PCSK9-inhibitor therapy leads to changes in immune cell gene expression and function: A subset analysis of the PACMAN-AMI trial

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Introduction: The PACMAN-AMI trial showed a significant reduction of plaque atheroma volume by alirocumab on top of high-intensity statin therapy as compared to placebo in 265 patients undergoing serial multi-vessel imaging after acute myocardial infarction (AMI). Neutrophils and the formation of neutrophil extracellular traps (NETs) have been implicated in the development and progression of atherosclerosis. High levels of low-density lipoprotein (LDL) have been associated with neutrophil activation.

Objectives: This study aimed to determine the influence of aggressive LDL-lowering on neutrophil activation, and immune cell gene expression of pro-inflammatory markers in a subset of the PACMAN-AMI cohort.

Methods: Neutrophils and peripheral blood mononuclear cells (PBMC) of 38 patients were isolated by serial density-gradient centrifugation to assess relative gene expression of IL-1β, IL-6, TNFα, X-box binding protein (XBP) 1, caspase 1 (CASP1), and NLRP3 by quantitative PCR. Neutrophils were stimulated to undergo NET formation using a calcium ionophore in the presence or absence of pro-protein convertase subtilisin/kexin type 9 (PCSK9), detecting the release of double-stranded (ds)DNA. Plasma samples were screened for dsDNA, citrullinated histone H3, neutrophil elastase, and myeloperoxidase. Healthy controls (n=30) were recruited during routine check-ups in a health and prevention center.

Results: After one year of double-blind injections, the probability to undergo NET formation in vitro was still higher in AMI patients than in healthy controls. Considering randomized treatment, only patients injected with alirocumab showed significantly higher NET release than controls. Additional stimulation with the alirocumab target PCSK9 could reduce NET formation in all cohorts. This effect was significantly higher in patients treated with alirocumab. Relative gene expression of CASP1, IL-1β, IL-6, and XBP 1 was higher in patients treated with alirocumab. There was no difference in plasma NET markers comparing healthy controls and both treatment arms.

Conclusion: Our data indicate that PCSK9 might have effects beyond its role in cholesterol metabolism influencing immune cell activation, which could mask effects of LDL lowering. Treatment does not directly translate to higher plasma NET levels without an additional inflammatory stimulus disrupting the steady state.