Journal of Pharmaceutical Analysis 14 (2024) 100998

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Original article

Circulating memory T cells and TCF1$^+$ T cells aid in diagnosis and monitor disease activity in vitiligo

Xinju Wang [1], Jianru Chen [1], Wei Wu [1], Jinrong Fan, Luling Huang, Weiwei Sun, Kaiqiao He, Shuli Li **, Chunying Li *

Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, 710032, China

ARTICLE INFO

Article history:
Received 19 December 2023
Received in revised form
28 March 2024
Accepted 5 May 2024
Available online 9 May 2024

Keywords:
Vitiligo
Biomarkers
TCF1
Immune memory
T lymphocytes

ABSTRACT

Vitiligo is an immune memory skin disease. T-cell factor 1 (TCF1) is essential for maintaining the memory T-cell pool. There is an urgent need to investigate the characteristics of peripheral memory T-cell profile and TCF1$^+$ T-cell frequencies in patients with vitiligo. In this study, 31 patients with active vitiligo (AV), 22 with stable vitiligo (SV), and 30 healthy controls (HCs) were included. We measured circulating memory and TCF1$^+$ T-cell frequencies using flow cytometry. The Spearman's rank test was used to evaluate the correlation between cell frequencies and disease characteristics. Receiver operating characteristic curves (ROC) were constructed to investigate the discriminative power of the cell subpopulations. Circulating CD4$^+$ and CD8$^+$ terminally differentiated effector memory T-cell (T_{EMRA}) frequencies were significantly higher in the AV group than in HCs ($P < 0.05$). TCF1$^+$ T-cell subpopulations were widespread increased in patients with vitiligo ($P < 0.05$). After adjusting for potential confounders, CD8$^+$ and CD4$^+$ central memory (T_{CM}) cells, and CD8$^+$ T_{EMRA} were correlated with disease activity ($P < 0.05$). The combined diagnostic value of the four (naïve, effector memory, T_{CM}, and T_{EMRA}) CD8$^+$TCF1$^+$ T-cell subsets was relatively high (area under the ROC curve (AUC) = 0.804, sensitivity = 71.70%, specificity = 83.34%), and the CD8$^+$ T-cell subsets combination performed well in discriminating disease activity (AUC = 0.849, sensitivity = 70.97%, specificity = 90.91%). We demonstrated an altered circulating memory T-cell profile and increased TCF1$^+$ T-cell percentage in patients with vitiligo. T-cell subpopulations had a strong value for vitiligo diagnosis and activity evaluation. This evidence presents a potential new pharmacological target for inhibiting autoimmunity that leads to vitiligo.

1. Introduction

Vitiligo is a chronic and relapsing autoimmune disease characterized by cutaneous depigmentation caused by melanocyte depletion [1]. Vitiligo affects approximately 0.5%–2% of the world population and markedly impairs patients' quality of life [2,3]. Approximately 40% of patients with vitiligo experience relapse within 1 year after discontinuing treatment, which is psychologically and financially burdensome for them [4]. Therefore, identifying clinical biomarkers for monitoring disease stages and developing novel therapeutic targets are urgently needed.

Vitiligo lesions often recur at local or distant locations. Indeed, vitiligo is considered an immune memory skin disease, which requires memory T-cells to continuously differentiate into effector T-cells, leading to melanocyte destruction [5,6]. The dysregulation of memory T-cells has been implicated in the maintenance and relapse of vitiligo [7]. Local memory T-cells in tissues and lymph nodes should be analyzed, however, obtaining these cells from patients is challenging. Thus, circulating memory T-cell subpopulations may be a promising substitute for assessing immune response dysfunction, considering the recycling of memory T-cells between peripheral blood and the tissue. Previous research has found decreased frequencies of circulating CD4$^+$ and CD8$^+$ helper and cytotoxic effector memory T-cell (T_{EM}) subsets in patients with vitiligo, suggesting a possible process by which these subsets migrate to the skin lesions and exert pathogenic effector functions [8]. The characteristics of circulating memory T-cell profiles in patients with vitiligo are unclear and require further investigation.

T-cell factor 1 (TCF1) is a major transcription factor that regulates the stem-like properties of naïve and memory T-cells [9]. TCF1$^+$ T-cells exhibit stem-like properties with the ability to self-renew and give rise to TCF1$^-$ terminal effector T-cells [10]. Current research has shown that TCF1$^+$CD8$^+$ T-cells are essential for

* Corresponding author.
** Corresponding author.
E-mail addresses: lishli@fmmu.edu.cn (S. Li), lichying@fmmu.edu.cn (C. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jpha.2024.100998

the long-term maintenance of the T-cell responses in tumors and viral infectious diseases [11,12]. A higher frequency of $TCF1^+CCR7^-CD8^+$ T-cells indicates a better response to immune checkpoint therapy [13]. In chronic viral infections, $CD8^+$ T cells preferentially differentiate into $TCF1^+$ T memory-like cells for fighting against long-term infection [14]. In type 1 diabetes, $TCF1^+CD8^+$ T cells in pancreatic draining lymph nodes were identified to acquire the potential of long-term self-renewal and differentiation into $TCF1^-$ T cells to destroy pancreatic beta cells [15]. Therefore, we hypothesize that the persistent immune abnormalities in patients with vitiligo might be related to the abnormalities in $TCF1^+$ T-cells.

Herein, we reported, for the first time, the distribution characteristics of circulating memory T-cell subsets and the proportion of $TCF1^+$ T-cell populations in patients with vitiligo. Furthermore, we analyzed their correlation with disease severity and activity and subsequently identified their potential utility in assessing vitiligo activity. This study provided novel insights into the pathogenesis of vitiligo.

2. Patients and methods

2.1. Study participants

This study enrolled 31 patients with active vitiligo (AV), 22 patients with stable vitiligo (SV), and 30 healthy controls (HCs). The sample size was calculated using the Sample Size Program (http://hedwig.mgh.harvard.edu/sample_size/size.html). We assumed a significance level of 0.05, power of 0.8, and minimal detectable difference of 0.4. A minimum sample size of 52 patients was deemed necessary for this study. All samples were collected with patients' consent, and a written informed consent form was singed. The experimental protocol was designed and executed according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Xijing Hospital, Fourth Military Medical University (Approval number: KY20172030-1). None of the patients had received systemic immunomodulatory therapies in the preceding 12 weeks. Individuals engaging in smoking or alcohol consumption, patients who were pregnant or lactating, as well as those with coexisting or previous infections, cancer, or endocrine disorders were excluded from the study. Three dermatologists clinically diagnosed vitiligo based on the consensus on the diagnosis and treatment of vitiligo in China [16]. Disease severity was measured using affected body surface area (BSA) involved [17]. Disease activity was assessed based on the vitiligo disease activity (VIDA) score, clinical features, the Koebner phenomenon, and wood lamp examination [16]. HCs were age-, sex-, and ethnicity-matched volunteers with no personal history of malignancy, autoimmune disease, or recurrent/chronic inflammatory disease. All the participants were of Asian race and Chinese Han ethnicities.

2.2. Sample processing

Peripheral blood samples were obtained from each patient and healthy donors (Xijing Hospital, Xi'an, China). The peripheral blood mononuclear cells (PBMCs) from patients and HCs were isolated using Ficoll gradient centrifugation (DAKEWE, Shenzhen, China). Blood samples from patients were diluted with an equal volume of phosphate-buffered saline (PBS) (Solarbio, Beijing, China), layered onto Ficoll medium, and subjected to centrifugation at 2,000 rpm for 20 min at 25 °C. A high-speed refrigerated centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) was used for the centrifugation. Subsequently, PBMCs were carefully collected, washed twice with PBS, and maintained in complete Roswell Park Memorial Institute (RPMI) 1640 medium (Corning, Shenzhen, China) supplemented with 10% fetal bovine serum (FBS; Procell, Wuhan, China) and 0.1% penicillin-streptomycin antibiotics (Invitrogen, Carlsbad, CA, USA).

2.3. Flow cytometry

PBMCs were stained with CD3-FITC, CD4-AF700, CD8-BV605, CD45RA-APC, CCR7-BV421, and TCF1-PE. All monoclonal antibodies were purchased from BioLegend (San Diego, CA, USA). For dead cell staining, the Zombie UV™ Fixable Viability Kit (BioLegend, San Diego, CA, USA) was used. The samples were stained according to standard flow cytometry protocols. The cells were surface-stained for 30 min at room temperature (RT) in the dark and washed with PBS (1,300 rpm, 10 min, 4 °C). Following this, the cells were fixed for 30 min using fixation/permeabilization solution (Thermo Fisher Scientific), and then permeabilized with a permeabilization buffer (Thermo Fisher Scientific) for 30 min at RT in the dark. Subsequently, the cells were stained with intracellular antigens (TCF1-PE) for 1 h at RT away from light, and washed with PBS (1,300 rpm, 10 min, 4 °C). Unstained and single antibody-stained PBMCs were used as negative controls. Flow cytometry samples were analyzed using the BD LSR-Fortessa cell analyzer (BD Biosciences, San Jose, CA, USA). Data were analyzed using FlowJo 10 software (FlowJo LLC, Ashland, OR, USA) (Fig. S1).

2.4. Statistical analyses

All data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS Version 26 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 9 software (GraphPad, La Jolla, CA, USA). Normally distributed continuous variables were compared using the unpaired *t*-test, and non-normally distributed continuous variables were assessed using the Mann–Whitney *U* test. Correlations were evaluated using Spearman's rank correlation test. Adjustments for possible confounding factors were performed using multivariate logistic regression. Receiver operating characteristic (ROC) curves were constructed, and the area under the ROC curves (AUC) was used as a measure of discriminative power. Differences at *P*-value <0.05 were statistically significant, and those at $P < 0.01$ were considered greatly statistically significant.

3. Results

3.1. Patient characteristics

We included a total of 53 patients with vitiligo, including 31 patients with AV and 22 patients with SV, along with 30 HCs. The groups were well-matched in terms of age and sex. Detailed characteristics of patients with vitiligo and HCs are presented in Table 1.

3.2. Circulating T-cell profile and frequencies of $TCF1^+$ T-cell subpopulations in vitiligo

Based on the expression of CCR7 and CD45RA, T-cells were phenotypically categorized into naïve precursors (T_N, $CD45RA^+CCR7^+$), central memory (T_{CM}, $CD45RA^-CCR7^+$), T_{EM} ($CD45RA^-CCR7^-$), and terminally differentiated effector memory T-cells (T_{EMRA}, $CD45RA^+CCR7^-$) [18]. Flow cytometry analysis revealed that the frequencies of circulating $CD4^+$ T_{EM}, $CD4^+$ T_{EMRA}, and $CD8^+$ T_{EMRA} cells were significantly higher in patients with AV than in HCs ($P = 0.005$, $P = 0.010$, and $P < 0.001$, respectively; Fig. 1). Furthermore, compared with the SV group, the proportions of $CD4^+$ and $CD8^+$ T_{CM} cells were significantly increased in the AV group ($P = 0.035$ and $P = 0.010$, respectively). These results suggested that

Table 1
Clinical characteristics of patients with vitiligo.

Characteristics	Active vitiligo ($n = 31$)	Stable vitiligo ($n = 22$)	Healthy control ($n = 30$)
Gender (Male/Female)	16/15	11/11	15/15
Age (mean (SD), year)	37.3 (9.7)	32.0 (8.8)	36.3 (8.9)
Age at onset (mean (SD), year)	26.0 (13.0)	21.2 (11.2)	–
Disease duration (mean (SD), year)	10.6 (9.4)	10.3 (5.9)	–
Affected BSA (mean (SD), %)	5.4 (6.8)	2.0 (2.2)	–
Associated autoimmune disease (n (%))	1 (3.2)	1 (4.5)	–
FH vitiligo (n (%))	4 (12.9)	4 (18.2)	–
FH other autoimmune (n (%))	2 (6.5)	1 (4.5)	–
VIDA score (n (%))			
+4: active, in the past 6 week	14 (45.2)	0 (0)	–
+3: active, in the past 3 month	9 (29.0)	0 (0)	–
+2: active, in the past 6 month	4 (12.9)	0 (0)	–
+1: active, in the past 1 year	4 (12.9)	0 (0)	–
0: stable for at least 1 year	0 (0)	16 (72.7)	–
−1: stable, for at least 1 year and spontaneous repigmenting	0 (0)	6 (27.3)	–

BSA: body surface area; FH: family history; VIDA: vitiligo disease activity; SD: standard deviation.

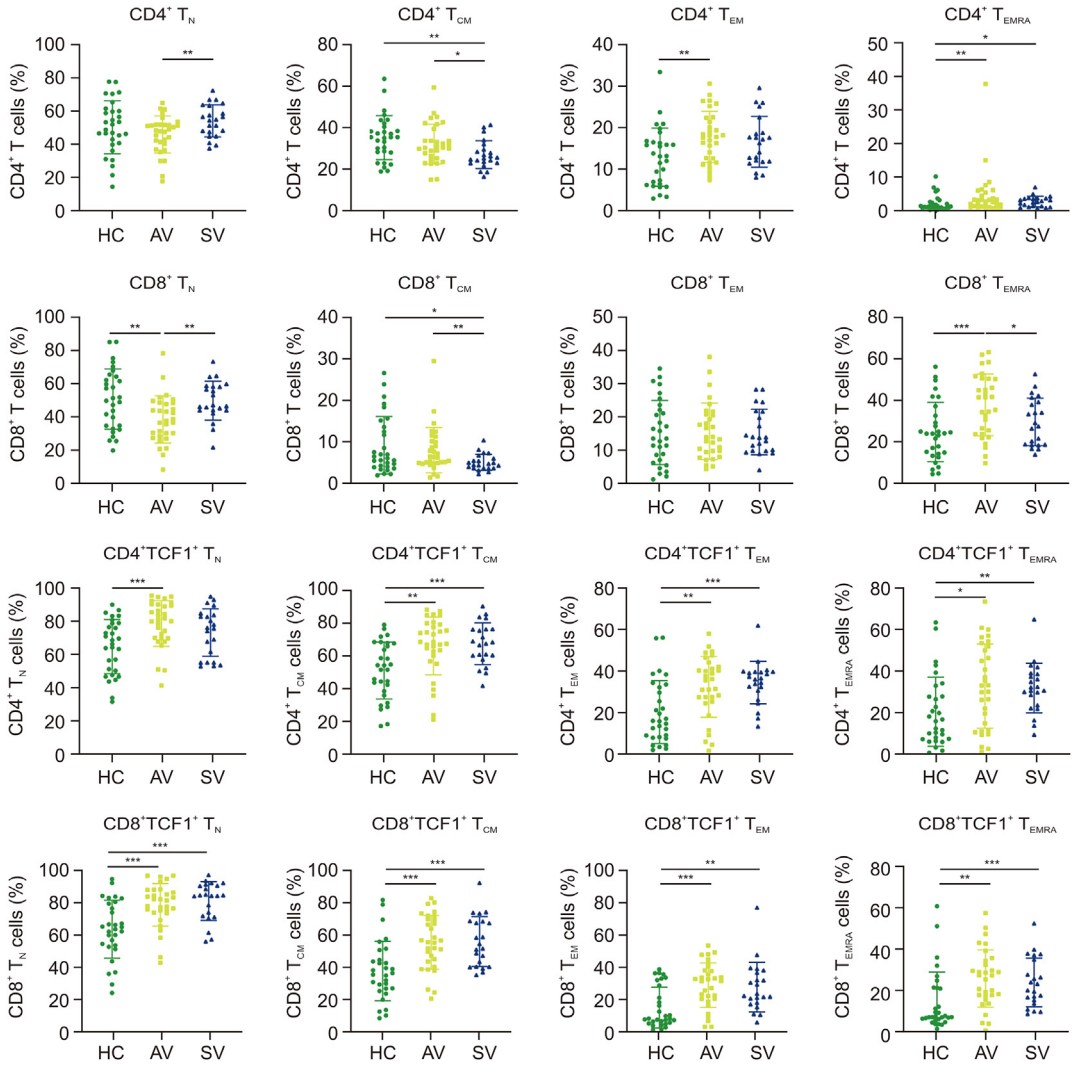

Fig. 1. Peripheral T-cell subpopulations and T-cell factor 1 (TCF1) expression in active vitiligo (AV) and stable vitiligo (SV). Peripheral blood mononuclear cells from healthy controls (HC; $n = 30$), AV ($n = 31$), and SV ($n = 22$) were stained for multiparameter flow cytometry. The frequencies of naïve precursors (T_N), central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated effector memory (T_{EMRA}) T-cells in $CD4^+$ and $CD8^+$ T-cells were analyzed. The frequencies of $TCF1^+$ T-cells in different $CD4^+$ and $CD8^+$ T-cell subpopulations were identified. Data were displayed as mean ± standard deviation (SD). P values were calculated using Mann–Whitney U test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

these subsets were involved in the progression of vitiligo. We also investigated TCF1$^+$ T-cell frequencies in naïve and memory T-cells, which revealed remarkably elevated proportions of TCF1$^+$ T-cells in most subsets of both CD4$^+$ and CD8$^+$ T-cells in the AV and SV groups compared with those in HCs ($P < 0.05$). This indicated that the proportions of TCF1$^+$ circulating CD4$^+$ and CD8$^+$ T-cells were higher in patients with vitiligo than in HCs.

3.3. Relationship between T-cell subpopulations and vitiligo characteristics

We further explored the correlation between memory T-cell subsets and the clinical characteristics of patients with vitiligo. Both CD8$^+$ T_{CM} ($r = 0.326$, $P = 0.017$) and CD4$^+$ T_{CM} ($r = 0.323$, $P = 0.018$) cell frequencies were positively correlated with BSA in patients with vitiligo. Meanwhile, the proportions of CD8$^+$ T_{CM} ($r = 0.362$, $P = 0.008$), CD8$^+$ T_{EMRA} ($r = 0.336$, $P = 0.014$), and CD4$^+$ T_{CM} ($r = 0.321$, $P = 0.019$) cells were positively correlated with the VIDA scores in patients with vitiligo, with CD8$^+$ T_N ($r = -0.426$, $P = 0.001$) and CD4$^+$ T_N ($r = -0.271$, $P = 0.049$) cells negatively correlated with the VIDA scores (Table 2, and Fig. S2).

Furthermore, we used multivariate logistic regression models to eliminate the influence of the affected BSA. Disease activity was divided into three grades based on the VIDA score: very active (VIDA score = 4), mild-to-moderate active (VIDA score = 3/2/1), and stable (VIDA score = 0/−1). The association between CD8$^+$ T_{EMRA} cell frequency and disease activity was significantly influenced by the affected BSA in patients with vitiligo. Our results showed that CD8$^+$ T_{CM}, CD8$^+$ T_{EMRA}, and CD4$^+$ T_{CM} cell frequencies were significantly associated with disease activity, after adjusting for BSA ($P = 0.020$, $P = 0.008$, and $P = 0.042$, respectively; Table 3). Notably, the levels of TCF1$^+$ T-cell subsets were not significantly associated with disease activity or severity. These correlations suggested that memory T-cell subsets served as potential biomarkers of disease activity. Combined with the previous observation of elevated circulating TCF1$^+$ T-cell frequencies in vitiligo, our correlation analysis indicated that patients with vitiligo had elevated levels of circulating TCF1$^+$ T-cell subsets, regardless of disease activity and severity.

3.4. Capabilities of T-cell subpopulations in vitiligo diagnosis and assessment

Next, we used ROC analysis to analyze whether memory T-cell subpopulation levels could discriminate patients with vitiligo from HCs and to assess disease activity. All eight TCF1$^+$ T-cell subpopulations showed potential diagnostic value, with AUC > 0.7 (Table 4). The top four cell subsets were CD8$^+$TCF1$^+$ T_{CM} (AUC = 0.779), CD8$^+$TCF1$^+$ T_N (AUC = 0.774), CD8$^+$TCF1$^+$ T_{EM} (AUC = 0.761), and CD8$^+$TCF1$^+$ T_{EMRA} (AUC = 0.758). The combination of the four subsets had a good predictive value (AUC = 0.804, sensitivity = 71.70%, specificity = 83.34%), suggesting that the combination of CD8$^+$TCF1$^+$ T cell subsets could improve the diagnostic accuracy. To assess the disease stage, we compared the top four cell subpopulations with the largest AUC values, including CD8$^+$ T_N (AUC = 0.749), CD8$^+$ T_{CM} (AUC = 0.710), CD4$^+$ T_N (AUC = 0.691), and CD4$^+$ T_{CM} (AUC = 0.680) (Table 5). The combination of CD8$^+$ naïve and all three memory T-cell subsets performed well in discriminating disease activity (AUC = 0.849, sensitivity = 70.97%, specificity = 90.91%). Notably, the combination of only two subsets (CD8$^+$ T_N and CD8$^+$ T_{CM}) achieved a relatively high predictive value (AUC = 0.818, sensitivity = 74.19%, specificity = 81.82%). These analyses revealed that TCF1$^+$ T-cell subsets had good discriminatory ability in vitiligo diagnosis and that circulating CD8$^+$ memory T-cell subsets can serve as promising biomarkers for disease activity.

Table 2
Correlation analysis of T-cell subpopulations and vitiligo characteristics.

Parameters	Disease duration		BSA		VIDA score	
	r	*P*	*r*	*P*	*r*	*P*
CD8$^+$ T_N	−0.097	0.491	−0.081	0.566	−0.426	0.001*
CD8$^+$ T_{CM}	−0.086	0.541	0.326	0.017*	0.362	0.008*
CD8$^+$ T_{EM}	0.051	0.715	−0.031	0.824	−0.051	0.717
CD8$^+$ T_{EMRA}	0.059	0.676	−0.018	0.897	0.336	0.014*
CD4$^+$ T_N	−0.003	0.984	−0.259	0.062	−0.271	0.049*
CD4$^+$ T_{CM}	−0.075	0.595	0.323	0.018*	0.321	0.019*
CD4$^+$ T_{EM}	−0.006	0.964	−0.015	0.915	−0.002	0.988
CD4$^+$ T_{EMRA}	−0.031	0.827	0.006	0.964	0.034	0.809

BSA: body surface area; VIDA,: vitiligo disease activity; T_N: naïve T cell; T_{CM}: central memory T cell; T_{EM}: effector memory T cell; T_{EMRA}: terminally differentiated effector memory T cell.
*$P < 0.05$.

Table 3
Multivariate models investigating the association of CD8$^+$ T_{CM}, CD8$^+$ T_{EMRA}, CD4$^+$ T_{CM}, and disease activity.

Independent variable	Coefficient	Wald test	OR (95% CI)	*P*
Model 1				
BSA	0.063	1.705	1.065 (−0.032, 0.159)	0.192
CD8$^+$ T_{CM}	0.190	5.431	1.209 (0.030, 0.350)	0.020*
Model 2				
BSA	0.115	4.826	1.122 (0.012, 0.218)	0.028*
CD8$^+$ T_{EMRA}	0.055	7.026	1.057 (0.014, 0.096)	0.008*
Model 3				
BSA	0.074	2.263	1.077 (−0.023, 0.171)	0.132
CD4$^+$ T_{CM}	0.064	4.129	1.067 (0.002, 0.127)	0.042*

BSA: body surface area; OR: odds ratio; CI: confidence interval; T_{CM}: central memory T cell; T_{EMRA}: terminally differentiated effector memory T cell.
*$P < 0.05$.

4. Discussion

We demonstrated that the proportion of circulating TCF1$^+$ T-cells was higher in patients with vitiligo than in HCs. After adjusting for latent factors, CD8$^+$ T_{CM}, CD8$^+$ T_{EMRA}, and CD4$^+$ T_{CM} cell proportions were significantly and positively correlated with disease activity. Importantly, we discovered that CD8$^+$ T-cell subsets added predictive value to vitiligo diagnosis and activity, indicating the clinical significance of circulating memory and TCF1$^+$ T-cell subsets in vitiligo assessment.

Vitiligo is a chronic autoimmune disease caused by the synergistic contributions of CD4$^+$ and cytotoxic CD8$^+$ T-cells [19]. The recurrence of vitiligo at the same site after treatment discontinuation strongly indicates the involvement of immune memory. The presence of resident memory T (T_{RM}) cells, a long-lived and non-migratory subpopulation of memory cells, is considered a crucial factor in disease recurrence [6]. T_{RM} induces apoptosis in melanocytes through direct secretion of interferon-γ (IFN-γ), perforin, and granzyme B, while also expressing CXCL9 and CXCL10 to recruit circulating T-cells and elicit synergistic damage [20]. A recent study demonstrated a significant increase in both the number and proportion of CD4$^+$ and CD8$^+$ T_{RM} within the perilesional, lesional, and non-lesional areas in patients with generalized vitiligo [21]. This study demonstrates that the aberrant memory T-cells in patients with vitiligo exhibit functional and quantitative systemic dysregulation. Notably, a previous study showed that significant repigmentation could be achieved by selectively depleting circulating memory T-cells in mouse models of vitiligo. Additionally, inhibiting circulating T-cell migration to the skin using the chemokine receptor

Table 4
Diagnostic values of T-cell subpopulations in patients with vitiligo.

Parameters	AUC	Sensitivity (%)	Specificity (%)	95% CI	*P*
$CD8^+TCF1^+$ T_N	0.774	84.91	66.67	0.667–0.880	< 0.001
$CD8^+TCF1^+$ T_{CM}	0.779	71.70	76.67	0.668–0.890	< 0.001
$CD8^+TCF1^+$ T_{EM}	0.761	90.57	60.00	0.648–0.874	< 0.001
$CD8^+TCF1^+$ T_{EMRA}	0.758	84.91	70.00	0.638–0.878	< 0.001
$CD4^+TCF1^+$ T_N	0.708	50.94	80.00	0.595–0.821	0.002
$CD4^+TCF1^+$ T_{CM}	0.748	77.36	66.67	0.641–0.856	< 0.001
$CD4^+TCF1^+$ T_{EM}	0.757	83.02	66.67	0.642–0.871	< 0.001
$CD4^+TCF1^+$ T_{EMRA}	0.708	64.15	76.67	0.588–0.828	0.002
Combinations					
$CD8^+TCF1^+$ T_N + $CD8^+TCF1^+$ T_{CM} + $CD8^+TCF1^+$ T_{EM} + $CD8^+TCF1^+$ T_{EMRA}	0.804	71.70	83.34	0.702–0.906	< 0.001
$CD4^+TCF1^+$ T_N + $CD4^+TCF1^+$ T_{CM} + $CD4^+TCF1^+$ T_{EM} + $CD4^+TCF1^+$ T_{EMRA}	0.767	81.13	73.34	0.654–0.880	< 0.001

AUC: area under the receiver operating characteristic curve; CI: confidence interval; TN: naïve T cell; T_{CM}: central memory T cell; T_{EM}: effector memory T cell; T_{EMRA}: terminally differentiated effector memory T cell.

Table 5
The value of T-cell subpopulations for assessing disease activity of vitiligo.

Parameters	AUC	Sensitivity (%)	Specificity (%)	95% CI	*P*
CD8					
$CD8^+$ T_N	0.749	65.52	81.82	0.616–0.883	0.002
$CD8^+$ T_{CM}	0.710	83.87	54.55	0.569–0.850	0.010
Combinations					
$CD8^+$ T_N + $CD8^+$ T_{CM}	0.818	74.19	81.82	0.704–0.932	< 0.001
$CD8^+$ T_N + $CD8^+$ T_{CM} + $CD8^+$ T_{EM} + $CD8^+$ T_{EMRA}	0.849	70.97	90.91	0.744–0.954	< 0.001
$CD8^+TCF1^+$ T_N + $CD8^+TCF1^+$ T_{CM} + $CD8^+TCF1^+$ T_{EM} + $CD8^+TCF1^+$ T_{EMRA}	0.575	93.55	45.45	0.401–0.749	0.357
CD4					
$CD4^+$ T_N	0.691	83.87	54.55	0.545–0.836	0.019
$CD4^+$ T_{CM}	0.680	77.52	50.00	0.534–0.827	0.026
Combinations					
$CD4^+$ T_N + $CD4^+$ T_{CM}	0.714	61.29	72.73	0.575–0.853	0.008
$CD4^+$ T_N + $CD4^+$ T_{CM} + $CD4^+$ T_{EM} + $CD4^+$ T_{EMRA}	0.723	70.97	72.73	0.585–0.861	0.006
$CD4^+TCF1^+$ T_N + $CD4^+TCF1^+$ T_{CM} + $CD4^+TCF1^+$ T_{EM} + $CD4^+TCF1^+$ T_{EMRA}	0.748	77.42	68.18	0.605–0.891	0.002

AUC: area under the receiver operating characteristic curve; CI: confidence interval; TN: naïve T cell; TCM: central memory T cell; T_{EM}: effector memory T cell; T_{EMRA}: terminally differentiated effector memory T cell.

$S1P_1$ inhibitor, FTY720, has also been effective [5]. Although these treatments did not affect T_{RM}, they suggested that circulating memory T cells could potentially serve as a therapeutic strategy for vitiligo.

Previous studies have found differences in peripheral circulating T-cell subsets in patients with vitiligo compared with HCs, while few studies have investigated circulating memory T-cell profile [8,22]. We found that peripheral T-cell populations were skewed toward memory and terminal effectors in AV. T_{EMRA} cells exert competent effector functions in the immune response [23]. Therefore, the increase in circulating T_{EMRA} frequencies in AV might suggest an active cellular immune response. Moreover, correlation and multivariate logistic regression analyses showed that $CD8^+$ T_{EMRA}, $CD8^+$ T_{CM}, and $CD4^+$ T_{CM} were positively correlated with disease activity. T_{CM} cells proliferate rapidly and differentiate into effector T-cells upon antigen re-encounter, which is associated with active immune responses [24]. The positive correlation between T_{CM} frequencies and disease activity again demonstrated an increased immune response in patients with AV. Moreover, the observed low-frequency circulating T_{CM} cells in the SV group may have resulted from clonal shrinkage [25]. Overall, these findings suggest that circulating memory T-cell levels correlate disease activity, which may help in the early assessment of vitiligo activity.

Clinical guidelines recommend different treatments for AV and SV [26]. Accurate identification of the active stage of vitiligo is crucial to prevent its progression. However, self-reported depigmentation remains the primary assessment in clinical practice [27,28]. Moreover, alarmins (S100B, S100A9, and HMGB1), oxidative stress markers (total antioxidant capacity (TAC), malondialdehyde (MDA), and 8-Hydroxy-2'-deoxyguanosine (8-OHdG)), soluble CD molecules (sCD25, and sCD27), and chemokines (CXCL9, and CXCL10) can be used as biomarkers for vitiligo [29–33]. However, these biomarkers are secreted by various cell types, indicating that several factors may affect their peripheral levels. Moreover, a certain amount of time is required for their release and metabolism to occur, which results in a time lag when detecting their levels. In contrast, circulating memory T-cell subsets are relatively stable and less affected by external factors. In addition, they can directly reflect the immune system status and indicate the anti-melanocyte T-cell response in patients with vitiligo, providing unique advantages as biomarkers of vitiligo. A study on coronavirus disease 2019 has revealed a significantly higher proportion of $CD8^+$ T_{EM} cells in mildly ill patients than in severely ill patients, indicating successful expansion and differentiation of memory T-cells [34]. Therefore, the identification of T-cell subpopulations in peripheral blood represents an objective and convenient approach that can be considered a potential biomarker of disease outcome and control. Our research revealed that the combination of $CD8^+$ memory T-cell subsets could monitor disease activity with high sensitivity and specificity, which would greatly improve the current state of subjective assessment of vitiligo activity. Further refinement and external validation are required to clarify their practicality in patients with vitiligo.

In our study, we demonstrated significantly elevated $TCF1^+$ T-cell levels in circulating naïve and memory T-cells in both the AV and SV groups, indicating that memory T-cell subsets maintain a high differentiation potential and are poised to generate effector T-cells to execute melanocytes. Importantly, TCF1 is highly expressed in naïve T-cells and is gradually downregulated as naïve T-cells differentiate into memory T-cells and effector T-cells along their differentiation trajectories [35]. Our results also confirmed this finding.

Notably the differentiation activity of $TCF1^+$ T-cells may differ depending on the disease state. Memory T-cell differentiation occurs in a stepwise manner via a linear or partly progressive model [36,37]. $TCF1^+$ T-cells can divide asymmetrically to give rise to the $TCF1^-$ T-cell population while maintaining their population stability during differentiation [35]. As we have mentioned, T-cell populations were skewed toward a terminal effector memory profile in AV, while T_{EMRA} cell proportions in the SV group were comparable to those in HCs. We hypothesized that the increased frequency of T_{EMRA} subsets in the AV group might rely the progressive activities of $TCF1^+$ T-cell proliferation and differentiation. Conversely, during the stable phase of vitiligo, $TCF1^+$ progenitors may become more inclined to contribute to the maintenance of the $TCF1^+$ memory cell pool through self-renewal. Therefore, blocking $TCF1^+$ T-cell differentiation may be a promising approach to control vitiligo progression.

In the pathogenesis of vitiligo, melanocyte-specific cytotoxic T-cells are pivotal in mediating the destruction of melanocytes [38]. However, only a limited repertoire of melanocyte antigens, such as gp100 and melan-A, have been implicated as targets for T-cells, with the precise antigenic determinants remaining unspecified due to technical constraints [39]. Therefore, the utilization of techniques such as tetramers for the monitoring or capturing melanocyte-specific $CD8^+$ T-cells in patients with vitiligo remain unavailable. In this study, we examined the overall level of memory T-cell population, which was effectively reflected disease progression. Additionally, when selecting potential indicators for vitiligo diagnosis and disease assessment, we considered their clinical applicability as an evaluation criterion. Further follow-up studies are needed to help develop an appropriate test for melanocyte-specific T-cell subsets.

There are some limitations in our study. First, *in vivo* or *in vitro* experiments are needed to verify the regulatory mechanism of TCF1 in vitiligo. Additionally, we were unable to evaluate the sustainability of $TCF1^+$ T-cell frequencies over time to determine whether the change was a long-term anomaly, due to the lack of clinical follow-up data.

5. Conclusion

Our study demonstrated significantly elevated frequencies of circulating T_{EMRA} cells in patients with AV. Both patients with AV and SV exhibited elevated frequencies of circulating $TCF1^+$ T-cell subpopulations. In addition, memory T-cell subsets exhibited promising performance in assessing disease progression, thereby offering practical clinical value for disease monitoring. A moderate reduction in the proportion and differentiation activity of $TCF1^+$ T-cells may be a potential therapeutic strategy for prolonged disease control in patients with vitiligo.

Data availability statement

The data supporting the findings of this study are available in the article and its supplementary materials. And any additional data are available from the corresponding author, Shuli Li, upon reasonable request.

CRediT authorship contribution statement

Xinju Wang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Jianru Chen:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Wei Wu:** Data curation, Methodology, Supervision, Validation, Writing – review & editing. **Jinrong Fan:** Formal analysis, Investigation, Writing – original draft. **Luling Huang:** Formal analysis, Investigation. **Weiwei Sun:** Formal analysis, Investigation. **Kaiqiao He:** Formal analysis, Investigation. **Shuli Li:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. **Chunying Li:** Funding acquisition, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We express our gratitude to the patients and healthy volunteers who agreed to contribute their blood samples for our study. This research was funded by the National Natural Science Foundation of China (82222059, 82173416, and 81930087).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2024.100998.

References

[1] K. Ezzedine, V. Eleftheriadou, M. Whitton, et al., Vitiligo, Lancet 386 (2015) 74–84.
[2] M.W. Linthorst Homan, P.I. Spuls, J. de Korte, et al., The burden of vitiligo: Patient characteristics associated with quality of life, J. Am. Acad. Dermatol. 61 (2009) 411–420.
[3] K. Boniface, J. Seneschal, M. Picardo, et al., Vitiligo: Focus on clinical aspects, immunopathogenesis, and therapy, Clin. Rev. Allergy. Immunol. 54 (2018) 52–67.
[4] M. Cavalié, K. Ezzedine, E. Fontas, et al., Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: A randomized, double blind, placebo-controlled study, J. Invest. Dermatol. 135 (2015) 970–974.
[5] J.M. Richmond, J.P. Strassner, M. Rashighi, et al., Resident memory and recirculating memory T cells cooperate to maintain disease in a mouse model of vitiligo, J. Invest. Dermatol. 139 (2019) 769–778.
[6] R.L. Riding, J.E. Harris, The Role of Memory $CD8^+$ T Cells in Vitiligo, J. Immunol. 203 (2019) 11–19.
[7] K. Boniface, C. Jacquemin, A.S. Darrigade, et al., Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3, J. Invest. Dermatol. 138 (2018) 355–364.
[8] C. Martins, A.S. Darrigade, C. Jacquemin, et al., Phenotype and function of circulating memory T cells in human vitiligo, Br. J. Dermatol. 183 (2020) 899–908.
[9] X. Zhao, Q. Shan, H.H. Xue, TCF1 in T cell immunity: A broadened frontier, Nat. Rev. Immunol. 22 (2022) 147–157.
[10] I. Siddiqui, K. Schaeuble, V. Chennupati, et al., Intratumoral $Tcf1^+PD\text{-}1^+CD8^+$ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy, Immunity. 50 (2019) 195–211.e10.
[11] B.C. Miller, D.R. Sen, R. Al Abosy, et al., Subsets of exhausted $CD8^+$ T cells differentially mediate tumor control and respond to checkpoint blockade, Nat. Immunol. 20 (2019) 326–336.
[12] R.L. Rutishauser, C.D.T. Deguit, J. Hiatt, et al., TCF-1 regulates HIV-specific $CD8^+$ T cell expansion capacity, JCI Insight 6 (2021), e136648.
[13] R. Maniar, P.H. Wang, R.S. Washburn, et al., Self-renewing $CD8^+$ T-cell abundance in blood associates with response to immunotherapy, Cancer Immunol. Res. 11 (2023) 164–170.
[14] L.M. Snell, B.L. MacLeod, J.C. Law, et al., $CD8^+$ T cell priming in established chronic viral infection preferentially directs differentiation of memory-like cells for sustained immunity, Immunity 49 (2018) 678–694.e5.
[15] S.V. Gearty, F. Dündar, P. Zumbo, et al., An autoimmune stem-like CD8 T cell population drives type 1 diabetes, Nature 602 (2022) 156–161.
[16] T. Lei, A. Xu, T. Gao, et al., Consensus on the diagnosis and treatment of vitiligo in china (2021 Revision), Int. J. Dermatol. Venereol. 4 (2021) 10–15.
[17] G.R. Kanthraj, C.R. Srinivas, S.D. Shenoi, et al., Comparison of computer-aided design and rule of nines methods in the evaluation of the extent of body involvement in cutaneous lesions, Arch. Dermatol. 133 (1997) 922–923.
[18] F. Sallusto, D. Lenig, R. Förster, et al., Two subsets of memory T lymphocytes with distinct homing potentials and effector functions, Nature 401 (1999) 708–712.
[19] J.M. Richmond, J.P. Strassner, K.I. Essien, et al., T-cell positioning by chemokines in autoimmune skin diseases, Immunol. Rev. 289 (2019) 186–204.
[20] F. Shah, S. Patel, R. Begum, et al., Emerging role of tissue resident memory T cells in vitiligo: From pathogenesis to therapeutics, Autoimmun. Rev. 20 (2021), 102868.

[21] F. Shah, P.S. Giri, A.H. Bharti, et al., Compromised melanocyte survival due to decreased suppression of $CD4^+$ & $CD8^+$ resident memory T cells by impaired T_{RM}-regulatory T cells in generalized vitiligo patients, Exp. Dermatol. 33 (2024), e14982.
[22] T. Czarnowicki, H. He, A. Leonard, et al., Blood endotyping distinguishes the profile of vitiligo from that of other inflammatory and autoimmune skin diseases, J. Allergy Clin. Immunol. 143 (2019) 2095–2107.
[23] C.M. Mousset, W. Hobo, R. Woestenenk, et al., Comprehensive phenotyping of T cells using flow cytometry, Cytometry A. 95 (2019) 647–654.
[24] E.J. Wherry, V. Teichgräber, T.C. Becker, et al., Lineage relationship and protective immunity of memory CD8 T cell subsets, Nat. Immunol. 4 (2003) 225–234.
[25] J.J. Goronzy, C.M. Weyand, T cell development and receptor diversity during aging, Curr. Opin. Immunol. 17 (2005) 468–475.
[26] V. Eleftheriadou, R. Atkar, J. Batchelor, et al., British Association of Dermatologists guidelines for the management of people with vitiligo 2021, Br. J. Dermatol. 186 (2022) 18–29.
[27] N. van Geel, L. Depaepe, V. Vandaele, et al., Assessing the dynamic changes in vitiligo: reliability and validity of the Vitiligo Disease Activity Score (VDAS) and Vitiligo Disease Improvement Score (VDIS), J. Eur. Acad. Dermatol. Venereol. 36 (2022) 1334–1341.
[28] N. van Geel, T. Passeron, A. Wolkerstorfer, et al., Reliability and validity of the Vitiligo Signs of Activity Score (VSAS), Br. J. Dermatol. 183 (2020) 883–890.
[29] K. He, W. Wu, X. Wang, et al., Circulatory levels of alarmins in patients with non-segmental vitiligo: Potential biomarkers for disease diagnosis and activity/severity assessment, Front. Immunol. 13 (2022), 1069196.
[30] S. Li, W. Dai, S. Wang, et al., Clinical significance of serum oxidative stress markers to assess disease activity and severity in patients with non-segmental vitiligo, Front. Cell. Dev. Biol. 9 (2021), 739413.
[31] R. Speeckaert, J. Lambert, N. van Geel, Clinical significance of serum soluble CD molecules to assess disease activity in vitiligo, JAMA Dermatol. 152 (2016) 1194–1200.
[32] J.P. Strassner, M. Rashighi, M. Ahmed Refat, et al., Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity, J. Am. Acad. Dermatol. 76 (2017) 847–855.e5.
[33] X.X. Wang, Q.Q. Wang, J.Q. Wu, et al., Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo, Br. J. Dermatol. 174 (2016) 1318–1326.
[34] I. Odak, J. Barros-Martins, B. Bošnjak, et al., Reappearance of effector T cells is associated with recovery from COVID-19, EBioMedicine 57 (2020), 102885.
[35] R. Kratchmarov, A.M. Magun, S.L. Reiner, TCF1 expression marks self-renewing human $CD8^+$ T cells, Blood Adv. 2 (2018) 1685–1690.
[36] M.C. van Aalderen, M. van den Biggelaar, E.B.M. Remmerswaal, et al., Label-free analysis of $CD8^+$ T cell subset proteomes supports a progressive differentiation model of human-virus-specific T cells, Cell Rep. 19 (2017) 1068–1079.
[37] P. Durek, K. Nordström, G. Gasparoni, et al., Epigenomic profiling of human $CD4^+$ T cells supports a linear differentiation model and highlights molecular regulators of memory development, Immunity 45 (2016) 1148–1161.
[38] J.G. van den Boorn, D. Konijnenberg, T.A. Dellemijn, et al., Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients, J. Invest. Dermatol. 129 (2009) 2220–2232.
[39] B. Palermo, R. Campanelli, S. Garbelli, et al., Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: The role of cellular immunity in the etiopathogenesis of vitiligo, J. Invest. Dermatol. 117 (2001) 326–332.