ORIGINAL REPORT

HLA-DR Helps to Differentiate Erythrodermic Cutaneous T-cell Lymphoma from Erythrodermic Inflammatory Dermatoses in Flow Cytometry

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Differential diagnosis of erythroderma is challenging in dermatology, especially in differentiating erythrodermic cutaneous T-cell lymphoma from erythrodermic inflammatory dermatoses. This study retrospectively reviewed the peripheral blood flow cytometric results of 73 patients diagnosed with erythroderma at Peking University First Hospital from 2014 to 2019. The flow cytometry antibody panel included white blood cell markers, T-cell markers, B-cell markers, T-cell activation markers, and T helper cell differentiation markers. Features of the cell surface antigens were compared between 34 patients with erythrodermic cutaneous T-cell lymphoma and 39 patients with erythrodermic inflammatory dermatoses. The percentage of HLA-DR+/CD4+T cells was the most pronounced marker to distinguish erythrodermic cutaneous T-cell lymphoma from erythrodermic inflammatory dermatoses, with a threshold of 20.85% (sensitivity 96.77%, specificity 70.37%, $p = 0.000$, area under the curve (AUC) 0.882), suggesting its potential capability in the differential diagnosis of erythrodermic cutaneous T-cell lymphoma from erythrodermic inflammatory dermatoses. Moreover, in contrast to erythrodermic inflammatory dermatoses, the percentage of Th17 cells was significantly downregulated in erythrodermic cutaneous T-cell lymphoma ($p = 0.001$), demonstrating a dysregulated immune environment in erythrodermic cutaneous T-cell lymphoma.

Key words: erythroderma; erythrodermic cutaneous T-cell lymphoma; erythrodermic inflammatory dermatoses; flow cytometry; HLA-DR; T helper 17 cells.

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SIGNIFICANCE

Erythroderma is a severe clinical condition that can arise from a variety of diseases. It is quite difficult to differentiate erythrodermic cutaneous T-cell lymphoma from erythrodermic inflammatory dermatoses. This study retrospectively reviewed the peripheral blood flow cytometric results of 73 patients diagnosed with erythroderma. Decreased HLA-DR expression of CD4+ T cell was found to be a potential marker that could be used together with classical markers in distinguishing erythrodermic cutaneous T-cell lymphoma from erythrodermic inflammatory dermatoses. In addition, the peripheral blood of the patients with erythrodermic cutaneous T-cell lymphoma showed a dysregulated immune milieu and abnormal immunophenotype of T cells.

Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common types of primary cutaneous T-cell lymphoma (CTCL) (1, 2). Both SS and advanced MF may present as generalized erythroderma (erythrodermic CTCL, E-CTCL), associated with a progressive clinical course and poor prognosis (2–4). Diagnosis of E-CTCL is difficult due to its clinical resemblance to erythrodermic inflammatory dermatoses (EID), which is caused by inflammatory disorders, including drug eruption, eosinophilic dermatoses (ED), eczema, etc. Skin biopsy lacks sensitivity in diagnosing E-CTCL, and peripheral blood analysis by flow cytometry has increasing importance in establishing a diagnosis (5–8).

Flow cytometry is a useful tool to detect neoplastic cell subsets in the peripheral blood of patients with advanced CTCL and is thus mainly applied to evaluate blood classification in disease staging. Flow cytometry for blood classification has been introduced into the CTCL tumour-node-metastasis-blood (TNMB) classification system since 2007 (9). Neoplastic T cells typically express mature T helper cell markers, including TCRαβ, CD2, CD3, CD4, and CD45RO, with loss of CD5, CD7, and CD26 on different levels (7, 10). Previous studies differentiating malignant from benign erythroderma have relied mainly on the flow cytometry criteria used in B2 staging, including the CD4/CD8 ratio and loss of CD7 or CD26 (10). A CD4/CD8 ratio of 10 or more occurs in approximately 80% of cases of SS and occasionally in cases of benign erythroderma (4). A percentage of CD4+CD7– cells of at least 40% and/or a percentage of CD4+CD26– cells of at least 30% lymphocytes (LC) have been used as tentative diagnostic criteria in several

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studies (4, 10–13). Another study suggested that a loss of CD26 (≥80% CD4+T cells) and/or a loss of CD7 (≥40% CD4+T cells) could be applied in the differential diagnosis of SS vs EID (14). However, only a minority of patients with E-CTCL fulfilled the B2 classification, while a portion of patients with E-CTCL, predominantly patients with erythrodermic MF (4), showed only mild peripheral blood involvement, restricting the sensitivity of the above criteria.

Accumulating evidence indicates that CTCL is associated with immune dysregulation and aberrant T-cell activation, especially in advanced stages. HLA-DR, one of the major histocompatibility complex (MHC) class II proteins, is normally expressed on antigen-presenting cells, B cells and activated T cells. Compared with tumour-infiltrating lymphocytes, the phenotype of MF tumour cells was found to be much more heterogeneous with varied HLA-DR expression when disease progression occurs (15). Advanced CTCL displays a T helper (Th) 2 immunophenotype with increased production of interleukin (IL)-4, IL-5, and IL-13 (16). In a portion of patients with SS, malignant T cells express Th17 cytokines (17), whereas some reports found that IL-17 reduction was associated with disease progression (18, 19). It is not known if these aberrancies help to distinguish malignant from benign erythroderma.

This study retrospectively analysed the peripheral blood of 73 patients with erythroderma using a flow cytometry panel covering T-cell activation and Th-cell differentiation markers. The aim of the study was to identify immunological features that contribute to differential diagnosis of patients with E-CTCL vs those with EID.

MATERIALS AND METHODS

This single-institution cohort study was a retrospective review of 73 patients presenting with erythroderma (34 E-CTCL vs 39 EID) at Peking University First Hospital between 2015 and 2019. The study was approved by the ethics committee of Peking University First Hospital (number 2021-478). E-CTCL was diagnosed and staged by the ISCL/EORTC criteria (9, 20). Flow cytometry analyses of the peripheral blood samples were obtained at the time of initial referral and were evaluated by at least 2 senior doctors.

Peripheral blood samples were collected, prepared, and analysed by a standard red blood cell lysis method (21). Eight-colour flow cytometry (FACS Canto II cytometer, BD Biosciences, San Jose, CA, USA) was performed. The flow cytometry panel included white blood cell markers (CD45), T-cell markers (CD2, CD3, CD4, CD5, CD7, CD8, CD26, CD45RA, CD45RO, TCRαβ and TCRγδ), B-cell markers (CD19), T-cell activation markers (CD25 and HLA-DR), and Th-cell differentiation markers (CXCR3, CCR4 and CCR6) (22).

Statistical analysis

Statistical analysis was performed with SPSS version 23 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8 (Dotmatics, Boston, MA, USA). Continuous variables are presented as mean and standard deviation (SD) or median and interquartile range (IQR). Categorical data are shown as percentages. Comparisons were performed using the Mann–Whitney *U* test, Kruskal–Wallis test, χ^2 or Fisher's exact t-tests. Kaplan–Meier curves were used for survival analysis. Receiver operating characteristic (ROC) curve analysis was performed to determine cut-off values. Statistical significance was considered with a 2-sided $p<0.05$.

RESULTS

Patients' characteristics

The patient demographic information is described in **Table I** and **Fig. 1**. More detailed clinical information is listed in Table SI. The EID group had a higher ratio of men than the E-CTCL group ($p=0.014$). Of the 73 patients, there were 26 with MF (35.6%) and 8 with SS (11.0%, 4 SS preceded by MF) belonging to E-CTCL, whereas the other 39 patients were diagnosed with EID, including 12 drug eruptions (16.4%), 8 eosinophilic dermatoses (11.0%), 9 eczemas (12.3%), 7 idiopathic cases (9.6%), 1 pityriasis rubra pilaris (PRP, 1.4%), 1 lichen planus (LP, 1.4%), and 1 psoriasis (1.4%) (Fig. 1A). The mean age of the EID group (61.8 ± 15.8 years) was older than that of the E-CTCL group (49.8 ± 17.5 years, $p=0.003$). In the E-CTCL group, 24 (70.6%) patients were at stage III, and 10 (29.4%) patients were at stage IV (Table SI). Only 8 (23.5%) patients with E-CTCL met the B2 criteria in the peripheral blood, 11 (32.4%) patients were diagnosed as B1, and 15 (44.1%) patients were at the B0 stage. The white blood cell (WBC) count showed greater variability in the E-CTCL group (2.99–95.26×10^9/L) than in the EID group (3.88–21.68×10^9/L), although the median WBC (E-CTCL 8.00×10^9/L vs EID 8.91×10^9/L) did not vary between the 2 groups ($p=0.537$). The median levels of lactate dehydrogenase (LDH) were comparable in the 2 groups (E-CTCL 244 IU/L vs EID 261 IU/L, $p=0.725$), but there was an extreme value (1,120 IU/L) in E-CTCL. The median follow-up duration was longer in EID than in E-CTCL to exclude the possibility of misdiagnosis (EID 41 months vs E-CTCL 7.5 months, $p=0.000$). During the follow-up period, 7 patients with E-CTCL

Table I. Demographic and clinical characteristics of patients with erythroderma

	E-CTCL ($n=34$)	EID ($n=39$)	p-value
Sex, n (%)			**0.014**
Male	20 (58.8)	33 (84.6)	
Female	14 (41.2)	6 (15.4)	
Age at diagnosis, years, mean (SD)	49.8 (17.5)	61.8 (15.8)	**0.003**
WBC, 10^9/L, median (range)	8.00 (2.99–95.26)	8.91 (3.88–21.68)	**0.537**
LDH, IU/L, median (range)	244 (138–1120)	261 (151–616)	**0.725**
Outcome, n (%)			**0.176**
Alive	21 (61.8)	24 (61.5)	
Died	7 (20.6)	3 (7.7)	
Not known	6 (17.6)	12 (30.8)	
Follow-up duration, months, median (range)	7.5 (1–34)	41 (3–80)	**0.000**

E-CTCL: erythrodermic cutaneous T cell lymphoma; EID: erythrodermic inflammatory dermatoses; SD: standard variation; WBC: white blood count; LDH: lactate dehydrogenase. Bold numbers indicate statistical significance $p<0.05$.

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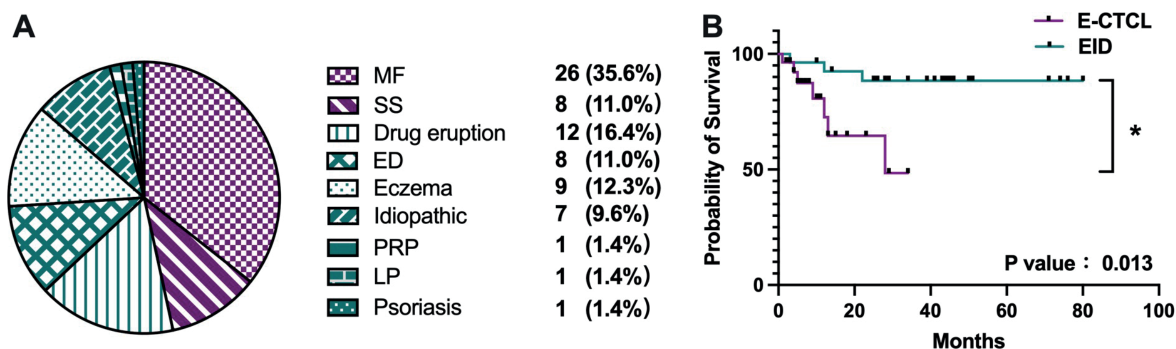

Fig. 1. Overview of 73 patients diagnosed with erythroderma. (A) Distribution of the causes of erythroderma. MF: mycosis fungoides; SS: Sézary syndrome; ED: eosinophilic dermatoses; PRP: pityriasis rubra pilaris; LP: lichen planus. (B) Overall survival of the patients with erythrodermic cutaneous T-cell lymphoma (E-CTCL) vs those with erythrodermic inflammatory dermatoses (EID) (* $p < 0.05$).

died from disease progression, and 3 patients with EID died with systemic diseases or unknown reason (Table SI). Kaplan–Meier survival analysis demonstrated better overall survival (OS) of the EID group than of the E-CTCL group, confirming the unfavourable prognosis of E-CTCL (Fig. 1B, $p=0.013$). Collectively, these data suggested that WBC and LDH could not distinguish between benign and malignant erythroderma.

B2 blood rating markers, including the CD4/CD8 ratio and loss of CD7 or CD26, lack sensitivity in differentiating malignant and benign erythroderma

To explore the flow markers that distinguish E-CTCL from EID, this study first evaluated the markers previously reported in blood ratings of CTCL. The absolute CD4/CD8 ratio was compared between the EID and E-CTCL groups, but no significant difference was found (Tables SII and SIII, $p=0.144$). The results showed that 17.6% (6/34) of the patients with E-CTCL had a CD4/CD8 ratio > 10, compared with 2.6% (1/39) of the patients with EID (sensitivity 17.6%, specificity 97.4%, $p=0.074$, **Table II**). One EID patient with CD4/CD8 > 10 was diagnosed with drug eruption, but no positive T-cell clone was revealed in her peripheral blood.

Phenotypically abnormal malignant T-cell populations usually feature a loss of CD7 and/or CD26 (10). In the current study, as shown in Tables SII and SIII, no evident discrepancies in the absolute percentage values of CD7–/CD4 + ($p=0.478$), CD26–/CD4+($p=0.135$), CD4+CD7–/LC ($p=0.239$), and CD4+CD26–/LC ($p=0.450$) were found between the EID and E-CTCL patients. Next, this study evaluated the previously suggested criteria for erythroderma differentiation (4, 11, 14). Within the lymphocyte gate, CD4+CD7–/LC ≥ 40% was exclusively observed in 25.9% (7/27) of the patients with E-CTCL ($p=0.009$) with a specificity of 100%, but no obvious difference in CD4+CD26–/LC ≥ 30% was found between the 2 groups (sensitivity 37.0%, specificity 77.4%, $p=0.228$, Table II). Furthermore, in the CD4+T-cell population, CD26–/CD4 + T cells > 80% were found in 37% (10/27) of the patients with E-CTCL compared with 6.5% (2/31) of the patients with EID, demonstrating a high specificity of 93.5% in differential diagnosis ($p=0.004$), while CD7–/CD4+ ≥ 40% was not significantly discrepant (sensitivity 33.3%, specificity 87.1%, $p=0.063$, Table II). Therefore, although the CD4/CD8 ratio and loss of CD7 and CD26 could aid in the differential diagnosis of benign and malignant erythroderma, their low sensitivity and imperfect specificity hindered practicability during clinical work.

The percentages of eosinophil, CD45RO, CD25, and HLA-DR showed differential expression between benign and malignant erythroderma

To identify the flow cytometric features of benign and malignant erythroderma, other cell surface markers were assessed, including CD45, CD2, CD3, CD5, CD19, CD25, CD45RA, CD45RO, TCRαβ, TCRγδ and HLA-DR (Tables SII and SIII). Under CD45 and SSC gate, the population of eosinophil (EO, **Fig. 2**A) was higher in the patients with EID (median 7.30%, IQR 2.60–16.50%) than in the patients with E-CTCL (median 3.95%, IQR 2.08–7.13%) ($p=0.025$). CD25 (Fig. 2B), a transmembrane protein present on activated CD4+T cells (23), was upregulated in EID (median 48.30%, IQR 32.65–66.75%) compared with E-CTCL (median

Table II. Overview of the classical flow cytometry markers in differentiating erythrodermic cutaneous T cell lymphoma (E-CTCL) from erythrodermic inflammatory dermatoses (EID)

Markers	E-CTCL, *n*/total (%)	EID, *n*/total (%)	Sensitivity %	Specificity %	*p*-value
CD4/CD8 ratio ≥ 10%	6/34 (17.6)	1/39 (2.6)	17.6	97.4	0.074
CD4+CD7–/lymphocyte ≥ 40%	7/27 (25.9)	0/31 (0)	25.9	100.0	0.009
CD4+CD26–/lymphocyte ≥ 30%	10/27 (37.0)	7/31 (22.6)	37.0	77.4	0.228
CD7–/CD4+ ≥ 40%	9/27 (33.3)	4/31 (12.9)	33.3	87.1	0.063
CD26–/CD4+ ≥ 80%	10/27 (37.0)	2/31 (6.5)	37.0	93.5	0.004

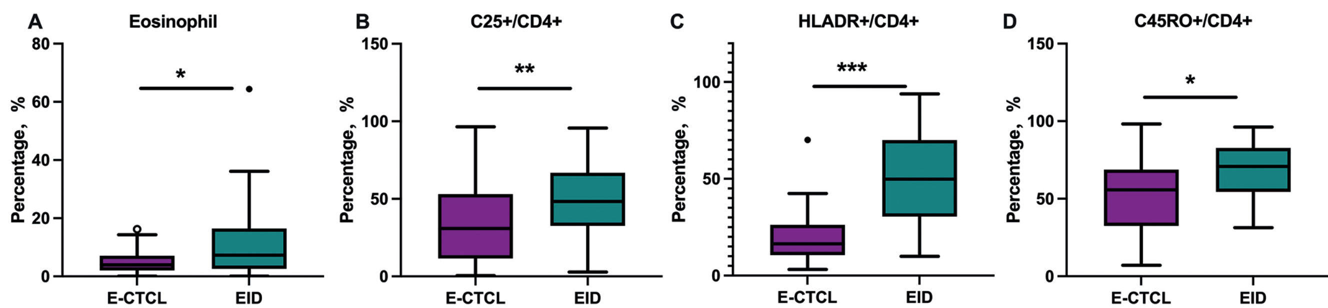

Fig. 2. Comparison of the 4 distinguished cell surface markers between benign and malignant erythroderma, including (A) eosinophils, (B) CD25, (C) HLA-DR/CD4+, and (D) CD45RO+/CD4+ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

30.90%, IQR 11.58–53.10%, $p=0.009$). In comparison with the patients with E-CTCL (median 16.40%, IQR 10.60–26.20%), the expression of HLA-DR in CD4+T cells was significantly higher in the patients with EID (median 49.80%, IQR 30.50–70.00%, $p=0.000$, Fig. 2C). Although malignant T cells always demonstrate a CD45RO+ immunophenotype (10), the percentage of CD45RO+/CD4+T cells (Fig. 2D) in the patients with E-CTCL (median 55.70%, IQR 32.53–68.75%) was lower than that in the patients with EID (median 70.80%, IQR 54.40–82.70%, $p=0.012$). These results suggested a dysregulated immune status of malignant T cells. Meanwhile, the loss of pan T-cell markers, including CD2 ($p=0.243$) and CD5 ($p=0.859$), showed no remarkable distinction between the 2 groups. Collectively, these data showed that loss of pan T-cell markers could be seen in both benign and malignant erythroderma, and the percentages of EO, CD45RO, and activation markers on CD4+T cells (CD25 and HLA-DR) were significantly higher in the patients with EID than in those with E-CTCL.

HLA-DR served as a potential flow marker in differentiating malignant and benign erythroderma

Given that HLA-DR showed the lowest *p*-value when comparing E-CTCL and EID, further analyses were performed to evaluate its potential as a marker in differentiating malignant and benign erythroderma. First, using B0-1 stage E-CTCL as a comparator, the percentage of HLA-DR on CD4+T cells showed a tendency to decrease in the B2 stage, although the *p*-value was not significant (B0-1: $n=19$, median 18.70%, IQR 14.30–31.90%; B2: $n=8$, median 10.68%, IQR 3.75–19.6%; $p=0.098$; **Fig. 3**A). To further explore whether HLA-DR expression is downregulated in malignant T cells, the current study analysed the transcription levels of HLA-DR in isolated CD4+T cells from 6 patients with SS and 8 healthy controls based on a microarray study from our previous study (24) (sequenced by Agilent Whole Human Genome Oligo microarrays G4112F). *HLA-DRB1* represents the most abundant transcript encoding the HLA-DR protein (25). According to the microarray dataset, the mRNA expression level of *HLA-DRB1* was significantly decreased in the CD4+T cells of SS (SS: $n=6$, median 6,177, IQR 4,515–7,725; Control $n=8$, median 17,503, IQR 8,563–26,285; $p=0.005$; Fig. 3B), confirming the HLA-DR decrease in E-CTCL. Next, ROC curve analysis suggested a threshold of HLA-DR+/CD4+T cells of 20.85% to differentiate malignant erythroderma from benign erythroderma ($p=0.000$, AUC 0.882, Fig. 3C), with a sensitivity of 96.77% and

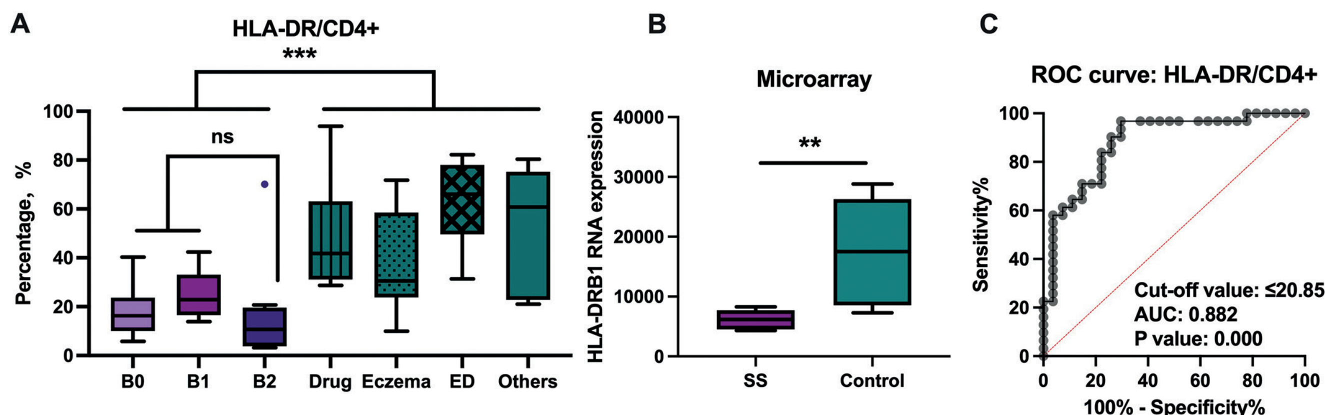

Fig. 3. HLA-DR expression in cutaneous T-cell lymphomas (CTCLs) and benign conditions. (A) Percentages of HLA-DR/CD4+ cells in the different stages of erythrodermic cutaneous T-cell lymphoma (E-CTCL) and in the different subtypes of erythrodermic inflammatory dermatoses (EIDs) (ns: $p>0.05$, ***$p<0.001$). ED: eosinophilic dermatoses; Others: include idiopathic erythroderma, pityriasis rubra pilaris, lichen planus, and psoriasis. (B) Transcription levels of HLA-DR in CD4+ T cells from 6 patients with Sézary syndrome (SS) vs 8 healthy controls (**$p<0.01$). (C) Receiver operating characteristic (ROC) curve analysis of HLA-DR/CD4+ to distinguish E-CTCL from EID.

a specificity of 70.37%. Combined with HLA-DR, the specificity of classical criteria could improve to 100% for the differential diagnosis of E-CTCL from EID, although their sensitivity decreased a little (as shown in Table SV; sensitivities: CD4/CD8 17.6%; CD4+CD7–≥40%: 22.2%; CD4+CD26–≥30%: 29.6%; CD7–/CD4+≥40%: 25.9%; CD26–/CD4+≥80%: 29.6%). To summarize, the T-cell activation marker HLA-DR was decreased in the peripheral CD4+T cells of the patients with E-CTCL, and HLA-DR loss may contribute to the differentiation of E-CTCL from EID.

T helper cell markers in benign and malignant erythroderma

Previous studies have evidenced that the Th2 cytokine profile dominates in the peripheral blood of advanced CTCL, correlating with the T-cell immunophenotypic changes (16). The current study used the cell surface antigens to identify the percentages of Th1 (CXCR3+), Th2 (CCR4+CCR6–) and Th17 cells (CCR4+CCR6+) in the CD4+T cell population, aiming to assess Th-cell differentiation status between benign and malignant erythroderma (22). As **Fig. 4**A and Table SIV showed, there was no significant difference in the percentage of Th1 cells between the 2 groups (E-CTCL median 24.00%, IQR 12.00–36.10%; EID median 28.50%, IQR 20.60–33.60%; $p=0.498$). Although the patients with B2 stage E-CTCL (median 69.50%, IQR 13.71–77.50%) had a higher Th2 percentage than the patients with B0-1 stage E-CTCL (median 14.20%, IQR 8.86–20.40%), the patients with benign and malignant erythroderma had comparable levels of Th2 cells (E-CTCL median 14.60%, IQR 8.86–34.90%; EID median 14.20%, IQR 9.87–31.20%; $p=0.585$, Fig. 4B, Table SIV). It is noteworthy that the Th17 percentage was distinctly lower in the E-CTCL group (median 10.70%, IQR 3.17–14.30%) than in the EID group (median 17.90%, IQR 10.70–26.20%) ($p=0.001$, Fig. 4C, Table SIV). Furthermore, compared with the patients with B0–B1 stage E-CTCL (median 12.10%, IQR 5.61–14.80%), the patients with B2 stage E-CTCL (median 2.69%, IQR 1.95–11.30%) had a lower level of Th17 cells ($p=0.019$, Fig. 4C, Table SIV), indicating a stage-dependent decrease in the Th17 profile in the peripheral blood of CTCL patients. Hence, the current data showed that the percentage of Th2 cells failed to differentiate E-CTCL from EID, while a lower Th17 percentage was noted in E-CTCL, suggesting a distinct immune milieu between the 2 groups.

DISCUSSION

Erythroderma always causes diagnostic dilemmas in dermatology. E-CTCL, although accounting for less than 5% of erythroderma cases, frequently poses diagnostic and therapeutic challenges for dermatologists during clinical work (26). The current study evaluated the efficacy of a series of flow cytometry markers in differentiating E-CTCL from EID and identified HLA-DR as a potential novel indicator in the differential diagnosis of benign and malignant erythroderma.

Previous studies used flow cytometry criteria of B2 blood rating in CTCL to differentiate malignant erythroderma. However, several limitations on these criteria have restricted their clinical application. First, in line with previous finding (4), only 6 out of the 10 (60%) B2-stage E-CTCL patients reached CD4/CD8≥10 in the current study cohort, but a patient with drug-induced erythroderma had a ratio of CD4/CD8>10 (27). Secondly, all criteria involving loss of CD7 and CD26 in the current cohort demonstrated low sensitivities. Only CD4+CD7–≥40% and CD26–/CD4+≥80% could distinguish malignant erythroderma from benign erythroderma with relatively high specificities, which is in line with previous research (14, 28). Therefore, these criteria were not robust enough to differentiate E-CTCL from EID.

HLA-DR is an activation marker of lymphocyte, but its role in the pathogenesis of MF remains unknown. Previous studies have demonstrated that high HLA-DR expression is associated with a good prognosis in cancer (29–31). Margaret R. Dunne proposed that, in colorectal cancer, loss of HLA-DR expression by tumour cells resulted in ineffective antigen presentation to T

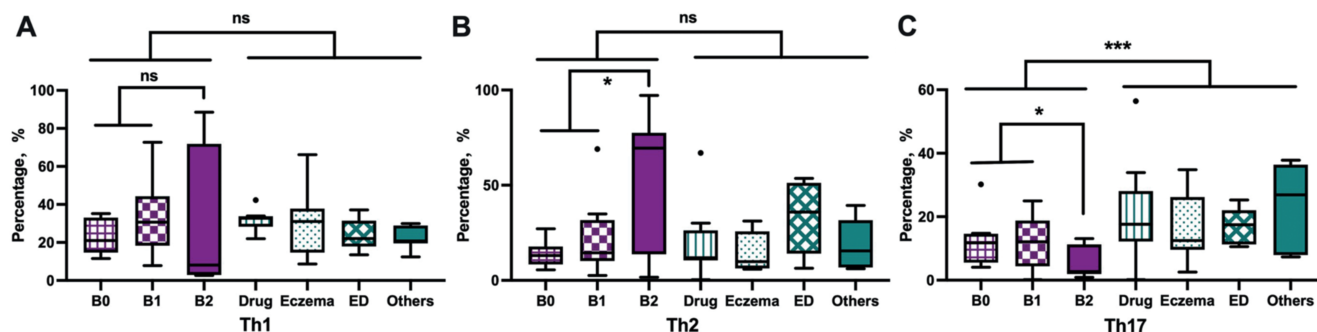

Fig. 4. Percentages of T helper cells in the different stages of erythrodermic cutaneous T-cell lymphomas (E-CTCLs) and in the different subtypes of erythrodermic inflammatory dermatoses (EIDs), including (a) Th1, (b) Th2, and (c) Th17 (ns: $p>0.05$, *$p<0.05$, ***$p<0.001$). ED: eosinophilic dermatoses; Others: include idiopathic erythroderma, pityriasis rubra pilaris, lichen planus, and psoriasis.

cells, thus impairing the anticancer immune response (30). Morihiro Higashi also reported that loss of HLA-DR expression in diffuse large B-cell lymphoma cells could decrease the infiltrated reactive T cells in the tumour microenvironment, contributing to escape from immunosurveillance (32). The current study detected lower expression of HLA-DR on CD4+T cells in E-CTCL than in EID. HLA-DR expression in the E-CTCL patients with B2 stage was much lower than that in the patients with E-CTCL with inapparent blood involvement. The current study next determined a cut-off value of HLA-DR expression for differentiating malignant erythroderma from benign erythroderma. Compared with classical markers, HLA-DR may help to better differentiate E-CTCL with minimal blood involvement from EID. Moreover, combination with HLA-DR, the specificity of classical markers to distinguish E-CTCL from EID could improve to 100%. HLA-DR may be a useful adjunct in the differential diagnosis of E-CTCL from EID, although validations by independent cohorts with large sample sizes are warranted. These findings suggested HLA-DR loss as an immunophenotypical feature in malignant erythroderma, making HLA-DR a potential diagnostic marker.

With respect to the Th classification of CD4+T cells in the peripheral blood, previous literature has shown that Th2 phenotypic cells are dominant in the peripheral blood of patients with advanced CTCLs with increased production of IL-4, IL-5 and IL-13 (33), which was confirmed by the current study data in the comparison of patients with B0-1 and B2 stage E-CTCL. However, patients with EID may also display a Th2 immunophenotypic milieu, especially in diseases belonging to the type 2 inflammatory dermatoses (34, 35). The higher percentage of EO in the patients with EID in the current cohort may also reflect this phenomenon. Interestingly, the current study found that the percentage of Th17 cells was lower in E-CTCL than in EID, and lowest in the patients with advanced CTCL. This result is consistent with a recent study demonstrating distinctively lower IL-17 mRNA expression levels in the blood of patients with MF than in healthy controls (36). The lower Th17 percentage in patients with advanced E-CTCL might reflect a dysregulated host immune milieu or an abnormal immunophenotype of tumour cells as the disease is exacerbated. However, the absolute Th17 percentage is highly overlapping between the patients with E-CTCL and those with EID, ruling out its capability as a diagnostic marker.

Thus, the main limitation of the current study is its single-centre nature and lack of an independent validation cohort, which requires external validation with larger cohorts to generalize its conclusion. In addition, surface markers were used for the identification of primary Th-cell subsets, which are easily applicable in clinical practice, but require more verification in future clinical work.

In conclusion, this study of a clinical cohort with long-term follow-up provides novel information for the differential diagnosis of E-CTCL from EID, in which HLA-DR was suggested as a novel marker in aiding classical markers to distinguish E-CTCL from EID.

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The study was approved by the ethics committee of Peking University First Hospital (number 2021-478). All participants provided written informed consent.

Supplementary materials are available on request from the corresponding authors.

The authors have no conflicts of interest to declare.

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