

## SCIENTIFIC LETTERS

### Plasma fibrinogen, soluble P-selectin, and von Willebrand factor in aortic valve disease: evidence for abnormal haemorrhology, platelet activation, and endothelial dysfunction

Haemodynamic studies have shown that diseased cardiac valves, whether stenosed or incompetent, create regions of increased turbulence and shear stresses that are large enough to damage the vascular endothelium and cellular blood elements, leading to abnormal haemorrhology, platelet activation, and endothelial dysfunction.<sup>1</sup> For example, the intensity of turbulence in patients with pure aortic stenosis (AS) may be 10 times greater than normal while the intensity of turbulence in patients with pure aortic regurgitation (AR) may be three times greater than normal.<sup>1</sup>

We hypothesised that patients with aortic valve disease may show abnormal haemorrhology, platelet activation, and endothelial dysfunction, that may increase their risk of thromboembolism. These abnormalities may perhaps reflect haemodynamic changes resulting from AS or AR, in particular their respective severity. To test our hypothesis, we measured plasma concentrations of soluble P-selectin (sP-sel, a marker for platelet activation<sup>2</sup>), von Willebrand factor (vWf, a marker for endothelial cell dysfunction<sup>3</sup>) and fibrinogen (as an index of haemorrhology and a clotting factor), in 61 patients with moderate to severe aortic valve disease in sinus rhythm.

We recruited consecutive patients attending outpatient clinics or admitted to our regional referral cardiothoracic unit with primary (native) aortic valve disease. We excluded patients with atrial fibrillation, patients on warfarin, statins or hormone replacement therapy, those with double valve disease (namely, mitral and aortic valve disease) and associated medical conditions known to influence the markers under investi-

gation (including coronary artery or peripheral artery disease, cerebrovascular disease, diabetes mellitus, hypertension, etc). Clinical assessment included transthoracic echocardiography to ascertain the transvalvar peak velocity and gradient across the aortic valve in patients with AS, and Doppler echocardiography to assess the severity of aortic regurgitation. Venous blood was obtained (atraumatically) from all eligible patients into 0.105 M sodium citrate vacutainer tubes. The blood samples were immediately centrifuged at 3000 rpm for 20 minutes at 40°C and the plasma separated and stored at -70°C until analysed for the three plasma markers under investigation. Plasma samples obtained were analysed in batches for sP-sel (ELISA, R&D Systems, UK), vWf (ELISA, Dako, Denmark), and fibrinogen (g/l) modified Clauss assay using thrombin (Pacific Haemostasis, California, USA). Baseline blood parameters were compared to 61 age and sex matched healthy controls in sinus rhythm.

We studied 61 patients (mean (SD) age 65 (12) years, 36 males) with sinus rhythm (table 1). Patients with aortic valve disease had significantly higher concentrations of plasma fibrinogen (3.5 (1.1) v 3.0 (0.6) g/l,  $p = 0.01$ ) and vWf (120 (32) v 93 (29) IU/dl,  $p = 0.00008$ ), when compared to healthy controls. There was a trend towards higher sP-sel concentrations (76 (33) v 94 (39) ng/ml,  $p = 0.06$ ), which was of borderline significance. When concentrations of the three markers were compared between patients with AR and those with AS (table 1), there were no significant differences in the plasma concentrations of fibrinogen ( $p = 0.3$ ), vWf ( $p = 0.3$ ) or sP-sel ( $p = 0.4$ ) between the two groups.

There was a significant positive correlation between age and plasma concentrations of fibrinogen (Pearson's  $r = 0.3$ ,  $p = 0.01$ ) and vWf ( $r = 0.4$ ,  $p = 0.002$ ) but not sP-sel ( $r = -0.09$ ,  $p = 0.5$ ), suggesting that plasma concentrations of fibrinogen and vWf increased with increasing age of patients. There were no differences in the plasma concentrations of fibrinogen (3.9 (1.1) v 3.5 (1.2) g/l,  $p = 0.09$ ), sP-sel (79 (39) v 74 (26) ng/ml,  $p = 0.6$ ) or vWf (126 (31) v 123 (38) IU/dl,  $p = 0.8$ ) between patients presenting in New York

Heart Association (NYHA) functional class III or IV and those in NYHA class I and II.

In patients with AR there was a significant positive correlation between the severity of AR and plasma concentrations of fibrinogen (Spearman's  $r = 0.5$ ,  $p = 0.04$ ) but not vWf ( $r = 0.2$ ,  $p = 0.4$ ) and sP-sel ( $r = 0.04$ ,  $p = 0.9$ ), suggesting that severe AR was associated with higher concentrations of fibrinogen compared to moderate AR. In patients with AS, there was no significant correlation between the transvalvar aortic gradient and plasma concentrations of fibrinogen ( $r = -0.5$ ,  $p = 0.8$ ), sP-sel ( $r = 0.2$ ,  $p = 0.9$ ), and vWf ( $r = 0.03$ ,  $p = 0.9$ ).

In patients presenting with severe aortic valve disease, stepwise regression analysis showed that the type of valve lesion (that is, aortic stenosis or regurgitation,  $p = 0.03$ ) and age ( $p = 0.00067$ ) (and hence patients selected to receive mechanical or biological implants ( $p = 0.01$ )), were independent predictors for raised plasma concentrations of vWf. There were no independent predictors for plasma fibrinogen and sP-sel concentrations.

In the present study, we have shown that patients with aortic valve disease have significantly higher concentrations of plasma fibrinogen and vWf, when compared to healthy age and sex matched controls, suggesting endothelial dysfunction and abnormal haemorrhology in these patients. There was an increase of sP-sel concentrations of borderline significance, which suggests that some platelet activation may perhaps be present. These observations suggest that aortic valve disease may confer a hypercoagulable state, and confirms our previous population controlled study demonstrating increased plasma fibrinogen concentrations in patients with aortic stenosis.<sup>4</sup>

Plasma fibrinogen (a plasma protein and clotting factor) may predispose to thrombus formation by increasing fibrin turnover, causing platelet aggregation and promoting stasis. The higher plasma concentrations of fibrinogen in patients with aortic valve disease, compared to healthy controls, are in keeping with increased plasma viscosity and abnormal rheology of blood flow in moderate and severe aortic valve disease.<sup>4</sup> Furthermore, there was a significant positive correlation between severity of AR and plasma fibrinogen concentrations. As severe AR is associated with left ventricular dysfunction, this correlation suggests that severe AR may perhaps be associated with increased stasis and hypercoagulability.

The raised concentrations of vWf in patients with AS suggests increased endothelial damage in these patients, perhaps secondary to increased turbulence of flow distal to the stenosed valve. Stasis proximal to the diseased valve may also have contributed to a degree of endothelial dysfunction.<sup>1</sup> Likewise, in patients with AR, the higher plasma concentrations of vWf are in keeping with the hypothesis that the regurgitant jet of AR may result in increased endothelial damage or dysfunction, caused by regions of increased turbulence and shear stresses within the left ventricle.

Nevertheless, the positive correlation between plasma fibrinogen and vWf concentrations and age suggests that age, in addition to severity of aortic valve disease, may play a significant role in predisposing patients to a prothrombotic or hypercoagulable state and thrombogenesis, especially since the incidence of stroke rises exponentially with age.<sup>5</sup>

Table 1 Characteristics of patients admitted with aortic valve disease and comparisons between patients with aortic stenosis (AS) and aortic regurgitation (AR)

	Whole cohort (n = 61)	AS (n = 44)	AR (n = 17)		AS v AR p value
Clinical parameters*					
Mean (SD) age (years)	65 (12)	68 (10)	59 (14)	t = -2.6	0.01
Males:females	36:25	23:21	13:4	$\chi^2 = 2.97$ ; df = 1	0.08
Mean (SD) body surface area	1.8 (0.2)	1.8 (0.2)	1.8 (0.2)	t = -0.5	0.6
Aetiology					
Calcification	69 (42)	89 (39)	18 (3)		
Rheumatic	8 (5)	7 (3)	12 (2)		
Myxomatous	13 (8)	-	47 (8)		
Others	11 (6)	5 (2)	25 (4)		
NYHA class					
I and II	62 (38)	43 (24)	82 (14)	$\chi^2 = 4.04$ ; df = 1	0.04
III and IV	38 (23)	57 (20)	18 (3)		
Antithrombotic treatment					
Aspirin	41 (25)	48 (21)	14 (4)	$\chi^2 = 2.9$ ; df = 1	0.08
Echocardiographic data					
Mean (SD) transvalvar gradient	-	74 (34)	-		
Severity of AR					
Moderate	-	-	35 (6)		
Severe	-	-	65 (11)		
Mean (SD) plasma concentrations of coagulation markers					
Plasma fibrinogen (g/l)	-	3.6 (0.6)	3.4 (1.3)		0.3
Soluble P-selectin (ng/ml)	-	80 (37)	92 (32)		0.3
von Willebrand factor (IU/dL)	-	127 (31)	120 (42)		0.4

\*Values are percentages (numbers) unless otherwise specified

Age was also an independent predictor for vWf concentrations on stepwise multiple regression analysis. There was, however, no significant association between the degree of clinical deterioration (as indicated by NYHA class) and plasma concentrations of these markers.

Since initiation of thrombus formation at the site of diseased valves has been ascribed to endothelial dysfunction and increased coagulability, the present study supports the view that diseased aortic valves, whether AS or AR, may be "traumatic" to the vascular endothelium (or endocardium), resulting in a hypercoagulable state which may contribute to the risk of thromboembolism in these patients.

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### Raised interleukin 6 concentrations as a predictor of postangioplasty restenosis

The most common pathogenesis of coronary artery disease is atherosclerotic plaque formation in the coronary arteries leading to narrowing of the blood vessel and impairment of blood flow.<sup>1,2</sup> Therapeutic measures are aimed at revascularisation and increased blood flow. While coronary angioplasty is widely used today, one shortcoming of this

procedure is the occurrence of restenosis in which the lesion regenerates in approximately 30% of patients within three months, necessitating reoperation.<sup>3</sup> Although the pathogenic mechanisms of restenosis have been extensively studied in recent years, the underlying mechanisms are still not fully understood but are thought to be associated with neointimal formation as part of an inflammatory response to vascular injury.

Of the inflammatory mediating substances, the interleukins are the most well characterised. Interleukin 6 (IL-6) is the predominant determinant for production of acute phase proteins (for example, C reactive protein), and shows proinflammatory properties in addition to cellular effects (for example, smooth muscle hyperplasia) which are associated with restenosis. IL-6 concentrations which are raised in unstable angina are also raised after angioplasty, suggesting that IL-6 may therefore be a sensitive marker reflective of the postprocedure initial inflammatory response, and also a possible predictor of later restenosis.<sup>4,5</sup> A relation between IL-1 concentrations and luminal re-narrowing has been shown.<sup>6</sup> We conducted a prospective study to assess whether circulating IL-6 concentrations are associated with restenosis.

In 20 patients with stable angina pectoris undergoing elective coronary angioplasty, IL-6 concentrations were measured before, immediately after, and at one and six hours postprocedure by an enzyme immunoassay (Fujirebio, Tokyo, Japan); a coronary angiogram was done six months postprocedurally to assess the presence of restenosis (> 50% lumen loss).

Of the 20 patients examined, nine showed restenosis. Mean (SD) circulating baseline IL-6 concentrations did not differ regardless of presence of restenosis, being within the normal range for either group (3.3 (2.9) v 1.9 (0.7) pg/ml for non-restenosis v restenosis, respectively; normal reference 2.8 (1.6) pg/ml). Patients with restenosis, however, showed significant ( $p < 0.05$ ) increases in IL-6 concentrations at one hour ( $p = 0.03$ ) and six hours ( $p < 0.01$ ) postprocedure with 3.6 and 4.4-fold increases, respectively. Circulating IL-6 concentrations at six hours postprocedure for the group with restenosis also exceeded the normal range (> 2 SD). In contrast, the non-restenosis group did not show significant increases at any time point (fig 1). There was no significant difference in prevalence of coronary risk factors, including hypertension, hyperlipidemia, diabetes mellitus, and gout as well as previous myocardial infarction and past coronary bypass, between the groups with and without restenosis.

The finding that postprocedure IL-6 concentrations are increased in patients with restenosis following coronary angioplasty suggests that IL-6 may be a predictive marker

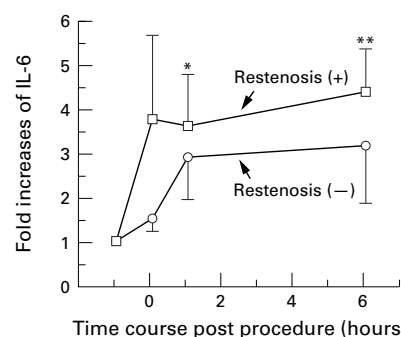


Figure 1 Circulating IL-6 concentrations in patients with and without restenosis. Graph shows fold increases of IL-6 concentrations at measured time point against baseline. Error bars show SEM. \* $p < 0.05$  against baseline; \*\* $p < 0.01$  against baseline.

of ensuing restenosis. Although the number of patients examined was small and the specificity of the IL-6 response to the coronary artery unclear, based on our preliminary findings targeted anti-inflammatory treatment may be warranted as an adjunct for patients with raised IL-6 concentrations following angioplasty. Further large scale studies should clarify the role of IL-6 concentrations as a predictive and therapeutic marker of restenosis.

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