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Differential distribution and prognostic value of CD4⁺ T cell subsets before and after radioactive iodine therapy in differentiated thyroid cancer with varied curative outcomes

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Differentiated thyroid cancer is the most frequently diagnosed endocrine tumor. While differentiated thyroid cancers often respond to initial treatment, little is known about the differences in circulating immune cells amongst patients who respond differently. A prospective study of 39 patients with differentiated thyroid cancer was conducted. Serum thyroglobulin levels and thyroid and immunological functions were tested before and after radioactive iodine treatment (RAIT). Efficacy assessments were performed 6 to 12 months after radioactive iodine treatment. Most patients showed an excellent response to radioactive iodine treatment. Before radioactive iodine treatment, the excellent response group had considerably fewer circulating CD4⁺ T cell subsets than the non-excellent response group. Both the excellent response and non-excellent response groups had considerably lower circulating CD4⁺ T lymphocyte subsets 30 days after radioactive iodine treatment, but those of the excellent response group were still lower than those of the non-excellent response group. All circulating CD4⁺ T cell subsets in the excellent response group rose by varying degrees by the 90th day, but only Treg cell amounts increased in the non-excellent response group. Interestingly, in the non-excellent response group, we noticed a steady drop in Th1 cells. However, the bulk of circulating CD4⁺ T cell subsets between the two groups did not differ appreciably by the 90th day. Finally, we discovered that CD4⁺ T cell subsets had strong predictive potential, and we thus developed high-predictive-performance models that deliver more dependable prognostic information. In conclusion, in individuals with differentiated thyroid cancer, there is great variation in circulating immune cells, resulting in distinct

treatment outcomes. Low absolute CD4⁺ T cell counts is linked to improved clinical outcomes as well as stronger adaptive and resilience capacities.

KEYWORDS

differentiated thyroid cancer, efficacy assessment, CD4⁺ T cell subsets, radioactive iodine therapy, prognosis

1 Introduction

Thyroid cancer is the most frequently diagnosed endocrine cancer, with an annual incidence of 8–9 cases per 100,000 persons worldwide. Differentiated thyroid cancer (DTC) accounts for more than 90% of all thyroid cancers (1). DTC has become more widespread in the last 10 years as imaging technology has improved, and people's lifestyles have changed (2–4).

The most commonly utilized first-line treatment option for individuals with DTC is radioactive iodine therapy (RAIT) following complete thyroidectomy (5). In addition, the American Thyroid Association recommends that DTC patients, particularly those at intermediate and high risk, undergo long-term active follow-up after RAIT (6). At 6–12 months, the initial therapeutic response should be evaluated. Serum thyroglobulin (Tg) levels in the blood and lymph node invasion are two major predictors of tumor development that must be monitored during long-term follow-up of DTC patients who have received RAIT (7–9). Although this management strategy has proven effective, some patients continue to face tumor recurrence and metastasis, which substantially influences survival (2, 10–14). Previous research has suggested that thyroglobulin (Tg) or preoperative anti-thyroid peroxidase antibody (TPOAb) levels are prognostic indicators for DTC recurrence (7, 15). However, their predictive power is limited. This necessitates deeper investigations into novel predictive tools.

Cancer immune surveillance mechanisms can eliminate aberrant cells from the body, preventing tumor formation. Tumor incidence and development in the body result from a series of dynamic and complicated interactions between the immune system and tumor cells, including clearance, balance, and escape (16–18). Tumors are the result of immunological escape. In addition, tumor cells that developed from normal cells appear to have a higher chance of developing immune evasion mechanisms, including tumor cell self-modification, alterations in metabolism, and changes in the tumor microenvironment. At a certain point, the body's anti-tumor immune system will fail in preventing further development, and tumor cells will grow and divide uncontrollably (19). Thus, it is important to increase T cell recognition of tumor cells for anti-tumor immunity.

CD8⁺ cytotoxic T cells, CD4⁺ T helper cells, and dendritic cells are important biological mediators of cancer immune surveillance. CD4⁺ T cells primarily consist of helper T (Th) cells, which adhere to the non-polypeptide portion of major histocompatibility complex class II molecules and participate in signal transduction using T cell antigen receptors to identify antigens, thereby activating CD8⁺ T cells (20–22). CD8⁺ T cells can release IFN- γ to destroy tumor cells directly (23). These findings indicate that CD4⁺ T cells are important in anticancer immunity (24–26). Currently, tumor immunotherapies seek to activate the body's immune system to produce a potent anti-tumor immune response. However, current methods only show good outcomes in a limited number of patients (27–29). This might be due to a lack of understanding of the distribution and function of CD4⁺ T cell subsets (Th1, Th2, Th17, and regulatory T cells (Treg cells)) in the tumor microenvironment and circulatory system (30, 31).

Therefore, the purpose of this study was to examine the differences in the dynamic distribution of circulating CD4⁺ T cell subsets before and after RAIT in DTC patients who had different therapeutic outcomes, as well as to assess the diagnostic and predictive potential of CD4⁺ T cell subsets (before and after RAIT) relative to serum Tg before RAIT. Furthermore, we designed and evaluated multiple predictive models to improve the prognosis of DTC patients.

2 Materials and methods

2.1 Patients

Between January 2021 and August 2021, 79 participants consisting of 39 DTC patients and 40 age- and sex-matched healthy controls (HC) were recruited from Shanxi Medical University's First Hospital. According to the EANM guide, all DTC patients were treated with thyroidectomy and radioactive iodine therapy (RAIT). Patients with acute inflammatory or autoimmune disorders, chronic inflammation or other malignancies, or other conditions that might cause altered immunity were excluded from this study. In the three months before recruitment, none of the patients had received

glucocorticoids or immunosuppressive medicines. All patients had peripheral blood samples drawn, and thyroid and immune function tests were performed on day 0 before RAIT, as well as on 30 and 90 days after RAIT. Free T3 (FT3), free T4 (FT4), thyroid-stimulating hormone (TSH), and thyroglobulin (Tg) levels were all assessed using thyroid function assays (SN-697, Shanghai Nuclear Research Institute Rihuan Photoelectric Instrument Co., Ltd, China). Immune function testing included evaluating CD4⁺ T cell subset levels, using the detection methods described in the following section (Figure 2A). Venous blood was taken prior to RAIT for standard analyses of lymphocytes, neutrophils, four blood lipids (triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C)), and liver function. The institutional review board of Shanxi Medical University's First Hospital in China approved this study.

2.2 Flow cytometry

Flow cytometry was used to measure the total number of lymphocytes in each of the subpopulations (CD3⁺ T/CD4⁺ T/CD8⁺ T/B/NK cells) which were isolated from peripheral blood samples. Blood cells were collected by flow cytometry using a BD FACSCalibur platform (BD Biosciences, Franklin Lakes, NJ, USA) and quantified using MultiSET software (BD Biosciences) after 50 μ L of EDTA-anticoagulated venous blood was placed into A and B Trucount tubes (BD Biosciences). The CD3⁺/CD4⁺/CD8⁺ T cell subsets were identified using the conjugated monoclonal antibodies fluorescein isothiocyanate (FITC)-CD3, allophycocyanin (APC)-CD4, peridinin chlorophyll protein (PerpCP)-CD45, and phycoerythrin (PE)-CD8 (20210301; Beijing Tongsheng Shidai Biotech Co., Ltd, Beijing, China). For B and NK cell populations, the monoclonal antibodies FITC-CD3, APC-CD19, PerpCP-CD45, and PE-CD16+CD56 were used (20210726; Beijing Tongsheng Shidai Biotech Co., Ltd.). CD4⁺ T cell subsets (Th1/Th2/Th17/Treg cells) were then detected and Th1/Th2/Th17 cells were stimulated with PMA and ionomycin. These cells were then labeled with FITC-conjugated anti-CD4 antibodies (20210604; Beijing Tongsheng Shidai Biotech Co., Ltd.), fixed, permeabilized using 1 mL freshly prepared fixation/permeabilization buffer, and then stained with APC-conjugated anti-IFN- γ (C7048040521503; Beijing Tongsheng Shidai Biotech Co., Ltd.)/PE-conjugated IL-4 (C7048040521503; Beijing Tongsheng Shidai Biotech Co., Ltd.)/PE-conjugated IL-17A antibodies (0314403; Beijing Tongsheng Shidai Biotech Co., Ltd.). Cell surfaces were stained with FITC-conjugated anti-CD4 and APC-conjugated anti-CD25 (0139110; Beijing Tongsheng Shidai Biotech Co., Ltd.), before being fixed and permeabilized in a 4°C incubator with 1 ml Cytotfix/Cytoperm reagent. This was followed by staining

with anti-Foxp3-PE (2429732; Beijing Tongsheng Shidai Biotech Co., Ltd.) for 30 min, according to the manufacturer's protocol for Treg cells. CellQuest software version 6 was used to compute the proportion of each subpopulation, and the absolute number of cells in each subpopulation was estimated as follows: absolute cell number = percentage of positive cells in each subgroup \times absolute counts of CD4⁺ T cells (cells/ μ L).

2.3 Response assessment

All patients were assessed for treatment effectiveness within 6–12 months of receiving RAIT. According to the 2015 American Thyroid Association guidelines, patients were classified as: (I) excellent response (ER) (i.e., inhibitory Tg < 0.2 μ g/L or stimulatory Tg < 1 g/L, negative imaging examination); (II) indeterminate response (IDR) (i.e., 0.2 g/L \leq inhibitory Tg < 1 g/L or 1 g/L \leq stimulatory Tg < 10 g/L, negative imaging examination); (III) biochemical incomplete response (BIR) (i.e., 1 g/L < inhibitory Tg or 10 g/L < stimulatory Tg, negative imaging examination); or (IV) structural incomplete response (SIR) (i.e., structural disease testing independent of Tg and TgAb results).

All individuals in this study were separated into two groups: efficacy response (ER) or non- efficacy response (NER), which included IDR, BIR, and SIR classified patients.

2.4 Statistical analysis

All statistical analyses were carried out using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and/or GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA). Simple and relative frequencies were used for analysis of categorical variables. Median, average, standard deviation, and range values were used for analysis of continuous variables. Continuous variable data were tested for normality using the normality test. Student's *t*-tests and the Mann-Whitney U tests were used for normally distributed continuous data and non-normally distributed continuous variables, respectively. Bonferroni correction was used to correct for multiple variables. One-way ANOVA was used for normally distributed continuous variables when comparing three or more populations, and the Kruskal-Wallis rank-sum test was used for non-normally distributed continuous variables. Repeated measures ANOVA tests were employed for factors that change over time. To compare pre- and post-treatment results, paired *t*-tests were performed. The predictive efficacy of Tg and CD4⁺ T cell subsets was assessed using univariate logistic regression analysis. Predictive ability was assessed using multiple regression analysis. To distinguish ER from NER, receiver operating characteristic (ROC) curves were drawn, and the optimal cut-off value for each index was selected. The area

under the curve (AUC) was determined using a 95% confidence interval (CI). Statistical significance was set at $p < 0.05$.

3 Results

3.1 Comparison of clinical data

The clinical data of the two groups are summarized in **Table 1**. The ER group consisted of 21 patients with an average age of 43.95 ± 12.54 years, 71.4% ($n = 15$) of whom were female. The NER group included 18 patients with an average age of 40.77 ± 2.24 years, with 61.12% ($n = 11$) being female. There was no significant difference between the two groups in terms of age, gender, tumor volume, number of lesions, BMI, or partial testing ($p > 0.05$; **Table 1**). Furthermore, there were no statistically significant changes between the two cohorts in the four blood lipids (TC, TG, HDL-C, and LDL-C), lymphocytes, neutrophils, ALT, or AST ($p > 0.05$; **Figure S1**). Several studies have shown that these measures are strongly associated with thyroid cancer (32–34); however, we did not find significant differences between the two cohorts.

Comparative analysis of thyroid function and thyroglobulin levels in efficacy response and non-efficacy response groups before and after radioactive iodine therapy (RAIT)

To exclude the possibility of TSH-suppressing treatment influencing the study, we compared thyroid function in the two

groups before and after RAIT. Serum FT3 and FT4 levels in the ER and NER groups rose swiftly after treatment with levothyroxine sodium pills, whereas TSH levels quickly decreased, and thereafter, thyroid function stabilized (**Figures 1A–C**). Simultaneously, no significant changes in thyroid function were identified between the two groups ($p > 0.05$; **Figures 1A–C**). The variations in serum Tg levels in the two groups were also studied before and after therapy. The results indicated that Tg levels in all participants fell immediately and stabilized following therapy. From 30 days to 180 days after RAIT, patients in the ER group showed significantly lower Tg levels than those in the NER group ($p < 0.05$; **Figure 1D**). Although there was no significant difference in Tg levels between the two groups before RAIT, serum Tg levels in the NER group tended to be higher than that in the ER group. As a result, lower baseline Tg levels are closely linked to RAIT effectiveness.

3.3 Comparative analysis of the distribution of CD4⁺ T cell subsets in pre-RAIT ER, NER, total DTC, and HC groups

In all DTC patients (ER + NER), the proportion of Th17 cells ($p < 0.05$; **Figure S2C**) was significantly higher, while the proportion of Treg cells ($p < 0.001$; **Figure S2D**) was significantly lower, compared to the HC group. The proportions of Th1 and Th2 cells were not significantly different between the two groups ($p > 0.05$; **Figures S2A, B**). The proportion of Treg cells was significantly lower in the ER group compared with that of the HC group ($p = 0.001$; **Figure**

TABLE 1 Comparison of general clinical data of the two groups of patients.

	ER (n=21)	NER (n=18)	P
Age (year), median (range)	46 (20-77)	37.5 (24-59)	0.386
Tumor size (cm), mean \pm SD	1.35 \pm 0.89	1.31 \pm 1.02	0.908
Gender, n (%)			0.734
Male	6 (28.57)	7 (38.88)	
Female	15 (71.43)	11 (61.12)	
Number of lesions, n (%)			0.748
Single lesion	10 (47.61)	7 (38.88)	
Multiple lesions	11 (52.39)	11 (61.12)	
BMI	20.19 \pm 3.11	21.01 \pm 4.29	0.497
Leukocyte ($10^9/L$), mean \pm SD	5.30 \pm 1.16	5.81 \pm 1.39	0.224
Red blood cells ($10^{12}/L$), mean \pm SD	4.80 \pm 0.55	5.01 \pm 0.42	0.178
Hemoglobin (g/L), mean \pm SD	141.09 \pm 21.24	146.8 \pm 19.69	0.39
Urea (mmol/L), mean \pm SD	3.72 \pm 1.05	4.04 \pm 1.29	0.392
Cr ($\mu\text{mol/L}$), mean \pm SD	70.94 \pm 11.14	71.17 \pm 17.98	0.962
UA ($\mu\text{mol/L}$), mean \pm SD	309.00 \pm 86.14	364.00 \pm 112.97	0.093

BMI, Body Mass Index; Cr, creatinine; UA, uric acid; ER, excellent response; NER, non-excellent response. These blood parameters are reference baseline values.

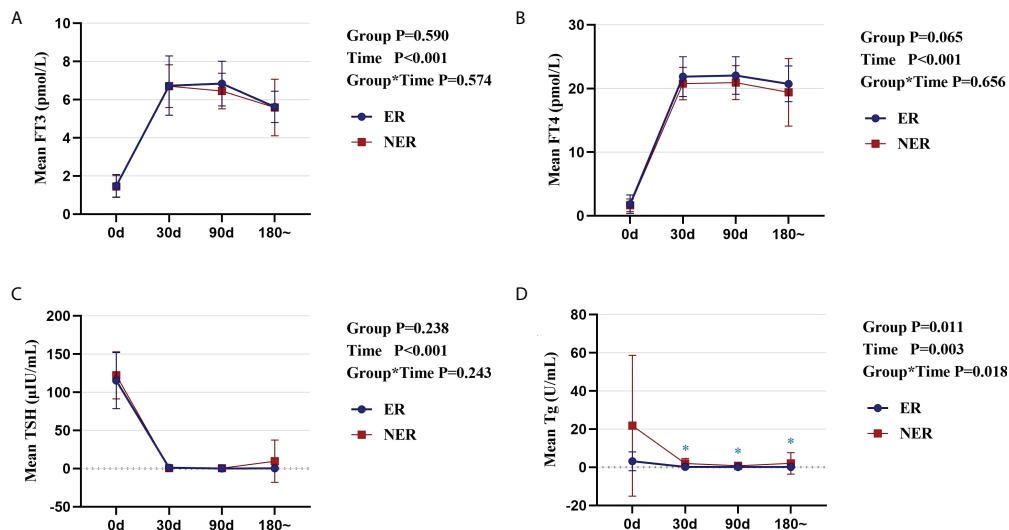


FIGURE 1

Comparative analysis of the dynamic changes and differences in thyroid function and Tg in ER and NER groups before and after RAIT. Changes in serum FT3 (A), FT4 (B), TSH (C), and Tg (D) in the two groups of patients before and after RAIT. ER, excellent response; NER, non-excellent response; RAIT, radioactive iodine treatment; Tg, serum thyroglobulin; d, day; 180~, expressed as 6 months to 1 year after RAIT.

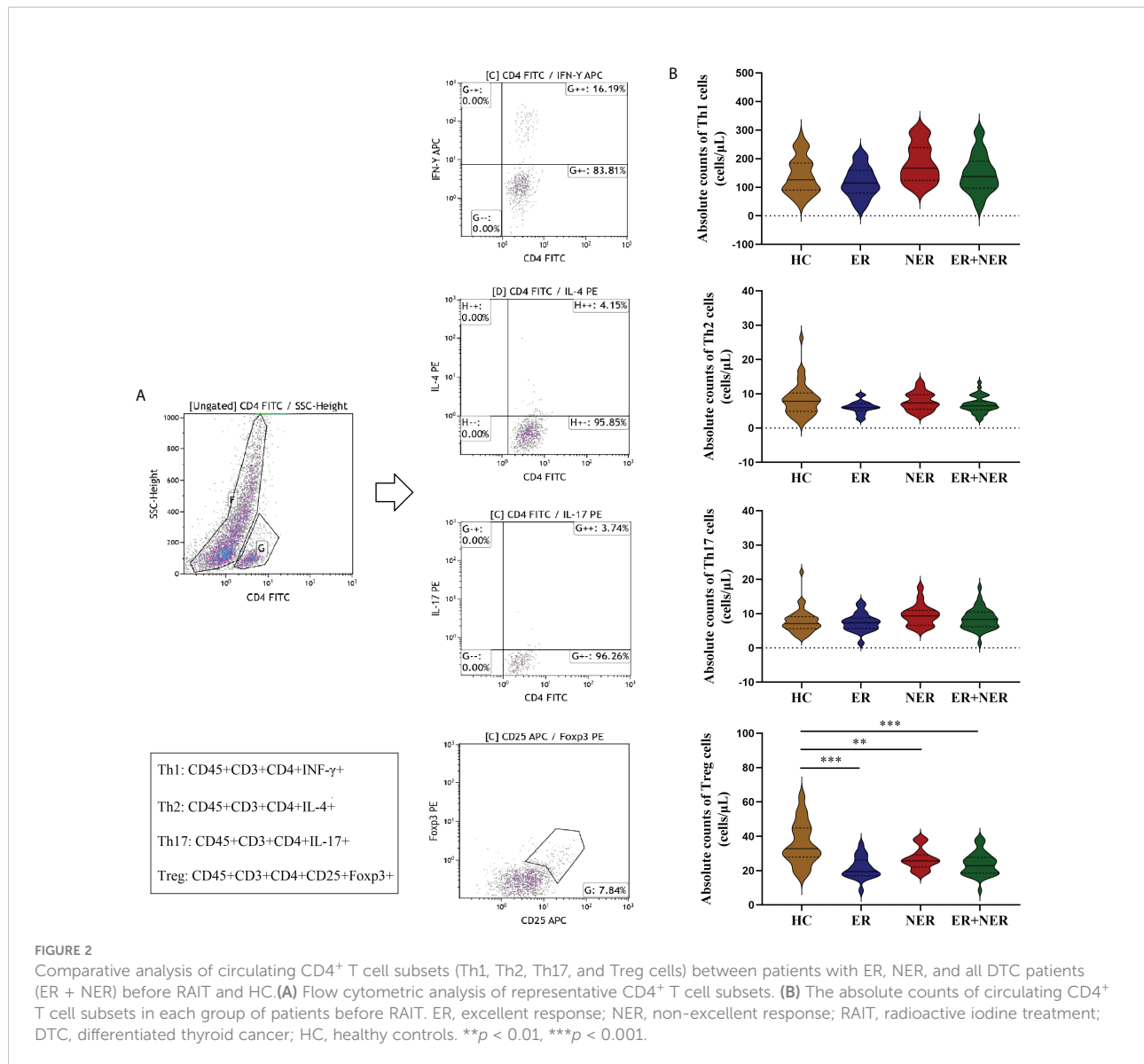
S2D). The proportion of Th17 cells was significantly higher in the NER group compared to the HC group ($p = 0.037$; Figure S2C), whereas that of Treg cells was significantly lower ($p = 0.013$; Figure S2D). Furthermore, relative to the HC group, the proportion of Th1 cells in the NER group was significantly higher ($p = 0.005$; Figure S2A). The proportional distribution of CD4⁺ T cell subsets in the ER and NER groups was compared, and it was discovered that there was no significant difference between the two groups ($p > 0.05$; Figure S2). We then examined how the absolute levels of circulating CD4⁺ T cell subsets differed between the different patient groups. In all DTC patients, we found a substantial decrease only in the absolute numbers of circulating Treg cells relative to those in the HC group ($p < 0.05$; Figure 2B). This may be related to tumor burden.

3.4 Dynamic observation of the distribution characteristics of CD4⁺ T cell subsets before and after RAIT in ER and NER groups

Compared with pre-RAIT, the proportions of circulating CD4⁺ T cell subsets in ER, NER, and all DTC patients were not significantly altered 30 d after RAIT ($p > 0.05$; Figures S3A–D). The proportion of circulating Th1 cells in ER, NER, and all DTC patients was significantly reduced 90 days after RAIT ($p < 0.05$, Figure S3A). The proportion of circulating Th2 cells in NER was

also significantly reduced 90 days after RAIT ($p < 0.05$; Figure S3B). We also compared and analyzed the proportional differences of circulating CD4⁺ T cell subsets between ER and NER patients. Before RAIT, NER patients had a much larger proportion of Th1 cells than ER patients ($p = 0.041$; Figure S3E). 30 days after RAIT, the proportion of Th1 cells in the two groups remained significantly different ($p = 0.019$; Figure S3E). During the 90 days after RAIT, numbers of circulating Th1 cells of all participants increased steadily over time before dramatically decreasing.

Subsequently, we used dynamic analysis to analyze changes in absolute circulating CD4⁺ T cell subset numbers before and after RAIT. The absolute numbers of all circulating CD4⁺ T cell subsets in both the ER and total DTC groups were significantly lower 30 days after RAIT than those pre-RAIT ($p < 0.05$; Figures 3A–D). In the NER group, the absolute numbers of circulating Th1, Th17, and Treg cells were significantly lower 30 days after RAIT than pre-RAIT ($p < 0.05$; Figures 3A, C, D). 90 days after RAIT, the absolute numbers of circulating Treg cells in the ER and total DTC groups were significantly greater than those 30 days after RAIT ($p < 0.05$; Figure 3D). 90 days after RAIT, the absolute counts of circulating Th1, Th2, and Treg cells in the NER group and total DTC groups were significantly lower than those before RAIT (both $p < 0.05$; Figures 3A–C). Surprisingly, we discovered that the absolute Th1 and Th17 cell counts in the ER group were lower 90 days after RAIT than before RAIT ($p < 0.05$; Figures 3A, C), but we observed a recovery trend for both cell types. This indicated that in the ER group, circulating CD4⁺ T cells had begun to recover to

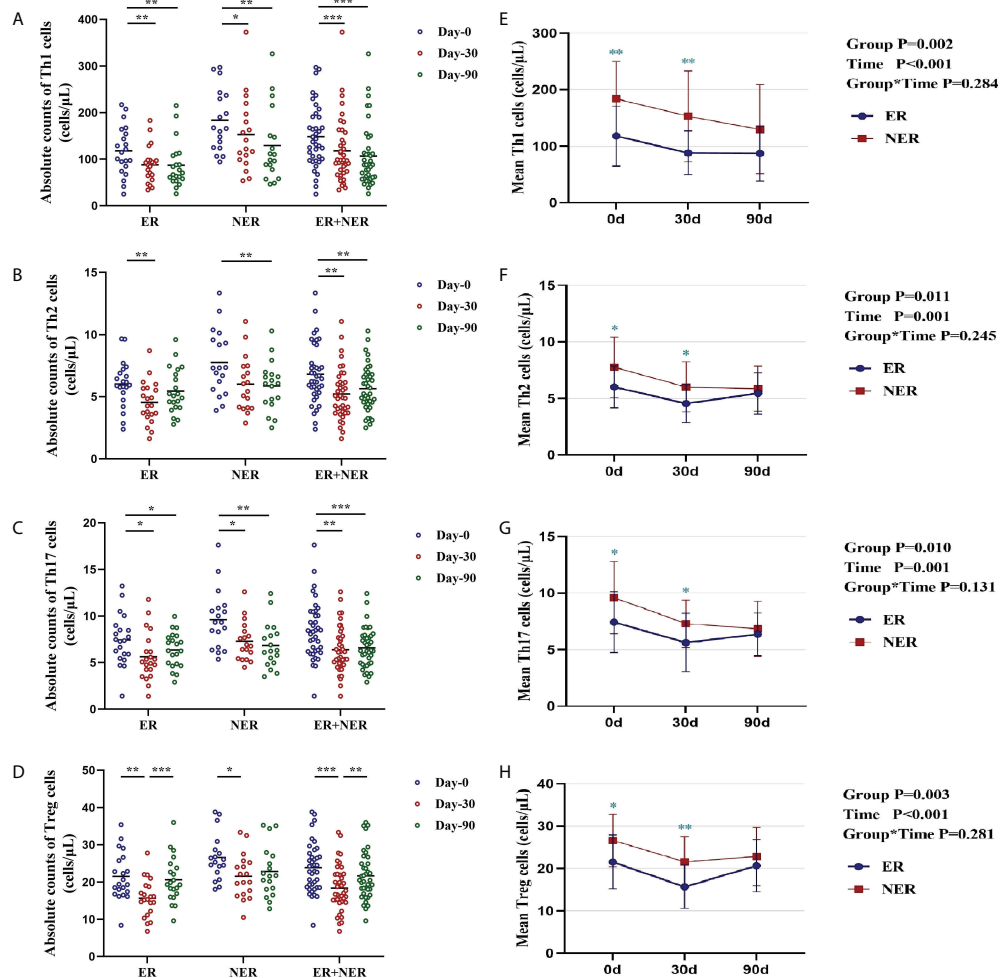


pre-RAIT levels. This might be a key factor underlying better prognoses in ER patients. In the NER group, however, the majority of circulating CD4⁺ T cell subsets had not recovered, which might be due to persistent tumors and/or metastases during RAIT (Figures 3A–D). Next, we investigated the differences in the absolute CD4⁺ T cell counts between the ER and NER groups. Results indicated that numbers of the CD4⁺ T cell subsets in the ER group were considerably lower than those in the NER group before RAIT ($p < 0.05$; Figures 3E–H). Additionally, 30 days after RAIT, those of the ER group remained significantly lower than those of the NER group ($p < 0.05$; Figures 3E–H). The absolute counts of circulating CD4⁺ T cell subsets did not differ significantly between the two groups 90 days after RAIT (both $p > 0.05$; Figure 3E–H). This shows that whereas the baseline numbers of circulating CD4⁺ T cell subsets

in the ER group were much lower than those of the NER group, each circulating CD4⁺ T cell subgroup had a greater potential to adapt and recover in the ER group. This might be because patients in the NER group had non- or low-functioning CD4⁺ T cell subsets, and/or CD4⁺ T cell subsets which were resistant to the body's immune regulatory systems. The molecular pathways underlying these results should be investigated further.

3.5 Comparative analysis of the predictive value of CD4⁺ T cell subsets and Tg levels

Univariate analysis revealed that the effectiveness responses (ER and NER) of RAIT were significantly associated with each



CD4⁺ T cell subgroup before and 30 days after RAIT in the entire population (*p* < 0.05; Table 2). We did not conduct univariate analyses on CD4⁺ T cell subsets 90 days after RAIT since there were no significant differences between the two groups. Our data also revealed that efficacy response was significantly associated with pre-RAIT Tg levels ($\beta = 0.107$, *p* = 0.024; Table 2), which is consistent with prior findings (14).

3.6 Construction and evaluation of multiple predictive models

Using a multivariate approach, we evaluated the absolute number of immune cells both before and after RAIT as independent risk variables for RAIT success. Then, several

prediction models were developed and assessed using Tg together with other risk variables.

3.6.1 Predictive model based on pre-RAIT CD4⁺ T cell subset absolute count and Tg levels

Even after controlling for the interaction between these two factors, Tg levels and absolute pre-RAIT Th1 cell counts were found to be independently associated with RAIT success using logistic multiple regression analysis (Figure 4A). The optimum cut-off values separating ER and NER patients, according to ROC curve measurement, were 5.82 g/L (AUC = 0.651, $\beta = 0.099$) and 100.73 cells/L (AUC = 0.775, $\beta = 0.073$). The ROC curve findings showed that the combined model including pre-RAIT CD4⁺ T cell subset

TABLE 2 Univariate analysis of prognosis in DTC patients.

Variable	β	Wald	OR	95% CI	P Value
Tg	0.107	5.116	1.113	1.014-1.221	0.024
Th1	0.019	7.264	1.019	1.005-1.033	0.007
Th2	0.362	4.522	1.436	1.029-2.004	0.033
Th17	0.275	4.204	1.316	1.012-1.712	0.04
Treg	0.135	4.92	1.145	1.016-1.291	0.027
Th1-30	0.022	6.735	1.022	1.005-1.039	0.009
Th2-30	0.409	4.309	1.505	1.023-2.214	0.038
Th17-30	0.311	3.999	1.365	1.006-1.851	0.046
Treg-30	0.204	6.979	1.226	1.054-1.427	0.008

Tg, serum thyroglobulin; Th1-30, Th2-30, Th17-30, and Treg-30 represent quantification of CD4⁺ T cell subsets on day 30 after RAIT.

count and Tg levels (Model 1; AUC = 0.905, β = 0.048) had a better predictive capacity than the two indicators alone (Figure 4B).

3.6.2 Predictive model based on CD4⁺ T cell subset absolute count 30 days after RAIT and Tg levels

According to logistic multiple regression analysis, the absolute numbers of Treg cells 30 days after RAIT and Tg levels were independently related to RAIT success. The ROC curve revealed that the appropriate cut-off for the absolute Treg cell count 30 days after RAIT was 19.04 cells/L (AUC = 0.791, β = 0.073). Furthermore, the ROC curve findings revealed that the combined model including CD4⁺ T cell subset absolute count 30 days after RAIT and Tg levels (Model 2; AUC = 0.918, β = 0.043) had a better predictive ability than each index individually (see Figure 4D).

3.6.3 Predictive model based on Th1 before RAIT, Treg 30 days after RAIT, and Tg levels

According to a logistic multiple regression analysis, Tg levels and absolute Treg numbers 30 days after RAIT were independently associated with RAIT success (Figure 4). Considering the predictive power of Model 1, we made a combined prediction model using pre-RAIT Th1 levels, Treg levels 30 days after RAIT, and Tg levels (Model 3), which had better predictive power (AUC = 0.952, β = 0.03) than the individual predictors and Models 1 and 2 (see Figure 4F).

4 Discussion

The distribution of circulating CD4⁺ T cells before and after RAIT in DTC patients with various treatment outcomes was dynamically analyzed for the first time, to the best of our knowledge, in this study. We also compared differences in general patient data between the two patient groups.

First, the impact of TSH-suppressing treatment in the two patient groups was investigated. There were no significant

differences in the levels of FT3, FT4, or TSH between the two groups. This confirmed that the study's findings were not impacted by differential TSH levels. In addition, a comparison of Tg levels between the ER and NER groups revealed that lower baseline Tg levels were directly connected to the efficiency of RAIT (14, 35, 36).

We examined the responses of circulating CD4⁺ T cell subsets to RAIT in 39 DTC patients with varying therapeutic outcomes. A prior study discovered that the proportion of circulating Treg cells in DTC patients was considerably higher one month after RAIT compared to before RAIT (37). This, however, contradicts our observations. This might be due to the small sample size of our study as well as the high variability. The absolute numbers of all circulating CD4⁺ T cell subsets were reduced by various degrees in both ER and NER groups 30 days following RAIT, indicating that RAIT may induce some immune suppression; however, this effect is transient and indiscriminate. We discovered that 90 days after RAIT, the circulating CD4⁺ T cell subsets in the ER group had mostly recovered to pre-RAIT levels compared with those in the NER group which did not show similar recovery trends. This shows that circulating CD4⁺ T cells in ER patients are more adaptable and resilient, likely because patients in the NER group had a higher proportion of non- or hypofunctioning CD4⁺ T cell subsets, as well as immune system resistance. Surprisingly, we discovered that 90 days after RAIT, the absolute amount of circulating Th1 cells in the NER group continued to fall. These findings are consistent with the role of Th1 cells in anti-tumor immunity (38–41). Additionally, this study discovered that changes in circulating CD4⁺ T cell subsets in the total DTC group were the result of a combination of trends in both ER and NER patients. This implies that the reported immunological phenomena do not adequately clarify the immune condition of DTC patients if treatment effectiveness is not differentiated. Exploring the distribution patterns of circulating CD4⁺ T cell subsets in DTC patients with various treatment outcomes is thus beneficial for targeted and precise immunotherapy.

Simultaneously, we looked at the differences in circulating CD4⁺ T cell subset distribution between the two groups of

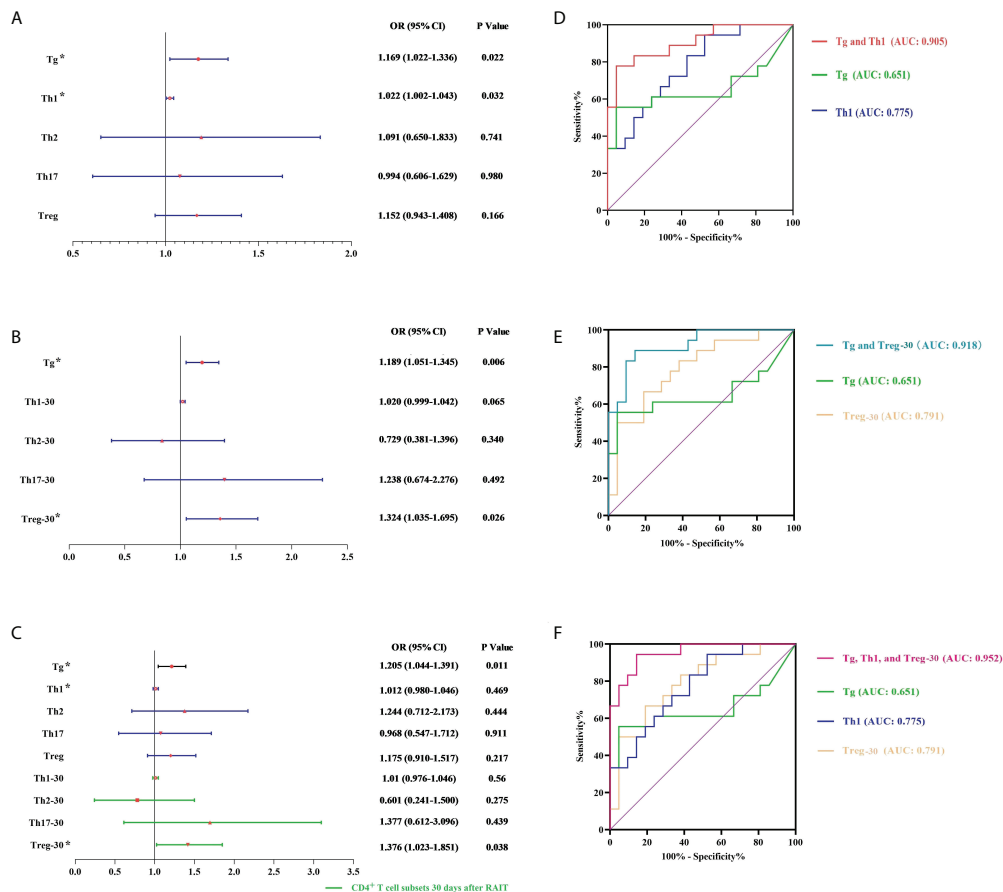


FIGURE 4

Multivariate logistic analysis and receiver operating characteristic (ROC) analysis. (A, D) Model 1 is based on pre-RAIT Tg and absolute counts of pre-RAIT CD4⁺ T cell subsets. (B, E) Model 2 is based on pre-RAIT Tg and absolute counts of CD4⁺ T cell subsets 30 d after RAIT. (C, F) Model 3 is based on pre-RAIT Tg and absolute counts of CD4⁺ T cell subsets before and 30 d after RAIT. RAIT, radioactive iodine treatment; Tg, serum thyroglobulin. * represents the parameters of the model establishment.

patients before and after RAIT. Before and 30 days after RAIT, our findings revealed substantial disparities in circulating immune cells, and patients with lower absolute numbers of CD4⁺ T cell subsets had a better prognosis, consistent with previous studies (42). Patient prognosis is much poorer when CD4⁺ T cells are heavily proliferated, which might imply the existence of highly inflammatory residuals or recurring foci of highly inflammatory malignancies which lack specific immunogenic targets.

The importance of investigating absolute immune cell numbers is emphasized in this work. As opposed to proportions, absolute quantitative research can more accurately and comprehensively depict the state of immunological alterations. This has been demonstrated in several immunological disorders (43–45).

Finally, we developed and tested three different predictive models. When compared with the individual metrics, the performance of the three predictive models were superior. Model 3 had a greater AUC than the first two, indicating

greater predictive power. However, we recommend the first two models due to their economic benefits. Model 1 is easier to manage, but Model 2 gives predictive data as well as the immunological state of patients following RAIT. Therefore, clinicians can select the best-suited model for their requirements.

This study has some limitations: 1) the sample size is small; 2) the present study does not rule out the effects of hypothyroidism before RAIT on the immunological function of DTC patients; 3) the study did not perform further study of tumor tissues and tumor microenvironments. Therefore, prospective, large-sample, multi-center investigations are needed in the future, and the impact of pre-RAIT hypothyroidism on DTC patient immunological function should not be ruled out.

In summary, our data demonstrate, for the first time, that circulating immune cell profiles in DTC patients are highly heterogeneous. These differences can be exploited to predict patient prognosis and develop more targeted treatment

regimens. Fewer absolute counts of circulating immune cells are linked to improved clinical outcomes as well as stronger adaptive and resilience capacities. Moreover, differences in the tumor microenvironment appear to play an integral role (46–50). As a result, more research is needed to differentiate individuals with high absolute T cell counts from those with low absolute T cell counts. In the future, single-cell sequencing and gene expression profiling approaches should be coupled to properly characterize the distinctions between the two. Furthermore, in DTC patients, RAIT may produce transient and indiscriminate radiation damage to circulating immune cells, which may have implications that we do not currently understand. Finally, we built various predictive models using the high predictive power of CD4⁺ T cell subsets to offer more accurate prognostic information.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The institutional review board of Shanxi Medical University's First Hospital in China approved this study. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors contributed to data acquisition. Z-YS and S-XZ provided the study design. C-HL, Z-HC, DF, YX, L-XW, and K-YL performed the sample collection and completed the patient communication. Z-FW contributed to the discussion. Z-YS analyzed the data and wrote the manuscript. X-FL, H-YL, and S-JL critically revised the manuscript. All authors were responsible for data analysis and manuscript preparation and approve its submission to this journal.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2022.966550/full#supplementary-material>

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