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Inflammation and Calcification in Calcific Aortic Valve Disease in Patients with and without Diabetes

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Calcific aortic valve disease (CAVD) is the most common valvular heart disease and is thought to evolve from inflammatory pre-conditions in the valve. Potentially, osteogenic differentiation of valvular interstitial cells (VICs) and myofibroblasts may lead to bone-like formation of calcification. Diabetes mellitus type II (DMII) is considered to be associated with the pathogenesis of CAVD. However, the connection between DMII and CAVD still remains to be elucidated.

The present study is based upon the hypothesis that signs of inflammation and calcification are increased in diabetic compared to non-diabetic CAVD patients. Therefore, 45 CAVD human aortic valve samples were examined macroscopically with gross measurements of calcified areas and by the use of immunohistochemistry and immunofluorescence staining. Alizarin red (calcium deposition) as well as early (Runx2, ALP) and mature (osteopontin, osteocalcin) calcification markers were chosen for evaluation of calcification, Ostesense served as an indicator of microcalcification. Annexin II, V and VI were assessed in regard to their potential role in microcalcification. Prior to this study, annexins have not been investigated in human heart valves. Inflammation was measured by infiltration of lymphocytes ($CD4^+$ and $CD8^+$ T cells), macrophages ($CD68^+$ cells) and expression of the pro-inflammatory protein S100A9. All stainings were quantified by measuring the percentage of positively stained area.

Runx2 and ALP expression was significantly elevated in diabetic patients suggesting increased calcification formation with more cells undergoing osteogenic differentiation. Microcalcification was also augmented, as detected by Osteosense. Annexin II and VI expression correlated with increased microcalcification which might indicate a potential participation of annexins in the formation of microcalcification in CAVD. Diabetic patients showed significantly larger macroscopically visible calcification areas and calcium deposits, as indicated by alizarin red. Mature calcification markers (osteocalcin, osteopontin) were expected to be in equal measure elevated but no difference between diabetic and non-diabetic patients was identified. These findings contrast strongly with the increased macroscopic calcification detected in diabetic patients thus questioning the viability of these markers in the context of

aortic stenosis. Surprisingly, significantly *de*creased expression of S100A9 and the presence of fewer macrophages in aortic valves of diabetic patients suggest a *reduction* of inflammation in these patients. Likewise, evaluation of lymphocytes revealed a *de*crease of $CD4^+$ T cells in diabetic patients whereas $CD8^+$ T cell count was similar in both groups.

It can be concluded that there is more calcification in diabetic compared to non-diabetic patients. Considering the generally accepted theory of an inflammation-dependent mechanism of calcification, this data suggests that at the time of valve replacement surgery, diabetic patients are in a more advanced disease stage than non-diabetic patients, in whom inflammation is still more abundant.

As no drugs are available for prevention or deceleration of disease progression, surgical or interventional aortic valve replacement remains the only beneficial treatment option for AS patients. Further studies directed to clarifying pathologic mechanisms of CAVD could enable the development of novel targeted pharmaceuticals. Eventually, disease progression may be prevented if intervention is carried out in a stage of early microcalcification. Therefore, novel imaging technologies detecting early stages of microcalcification *in vivo* are highly desirable.

While it is widely recognized that DMII constitutes a relevant risk factor for CAVD, this study was the first to investigate the impact of DMII on inflammation and calcification in CAVD tissue samples. This examination emphasized the theory of inflammation-dependent calcification which warrants further studies on mechanisms underlying this process. As the impact of DMII on CAVD was found to be considerable, this study suggests clinical evaluation of screenings for CAVD in diabetic patients, as this group does not only show a higher risk of CAVD development but also appears to have a substantially accelerated course of disease.