

Low Circulating Monocyte Count is Associated with Severe Aortic Valve Stenosis

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ABSTRACT: **Background:** The pathophysiology of aortic stenosis (AS) involves inflammatory features including infiltration of the aortic valve (AV) by activated macrophages and T cells, deposition of lipids, and heterotopic calcification.

Objectives: To evaluate the correlation between white blood cell (WBC) differential count and the occurrence and progression of AS.

Methods: We identified in our institutional registry 150 patients with AS who underwent two repeated echo studies at least 6 months apart. We evaluated the association between the average of repeated WBC differential counts sampled during the previous 3 years and subsequent echocardiographic AS indices.

Results: There was no significant difference in total WBC, lymphocyte or eosinophil count among mild, moderate or severe AS groups. There was a progressive decrease in monocyte count with increasing AS severity ($P = 0.046$), more prominent when comparing the mild and severe groups. There was a negative correlation between AV peak velocity or peak or mean gradient and monocyte count in the entire group ($r = -0.31, -0.24, \text{ and } -0.25$ respectively, all $P \leq 0.01$). Similar partial correlations controlling for age, gender, hypertension, smoking, dyslipidemia and ejection fraction remained significant. The median changes over time in peak velocity and peak gradients in AS patients were 0.44 (0–1.3) m/sec/year and 12 (0–39) mmHg/year, respectively. There was no correlation between any of the WBC differential counts and the change in peak velocity or peak gradient per year.

Conclusions: Severe AS is associated with decreased total monocyte count. These findings may provide further clues to the mechanism underlying the pathogenesis of aortic stenosis.

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total cholesterol levels in patients with aortic stenosis involving three-cuspid aortic valves compared to patients without aortic stenosis [8]. Moreover, patients with familial homozygous hyperlipidemia usually develop calcific depositions on their aortic valve cusps at a very young age [9].

Growing evidence points towards a “response to injury” mechanism as the cause of degenerative calcific aortic valve disease, similar to what has been described for vascular atherosclerosis. The hallmark of end-stage aortic stenosis is calcification which occurs predominantly on the aortic surface of the valve [10]. In a recent study [11], fluorodeoxyglucose positron emission tomography uptake was assessed in patients with varying degrees of aortic stenosis. FDG uptake was increased in mild and moderately stenotic valves but not in severely calcified stenotic valves, implying that the inflammatory process diminishes in the late stages of aortic stenosis.

Aortic valves at different stages of the disease have histologic components seen also in atherosclerotic plaques, such as macrophages, foam cells, T lymphocytes, lipoproteins, heterotopic calcification, and bone tissue [3,10,12–15]. The deposition of lipids and other compounds in the aortic valve activate valvular myofibroblasts, resulting in their osteoblastic differentiation and setting the stage for extracellular matrix remodelling and neovascularization and ultimately leading to active calcification [3,4–6,10,12]. Inflammatory infiltration of activated macrophages and T cells, as well as cytokine release, have been reported in stenotic aortic valves [3,12,15], and enhanced levels of mRNA of inflammatory leukotriene pathway enzymes are found in thickened stenotic aortic valves and correlate with stenosis severity [16].

Given the association between inflammation and the pathophysiology of aortic stenosis, our objective was to test the hypothesis that white blood cell differential counts averaged over an extended period are associated with aortic stenosis occurrence and progression.

PATIENTS AND METHODS

We identified in our institutional echocardiography lab registry 150 consecutive patients with aortic stenosis who had two

Previous studies have shown that aortic stenosis is not simply a passive degenerative process but rather represents an active chronic inflammatory process with risk factors similar to those of atherosclerosis [1–7]. Hyperlipidemia is a well-established risk factor, as exemplified by higher levels of serum

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FDG = fluorodeoxyglucose

or more repeated echo studies at least 6 months apart during the period 2007 to 2010. We then accessed their electronic outpatient records and collected clinical data including all blood counts taken during the 3 years that preceded the earliest echocardiography study for each patient (referred to here as the “index echo study”), medical history, medication use at the time of the index echo study, and all repeated echocardiography data. For the control group we identified comparative consecutive elderly patients who underwent echocardiography at our institution and in whom aortic stenosis was excluded. We excluded patients with malignant or chronic inflammatory disease, patients who had been on chronic steroid or immunosuppressive treatment, and blood counts that were higher or lower than 2 standard deviations of all the collective blood counts. In patients who underwent aortic valve replacement by open surgery, percutaneous catheterization or percutaneous balloon valvuloplasty, any echocardiograms performed after such procedures were excluded. We also excluded patients for whom at least two blood counts taken at least 6 months apart were not available. After application of the exclusion criteria, we included for our analysis a total of 185 patients: 136 patients with aortic stenosis and 49 control patients without.

All repeated WBC differential counts over 3 years preceding the index echo study for each patient were averaged and these averaged values were used in the final analysis. The study was approved by our Institutional Ethics Committee. The authors had full access to the data and take responsibility for its integrity.

TRANSTHORACIC ECHOCARDIOGRAPHY

All echocardiographic studies were performed at our institution using commercially available ultrasonographic instruments. Standard M-mode, two-dimensional, continuous-wave Doppler of the aortic, mitral and tricuspid flow as well as pulsed-wave Doppler examination of the left ventricular outflow tract and mitral inflow were performed in each subject in accordance with the guidelines of the American Society of Echocardiography [17]. The morphology of the aortic valve was assessed in short and long-axis echocardiographic views. Aortic valve area was calculated by the continuity equation. Aortic stenosis severity was graded as mild, moderate or severe according to guidelines [17,18]. Ejection fraction was determined by the visual estimate method.

WBC COUNT ASSESSMENT

Complete blood counts, including a WBC differential, were performed using hematology analyzer Pentra 120 Retic (ABX Diagnostic, Montpellier, France).

STATISTICAL ANALYSIS

Continuous data are presented as medians and interquartile ranges (25th–75th percentiles) for skewed distributed variables

or as mean \pm SD when normally distributed, and categorical data are presented as absolute numbers and respective percentages. All *P* values were two-tailed and differences were considered significant if *P* values were ≤ 0.05 . Non-Gaussian distributed continuous parameters were analysed with non-parametric Mann-Whitney (for two-group comparison) or Kruskal-Wallis test (for multiple group comparison). Normally distributed continuous parameters in multiple subgroups were compared by the ANOVA test. Bonferroni post hoc correction was used for significant differences. Categorical data were compared with the χ^2 test.

In the second step, correlation analyses between echocardiographic parameters related to aortic stenosis and WBC counts were provided using Pearson correlation coefficients. Partial correlation analysis was performed for significant correlations controlling for age, gender, ejection fraction and aortic valve calcification.

Receiver-operating characteristic curve analyses were used to determine the ability of WBC absolute and differential counts to distinguish between patients who had an increase in peak aortic jet velocity above 0.3 m/sec within 1 year and those whose peak aortic jet velocity change was below this cutoff point, which denotes rapid hemodynamic aortic stenosis progression [19].

RESULTS

Patient characteristics are outlined in Table 1. Patients with aortic stenosis were older and more likely to be female than patients in the control group (without aortic stenosis). There were no differences in any of the background illnesses or medications between these two groups. Patients with aortic stenosis were more likely to have mitral annular calcification, thicker interventricular septum and higher pulmonary artery systolic pressure than non-aortic stenosis patients. Aortic stenosis severity correlated positively with increased age and interventricular septal thickness.

Peak and mean aortic valve gradients were 23 mmHg (range 19–28), 39 mmHg (36–44) and 77 mmHg (66–94) ($P < 0.001$), and 13 mmHg (10–15), 22 mmHg (20–27) and 48 mmHg (40–58) ($P < 0.001$) in mild, moderate and severe aortic stenosis, respectively. The calculated aortic valve area was 1 cm² (0.8–1.1) in moderate aortic stenosis and 0.7 cm² (0.6–0.8) in severe aortic stenosis. The median interval between the index echo study and the repeated study was 7.8 months (6.2–12.1). This interval tended to be shorter in the severe aortic stenosis group; however, this difference was not statistically significant between the three groups ($P = 0.12$).

RELATIONSHIP OF WBC DIFFERENTIAL COUNTS AND AORTIC STENOSIS INDICES

The median number of WBC counts available for each patient was 3. There were no differences in the total WBC, neutro-

WBC = white blood cells

Table 1. Baseline characteristics of patients with aortic stenosis and control group

	Control	Aortic stenosis			P†	P‡
	No AS (n=49)	Mild (n=42)	Moderate (n=39)	Severe (n=53)		
Age (yr)	68 (60–75)	76 (69–80)	76 (67–81)	78 (72–86)	< 0.001*	0.07
Female	10 (20%)	22 (54%)	24 (61%)	32 (60%)	< 0.001*	0.7
Medical history						
IHD	28 (56%)	24 (58%)	15 (39%)	24 (46%)	0.4	0.2
HTN	39 (78%)	31 (76%)	32 (84%)	40 (77%)	1.0	0.6
DM	15 (30%)	22 (54%)	16 (42%)	18 (35%)	0.13	0.2
Dyslipidemia	37 (74%)	36 (88%)	33 (87%)	42 (81%)	0.13	0.6
Smoker	4 (8%)	4 (10%)	4 (10%)	10 (19%)	0.4	0.3
CRF	7 (14%)	13 (32%)	16 (42%)	11 (22%)	0.02	0.1
Medications						
ACEI	26 (59%)	19 (47%)	19 (27%)	32 (61%)	0.6	0.35
Beta-blocker	33 (73%)	26 (65%)	21 (57%)	28 (54%)	0.08	0.5
Calcium channel blocker	12 (29%)	9 (22%)	15 (39%)	19 (36%)	0.7	0.2
Statin	37 (79%)	28 (70%)	25 (66%)	34 (65%)	0.14	0.8
Echo						
MAC					< 0.001*	0.08
0	43 (88%)	23 (57%)	14 (38%)	14 (27%)		
1	3 (6%)	8 (20%)	12 (32%)	15 (28%)		
2	2 (4%)	2 (5%)	4 (11%)	9 (17%)		
IVS (mm)	11 (10–13)	12 (11–13)	13 (12–14)	13 (13–14)	< 0.001*	0.001*
LVEDD (mm)	47 (44–52)	49 (44–56)	49 (46–54)	48 (43–53)	0.46	0.36
PASP (mmHg)	34 (27–40)	38 (35–45)	41 (35–46)	43 (35–51)	0.002*	0.3

*Statistically significant ($P < 0.05$)

†Value for comparison between control patients and AS patients

‡Value for comparison between AS severity groups only

ACEI = angiotensin-converting enzyme inhibitor, MAC = mitral annulus calcification (0 = none, 1 = mild, 2 = moderate/severe), IVS = interventricular septum thickness, LVEDD = left ventricular end-diastolic diameter, PASP = pulmonary artery systolic pressure

Table 2. Mean white blood cell differential counts

	Control	Aortic stenosis			P†	P‡
	No AS (n=49)	Mild (n=42)	Moderate (n=39)	Severe (n=53)		
WBC	7.3 ± 1.5	7.4 ± 1.5	7.2 ± 1.7	7.1 ± 1.5	0.9	0.7
PMN	4.3 ± 0.9	4.4 ± 1.3	4.4 ± 1.2	4.4 ± 1.2	0.8	0.9
Lymphocytes	2.1 ± 0.6	2.2 ± 0.8	1.9 ± 0.7	2 ± 0.5	0.2	0.2
Monocytes	0.5 ± 0.11	0.51 ± 0.1	0.49 ± 0.14	0.44 ± 0.09	0.5	0.046*
Eosinophils	0.2 ± 0.11	0.21 ± 0.1	0.23 ± 0.1	0.25 ± 0.13	0.33	0.5

All values represent mean of absolute counts ± SD. Units are 10⁶/ml

*Statistically significant ($P < 0.05$)

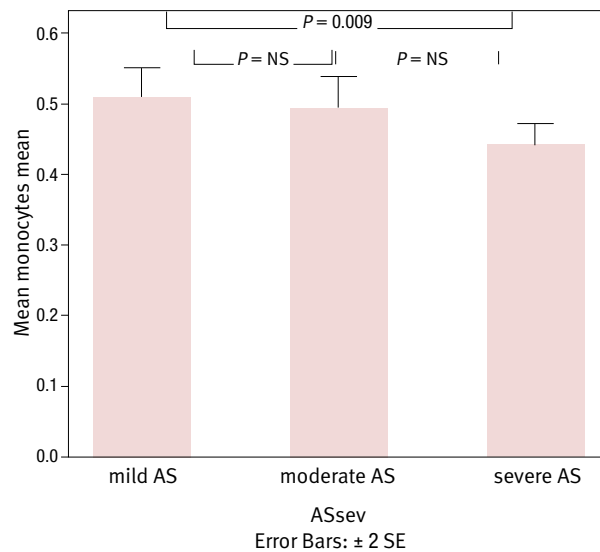
†Value for comparison between control patients and AS patients

‡Value for comparison between AS severity groups only

WBC = white blood cells, PMN = polymorphonuclear cells

phil, lymphocyte or eosinophil count between the patients with aortic stenosis and patients without or among the aortic stenosis subgroups [Table 2]. There was a significant difference in the monocyte count among these subgroups. As shown in Figure 1, there was a progressive decrease in the monocyte count with severe disease, with a significant difference observed between the mild and severe subgroups

Figure 1. Bar graph of absolute monocyte count (units are 10⁶/ml) by AS severity groups. Comparisons between every two groups are marked



(and a similar trend albeit non-significant between the mild-moderate and moderate-severe subgroups). Figure 2 shows a pronounced negative correlation between aortic valve peak or mean gradient or peak velocity and monocyte count in the pooled aortic stenosis patients ($r = -0.24, P = 0.01$; $r = -0.25, P = 0.01$; $r = -0.31, P = 0.014$, respectively).

A similar negative correlation is noted for aortic valve mean gradient and neutrophil count ($r = -0.26, P = 0.04$) but not for the other severity indices and neutrophil count. No correlation was seen between aortic stenosis indices and total WBC, lymphocytes or eosinophils ($P > 0.05$ for all).

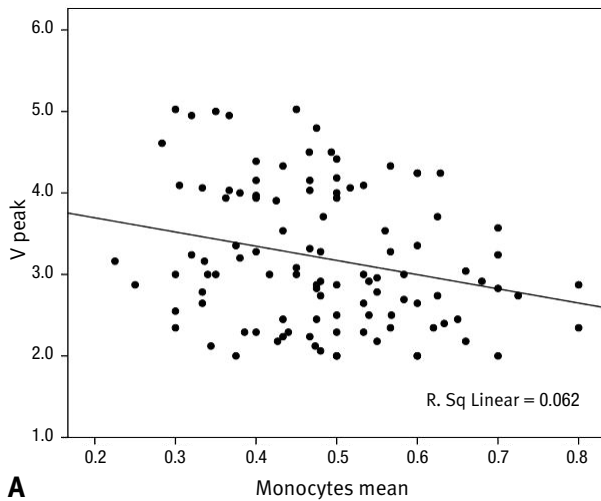
In addition, we examined the bivariate partial correlation between monocyte count and aortic valve peak or mean gradient or peak velocity controlling for age, gender, hypertension, smoking, hyperlipidemia and ejection fraction, and found persistence of the significant negative correlation in all cases ($r = -0.29, -0.27$ and -0.3 respectively; all P values < 0.05).

RELATIONSHIP OF WBC DIFFERENTIAL COUNTS AND AORTIC STENOSIS PROGRESSION

The median changes in peak velocity and peak gradients in aortic stenosis patients were 0.44 (range 0–1.3) m/sec/year and 12 (0–39) mmHg/year, respectively. There was no correlation between any of the WBC differential counts and the calculated change in peak velocity or peak gradient per year.

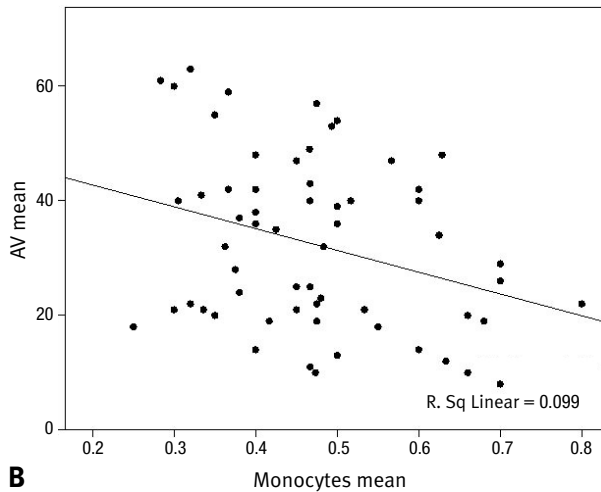
We also performed receiver-operating characteristic curve analyses for the ability of WBC differential counts to distinguish between patients who had an increase in peak aortic jet velocity above 0.3 m/sec within 1 year and those whose peak aortic jet velocity change was below this cutoff point, and found no role

Figure 2. [A] Scatterplot of index echo aortic valve peak velocity (in m/sec) by absolute monocyte count (units are $10^6/m$) in all patients with aortic stenosis



A

[B] Scatterplot of index echo aortic valve mean gradient (in mmHg) by absolute monocyte count (units are $10^6/m$) in all patients with aortic stenosis



B

for any of the WBC types for this purpose (area under the curve was close to 0.5 in all cases and *P* values were not significant).

DISCUSSION

In the present study we found a negative correlation between monocyte count and aortic valve peak and mean gradients and between monocyte count and aortic stenosis grade. This correlation appears to persist when controlling for age, gender, hypertension, smoking, dyslipidemia and left ventricular ejection fraction.

We did not find an association in our overall population-based study between averaged WBC differential counts and aortic stenosis progression over time. To our knowledge there are no data on the association between peripheral blood WBC differential count and aortic stenosis severity and progression.

The histological analysis of aortic stenosis valvular lesions at different stages of the disease shows similarities to atherosclerotic plaque evolution. This may be the result of active biologic processes of chronic inflammation and tissue repair, similar to the processes involved in atherosclerosis.

The monocyte is considered a key cellular player in atherogenesis and its transition to macrophage is a prerequisite to the initial and subsequent stages of the process. Therefore, the negative association between monocyte count and aortic stenosis severity is apparently surprising given the fact that aortic stenosis and atherosclerosis share similar histological features and risk factor profile [1-7]. However, aortic stenosis and atherogenesis are not identical processes. The extent of calcification is greater in the aortic valve than in the coronary artery wall and only a few smooth muscle cells are present in the aortic valve leaflets. In atherosclerosis, plaque destabilization is the key factor in adverse prognosis (myocardial infarction occurrence), whereas in aortic stenosis the shear bulk of the lesion correlates with hemodynamic severity and clinical symptoms.

A large community-based observational study did not find any association between aortic valve sclerosis and isolated blood counts, including WBC differential counts [20]. We also did not observe a difference in WBC differential counts in patients with mild aortic stenosis as compared to controls.

Diehl et al. [21] found that patients with severe aortic stenosis had increased numbers of CD 11b+ activated monocyte cells in their blood compared with controls as well as higher numbers of platelet microparticles conjugated to monocytes compared to controls. The total number of leukocytes was similar in aortic stenosis patients and controls in this study. In our study we looked at the average of WBC differential counts over 3 years before the first echo study. We found significantly lower monocyte counts during this time frame in patients with severe aortic stenosis compared to patients with mild disease. However, our study does not determine causality between low monocyte count and aortic stenosis severity.

One plausible mechanism for these observations is that monocytes are depleted from the peripheral blood due to long-standing migration into the degenerated aortic valves where they transform into active macrophages and play an important role in the pathophysiology of valve calcification. This hypothesis is supported by the recent finding that in aortic valves excised from patients with severe calcific aortic valves undergoing aortic valve replacement, the presence of an inflammatory process within the aortic tissue was independently related to the remodeling process and the peak transaortic gradient, and the density of WBC within the aortic tissue tended to correlate with the progression

rate of aortic stenosis [22]. We found a very strong negative correlation in the severe aortic stenosis subgroup between the mean monocyte count and aortic valve peak or mean gradients or peak velocity. This suggests that the monocyte chemotaxis to the stenotic aortic valves is a gradual and self-perpetuating process that contributes significantly to aortic stenosis progression.

Another explanation can be suggested from animal studies [23] and recent studies in patients with stable coronary disease that examined the correlation between monocyte count assessed by Coulter analysis and collateral flow [24,25]. A significant positive correlation between the monocyte count and collateral development was observed in non-diabetics as well as diabetics, suggesting reparative and arteriogenic functions of monocytes in some conditions. It is possible that in patients with severe aortic stenosis, there is an insufficient number of subgroups of monocytes that are related to a reparative process.

Our study is limited by its retrospective nature and by the variable intervals between the different blood count samplings in the individual patients. Nonetheless, we believe that careful application of the selection criteria and the averaging of repeated blood counts over an extended period counterbalanced this drawback. We used automatic Coulter identification of monocytes, but this assessment may not be accurate enough and may identify other cellular subsets as well, such as endothelial progenitor cells. Future studies may need to identify cellular subsets by more accurate techniques such as flow analysis.

In conclusion, we found a negative association between monocyte count and aortic stenosis severity. These findings may provide further clues to the mechanism underlying the pathogenesis of aortic stenosis. A larger scale prospective study is needed to evaluate the clinical implications of these findings.

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