

# Correlations between alterations of T-helper 17 cells and treatment efficacy after concurrent radiochemotherapy in locally advanced cervical cancer (stage IIB–IIIB): a 3-year prospective study

Yi Liu<sup>1,2,3</sup>, Qiu-Fen Guo<sup>3</sup>, Jin-Long Chen<sup>3</sup>, Xi-Rui Li<sup>4</sup>, Fei Hou<sup>5</sup>, Xiao-Yan Liu<sup>6</sup>, Wen-Jing Zhang<sup>1</sup>, Yan Zhang<sup>7</sup>, Fu-Feng Gao<sup>3</sup>, You-Zhong Zhang<sup>1</sup>, Bao-Xia Cui<sup>1</sup>, Nai-Fu Liu<sup>3</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Qilu Hospital, Shandong University, Ji'nan, Shandong 250012, China;

<sup>2</sup>Medical Management Service Center of Health Commission of Shandong Health commission, Ji'nan, Shandong 250014, China;

<sup>3</sup>Department of Gynecological Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Ji'nan, Shandong 250117, China;

<sup>4</sup>Department of Medical Record, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Ji'nan, Shandong 250117, China;

<sup>5</sup>Department of Gynecological Oncology, Jinan Maternal and Child Care Hospital, Ji'nan, Shandong 250012, China;

<sup>6</sup>Department of Gynecological Oncology, Taian Second Hospital of Traditional Chinese Medicine, Taian, Shandong 271000, China;

<sup>7</sup>Department of Gynecology, Weifang People's Hospital, Weifang, Shandong 261000, China.

## Abstract

**Background:** Recently, T-helper 17 (Th17) cells have been proved to play an important role in promoting cervical cancer. But, till now, few study has been carried out to understand the involvement of these cells in efficacy of anti-tumor treatments. This study aimed to investigate the alterations in the percentage of circulating Th17 cells and related cytokines in locally advanced cervical cancer (LACC) patients before and after concurrent chemoradiotherapy (cCRT) and to analyze the correlations between the alterations in Th17 cells and treatment efficacy.

**Methods:** A prospective study with 49 LACC (International federation of gynecology and obstetrics [FIGO] stage IIB–IIIB) patients and 23 controls was conducted. Patients received the same cCRT schedule and were followed up for 3 years. Circulating Th17 cells (CD3+CD8– interleukin [IL]-17+ T cells) and related cytokines IL-17, transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-10, IL-23, IL-6, and IL-22 were detected before and after cCRT. Correlations between alterations of circulating Th17 cells and treatment efficacy were analyzed. Kaplan-Meier analysis was used for overall survival (OS) and progression-free survival (PFS).

**Results:** We found that 40 patients finished the entire cCRT schedule and met the endpoint of this study. The percentage of circulating Th17 cells in the LACC patients was higher than that in the controls, and it significantly decreased after cCRT ( $P < 0.05$ ). After cCRT, patients were divided into two groups based on the average of the Th17 cells declined. The subgroup of patients with a prominent decrease in circulating Th17 cells after cCRT had a higher treatment efficacy and longer PFS and OS times. Compared with the control patients, LACC patients had higher IL-6, IL-10, IL-22, TGF- $\beta$  levels and a lower IL-23 level ( $P < 0.05$ ). After cCRT, IL-6, IL-10, IL-17, IL-23 level significantly increased and TGF- $\beta$  level significantly decreased compared with the levels before cCRT ( $P < 0.05$ ).

**Conclusion:** Circulating Th17 cells in the LACC patients (FIGO stage IIB–IIIB) were higher than those in the controls, but they generally decreased after cCRT. A more pronounced decrease in circulating Th17 cells after cCRT was correlated with better therapeutic effect and longer PFS and OS times.

**Keywords:** Locally advanced cervical cancer; Concurrent chemoradiotherapy; Th17 cells; Cytokines; Treatment efficacy

## Introduction

Cervical cancer is the second leading cause of cancer death worldwide in young women.<sup>[1]</sup> In contrast to its decreasing incidence trend in America, the incidence and mortality of

cervical cancer have been increasing in China.<sup>[2]</sup> These different trends obviously reflect the increasing prevalence of high-risk human papillomavirus (HPV) infections and the lack of HPV vaccines in China. Almost all cervical

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**Correspondence to:** Dr. Nai-Fu Liu, Department of Gynecological Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, 440 Jiyan Road, Ji'nan, Shandong 250012, China  
E-Mail: liunaifu@126.com

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cancers are initiated by infection with high-risk strains of HPV. Persistent infection with HPV induces immune dysfunction and a chronic inflammatory response, which have been proven to contribute to tumor formation, growth and progression.<sup>[3,4]</sup>

T-helper 17 (Th17) cells are recognized as a particular subset of CD4+ T helper lymphocytes, which are characterized by high production of interleukin (IL)-17. In addition, they also produce other inflammatory cytokines, such as IL-21, IL-22, and transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>[5]</sup> It has been extensively confirmed that Th17 cells and their related cytokines are involved in wound healing, tissue regeneration, and carcinogenesis.<sup>[6,7]</sup> Although the accumulation of Th17 cells has been observed in many different types of tumors,<sup>[8-11]</sup> their role in tumor immunity remains controversial due to their high degree of plasticity. Th17 cells have reported to be associated with the promotion of tumor growth (by inducing angiogenesis via IL-17) and anti-tumor immune responses.<sup>[5-7]</sup>

Several studies have been carried out to investigate the involvement of Th7 cells in cervical cancer.<sup>[12-15]</sup> One of our previous studies confirmed that Th17 cells infiltrated into cervical intraepithelial neoplasia (CIN) and cervical cancer tissues, and they are associated with cancer progression.<sup>[12]</sup> Thus far, no study has focused on the alterations in Th17 cells and related cytokines during the treatment for patients with locally advanced cervical cancer (LACC) (International federation of gynecology and obstetrics [FIGO] stages IIB–IIIB).

Treatment of LACC has undergone refinement in the last two decades. Concurrent chemoradiotherapy (cCRT) is currently recognized as the standard treatment protocol for LACC patients.<sup>[16]</sup> The purpose of this study was to investigate the variations in Th17 cells and their related cytokines during cCRT, and to elucidate correlations between these alterations and treatment efficacy. In this prospective study, we primarily detected the percentage of circulating Th17 cells and related cytokines in LACC patients before and after cCRT and compared them with control patients. Secondly, we collected data on treatment efficacy, including the response rate and 3-year prognosis, and then analyzed the correlation between variations in Th17 cells and treatment efficacy.

## Methods

### Ethical approval

This study was approved by the Ethics Committee on Scientific Research of Shandong Cancer Hospital and Institute Affiliated to Shandong University (SHTHEC20120306). Written informed consent was obtained from all participants.

### Patients and controls

Between June 2017 and February 2018, 49 patients (aged 33–73 years, median 53.5 years old) with LACC were eligible for this study. Eligibility criteria included newly diagnosed patients with cervical neoplasms, stage IIB–IIIB, according to the FIGO 2019, without significant comorbid-

ities and suitable for cCRT. Stage IV patients were excluded due to their poor health condition and diagnosis. No patients had received previous surgery, radiotherapy, chemotherapy, or other oncologic treatment before enrollment in this study.

Twenty-three healthy women with normal results on Papanicolaou smears (thinprep cytologic test) and high-risk HPV DNA tests served as controls (aged 32–72 years, median 49.1 years old) without any other disease. All LACC patients and controls are from Shandong Cancer Hospital and Institute.

### Treatments and follow-up

All LACC patients received the same cCRT schedule recommended by the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Cervical Cancer (2017). The schedule includes platinum-based chemotherapy (cisplatin/carboplatin plus paclitaxel), external beam radiotherapy (EBRT), and high-dose-rate integrated 3D after-loading brachytherapy (Iridium-192 source).

EBRT covered the primary tumor, entire uterus, cervix, parametrial tissues, the upper half of the vagina, and the gross pelvic and pelvic lymph node drainage area. The total radiation dose was 50 Gy/25 fractions. Patients with para-aortic lymph node metastasis received an additional dose of 10 to 15 Gy by intensity modulated radiation therapy or an extended field in EBRT. Computed tomography-guided-3D after-loading brachytherapy made Point A 25 to 30 Gy/5 to 6 fractions in total.

Two weeks after completion of cCRT, the tumor response was evaluated by gynecologic examination and computed tomographic imaging. Patients were followed up every 6 months during the first 3 years after completion of the treatment. The treatment, physical examination, assessment of response to cCRT, and follow-up were completed by the same group of gynecological oncologists. The response was evaluated according to the Response Evaluation Criteria in Solid Tumors.

Progression-free survival (PFS) and overall survival (OS) were calculated from recruitment to the date of progressive disease and death or last follow-up, respectively. PFS and OS were calculated at 6, 12, 24, and 36 months. Kaplan-Meier analysis were used for OS and PFS.

### Flow cytometric analysis of Th17 cells in peripheral blood

Peripheral blood was sampled from the LACC patients before and after completion of treatment. The percentage of circulating Th17 cells in the controls (control) and LACC patients before (pre-cCRT) and after treatment (post-cCRT) were compared. We calculated the descending rate (DR) of circulating Th17 cells in each patient by the following formula. Patients were divided into two groups based on the average of DR.

$$DR = (P_{pre} - P_{post}) / P_{pre}$$

P<sub>pre</sub>: percentage of circulating Th17 cells before treatment; P<sub>post</sub>: percentage of circulating Th17 cells after treatment.

**Table 1: The clinical characteristics of the cervical cancer patients (n = 40).**

Characteristics	Category	Number	Percentage (%)
Age (years)	<50	16	40.0
	≥50	24	60.0
FIGO stage	IIB	19	47.5
	IIIA	10	25.0
	IIIB	11	27.5
Histology	SCC	33	82.5
	ADC/ADSC	4	10.0
	SCC-carcinosarcoma	1	2.5
	Undifferentiated carcinoma	2	5.0
Tumor differentiation	Well	26	65.0
	Moderate	4	10.0
	Poor	10	25.0

ADC: Adenocarcinoma; ADSC: Adenosquamous carcinoma; FIGO: International federation of gynecology and obstetrics; SCC: Squamous cell carcinoma.

Heparinized peripheral whole blood (2 mL) with an equal volume of normal saline was isolated in lymphocyte isolation fluid (4 mL) by centrifugation ( $167.7 g \times 5 \text{ min}$ ). Then, the lymphocytes obtained were rinsed, resuspended, and incubated in Roswell Park Memorial Institute medium (RPMI) 1640, including 1.0  $\mu\text{g/mL}$  phorbol myristate acetate, 25 ng/mL of ionomycin, and 1.7  $\mu\text{g/mL}$  monensin, for 4 h at 37°C, 5% CO<sub>2</sub>. The incubated lymphocytes were stained with 5  $\mu\text{L}$  PE-Cy5-conjugate anti-human CD8 and 5  $\mu\text{L}$  fluorescein isothiocyanate (FITC)-conjugated anti-human CD3 monoclonal antibodies (ThermoFisher Scientific, Waltham, MA, USA) for 15 min at room temperature in the dark. After fixation, 100  $\mu\text{L}$  permeabilization buffer and 5  $\mu\text{L}$  PE-conjugated anti-IL17 monoclonal antibody were added into each sample and incubated for 15 min at room temperature in the dark. Meanwhile, isotype controls were made by using the same method to correct for any compensation and to confirm the antibody specificity. The stained lymphocytes were analyzed by flow cytometry (FACS Calibur BD, Philadelphia, PA, USA) and Flowjo X-V10 software (Tree Star, IBM Corp., NJ, USA).

### Enzyme linked immunosorbent assay (ELISA) in peripheral blood

The serum was extracted after the peripheral blood was sampled and centrifuged ( $167.7 g \times 5 \text{ min}$ ), then stored at  $-80^\circ\text{C}$  until analyzed. ELISA assays were performed to determine the levels of IL-17, TGF- $\beta$ , IL-10, IL-23, IL-6, and IL-22 according to the instructions of the manufacturers of the kits (CUSABIO, Wuhan, China). All of the samples were measured in duplicate.

### Statistical analysis

Categorical variables were reported as numbers and percentages, and quantitative variables as means and standard deviations or medians and quartiles. Differences between groups were compared by *t*-test or Mann-Whitney *U* test. Differences between paired groups were compared by paired *t*-test or Wilcoxon paired signed-rank test. The correlation between the two groups was analyzed

**Table 2: Treatment efficacy and survival time after cCRT treatment of patients with LACC (n = 40).**

Item	Number	Percentage (%)
Treatment efficacy		
Complete remission	33	82.5
Partial remission	3	7.5
Stable disease	1	2.5
Progressive disease	3	7.5
Progress-free survival		
6 months	32	80.0
12 months	31	77.5
24 months	28	70.0
36 months	28	70.0
Overall survival		
6 months	38	95.0
12 months	34	85.0
24 months	32	80.0
36 months	28	70.0

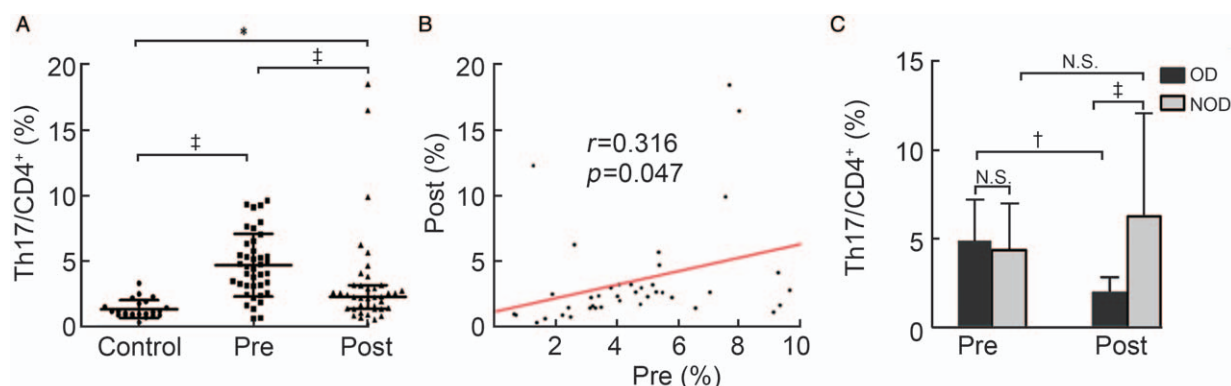
cCRT: Concurrent chemoradiotherapy; LACC: Locally advanced cervical cancer.

by Pearson correlation analysis. The Log rank test compared the survival curves. All analyses were performed by SPSS 19.0 software (IBM Corp., NY, USA).  $P < 0.05$  was considered statistically significant.

## Results

### Treatment efficacy and prognosis

Of the 49 patients eligible for the study, 40 patients (the clinical characteristics were shown in Table 1) finished the entire cCRT schedule and met the endpoint of this study (died or completed the 3 years follow-up). Five patients did not complete the cCRT, and four patients were lost to follow-up. The treatment efficacy to cCRT and survival time were shown in Table 2. PFS at 6, 12, 24, and 36 months was 80.0%, 77.5%, 70.0%, and 70.0%, respectively. OS at 6, 12, 24, and 36 months was 95.0%, 85.0%, 80.0%, and 70.0%, respectively. Twelve patients died



**Figure 1:** Variations of Th17 cells in LACC patients. (A) Percentage of circulating Th17 cells in LACC patient pre-/post-concurrent chemoradiotherapy and control. (B) Correlation of circulating Th17 cells in LACC patients before and after cCRT. (C) Percentage of Th17 cells before and after cCRT in OD group and NOD group. \* $P < 0.05$ , † $P < 0.01$ , ‡ $P < 0.001$ . cCRT: Concurrent chemoradiotherapy; LACC: Locally advanced cervical cancer; NOD: Non-obviously decreasing group; N.S.: Non-significant; OD: Obviously decreasing; Th17: T-helper 17; Pre: Pre-cCRT; Post: Post-cCRT.

within 3 years of follow-up, which was attributed to cerebral hemorrhage ( $n=1$ ), chronic ischemic heart disease ( $n=1$ ), and cancer progression ( $n=10$ ).

### Variations of circulating Th17 cells

As shown in Figure 1A, the median percentage of circulating Th17 cells (CD3+CD8- IL17+ T cells) in pre-cCRT patients (4.54% [3.13%–6.23%]) was significantly higher compared with that in the control groups ( $P < 0.001$ ). After cCRT, the percentage of circulating Th17 cells significantly decreased ( $P < 0.001$ ). However, it was still higher than that in the controls ( $P < 0.05$ ). We found that there was a correlation between the circulating Th17 cells in each LACC patients before and after cCRT ( $r=0.316$ ,  $P=0.047$ ) [Figure 1B]. It indicated that 31 of 40 patients exhibited a decrease variation trend, nine of 40 patients experienced an increasing percentage of circulating Th17 cell.

The DR of circulating Th17 cells in each patient ranged from  $-140.60\%$  to  $88.39\%$ , and the average was  $28.88\%$ . The patients with a DR over  $28.88\%$  were assigned to the obviously decreasing group (OD group,  $n=27$ ), and the other patients were assigned to the non-obviously decreasing group (NOD group,  $n=13$ ). As shown in Figure 1C, there was no significant difference in the percentage of Th17 cells before treatment between the OD and NOD groups ( $P=0.587$ ). After cCRT, the percentage of Th17 cells was lower in the OD group than that in the NOD group ( $P=0.026$ ). A typical dot plot of the percentage of Th17 cells in the representative LACC and control patients is shown in Figure 2.

### Correlation between variations of Th17 cells and treatment efficiency

Given that nine of 40 patients experienced an increasing percentage of circulating Th17 cells, we speculated that the inconsistent variations correlated with different treatment responses. Treatment efficacy, PFS, and OS were compared

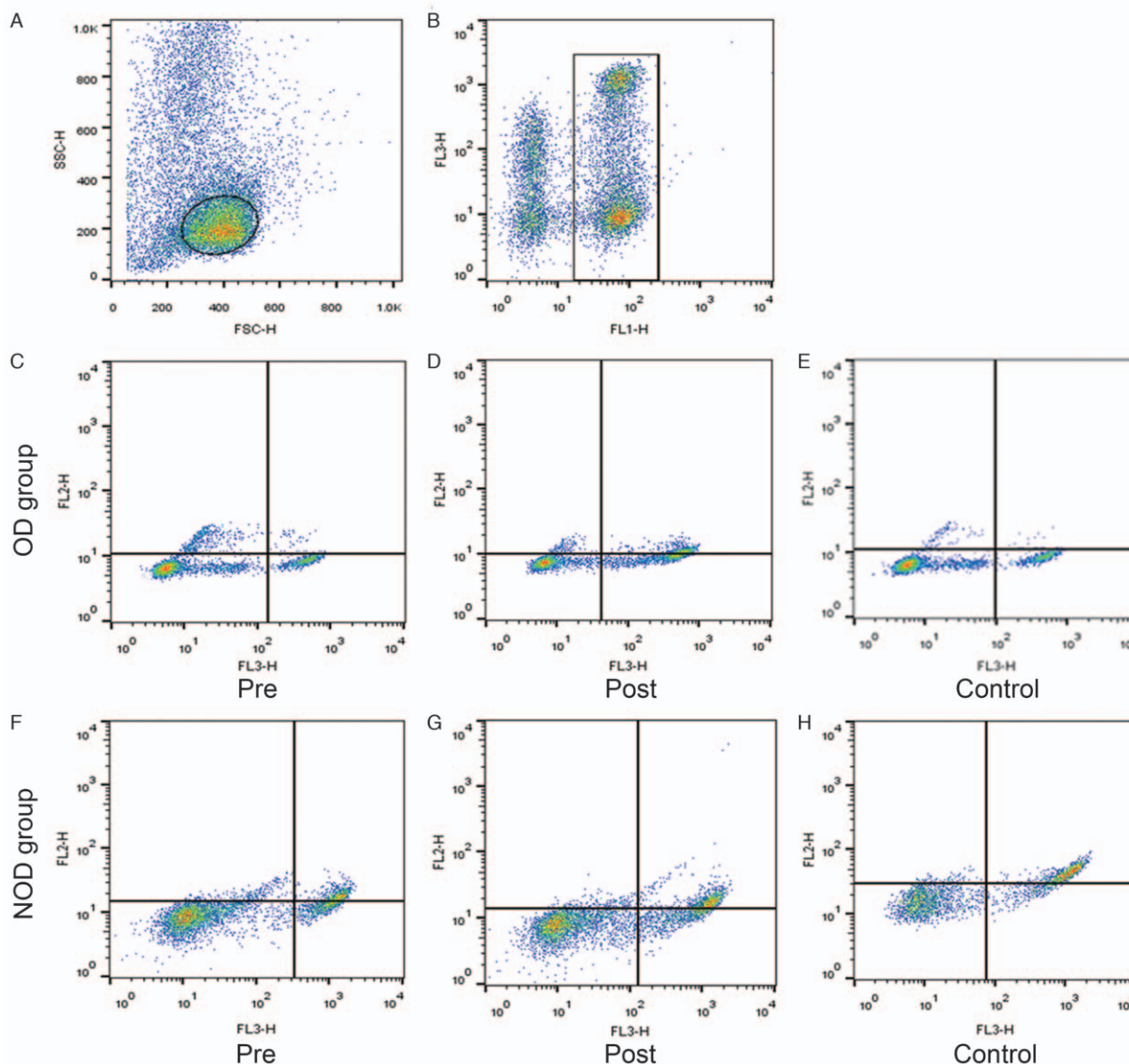
between the OD and NOD groups. These results as shown in Figure 3A and 3B indicated that an obvious decrease in circulating Th17 cells after cCRT was correlated with better treatment efficiency. PFS and OS were higher in the OD group than those in the NOD group [Figure 3C and 3D]. Kaplan-Meier curves for the patients showed a significant difference in PFS and OS between OD group *vs.* NOD group (log-rank test, both  $P < 0.05$ ). Overall, patients that in OD group had a significantly higher PFS ( $P=0.019$ ) and OS ( $P=0.02$ ) than patients in NOD group.

### Variations of Th17 cell-associated cytokines

As shown in Table 3, all of the cytokines in the pre-cCRT group were compared with those in the post-cCRT and control groups. Compared with the control patients, pre-cCRT patients had higher IL-6, IL-10, IL-22, TGF- $\beta$  levels and a lower IL-23 level [\* in Table 3]. After cCRT, IL-6, IL-10, IL-17, IL-23 level significantly increased and TGF- $\beta$  level significantly decreased compared with the levels before cCRT ( $P < 0.05$ ) [† in Table 3]. The cytokines were further compared between the OD and NOD groups to understand the association between the cytokines and the Th17 cells. Compared with OD group, IL-22 and IL-23 levels in NOD group were higher, while TGF- $\beta$  was lower before treatment. [‡ in Table 3]. After cCRT, the IL-10 and IL-17 levels in the OD group were higher than those in the NOD group which were statistically significant [§ in Table 3].

### Correlation between variations of Th17 cells and associated cytokines

We analyzed the correlation between Th17 cells and associated cytokines. As shown in Figure 4, we found that there were respectively positive correlations which existed between Th17 cells and IL-17 ( $r=0.493$ ,  $P=0.001$ ), IL-22 ( $r=0.622$ ,  $P < 0.001$ ), IL-23 ( $r=0.347$ ,  $P=0.028$ ), TGF- $\beta$  ( $r=0.358$ ,  $P=0.023$ ) before cCRT. After cCRT, we analyzed the relationships between the decreased percentage of Th17 cells and the numbers of associated cytokines increased/decreased. It indicated that before and after cCRT



**Figure 2:** Levels of circulation Th17 cells in pre-/post-cCRT and control. (A) Lymphocytes were gated by flow cytometry. (B) CD3+ T cell subsets were gated by flow cytometry. Plots in intern box represented CD3+ T cells. (C–E) Proportion of Th17 to CD3+CD8- T (CD4+) subsets in representative pre-cCRT, post-cCRT of OD group and controls. (F–H) Proportion of Th17 to CD3+CD8- T (CD4+) subsets in representative pre-cCRT, post-cCRT of NOD group and controls. cCRT: Concurrent chemoradiotherapy; NOD: Non-obviously decreasing group; OD: Obviously decreasing; Th17: T-helper 17; Pre: Pre-cCRT; Post: Post-cCRT.

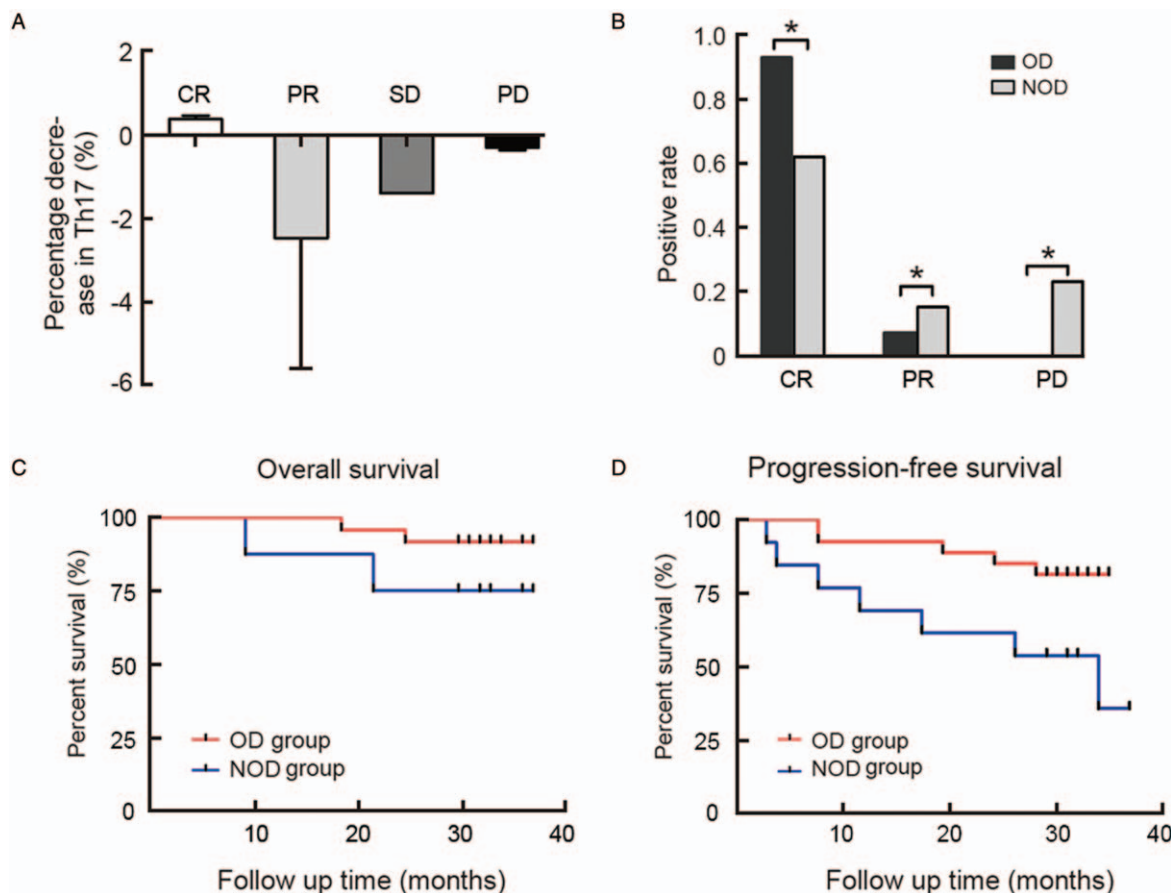
the difference of Th17 cell changes was positively correlated with the difference of IL-17 ( $r = 0.453$ ,  $P = 0.003$ ), IL-22 ( $r = 0.528$ ,  $P < 0.001$ ), IL-6 ( $r = 0.399$ ,  $P = 0.011$ ), IL-10 ( $r = 0.362$ ,  $P = 0.022$ ), and TGF- $\beta$  ( $r = 0.431$ ,  $P = 0.005$ ) changes [Figure 5].

## Discussion

The current 3-year prospective study initially investigated the variations in circulating Th17 cells during cCRT for LACC patients (FIGO stages IIB–IIIB). Meanwhile, a preliminary analysis was performed to investigate the correlation between variations in Th17 cells and treatment efficacy, including the responses and the short- to mid-term prognosis. The principal findings of this study are as follows: (1) the percentage of circulating Th17 cells tended to decrease after cCRT overall. However, some patients

experienced increases in circulating Th17 cells after treatment; (2) an obvious decrease in Th17 cells after cCRT correlated with a better treatment efficiency.

In China, advanced cervical cancer is common at the time of diagnosis. Management of LACC has undergone refinement in the last two decades. Although novel treatment approaches have been proved to have positive effects, such as adjuvant chemotherapy after cCRT,<sup>[17]</sup> novel agents targeting molecular pathways<sup>[18,19]</sup> and immune check point inhibitors,<sup>[20]</sup> cCRT is still the standard treatment approach for LACC patients. Reported 5-year OS varies from 30% to over 60% depending on the stage. The standard cCRT protocol was adopted for LACC patients at our hospital in the year 2000. In the present study, 3-year OS and PFS were both 70%, which are consistent with parallel studies.<sup>[21–23]</sup>



**Figure 3:** Variations of circulating Th17 cells in OD and NOD group after cCRT. (A) Relationship between variations of Th17 cells and treatment efficiency after cCRT. (B) The positive rate of different classifications (CR, PR and PD) of treatment efficiency after cCRT between the OD and NOD groups. (C–D) OS and PFS compared between the OD and NOD groups. \**P* < 0.001. cCRT: Concurrent chemoradiotherapy; NOD: Non-obviously decreasing group; OD: Obviously decreasing; Th17: T-helper 17; CR: Complete response; PR: Partial response; PD: Progressive disease; SD: Stable disease.

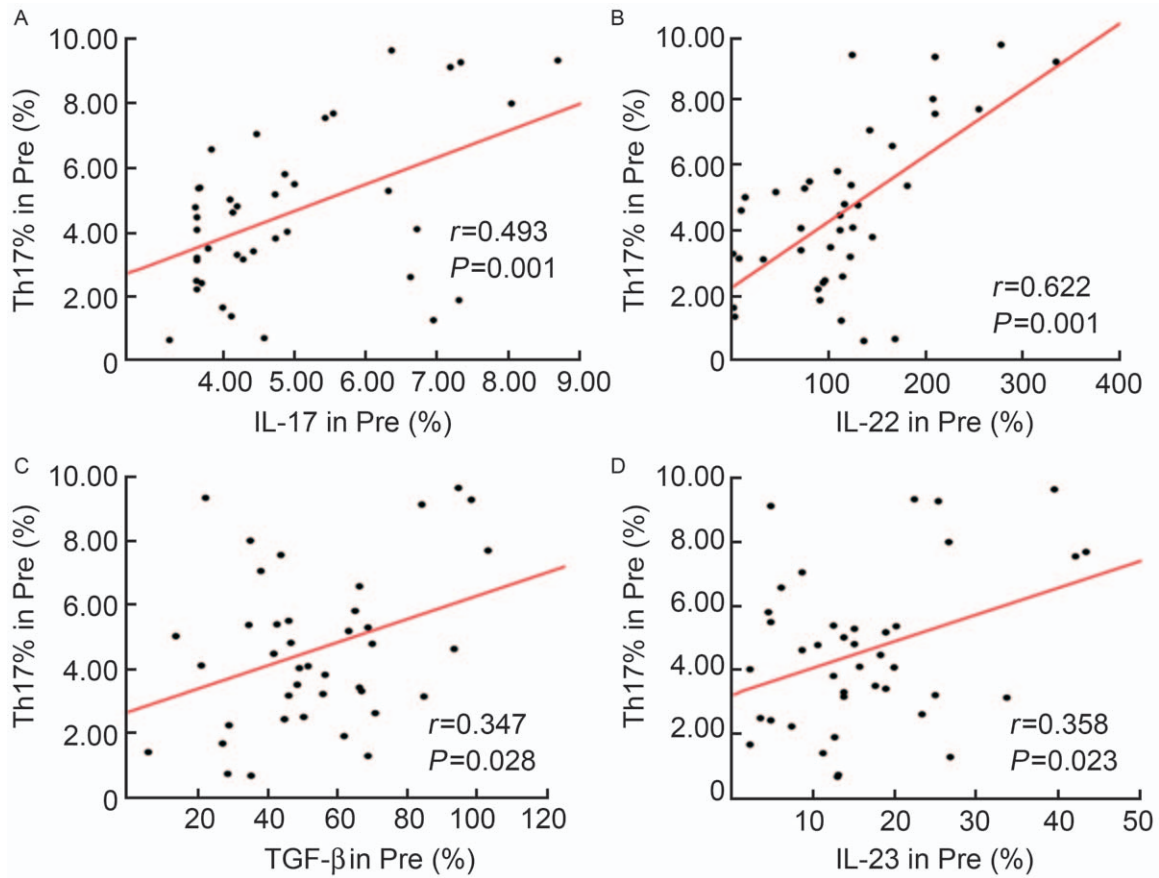
**Table 3: Correlation between variations of Th17 cells and associated cytokines in different groups.**

Cytokine	CON (n=23)	LACC (n=40)		OD Group (n=27)		NOD Group (n=13)	
		Pre-cCRT	Post-cCRT	Pre-cCRT	Post-cCRT	Pre-cCRT	Post-cCRT
IL-17 (pg/mL)	4.19 (3.85, 4.36)	4.91 (3.67, 6.12)	6.02 (4.60, 6.72) <sup>†</sup>	4.19 (4.10, 4.81)	5.32 (4.91, 6.78)	4.73 (3.64, 6.79)	4.57 (3.66, 5.36) <sup>§</sup>
IL-22 (pg/mL)	26.34 (18.18, 38.76)	72.33 (9.55, 114.93) <sup>*</sup>	165.27 (117.60, 209.30)	97.05 (33.41, 125.84)	169.44 (114.60, 266.57)	137.22 (113.29, 195.31) <sup>‡</sup>	146.71 (87.37, 175.04)
IL-23 (pg/mL)	24.01 (17.59, 38.20)	13.74 (4.80, 17.59) <sup>*</sup>	26.58 (16.31, 45.98) <sup>†</sup>	13.74 (7.99, 17.27)	20.15 (15.67, 42.09)	19.89 (12.78, 26.67) <sup>‡</sup>	15.03 (10.11, 30.83)
TGF-β (ng/mL)	6.93 (6.08, 8.86)	110.09 (81.74, 137.04) <sup>*</sup>	49.57 (39.58, 66.63) <sup>†</sup>	116.31 (95.51, 148.47)	45.88 (21.41, 64.63)	37.30 (30.01, 92.72) <sup>‡</sup>	43.63 (34.98, 65.20)
IL-6 (pg/mL)	4.77 (3.70, 8.15)	10.05 (3.75, 11.72) <sup>*</sup>	15.56 (6.65, 21.04) <sup>†</sup>	10.05 (3.77, 13.40)	13.33 (5.73, 20.60)	10.06 (4.73, 11.90)	10.10 (6.88, 13.87)
IL-10 (pg/mL)	6.93 (5.97, 10.47)	10.40 (6.89, 13.86) <sup>*</sup>	19.21 (13.86, 43.36) <sup>†</sup>	10.33 (7.67, 21.73)	15.40 (13.59, 43.36)	12.77 (8.69, 18.49)	13.04 (8.08, 17.78) <sup>§</sup>

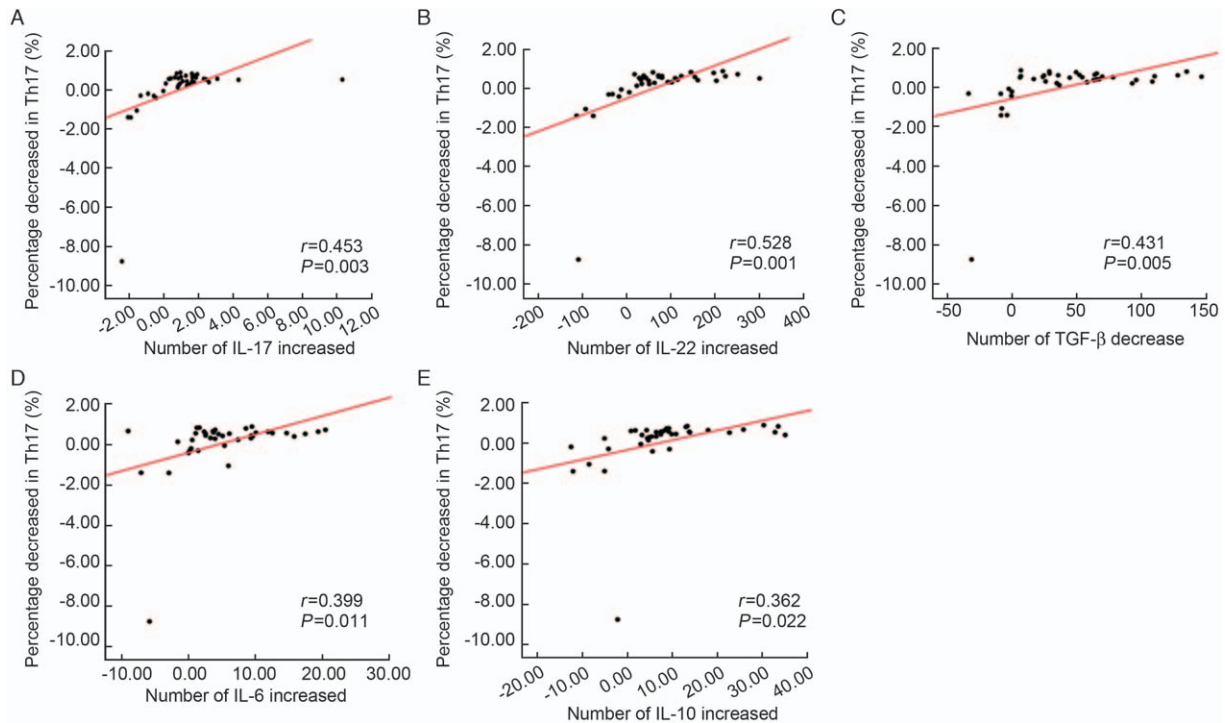
Data are provided as median (interquartile range). \* Control vs. LACC patients before cCRT, *P* < 0.05; <sup>†</sup> LACC patients after vs. before cCRT, *P* < 0.05; <sup>‡</sup> Pre-cCRT OD group vs. NOD group, *P* < 0.05; <sup>§</sup> Post-cCRT OD group vs. NOD group, *P* < 0.05. CON: Control; IL: Interleukin; LACC: Locally Advanced Cervical Cancer; NOD Group: Non-obviously Decreasing Group; OD Group: Obviously Decreasing Group; Pre-cCRT: Before Concurrent Chemoradiotherapy; Post-cCRT: After Concurrent Chemoradiotherapy; TGF-β: Transforming Growth Factor-β.

Our previous studies demonstrated that Th17 cells increased in cervical cancer (FIGO stage I–IIA) and CIN (CIN I–III), playing critical roles in cancer progression and angiogenesis.<sup>[12–14]</sup> Similar results have been confirmed in

other studies.<sup>[24]</sup> In the present study, we detected the percentage of circulating Th17 cells in LACC patients (FIGO stage IIB–IIIB) before and after cCRT. As expected, the percentage of circulating Th17 cells was higher in



**Figure 4:** Correlation of Th17 cells and associated cytokines before cCRT. (A–D) Correlation of Th17 cells and IL-17, IL-22, TGF-β, IL-23 before cCRT. cCRT: Concurrent chemoradiotherapy; IL: interleukin; TGF-β transforming growth factor-β; Th17: T-helper 17; Pre: Pre-cCRT; Post.



**Figure 5:** Correlation between the changes of Th17 cells and associated cytokines before and after cCRT. (A–E) Correlation between the changes of Th17 cells and IL-17, IL-22, TGF-β, IL-6, IL-10 before and after cCRT. cCRT: Concurrent chemoradiotherapy; IL: interleukin; TGF-β transforming growth factor-β; Th17: T-helper 17.

LACC patients than that in controls. After cCRT, they showed a decreasing trend overall. However, nine of 40 patients experienced an increase in circulating Th17 cells. To the best of our knowledge, this is a rare study focusing on Th17 cells in LACC patients, and no previous study has reported variations in Th17 cells during cCRT or other treatments for patients with LACC.

A group of Th17 cell-related cytokines were also examined and analyzed in this study, including IL-6, IL-10, IL-17, IL-22, IL-23, and TGF- $\beta$ . These cytokines are necessary for the differentiation and function of Th17 cells. Researchers have shown that the differentiation of CD4+ T cells into the Th17 subset is induced by IL-6, IL-23, TGF- $\beta$ . Then, the Th17 cells produce IL-10, IL-21, IL-22, IL-26, and IL-17. In addition, Th17 cells can transdifferentiate into Th1 or Treg cells.<sup>[25-27]</sup> Sufficient expression of TGF- $\beta$  and IL-6 induces the conversion of Treg cells toward Th17 cells, while low amounts of TGF- $\beta$ , IL-12, and IL-23 induce conversion of Th17 toward Th1 cells.<sup>[5,7,25,26]</sup> Furthermore, IL-23, a member of the IL-12 family, is necessary for the phenotype, expansion, pathogenic function, and cytokine release of Th17 cells.<sup>[27-29]</sup>

In the present study, we detected pre-cCRT patients had higher IL-6, IL-10, IL-22, TGF- $\beta$  levels and a lower IL-23 level. After cCRT, all the cytokines increased and it was statistically significant except for IL-22, and TGF- $\beta$  level significantly decreased. These complex alterations in cytokines indicate an integrated regulation of Th17 cells and the corresponding effects.

Given that the circulating Th17 cells presented a decreasing trend overall while nine of 40 patients experienced an increase in Th17 cells after cCRT, we speculated that variations in Th17 cells during cCRT were correlated with treatment efficacy. Consequently, we compared the response rate, PFS, and OS between patients with and without obviously decreased numbers of Th17 cells. The results proved our speculation and demonstrated that an obvious decrease in circulating Th17 cells after cCRT was correlated with a better treatment efficacy, PFS, and OS and that patients experiencing a non-obvious decrease or even an increase in Th17 cells were inclined to have a poor prognosis.

A correlation between variations in Th17 cells and the treatment efficacy of cCRT has not been reported in other studies so far. These results prompted speculations that variations in Th17 cells could be used as a prognostic factor in LACC. Identification of LACC patients at high-risk of tumor recurrence after cCRT helps to select patients for close surveillance and more aggressive treatment, such as adjuvant chemotherapy, targeted agents, and so on. Post-treatment hematological parameters, particularly neutrophil-to-lymphocyte ratio, have been reported as a prognostic indicator in patients with LACC who received cCRT.<sup>[22]</sup> Qing *et al* reported that the number of intratumoral Th17 cells, together with lymph node status and lymph-vascular space invasion, were independent predictors of local recurrence in cervical cancer patients (FIGO stages IIA–IIB) who underwent radical resection.<sup>[30]</sup> In addition to Th17 cells, several related cytokines,

including IL-17, IL-22, and IL-23, could also be regarded as prognostic factors of LACC after cCRT due to the marked difference between OD and NOD patients. It is a pity that the prognostic value of Th17 cells, related cytokines, and other factors were not evaluated due to the small sample size of this study. Further studies with a larger sample size and a longer observation period are necessary to confirm the prognostic effect of variations in circulating Th17 cells and related cytokines in patients with LACC after cCRT.

There were several limitations to this study. First, this is a single-center study and the sample size is small, which may cause selection bias. In addition, the wide range of LACC stages enrolled may have affected the results. Second, we only detected Th17 cells and related cytokines before and after cCRT. Blood samples were not obtained at various time points during cCRT. More frequent sampling could better reflect variations in circulating Th17 cells in response to cCRT. Third, the follow-up lasted only 3 years. A 5-year follow-up may more clearly reveal correlations between Th17 cells and treatment efficacy. Therefore, a multicenter clinical study with a large sample size, more frequent detection of Th17 cells and related cytokines during treatment, and 5 years of follow-up is necessary to confirm our results.

Despite these limitations, the present study provided some initial and significant findings about the variations in circulating Th17 cells and related cytokines before and after cCRT and correlations between the variations and treatment efficacy in patients with LACC.

In summary, this prospective 3-year study found that circulating Th17 cells generally decreased after cCRT in patients with LACC (FIGO stages IIB–IIIB), while some patients experienced only a minor decrease or even an increase in Th17 cells after treatment. Th17 cell-related cytokines varied accordingly. An obvious decrease in circulating Th17 cells after cCRT correlated with higher treatment efficacy and longer PFS and OS times. Variations in circulating Th17 cells after cCRT may be useful for predicting long-term outcomes.

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### Conflicts of interest

None.

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