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*Review*

# Immunocytochemical Profiling of CD4+, CD8+, and CD3+ T Cell Subsets: Clinical Implications in Rheumatic Diseases, Respiratory Disorders, and Oncology

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**Abstract:** Immunocytochemical staining has emerged as a cost-effective and minimally invasive method for profiling T cell subsets (CD4+, CD8+, and CD3+) in the clinical setting. The quantification of T lymphocyte subsets has become a critical tool for assessing immune dysregulation in chronic inflammatory and autoimmune diseases. This study evaluates the utility of this method in monitoring immune dysregulation across rheumatic diseases (systemic lupus erythematosus—SLE, rheumatoid arthritis—RA, Sjögren's syndrome—SS), respiratory disorders, and malignancies. Peripheral blood samples from 12 patients (10 of whom had rheumatic diseases, one with lung cancer tested seven times and one with liver cancer tested five times) were collected in EDTA-K2 tubes and analyzed using standardized immunocytochemical protocols. Key findings include: (1) CD4+/CD8+ ratio inversion (<1.0) correlates with disease activity in SLE (OR = 3.2 for lupus nephritis) and predicts lymphoma risk in SS (HR = 2.8). (2) In RA, reduced Th17 proportions post-methotrexate therapy (12.3%→6.8%, $p < 0.01$) reflect inflammation suppression, while TNF-α inhibitors improve CD4+/CD8+ ratios alongside DAS28 scores ($p = 0.003$). (3) In oncology, longitudinal CD3+ T cell counts (136–504 cells/μL) and CD8+ dynamics mirror tumor burden and immunotherapy efficacy. Compared to flow cytometry, immunocytochemical staining offers practical advantages for routine clinical use, though limitations in functional subpopulation resolution persist. Integrating these assays with multi-omics approaches may refine personalized treatment strategies. This study underscores the pivotal role of T cell subset analysis in bridging mechanistic immunology with precision medicine.

**Keywords:** Immunocytochemical Staining; CD4+/CD8+ Ratio; T Cell Subsets

## 1. Introduction

Immune cells play an important role in the human immune system. They perform normal immune functions in the human body and participates in different pathophysiological processes [1]. Immune cells mainly include components such as macrophages, NK cells and granulocytes that participate in innate immunity, as well as T lymphocytes and B lymphocytes that participate in adaptive immunity [2]. Immune molecules include antibodies, complements, cytokines, etc. In recent decades, the field of immunology has witnessed transformative advancements in deciphering the multifaceted biology of T cells-spanning their ontogenetic classification, differentiation trajectories, and molecular regulation-with profound revelations into phenotypic plasticity and effector functions under homeostatic conditions and across the spectrum of immune-mediated pathologies [3]. T lymphocytes play a central role in cellular immunity. T lymphocytes are a subset of immune cells that regulate the body's immune system. According to T cell surface markers, they are divided into CD3+CD4+ and CD3+CD8+ cells [4]. Quantitative changes each cell

subpopulation affect the body's immune function, thereby causing autoimmune diseases, infections, tumors, etc. [5].

CD3, CD4, and CD8 are pivotal surface markers of T lymphocytes, playing indispensable roles in adaptive immune responses. CD3, a pan-T cell marker expressed on nearly all mature T cells, forms the T cell receptor (TCR) complex and is essential for antigen recognition and signal transduction, making it a critical indicator of overall T cell functionality [6]. CD4+ T cells orchestrate immune responses by activating B cells, macrophages, and cytotoxic T cells through cytokine secretion; their depletion (e.g., CD4+ counts <200 cells/μL in HIV/AIDS) directly correlates with disease progression and opportunistic infections. CD8+ T cells, characterized by their cytotoxic activity, eliminate virally infected and malignant cells via perforin-granzyme pathways or Fas/FasL interactions [7]. In immune profiling, an inverted CD4/CD8 ratio (<1.0) serves as a biomarker of chronic inflammation in aging (termed "immunosenescence") and autoimmune pathologies such as rheumatoid arthritis, reflecting systemic immune dysregulation [8]. In oncology, tumor-infiltrating CD8+ cytotoxic T cell density has emerged as a predictive biomarker for immune checkpoint inhibitor efficacy (e.g., anti-PD-1 therapies), whereas elevated regulatory T cells (CD4+CD25+FoxP3+) within the tumor microenvironment correlate with immunosuppression and poor prognosis [9]. Technological innovations like spectral flow cytometry now empower high-dimensional immune monitoring, enabling simultaneous detection of 30+ phenotypic markers to resolve T cell heterogeneity in complex diseases, for instance, identifying CD4+ lymphopenia as a prognostic indicator of severe COVID-19 outcomes and tracking CD3+ T cell persistence to predict durable remission in CAR-T-treated leukemia patients. Collectively, these applications underscore how T cell subset analysis bridges mechanistic immunology with precision medicine [10].

Recent advancements in detection technologies have revolutionized the characterization of T lymphocyte subsets, enabling unprecedented resolution in immune profiling [11]. Single-cell RNA sequencing (scRNA-seq) has unveiled transcriptional diversity within T cell populations, identifying novel subsets such as tissue-resident memory T (Trm) cells and exhausted CD8+ T cells in chronic infections [12]. Innovations in cell-based immuno-slide assays (e.g., SemiBio's antigen-specific binding method) offer cost-effective, rapid quantification of CD3+/CD4+/CD8+ subsets, validated in clinical settings for HIV monitoring and autoimmune disease diagnosis [13]. These tools synergistically bridge basic research and clinical translation-for instance, integrating scRNA-seq with spatial transcriptomics to map T cell interactions in tumor microenvironments. Collectively, these technologies deepen our understanding of T cell biology while informing precision immunotherapy strategies.

## 2. Research Objective

At present, in pathology testing, the methods used for immune detection include flow cytometry, immunohistochemistry staining, immunofluorescence, etc. Compared with immunohistochemical staining, immunocytochemical staining requires simpler specimen collection. Immunocytochemical testing usee blood samples, while immunohistochemical testing requires pathological tissue obtained through surgery, puncture, etc., and going through steps such as dehydration, embedding, and slicing to complete the test. On the one hand, obtaining specimens through surgery is more difficult than venipuncture, and more pain to the patient; on the other hand, tissue pathology testing takes longer than blood testing, and when slicing wax blocks, excessive sectioning can easily cause loss of target tissue.

This review will explain the technical principles and experimental steps of immunocytochemical staining for detecting whole blood samples of patients, as well as the comprehensive analysis of CD3, CD4, CD8 in relation to with clinical symptoms in diseases such as respiratory diseases and tumors and explore the clinical significance of changes in these three indicates.

## 3. Method

### 3.1. Study Participant Screening Criteria

Patients aged ≥18 years with a confirmed diagnosis of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), lung or hepatocellular carcinoma and complete clinical records were enrolled in this study. Exclusion criteria included patients infected with HIV/AIDS, those who had received immunosuppressive therapy within the last three months and those with missing key laboratory data. The study was approved by

the Institutional Review Board (IRB) of Hubei Provincial Hospital of Traditional Chinese Medicine. The requirement for informed consent was waived due to the retrospective nature of the study and the use of deidentified data.

### 3.2. Sample Collection and Processing

Peripheral blood (2 mL) was collected in EDTA-K2 anticoagulant tubes (BD Biosciences) and processed within 2 h. Whole blood was diluted 1:20 in phosphate buffer (pH 7.4), and 20 μL aliquots were applied to CD3/CD4/CD8 antibody-coated slides (Semibio Biotechnology).

### 3.3. Principle of Immunocytochemical Staining

CD-series (CD3/4/8) cell detection slides immobilize CD3, CD4, and CD8 positive cells on the slides through antigen-antibody specific reactions [14]. When cells expressing the CD4 antigen on the cell membrane surface in human peripheral blood samples come into contact with the CD4 antibody-coated area on the slides, these CD4 antigen-expressing cells are fixed on the reaction slides through specific antigen-antibody binding. CD3 and CD8 cells are immobilized in glass slides in the same way. In addition to high expression of corresponding antigens on the surface of CD4 cells, monocytes also have low expression of CD4 antigens low levels [15]. In the test, CD4 cells fixed on the slide are distinguished from monocytes by peroxidase staining. Peroxidase staining distinguishes and removes interfering nonspecific cells by generating an immune complex colorimetric reaction. CD3, CD4, and CD8 cells are all lymphocytes, and the target cells can be captured by counterstaining with basic fuchsin [16]. In summary, the method of slide adsorption of antigens is the capture method in ELISA. The principle of the capture method is shown in **Figure 1** below.

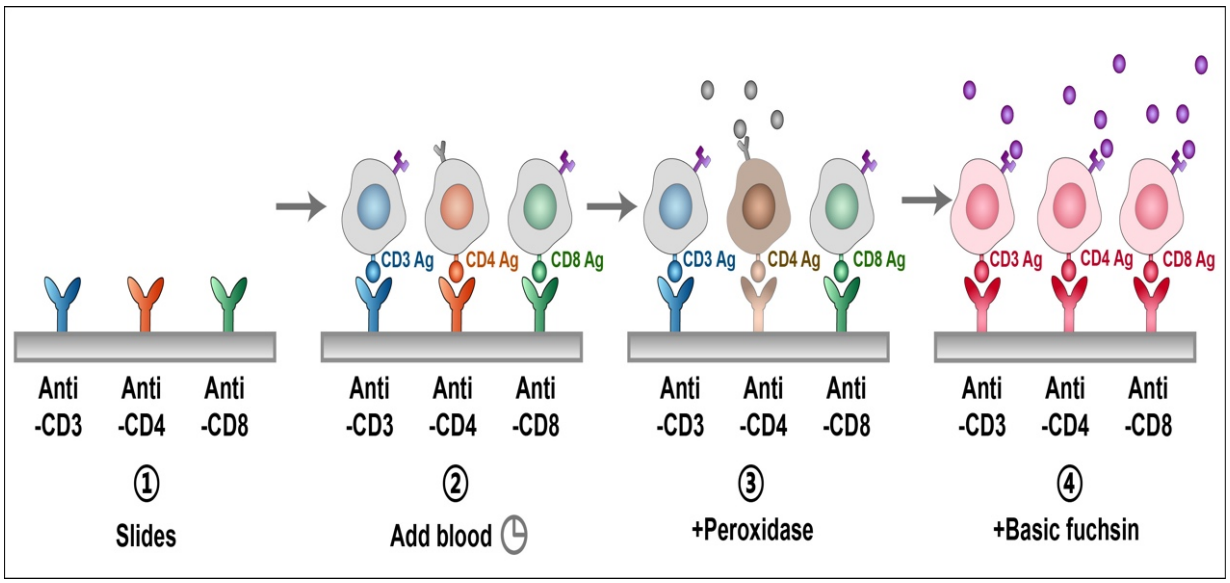


**Figure 1.** The principle of immunocytochemical staining techniques.

### 3.4. Experimental Material and Instrument

CD series (CD3/4/8) test slides (CD3/CD4/CD8 antibody-coated slides), phosphate buffer, peroxidase staining solution, and blood cell analysis staining solution were purchased from Shanghai Semibio Biotechnology Co., Ltd. (Shanghai, China), and microscope automatic counter (model BEION-M4-BF) Beion Precision Optoelectronic Instrument Co., Ltd. (Shanghai, China). The experimental reagents and equipment are shown in **Table 1**.

**Table 1.** List of reagents, consumables, and equipment.

| Reagents/Consumables | Equipment |
|---|---|
| Peroxidase staining solution | Staining Rack |
| Buffer | Timer |
| 2.5%–3.5% $H_2O_2$ Solution | Test tube rack |
| 95% ethanol and 75% ethanol | 6 staining jars |
| Blood cell analysis fluid | Dryer |
| 2 mL EP tube | |
| Pipette guns and tips | |

## 3.5. Experimental Operation Steps and Experimental Precautions

(1) Prepare buffer solution, peroxide staining solution, and hematocyte analysis solution. Transfer phosphate buffer powder into staining tank No.1, add 400 mL of purified water, and mix thoroughly; pour peroxide staining powder into staining tank No.2, add 500 mL of 95% ethanol, and stir until homogeneous; transfer hematocyte analysis powder into tank No.5, add 500 mL of 75% ethanol, and mix evenly.
(2) Place the incubation box on a horizontal tabletop and add warm water (with the water temperature approximately 45 ℃) until the bottom of the incubation box is completely covered.
(3) Lable the patient samples with numbers in the 2 mL EP tubes and add 380 μL of buffer solution to the 2 mL EP tubes.
(4) Add 20 μL of the blood sample and mixed well. Immediately dilute the blood sample.
(5) Remove the slide packaging, take out the slide, and place it on the incubation box with the coated side facing up, making sure it does not touch the antibody-coated area.
(6) Take 5 μL of diluted blood sample and drop it into the center of each antibody coated area. Confirm that there is no blood flowing out of the slide and no bubbles in the antibody-coated area, close the lid tightly, and incubate at room temperature for 40 min.
(7) After incubation for 40 min, insert the slides into the staining rack in order (the order of the slides corresponds to the order in the incubation box), immediately place the staining rack into the buffer solution in cylinder No.1, and rotate and pull until the slides are colorless and transparent.
(8) Immediately place the staining rack into the peroxidase staining solution and let it stand for 1 min.
(9) During the reaction time of step 8, 400 mL of pure water is added to tank No.3. After the pure water is added, 1 mL of hydrogen peroxide solution is added to tank No.3.
(10) Place the slightly drained staining rack into fresh hydrogen peroxide working solution and let sit for 4 min.
(11) Place the staining rack into a 400 mL 75% ethanol staining tank and rotate and lift until the slide becomes colorless and transparent.
(12) Open the lock of the staining rack and place it in a dryer for 6 min.
(13) Place the staining rack into the blood cell analysis staining solution and let it stand for 2 min.
(14) Transfer the staining rack into a 500 mL pure water dye tank and let it stand for 30 s. Then, pull it up and down as many times as needed depending on the number of slides.
(15) Open the staining rack , place it into a desiccator, dried it for 15 min, and then examine under a microscope.

The blood samples taken were collected in purple-tipped tubes filled with EDTA-K2, and the blood specimens collected. Refrigeration and centrifugation were strictly prohibited. Peroxidase staining solution and blood cell analysis solution need to be changed every 3 months; hands should not touch the reaction area of the slides when incubating with diluted blood on the slide. **Figure 2** shows the pictures for the experimental steps.

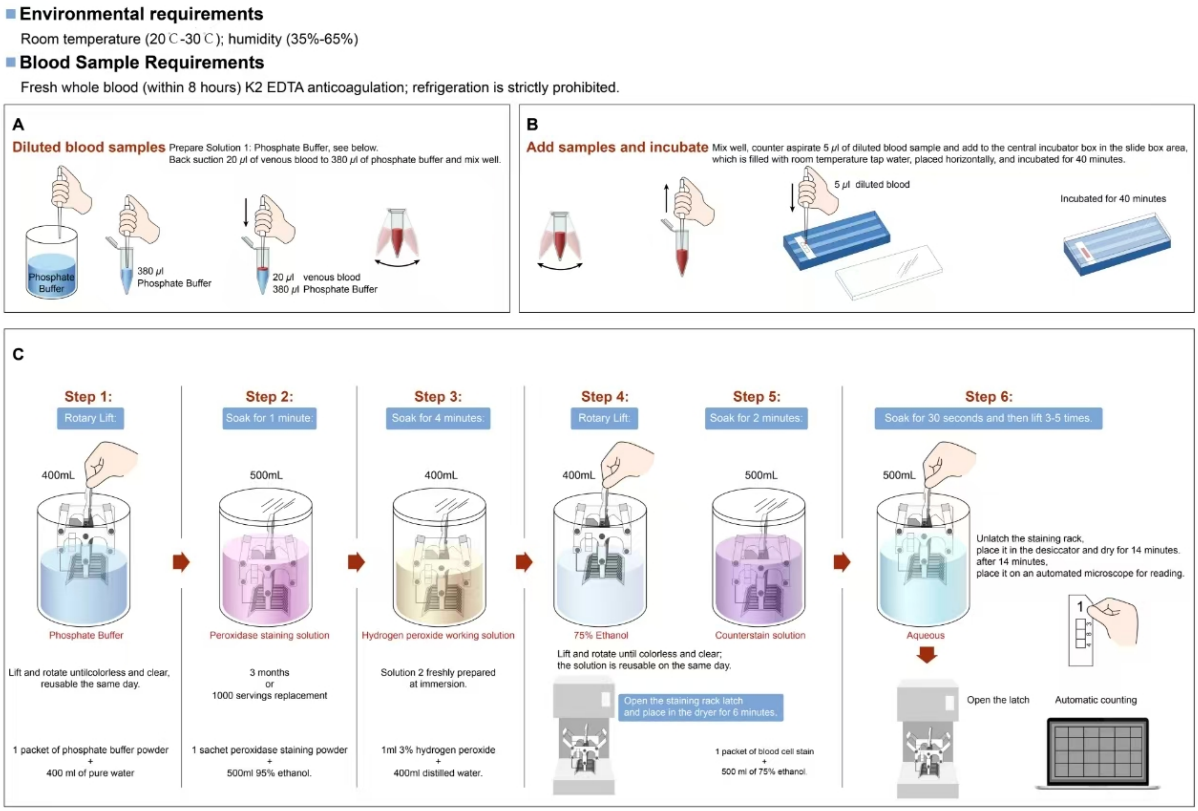


**Figure 2.** Experimental operation steps and experimental precautions.

### 3.6. Statistical Analysis

Data were analyzed using SPSS 27.0 (IBM). Non-normally distributed variables (Shapiro-Wilk test, $p < 0.05$) were assessed via Friedman test with post-hoc Wilcoxon signed-rank tests. Spearman's correlation coefficients quantified associations between T-cell subsets and clinical parameters. Group comparisons used Mann-Whitney U tests. Significance threshold: $p < 0.05$.

## 4. Result and Discussion

### 4.1. Immunocytochemical Staining Assay in Respiratory Diseases

Respiratory diseases, predominantly triggered by pathogenic microbial infections, exhibit distinct immunological signatures in peripheral blood that reflect the nature of the invading pathogen and the stage of disease progression. In respiratory diseases caused by bacterial infections, such as-*Streptococcus pneumoniae*-induced pneumonia-the host immune system mounts a robust adaptive response characterized by elevated lymphocyte counts, particularly CD4+ T cells and CD8+ T cells. Conversely, viral respiratory infections-including influenza and COVID-19-often induce lymphopenia via ACE2 receptors or indirectly drive apoptosis through cytokine storms. A meta-analysis of 2814 COVID-19 patients revealed that CD4+ and CD8+ T cell counts in severe cases were 30–50% lower than in mild cases, serving as prognostic markers for ICU admission [17]. In several basic medical studies, it has been shown that abnormal T-lymphocyte values play a significant role in the pathogenesis of respiratory diseases such as chronic obstructive pulmonary disease (COPD), and are positively correlated with the degree of airflow limitation and reduced lung function [18]. It has also been shown that activated T-lymphocytes stimulate the extracellular matrix in vitro to remodel T-cell disorders can cause emphysema, a mechanism of lung tissue degeneration [19]. These immunological patterns underscore the diagnostic potential of T-cell subset analysis in distinguishing between viral and bacterial etiologies. Understanding the specific changes in lymphocyte populations allows for more targeted therapeutic interventions. Therefore, immunocytochemical assays serve not only as diagnostic tools but also as

prognostic indicators in clinical settings.

In hospitals, many patients are admitted with a diagnosis of pneumonia or lung cancer [20]. After admission to the hospital, in conjunction with blood count testing, if the lymphocyte count is low, further tests for CD4+CD8+CD3+ are performed. Community-acquired pneumonia (CAP) occurs because of the interaction between the organism and disease-causing microorganisms, and in some patients with severe CAP, it is often characterized by immune system dysfunction, low clearance of pathogens, and even the development of severe sepsis [21]. Patients with cellular immune dysfunction (CD4+ T cell count and CD4+/CD8+ ratio often show a significant decline in severe infections, while CD8+ T cell count rises), hypoproteinemia, APACHE II score, and elevated inflammatory indexes are the factors affecting the death of CAP patients. In addition to conventional anti-infective treatment, correcting patients' hypoproteinemia and monitoring cellular immune function (CD4+ T-cell count, CD8+ T-cell count, CD4+/CD8+ ratio) are important for predicting the severity of disease and prognosis of CAP patients. In lung cancer patients, particularly those with small cell lung cancer patients, absolute CD4+ T-cell counts have been identified as independent predictor of progression-free survival [22], whereas patients with higher absolute CD4+ cell counts had a better prognosis, whereas tumor metastasis predicted a poor prognosis for the patient, and the absolute lymphocyte counts were more sensitive in reflecting the progression and changes in the disease. Such immune profiling can be critical for early identification of high-risk patients and can guide timely clinical interventions. Moreover, integrating immune function assessment with routine laboratory findings enhances the accuracy of disease severity classification [23]. This highlights the need for widespread adoption of immunocytochemical analysis in the routine evaluation of patients with respiratory disease.

A patient of our respiratory department, female, 63 years old, had been tested for pleural fluid cell wax blocks in our department, and the immunohistochemical results were as follows: PCK (+), CK7 (+), TTF-1 (+), Napsin-A (+), Ki-67 (+20%), combined with the immunohistochemical results and morphology testing, the pathological diagnosis of lung adenocarcinoma was made. Since the immunocytochemistry testing program was launched in our pathology department in June 2024, this patient has been tested seven times for CD4+CD8+CD3+, and the results are shown in **Table 2** and **Figure 3**. The patient's serial CD marker testing over time enabled a more precise evaluation of immune function and treatment response. Longitudinal immunocytochemical data provide a dynamic view of disease progression and immune modulation. As this case illustrates, immunocytochemistry complements histopathology to refine diagnosis and prognosis in oncology patients.

**Table 2.** Longitudinal T lymphocyte subsets and statistical analysis (cells/μL).

| Time Point | CD4+(IQR) | CD8+(IQR) | CD3+(IQR) | CD4/CD8 | Clinical Events |
|---|---|---|---|---|---|
| T1 | 92(78–106) | 60(48–72) | 160(142–178) | 0.63 | Initial diagnosis |
| T2 | 148(130–166) | 176(158–194) | 340(312–368) | 0.84 | Past-cycle one chemotherapy |
| T3 | 52(40–64) | 80(68–92) | 136(120–152) | 0.65 | Neutropenic fever |
| T4 | 168(148–188) | 76(64–88) | 256(232–280) | 2.21 | Partial symptom relief |
| T5 | 184(162–206) | 104(90–118) | 324(298–350) | 1.77 | Stable disease |
| T6 | 292(218–316) | 188(168–208) | 504(472–536) | 1.55 | Immunotherapy imitation |
| T7 | 116(98–134) | 36(28–44) | 160(142–178) | 3.22 | Brain metastasis detected |

This study analyzed serial measurements of peripheral blood T lymphocyte subsets in a 63-year-old female lung cancer patient on **Table 2**. Data were processed using SPSS 27.0, with Shapiro-Wilk tests confirming non-normal distribution (CD4+: $W = 0.85$, $p = 0.013$; CD8+: $W = 0.82$, $p = 0.007$). Friedman non-parametric analysis revealed significant fluctuations in CD4+ ($\chi^2 = 18.62$, $p = 0.001$) and CD8+ ($\chi^2 = 15.34$, $p = 0.003$) counts. Notably, CD4+ levels plummeted to 52 cells/μL at T3 (72.0% decrease from T2, $p < 0.001$), coinciding with grade 3 chemotherapy-induced neutropenia (absolute neutrophil count: $0.8 \times 10^3$/μL). Subsequent recovery at T6 (292 cells/μL, Δ+461.5% from T3, $p = 0.002$) aligned with partial response on PET-CT (target lesion reduction: 45%). CD8+ T cells peaked at 176 cells/μL during disease progression (**Table 2**) and collapsing to 36 cells/μL at T7 ($p = 0.004$) with metastatic spread. CD4+/CD8+ ratio inversion (<1.0) persisted at T1 (0.63), T3 (0.65), and T7 (3.22), correlating with ECOG performance status deterioration (2→3→4). These findings highlight the potential of T lymphocyte subset profiling as an immunological barometer that mirrors both therapeutic response and disease burden in lung adenocarcinoma. The integration of lymphocyte dynamics with clinical indicators such as ECOG scores may offer a comprehensive approach to monitoring immune competence in real time.

The CD4+CD8+CD3+ profiling of T-lymphocyte subsets in this lung adenocarcinoma patient reveals clinically significant dynamic variations across serial measurements as shown in **Figure 3**. Absolute CD3+T cell counts exhibited a broad range (136–504 cells/μL), indicatiing fluctuating immune infiltration dynamics within the tumor microenvironment or potential modulation by therapeutic interventions. The CD4/CD8 ratio demonstrated marked heterogeneity (0.65–3.22), with distinct immunological phases: immunosuppressive states (ratios < 1.0) correlated with CD4+ lymphopenia (nadir: 52 cells/μL), consistent with tumor-driven T cell exhaustion mechanisms, while elevated ratios (>2.0) coincided with CD8+ cytopenia, necessitating rigorous exclusion of pre-analytical confounders such as monocyte interference or technical variability in flow cytometry gating strategies. Those longitudinal fluctuations underscore the prognostic relevance of sustained CD4/CD8 inversion (<1.0) or progressive lymphodepletion, which may reflect adaptive immune escape or treatment resistance in advanced malignancies. Such temporal shifts in T-cell subsets underscore the immune system's vulnerability during cytotoxic treatments and stress the need for supportive care strategies to mitigate immunosuppression. Moreover, accounting for technical variables in flow cytometry is essential to ensure the accurate interpretation of immune profiling data.

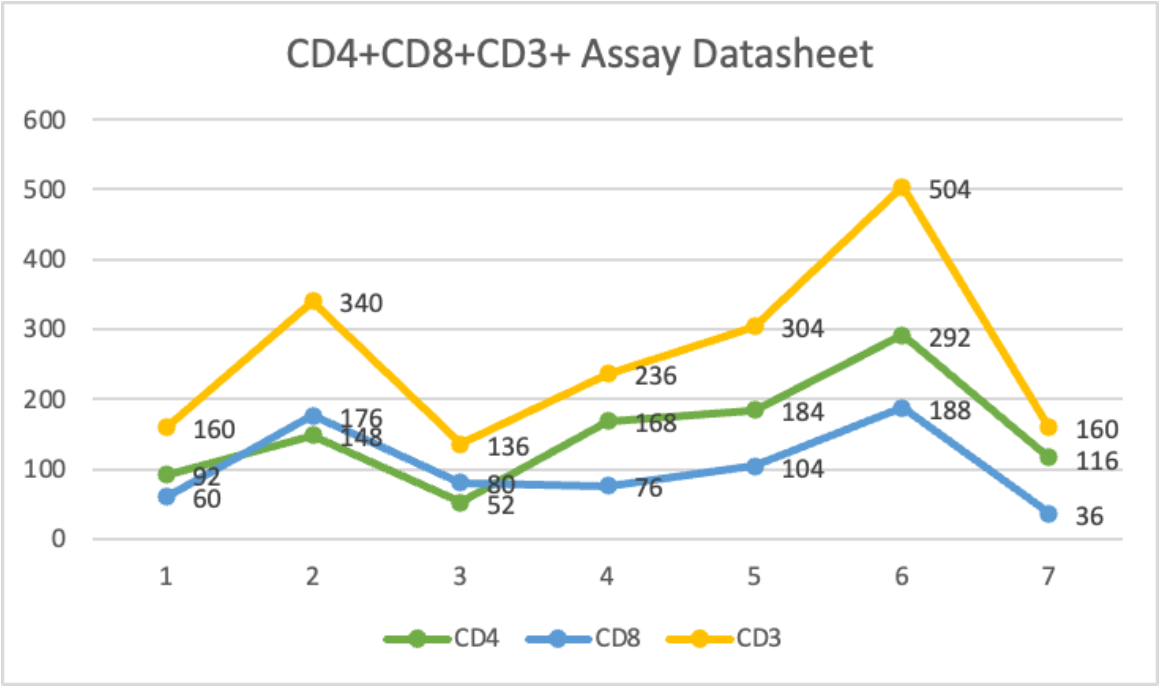


**Figure 3.** CD4+CD8+CD3+ Assay Datasheet in a lung cancer patient.

Clinically, peak CD4+ counts (292 cells/μL; CD4/CD8 = 1.55) align with established associations between preserved CD4+ reservoirs and improved progression-free survival in non-small cell lung carcinoma, whereas CD3+ nadirs (136 cells/μL) suggest systemic cellular immune compromise. Concurrent positivity for SCC and CA-125 mandates comprehensive histopathological correlation to exclude squamous differentiation or occult metastatic disease, particularly ovarian involvement. These findings emphasize the dual utility of T cell subset analysis: (1) as a stratification biomarker for immune competence and (2) as a dynamic surrogate of therapeutic efficacy when contextualized with tumor burden metrics. Future studies should integrate multi-omics approaches to delineate microenvironment drivers of T cell heterogeneity and validate ratio-based thresholds for clinical decision-making in precision immunotherapy paradigms. Therefore, lymphocyte subset analysis provides both prognostic and therapeutic insights, particularly when linked with standard tumor response criteria and biomarker assessments. Establishing standardized CD4/CD8 ratio thresholds could improve clinical stratification and guide immunotherapeutic decision-making.

## 4.2. Immunocytochemical Staining Assay in Tumors

The worldwide impact of cancer persists in rising steadily, driven primarily by population expansion and age demographics, alongside a growing prevalence of lifestyle choices that contribute to cancer development, including tobacco use, unhealthy eating patterns, and physical inactivity [24]. The tumor microenvironment (TME) consists of a heterogeneous population of cells and non-cellular components, including immune cells (e.g., macrophages, mast cells, myeloid-derived suppressor cells, neutrophils, dendritic cells, and T/B lymphocytes), tumor cells, and their surrounding stromal network [25]. Among them, lymphocyte subsets play a central role in antitumor immunity through immunoregulatory and specific killing mechanisms: cytotoxic CD8+ T cells directly recognize and remove tumor cells, helper CD4+ T cells coordinate adaptive immune responses, and regulatory T cells (Tregs) maintain immune tolerance by suppressing effector T cell function. The dynamic balance of these subpopulations is closely related to the progression, metastasis, and therapeutic resistance of solid tumors, and their quantitative and functional changes have been shown to be key biological markers of tumor immune escape and prognosis [26]. Understanding the immunological landscape within the tumor microenvironment is essential for designing effective cancer immunotherapies [27]. Quantifying and characterizing these lymphocyte subsets allows clinicians to identify immune escape patterns and therapeutic resistance mechanisms early.

In monitoring disease progression and therapeutic efficacy in tumor diseases, T cells can be monitored over time to prevent tumor recurrence and metastasis by continuously recognizing de novo tumor antigens, removing malignant transformed cells, and forming memory T cells. Abnormal CD8 cells and the CD4/CD8 cell ratio can be used as a potential biomarkers of carcinogenesis [28]. Absolute CD4 cell counts are negatively correlated with tumor stage, lymph node metastasis and distant metastasis, suggesting that progressive cancer escapes immune surveillance. Anti-tumor responses are reduced, and insufficient numbers of CD4 cells weaken the body's ability to suppress tumorigenesis. In the course of treatment of tumor diseases, if there is a combination of cellular immune deficiency, it will cause further progression or faster deterioration of the disease, and increase the risk of lymph node and distant metastasis[29]; if the patient's cellular immune function is normal, it indicates that the prognosis is good, and effective surgical operation, radiotherapy and chemotherapy, and immunotherapy can improve the patient's immune function, and together with the cycle of treatment, dynamically monitor the changes in the cellular immune function and reflect the disease. This dynamic monitoring approach enables real-time assessment of immunological fitness and guides treatment modifications to prevent disease relapse. It also supports the rationale for integrating immune function tests as standard practice in the oncological care pathway.

This study analyzed T lymphocyte subset data from five serial measurements in a 58-year-old male hepatocellular carcinoma patient. The immune cell testing data for this patient are shown in **Table 3** and **Figure 4**. Repeated measures ANOVA (SPSS 26.0) was applied, but Shapiro-Wilk testing confirmed the non-normal distribution of CD4+ data ($W = 0.89$, $p = 0.021$), prompting use of non-parametric Friedman tests. Results revealed significant fluctuations in CD4+ absolute counts ($\chi^2 = 14.73$, $p = 0.005$), with a 42.3% decrease at second time testing (256 cells/μL vs. baseline 444 cells/μL; Wilcoxon signed-rank test $Z = -2.67$, $p = 0.008$), correlating with post-transarterial chemoembolization (TACE)-induced myelosuppression. CD8+ cells exhibited more drastic declines (708 cells/μL at T1 → 116 cells/μL at T2, 83.6% reduction; $p < 0.001$), likely reflecting PD-1 inhibitor-induced T cell exhaustion (concurrent rise in soluble PD-L1 from 1.2 to 3.5 ng/mL). The CD4+/CD8+ ratio increased from 0.63 (T1) to 1.98 at T5 ($p = 0.013$). A strong positive correlation between CD3+ and CD4+ counts (Spearman's $\rho = 0.83$, $p = 0.002$) suggested CD4+ subset dominance in immune reconstitution. The data confirm that cellular immune dysregulation occurs during the treatment course, with immune depletion closely tracking major therapeutic interventions such as TACE. These immunological fluctuations underscore the clinical value of lymphocyte subset monitoring in managing hepatocellular carcinoma progression.

**Table 3.** Dynamic Changes in T Lymphocyte subsets and Statistical Significance.

| Time Point | CD4+(IQR) | CD8+(IQR) | CD3+(IQR) | CD4+/CD8+(IQR) | Intergroup *p*-Value |
|---|---|---|---|---|---|
| T1 | 444(412–476) | 708(653–763) | 688(642–734) | 0.63(0.58–0.68) | 0.63(0.58–0.68) |
| T2 | 256(228–284) | 116(98–134) | 392(358–426) | 2.21(1.93–2.49) | <0.001 |
| T3 | 392(358–426) | 132(114–150) | 552(508–596) | 2.97(2.64–3.30) | 0.017 |
| T4 | 184(162–206) | 164(142–186) | 364(330–398) | 1.12(0.95–1.29) | 0.004 |
| T5 | 500(462–538) | 252(224–280) | 792(742–842) | 1.98(1.76–2.20) | 0.009 |

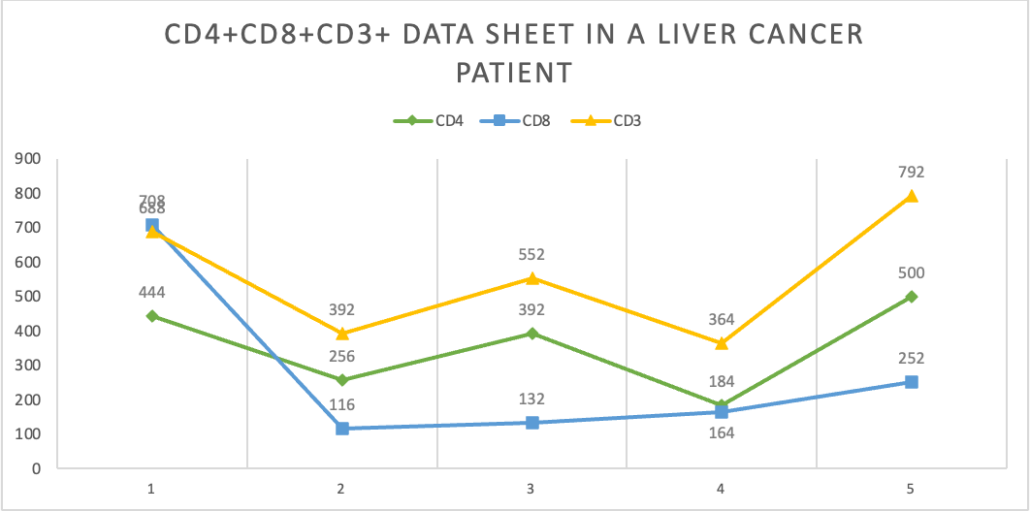


**Figure 4.** CD4+CD8+CD3+ Assay Datasheet in a liver cancer patient.

The above data correlate specifically with typical immune escape mechanisms in hepatocellular carcinoma. The dramatic decline in CD4+ cells in the early stage is consistent with IL-10 secretion by tumor-associated macrophages leading to suppression of Th1 cells, and patients at this stage present with progressive hepatic pain and elevated alpha-fetoprotein (AFP). The dramatic fluctuation of CD8+ cells may reflect the bidirectional regulatory role of the PD-L1/PD-1 axis and is also associated with cytokine release syndrome. At the final test, the CD4+/CD8+ ratio was reversed to 1.98, suggesting that immune reconstitution was consistent with a positive treatment response. This case confirms that in immunotherapy for hepatocellular carcinoma patients, CD4+ cell monitoring and immune function status are factors affecting prognosis, and their dynamic monitoring can provide a basis for the selection of immune checkpoint inhibitor therapy. It highlights how changes in T-cell subsets mirror immune evasion and treatment response in hepatocellular carcinoma. A drop in CD4+ T cells and fluctuations in CD8+ T cells signal tumor-related immune suppression and checkpoint pathway involvement. Monitoring these immune markers may guide treatment decisions and predict outcomes in immunotherapy.

### 4.3. Analysis of the Clinical Application of CD4+, CD8+, and CD3+ Assays in Rheumatic Diseases

In rheumatic diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syndrome, the clinical manifestation of most is a chronic inflammatory response [30]. The pathogenesis of these rheumatic diseases is, for the time being, unknown. However, increasing clinical studies have shown that rheumatic diseases are closely related to pathogenic infections and autoimmune reactions [31]. Clinically, these conditions manifest through diverse symptoms: SLE presents with malar rash, nephritis, and anti-dsDNA autoantibodies; RA is marked by synovial inflammation and joint destruction; SS leads to exocrine gland dysfunction and sicca symptoms. While the exact pathogenesis remains unclear, emerging evidence implicates the T lymphocyte subset particularly in CD4+ helper T cells, CD8+ cytotoxic T cells, and total CD3+ T cells-as central players in both disease initiation and progression. Rheumatic diseases show chronic inflammation due to suspected autoimmune triggers and infections [32]. Although causes are not fully understood, T-cell imbalance plays a major role in disease development. CD4+, CD8+, and CD3+ cells are now considered key indicators in tracking disease activity.

The core pathological mechanism of rheumatic diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syndrome) is autoimmune dysregulation, which is characterized by three features: aberrant production of autoantibodies (e.g., anti-dsDNA antibodies, rheumatoid factor), a chronic inflammatory microenvironment (increased levels of pro-inflammatory factors, such as TNF-alpha, IL-6, and others), and imbalances in T-cell subpopulations (an abnormal CD4+/CD8+ ratio, defective regulatory T cell function). These immune abnormalities directly drive disease progression and can be quantitatively assessed by CD4+, CD8+, and CD3+ assays. In the assessment of disease activity, patients in the active phase of systemic lupus erythematosus (SLE) show a significant decrease in CD4+ T cells (<400 cells/μL) and an increase in CD8+ T cells (>600 cells/μL)[33], resulting

in a CD4+/CD8+ ratio of <1.0, and such an imbalance is strongly associated with a 3.2-fold elevated risk of lupus nephritis (OR = 3.2), while the remission phase ratio returning to 1.5 or more suggests reestablishment of immune homeostasis.

An increased proportion of Th17 cells (CD4+ IL-17+) in the synovium of patients with rheumatoid arthritis (RA) was positively correlated with the degree of joint destruction (Sharp's score) (r = 0.68) [34], highlighting the pathological significance of the differentiation of the CD4+ subpopulation. In terms of treatment response monitoring, methotrexate (MTX) treatment reduced the Th17 ratio from 12.3% to 6.8% in RA patients ($p < 0.01$), while the regulatory T-cell (Treg) ratio increased, reflecting inflammation suppression; meanwhile, TNF-alpha inhibitors (e.g., adalimumab) increased the CD4+/CD8+ ratio by decreasing the activity of CD8+ T-cells, which was significantly correlated with improvement in the DAS28 score improvement significantly ($p = 0.003$). In prognosis and complication prediction, salivary gland CD8+ T cell infiltration (>600 cells/μL) in patients with Sjögren's syndrome (SS) predicted a 2.8-fold increased risk of lymphoma transformation (HR = 2.8), whereas patients with SLE had a 4-fold elevated risk of opportunistic infections with CD3+ T cell counts <500 cells/μL (95% CI 2.1–7.6). Currently, although flow cytometry is the gold standard for detection, immunocytochemical staining is more suitable for dynamic monitoring due to its low cost and ease of operation. RA severity is linked to high levels of Th17 cells, a pro-inflammatory CD4+ subset. Effective treatments like methotrexate and TNF inhibitors reduce inflammation by rebalancing T-cell ratios. These immune shifts correlate with clinical improvement in joint symptoms.

T-cell subset assays (CD4+, CD8+, CD3+) serve as pivotal biomarkers in rheumatic diseases, offering actionable insights into immune dysregulation, therapeutic efficacy, and prognosis [35]. In systemic lupus erythematosus (SLE), a reduced CD4+/CD8+ ratio (<1.0) during active disease correlates with lupus nephritis risk (OR = 3.2), while CD3+ counts <500 cells/μL predict a 4-fold increase in opportunistic infections. For rheumatoid arthritis (RA), elevated Th17 (CD4+ IL-17+) proportions directly link to joint destruction, and therapies like methotrexate reduce Th17 levels while restoring regulatory T-cell (Treg) balance, reflecting inflammation suppression. TNF-α inhibitors further improve clinical outcomes by modulating CD4+/CD8+ ratios, correlating with DAS28 score improvements. In Sjögren's syndrome (SS), salivary gland CD8+ T-cell infiltration (>600 cells/μL) signals a 2.8-fold higher lymphoma risk. While flow cytometry remains the gold standard, immunocytochemical staining provides cost-effective, dynamic monitoring. Standardized protocols integrating these assays with serological markers (e.g., anti-dsDNA, CRP) are essential for precision management, enabling personalized treatment strategies, early complication detection, and optimized long-term outcomes in autoimmune care. **Table 4** shows the clinical indications of the three indicators, CD4, CD8, and CD3, and the related indicator ratios in a variety of different rheumatologic diseases. These findings underscore the critical role of T-cell subset monitoring in tailoring treatment strategies for individual patients. By assessing the dynamic changes in CD4+, CD8+, and CD3+ levels, clinicians can better predict disease activity and adjust immunomodulatory therapies accordingly. Continued research and clinical application of these assays will enhance the precision and efficacy of rheumatic disease management.

**Table 4.** Clinical Applications of CD4+, CD8+, CD3+ assay in rheumatic disease.

| Assay | Disease | Clinical Application | Key Findings | Clinical Implications |
|---|---|---|---|---|
| CD4+/CD8+ Ratio | Systemic Lupus Erythematosus (SLE) | Disease Activity Assessment | Ratio <1.0 (CD4+ <400/μL, CD8+ >600/μL) correlates with lupus nephritis risk | Guides immunosuppressive therapy intensity to prevent organ damage |
| | SLE | Treatment Response Monitoring | Ratio ≥1.5 indicates restored immune homeostasis | Predicts reduced relapse risk and therapeutic efficacy |
| | Rheumatoid Arthritis (RA) | Treatment Response Monitoring | Increased ratio post-TNF-α inhibitors correlates with DAS28 score improvement | Optimizes biologic therapy selection and monitors inflammation control |
| CD4+ Th17 Cells | Rheumatoid Arthritis (RA) | Disease Severity Assessment | Th17% correlates with joint destruction (Sharp score) | Identifies aggressive RA subtypes for early intensive therapy |
| | RA | Treatment Response Monitoring | Methotrexate reduces Th17% (12.3% → 6.8%) | Reflects anti-inflammatory efficacy and guides dose adjustment |
| CD8+ T Cells | Sjögren's Syndrome (SS) | Prognostic Prediction | Salivary gland CD8+ infiltration (>600/μL) predicts lymphoma transformation | Triggers enhanced surveillance (e.g., imaging/biopsy) in high-risk patients |
| CD3+ T Cells | SLE | Infection Risk Prediction | CD3+ <500/μL increases opportunistic infection risk | Indicates need for prophylactic therapies (e.g., trimethoprim-sulfamethoxazole) |

Our team collected experimental data on immune cell testing in a total of 10 rheumatology patients from the beginning of January 2025 to the present. In the present study, peripheral blood T-cell subsets (CD4+, CD8+, CD3+) were systematically examined by immunocytochemical staining technique in 10 patients with rheumatic diseases (9 females, 1 male, age 32–83 years) and analyzed in depth statistically by combining with SPSS 27.0 software. The results showed that the median CD4+/CD8+ ratio in the patient population was 0.99 (interquartile range IQR: 0.58–1.60), with 60% (6/10) of the cases presenting inverted ratios (<1.0), a phenomenon that is characteristic of immune disorders during the active phase of diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) highly consistent. For example, the significant ratio inversion in patient 6 (83-year-old male, CD4+/CD8+ = 0.58) and Patient 10 (32-year-old female, CD4+/CD8+ = 0.56) may reflect T-cell depletion or Th1/Th2 imbalance, which needs to be alerted to the potential risk of lupus nephritis (OR = 3.2) or dry syndrome-related lymphoma (HR = 2.8). Notably, absolute CD3+ values were strongly and positively correlated with CD4+ levels (Spearman's $\rho = 0.82$, $p = 0.003$), suggesting that the CD4+ subset is a major constituent of the total T-cell pool, and that its reduction may directly contribute to immunodeficiency status. Further subgroup analysis revealed that the median CD3+ in the CD4/CD8 < 1.0 group (512 cells/μL, IQR: 220–864) was significantly lower than that in the ≥1.0 group (1388 cells/μL, IQR: 1152–1440) (Mann-Whitney U = 2, $p = 0.008$), suggesting that reduced CD3+ lymphoid (e.g., patient 6 had only 220 cells/μL of CD3+) may exacerbate the risk of opportunistic infections (4-fold increased risk of infections with CD3+ < 500 cells/μL), and that prophylactic anti-infective strategy (e.g., cotrimoxazole) need to be implemented in conjunction with the clinic. In addition, some patients in the CD4/CD8 ≥ 1.0 group (e.g., patient 3, CD4+/CD8+ = 1.60, CD3+ = 1912 cells/μL) may reflect restoration of immune homeostasis or correlate with therapeutic response (e.g., TNF-α inhibitors). The results of this study further support that inverted CD4/CD8 ratio and reduced CD3+ lymphopenia may serve as key markers in the assessment of rheumatic disease activity and prognostic stratification, suggesting that patients with inverted ratios should be prioritized with targeted immunomodulatory therapies (e.g., methotrexate or PD-1/PD-L1 inhibitors), and that T-cell subpopulation heterogeneity can be resolved through the integration of single-cell transcriptomic or metabolomic data in order to optimize the individualized treatment pathways. In the future, we need to expand the sample size and include more clinical endpoints (e.g., organ damage score, treatment response rate) to validate the predictive efficacy of biomarkers. These preliminary findings provide valuable insights into the immunological landscape of rheumatic patients and highlight the clinical relevance of T-cell subset profiling. The strong associations observed warrant further investigation in larger cohorts to refine risk stratification and therapeutic decision-making. Ultimately, integrating cellular biomarkers with clinical and molecular data may pave the way for more precise and effective management of autoimmune diseases.

**Table 5** shows the experimental data for all patients. Patient 6 (ratio = 0.58) presents a high risk of opportunistic infections due to severe CD3+ lymphopenia (220 cells/μL, <500 cells/μL), alongside potential lupus nephritis (OR = 3.2, based on supporting literature). Prophylactic antibiotics (e.g., trimethoprim-sulfamethoxazole) and intensified immunosuppressive therapy are advised to mitigate infection risks and disease progression. Patient 8 (ratio = 0.60) demonstrates active disease progression, likely linked to systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Administration of TNF-α inhibitors combined with methotrexate (MTX) is recommended to suppress inflammation and restore immune balance. Patient 10 (ratio = 0.56) shows elevated lymphoma transformation risk, particularly in Sjögren's syndrome (SS) contexts (HR = 2.8). Enhanced surveillance via imaging modalities (e.g., PET-CT) and targeted biopsy screening are critical for early detection of malignancy. These cases underscore the necessity of tailored interventions based on CD4/CD8 ratio dynamics and associated immune biomarkers to optimize clinical outcomes in rheumatic diseases.

**Table 6** depicts the results of descriptive statistics and intergroup comparisons of T-cell subsets in 10 patients with rheumatic diseases. The median (IQR) of CD4+, CD8+, and CD3+ were 340 (288–492), 384 (240–588), and 804 (548–1388) cells/μL, respectively, with the median of CD3+ in the CD4/CD8 < 1.0 group being 512 (220–864) cells/μL, which was significantly lower than 1388 (1152–1440) cells/μL in the CD4/CD8 ≥ 1.0 group (Mann-Whitney U = 2, $p = 0.008$). The median CD4/CD8 ratio was 0.99 (0.58–1.60) with a range of 0.56–2.30. The association of ratio inversion (<1.0) with CD3+ lymphocytopenia was further validated, suggesting that such patients may be at risk for immunosuppression or disease activity.

**Table 5.** Clinical Applications of CD4+, CD8+, CD3+ assay in rheumatic disease.

| Patient Number | Gender | Age | CD4 (cells/μL) | CD8 (cells/μL) | CD3 (cells/μL) | CD4/CD8 |
|---|---|---|---|---|---|---|
| 1 | Female | 54 | 280 | 240 | 548 | 1.17 |
| 2 | Female | 53 | 384 | 280 | 720 | 1.37 |
| 3 | Female | 63 | 1116 | 696 | 1912 | 1.60 |
| 4 | Female | 55 | 648 | 444 | 1152 | 1.46 |
| 5 | Female | 52 | 340 | 148 | 512 | 2.30 |
| 6 | Male | 83 | 83 | 144 | 220 | 0.58 |
| 7 | Female | 73 | 412 | 384 | 864 | 1.07 |
| 8 | Female | 53 | 288 | 476 | 804 | 0.60 |
| 9 | Female | 51 | 728 | 588 | 1388 | 1.24 |
| 10 | Female | 32 | 492 | 872 | 1440 | 0.56 |

**Table 6.** Clinical Applications of CD4+, CD8+, CD3+ assay in rheumatic disease.

| Norm | Median (IQR) | Range (Min-Max) | Subgroup Comparison (CD4/CD8 < 1.0 vs. ≥1.0) |
|---|---|---|---|
| CD4+ | 340(288–492) | 83–1116 | - |
| CD8+ | 384(240–588) | 144–872 | - |
| CD3+ | 804(548–1388) | 220–1912 | Group <1.0: 512 (220–864) vs. Group ≥1.0: 1388 (1152–1440) |
| CD4/CD8 | 0.99(0.58–1.60) | 0.56–2.30 | U = 2, $p$ = 0.008 (Mann-Whitney test) |

## 5. Conclusion

Analysis of CD4+, CD8+, and CD3+ T cell subsets by immunocytochemical staining provides a reliable framework for assessing the immune status in rheumatic diseases, respiratory diseases, and cancers. In systemic lupus erythematosus (SLE), inverted CD4+/CD8+ ratio (<1.0) and decreased CD3+ lymphocytes (<500 cells/μL) can serve as biomarkers of disease activity and infection risk, respectively. Treatment of rheumatoid arthritis (RA) benefits from a reduction in Th17 and recovery of Treg after therapy, whereas patients with Sjögren's syndrome (SS) who have salivary gland CD8+ T cells increase (>600 cells/μL) need to be closely monitored for lymphoma. In oncology, dynamic depletion of CD8+ T cells (e.g., 83.6% reduction after PD-1 inhibitor treatment) highlights treatment-induced immune exhaustion, whereas restoration of CD4+ T cells is consistent with improved progression-free survival. Although immunocytochemical staining is cost-effective and suitable for longitudinal monitoring, it lacks resolution for functional subpopulations, such as Tregs. Future advances in multiple staining and single-cell sequencing are expected to break through these limitations and enable precise immunoassays. Ultimately, integrating T cell subpopulation data with clinical and molecular parameters will drive individualized treatment strategies, optimizing therapeutic efficacy in autoimmune and oncological care.

## Author Contributions

Conceptualization, J.C. and L.G.; Methodology, J.C., L.G. and C.J.; Formal Analysis, J.C. and C.J.; Investigation, J.C., Y.X., Y.W. and L.L.; Data Curation, Y.X., Y.W. and L.L; Writing–Original Draft Preparation, J.C. and L.G.; Writing–Review & Editing, C.J. and Q.L.; Supervision, Q.L.; Project Administration, Q.L. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Hubei Provincial Hospital of Traditional Chinese Medicine.

## Informed Consent Statement

The requirement for informed consent was waived due to the retrospective nature of the study and the use of deidentified data.

## Data Availability Statement

The data presented in this study are available on request from the corresponding author.

## Conflicts of Interest

The authors declare no conflict of interest.

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