International Journal of Cardiology 218 (2016) 181–187

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Circulating regulatory T cells in patients with aortic valve stenosis: Association with disease progression and aortic valve intervention

Sara Shimoni *[,1,2], Iris Bar [1,2], Valery Meledin [1,2], Gera Gandelman [1,2], Jacob George [1,2]

The Heart Institute, Kaplan Medical Center, Rehovot, Israel

ARTICLE INFO

Article history:
Received 15 April 2016
Accepted 12 May 2016
Available online 14 May 2016

Keywords:
Aortic valve stenosis
Regulatory T cells
Immune system
Progression

ABSTRACT

Background: Severe aortic valve stenosis (AS) accounts for considerable morbidity and death, especially in older patients. There is increasing evidence to suggest a role for immune modulating cells in aortic valve (AV) degeneration. Regulatory T cells (Tregs) tune down inflammation. We aimed to study the levels of circulating Tregs in patients with AS and to assess their association with disease progression.

Method and results: The number of Tregs (CD4 + CD25 + Foxp3+) was determined by flow cytometry in 229 patients with AS and a control group of 69 patients. Tregs were significantly higher in patients with AS compared to the control group (1.64 ± .61% vs 1.13 ± 0.97%, $p = 0.04$). In the logistic regression analysis, adjusted for baseline characteristics, only the hemoglobin level and Treg percent correlated with the presence of AS (OR 0.642 95% CI 0.512–0.805, $p < 0.001$ and OR 1.411, 95% CI 1.080–1.844, $p = 0.011$, respectively). One hundred patients underwent 2 echocardiographic studies during follow-up. The median decrease in AV area (AVA) was 0.1 cm^2/year. A borderline association was observed between Tregs and AVA progression ($r = 0.19$, $p = 0.054$). In a subgroup of 68 patients with severe AS, the association between Tregs and AVA progression was significant ($r = 0.374$, $p = 0.0017$). In addition, a drop in Treg levels was observed 3–6 months after AV-intervention (1.86 ± 1.6% vs 1.04 ± 1.8%, $p = 0.0005$).

Conclusions: Circulating Tregs are elevated in patients with AS. The levels of Tregs are higher in patients with severe AS and accelerated progression of valve narrowing. These results may help to identify AS patients with accelerated disease progression and possibly in need for earlier intervention.

1. Introduction

Degenerative aortic valve (AV) stenosis (AS) is the most common valvular disease and increases in prevalence with age [1]. Severe aortic valve stenosis accounts for considerable morbidity and death, especially in older patients. Aortic valve stenosis is the primary indication for valve replacement in western countries, and the number continues to increase as the population grows older. However, despite improved outcome due to valvular interventions, AS continues to be a prevalent disease with a substantial morbidity and mortality and with no effective treatment strategy to inhibit the progression of AS.

AS was attributed for centuries to a passive wear and tear process with passive deposition of calcium–phosphate complexes on the injured valve surface. However, AS is now recognized as an active inflammatory and potentially modifiable pathology, with similarities to atherosclerosis [2,3]. There is increasing evidence to suggest a role for immune modulatory cells in AV thickening and calcifications [4]. Wu et al. showed that the lymphocytic infiltration in calcific aortic stenosis consists clonally expanded T cells [5]. Some of the T cell clones were of CD8 lineage suggesting that these cells are likely to participate in the pathogenesis of AV calcifications and are not a secondary response. Another study, by Winchester et al., identified increased levels of circulating activated CD8 cells and memory effector cells in patients with calcified valves, suggesting that a systemic adaptive immune response may be associated with AV calcifications [6].

Atherosclerosis is considered an inflammatory disorder with both innate and adaptive immune responses involved in the disease processes. Since Tregs are capable of regulating these immune responses they received an increasing attention in atherosclerosis [7,8]. Low levels of circulating Tregs were reported in rheumatic valvular disease [9]. However, autoimmunity plays an essential role in the pathogenesis of rheumatic valvular disease. The pathogenesis of degenerative aortic stenosis differs from rheumatic disease. We hypothesized that Tregs are associated with aortic valve degeneration. The aim of the present study is to study the levels of circulating regulatory T cells and patients with

Abbreviations: AS, aortic stenosis; AV, aortic valve; AVR, aortic valve replacement; AVA, aortic valve area; CAD, coronary artery disease; IL-10, interleukin 10; LVEF, left ventricular ejection fraction; NYHA, New York heart association; TGF-β, Transforming growth factor beta; Tregs, regulatory T cells.

* Corresponding author at: Kaplan Medical Center, POB 1, Rehovot 76400, Israel.
E-mail address: Sara_s@clalit.org.il (S. Shimoni).
[1] Affiliated to the Hebrew University and Hadassah Medical School, Jerusalem, Israel.
[2] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.ijcard.2016.05.039
0167-5273/

various stages of aortic stenosis and to assess correlation with disease progression. We also aimed to assess the change in Tregs after aortic valve intervention.

2. Methods

2.1. Patients

In this observational case control study we included 243 patients with AS who were followed in the valvular disease clinics in Kaplan Medical Center between July 2011 and December 2013. Patients were compared with 75 patients with similar atherosclerotic risk factor profile who had no significant valvular disease and were included in the study the same time period. The control group included patients who were referred to coronary angiography for chest pain evaluation or patients with atrial arrhythmia referred to echocardiography. Since AS patients have a high prevalence of concomitant coronary artery disease and quite a few have atrial arrhythmia, mainly atrial fibrillation, the control group was designed to include these factors. The sample size calculation was based on the data from previous studies [10,11] with an error of 0.05 and a power of 0.8. By assuming a difference of 30% in the Tregs values, resulted in a group size of 68 patients in the group of AS and in the control group. Since we included patients with mild, moderate and severe AS, the AS group was significantly larger. Patients with a rheumatic disease were excluded. Patients with mitral stenosis and more than mild mitral regurgitation were excluded. Patients with a history of acute coronary syndrome or revascularization in the previous 3 months or any type of malignant or hematologic disorder were also excluded. The patients underwent a clinical evaluation, and echocardiography and blood sample was drawn at the beginning of the study. The study was approved by the institutional ethics committee and all patients provided written informed consent.

Risk factors were assessed in all patients based on their medical records. Diabetes mellitus was defined as hyperglycemia requiring pharmacologic therapy; hypertension was diagnosed as either a systolic or a diastolic increase in blood pressure (>140/90 mm Hg) or use of antihypertensive therapy; hypercholesterolemia was defined as a total cholesterol level of greater than 200 mg/dL or use of lipid-lowering agents; and cigarette smoking as being an active smoker or having a smoking history of at least 10 pack-years. Coronary artery disease (CAD) was defined as a history of an acute coronary syndrome or presence of CAD on coronary angiography.

2.2. Follow-up

Patients were followed in the valvular clinic. The last follow-up date was January 2014. The indication for valve intervention was determined by patient's symptoms and co-morbidities, based on standard guidelines [12]. Clinical and echocardiographic evaluation was performed every 6 or 12 months based on patients' symptoms and AS severity. Assessment of AS progression was done based on echocardiographic aortic valve area change [13]. This was done since in some patients there was a decrease in the left ventricular function during follow-up and the change in peak velocity and peak gradient was not reflecting the progression in AS. Patients who underwent intervention and were stable, with no inflammatory or infections disease, had another blood test for Tregs 3–6 months after intervention.

2.3. Echocardiography studies

Transthoracic echocardiography including the assessment of the aortic valve was performed according to the established guidelines [14,15]. Left ventricular volumes and left ventricular ejection fraction were assessed by modified Simpson's method. Left ventricular mass was assessed using the Devereux formula. Aortic valve area (AVA) was calculated using the continuity equation [16]. The same left ventricular outflow tract diameter was used in patients with several studies during follow-up. Mild, moderate and severe AS was defined as the valve area of 1.5–2.0 cm^2, 1.0–1.5 cm^2 and less than 1.0 cm^2, respectively.

2.4. Quantification of circulating regulatory T cells by flow cytometry

Peripheral blood was drawn from patients with AS and control donors. Peripheral blood mononuclear cells (PBMCs) were stained with FITC-labeled anti-CD4 (Miltenyi Biotech, Bergisch Gladbach, Germany) and APC-labeled anti-CD25 (Miltenyi Biotech) as previously described [17]. After incubation of 30 min, cells were incubated with a fixation solution, washed, and resuspended in a permeabilization solution (FCM, Santa cruz biotech). Fixated and permeabilized cells were stained with PE-labeled anti- Foxp3 (Miltenyi biotech). Isotype-matched controls were used to determine the background. Stained cells were analyzed on a FACSCalibur flow cytometer, using CellQuest software (BD biosciences).

2.5. Statistical analysis

Continuous data are presented as medians and interquartile ranges (25th–75th percentiles) for skewed distributed variables or as mean ± SD when normally distributed. Categorical data are presented as absolute numbers with their respective percentages. Chi-square test was used for categorical variables and Student's *t* test for continuous variables. One way Anova test was used for the comparison of Tregs in patients with different AS severities. Logistic regression analysis was performed in order to adjust the differences between the groups in regard to baseline characteristics. The prediction of AS progression by Treg levels was assessed by ROC curves which were summarized by the AUC (area under the ROC curve). Paired *t* test was used to compare Treg levels before and after intervention. All the above analyses were considered significant at $p \leq 0.05$. Analyses were performed with IBM SPSS version 21.

3. Results

3.1. Patients' characteristic

The initial study group included 318 patients, all Caucasians. Treg levels could not be evaluated in 20 patients due to technical problems. The study patient population included 229 consecutive patients (median age 78 years, 95 male) with AS who were followed in the valvular heart clinic. A control group included 69 stable patients (median age 72 years, 47 male) with no valvular disease. Thirty-eight, 51 and 140 patients had mild (mean AVA 1.6 ± 0.16 cm^2), moderate (mean AVA 1.1 ± 0.15 cm^2) and severe (mean AVA 0.7 ± 0.17 cm^2) AS, respectively. The etiology of AS was degenerative in 207 patients and bicuspid aortic valve in 22 patients. Patient baseline characteristics are outlined in Table 1. Patients with AS were older, more females and higher frequency of hypertension. Patients with AS were also treated more frequently with beta receptor blocking agents. Hemoglobin levels were lower in patients with AS.

3.2. T cells and Tregs in patients with AS

The levels of circulating T cells and Tregs in patients with AS and control group are shown in Fig. 1. The levels of Tregs (CD4 + CD25 + Foxp3+) were significantly higher in patients with AS compared to the control group (1.64 ± 1.61% vs 1.13 ± 0.97%, $p = 0.04$, Fig. 1a). There was no difference in Treg levels between patients with mild, moderate and severe AS ($p = 0.18$, Fig. 1b). The percent of CD25 + and CD4 + CD25 + cells was also higher in patients with AS compared to the control group ($p = 0.007$ and $p = 0.04$, respectively). There was no difference in there percent