Histological evidence for impaired myocardial perfusion reserve in severe aortic stenosis

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Myocardial perfusion reserve is impaired in aortic stenosis (AS) even in the absence of epicardial coronary stenosis (1), which may be due to reduced capillary density and endothelial dysfunction (2,3). However, the relationship between histopathological abnormalities of the vasculature and myocardial perfusion reserve on CMR has never been examined in severe AS. Understanding the pathophysiological mechanisms is important for advancing therapeutic strategies in AS - treatments that could potentially improve endothelial function may be beneficial in delaying the onset of symptoms in asymptomatic AS, or for symptomatic patients who are not candidates for aortic valve intervention. We sought to characterise microvascular abnormalities in the myocardium of patients with severe AS and examine their relationship with myocardial perfusion reserve as assessed by stress perfusion cardiovascular magnetic resonance (CMR).

Fourteen patients (mean age 71±7, 79% male) with normal coronary angiogram, scheduled for aortic valve replacement (AVR) were recruited. Patients with left ventricular (LV) ejection fraction <50%, uncontrolled hypertension, contraindications to CMR, significant coronary artery disease and underlying cardiomyopathy were excluded. Heart samples from nine age- and sex- matched subjects who suffered from non-cardiac deaths served as controls. The hearts were macroscopically and microscopically normal as assessed by an expert cardiac pathologist. AS patients underwent CMR scanning for cardiac function, mass, adenosine stress perfusion (n=12, 2 patients did not tolerate adenosine) and late gadolinium enhancement (LGE). Ten patients had a follow-up CMR scan 6 months post AVR. Myocardial biopsies were obtained from the basal anteroseptum during AVR. Control tissues were obtained from identical locations. Two consecutive sections were stained with smooth muscle actin (SMA) antibody and anti-CD31 for endothelium. CD31 and SMA antibodies stained images were quantified over an average of 10 power fields. Additionally, qualitative

assessment of presence of both SMA+ve and CD31+ve were undertaken for the same section of tissue.

As expected, AS patients (all tricuspid aortic valve) had concentric LV hypertrophy with increased LV mass index. There was high prevalence of myocardial fibrosis (n=12, 86%) on qualitative LGE assessment. When compared to controls, severe AS had reduced myocardial vessel count as shown by reduced total quantity of SMA+ve vessels (AS: 135±75/mm^2 vs control 386±155/mm^2, $p<0.001$) and CD31+ve (AS: 500±251/mm^2 vs control: 786±236/mm^2, p=0.01), Figure 1. On qualitative assessment, there was marked loss of endothelial cells (absence of CD31+ve) but intact smooth muscle (SMA+ve), lining the arterioles in severe AS when compared to controls. Patients with an aortic valve area (AVA) ≤0.8cm^2 (n=6) had greater endothelial loss compared to those with an AVA >0.8 and ≤1.0cm^2 (n=8); (AVA >0.8: 1.23±0.40% vs 2.77±1.11% AVA >0.8 and ≤1.0cm^2, p=0.007). The total area of CD31+ve endothelium correlated with AVA (r=0.58, p=0.03). In the 12 patients who underwent adenosine stress perfusion, mean myocardial perfusion reserve index (MPRI) was 0.88±0.24. This was less than two standard deviations from our previously published normal MPRI range (1.7±0.3) from healthy volunteers of similar age and gender (1), indicating severe impairment in myocardial perfusion reserve in the AS patients enrolled in this study. Endothelial loss and not vascular density also correlated with reduced MPRI, r=0.66, p=0.019 (Figure 1), which improved significantly post AVR (from 0.95±0.17 to 1.50±0.43, p=0.018).

In this study, we have shown that the myocardium of patients with severe AS is not only characterised by capillary rarefaction but also endothelial cell loss. The extent of endothelial cell loss was associated with increasing severity of AS. Vascular endothelial loss, and not capillary rarefaction, correlated best with myocardial perfusion reserve.

The vascular endothelium plays an important homeostatic role in maintaining vasomotor tone by regulating both nitric oxide synthesis and release which in turn causes smooth muscle relaxation and vasodilation (4). Therefore, the loss of endothelium is likely to contribute to the impaired vasoreactivity and attenuated perfusion reserve seen in severe AS. Although myocardial biopsies post AVR were not available for a repeat histological assessment, myocardial perfusion reserve on CMR considerably improved post AVR. This suggests that the process of endothelial cell loss may be reversible and presents an opportunity for further research.

Furthermore, although the onset of symptoms in severe AS is an indication for AVR, the detection of microvascular dysfunction could potentially help select individuals more likely to benefit from AVR as an alternative to the current symptom-based approach. In addition, given that AVR may be associated with operative risks, therapies that slow progression of disease or delay the onset of symptoms could be beneficial. Indeed, treatments found to be protect endothelial function, such as renin-angiotensin aldosterone system blockade, have been shown to slow the progression of AS (5). Future studies targeting the preservation of endothelial function, and in turn myocardial perfusion reserve, could therefore be important given its potential to delay symptom-onset in severe AS and reduce the need for aortic valve intervention.

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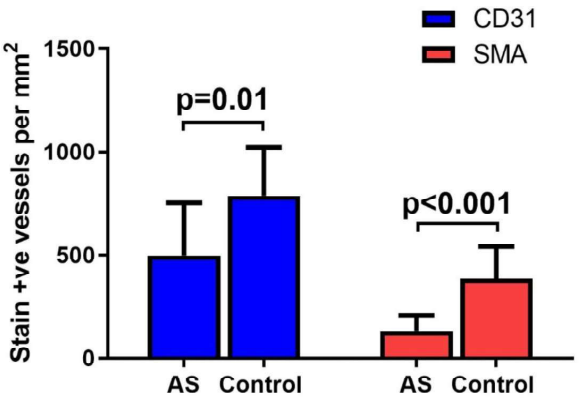
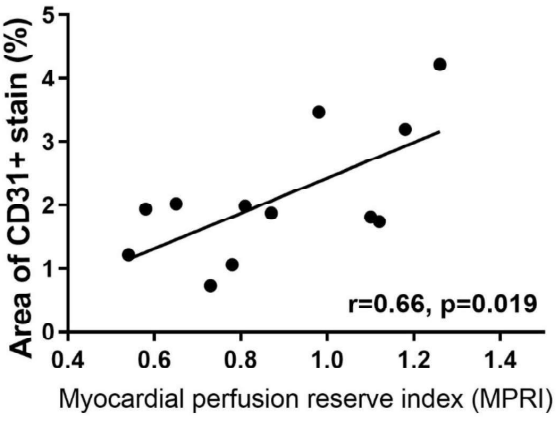
Figure legend

Figure 1. A - Bar chart shows reduced number of vessels in aortic stenosis (AS) when compared to control (samples from non-cardiac death). **B** - Relationship between CD31+ve endothelium with myocardial perfusion reserve index (MPRI). **C** - Consecutive SMA and CD31 staining of myocardial samples. Red arrows show an example of high power magnification showing absence of CD31 staining in the same arteriole identified by SMA+ve indicating endothelial cell loss in AS.

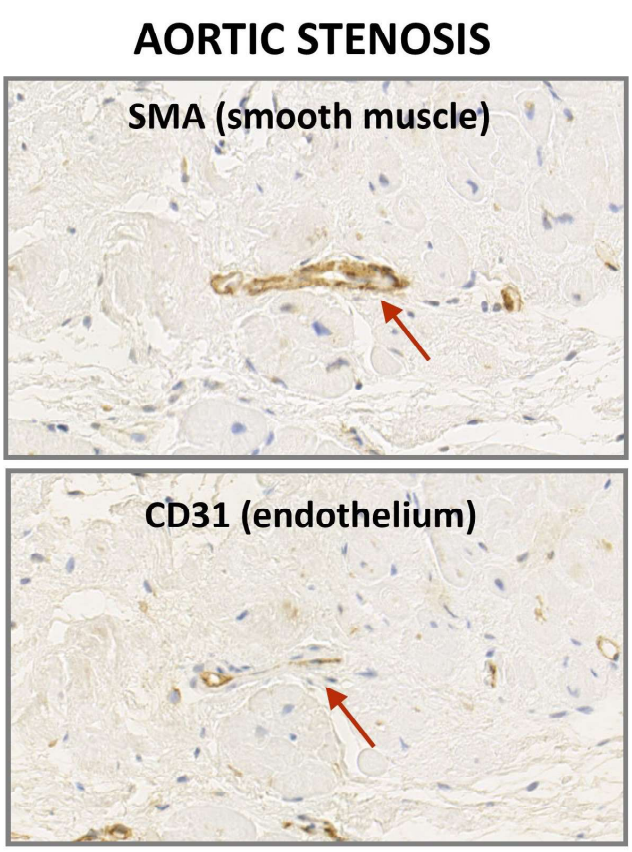
A


CD31
SMA
Stain +ve vessels per mm2
1500
1000
500
0
p=0.01
p<0.001
AS
Control
AS
Control

B


Area of CD31+ stain (%)
5
4
3
2
1
0
r=0.66, p=0.019
0.4
0.6
0.8
1.0
1.2
1.4
Myocardial perfusion reserve index (MPRI)

C


AORTIC STENOSIS
SMA (smooth muscle)
CD31 (endothelium)

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Article entitled: Histological evidence for impaired myocardial perfusion reserve in severe aortic stenosis

Manuscript number: JIMG040219-0429-T

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