TLR2 signalling orchestrates neutrophil activation in acute coronary syndrome with intact fibrous cap – results from the OPTICO-ACS study

D. Meteva[1], C. Seppelt[1], Y. Abdelwahed[1], R. Himanshu[2], A. Lauten[3], B. Staehli[4], M. Reinthaler[1], U. Rauch-Kroehnert[1], A. Haghikia[1], D. Sinning[1], C. Skurk[1], M. Joner[2], D. Leistner[1], U. Landmesser[1], N. Kraenkel[1]

[1]Charite - Campus Benjamin Franklin, Cardiology, Berlin, Germany; [2]Deutsches Herzzentrum Muenchen Technical University of Munich, Cardiology, Munich, Germany; [3]Helios Klinikum, Cardiology, Erfurt, Germany; [4]University Hospital Zurich, Cardiology, Zurich, Switzerland

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Background: Neutrophil granulocytes are key players of the innate immunity, participating in the initiation and progression of atherosclerosis. However, the exact mechanisms of neutrophil activation after acute coronary syndrome (ACS) are poorly understood, especially in the context of the two predominant ACS-causing pathophysiologies - ACS with intact fibrous cap (IFC-ACS) and ACS with ruptured fibrous cap (RFC-ACS). Therefore, the current study focuses on immunophenotyping and ex-vivo functional characterization of neutrophils with regard to the molecular differences between IFC-ACS and RFC-ACS.

Methods: Using high-resolution optical coherence tomography (OCT) of the ACS-causing culprit lesion and re-evaluation by a second OCT-core lab, thirty-two IFC-ACS-patients were matched to thirty-two RFC-ACS-patients by gender, age and diabetes. Local and systemic blood samples were obtained from the site of the ACS-causing culprit lesion (LOC) and from the arterial sheath (SYS), respectively. Neutrophil abundance and surface marker expression were quantified by flow cytometry. Fresh neutrophils were isolated for functional analysis and ex-vivo assessment of cell-toxicity in a co-culture with human aortic endothelial cells (HAECs). Neutrophil secretion of active MMP9 was evaluated by fluorescence-based zymography in supernatants of isolated neutrophils and in patients' plasma samples.

Results: Neutrophils of patients with IFC-ACS show significantly higher expression of the toll-like receptor 2 (TLR2) in comparison to RFC-ACS-derived neutrophils (LOC: 1991±492.8 vs. 1615±440.2; p=0.01; SYS: 2062±464.4 vs. 1670±525.1; p=0.0056). Ex-vivo TLR2-stimulation of local neutrophils in patients with IFC-ACS led to increased toxicity of their secretome and aggravated endothelial cell death in co-culture, as compared to neutrophils from RFC-ACS patients (+59% dead HAECs, IFC-LOC vs. RFC-LOC; p=0.0078). Furthermore, TLR2-stimulation using Pam3CSK4 triggered higher activity rates of MMP9 exclusively in local neutrophils of IFC-ACS-patients (+38.9%±6.1% in IFC-LOC vs. RFC-LOC; p=0.0154). This effect was reversed in IFC-ACS-derived neutrophils being pre-treated with an anti-TLR2 neutralizing antibody (–58.4% ±5.2%, IFC-LOC-anti-TLR2 vs. IFC-LOC-vehicle; p=0.0069). Additionally, MMP9 activity was higher in plasma obtained from the culprit site of IFC-ACS patients (74.1 U/ml ±4.1 vs. 70.0 U/ml ±5.1, IFC-LOC vs. RFC-LOC; p=0.01).

Conclusion: The current study demonstrates novel TLR2-dependant neutrophil activation patterns at the coronary culprit lesion of IFC-ACS, leading to higher endothelial cell toxicity and MMP9 activity. Further studies need to assess whether a temporary blockade of TLR2 activation could be a possible therapeutic target in the era of personalized medicine.