

Increased Numbers of Circulating Th22 and Th17 Cells in Children With Kawasaki Disease

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Abstract

T-helper (Th) 17 and Th22 cells are critical for the pathogenic process of Kawasaki Disease (KD). A total of 43 children with freshly diagnosed KD and 20 healthy controls (HC) were quantified for the numbers of Th17, Th22 and Th1 cells by flow cytometry. The concentrations of serum IL-17, IL-22, IL-6, IFN- γ and TNF- α were examined by ELISA. Compared to those in the HC, significantly increased numbers of Th17 and Th22 cells, but not Th1 cells, and higher levels of serum IL-17 and IL-22, but not IFN- γ , were found in KD patients. Stratification analysis indicated the numbers of both Th17 and Th22 cells and the concentrations of serum IL-17 and IL-22 in KD patients with coronary artery lesions (CAL) were significantly greater than that in those with noncoronary artery lesions (NCAL). Treatment with the intravenous immunoglobulin (IVIG) therapy significantly decreased numbers of Th22 and Th17 cells as well as the serum concentrations of IL-22 and IL-17 in KD patients. The concentrations of serum IL-22 and IL-17 were correlated positively with C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values as well as N-terminal pro-brain natriuretic peptide (NT-proBNP) in those patients respectively.

Conclusion: Our study provided direct evidence that Th22 and Th17 cells might contribute to the pathogenesis of KD.

What Is Known

KD is characterized by an aberrant immune response.

What is NEW:

We demonstrate that increased numbers of Th22 and Th17 Cells in KD.

Th22 and Th17 cells collaborate and contribute to the formation of coronary aneurysms.

Introduction

Kawasaki disease (KD), also known as Kawasaki syndrome, is an acute, self-limited febrile vasculitis characterized by high spiking fever persisting, congestive oral mucosa, bilateral conjunctivitis and edematous extremity [1–2]. Furthermore, KD is the most common cause of acquired cardiac disease, especially coronary artery aneurysms in children [3]. Although KD has been studied for almost half a century, the pathogenic mechanism of KD is still unclear. Moreover, while most children with KD respond well to intravenous immunoglobulin (IVIG), roughly one-quarter of the children meeting clinical criteria will go on to have coronary artery inflammation, including aneurysms [4]. Hence, further illustration of the mechanism of KD is a crying need to find a therapeutic for KD treatment clinically.

Both the innate and the adaptive immune systems plays an important in the pathogenic process of KD [5]. The early event of visualized mmune dysfunction is the activation of natural immune system represented as the elevated numbers of activated monocytes and increased levels of tumor necrosis

factor (TNF)- α and interleukin (IL)-6 [6]. Subsequently, it is generally believed that autoreactive T cell and their inflammatory cytokines play a major role in the development of KD [7]. T helper (Th) 17 cells, a recently identified lineage of CD4+ Th cells, predominantly produce IL-17A. Th17 cell and IL-17A have been shown to participate in host defense responses and inflammatory diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Graves' disease (GD), and Crohn's disease (CD) [8–11]. A recent report found greater numbers of Th17 cells and higher levels of serum IL-17 in the acute stage of KD, and that elevated Th17 cells might be associated with tissue damage and coronary artery aneurysm formation [7, 12]. However, there is little information about whether Th17 cells are associated with the formation of coronary artery aneurysm in KD patients.

More notably, a newly identified T-cell subset, named Th22 cells, has been confirmed to have the ability to activate signal transduction and transcription 3 (STAT3) by secreting interleukin (IL)-22 [13]. IL-22, originally termed as an IL-10-related T-cell-derived-inducible factor, enhance innate immunity and promote epithelial cell proliferation and tissue. In contrast, IL-22 also act as regulator in the pathogenesis of RA and SLE [14–15]. Furthermore, recent studies have reported that IL-22 may function as a biphasic cytokine: protective and regenerative in steady state while amplifying proinflammatory signals given by TNF- α [16], which can exacerbate coronary vascular damage. in KD. However, the specific mechanism of Th22 cells in the formation of coronary aneurysms in KD is still unclear.

Here, in the current study, we tried to further expose the specific mechanism underlying hyperactivation of Th22 and Th17 during the different stages of KD. We characterized the numbers of circulating Th22, Th17 and Th1 cells by flow cytometry, and measured the serum concentration of inflammatory cytokines by ELISA in 43 patients with freshly diagnosed KD. Moreover, we further analyzed the relationship of the numbers of Th22/Th17/Th1 cells with the clinical parameter in these KD patients. Our data suggestd that increased numbers of Th17 and Th22 cells might be contributed to the pathogenic process of KD in Chinese children.

Material And Methods

Patients and samples

A total of 43 newly diagnosed children with KD were admitted by the Pediatric outpatient department, the Second People's Hospital of Changzhou, China. Another 20 age-, gender-and ethnicity-matched healthy controls (HCs), who had no a history of any chronic inflammatory disease, were recruited during the same period. According to the international criteria of the 2004 American Heart Association (AHA) statement individual children with KD were newly diagnosed[1]. The severity of coronary artery dilation in those patients were assessed using the Z-score, and a Z-score > 2 was defined as a coronary artery abnormality (CAL $^+$) [17]. 17 of among the KD children were diagnosed with coronary artery lesions according to the Z-score. All patients with any of other autoimmune diseases, recent infection or those who had received immune suppressive therapies or glucocorticoid therapies were excluded from the study. All children with KD were the administration of intravenous immunoglobulin at a dose of 2 g/kg for 1 day and oral aspirin

at a dose of 30–50 mg/kg per day. Then these children with KD were in remission, at which stage all patients had been afebrile for at least 48 h. The study was approved by the Ethical Committee of the NanJing medical University, and all children signed an informed consent form.

Clinical examination

The clinical data of each subject, including age, sex, and laboratory tests, was collected from the hospital records. Individual subjects were subjected to routine laboratory tests for C-reactive protein (CRP), erythrocyte sedimentationrate (ESR), N-terminal pronatriuretic peptide (NT-proBNP), and the concentrations of serum immunoglobulins (Ig)G, M, A and complement (C) 3, 4 by a biochemistry automatic analyzer (Siemens Healthcare Diagnostics Products, GmbH, Germany).

Flow cytometric analysis of Th22/Th17/Th1-related cytokine staining

Peripheral blood mononuclear cells (PBMCs) of children with Kawasaki disease were stimulated in duplicate with .0 mg/mL of ionomycin (Sigma, St. Louis, MO, USA) and 50 ng/mL of phorbol myristate acetate (PMA) in RPMI 1640 medium at room temperature in a humidified incubator with 95% air and 5% carbon dioxide for 2 hours and then cultured for another 4 hours in the presence of 0.5 mg/mL of brefeldin A (BFA, Sigma). The frequency of CD3⁺CD4⁺IL-17⁺Th17, CD3⁺CD4⁺IL-22⁺Th22 and CD3⁺CD4⁺IFN-γ⁺Th1 cells in individual samples were determined by flow cytometry.

Enzyme-linked Immunosorbent assay for Th22/Th17/Th1-related cytokine

The IL-6, TNF- α , IFN- γ , IL-17 and IL-22 concentration in the peripheral blood serum was measured using an Enzyme-linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions (BOSTER, Wuhan, China) .

Statistical analysis

Data presentation and analysis were performed with GraphPad Prism 7.0 software (Statcon, Witzenhausen, Germany). Data were presented as means ± standard error of the mean (S.E.M.). Mann-Whitney test and Wilcoxon signed rank test were used to test the probability of significant differences between samples. The Spearman rank correlation test were used to the relationship between variables. A two-side P value < 0.05 was considered statistically significant.

Results

Demographic characteristics

The demographic characteristics of KD patients and HC were summarized in Table 1. As expected, the WBC, lymphocyte and Lymphocytes counts and the levels of serum ESR and CRP in the patients were significantly higher than that in the HC ($P < 0.001$). Furthermore, the concentrations of serum NT-proBNP

in KD patients with CAL were significantly higher than that in KD group with NCAL ($P < 0.001$). In contrast, there was no significant difference in the distribution in the gender and age, and the levels of serum IgG, IgM, IgA, C3 and C4 among the different groups of patients and HCs ($P > 0.001$).

Table 1
The demographic and clinical characteristics of subjects.

Parameters	CAL-KD	NCAL-KD	HC
NO.	17	26	20
Age(years)	3.5 ± 1.8	3.2 ± 2.4	2.9 ± 1.8
Gender:female/male	9/8	14/12	11/9
WBC($\times 10^9/L$)	$14.45 \pm 2.37^*$	$14.46 \pm 2.98^*$	5.08 ± 3.2
Neutrophils($\times 10^9/L$)	$10.56 \pm 1.76^*$	$10.22 \pm 2.86^*$	2.62 ± 1.34
Lymphocytes($\times 10^9/L$)	$3.25 \pm 1.39^*$	$3.11 \pm 1.54^*$	2.12 ± 0.74
ESR(mm/h)	$59.77 \pm 17.28^*$	$55.77 \pm 16.91^*$	6.53 ± 2.62
CRP(mg/L)	$52.34 \pm 17.50^*$	$51.4 \pm 17.62^*$	2.12 ± 0.83
NT-proBNP (ng/L)	$1210.9 \pm 152.07^{*\#}$	855.96 ± 120.49	ND
IgG(g/L)	9.89 ± 2.33	9.06 ± 5.6	ND
IgM(g/L)	1.29 ± 0.47	1.20 ± 0.49	ND
IgA(g/L)	2.04 ± 0.69	1.95 ± 0.83	ND
C3(g/L)	1.18 ± 0.26	1.12 ± 0.19	ND
C4(g/L)	0.26 ± 0.06	0.25 ± 0.07	ND

Data shown are real case number or mean \pm SD.

Normal values: White blood cell counts (WBC): $4\text{--}10 \times 10^9/L$; Erythrocyte sedimentation rate (ESR): 0–20mm/h; C-reactive protein (CRP):0–5 mg/L; N-terminal pronatriuretic peptide (NT-proBNP): <300pg/ml; IgG: 7–16 g/L; IgM: 0.7–4.6 g/L; IgA:0.4–2.3 g/L; Complement component 3(C3):0.80–1.40g/L; Complement 4(C4): 0.20–0.70g/L; ND: not determined. HC, healthy control; CAL-KD, Coronary Artery Lesions Kawasaki disease. * $P < 0.05$ vs HC; # $P < 0.05$ vs. NCAL-KD .

Increased numbers of Th22 and Th17 cells in KD children

We first characterized the levels of different subset of effector CD3 $^+$ CD4 $^+$ T cells in KD patients by flow cytometry analysis. As shown in Fig. 1a, KD patients with CAL had significantly increased numbers of CD3 $^+$ CD4 $^+$ IL-22 $^+$ Th22 and CD3 $^+$ CD4 $^+$ IL-17 $^+$ Th17 cells compared to KD patients with NCAL and HCs

[Th22 (1.62 ± 0.29) $\times 10^4$ versus ($1.10 \pm 0.27\%$) $\times 10^4$ versus ($0.85 \pm 0.21\%$) $\times 10^4$, $P < 0.05$, Th17 ($2.80 \pm 0.78\%$) $\times 10^4$ versus ($2.07 \pm 0.66\%$) $\times 10^4$ versus ($2.07 \pm 0.41\%$) $\times 10^4$, $P < 0.05$]. Furthermore, KD patients with NCAL had significantly increased numbers of CD3 $^+$ CD4 $^+$ IL-22 $^+$ Th22 and CD3 $^+$ CD4 $^+$ IL-17 $^+$ Th17 cells compared to HCs ($P = 0.004$, Fig. 1B; $P = 0.033$, Fig. 1C; respectively). However, we did not find a significant difference in the numbers of CD3 $^+$ CD4 $^+$ IFN- γ $^+$ Th1 cells among the different groups of patients and HCs [(24.80 ± 4.15) $\times 10^4$ versus (22.22 ± 5.12) $\times 10^4$ versus (25.33 ± 4.84) $\times 10^4$, $P > 0.05$].

Higher concentrations of serum IL-22, IL-17A and TNF- α were present in KD children

To determine the function of different subsets of IL-22- and IL-17A-producing CD4 $^+$ T-cells, we measured the concentrations of serum IL-22, IL-17A and IFN- γ in the patients. We found that the concentrations of serum IL-22 and IL-17A in KD patients with CAL were significantly higher than that in KD patients with NCAL and HCs [IL-22 (18.59 ± 4.15) versus (13.65 ± 5.77) versus (8.57 ± 1.69), $P < 0.05$, IL-17 (78.51 ± 22.37) versus (53.16 ± 16.35) versus (40.74 ± 11.68), $P < 0.05$]. However, we did not find a significant difference in the concentrations of serum IFN- γ among the different groups of patients and HCs [(13.19 ± 4.23) versus (11.76 ± 2.77) versus (11.46 ± 3.96), $P > 0.05$]. TNF- α is necessary for exacerbation of vascular injury in KD. We further analyzed the levels of TNF- α in the serum of KD patients and found a higher level of serum TNF- α in KD patients with CAL, compared to KD patients with NCAL and HC [(14.36 ± 3.85) versus (7.78 ± 3.21) versus (5.25 ± 2.01), $P < 0.05$]. In addition, the concentrations of serum IL-6 in KD patients were significantly higher than that in the HCs [(8.96 ± 2.39) versus (8.49 ± 2.29) versus (3.73 ± 1.24), $P < 0.05$].

Th22 cells were associated with serum Th17 and TNF- α in KD children

We analyzed the relationship among the numbers of circulating Th22, Th17 and TNF- α in KD patients. We found that the numbers of Th22 cells were significantly positively correlated with the numbers of Th17 cells in KD patients with CAL ($R = 0.494$, $P = 0.044$, Fig. 3A). Further analysis revealed that the numbers of circulating Th22 and Th17 cells were correlated positively with the levels of serum IL-22 and IL-17A in KD patients with CAL, respectively ($R = 0.647$, $P = 0.005$, Fig. 3B; $R = 0.774$, $P = 0.003$, Fig. 3D). In addition, the numbers of circulating Th22 cells were correlated positively with the levels of serum TNF- α in KD patients with CAL ($R = 0.756$, $P < 0.001$, Fig. 3C). Moreover, KD patients with NCAL also showed the same trend ($R = 0.522$, $P = 0.006$, Fig. 3E; $R = 0.676$, $P = 0.002$, Fig. 3F; $R = 0.643$, $P < 0.001$, Fig. 3G; $R = 0.491$, $P = 0.011$, Fig. 3H; respectively).

Th22 and Th17 cells were associated with clinical parameters in KD children

To determine the pathogenic role of Th22 and Th17 cells in the development of KD, we analyzed the potential association of the levels of these CD3 $^+$ CD4 $^+$ T cell-related cytokines with the values of clinical parameters in KD patients. We found that the concentrations of serum IL-22 and IL-17 were correlated

positively with the concentrations of ESR, CRP and NT-proBNP in KD patients with CAL, respectively ($R=0.653, P=0.005$; $R=0.506, P=0.038$; $R=0.609, P=0.009$, Fig. 4A; $R=0.743, P<0.001$; $R=0.837, P<0.001$; $R=0.597, P=0.011$, Fig. 4B; respectively). Moreover, KD patients with NCAL also showed the same trend ($R=0.753, P<0.001$; $R=0.504, P=0.009$; $R=0.406, P=0.039$, Fig. 4C; $R=0.561, P=0.003$; $R=0.444, P=0.023$; $R=0.492, P=0.011$, Fig. 4D; respectively).

Altered numbers of Th22/Th17 cells and related cytokines in KD children after treatment

Finally, we tested how the treatment with intravenous immunoglobulin (IVIG) affected the numbers of Th22/Th17 cells and the concentrations of serum IL-22, IL-17A and TNF- α in the patients. We found that the numbers of Th22 and Th17 cells were significantly lower than that before the treatment ($P<0.001$ and $P<0.001$, Fig. 5A; $P<0.001$ and $P=0.005$, Fig. 5B; respectively). Similarly, the concentrations of serum IL-22 and IL-17A in those patients with CAL and NCAL after the treatment were also significantly lower than that before the treatment ($P<0.001$ and $P<0.001$, Fig. 5A; $P=0.003$ and $P=0.008$, Fig. 5B; respectively). Moreover, the concentrations of serum TNF- α in those patients with CAL and NCAL after the treatment were lower than that before the treatment ($P<0.001$, Fig. 5A; $P=0.009$, Fig. 5B; respectively).

Discussion

It is well known that abnormal activation of effector CD3 $^+$ CD4 $^+$ T helper cells play an important role in the pathogenesis of KD [7, 12], this specific mechanism remain unknown. In this study, we found that KD patients had greater numbers of circulating Th17 cells, which were consistent with previous studies [7]. However, our finding were different from another report that displayed an equivalent levels of circulating Th17 cells [18]. Conflicting results may be due to enrollment of patients. Moreover, part of the factors for these inconsistencies are the identification methods and surface markers of Th17 cell. Indeed, the pathogenic mechanism of Th17 cells in the development of KD rely on the phase of their disease. Moreover, the concentrations of serum IL-17A were significantly higher in KD patients, consistent with a previous study [7]. More importantly, we found that circulating Th17 cells were positively correlated with serum IL-17A in those children. These suggest that Th17 cells are major producers of IL-17A in KD patients. Based on our data, serum IL-17A were positively correlated with ESR and CRP, indicating that Th17 cell contribute to innate immune responses. More importantly, our data showed that a higher expression levels of Th17 cells and serum IL-17A in KD patients with CAL were positively correlated with the concentrations of NT-proBNP, which is a hallmark pathological of CAL [19], speculating Th17 cells are involved in the formation of coronary aneurysms. This possible mechanism is that the proinflammatory cytokine IL-17A promotes neutrophils and monocytes to secrete the proinflammatory cytokines such as IL-6 and TNF- α [20]. These findings might provide a new perspective on the mechanism of CAL formation.

Like Th17 cells, Th22 cells are also associated with numerous autoimmune diseases, such as SLE, RA and ankylosing spondylitis (AS) [14–15, 21]. However, the underlying specific mechanism of Th22 cells in the pathogenesis of KD is still unclear. In this study, we detect that the numbers of circulating Th22 cells

and the serum levels of IL-22 were significantly greater in KD patients compared to those in the HC. These results extend previous observations and support the notion that Th22 cells, like Th17 cells, also play an important role in the pathogenesis of KD. It is possible that the inflammatory environment in the development of KD preferably promotes the differentiation of naive CD4⁺ T helper cells into Th22 and Th17 cells [22]. Moreover, we found that circulating Th22 cells were positively correlated with serum IL-22, suggesting that IL-22 is predominantly released by Th22 cells in KD children. More importantly, serum IL-22 were positively linearly correlated with ESR and CRP at the same time, indicating that Th22 cells might serve as a potential marker for indicating disease activity or prognosis of KD. Furthermore, we found that a higher expression level of Th22 cells and serum IL-22 were positively correlated with the concentrations of NT-proBNP in CAL⁺KD patients, suggesting that Th22 cells collaborate and contribute to the formation of coronary aneurysms. Notably, our study showed that circulating Th22 cells in KD patients were positively correlated with serum TNF- α , which were an independent risk factor associated with a significantly longer median time to recovery of CALs [23]. Therefore, the current data provide a possibility that Th22 cells might induces the secretion of TNF- α , which further cause the occurrence of CAL. We are interested in further investigating the specific mechanism of Th22 cells in the process of CAL.

Treating affected patients with IVIG has been demonstrated to control symptoms and inhibit coronary aneurysm formation effectively in KD patients with CAL [24]. The possible mechanism of action of IVIG in the treatment of KD is still unclear. We found that the treatment with IVIG reduced significantly the numbers of circulating Th22 and Th17 cells as well as the serum levels of IL-22 and IL-17 in KD patients. It is possible that immunosuppressants mainly induce the proliferation of naive T cells to regulatory T cells, rather than effector T cells. Moreover, the treatment with IVIG also reduced significantly the serum levels of TNF- α in KD patients. Consequently, our data reveal that treatment with IVIG treatment can effectively regulate the imbalance between effector T and regulatory T cells in KD patients.

In summary, our data showed significantly increased numbers of Th22 and Th17 cells, but not Th1 cells, in KD patients. More importantly, higher levels of serum Th22-type cytokines (IL-22) and Th17-type cytokines (IL-17) were positively correlated with the ESR, CRP and NT-proBNP. Meanwhile, treatment with IVIG not only relieved clinical symptoms quickly and effectively, but also reduced the numbers of Th22 and Th17 cells and their cytokines in KD patients. The presented data might aid in developing a new and effective treatment method for use in the children who have already developed coronary artery lesions. However, we recognized the limitations of our study, such as a relative small sample size and the lack of mechanism research of Th22 and Th17 cells in the pathogenesis of KD. Hence, further longitudinal studies of the specific mechanism of Th22 and Th17 cells in the pathogenesis of KD with a bigger population are urgently needed.

Abbreviations

Th22 cell

T-helper (Th) 22 cell

Th17

T-helper (Th) 17 cell
KD
Kawasaki Disease
CAL
Coronary artery lesions
NCAL
noncoronary artery lesions
IVIG
Intravenous immunoglobulin
ESR
Erythrocyte sedimentation rate
CRP
C-reactive protein
NT-proBNP
N-terminal pro-brain natriuretic peptide.

Declarations

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Authors' Contributions

Zhang Liwen and Ma Liang contributed equally to this work. Wan Yu designed the experiments and analyzed data. Zhang Liwen wrote the main manuscript text. Huang Zhiying and Xue Mei performed the experiments and prepared the figures. Zhang Xiaoyu and Wang Fei provided samples. Zhao Xuan provided conceptual and technical advices; and all authors reviewed the manuscript.

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Data availability All data and material will be available upon request.

Code availability Not applicable.

Ethics approval This study was approved by the Ethical Committee of the NanJing medical University.

Consent to participate All children signed an informed consent form.

Consent for publication All volunteers agreed that study results be published as long as volunteer's anonymity was maintained.

Conflict of Interests All authors declared there were no conflict interests involved.

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Figures

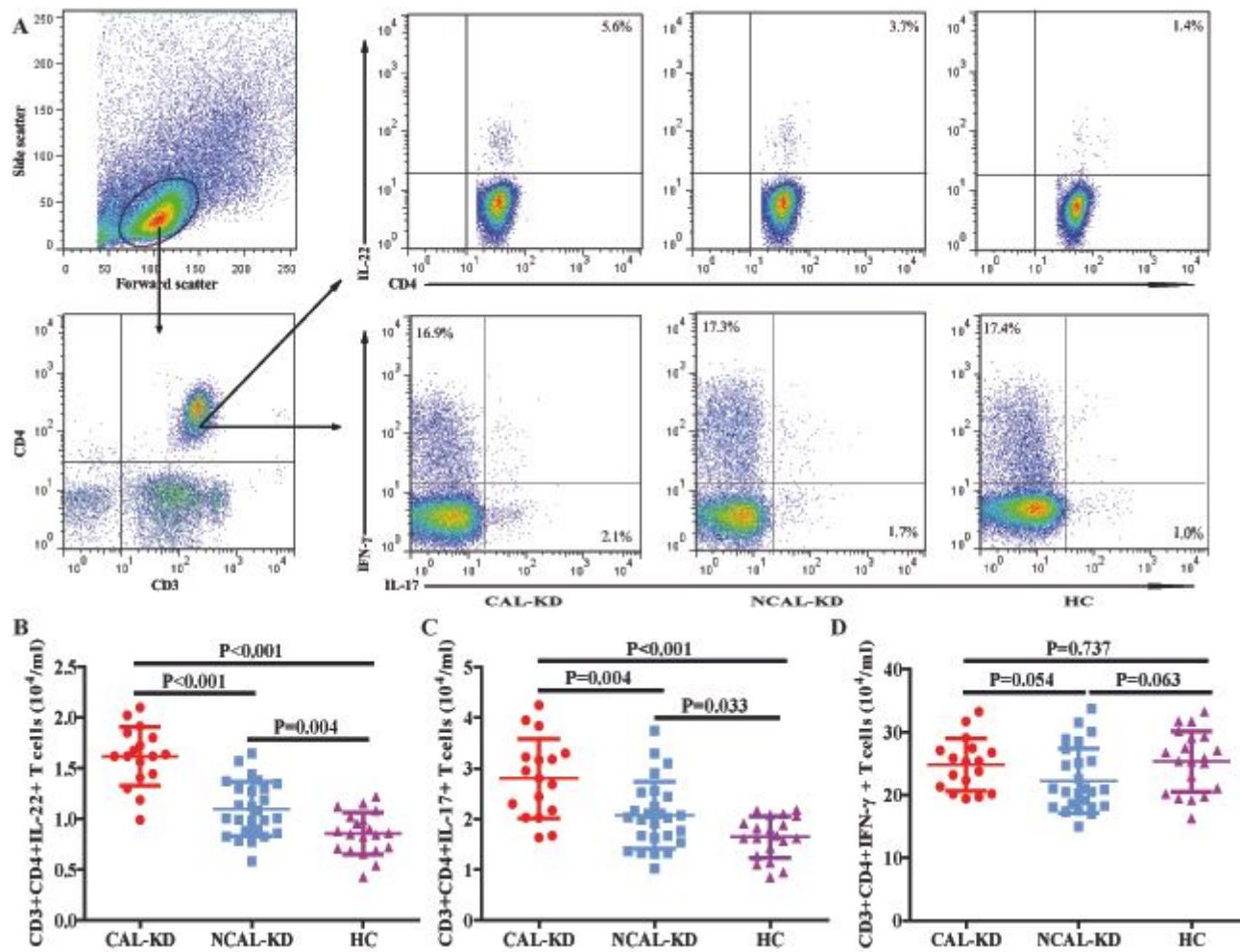


Figure 1

FACS analysis of circulating Th22/Th17/Th1 cells in KD children. Peripheral blood mononuclear cells 5*10⁵/tube were isolated and stained with PerCP-anti-CD4, PE-Cy5-anti-CD3 and isotype controls. Then these cells were fixed and permeabilized, followed by intracellular staining with PE-anti-IL-22 PE-Cy7-anti-IFN- γ and FITC-anti-IL-17. The frequency of CD3+CD4+IL-17+Th17, CD3+CD4+IL-22+Th22 and CD3+CD4+IFN- γ +Th1 cells were gated on CD3+CD4+ cells by flow cytometry analysis and at least about 50,000 events were analyzed for each sample. According to the frequency of different types of CD3+CD4+T cells and the total numbers of PBMCs, the numbers of CD3+CD4+IL-22+Th22, CD3+CD4+IL-17+Th17 and CD3+CD4+IFN- γ +Th1 cells were calculated. (A). Flow cytometry analysis; (B). The numbers

of CD3+CD4+IL-22+Th22 cells; (C). CD3+CD4+IL-17+Th17 cells; (D). CD3+CD4+IFN- γ +Th1 cells. Data shown are representative charts of Th22-Th17-Th1 cells from different groups of patients (n=17 for the patients with CAL, n=26 for the patients with NCAL and n=20 for the HC).

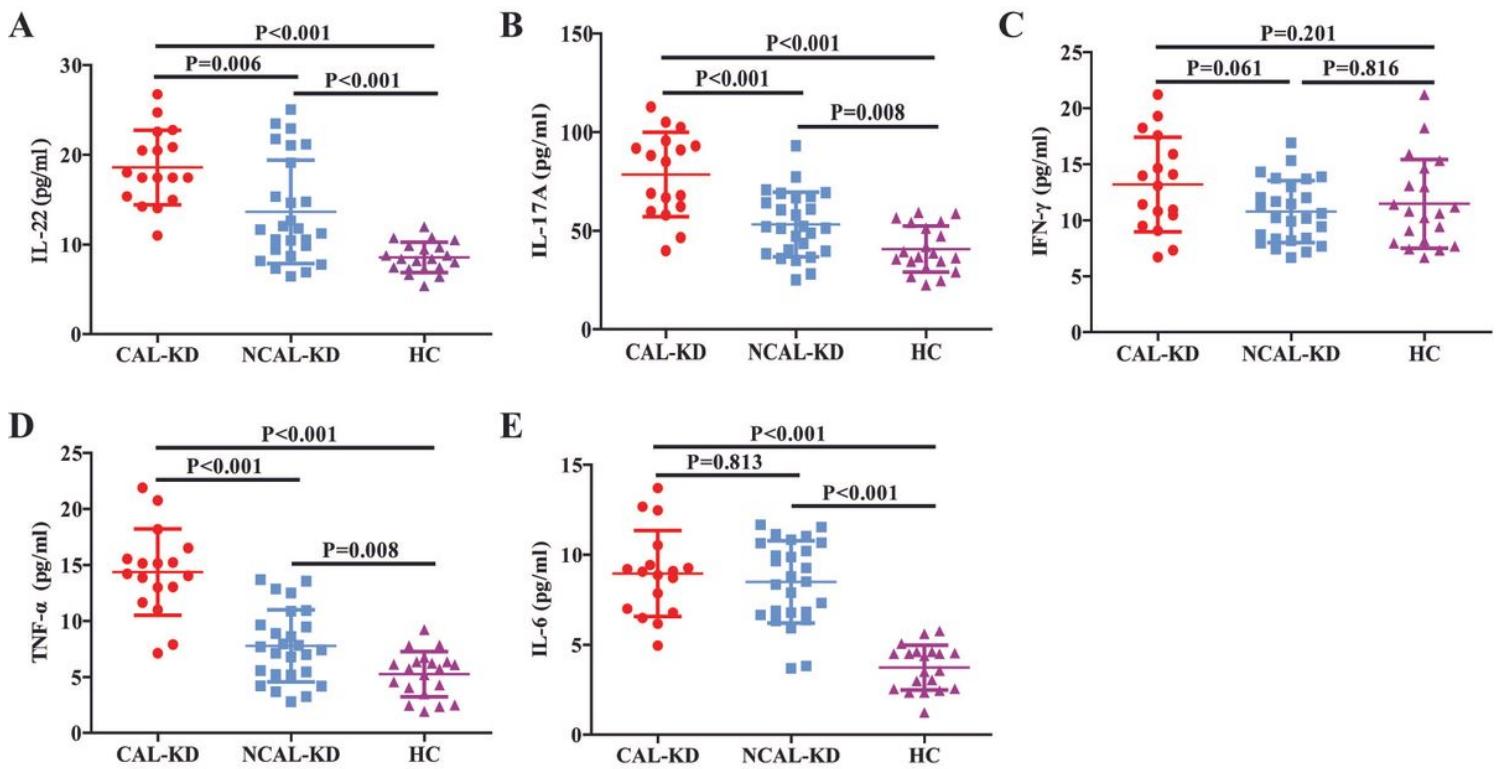


Figure 2

ELISA analysis of the concentrations of serum cytokines in KD children. The serum concentrations of IL-17, IL-22, TNF- α , IL-6, and IFN- γ in individual subjects were measured by ELISA. (A). Concentrations of serum IL-22; (B).Concentrations of serum IL-17; (C). Concentrations of serum IFN- γ ; (D). Concentrations of serum TNF- α ; (E). Concentrations of serum IL-6. Data shown are serum cytokines from different groups of patients (n=17 for the patients with CAL, n=26 for the patients with NCAL, and n=20 for the HC). The horizontal lines indicate the median values for each group.

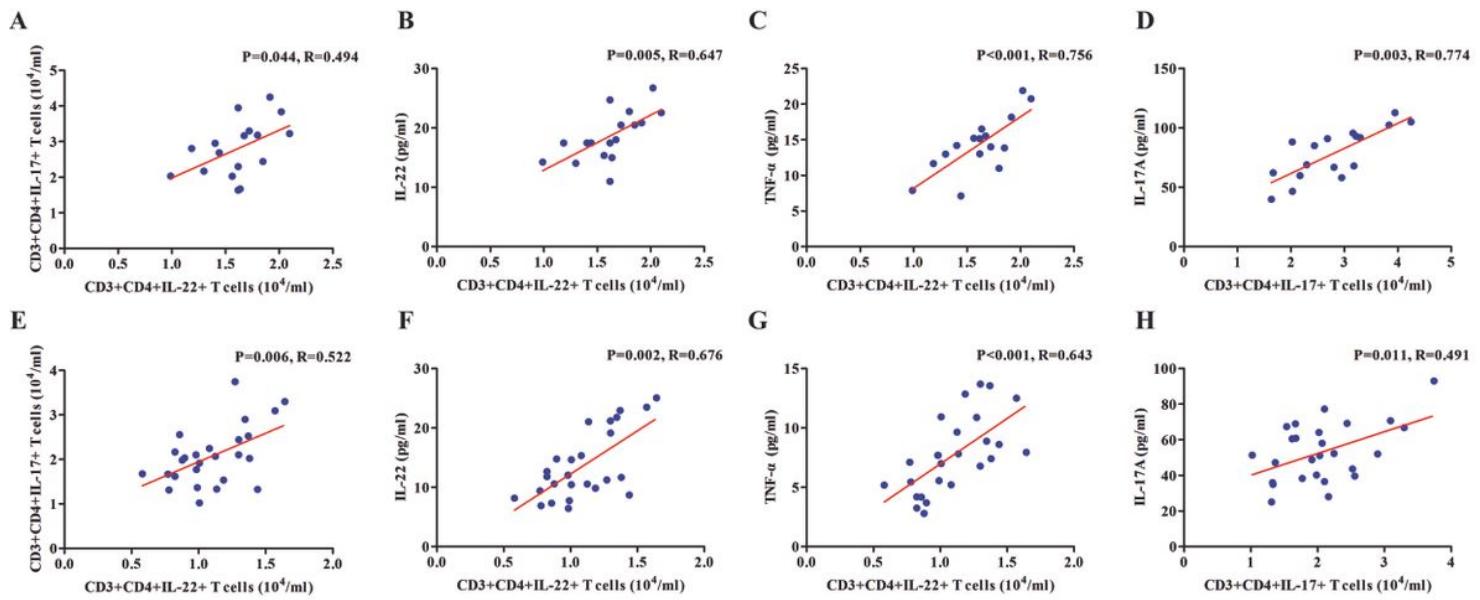
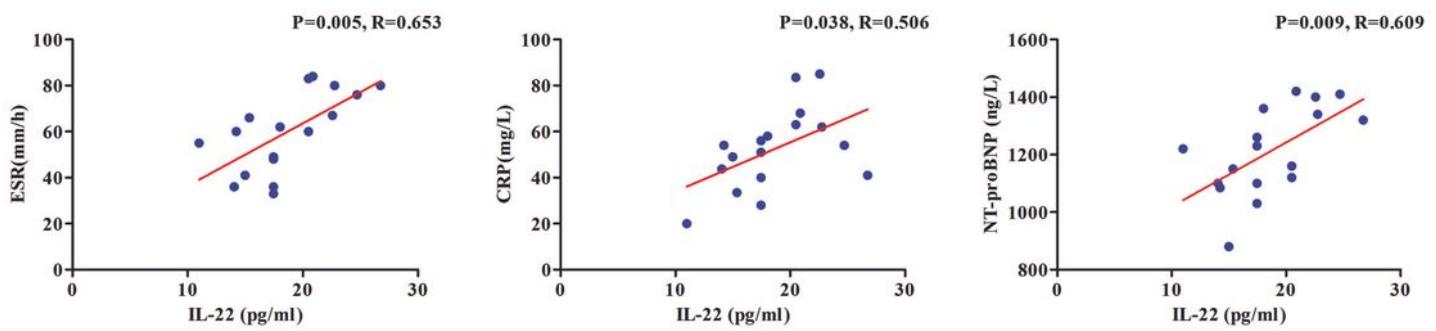
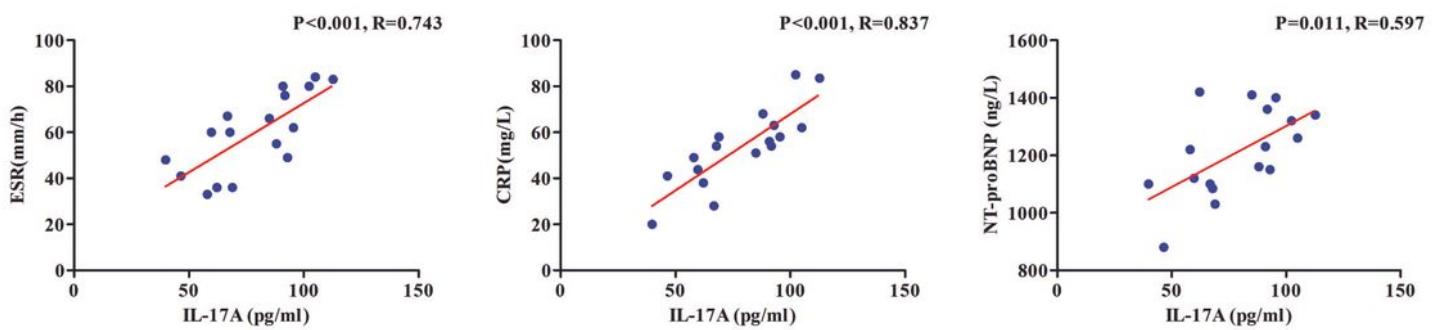
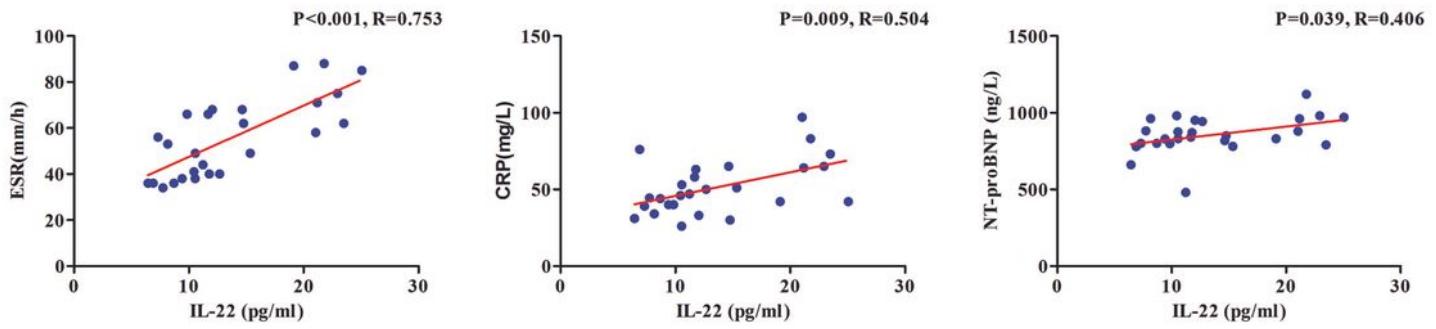
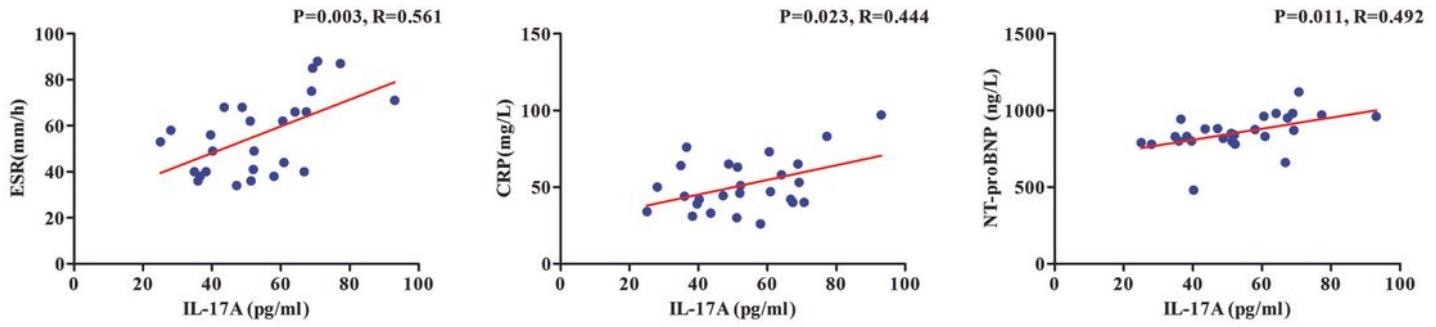
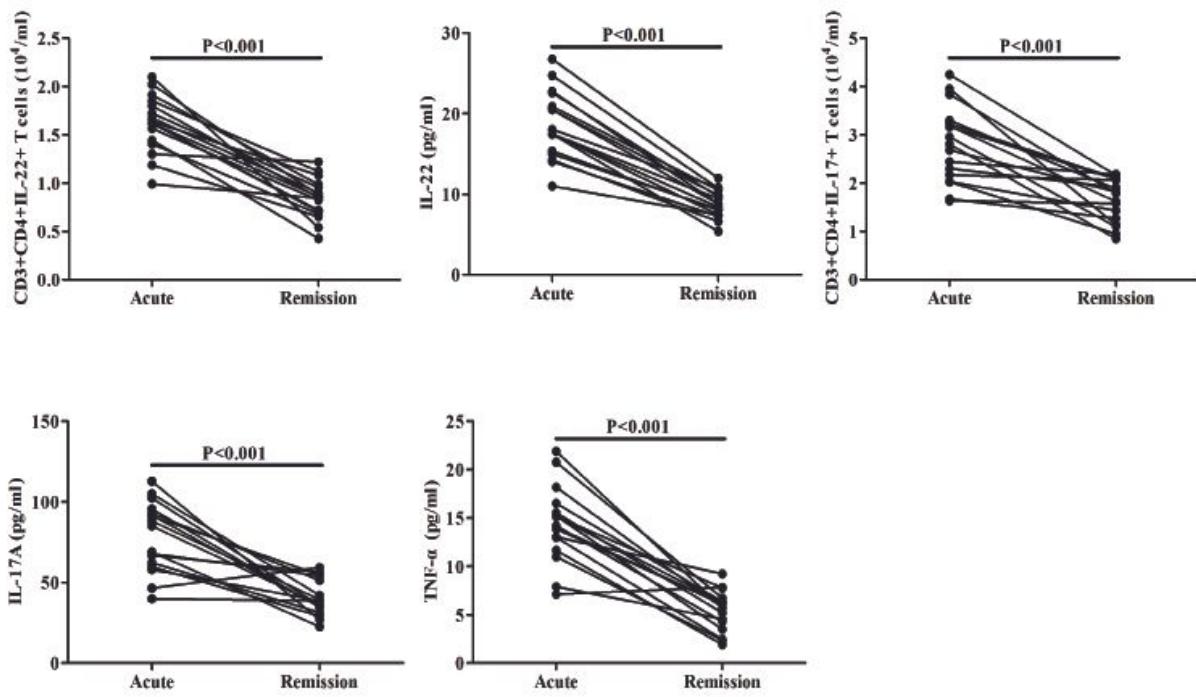
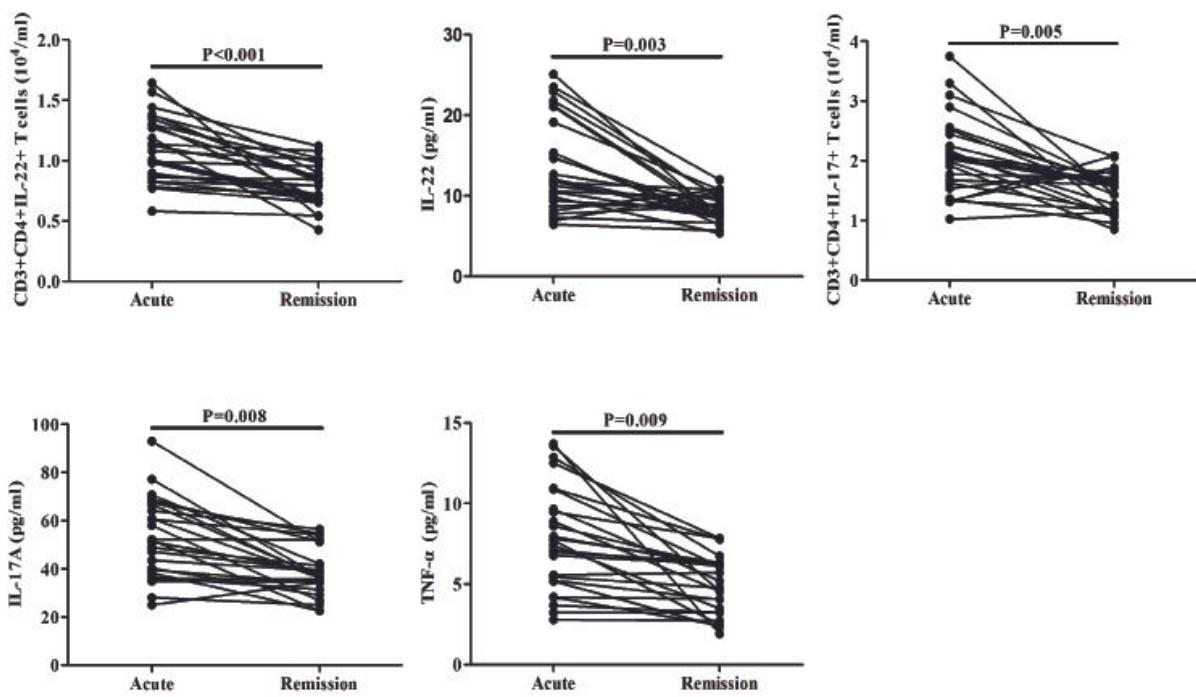


Figure 3

Correlation among the numbers of Th22/Th17 cells and the serum concentrations of cytokines in KD children. Correlation between the numbers of CD3+CD4+IL-22+Th22 cells and the numbers of CD3+CD4+IL-17+Th17 cells (A), serum IL-22 (B), serum TNF- α (C) in patients with CAL; Correlation between the numbers of CD3+CD4+IL-17+ Th17 cells and serum IL-17A (D) in patients with CAL; Correlation between the numbers of CD3+CD4+IL-22+Th22 cells and the numbers of CD3+CD4+IL-17+ Th17 cells (A), serum IL-22 (B) and serum TNF- α (C) in patients with NCAL; Correlation between the numbers of CD3+CD4+IL-17+Th17 cells and serum IL-17A (D) in patients with NCAL.

A**B****C****D****Figure 4**

Correlation between serum levels of Th22/Th17/Th1 cell-related cytokines and the values of disease activity parameters in KD patients. Correlation between serum levels of IL-22 (A), IL-17A (B) and ESR/CRP/NT-proBNP in KD patients with CAL; Correlation between serum levels of IL-22 (C), IL-17A (D) and ESR/CRP/NT-proBNP in KD patients with NCAL.

A**B****Figure 5**

The effects of IVIG therapy on the numbers of Th22/Th17 cells and serum IL-22, IL-17A and TNF- α in KD patients. The numbers of CD3+CD4+IL-22+Th22, CD3+CD4+IL-17+Th17 and the levels of serum IL-22, IL-17A and TNF- α in CAL (A) and NCAL (B) patients pre- and posttreatment.