

## “Cardiovascular Diseases (CVDs) Patients with Hypertrophic Epicardial Adipose Tissue (EAT) Has a Microbiome Core Associated to Innate Immunity Activation”

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**Introduction:** The epicardial adipose tissue (EAT) is a visceral fat surrounding myocardium with emergent role in heart metabolism due to its anatomical contiguity to cardiac organ. In case of EAT mass increase, dysfunctional adipocytes release pro-inflammatory adipokines and activate immune cells, including macrophages, developing a pro-inflammatory environment contributing to the severity and progression of cardiovascular diseases (CVDs). Notably is that endogenous alteration of host tissue microbiome can promote innate immunity responses, participating to the amplification of local inflammatory environment. Due to the role of activated macrophages in tissue repair and anti-bacteria responses, our aim was to investigate in CVDs patients if an increase in EAT mass can be associate to local microbiome host variation and to the activation of molecular patterns associate to innate immunity responses.

**Methods:** EAT biopsies were collected during open heart surgery from 23 CVDs patients. Patients were stratified according to EAT cut off value of 7mm, as marker of hypertrophy (in: CVDs < 7mm and CVDs ≥ 7mm). Microarray assays are performed to evaluate the molecular patterns of EAT biopsies associated to macrophage activation and related-cytokine release. mRNA levels of CD14, CD163, CD163L, TLRs, NF-Kb, APC-1 mRNA as genes involved in macrophages activation; and IL-1, IL-6, IL-12, IL-8 and TNFα as the main cytokines released by them, were measured. In all patients from each group, EAT microbiome composition was determined using next-generation sequencing technology.

**Results:** In EAT, mRNA expression of gene involved in macrophage activation and related pro-inflammatory cytokines resulted significantly higher in CVDs patients with hypertrophic EAT. A core of bacterial genera (*Acinetobacter* spp, *Chryseobacterium* spp, *Comamonas* spp, *Corynebacterium* spp, *Delftia* spp, *Flavobacterium* spp, *Kocuria* spp, *Methylobacterium* spp, *Paracoccus* spp, *Pelomonas* spp, *Propionibacterium* spp, *Pseudomonas* spp, *Sphingomonas* spp, *Staphylococcus* spp, *Streptococcus* spp) was identified in both CVDs group patients; interestingly CVDs with EAT ≥ 7mm presented different predominant opportunistic species than CVDs patients with physiological EAT thickness.

**Conclusions:** Our study demonstrated the presence of a preserved bacterial core directly into EAT and we also verified that hypertrophic EAT biopsies presented more opportunistic pathogens species than biopsies with physiological EAT thickness. Furthermore, CVDs with EAT ≥ 7mm presented higher expression of genes associated to macrophages activations and innate immunity responses. Our results suggest that EAT local microbiome in CVDs patients changes in case of EAT mass increase and stimulates a pro-inflammatory environments also through macrophage activations.