Circulating MPs show a prothrombotic phenotype in patients with long-life exposure to high LDL levels and directly associate with lipid-rich atherosclerotic plaque burden

R. Suades[1], T. Padro[1], R. Alonso[2], P. Mata[2], L. Badimon[1]

[1] Cardiovascular Research Center (CSIC-ICCC), IIB-Sant Pau, Barcelona, Spain, [2] Fundación Jimenez Diaz, Madrid, Spain

Background

High plasma cholesterol levels are a risk factor for atherothrombosis and cardiovascular disease (CVD). Familial hypercholesterolemia (FH) is a monogenic disorder characterized by a reduced expression of the LDL receptor that leads to life-time exposure to high levels of LDL and the development of premature coronary artery disease. Circulating microparticles (cMPs) may play important roles in vascular function. High levels of cMPs have been associated with thrombosis, inflammation, and metabolic disorders. We hypothesize that cMPs could contribute to the increased atherothrombotic risk in heterozygous FH patients.

Aims

The present study aimed to investigate cell-associated thrombogenic markers in cMPs of hypercholesterolemic patients with genetic diagnosis of FH and subclinical atherosclerosis detected by aortic magnetic resonance imaging (MRI) under lipid-lowering therapy (LLT). The control was an age / gender / treatment-matched group of non-FH patients treated with LLT for secondary hypercholesterolemia.

Patients & Methods

- **Patients:** Clinically and genetically characterized FH and non-FH patients were from the SpAnish Familial hypErcHolEsterolaemiA cohoRT (SAFEHEART).

	FH patients (n=37)	Controls (n=37)	Statistics
Male / Female (n)	19/18	14/23	$P = 0.35$
Age (years)	47.0 (41.0-54.0)	53.0 (38.0-62.0)	$P = 0.51$
Body mass index (Kg/m^2)	25.8 (22.9-28.1)	27.0 (22.8-30.7)	$P = 0.21$
Hyperlipidemia (n, %)	37 (100%)	37 (100%)	$P = 0.99$
Total cholesterol (mg/dL)	223.0 (185.8-257.0)	210.0 (192.0-240.0)	$P = 0.48$
Triglyceride (mg/dL)	82.0 (64.8-141.0)	82.0 (64.0-120.0)	$P = 0.23$
LDL-cholesterol (mg/dL)	145.0 (124.3-175.0)	134.2 (109.0-159.6)	$P = 0.21$
LDLR-gene mutation (null/defective/unknown)	(21/15/1)	-	-

- **cMP isolation:** cMPs were obtained and washed from citrated platelet-free plasma (PFP) by a two high-speed centrifugation steps (20000×g, 30 min).
- **FACS analysis:** cMPs were identified, size characterized and quantitatively analyzed by flow cytometry for annexin V binding, specific blood cell activation surface markers and tissue factor (TF).
- **TF-procoagulant activity:** cMP-associated TF-PCA activity was measured by a functional assay determining the FVII-dependent FXa generation.

Results

1. Activated platelet markers in circulating microparticles

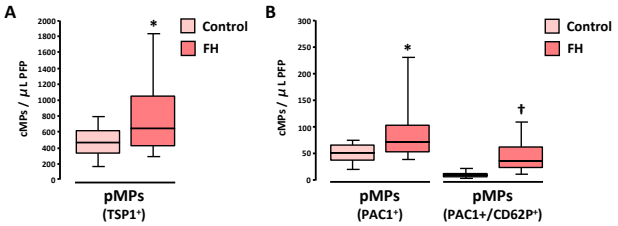

Figure 1. Levels of platelet-derived microparticles (pMPs) carrying activation markers: **(A)** thrombospondin-1 (TSP-1$^+$), and **(B)** activated $\alpha_{IIb}\beta_3$-integrin (PAC1$^+$) and activated $\alpha_{IIb}\beta_3$-integrin / P-selectin (PAC1$^+$/CD62P$^+$) in non-FH ad FH patients. Values given as cMPs / µL of platelet free plasma (PFP). Box plot show median (IQR). *p<0.005, †p<0.001, vs. control group (U-Mann Whitney test).

2. Tissue factor-bearing circulating microparticles

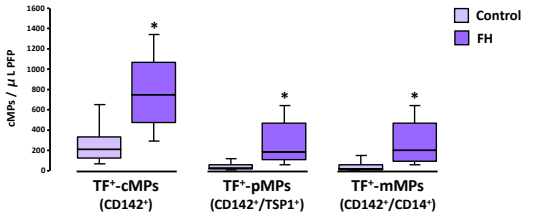

Figure 2. Levels cMPs exposing tissue factor (CD142$^+$, TF$^+$-cMPs), and TF with platelet (TSP1$^+$, TF$^+$-pMPs) and monocyte (CD14$^+$, TF$^+$-mMPs) markers in non-FH and FH patients. Values given as cMPs / µL of platelet free plasma (PFP). Box plot show median (IQR). *p<0.0001 vs. control group (U-Mann Whitney test).

3. Atherosclerotic plaque composition and cMPs in FH

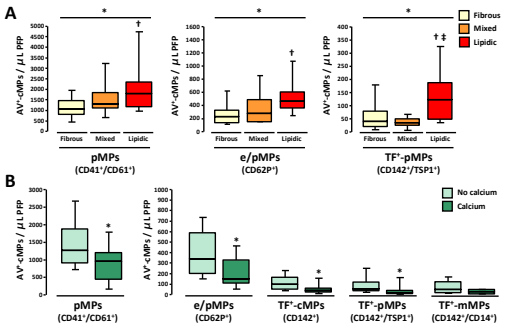

Figure 3. (A) Levels of annexin V-positive MPs (AV$^+$-cMPs) identified as pMPs (CD41$^+$/61$^+$), activated e/pMPs (CD62P$^+$), and TF$^+$-pMPs (CD142$^+$/TSP1$^+$) related to atherosclerotic plaque type. *p<0.05 (Kruskal Wallis), †p<0.05 vs fibrous and ‡p<0.05 vs mixed (U-Mann Whitney). **(B)** AV$^+$-cMPs levels of pMPs (CD41$^+$/61$^+$), activated e/pMPs (CD62P$^+$) and TF$^+$-cMPs (CD142$^+$) depending on plaque calcium content. Box plot show median (IQR). *p<0.05 vs. no calcium (U-Mann Whitney test). Values given as cMPs / µL of platelet free plasma (PFP).

4. cMP-TF activity and plaque burden

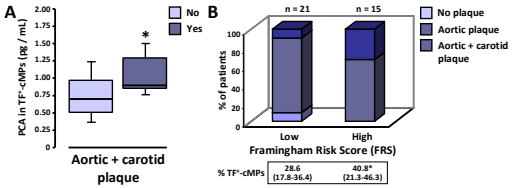

Figure 4. (A) Procoagulant activity (PCA) in TF$^+$-cMPs in regard to total atherosclerotic plaque burden (presence of carotid plus aortic atherosclerotic plaques). **(B)** Total atherosclerotic plaque burden divided into cardiovascular risk score subgroups (low and high Framingham Risk Score –FRS– at 10 years). Boxes indicate percentage of TF$^+$-cMPs in both groups. *p<0.05 (U-Mann Whitney test).

Conclusions

- Enhancement of shedding of cMPs in FH is not associated to the achievement of target LDL levels but to long-term exposure to high LDL.
- Circulating MPs showed a prothrombotic phenotype in patients with FH and directly associated with lipid-rich atherosclerotic plaque burden.

The specific increase in $\alpha_{IIb}\beta_3$-integrin$^+$ and TF$^+$-microparticles indicate that cMPs are active contributors in the on-going atherothrombotic process in FH patients and highlights the potential of TF-rich cMPs as a biomarkers of thrombotic risk and as attractive therapeutic target in FH patients.

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Prof. Lina Badimon. Cardiovascular Research Center. C/St. Antoni Ma. Claret, 167, 08025-Barcelona, Spain.*E-mail*: lbadimon@csic-iccc.org