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Short communication

Circulating cytokines predict severity of rheumatic heart disease☆

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ABSTRACT

Background: Rheumatic heart disease (RHD) is associated with inflammation that damages cardiac valves, often requiring surgical interventions. The underlying mechanisms involved in the disease progression are not completely understood. This study aimed to evaluate cytokine plasma levels in patients with RHD as possible markers of disease severity.

Methods and results: Eighty-nine patients with RHD, age of 41 years ±11.5 years, were prospectively enrolled. RHD severity was defined as valve dysfunction that required invasive intervention, either valve repair or replacement. Peripheral blood samples were collected from all patients for cytokine measurements. The patients were followed up to look at adverse clinical events defined as either the need for valve intervention or death.

At baseline, 64 (71.9%) patients had previously undergone valve intervention, whereas 25 patients had stable clinical presentation. Patients with severe RHD displayed higher levels of inflammatory cytokines than patients with stable disease. Cluster analysis showed segregation of severe and stable RHD based on IL-6/TNF-α and IL-6/IL-17A, respectively. IL-6 and TNF-α expression were positively correlated in severe but not in stable RHD patients.

During a median follow-up of 23 months, 16 patients (18%) had an adverse outcome. IL-10 at baseline (HR 1.24, 95% CI 1.08–1.43, p = 0.003), and IL-4 (HR 1.12, 95% CI 1.01–1.24, p = 0.041) were predictors of events during the follow-up.

Conclusions: High levels of cytokines are associated with severity of RHD. The co-regulated expression of IL-6 and TNF-α is associated with severe valve dysfunction, whereas high IL-10 and IL-4 levels predicted subsequently adverse outcome.

1. Introduction

Rheumatic heart disease (RHD) is associated with a progressive inflammatory reaction that leads to different degrees of valve dysfunction, and ultimately deadly outcomes [1].

Molecular mimicry between streptococcal antigens and human proteins, in association with an aberrant host immune response, is the cornerstone of RHD pathophysiology. The rate of progression of valvular lesions may be influenced by multiple factors, especially genetic predisposition and host's immune response [2]. Thus, cytokines involved in the activation of the immune response may play a role in determining RHD severity [3]. High tumor necrosis factor alpha (TNF-α), high interferon gamma (IFN-γ), and low interleukin 4 (IL-4) levels have been detected in the rheumatic valve tissue [2–4], supporting the hypothesis that an exacerbated inflammatory reaction induces progressive valve damage.

Despite this evidence, a clear association between circulating cytokines and RHD severity has not been demonstrated yet. Once valve lesions have developed, further progression to life-threatening heart failure may vary, and the identification of patients at risk for disease

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progression with subsequent need for intervention is essential. Thus, evaluation of circulating cytokine profile emerges as a valid strategy for biomarker identification and may have potential value for clinical risk stratification in RHD patients, which is currently lacking.

Therefore, this study was designed to determine the cytokine profile of patients in a wide spectrum of RHD severity, seeking association between cytokine profile and clinical presentation of RHD.

2. Methods

Our study initially selected 98 patients with RHD who were referred for management of RHD from March 2015 to May 2016. Patients with other inflammatory or systemic diseases that could independently alter the cytokine profile were excluded.

The study was approved by the institutional review board. Careful clinical examination was performed, focusing on clinical presentation, history of acute rheumatic fever (ARF), and RHD complications.

RHD severity was defined as the need for valve intervention, either valve repair or replacement prior to enrollment into the study. Indications for intervention followed the current guideline [5]. We classified severe RHD as stage C2 and D, and the others were considered as having stable disease [5]. The patients were followed up to look at adverse clinical events defined as a composite of mortality, or the need for valve intervention.

Echocardiography was performed to assess valve dysfunction, measurements and hemodynamic calculations. Cytokine were measured using a cytometric bead array containing interferon-gamma (IFN-γ), tumor necrosis factor-alfa (TNF-α), interleukin-17A (IL-17A), interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-4 (IL-4) and interleukin-2 (IL-2).

The measurements of patients with stable and severe RHD were compared using chi-square test, unpaired Student's *t*-test, Mann-Whitney test, as appropriate. Cox proportional hazards regression analyses were performed to assess the value of cytokine levels at baseline in predicting adverse outcome during the follow-up. Statistical analysis was performed using SPSS, version 21.0 (SPSS Inc., Chicago, Illinois). Cluster analysis and heatmaps were performed using the ClustVis software.

3. Results

The characteristics of the study population according to RHD severity are shown in Table 1. At baseline, 64 (71.9%) patients had previously undergone valve intervention, whereas 25 patients had stable clinical presentation. The age of onset of penicillin use was earlier in patients with stable than in those who had severe disease (23.9 ± 10.2 years versus 15.7 ± 10.5 years, p = 0.002).

Table 1
Baseline characteristics of the study population, stratified according to the severity of the valve disease.

Variable[a]	Stable RHD (n = 25)	Severe RHD (n = 64)	p value
Mean age (years)	40.5 ± 13.0	41.4 ± 11.0	0.736
Gender female (%)	17 (68.0%)	59 (92.0%)	0.004
History of ARF	16 (64.0%)	28 (43.8%)	0.086
Atrial fibrillation	5 (20.0%)	20 (31.2%)	0.289
Use of penicillin	23 (92.0%)	49 (76.6%)	0.096
Mean age of start of penicillin (years)	15.7 ± 10.5	23.9 ± 10.2	0.002
RHD in siblings	1 (4.0%)	5 (7.8%)	0.519
Recurrent pharyngotonsillitis	16 (64.0%)	37 (57.8%)	0.593
LV end-diastolic diameter (mm)	50.9 ± 5.9	48.4 ± 7.0	0.118
LV end-systolic diameter (mm)	33.2 ± 5.7	31.6 ± 4.9	0.196
LV ejection fraction (%)	64.0 ± 7.9	63.4 ± 7.0	0.717
LA volume index (mL/m^2)	51.1 ± 23.1	52.6 ± 10.5	0.860
SPAP (mm Hg)	38.8 ± 20.5	41.0 ± 14.3	0.608
Circulating cytokine levels (pg/ml)[b]			
IL-2	7.4 ± 0.4	9.6 ± 0.6	0.018
IL-4	9.8 ± 0.4	12.2 ± 0.8	0.007
IL-6	10.1 ± 1.9	19.6 ± 3.5	0.007
IL-10	7.1 ± 0.3	8.3 ± 0.5	0.366
IL-17A	9.0 ± 3.0	49 ± 15	0.019
TNF-α	4.8 ± 0.3	7.5 ± 0.6	0.017
IFN-gamma	4.0 ± 0.3	5.1 ± 0.3	0.026

ARF: acute rheumatic fever; RHD: rheumatic heart disease. LA: left atrium; LV: left ventricle; SPAP: systolic pulmonary artery pressure.
[a] Mean ± standard deviation.
[b] Mean ± standard error.

Concentration of inflammatory cytokines was higher in severe compared to stable disease (Table 1). However, analysis of the expression of the regulatory cytokine, IL-10, showed no difference between the groups (p = 0.366). Circulating cytokine analysis in severe RHD patients, stratified based on the median age of penicillin onset (19 years of age), showed that severe RHD patients who underwent intervention but started penicillin at an earlier age (lower than median) displayed higher IL-10 (p = 0.04) and higher IL-4 (p = 0.04), as compared to those who started penicillin later in life (IL-4 = 14 ± 10 and 11 ± 3 for early and late onset, respectively; IL-10 = 9 ± 5 and 7 ± 3 for early and late onset, respectively).

Cluster analysis showed distinct segregation patterns when comparing severe and stable groups. We observed that IL-17A and IL-6 clustered together in patients who had stable disease. In contract, IL-17A was alone in the hierarchy plot, while IL-6 grouped together with TNF-α, in severe disease. IL-2 and IL-4 clustered together and apart from the inflammatory cytokines in both groups, suggesting a co-production/regulation of these cytokines during RHD, regardless the outcome.

In patients with severe RHD, we observed a clustering of two cytokines with pathogenic potential, TNF-α and IL-6, in an independent tree, which did not occur in stable disease. Moreover, correlation analysis between expression of IL-6 and TNF-α showed a positive correlation of the expression of these cytokines in patients with severe but not stable disease (Fig. 1A). ROC analysis confirmed that concomitant analysis of IL-6 and TNF-α expression have a value that emerges as a possible biomarker of RHD severity, returning a specificity of 68% and a sensitivity of 70% (Fig. 1B).

During a median follow-up of 23 months (range, 3 to 37), 13 patients progressed with clinical worsening that warranted valve intervention, and 3 of the patients died. Analysis of cytokines showed that IL-10 at baseline was a prognostic predictor (hazard ratio [HR] 1.24, 95% confidence interval [CI] 1.08–1.43, p = 0.003), as well as IL-4 (HR 1.12, 95% CI 1.01–1.24, p = 0.041).

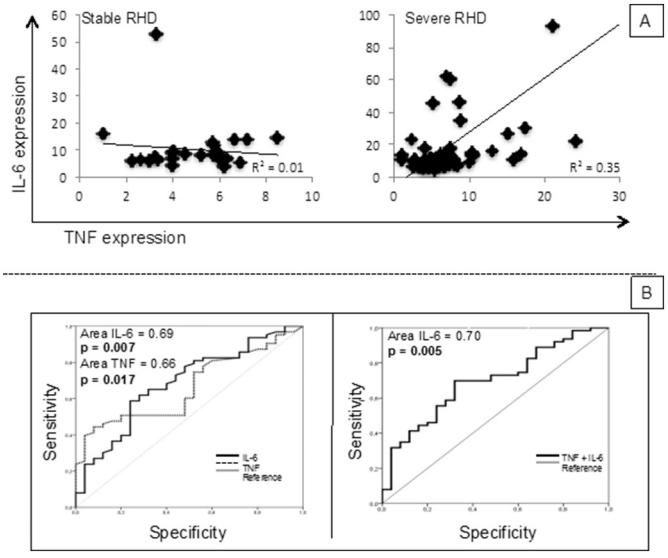

Fig. 1. IL-6 and TNF are associated with severe rheumatic heart disease (RHD). (A) Correlation analysis of IL-6 and TNF-α expression in patients with stable or severe rheumatic heart disease (RHD). Correlation plots of the expression of the inflammatory cytokines IL-6 and TNF-α are shown for each of the patient groups. Left panel shows a lack of correlation of the expression of the two cytokines in stable RHD patients; right panel shows a positive correlation of the expression of the two cytokines in severe RHD patients. (B) Receiver operating characteristic (ROC) curves for IL-6 and TNF-α expression in patients with rheumatic heart disease (RHD). Individual curves for IL-6 and TNF-α (left panel) and the curve resulting of the combination of IL-6 and TNF-α (right panel) are shown.

4. Discussion

RHD is the most common acquired heart disease in children and young adults in developing countries [1]. While the inflammatory nature of rheumatic fever-associated heart pathology is well-accepted, the underlying mechanisms involved in the development of severe forms are not completely understood [6]. Severe valve damage is the main cause of mortality, and currently there are no markers of disease progression and severity.

Studying the expression of inflammatory and anti-inflammatory cytokines in a large cohort of RHD patients stratified according to disease severity, we demonstrated that: (1) patients with severe RHD display higher expression of circulating pro-inflammatory cytokines, as compared to patients with stable disease, (2) IL-6/IL-17A and IL-6/TNF-α expression are associated with stable and severe disease, respectively, (3) while the inflammatory cytokines distinguish between stable and severe patients at baseline, IL-4 and IL-10 appeared as significant predictors of adverse outcome during the follow up.

Although previous studies demonstrated that cytokines are increased in RHD [7,8], our study shows that TNF-α and IL-6 are closely regulated and associated with the adverse outcome. An interesting point is that this association was not observed in the cluster plot for the patients without valve intervention, where IL-6 clustered together with IL-17A, and not with TNF-α. IL-6 is important for the induction of Th17 cells [9]. The association between IL-6 and IL-17A in patients with less severe RHD suggests that the development of Th17 cells, or at least the coordinated production of these cytokines, may be protective in RHD. It has previously been described that IL-17A is increased in RHD patients [10–13]. Despite the association of IL-17A with autoimmune processes, this cytokine has been associated with protection in other heart diseases [14].

IL-10 is a well-known modulatory cytokine and, while the reasons underlying the lack of difference in IL-10 between stable and severe patients may have several explanations, our results corroborate previous studies showing that its expression was not different between patients with ARF and established disease [7,15]. One possibility for this lack of difference is that IL-10 is expressed during all stages of RHD, as an attempt to control inflammation. However, the inflammatory cells of stable and severe patients might differently express IL-10 receptor. Alternatively, our study also showed that IL-10 was not tightly linked to any other cytokine, suggesting that the control of the inflammatory response in individuals with less severe disease might be attributed to other cytokines.

IL-6 is an important pro-inflammatory cytokine and a driving factor in the differentiation of T_H cells. Other studies showed that patients with RHD have higher secretion of IL-6 compared to patients with ARF [6,8,13]. This can be explained by the fact that this cytokine is produced a little after the inflammatory process has started, by macrophages, dendritic cells and mast cells, and in a small scale also by B-cells and $CD4^+$ T-cells. Our findings indicate that perhaps the pro-inflammatory response of IL-6 is generated initially in RHD, and its levels are maintained stable, independent of valve damage, and, depending on which population of cells the IL-6 stimulates, the prognosis of the RHD will vary from a stable progression, when IL-6 coordinates with IL-17A, or a more aggressive form of disease, when IL-6 coordinates with TNF-α.

TNF-α expression has been detected in valves from RHD patients [3,16]. However, despite its association with disease, use of TNF-α antagonists to treat RHD need to be defined [17]. While severe RHD manifests itself as a localized pathology, involving specifically the heart tissue, our data confirms that it has an important systemic immunological effect, allowing for the identification of circulating markers, which may present a practical alternative for clinical risk stratification.

5. Conclusions

High levels of inflammatory cytokines are associated with severity of RHD. The co-regulated expression of IL-6 and TNF-α is associated with a worse clinical presentation, and IL-4 and IL-10 are predictors of adverse outcome during the follow up. Employing the analysis of soluble molecules as markers of RHD severity provides a much-needed tool for risk stratification.

Conflict of interest

The authors declared that no conflict of interest exists.

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