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ORIGINAL ARTICLE

Imbalance of Follicular Double Positive and Follicular Double Negative T cells in Ulcerative Colitis Patients

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ABSTRACT

Background: *Double-positive (DP) and double-negative (DN) thymocytes are well-characterized stages of T cell development in the thymus. Disturbances in their levels have been previously observed in chronic inflammatory diseases and autoimmune disorders.* ***Objective:*** *This study aimed to analyze follicular DP and DN T cell levels in ulcerative colitis patients' peripheral blood and colon tissue biopsy and determine whether their levels correlate with disease severity.* ***Methodology****: This study involved 22 patients with ulcerative colitis (UC) and 18 healthy controls. Flow cytometry was used to measure the total levels of DP and DN T cells and their subsets in each participant's peripheral blood and colon tissue biopsy.* ***Results****: DP T cells were higher in tissue than in the blood samples. DN T cells were lower in tissue than the blood but higher in patients than control tissues. Differences were observed in the tissue levels of follicular DP and DN cells and their expression levels of CD154 compared with blood and in severe compared with non-severe cases.* ***Conclusion:*** *Levels of DP and DN follicular T cells are higher in the tissue of UC patients than in blood, with higher expression of CD154 in tissues. Levels of these cells are higher in more severe cases and show good efficiencies in predicting disease severity. Further research is needed to assess whether these cells have a role in the inflammatory process in UC.*

INTRODUCTION

Inflammatory bowel diseases (IBD) are a group of recurrent, long-term inflammatory diseases of the gastrointestinal system. The two main types of inflammatory bowel disease (IBD) are ulcerative colitis (UC) and Crohn's disease as reported by Spiceland et al.[1], Al-Bawardy et al. [2], Zhang et al.[3]

Studies indicate that aberrant interactions between T cells and other immune and non-immune cells are crucial in promoting the pathological processes of UC, even if the etiology and pathogenic processes of the disease are still unclear. T cell-derived cytokines are crucial mediators of these interactions.[4]

Before developing into either CD4+ or CD8+ (single positive) mature T cells, CD4+CD8+ double positive (DP) T cells were considered a developmental stage in the thymus. As a result, most T cells in peripheral blood and tissues have only expressed one of these co-receptors, which correlates to distinct functions. CD4 T cells serve as helpers in the immune response, while CD8 T cells primarily cytotoxically attack tumors or infected cells.[5]

Mature DP T cells, however, have been seen in peripheral blood and tissues in various contexts, including human malignancies. The contradicting research on the cytotoxic vs. immunosuppressive roles of DP T cells may suggest that these cells are diverse and/or have pleiotropic functions, which need further study in the context of each disease.[5] There's no hard evidence, but it's possible that poor thymic selection gave rise to auto-reactivity in CD4(hi) and CD8(hi) T cells.[6]

Some mature peripheral T cells are double-negative (DN) T cells. The origin and functions of DN T cells are a topic of discussion and not entirely established. While DN T cells in healthy individuals resemble a rare and heterogeneous T cell subpopulation, under several inflammatory conditions, the frequency of TCRαβ+ DN T cells is increased, and they also show different effector attitudes and infiltrate inflammatory tissues.[7] DN T cells can function as regulatory T cells (Tregs), which may prevent autoimmune diabetes, graft-versus-host disease, and allograft rejection.[8]

Chemokine receptor type 5 (CXCR5) plays a role in developing the B cell compartment and directing lymphocyte traffic within secondary lymphoid tissues.

Mice with deleted CXCR5 gene exhibit impaired B cell trafficking, inguinal node, Peyer's patch, and germinal center formation.[9] CD40 ligand (CD40L or CD154), a type II membrane protein transiently expressed on activated T cells and resembling TNF, plays a crucial role in B cell Ig synthesis, as well as in the activation and differentiation of monocytes and dendritic cells. Local synthesis of cytokines like TNF and an influx of activated lymphocytes into inflamed mucosa are characteristics of both Crohn's disease and ulcerative colitis. [10]

However, the contribution of CXCR5+CD4+CD8+ (follicular DP) and CXCR5+CD4-CD8- (follicular DN) T cells to the development of UC is so far lacking.

This study aimed to analyze the follicular DP and DN T cell levels in UC patient's peripheral blood and colon tissue biopsy compared with a control group and determine whether their levels correlate with disease severity.

METHODOLOGY

Study Design:

This case-control study was carried out from June 2022 to May 2023, at the Flow Cytometry Laboratory, South Egypt Cancer Institute, in collaboration with the Medical Microbiology & Immunology Department, Faculty of Medicine, Assiut University.

The study included 22 UC patients admitted to the Tropical Medicine and Gastroenterology Department, Al-Rajhi Liver Hospital, Assiut University. Inclusion criteria: Patients who diagnosed as IBD, based on clinical manifestation, radiographic, endoscopic, and histological criteria were included in the study. Exclusion criteria: Pregnant patients and patients having colorectal or any other cancers, intestinal parasitic infection, indeterminate colitis or Crohn's disease, patients receiving cyclosporine, non-steroidal anti-inflammatory drugs (NSAID), or any antibiotics within the last two weeks, and patients receiving any glucocorticoids or immunosuppressant within the previous three months were excluded from the study.

Also, 18 sex and age-matched healthy cases were enrolled as a control group. The control group was chosen from cases without clinical symptoms or signs associated with ulcerative colitis. Their endoscopic examination was normal and their serological marker for UC (ANCA, antineutrophil cytoplasmic antibody) was negative.

Complete history assessment and clinical examination were performed for all participants, including monitoring of vital signs, a dermatological, articular, and ophthalmological examination to check for extra intestinal manifestations of UC, an extensive abdominal examination, and a colonoscopy.

The Mayo Clinic scoring system was utilized to measure disease activity in UC patients. Rectal bleeding, stool frequency, physician assessment, and endoscopic appearance are the four components of this examination. A score of 3 to 5 indicates mildly active disease, 6 to 10 indicates moderately active disease, and a score of 11 to 12 indicates severely active disease [11]. All participants voluntarily gave written informed consent.

Complete blood count (CBC) was done for all participants, and the platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and neutrophil-to-platelet ratio (NPR) were calculated. These ratios are considered biomarkers for diagnosing and assessing disease activity in UC [12, 13, 14].

The Assiut University Faculty of Medicine's Medical Ethics Committee approved the study protocol (IRB no. 17101694).

Sample Collection and Processing

To evaluate the percentages of follicular DP and follicular DN T cells in human peripheral blood and the colon of patients with active UC compared to controls, two milliliters of whole blood samples and fresh colon tissue biopsy were collected from all participants and subjected to flow cytometry analysis.

A fresh tissue sample was transferred to a Petri dish and washed with 3 ml PBS (PH 7.4). Next, PBS was discarded, one ml PBS was added to the tissue, and the tissue was ground using a sharp scalpel. After proper tissue grinding, suspension (PBS with cells from tissue) was transferred into a 5 ml falcon tube. The tube was centrifuged at 2500 rpm for 1 min, and the supernatant was discarded. The tissue cell pellet was resuspended in 1 ml PBS, and a 40-micron Falcon mish filtered the suspension.

Flow Cytometry Analysis

A five-color flow cytometry analysis was carried out using a set of fluorochrome-labeled monoclonal antibodies against the surface markers [Phycoerythrin (PE)-conjugated anti-ICOS, Allophycocyanin (APC)-conjugated anti-CXCR5, APC-H7-conjugated anti-CD8, Fluorescein 6-isothiocyanate (FITC)-conjugated anti-CD154, Peridinin-Chlorophyll-Protein Cyanine 5.5 (PerCP-Cy5.5)-conjugated anti-CD4].

One hundred microliters of whole blood and one hundred micrograms of tissue were transferred into clean test tubes, and an RBC lysing solution was added. Then centrifugation was done at 2000 r.p.m for 1 min, and the supernatant was discarded. The pellet was resuspended in 1 ml phosphate-buffered saline (PBS). After centrifugation, ten microliters of each monoclonal antibody were added to the pellet, and the mixture was incubated for 15 min at 4^{O} C in the dark. Appropriate isotype controls were also prepared and processed similarly without adding the T cell monoclonal antibodies. Flow cytometric analysis was performed by the Becton Dickinson Biosciences (BD) fluorescence-activated cell sorter (FACSCanto ll) using the FACS

DIVA software (BD, USA). A minimum of 50000 events were collected for each sample analysis. Isotype control staining was used to exclude the nonspecific fluorescence.

Statistical Analysis

SPSS (Statistical Package for the Social Science, version 20, IBM, USA) was used to analyze the data. The Shapiro test was employed to determine if the data adhered to a normal distribution. Data was represented as mean ± standard deviation (SD). When comparing quantitative data with a normal distribution, the Student t-test was used. Number (n) and percentage (%) represented nominal data. Chi^2 testing was applied to this kind of data. Because the 95% confidence level was maintained, a *P* value was deemed significant if it was less than 0.05.

RESULTS

Demographic data of UC-infected patients:

Regarding mean age and sex, there were no significant differences between the two groups. The mean age of UC patients was 36.4±13.8 years compared to 34.4±12.0 years in the control group (p = 0.6). Fourteen patients (63.6%) were females, while 8 (36.8%) were males. On the other hand, 11 controls (61%) were females, and 7 (39%) were males (p = 0.87). It was found that 13 (59.1%) patients had mild/moderate disease severity, while 9 (40.9%) patients had severe disease.

Characterization of DP and DN T cell subsets in the studied groups in blood and colon tissue samples compared to controls

For blood samples, as shown in Table (1), the total level of DP T cells was higher in the UC patient group than in healthy controls (p = 0.02). While there was no significant difference in the percentage of active follicular DP T cells, their CD154 expression level was lower in UC patients than in controls (p = 0.007). Meanwhile, the frequency of active non-follicular DP T cells was higher in the UC patient group (p = 0.001), with no difference in their CD154 expression levels.

For DN T cells, there was no significant difference in either the total level of DN T cells or the active follicular DN T cells between both groups. On the other hand, active non-follicular DN T cells were higher in UC patients (p = 0.001). The expression of CD154 among both active follicular and active non-follicular DN T cells was lower in UC patients than HC (p < 0.0001 and 0.013, respectively).

Table 1: Comparison of the levels of double-positive (DP) and double-negative (DN) T cell subsets in blood samples of patients and controls

Cells	Patient group (n= 22)	Control group (n= 18)	*p*-value
Lymphocytes	29.67 ± 5.46	39.06 ± 8.24	<0.0001
CD4+CD8+ (DP) T cells (%)			
Total DP	0.37 ± 0.11	0.29 ± 0.09	0.02*
ICOS+CXCR5+ DP	32.54 ± 9.11	27.89 ± 7.75	0.095
ICOS+CXCR5+CD154+ DP	27.80 ± 5.67	33.46 ± 6.90	0.007*
ICOS-CXCR5+ DP	12.42 ± 3.61	14.33 ± 3.47	0.098
ICOS-CXCR5+CD154+ DP	48.45 ± 13.85	36.04 ± 11.24	0.005*
ICOS+CXCR5- DP	51.00 ± 15.28	34.67 ± 11.05	0.001*
ICOS+CXCR5-CD154+ DP	7.01 ± 2.36	6.85 ± 1.87	0.819
ICOS-CXCR5- DP	23.99 ± 7.63	37.80 ± 11.53	<0.0001
ICOS-CXCR5- CD154+ DP	40.32 ± 12.10	50.88 ± 13.22	0.014*
CD4-CD8- (DN) T cells (%)			
Total DN	50.38 ± 11.55	55.63 ± 7.23	0.102
ICOS+CXCR5+ DN	1.95 ± 0.63	1.82 ± 0.47	0.456
ICOS+CXCR5+CD154+ DN	22.57 ± 6.92	37.88 ± 10.61	<0.0001
ICOS-CXCR5+ DN	20.24 ± 6.54	7.01 ± 2.21	<0.0001
ICOS-CXCR5+CD154+ DN	7.10 ± 1.60	13.65 ± 3.90	<0.0001
ICOS+CXCR5- DN	22.39 ± 7.25	15.45 ± 4.33	<0.0001
ICOS+CXCR5-CD154+ DN	10.03 ± 3.31	12.91 ± 3.68	0.013*
ICOS-CXCR5- DN	26.61 ± 5.61	31.62 ± 6.43	0.012*
ICOS-CXCR5-CD154+ DN	16.54 ± 4.99	20.11 ± 6.47	0.056

As shown in Table (2), for colon tissue, the level of active non-follicular DP T cells was higher in UC patients than in controls (p < 0.0001). At the same time, there were no significant differences in the total DP T cells or the other DP T cell subsets. The total level of DN T cells was higher in UC patients than HC (p = 0.007*). The level of active non-follicular DN T cells expressing CD154 was lower in UC patients than in controls (p = 0.007). Other DN T cell subsets showed no significant differences between the two groups (table 2).

Table 2: Comparison of the levels of double-positive (DP) and double-negative (DN) T cell subsets in colon tissue samples of patients and controls

Cells	Study group (n= 22)	Control group (n= 18)	*P* value
Lymphocytes	44.72 ± 9.25	45.08 ± 9.21	0.903
CD4+CD8+ (DP) T cells (%)			
Total DP	23.40 ± 7.71	27.34 ± 7.46	0.111
ICOS+CXCR5+ DP	72.62 ± 10.81	75.55 ± 7.43	0.336
ICOS+CXCR5+CD154+ DP	98.21 ± 3.23	98.34 ± 5.17	0.923
ICOS-CXCR5+ DP	2.85 ± 0.86	3.66 ± 1.17	0.016*
ICOS-CXCR5+CD154+ DP	41.84 ± 11.09	48.09 ± 13.74	0.124
ICOS+CXCR5- DP	26.85 ± 7.61	17.86 ± 3.45	<0.0001
ICOS+CXCR5-CD154+ DP	87.46 ± 19.37	95.43 ± 10.13	0.123
ICOS-CXCR5- DP	4.04 ± 1.38	5.36 ± 1.62	0.008*
ICOS-CXCR5- CD154+ DP	41.00 ± 10.52	46.08 ± 12.56	0.172
CD4-CD8- (DN) T cells (%)			
Total DN	36.86 ± 10.90	28.25 ± 7.44	0.007*
ICOS+CXCR5+ DN	15.21 ± 4.95	17.90 ± 4.14	0.074
ICOS+CXCR5+CD154+ DN	88.62 ± 11.50	91.98 ± 6.62	0.279
ICOS-CXCR5+ DN	6.97 ± 1.70	12.19 ± 3.61	<0.0001
ICOS-CXCR5+CD154+ DN	34.47 ± 9.38	34.43 ± 10.28	0.989
ICOS+CXCR5- DN	36.28 ± 9.50	31.09 ± 7.83	0.071
ICOS+CXCR5-CD154+ DN	72.45 ± 16.87	84.73 ± 8.02	0.007*
ICOS-CXCR5- DN	16.80 ± 4.73	6.87 ± 1.68	<0.0001
ICOS-CXCR5-CD154+ DN	47.35 ± 10.75	55.97 ± 14.63	0.038*

Comparison between the blood and colon tissue levels of double-positive (DP) and double-negative (DN) T cell subsets in UC patients

As shown in table 3, the total level of DP T cells was higher in tissue samples than in blood samples of UC patients ($p < 0.0001$). Active follicular DP T cells were higher in tissue samples ($p < 0.0001$), while the opposite was observed in the active non-follicular DP T cells ($p < 0.0001$). Higher CD154 surface expression levels were observed among activated follicular and non-follicular DP T cells in tissue samples ($p < 0.0001$).

The total level of DN T cells was lower in the tissue than in the blood of UC patients ($p < 0.0001$). On the contrary, active follicular and non-follicular DP T cells and their expression of CD154 were higher in tissues ($p < 0.0001$).

Table 3: Comparison of the levels of blood and colon tissue levels of double-positive (DP) and double-negative (DN) T cell subsets in UC patients

Cells	Blood sample	Tissue sample	*P* value
Lymphocytes	29.67 ± 5.46	44.72 ± 9.25	<0.0001
CD4+CD8+ (DP) T cells (%)			
Total DP T cells	0.37 ± 0.11	23.40 ± 7.71	<0.0001
ICOS+CXCR5+ DP	32.54 ± 9.11	72.62 ± 10.81	<0.0001
ICOS+CXCR5+CD154+ DP	27.80 ± 5.67	98.21 ± 3.23	<0.0001
ICOS-CXCR5+ DP	12.42 ± 3.61	2.85 ± 0.86	<0.0001
ICOS-CXCR5+CD154+ DP	48.45 ± 13.85	41.84 ± 11.09	0.093
ICOS+CXCR5- DP	51.00 ± 15.28	26.85 ± 7.61	<0.0001
ICOS+CXCR5-CD154+ DP	7.01 ± 2.36	87.46 ± 19.37	<0.0001
ICOS-CXCR5- DP	23.99 ± 7.63	4.04 ± 1.38	<0.0001
ICOS-CXCR5-CD154+ DP	40.32 ± 12.10	41.00 ± 10.52	0.844
CD4-CD8- (DN) T cells (%)			
Total DN T cells	50.38 ± 11.55	36.86 ± 10.90	<0.0001
ICOS+CXCR5+ DN	1.95 ± 0.63	15.21 ± 4.95	<0.0001
ICOS+CXCR5+CD154+ DN	22.57 ± 6.92	88.62 ± 11.50	<0.0001
ICOS-CXCR5+ DN	20.24 ± 6.54	6.97 ± 1.70	<0.0001
ICOS-CXCR5+CD154+ DN	7.10 ± 1.60	34.47 ± 9.38	<0.0001
ICOS+CXCR5- DN	22.39 ± 7.25	36.28 ± 9.50	<0.0001
ICOS+CXCR5-CD154+ DN	10.03 ± 3.31	72.45 ± 16.87	<0.0001
ICOS-CXCR5- DN	26.61 ± 5.61	16.80 ± 4.73	<0.0001
ICOS-CXCR5-CD154+ DN	16.54 ± 4.99	47.35 ± 10.75	<0.0001

Comparison of the levels of DP and DN T cell subsets in blood and colon tissue of UC patients based on the disease severity assessed by the Mayo Score

Activated follicular DP and follicular DN T cells and their CD154 expression levels were greater in the blood and tissue of severe cases than in non-severe cases (p = 0.018, < 0.0001, < 0.0001, 0.003 in blood, and 0.002, 0.016, 0.001, 0.015 in tissue, respectively).

Evaluation of the different T cell subsets as possible predictors of severity in blood and colon tissue

As shown in Table (4) and Figures (1) and (2), the Receiver Operating Characteristics (ROC) curve has been used to evaluate the accuracy of employing the level of activated follicular DP and DN T cells and activated follicular DP and DN T cells expressing CD154 in blood and tissue as potential predictors of severity in UC patients. All cells have shown good predictive efficiencies.

Table 4: Receiver operating characteristics (ROC) curve of follicular DP and follicular DN T cells in blood and tissue for prediction of disease severity in UC patients

Blood T cell subsets	Cut-off	Sensitivity	Specificity	+PV	-PV	AUC
ICOS+CXCR5+ DP	> 25.3	100.00	87.50	93.3	100.0	0.920
ICOS+CXCR5+CD154+ DP	> 24.5	92.86	100.00	100.0	88.9	0.964
ICOS+CXCR5+ DN	> 1.7	92.86	100.00	100.0	88.9	0.996
ICOS+CXCR5+CD154+ DN	> 19.4	91.67	100.00	100.0	88.9	0.979
Tissue T cell subsets	**Cut-off**	**Sensitivity**	**Specificity**	**+PV**	**-PV**	**AUC**
ICOS+CXCR5+ DP	> 69.5	92.86	87.50	92.9	87.5	0.911
ICOS+CXCR5+CD154+ DP	> 99.3	92.86	87.50	92.9	87.5	0.853
ICOS+CXCR5+ DN	> 13.5	85.71	100.00	100.0	80.0	0.969
ICOS+CXCR5+CD154+ DN	> 89.8	92.86	100.00	100.0	88.9	0.929

PV predictive value, AUC area under the curve

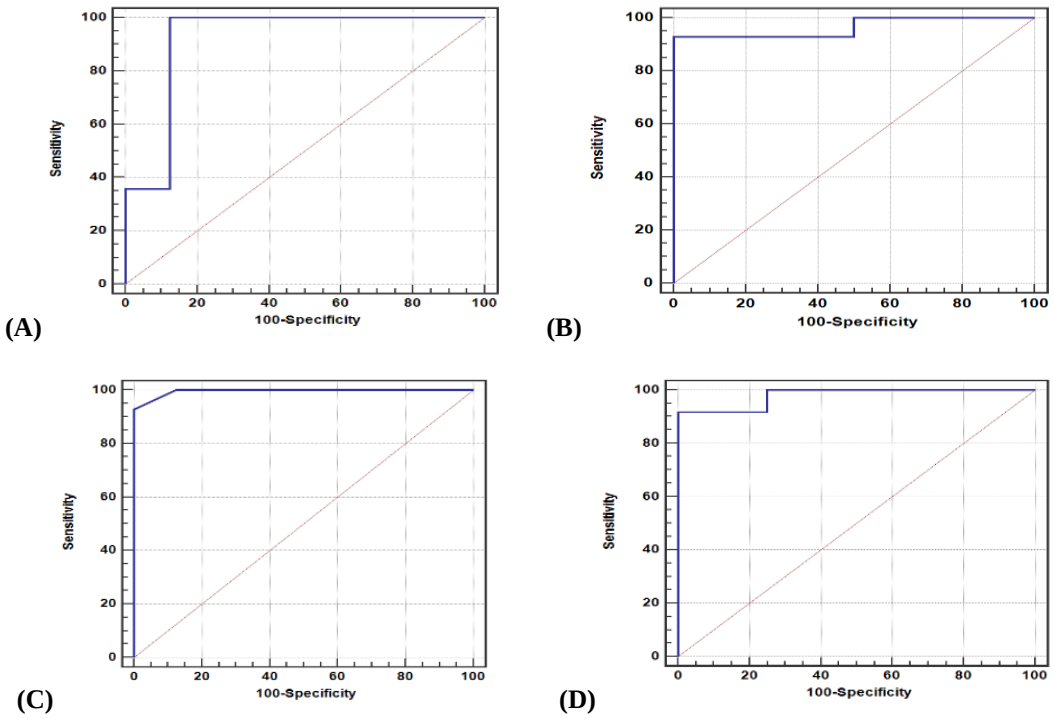
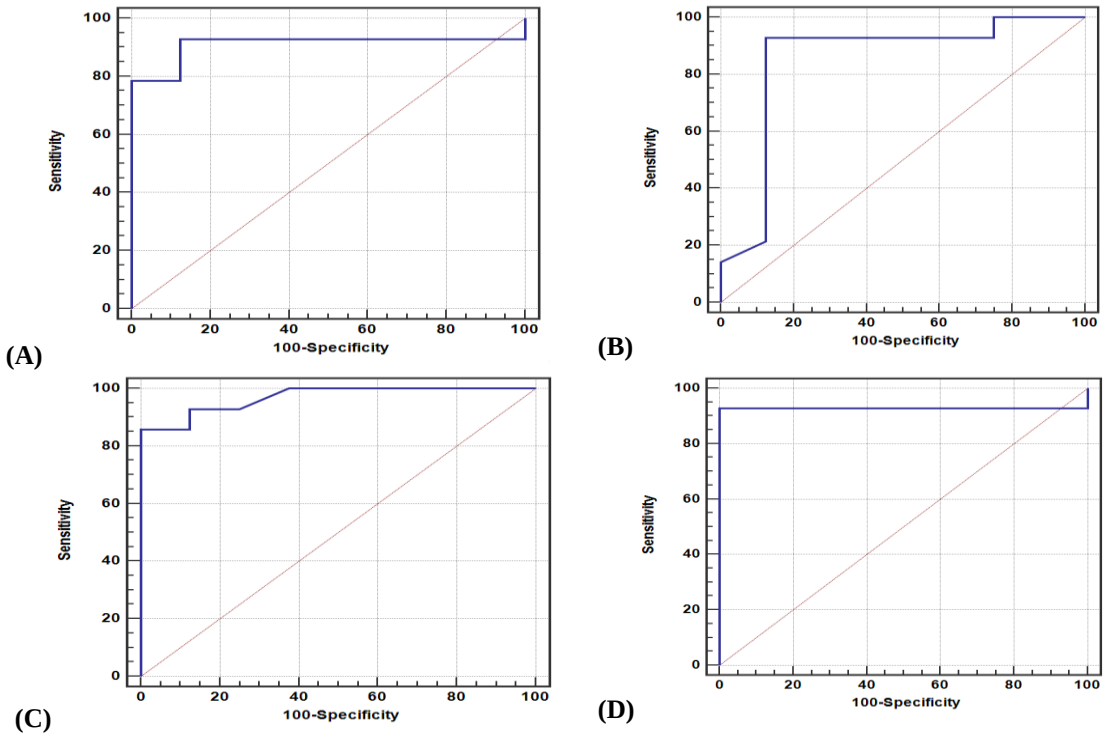


Fig. 1: Receiver operating characteristics (ROC) curve of follicular DP and follicular DN T cells in peripheral blood for severity prediction in UC patients. (A) ICOS+CXCR5+DP, (B) ICOS+CXCR5+CD154+DP, (C) ICOS+CXCR5+DN, (D) ICOS+CXCR5+CD154+DN



Fig, 2: Receiver operating characteristics (ROC) curve of follicular DP and follicular DN T cells in tissue for severity prediction in UC patients. (A) ICOS+CXCR5+DP, (B) ICOS+CXCR5+CD154+DP, (C) ICOS+CXCR5+DN, (D) ICOS+CXCR5+CD154+DN

DISCUSSION

Although the phenotype of CD4+ and CD8+ single-positive T cells has been the subject of numerous studies, little is known about the phenotype and functions of DP and DN T cells in autoimmune diseases[15,16], especially in UC patients.

Controversial findings regarding the function of the DP T cell subset have been published in the last decades. [17] Previous research has demonstrated changes in the DP T cells in various disorders, including cancer, inflammatory/autoimmune diseases, and infectious diseases. Some authors reported a pro-inflammatory activity, while others showed a down-regulatory one. The current study revealed higher levels of DP T cells in colon tissues than in the patient's blood, especially the activated follicular subset with higher expression of CD154. In severe cases, active follicular DP T cells and those expressing CD154 were higher. These findings suggest that these cells may play a role in colon involvement and UC progression. Additionally, their elevated levels demonstrated good predictive efficiency for UC severity. Therefore, controlling these cells' expression may help develop novel UC therapeutic approaches. Additionally, prior research indicates that CD40L up-regulation produces harmful cytokines in IBD and that patients may benefit from blocking CD40-CD40L interactions.[10]

Meanwhile, in the peripheral blood, the overall percentage of DP T cells was higher in the UC patient's group compared with the control. However, UC patients had higher percentages of activated non-follicular DP T cells in their colon tissue and blood than in the controls. There were no significant differences in the follicular DP T cell levels between both groups. More research is required to fully understand the function of the DP T cell subset in the UC inflammatory process.

Research on murine intra-epithelial lymphocytes (IELs) indicated that CD4+CD8αα+ DP T cells in the gut might carry out regulatory activities, as evidenced by their production of TGF-β and IL-10. These two cytokines reduce inflammation [13]. According to Wu *et al.* [18], DPT cells may play a key suppressive role in producing autoantibodies in SLE.

On the other hand, DP T cells in HIV-infected subjects were found to produce high levels of the proinflammatory cytokines IFN-γ and TNF[17,19,20]. Patients with Sjogren's syndrome and several autoimmune disorders like rheumatoid arthritis (RA) and multiple sclerosis (MS) have higher levels of DP T cells.[21-25]. In some inflammatory diseases, the existence of DP T cells is also suggested as a predictor for severity. Peripheral DP cells in systemic lupus erythematosus (SLE) are related to an increased risk of developing renal impairment [26]. Furthermore, DP T cell proliferation in RA is related to joint damage and frequent therapy escalation [25]. Graft versus host disease (GVHD) development is another example of DP T cell presence in inflammation. GVHD has been linked recently to establishing a DP T cell population that was not initially present in the graft [27].

Even though DN T cells account for a small fraction of the T-cell population, they are crucial in the pathogenesis of numerous diseases by regulating and suppressing essential inflammatory effector processes or inducing cell death and causing tissue damage [7]. Despite the lower levels of DN T cells in tissue than in the blood of UC patients, their levels in UC patient's tissues were significantly higher than in the control's tissues. Additionally, higher frequencies of activated follicular DN T cells with higher expression of CD154 were detected in colon tissue than in blood in UC patients. Their levels were also higher in the tissues and blood of severe cases. They showed good efficiencies in predicting UC severity, which might reflect their probable role in tissue damage and disease progression.

More research is required to understand how they influence the immunopathogenesis of UC. DN T cells are actively involved in chronic autoimmune inflammatory disorders, as evidenced by their tissue distribution and the effector cytokines they generate. Whether this population is a stable lineage of distinct subsets resembling the CD4+ T helper cell subset. To identify potential targets for interventional therapy, a deeper comprehension of the potential variability and flexibility of DNT cells is necessary [28].

It is commonly recognized that distinct lymphocyte subpopulations preferentially express and utilize distinct chemokine receptors for migration [29]. According to Wiener *et al.*, [30] CXCR5 plays a crucial role in the development of lupus through regulating B cell and double-negative T cell trafficking, particularly in the pathophysiology of lupus nephritis in murine models and systemic lupus erythematosus (SLE). Patients with SLE have more DN T lymphocytes in their peripheral blood. DN T cells invade inflammatory tissue, including the kidneys, release proinflammatory cytokines, and stimulate the synthesis of immunoglobulins. In mouse lupus, CXCR5 deficiency changes immune cell trafficking [31-33]. Furthermore, CXCL13 and its receptor CXCR5 have been linked to the pathophysiology of multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, primary Sjögren's syndrome, myasthenia gravis, and inflammatory bowel disease, according to recent investigations [34].

CONCLUSION

Levels of DP and DN follicular T cells are higher in the tissue of UC patients than in blood, with higher expression of CD154 in tissues. Levels of these cells are higher in more severe cases and show good efficiencies

in predicting disease severity. Further research is needed to assess whether these cells have a role in the inflammatory process in UC.

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