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

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## Abstract

# Background

T-helper (Th) 17 and Th22 cells are critical for the pathogenic process of Kawasaki Disease (KD).

# Methods

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.

# Results

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.

# Conclusions

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

## Background

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 seurm 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 information 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 paremeter 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.

# Results

# Patient 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($\times10^9$/L) | 14.45±2.37* | 14.46 ±2.98* | 5.08 ±3.2 |
| Neutrophils($\times10^9$/L) | 10.56±1.76* | 10.22±2.86* | 2.62±1.34 |
| Lymphocytes($\times10^9$/L) | 3.25±1.39* | 3.11±1.54* | 2.12±0.74 |
| ESR(mm/h) | 59.77±17.28* | 55.77±16.91* | 6.53±2.62 |
| CRP(mg/L) | 52.34±17.50* | 51.4±17.62* | 2.12±0.83 |
| NT-proBNP (ng/L) | 1210.9±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±SD. | | | |
| Normal values: White blood cell counts (WBC):4-10×$10^9$/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 (*P*<0.001 and *P*<0.001, Fig. 1B; *P*=0.004 and *P*<0.001, Fig. 1C; respectively). 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; respectively). However, we did not find a significant difference in the numbers of $CD3^+CD4^+$IFN-$\gamma^+$Th1 cells among the different groups of patients and HCs (P>0.05, Fig. 1D). Moreover, we also did not find a significant difference in the numbers

of $IL17^+IFN\text{-}\gamma^+$ or $IL17^+$ $IL22^+$ double positive T cells among the different groups of patients and HCs (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 (*P*=0.006 and *P*<0.001, Fig. 2A; *P*<0.004 and *P*<0.001, Fig. 2B; respectively). However, we did not find a significant difference in the concentrations of serum IFN-γ among the different groups of patients and HCs (P>0.05, Fig. 2C). 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 (*P*<0.001 and *P*<0.001, Fig. 2D; respectively). In addition, the concentrations of serum IL-6 in KD patients were significantly higher than that in the HCs (*P*<0.001 and *P*<0.001, Fig. 2E; respectively).

# 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). Morever, 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]. Moreover, we found an equivalent numbers of Th1 cells, which were inconsistent with previous studies [19]. 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 might contribute to innate immune responses [7]. 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 [20], speculating Th17 cells might involved in the formation of coronary aneurysms [12]. 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 [21]. 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, 22]. 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 may play an important role in the pathogenesis of KD [23]. More importantly, we found that Th22 cells were

correlated positively with that of Th17 cells in KD patients. Given that Th22 and Th17 development are positively regulated by IL-6 [11], it is possible that the inflammatory environment, where high levels of IL-6 are shown in our research, preferably promotes the differentiation of naive $CD4^+$ T helper cells into Th22 and Th17 cells in the development of KD [24, 25]. 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 [14] 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 might 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 [26]. 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 [27]. 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 [24]. 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.

# Conclusions

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.

# Methods

# Studied subjects

A total of 43 newly diagnosed children with KD were admitted by the Pediatric outpatient department, the Second People's Hospital of Changzhou, China. According to the international criteria of the 2004 American Heart Association (AHA) statement individual children with KD were newly diagnosed [1]. All children participated in the research were diagnosed with KD through at least 5 days fever and met at least 4 in the 5 clinical criteria or 3 of 5 criteria along with coronary abnormalities. 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. 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. The study was approved by the Ethical Committee of the NanJing medical University, and written informed consents were obtained from all children's parents.

# 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 intracellular cytokine staining

Peripheral blood mononuclear cells (PBMCs) of children with Kawasaki disease were stimulated in duplicate with 1.0 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). After being washed with PBS, the cells were characterized on a FACSCalibur (BD) and at least 20 000 events were analyzed. The numbers of different subsets of peripheral blood lymphocytes were calculated by total mononuclear leucocyte counts multiplied by the percentage of the subset of lymphocytes. The frequency of CD3+CD4+IL-17+Th17, CD3+CD4+IL-22+Th22 and CD3+CD4+IFN-γ + Th1 cells were determined by flow cytometry.

# Enzyme-linked Immunosorbent assay for IL-6, TNF-α, IFN-γ, IL-17 and IL-22

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.

# 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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### Availability of data and materials

All data used and analyzed during the present study will be available fromthe correspongding author on reasonable request.

### Authors' Contributions

Zhang Liwen andMa Liangcontributed equally to this work.Wan Yudesigned 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.

### Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the 1975Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval byEthics Committee of the Second People's Hospital of Changzhou. Written informed consents were obtained from all children’s parents.

### Consent for publication

Not applicable.

### Conflict of Interests

All authors declared there were no conflict interests invoved.

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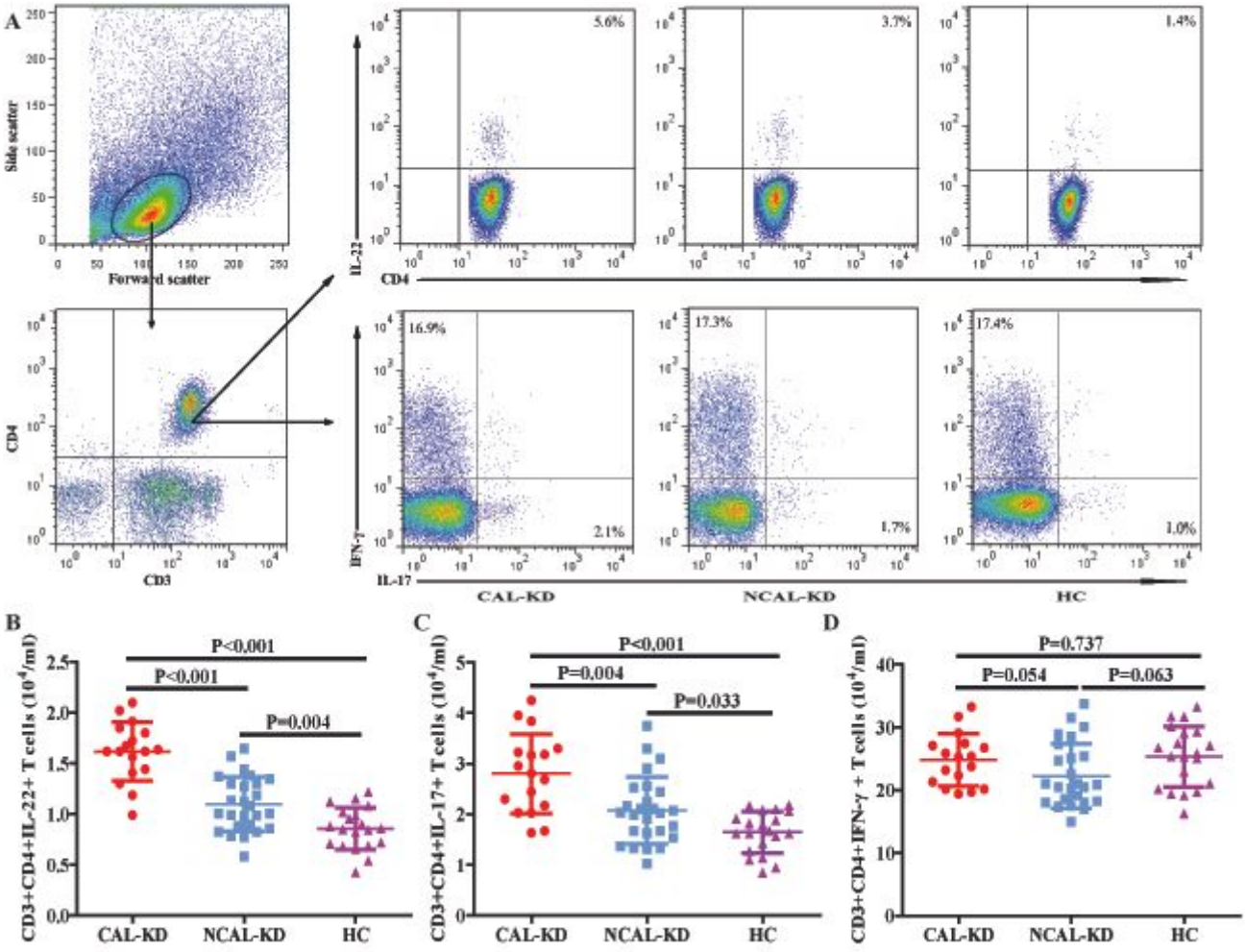
# Figures

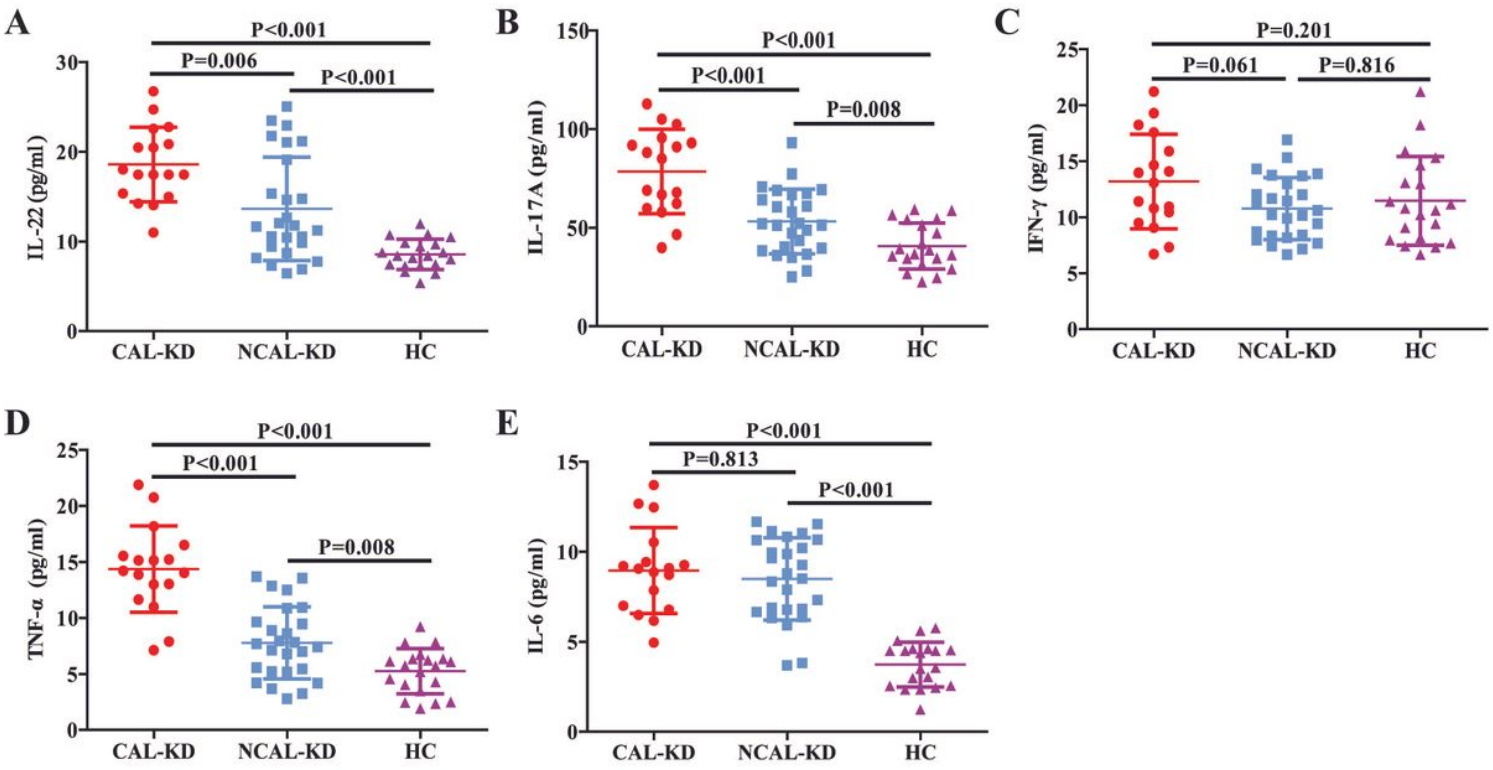



## Figure 1

### FACS analysis of circulating Th22/Th17/Th1 cells in KDchildren.

Peripheral blood mononuclear cells5*$10^5$/tubewere isolated and stained with PerCP-anti-CD4, PE-Cy5-anti-CD3and isotype controls. Then these cells were fixed and permeabilized, followed by intracellular staining withPE-anti-IL-22 PE-Cy7-anti-IFN-γ andFITC-anti-IL-17. The frequency of$CD3^+CD4^+$IL-$17^+$Th17, $CD3^+CD4^+$IL-$22^+$Th22 and $CD3^+CD4^+$IFN-$γ^+$Th1cells were gated on $CD3^+CD4^+$ cellsby 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-$γ^+$Th1cells were calculated . (A).Flow cytometry analysis; (B).The numbers of $CD3^+CD4^+$IL-$22^+$Th22cells;(C).$CD3^+CD4^+$IL-$17^+$Th17cells; (D).

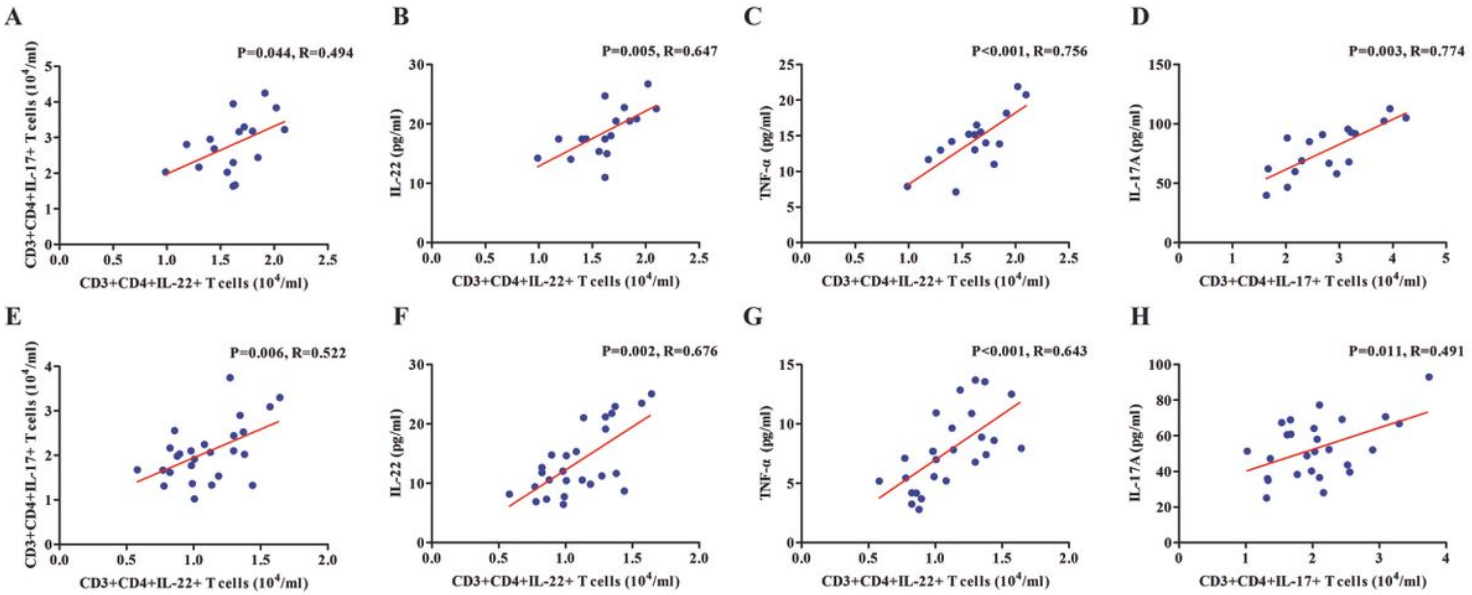
$CD3^+CD4^+IFN\text{-}\gamma^+$Th1cells. Data shown are representative charts of Th22-Th17-Th1 cells from different groups of patients (n=17 for the patients with CAL, n=26 for the patients with NCAL and n=20 for the HC).




## Figure 2

### ELISA analysis of the concentrations of serum cytokines in KD children.

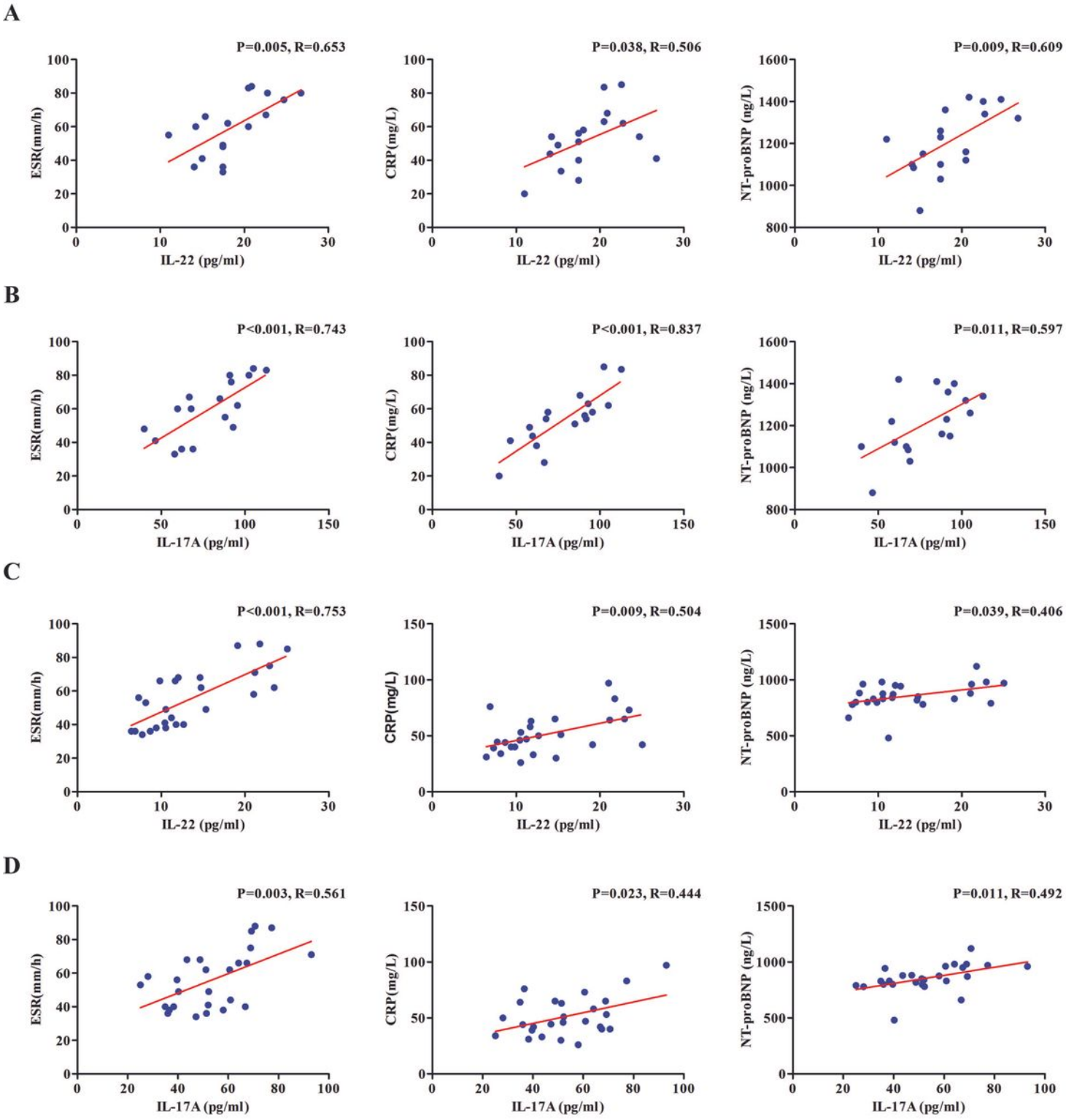
The serum concentrations of IL-17, IL-22, TNF-α,IL-6, and IFN-γ in individual subjectswere measured by ELISA. (A).Concentrations ofserum IL-22; (B).Concentrations ofserumIL-17; (C).Concentrations ofserum IFN-γ; (D). Concentrations ofserumTNF-α; (E). Concentrations ofserumIL-6.Data shown are serum cytokines 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.**

Correlationbetween the numbers of $CD3^+CD4^+IL\text{-}22^+$Th22 cells and the numbers of $CD3^+CD4^+IL\text{-}17^+$Th17cells(A), serum IL-22(B),serum TNF-α(C)in patients with CAL; Correlationbetween the numbers of $CD3^+CD4^+IL\text{-}17^+$ Th17cells and serum IL-17A (D) in patients with CAL;Correlationbetween the numbers of $CD3^+CD4^+IL\text{-}22^+$Th22 cells and the numbers of $CD3^+CD4^+IL\text{-}17^+$ Th17 cells(A), serum IL-22(B) and serum TNF-α (C) in patients with NCAL; Correlation between the numbers of $CD3^+CD4^+IL\text{-}17^+$Th17 cells and serum IL-17A (D) in patients with NCAL.

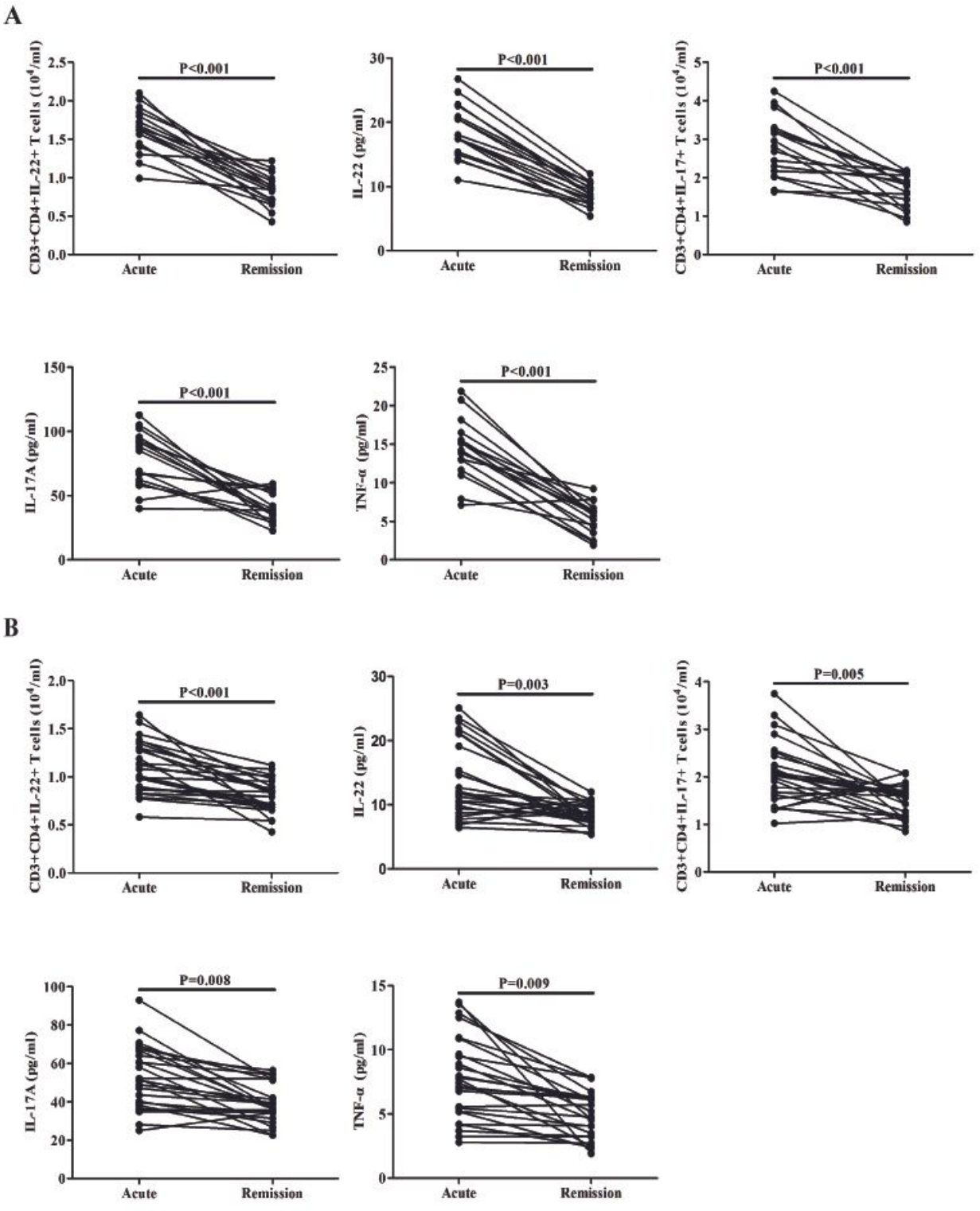



## Figure 4

**Correlation between serum levels of Th22/Th17/Th1 cell-related cytokines and the values of disease activity parameters in KDchildren.**

Correlation between serum levels of IL-22 (A), IL-17A (B)and ESR/CRP/NT-proBNPin KD patients with CAL; Correlation between serum levels of IL-22(C), IL-17A (D) and ESR/CRP/NT-proBNPin KD patients with

NCAL.




## Figure 5

**The effects of IVIG therapyon the numbers ofTh22/Th17 cells and serum IL-22, IL-17A and TNF-α in KD children.**

The numbers of$CD3^+CD4^+IL\text{-}22^+$Th22, $CD3^+CD4^+IL\text{-}17^+$Th17 and the levels of serum IL-22, IL-17A and TNF-α in CAL (A) and NCAL (B) patients pre- and posttreatment.