




Emostasi, infiammazione e immunità

# EXTRACELLULAR VESICLES AS BIOMARKERS IN FAMILIAL HYPERCHOLESTEROLAEMIA TREATED WITH EVINACUMAB

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**Introduction and Aims.** Familial hypercholesterolaemia (FH) is an inherited autosomal dominant disorder caused by the presence of variants in the genes that regulate low-density lipoprotein (LDL). Chronic elevation of LDL-cholesterol (LDL-C) levels predisposes patients to an increased cardiovascular risk. Growing evidence suggests that extracellular vesicles (EVs) contribute to vascular dysfunction, inflammation and atherogenesis through paracrine mechanisms and may reflect ongoing pathological processes in cardiovascular disease. The introduction of Evinacumab represents a novel therapeutic strategy for LDL-C reduction. This study aims to evaluate EVs in FH patients, in order to explore their potential role as biomarkers of disease activity and of the therapeutic response to Evinacumab treatment.

**Methods.** Seven patients with FH (4 males, 3 females; age range 9-83 years) and fifteen healthy age- and sex-matched controls were enrolled. Plasma EVs were isolated from platelet-poor plasma. EVs were quantified (absolute count, expressed as events/µL) and characterized using CytoFLEX flow cytometry at baseline and monthly for six months following initiation of Evinacumab treatment. Instrument standardization and size-based gating were performed using fluorescent polystyrene Gigamix beads (FSC and SSC), establishing an EV detection range of 0.1–0.9µm. EVs integrity was assessed using calcein-AM, membrane labelling was performed with a lipophilic dye and apoptotic EVs were identified by Annexin V staining. EV subpopulations were defined based on cell-specific surface markers: platelets ($CD41^+$, $CD62P^+$), leukocytes ($CD45^+$), monocytes ($CD14^+$), erythrocytes ($CD235a^+$), endothelial cells ($CD62E^+$), and apolipoprotein B–positive ($ApoB^+$).

**Results.** FH patients exhibited significantly higher levels of total EVs (lipophilic $dye^+$/calcein-$AM^+$/Annexin-$V^+$) and increased concentrations of $CD41^+$, $CD62P^+$, $CD45^+$, $CD235a^+$, $CD62E^+$, and $ApoB^+$ EVs compared with healthy controls ($p \leq 0.005$ for $CD41^+$ and $CD235a^+$; $p \leq 0.0001$ for all other subtypes). Evinacumab treatment was associated with a progressive reduction in endothelial, platelet and inflammatory EVs reaching statistical significance between the second to fifth treatment administrations ($p \leq 0.001$ vs baseline). $ApoB^+$ EV levels showed a significant decrease after the fourth infusion ($p \leq 0.001$).

**Conclusions.** Evinacumab treatment was associated with a reduction in endothelial- and ApoB-derived EVs and a partial decrease in inflammatory and platelet-derived EVs, suggesting an improvement in vascular and inflammatory status that was not fully normalized. These findings indicate a benefical effect of therapy in FH patients. Longitudinal studies are warranted to assess the durability of these effects. Overall, EVs may represent dynamic biomarkers of disease activity and therapeutic response in FH.