THE SIGNIFICANCE OF PLATELET MICROPARTICLES IN PATIENTS WITH CHRONIC HEPATITIS C AND THEIR ASSOCIATION WITH ANTIVIRAL TREATMENT AND SMOKING

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Introduction

Microparticles are heterogeneous submicron vesicles released from different cell types. They are divided in exosomes and microvesicles. Platelet-derived microparticles (PMPs) are shed from activated platelet membranes and constitute the most abundant microparticles in the blood circulation. The surface of PMPs is reported to be 100-fold more procoagulant than that of activated platelets. In their turn, PMPs activate platelets by transferring proinflammatory lipids. Elevated plasma PMPs levels are associated with atherothrombotic diseases such as coronary artery disease, or those predisposing to atherothrombosis such as type 2 diabetes, polycystic ovary syndrome etc.

Chronic hepatitis C (CHC) is associated with steatosis and insulin resistance/diabetes and many studies show an association between CHC and cardiovascular events and/or high risk factors for atherosclerosis. However, the mechanisms of CHC leading to atherogenic potential need further investigation.

The aims of the current study were first, to identify if patients with CHC had higher concentrations of PMPs compared to controls. Second, to examine if factors associated with atherosclerosis were related to PMPs levels. Third, to test if antiviral treatment had a beneficial effect on PMPs levels.

Material and Methods

28 CHC patients (13 G1/G4, 15 G3) were included, whereas 20 healthy volunteers (HV) and 20 patients with non-alcoholic fatty liver disease (NAFLD) were used as controls. Patients with cardiovascular diseases, diabetes, anticoagulant and antiplatelet treatment were excluded. All CHC patients were treated with pegylated-interferon/ribavirin. Twenty-four (86%) achieved sustained virological response (SVR). PMPs levels were determined by flow-cytometry (CD61-Annexin V) at baseline in all CHC patients and controls as well as at end of treatment (EOT) and 24 weeks post-treatment (SVR24) in all CHC patients.

Results

Characteristics of the patients

Twenty-four patients (86%) achieved SVR; two (7%) did not respond to treatment and 2 (7%) relapsed. Both non-responders and relapsers were considered as non-SVR cases. Three patients were treatment-experienced but achieved SVR. Six (86%) patients with genotype 1, 14 (93%) with genotype 3 and 4 (67%) with genotype 4 achieved SVR.

PMPs counts at baseline

Univariate analysis revealed that patients with high (≥500 counts/μL) compared to those with low (<500 counts/μL) PMPs were more frequently smokers (93% vs 36%, P=0.001). ALT levels showed a trend to be higher in cases with high than low PMPs levels (P=0.085). No other statistically significant difference in demographic or laboratory characteristics was observed between patients with low and high PMPs .

Median PMPs levels at baseline were higher in CHC patients (510 counts/μL) compared to NAFLD patients (166 counts/μL, P<0.001) and HV (316 counts/μL, P=0.007) (Fig. 1A). The median baseline PMPs levels were higher in smokers (n=18) compared to non-smokers (n=10) with CHC (794 vs 260 counts/μL, P=0.006) (Table 1). Higher PMPs were also found in smokers compared to non-smokers in patients with NAFLD (254 vs 108 counts/μL, P=0.05) and HV (436 vs 161 counts/μL, P=0.003). Among smokers from all groups, CHC patients had higher PMPs compared to NAFLD patients (P=0.001) and HV (P=0.024) (Fig. 1B). Similarly, among non-smokers from all groups, CHC patients had higher PMPs levels compared to NAFLD patients (P=0.014) and HV (P=0.075) (Fig. 1B).

PMPs levels during antiviral treatment

During antiviral treatment, PMPs levels declined significantly from baseline to both EOT (median decline: 372 counts/μL, P=0.035) and 24 weeks after EOT (SVR24) (median decline: 106 counts/μL, P=0.006) (Table 1, Fig.2A). PMPs levels showed a numerical reduction from EOT to SVR24, but this change did not reach statistical significance in all CHC patients (P=0.163).

The decline of PMPs levels during treatment was mainly observed in smokers, in whom PMPs declined significantly from baseline to EOT (P=0.004) and SVR24 (P=0.009) (Table 2, Fig. 2B), while no significant decline was again observed from EOT to SVR24 (P=0.382). In contrast, in non-smokers, there was no significant change of PMPs even between baseline and EOT (P=0.449) or SVR24 (P=0.600) (Table 1, Fig. 2B).

In relation to response to treatment, only patients who achieved SVR had a significant decline in PMPs from baseline to SVR24 (P=0.018). In this subgroup, PMPs declined from baseline to EOT with the change being close to statistical significance (P=0.062), which was reached in the change of PMPs from baseline to SVR24 (P=0.018). In contrast, in the patients without SVR, PMPs levels did not show any statistical difference from baseline to EOT (P=0.465) or SVR24 (P=0.180) (Table 1).

At SVR24, PMPs levels became similar between smokers and non-smokers CHC patients (Table 2, Fig. 2B). In addition, no significant difference was found in PMPs levels at EOT or SVR24 between CHC and NAFLD

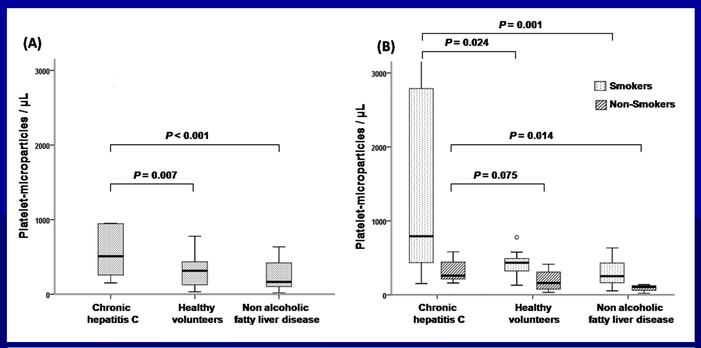

Fig. 1. Results of platelet microparticles' levels in 28 patients with chronic hepatitis C (CHC), 20 patients with non alcoholic fatty liver disease (NAFLD) and 20 healthy volunteers (HV). Boxes and whisker plots express medians, interquartile range and overall ranges. (A). All patients (B). Smokers vs. non smokers (the highest three values were omitted from the first box plot of 1A).

Table 1 Platelet microparticles' levels at different time points during antiviral treatment in chronic hepatitis C patients.

	Platelet Microparticles (counts/μL)			P values for Platelet Microparticles changes		
	Baseline	*EOT*	*SVR24*	*Baseline to EOT*	*EOT to SVR24*	*Baseline to SVR24*
All patients	510 (153-5184)	265 (57-1583)	186 (20-886)	0.035	0.163	0.006
Smokers	794 (153-5184)	298 (75-1408)	186 (20-886)	0.004	0.382	0.009
Non-smokers	260 (161-582)	186 (57-1583)	165 (54-772)	0.449	0.225	0.600
P*	0.006	0.837	0.910			
SVR	510 (153-5184)	265 (75-1583)	186 (20-886)	0.062	0.179	0.018
No SVR	509 (215-4369)	252 (57-914)	182 (165-198)	0.465	0.655	0.180
P**	0.874	0.538	1.000			
Genotype 1/4	493 (161-5184)	195 (57-1338)	178 (20-772)	0.398	0.686	0.025
Genotype 3	551 (153-4831)	298 (75-1583)	186 (42-886)	0.048	0.221	0.087
P***	0.928	0.731	1.000			

Quantitative values are expressed as median (range); EOT: End-of-Treatment; SVR24: 24 weeks post treatment; SVR, Sustained Virological Response.
*P values for comparisons between smokers and non-smokers; **P values for comparisons between patients with and without SVR; ***P values for comparison between patients with genotype 1/4 and patients with genotype 3

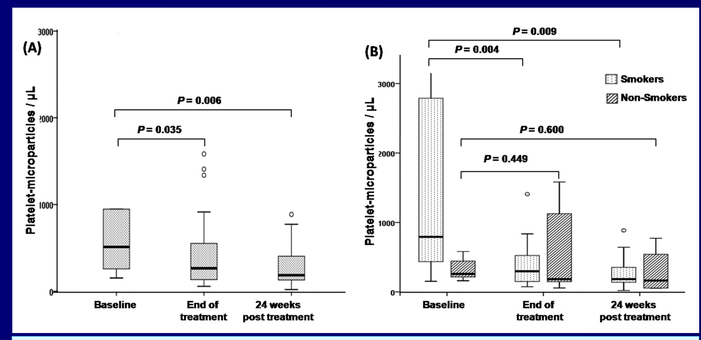

Fig. 2. Results of platelet microparticles' levels in 28 patients with chronic hepatitis C (CHC) at different time points during antiviral treatment. Boxes and whisker plots express medians, interquartile range and overall ranges. (A). All patients (B). Smokers vs. non smokers (the highest three values were omitted from the first box plot of 2A).

CONCLUSIONS

1. Platelet microparticles, a marker of platelet and endothelial cell activation, are elevated in patients with chronic hepatitis C (CHC) compared to those with non fatty liver disease and healthy volunteers.
2. The higher PMPs levels in CHC patients and particularly in smokers further support the atherosclerotic potential of CHC and suggest a potentially synergistic effect of smoking and CHC on the atherosclerotic process.
3. Since PMPs levels in CHC patients with sustained virological response do not differ from those in controls, the atherosclerotic potential of CHC seems to be abolished by effective antiviral treatment.

References

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