

## ORIGINAL ARTICLE

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# The Correlation between the Numerical Status of Th22 Cells and Serum Level of IL-22 with Severity of Ulcerative Colitis

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## ABSTRACT

Ulcerative colitis (UC) is a chronic relapsing inflammatory bowel disease, yet its etiology as well as pathogenesis remains poorly understood. There is increasing evidence that aberrant expression of CD4+T lymphocytes plays an essential role in the progression of different pathologies such as UC. This study aimed to evaluate the circulatory frequency of T-helper 22 (Th22), a subset of CD4+ T cells, and serum level of its signature cytokine, IL-22, in patients with UC.

Blood samples from 30 patients with UC and 30 controls (n=30) were tested for IL-22 level and circulatory Th22-cell count by ELISA and Flow cytometric analysis, respectively.

Our results revealed higher serum level of IL-22 as well as circulatory frequency of Th22 cells in patients with UC compared to those in healthy controls. Notably, effective factors on severity of the disease were age, Th22, IL-22, ESR and CRP.

We conclude that elevated circulating Th22 cells and their signature cytokine, IL-22, may be implicated in the pathogenesis of UC. These findings may provide preliminary experimental clues for the development of new therapies for UC and its severity judgment.

**Keywords:** Interleukin-22; Th22 cells; Ulcerative colitis

## INTRODUCTION

Ulcerative colitis (UC), a chronic relapsing inflammatory bowel disease (IBD), is a major threat of public health worldwide.<sup>1</sup>

It is characterized by periods of remission

punctuated by clinical exacerbations and frequent relapses in which 1% of the patients promote to cancer.<sup>2</sup> Previous studies have revealed important roles of different arms of immune system<sup>3</sup> such as IL-22 cytokine in the pathogenesis of IBD<sup>4</sup> and UC.<sup>5</sup> IL-22 is a unique cytokine produced by immune cells<sup>6</sup> and acts solely on non-immune tissue cells<sup>7</sup> such as intestinal epithelium<sup>8</sup> to increase their innate immunity, protect them from damage, and enhance their regeneration.<sup>8</sup> From the point of immune activities on epithelium, IL-22 induces the production of

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antibacterial products<sup>5,6,9</sup> as well as chemokines<sup>10</sup> and enhances migratory ability of epithelial cells.<sup>6,11</sup> IL-22 may also amplify the effects of IL-1 $\beta$ , TNF- $\alpha$ , IL-17 and IFN- $\gamma$  to some extent; thereby, it may enhance the pathogenic role of such cytokines.<sup>8</sup> Actually, IL-22 up-regulation has been reported in rheumatoid arthritis,<sup>12</sup> Crohn's disease,<sup>4</sup> psoriasis,<sup>6</sup> and atopic dermatitis,<sup>13</sup> whereas its serum down-regulation has been demonstrated in sarcoidosis and systemic lupus erythematosus.<sup>4</sup> Inducing antimicrobial, proliferative, and antiapoptotic effects, IL-22 helps tissue repair. All of such processes play a beneficial role in UC by enhancing intestinal barrier integrity and epithelial innate immunity.<sup>14</sup>

Th22 cells, a T-cell subset with novel functional profile, produce cytokines such as IL-22, IL-26, and IL-13 of which IL-22 is the most important regulating inflammatory responses in some autoimmune diseases.<sup>15</sup> Increased number of IL-22-positive cells in the lamina propria of UC mucosa<sup>5</sup> may promise some potential therapeutic effects of IL-22 in such pathology, i.e. treatment with recombinant IL-22 or gene delivery of IL-22 may alleviate tissue destruction during inflammatory responses.<sup>4</sup> From this point, Th22 cells provide a cellular target for therapeutic intervention and may resolve some unknown pathways in the control of inflammatory diseases.<sup>16</sup>

The current knowledge available about circulating Th22 and its related cytokines in IBD is often contradictory.<sup>17,18</sup> The development of other studies like ours using such measurements evaluating the prognostic accuracy of Th22 pathway is still preliminary. Having measured the frequency of circulating Th22 cells as well as serum level of IL-22 in a group of patients with UC, we correlated such quantities to clinical and pathological features of the disease. Such measurement may be a clue to promote therapeutic options as well as diagnostic markers of disease severity.

## MATERIALS AND METHODS

### Patients

Based on mean difference, a sample size of 30 patients with active UC and 30 healthy volunteers were enrolled. All subjects selected exclusively after signing the informed consent form approved by the local Ethical Committee (No. 92027). The study protocol conformed to the ethical guidelines of the

1975 Helsinki Declaration. Patients with active UC were diagnosed according to routine clinical, endoscopic and histopathological features. Healthy volunteers with no history of any inflammatory and autoimmune diseases were recruited as healthy controls from local Blood Donation Organization. Exclusion criteria were liver, kidney, rheumatoid, endocrine, cardiovascular, and metabolic diseases; cancer and a history of using any medications as well as smoking. Considering the anatomical site of endoscopic features of edema, erythema, mucosal friability, bleeding, erosions, ulcerations, and loss of the typical vascular pattern, we endoscopically categorized the extent of the disease into proctitis, proctosigmoiditis, left-sided colitis, and extensive colitis.<sup>19</sup> The severity of UC is defined as Truelove and Witts classification (Table 1).

### Specimens

Serum levels of IL-22 were measured by a commercial, sandwich type ELISA kit (e-Bioscience, USA) according to the manufacturer's instructions. The results were expressed as pg/mL. Venous blood samples of all subjects were collected in heparin-containing tubes. At the same time, serum was collected by centrifugation at 4°C, and then stored at -20°C until use.

### Flow Cytometric Analysis

Suspensions of 2 million cells/mL in RPMI-1640 medium containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin and 2 mM glutamine (Invitrogen, Carlsbad, CA, USA) were

**Table 1. Truelove and Witts classification of the severity of ulcerative colitis**

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#### Mild

<4 stools/day, without or with only small amounts of blood  
No fever  
No tachycardia  
Mild anemia  
ESR <30 mm/h

#### Moderate

Intermediate between mild and severe

#### Severe

>6 stools/day, with blood  
Fever > 37.5°C  
Heart rate > 90/min  
Anemia with Hgb level <75% of normal  
ESR >30 mm/h

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prepared from peripheral blood mononuclear cells (PBMCs) isolated on standard Ficoll-Hypaque. The cells were stimulated by 50 ng/mL phorbol myristate acetate (Sigma-Aldrich, USA) and 1 µg/mL ionomycin (Sigma-Aldrich) for 5 hours in the presence of 1mL Golgystop (BD-Bioscience, USA). After being washed once in phosphate-buffered saline (PBS), the cells were incubated in the dark at 4°C for 30 minutes to stain the surface with the anti-human CD4-FITC (eBioscience, USA). The cells were fixed, permeabilized (fixation-permeabilization buffer, eBioscience, USA) and incubated in the dark at 4° C for 30 minutes for intracellular staining with anti-human IL-22-PE (BD-Bioscience). Stained cells were analyzed by flowcytometric analysis using a BD FACS Calibur cytometer (BD Pharmingen, UAS) equipped with Cell Quest software.

### Statistical Analysis

Data were expressed as mean ±SD. The statistical indices of IL-22 levels as well as Th22 counts were analyzed using one-way Anova and chi-square tests. Simultaneous effects of various factors on severity of disease were analyzed by multinomial logistic regressions. Pseudo R-Square Cox and Snell and -2loglikelihood was determined as a criterion of

goodness-of-fit test, and  $p>0.2$  was considered for exclusion from the model.  $p$ -values<0.1 were considered statistically significant in the model. All analyses were performed using the SPSS 16 software (SPSS Inc, SPSS Inc., Chicago, USA).

## RESULTS

Values of the basic and clinical characteristics of the healthy individuals and patients at different severities are summarized in Table 2.

### Alteration of the Serum Level of IL-22 and Circulatory Number of Th22 Cells in UC Patients

Having investigated the alteration of serum levels of IL-22 and circulatory number of Th22 cells in UC patients, we found a significant increase of both markers compared to healthy controls ( $p\leq 0.001$ ) (Figures 1A and B). The more severe the disease, the more serum levels of IL-22 as well as circulatory number of Th22 cells.

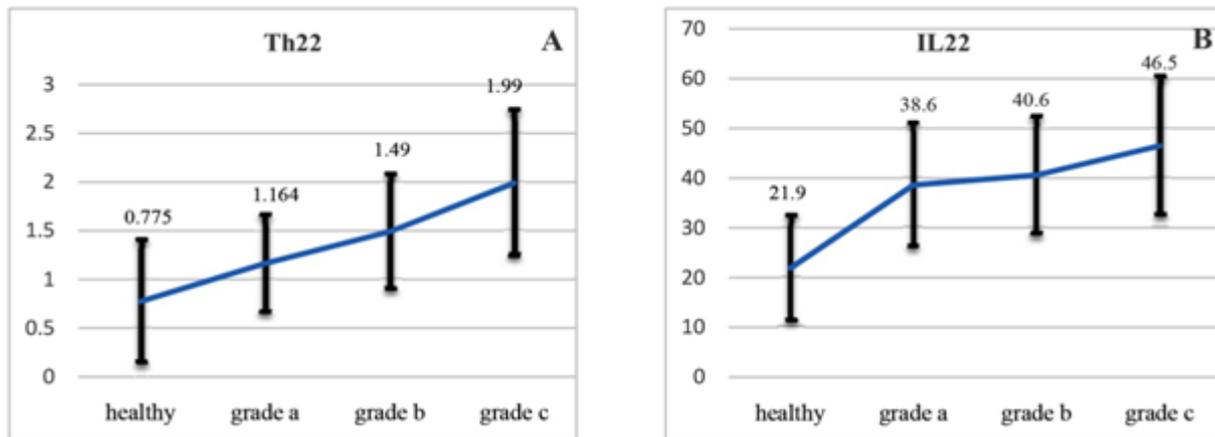
### Multivariate Analysis on Severity of UC

Using multinomial logistic regression analysis to investigate the effect of different factors on severity of UC, we found a model in which effective factors on

**Table 2. Distribution of basic and clinical characteristic of the ulcerative colitis patients and healthy individuals**

Parameters	Healthy (n=30)	Patients			p.value
		Mild (n=16)	Moderate (n=9)	Sever (n=5)	
Sex					
Male	10 (37.0%)	2 (7.4%)	5 (18.5%)	10 (37.0%)	0.266
Female	20 (60.6%)	3 (9.1%)	4 (12.1%)	6 (18.2%)	
Age (years)	22±9.96	38±10.95	27.78±12.02	32.5±14.37	0.007
Extention of diseases	-				-
Ulcerative proctitis		4 (80.0%)	1 (20.0%)	0 (0%)	
Proctosigmoiditis		1 (12.5%)	6 (75.0%)	1 (12.5%)	
Left-sided colitis		0 (0%)	2 (66.7%)	1 (33.3%)	
Total colitis		0 (0%)	0 (0%)	14 (100.0%)	
ESR (mm/h)					<0.001
<20	25 (69.4%)	1 (2.8%)	6 (16.7%)	4 (11.1%)	
≥20	5 (20.8%)	4 (16.7%)	3 (12.5%)	12 (50.0%)	
CRP(mg/L)					<0.001
<25	30 (75.0%)	3 (7.5%)		3 (7.5%)	
≥25	0 (0%)	13 (65.0%)		2 (10.0%)	
Th22	0.77±0.63	1.16±0.5	1.49±0.99	1.99±1.3	0.001
IL-22	21.9±10.6	38.6±14.02	40.6±11.9	46.6±12.57	<0.001

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**Figure 1. (A) The percentage of circulating Th22 cells in patients with active ulcerative colitis compared to healthy controls (B) The level of IL-22 of all subjects in the four groups**

UC severity were age, the number of circulatory Th22 cells, the serum level of IL-22, ESR and CRP (dependent variables) ( $p < 0.01$ ).  $-2\log$  likelihood from 166.3 in null model (the model with only constant) reached to 41.6 in saturated model ( $p < 0.001$ ). It means that such model yields a proper goodness-of-fit in which Cox and Snell pseudo R Square was 0.875 (Table 3). The negative indices of "normal ESR" and "normal CRP" in our model indicate that "normal values of ESR as well as CRP" makes the patients suffer from less severe forms of the disease.

### Sensitivity and Specificity of Circulatory Th22 Frequency and IL-22 Serum Levels in Determining of UC Severity

Using ROC curve and Area under Curve (AUC), we determined the sensitivity and specificity of both circulatory frequency of Th22 cells and serum levels of IL-22 as diagnostic markers of UC severity. In the best cutoff point of circulatory frequency of Th22 cells=0.885, sensitivity and specificity were 83.3% and 53.3%, respectively; and AUC was determined as 0.774 (Table 4).

**Table 3. Parameters of multinomial logistic regression model showing variables effective on ulcerative colitis severity**

Severity		B	Std. Error	Wald	df	Sig.	Exp (B)
Mild	age	16.373	101.307	0.026	1	0.872	1.290
	Th22	338.165	2442.430	0.019	1	0.890	7.299
	IL-22	16.134	113.073	0.020	1	0.887	1.016
	[ESR=0]	-967.627	938.009	1.064	1	0.302	0.000
	[CRP=0]	-610.503	6044.886	0.010	1	0.920	7.275
Moderate	age	16.222	101.306	0.026	1	0.873	1.109
	Th22	339.059	2442.430	0.019	1	0.890	1.784
	IL-22	16.103	113.073	0.020	1	0.887	9.852
	ESR (normal)	-957.232	938.001	1.041	1	0.307	0.000
	CRP (normal)	-613.639	6044.885	0.010	1	0.919	3.161
Severe	age	16.280	101.306	0.026	1	0.872	1.176
	Th22	339.556	2442.430	0.019	1	0.889	2.934
	IL-22	16.096	113.073	0.020	1	0.887	9.786
	[ESR=0]	-959.616	937.998	1.047	1	0.306	0.000
	[CRP=0]	-614.323	6044.885	0.010	1	0.919	1.595

**Table 4. Sensitivity and specificity of Th22 number cells and IL-22 levels in determining of ulcerative colitis severity**

Inflammatory markers	Cutoff point	Sensitivity	Specificity	PPV	NPV	AUC
Th22	0.885	83.3%	53.3%	61%	73.7%	0.774
IL22	27.1	86.7%	66.7%	72.2%	83.3%	0.868

## DISCUSSION

Recent findings have revealed that the imbalance of T lymphocytes plays an important role in the pathogenesis of UC.<sup>20</sup> Regarding Th22 subset of CD4+T cells, such imbalance has been demonstrated in several autoimmune diseases such as rheumatoid arthritis<sup>21</sup> and immune thrombocytopenic purpura.<sup>22</sup> Th22 cells produce IL-22 which is expressed also by Th17 and NK cells.<sup>23</sup> The foremost protective role of such cell populations via IL-22 production has been demonstrated in various chronic inflammatory diseases.<sup>8</sup> IL-22 mediates some crosstalk between immune and non-immune cells especially epithelial cells.<sup>24</sup> At such basis, the massive presence of IL-22 has been reported in affected tissues of inflammatory diseases such as autoimmune hepatitis and cholestatic liver disease,<sup>8</sup> psoriasis,<sup>6</sup> atopic dermatitis,<sup>7</sup> IBD,<sup>25</sup> rheumatoid arthritis,<sup>21</sup> cystic fibrosis,<sup>26</sup> and multiple sclerosis.<sup>27</sup> Moreover, elevated IL-22 expression has been observed in human infectious diseases such as Mycobacterium tuberculosis infection in which IL-22 levels are increased in the bronchoalveolar lavage fluid.<sup>28</sup>

A preventive role of IL-22 has been proposed in Crohn's disease.<sup>29</sup> In line with such concept, IL-22 gene delivery led to rapid amelioration of local intestinal inflammation through both STAT3-dependent expression of mucus-associated molecules and restitution of mucus-producing goblet cells in a mouse model of UC.<sup>30</sup> Moreover, a regulatory role of IL-22 in IBD has recently been proposed due to the ability of IL-22 to dampen systemic inflammatory response through the induction of lipopolysaccharide-binding protein.<sup>25</sup> In contrast, IL-22 antagonism might be a promising therapy for alleviation of inflammatory responses of the psoriasis.<sup>31</sup> Thus, the role of IL-22 in different epithelial cells like IBD may be still unclear and remains to be established.

In the present study, our results revealed that levels of circulating Th22 cell and IL-22 serum levels were

significantly increased in patients with UC. Our model also revealed that factors like age, the frequency of Th22 cells, serum levels of IL-22, ESR and CRP may have a positive correlation with severity of the disease. Therefore, we hypothesized that aberrant expression of Th22 pathway may play an important role in the pathogenesis of UC. It may be considered that the elevation of IL-22 serum levels as well as Th22 circulatory frequencies in our study could be one probable compensatory protective mechanism of tissue injury in UC. These findings may help to broaden our knowledge concerning the immunopathological role of these cells in the progression of UC.

The limitation of our study was lack of monitoring serum levels of IL-22 as well as circulatory frequencies of Th22 cells longitudinally. This limitation allowed just a cross-sectional analysis of such changes of only limited robustness. Secondly, functional assays which provide further information on the possible immunoregulatory mechanism of Th22 pathway were not performed.

We conclude that elevated circulating Th22 cells and their signature cytokine, IL-22, may be implicated in the pathogenesis of UC. Such implication may be related to disease alleviation. Therefore, these findings, provided that be confirmed by more studies with larger sample size, may provide preliminary experimental clues for the development of new therapies for UC and its severity judgment.

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