina, krzepnięcie krwi, płytki krwi, miażdżyca – czynniki ryzyka, uzależnienie od alkoholu, abstynencja.

Intravascular thrombus formation has a direct relation to atherosclerosis and its cardiovascular complications. This process is also involved in venous thromboembolic disease pathogenesis. It is known, that the main cause of thrombus formation are disturbances in balance between activators and inhibitors both of coagulation and fibrinolysis systems. The main factors responsible for arterial thrombosis are platelets activation as well as increased levels of fibrinogen, von Willebrand (vWf) and VII factors. To the new markers of increased cardiovascular events risk belong: high levels of protein C metabolism factors (thrombomodulin – TM, protein C, protein S, Leiden V factor), platelet receptors GPIIb/IIIa, plasminogen activator inhibitor type 1 (PAI-1), and lipoprotein(a) [1].

In heavy drinkers increase in cardiovascular events risk was observed (right arm of J-shaped curve illustrating relationships between quantity of drinking alcohol and coronary artery prevalence) [2]. During drinking period they had increased risk of haemorrhagic complications, as a result of liver injury, coagulation factors acetylation, or consumption in processes of disseminated intravascular coagulation [3]. Although the many studies provide evidence that alcohol and non-alcoholic compounds of alcoholic beverages affect haemostasis, there are little data concerning effect of alcohol withdrawal on blood coagulation. The results of epidemiological studies suggested that alcohol drinking cessation might increase risk of cardiovascular events (left arm of above mentioned J-shaped curve) in alcoholics. This process may be related to disturbances in platelets and plasma coagulation system, which are the main cause of acute cardiovascular syndromes. The authors found only a few reports concerning the state of haemostasis after heavy alcohol consump-

tion in chronic alcoholics [4, 5]. Because of this, using modern laboratory methods, the authors undertook the investigation, whose aim was to determine the levels of coagulation activation *in vivo* markers as well as other plasma coagulation and platelets parameters in alcohol dependent male patients during first two weeks after alcohol drinking cessation and after next four weeks and six-month-long abstinence period.

## Material and Methods

Forty-seven alcohol dependent male patients were studied. All were hospitalized in Addiction Treatment Unit, Department of Psychiatry, The Ludwik Rydygier Medical University in Bydgoszcz (Poland) in 1999 and 2000 for 8 weeks and treated with training to cope with alcohol craving. In all of them, alcohol dependence was diagnosed according to ICD-10 (International Classification of Diseases Tenth Revision) criteria. Eighty non-alcoholic males, who had not drink alcohol within one month before including into investigation, acted as control group. The inclusion criteria for the patients group were: male sex, age between 30–50 years, performance of alcohol dependence ICD-10 criteria, continued motivation and end of misuse period not longer than 14 days before the beginning of the study. The exclusion criteria were: acute and chronic inflammatory processes symptoms, the presence of the other diseases, which could have had an influence on the blood coagulation (for example clinically overt thrombotic process, liver or kidney failure, nephritic syndrome), psychotic or demential disorders, addiction to other substances than alcohol (except smoking), and any drugs taking. In all subjects the demographic and clinical data were estimated and presented in Table 1. Quantity of alcohol

**Table 1.** Demographic and clinical data of studied alcohol dependent patients**Tabela 1.** Demograficzna i kliniczna charakterystyka badanych osób

Variable (Wskaźnik)	Alcohol dependent patients (Pacjenci uzależnieni od alkoholu) n = 47	Control group (Grupa kontrolna) n = 18	p
Age – years (Wiek – lata)	40.8 ± 8.0	40.7 ± 7.0	0.95
Age of alcohol dependence onset – years (Wiek początku uzależnienia – lata)	22.2 ± 6.4	0	
Duration of alcohol dependence – years (Długość uzależnienia – lata)	17.7 ± 7.0	0	
MAST – score (Punktacja w skali MAST)	44.9 ± 21.5	0.8 ± 0.4	0.0001
Number of drinking days during 90 days before the study start – days (Liczba dni picia alkoholu w ciągu 90 dni przed badaniem)	50.9 ± 25.6	9.1 ± 6.8	0.0001
Number of standard drinks drunk during 90 days before the study start (Liczba standardowych drinków wypitych w ciągu 90 dni przed badaniem)	952.0 ± 670.5 (in average 142.8 g of pure ethanol per day) (średnio 142,8 g czys- tego etanolu dziennie)	17.3 ± 12.9	0.0001
Number of standard drinks drunk during 30 days before the study start (Liczba standardowych drinków wypitych w ciągu 30 dni przed początkiem badania)	259.1 ± 176.6 (in average 116.6 g of pure ethanol per day) (średnio 116 g czystego alkoholu dziennie)	0	
Smokers (Palący) – n, %	45 (96%)	13 (72%)	0.01
Mean daily nicotine dose (mg/d) – in smokers (Średnia dobowa dawka nikotyny u palących – mg/d)	28.6 ± 13.1	15.6 ± 9.2	0.03
Mean daily tar dose (mg/d) – smokers (Średnia dobowa dawka substancji smolistych u palących – mg/d)	337.0 ± 144.0	184.0 ± 113.5	0.03
γ-glutamyltransferase – U/l (γ-glutamylotransferaza – U/l)	78 ± 89.6	27.6 ± 14.7	0.015
Aspartate aminotransferase – U/l (Transaminaza asparaginianowa – U/l)	31.4 ± 36.6	25.0 ± 9.1	0.43
Alanine aminotransferase – U/l (Transaminaza alaninowa – U/l)	36.5 ± 33.8	37.8 ± 21.4	0.88
Systolic blood pressure – mm Hg (Ciśnienie tętnicze skurczowe – mm Hg)	114.3 ± 14.0	129.7 ± 18.7	0.001
Diastolic blood pressure – mm Hg (Ciśnienie tętnicze rozkurczowe – mm Hg)	75.3 ± 8.9	82.0 ± 8.4	0.012
BMI – kg/m <sup>2</sup>	25.0 ± 3.0	27.7 ± 3.7	0.002
WHR	0.97 ± 0.05	0.96 ± 0.07	0.72

Values are means ± SD, unless otherwise indicated:

MAST – Michigan Alcoholism Screening Test [24],

BMI – body mass index, calculated from weight (kilograms) to height (meters) quotient,

WHR – waist to hip ratio, calculated from waist to hip circumference quotient, unpaired *t*-Student test.

Dane przedstawiono jako średnia ± odchylenie standardowe, chyba że zaznaczono inaczej:

MAST – Michigan Alcoholism Screening Test, skala głębokości uzależnienia od alkoholu [24],

BMI – wskaźnik masy ciała, wyliczony z ilorazu masy ciała w kg przez wzrost w metrach podniesiony do drugiej potęgi,

WHR – wskaźnik talia-biodra, wyliczony z ilorazu obwodu w talii i biodrach, porównania średnich dokonano za pomocą testu *t*-Studenta dla zmiennych niezależnych.

drunk during 90 and 30 days before the beginning of the study was determined using WHO Timeline/IDS study and in standard drinks counted (1 standard drink = 13.6 g = 1 oz of pure ethanol). All patients were smokers before as well as during the study. During observation period all studied per-

sons did not take any drugs. The diet of all patients was hypolipemic, according to Second Joint Task Force of European and other Societies on Coronary Prevention [6].

Studied alcoholics were examined at the beginning of the study and after four weeks of abstinence.

About half of patients  $n = 27$  (57%) turned up to the third visit after six month observation, but only 18 (38%) remained abstinent during this period. During each examination in all studied people medical history, physical examination, abstinence keeping determination and blood sampling were made. Blood samples for determinations were taken after 14 hours of fasting, at the study start, after 4 weeks and 6 months of abstinence period. Blood sampling from antecubital vein was made between 8 and 9 am, after 15-minute rest in supine position with minimal stasis. Blood for plasma coagulation parameters determination was taken to the test tube with 3.8% sodium citrate in the ratio of 9 to 1. Just after a part of blood was centrifuged and obtained plasma was frozen in  $-80$  degrees Celsius (not longer than 6 months). It was determined: markers of thrombin *in vivo* generation (thrombin–antithrombin complexes, TAT complexes) concentration, using ELISA method and set manufactured by BEHRING

(Enzygnost TAT micro), according to producers instruction. Activity of antithrombin III, called recently simply antithrombin (AT), fibrinogen (fbg) concentration, prothrombin time, expressed as INR (International Normalized Ratio), activated partial thromboplastin time (aPTT), as well as platelets count and mean platelet volume (MPV) were determined immediately after blood sampling using routine laboratory method in certificated Regional Biziel's Hospital Diagnostic Laboratory (using laboratory analyser HEMOLAB). Normal laboratory ranges are given in Table 2.

Abstinence keeping was controlled during hospitalization on the basis of physical examination as well as alcohol presence in exhaled air and the biochemical markers of alcohol abuse level (activities of:  $\gamma$ -glutamyltransferase – GTP, aspartate aminotransferase – AST, alanine aminotransferase – ALT, determined every two weeks). After discharge from the Addiction Treatment Unit alco-

**Table 2.** Values of determined platelets and plasma coagulation parameters in alcoholics and in control group at the study start and after four weeks of observation period

**Tabela 2.** Wartości oznaczonych parametrów hemostazy płytkowej i osoczowej u pacjentów uzależnionych od alkoholu i w grupie kontrolnej na początku badania i po czterech tygodniach abstynencji

Variables (Wskaźnik)	Laboratory normal range (Norma laboratoryjna)	At the study start (Na początku badania)		After 4 weeks of observation (Po 4 tygodniach)	
		alcohol dependent patients (pacjenci uzależnieni) $n = 47$	control group (grupa kontrolna) $n = 18$	alcohol dependent patients (pacjenci uzależnieni) $n = 47$	control group (grupa kontrolna) $n = 18$
Platelets count (Liczba płytek) – G/l	150–400	$239.1 \pm 81.1$	$202.4 \pm 28.8$	$232.5 \pm 60.1$	$205.7 \pm 31.7$
MPV – fl	7.2–11.0	$8.8 \pm 0.8$	$9.1 \pm 0.9$	$9.2 \pm 0.7+$	$9.1 \pm 0.7$
TAT complexes (Kompleksy TAT) – mg/l	1–4.1	$17.5 \pm 20.7^*$	$3.1 \pm 4.3$	$19.0 \pm 27.8^*$	$3.3 \pm 4.5$
AT (Aktywność antytrombiny) – %	80–120	$108.4 \pm 19.6$	$112.5 \pm 51.6$	$101.0 \pm 15.5+$	$100.2 \pm 29.5$
Fibrinogen (Fibrynogen) – g/l	2–4	$3.85 \pm 1.0^{***}$	$2.88 \pm 0.7$	$3.5 \pm 0.7^{**};+$	$2.9 \pm 0.5$
INR	0.8–1.2	$0.9 \pm 0.7$	$0.9 \pm 0.7$	$0.94 \pm 0.07$	$0.94 \pm 0.07$
aPTT – s	29–37	$30.2 \pm 3.6$	$30.4 \pm 4.2$	$30.4 \pm 3.1$	$30.1 \pm 4.8$

Values are means  $\pm$  SD, unless otherwise indicated.

MPV – mean platelet volume, TAT-complexes – thrombin–antithrombin complexes, AT – antithrombin, INR – prothrombin time presented as International Normalized Ratio, aPTT – activated partial thromboplastin time.

Significance of difference between alcoholics and control groups was estimated using unpaired *t*-Student test, both in determination at the study start and after four weeks of abstinence: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; in comparison between determinations at the study start and after four weeks abstinence period in alcoholics group significance of difference was estimated using paired *t*-Student test: +  $p < 0.05$ .

Dane przedstawiono jako średnia  $\pm$  odchylenie standardowe, chyba że zaznaczono inaczej.

MPV – średnia objętość płytki krwi, kompleksy TAT – kompleksy trombina–antytrombiny, AT – antytrombina, INR – czas protrombinowy wyrażony jako Międzynarowy Znormalizowany Wskaźnik, aPTT – czas kaolinowo-kefalinowy, czas częściowej aktywacji tromboplastyny.

Znamienność różnicy średnich między grupą osób uzależnionych i grupą kontrolną oceniono za pomocą testu *t*-Studenta dla zmiennych niezależnych: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; do porównania średnich obserwowanych na początku badania i po 4 tygodniach abstynencji wykorzystano test *t*-Studenta dla zmiennych zależnych: +  $p < 0.05$ .

hol drinking was diagnosed on the basis of interview, the level of above mentioned biochemical markers of alcohol abuse, 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 mean  $\pm$  standard deviation and as percentage (%) of quantity. Normal distribution of variables using W Shapiro-Wilk test was assessed. Statistical significance of differences was determined using, respectively, unpaired Student's t-test, Fisher exact test and two-factorial ANOVA with two and three repetitions and Turkey post hoc test in statistical software STATISTICA PL 5.0.

## Results

At the beginning of the study start, in alcoholics the level of TAT-complexes was in average some times higher than in control group and upper level of normal range of reagent producer (Tab. 2). They also had significantly greater fibrinogen plasma concentration. After four weeks of abstinence period significant decrease of fbg and AT levels, as well as increase of MPV value were observed (Tab. 2). Because in the study blood was taken after the active drinking period during the first two weeks of abstinence, the results gave not clear information whether the changes reflect chronic alcohol abuse or alcohol withdrawal. By this reason, in spite of small number of subjects, the authors analyzed the levels of studied haemostatic parameters in patients, who turned up after six months observation and remained abstainers ( $n = 18$ , 38%). The authors compared also their results with obtained from patients who came at the third visit, but did not keep abstinence ( $n = 9$ , 19%). Abstinent patients after 6 months observation, in relation to relapsed subjects (Tab. 3), had larger platelets, higher INR value (longer prothrombin time) and lower TAT-complexes plasma concentration. This resulted from once more borderline (due to small patients number) decrease of INR and MPV values and increase of TAT complexes concentration in the second group (Tab. 3). Moreover, alcoholics, who remained abstinent within whole observation period, in relation to values at the beginning of the study, had significantly lower AT and fbg levels as well as borderline lower platelets count and higher INR value (longer prothrombin time) (Tab. 3).

The influence of some potentially important clinical factors (confounding factors differing alcoholics – Tab. 1) on studied haemostatic parameters levels was also analyzed. Using ANOVA method the authors did not find significant effect of consumed alcohol sort (beer, wine, vodka, or “non-beverage” alcohol defined as liquids containing alcohol but not aimed for consumption, such as glass-washing or defroster liquids), the level of liver function test (aspartate and alanine aminotransferase plasma activity in and above upper laboratory range level), body mass (BMI value below and above 25 kg/m<sup>2</sup>), and smoking on changes in studied haemostatic parameters. This suggests the isolated effect of alcohol drinking cessation on studied haemostatic parameters levels, independent of above mentioned confounding factors. In the investigation it was confirmed also by gradual changes in platelets count, MPV, AT, fbg, and INR levels along with abstinence period duration (Tab. 2) and once more increase of MPV value and TAT-complexes concentration as well as decrease of INR value after alcohol drinking relapse (Tab. 3).

## Discussion

In this investigation the authors studied the state of coagulation system in 47 alcohol dependent male patients after alcohol drinking cessation. The authors determined the levels of coagulation activation *in vivo* markers, TAT-complexes, which is a product of thrombin inhibition for antithrombin. The authors estimated also platelets count and mean platelet volume (MPV), which is often considered as a marker of raised platelets aggregation [7] as well as the levels of coagulation factor, fibrinogen, coagulation inhibitor, AT, and parameters of efficiency of intrinsic (INR) and extrinsic (aPTT) pathway of coagulation activation.

The results showed that in alcoholics during first two weeks after alcohol abuse period proceeded considerable blood coagulation activation expressed by greater than normal producer range concentrations of TAT-complexes (Tab. 2). This might imply procoagulant effect of alcohol abuse or alcohol withdrawal. The first suggestion came also from another increase of TAT-complexes level after six months observation in relapsed alcoholics (Tab. 3), and reference data. Trotti et al. [8] found that the levels of coagulation activation markers, such as TAT-complexes, prothrombin fragment F1+2, D-dimers, were higher in chronic alcohol abusers than in moderate drinkers. In spite of that McConnell's et al. [9] study showed that daily consumption of a single alcoholic beverage for 6 weeks did not change significantly the levels of



**Table 3.** Values of determined coagulation system parameters in alcohol dependent patients, who turned up to visit after six months (n = 27) divided in relation to abstinence status after six months of observation

**Tabela 3.** Wartości oznaczonych parametrów hemostazy płytkowej i osoczowej u pacjentów uzależnionych od alkoholu, którzy zgłosili się na kontrolną wizytę po 6 miesiącach obserwacji (n = 27), podzielonych w zależności od utrzymania abstynencji w tym okresie

Variable (Wskaźnik)	Abstinent (Abstynenci) n = 18	Relapsed (Pacjenci, którzy nie utrzy- mali abstynencji) n = 9	p
Platelets count (Liczba płytek krwi) – G/l	219.7 ± 51.4#	201.9 ± 75.4	0.47
MPV (fl)	9.3 ± 0.7	8.5 ± 0.8	0.007
TAT complexes (Kompleksy TAT) – mg/l	8.9 ± 11.0	31.0 ± 35.0	0.020
AT (Aktywność antytrombiny) – %	97.2 ± 22.1*	93.5 ± 21.4	0.69
Fibrinogen (Fibrynogen) – g/l	3.3 ± 0.61**	3.2 ± 0.37	0.64
INR	0.97 ± 0.09#	0.88 ± 0.08	0.014
aPTT – s	29.7 ± 3.1	29.8 ± 2.3	0.91

Values are means ± SD, unless otherwise indicated.

TAT complexes – thrombin–antithrombin complexes, AT – antithrombin, INR – prothrombin time presented as International Normalized Ratio, aPTT – activated partial thromboplastin time.

Unpaired *t*-Student test: in comparison between abstinent and relapsed alcoholics after 6 months abstinence in right-hand column (p), and paired Student *t*-test in comparison between values at the study start and after six months abstinence in alcoholics who remained abstinent during whole observation period: \* *p* < 0.05, \*\* *p* < 0.01; # *p* < 0.06.

Dane przedstawiono jako średnia ± odchylenie standardowe, chyba że zaznaczono inaczej.

MPV – średnia objętość płytki krwi, kompleksy TAT – kompleksy trombiny–antytrombiny, AT – antytrombina, INR – czas protrombinowy wyrażony jako Międzynarowy Znormalizowany Wskaźnik, aPTT – czas kaolinowo-kefalinowy, czas częściowej aktywacji tromboplastyny.

Znamienność różnicy średnich między grupą pacjentów, którzy utrzymali sześciomiesięczną abstynencję i pijących alkohol w tym okresie oceniono za pomocą testu *t*-Studenta dla zmiennych niezależnych (wartość *p* podana w prawej kolumnie tabeli), natomiast porównania wartości badanych wskaźników po 6 miesiącach obserwacji z wartościami z początku badania i po 4 tygodniach abstynencji dokonano za pomocą testu *t*-Studenta dla zmiennych zależnych: \* *p* < 0,05, \*\* *p* < 0,01; # *p* < 0,06.

such haemostatic parameters as vWf, prothrombin fragment F1+2, TAT complexes. Van Golde et al. [10] found also that the consumption of large amounts of wine does not influence the coagulation parameters levels, such as aPTT, TAT complexes, factors VII and VIII, and von Willebrand factor.

In spite of that procoagulative effect of alcohol abuse in presented study was negated by lack of TAT complexes significant decrease in abstinent patients during six months observation (Tab. 2), as well as by greatest AT and fbg levels at the study start, by their gradual, significant decrease during six months observation (Tab. 2 and 3), and lack of their level decrease due to consumption in activated coagulation processes in relapsed patients (Tab. 3). Contradictory to the hypothesis of procoagulative effect of alcohol abuse are also the results of epidemiological studies showing an increased risk of haemorrhagic stroke in alcoholics during drinking period [2, 11], and the results of majority of epidemiological studies presented negative relation between quantity of alcohol drinking and fbg

concentration [12, 13] and AT activity [13, 14]. These reports results are not compatible with observed in presented study the greatest fbg and AT levels at the study beginning, but these discrepancies might be a result of relatively late fbg and AT determination. This hypothesis supports the results of Wallestedt's et al. work [4], which showed a little (not significant) increase in fbg concentration and significant increase in AT activity between 1–3 day and 7–10 day after a period of heavy alcohol consumption in 19 chronic alcoholics. Kauhanen et al. [15] also found increased fbg concentration in heavy drinkers during hang-over and suggested that plasma fbg concentration appeared as one possible pathway to increased risk of cardiovascular death in men who frequently experience hangovers.

In the context of above mentioned considerations, the authors may interpret the results as showing procoagulative effect of alcohol withdrawal. In the work, it is suggested not only by increased (above set producer normal range) TAT-complexes concentration during six months obser-

vation period, but also by gradual increase of MPV value, which is a marker of accelerated platelets renewal [7]. Observed in the study the features of permanent slight increase in thrombin generation after alcohol drinking cessation might be probably due to endothelial cell dysfunction or cytokine acting, as well as insufficiency of fibrinolysis system in alcoholics during abstinence period, which was reported in literature [11]. Procoagulative effect of alcohol withdrawal, beside above quoted reports by Wallestedt's [4] and Kauhanen [15], results also from the epidemiological study showing increase in cardiovascular event risk in teetotallers (left arm of J-shaped curve) [11] and imply increase in atherosclerosis progression and its complications menace in alcoholics not only during drinking period but during abstinence too.

Described subjects, at the study beginning, simultaneously with the increased level of coagulation activation *in vivo* marker, had the greatest concentration of fbg, AT and the lowest INR value (Tab. 2). In the next weeks of abstinence period a gradual decrease in these parameters level was observed. This may suggest an effect of similar factors on chronic thrombin generation activation and increase in coagulation factors and inhibitors levels. Next, in the vicious circle mechanism, increased fbg concentration [15, 16] and shorter prothrombin time (lower INR value) [17] may independently facilitate thrombotic processes acceleration. On the other hand, these processes activation, expressed by increased TAT-complexes concentration, may lead to the rise of coagulation factors (fbg, prothrombin complex) and its inhibitors (AT) levels, as a result of chronic adaptation to the slight increased thrombin generation processes and chronic response to atherosclerosis progression [12, 14]. In spite of that Wallestedt et al. [4] suggested that increased level of coagulation factors and other protein in heavy drinkers may be a result of liver enzymes induction or decrease in the liver clearance. The first hypothesis might be confirmed by the simultaneous observation of the highest GTP activity (Tab.1) and the highest fbg level and prothrombin time shortening (the lowest INR value) at the study beginning (Tab. 2) and lower INR value in relapsed patients (Tab. 3). On the other hand, normal INR value in described patients imply that the patients had no severe liver dysfunction, which might affect the studied haemostatic parameters levels, both in the aspect of their synthesis and clearance [18].

At the study beginning the authors found also the highest platelets count and the lowest MPV (Tab. 2). In the next weeks of the observation period the authors observed gradual decrease in platelets count and increase in MPV value

(Tab. 2), which once again significantly decreased in relapsed patients (Tab. 3). MPV is the parameter whose value positively correlates with cardiovascular event prevalence [7] and, because the new platelets are greater than old, show indirectly, that in described alcoholics after four weeks observation period increased platelets renewal occurred. There were also reports of increase in megacaryocytes precursors (BFU-Mk and CFU-Mk) *in vitro* and megacaryocytes in bone marrow *in vivo* during early abstinence period, which may resulted in thrombocytosis. The other clinical meaning of this observation might be also that observed in the patients increased thrombin generation (TAT-complexes) during first two weeks abstinence period was not related to the platelets haemostasis disturbances in opposition to the determination after four weeks. In this context, mentioned changes in MPV value should be interpreted as an anti-platelet, cardioprotective effect of alcohol drinking and abuse (low MPV) and significant increase in platelets activity after four weeks abstinence (increase of MPV value). Marked prolongation of bleeding time, decrease in platelet adhesion to fibrillar collagen and collagen induced platelet aggregation were demonstrated in the previous investigations [19]. This anti-platelets effect disappeared after alcohol withdrawal [2, 11, 20].

Changes in platelets count, observed in the study, were seemingly different from reference data, which in alcoholics during drinking period and immediately after it described the thrombocytopenia due to hypersplenism, bone marrow injury, folic acid deficiency, and direct ethanol and acetaldehyde effect [20]. But in persons without severe organ injury between 5 and 21 days after alcohol drinking cessation increase in platelets count was observed. Normalisation of platelets count occurred only after 1–3 weeks of abstinence duration [20]. The described curve of changes in platelets count after alcohol drinking cessation might explain presented observations, because the subjects at the study beginning were examined during phase of platelets count increase.

The results may be a matter of great clinical importance implying increase in cardiovascular events risk in alcohol dependent patients after alcohol drinking cessation. During early abstinence period it was related to plasma coagulation activation and to platelets activation during next five months of observation. This hypothesis came from the results of observational and experimental works which showed that increased TAT-complexes [21], fbg [15, 16] concentrations, shorter prothrombin time [17], lower AT activity [14] and larger MPV [7] may predict cardiovascular events. Increased risk of cardiovascular death was also

reported in men who frequently experience hangovers [15, 22], in teetotallers and people who regular drink more than 3 drinks (the left and right arms of J-shaped curve) [11] or drink alcohol in binge pattern [23]. This suggest necessity to undertake interventional studies, especially in patients who start anti-alcohol relapse therapy, in order to estimate the effectiveness of no pharmacological methods (simultaneous smoking cessation, diet modification, exercise, body mass control) or anticoagulants, and/or anti-platelets drugs in cardiovascular events prevention after alcohol drinking cessation. Presented study suggests also usefulness of low MPV value as a biological marker of heavy alcohol consumption.

Presented study has some limitations. Firstly, the patients group was relatively small and because of drop-out (although typical for studies in alcoholics), it was finished only for fifty-seven percent of patients. In spite of that the authors obtained some significant differences. Secondly, studied alcoholics and control group were diversified in relation to factors potentially affecting haemostatic system such as smoking, body mass, age, kind and quantity of consumed alcohol, drinking pattern, length of alcohol dependence, and liver function (Tab. 1). However, it seems that on the base of following three determinations, and analysis, in which each patient was own control, and lack of

significant changes in the patients' lifestyle, besides drinking status, the authors were able to estimate an effect of alcohol abstinence on studied haemostatic parameters levels, considering the fact that the study was relatively short and the subjects unchanged their smoking status during whole study period. This same explanation had a potentially confounding factor of liver dysfunction, body mass, age and kind of drinking alcohol beverages. Thirdly, it should be noted too, that abstinence keeping was controlled without CDT determination, but in Poland this test is seldom available.

In conclusion, early abstinence period in alcohol dependent patients, both at the study start and in relapsed patients, was characterized by increased blood coagulation activation, expressed by greater TAT-complexes concentration. Increased thrombotic readiness after alcohol withdrawal was facilitated by greater fibrinogen concentration and lower INR value as well as induced increased platelets renewal and compensatory rise of coagulation inhibitors (AT) activity. Further studies are needed to explain the prognostic importance of observed haemostatic disturbances and to find methods, which might protect alcoholics during anti-relapse therapy against increased cardiovascular event risk suggested by epidemiological investigations (J-shaped curve).

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