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

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**Background**

High plasma cholesterol levels are a risk factor for atherosclerosis 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 hypercholesterolemia A cohort (SAFEHEART). The control was an age / gender / treatment-matched group of non-FH patients treated with LLT for secondary hypercholesterolemia.

- **Patients & controls:**
  - **FH patients (n=37)**
    - Male / Female (n): 19/18
    - Age (years): 47.0 (41.0-54.0)
    - Body mass index (kg/m²): 25.8 (22.9-28.3)
    - Hyperlipidemia (%): 37.0 (%100)
    - Total cholesterol (mg/dL): 223.0 (185.8-257.0)
    - LDL-cholesterol (mg/dL): 140.0 (124.3-175.0)
    - LDL-cholesterol mutation (nul/defective/unknown): 21/15/1

- **cMPS isolation:** cMPS were obtained and washed from citrated platelet-free plasma (PPP) by a two high-speed centrifugation steps (20000g x 30 min).

- **cMPS and 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:** cMPS-associated TF-PCA activity was measured by a functional assay determining the FVII-dependent FXa generation.

**Results**

1. Activated platelet markers in circulating microparticles

   ![Activated platelet markers in circulating microparticles](image1.png)

2. Tissue factor-bearing circulating microparticles

   ![Tissue factor-bearing circulating microparticles](image2.png)

3. Atherosclerotic plaque composition and cMPS in FH

   ![Atherosclerotic plaque composition and cMPS in FH](image3.png)

4. cMPS-TF activity and plaque burden

   ![cMPS-TF activity and plaque burden](image4.png)

**Conclusions**

- Enhancement of shedding of cMPS in FH is not associated to the achievement of target LDL levels but over 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.

**Acknowledgements**

- This work was supported in part by the SAFE-T project (Grant Agreement No 115003, IMI-JU; Salud Carlos III (CIBERobn CB06/03); CNIC-082008. RS is recipient of a predoctoral grant (P11-FI-09/00092, ISCIII).