Circulating CD31 reflects endothelial activity and is associated with a lower risk for cardiovascular complications

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\textbf{Background:} CD31 is a membrane receptor expressed by leucocytes, platelets and endothelial cells. CD31 regulates endothelial leukocyte transmigration and is key in maintaining endothelial homeostasis. An increased endothelial activation is suggested to contribute to atherosclerotic plaque inflammation but studies focusing on CD31 in atherosclerosis have provided evidence for both pro- and anti-atherosclerotic effects. However, which biological processes that are reflected by circulating soluble CD31 (sCD31) in human atherosclerosis remain unknown.

\textbf{Purpose:} Here, we aimed to explore 1) which biological processes circulating sCD31 reflects in the human atherosclerotic plaque and circulation 2) if sCD31 levels predicts cardiovascular death in individuals with carotid atherosclerosis and 3) if circulating sCD31 is causally associated to coronary artery disease (CAD) and ischemic stroke, in order to investigate its role as a biomarker of atherosclerotic disease and complications.

\textbf{Methods:} Cardiovascular associated plasma proteins (n=543) and carotid plaque proteins (n=197) were assessed by a proximity extension assay (Olink, SciLife) in the Carotid Plaque Imaging Project biobank. Plaques were phenotyped by immunohistochemistry. CD31 was located by immunofluorescence. Human carotid artery endothelial cells (HCAECs) were stimulated with IL-6 and TNF-\textgreek{a} to assess sCD31 release. National registries were used to identify cardiovascular death during follow up. All patients gave written informed consent. The study was approved by the local ethical review board and followed the declaration of Helsinki. Causal effects for sCD31 on stroke and CAD were examined by Mendelian randomization (MR). Summary statistics for GWAS of circulating CD31, coronary artery disease and stroke were retrieved from the SCALLOP study, the CARDioGRAMplusC4D and the MEGASTROKE consortia.

\textbf{Results:} Plasma sCD31 correlated to plaque sCD31, TNF-\textgreek{a}, MMP-2, MMP-9 levels and CD68\textgreek{+} plaque area. Plasma sCD31 also correlated to plasma CSF-1, MCP-1 and IL-8 levels. Clustering and enrichment analyses revealed that plasma sCD31 was associated to angiogenesis, cell migration and inflammatory responses. CD31 was pre-dominantly expressed on the plaque endothelium. TNF-\textgreek{a} and IL-6 induced HCAECs sCD31 release in vitro. Furthermore, low levels of circulating sCD31 predicted cardiovascular death and our MR analyses suggested that low circulating sCD31 levels were causally associated with a higher risk of stroke and CAD.

\textbf{Conclusions:} The present study shows that circulating sCD31 is released from endothelial cells due to inflammatory stimuli and reflects plaque inflammation. However, our follow-up data and MR analyses suggest that circulating sCD31 is associated with a lower risk for cardiovascular complications. This indicates that circulating sCD31 is not only a marker of an inflammatory response but also a marker of a functional endothelium, able to maintain the endothelial homeostasis.