

PD-1, S-100 and CD1a expression in pseudolymphomatous folliculitis, primary cutaneous marginal zone B-cell lymphoma (MALT lymphoma) and cutaneous lymphoid hyperplasia

Background: Pseudolymphomatous folliculitis is a lymphoid proliferation that clinically and histopathologically mimics primary cutaneous extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In this study, we assessed the diagnostic value of three immunohistochemical markers, programmed death-1 (PD-1), CD1a and S100.

Methods: We evaluated 25 cases of cutaneous lymphoid proliferations with established diagnoses, including 9 patients with pseudolymphomatous folliculitis, 11 with MALT lymphoma, and 5 with cutaneous lymphoid hyperplasia (CLH). The clinical, histopathologic and immunohistochemical characteristics were reviewed and three major characteristics assessed: (a) proportion of T cells expressing PD-1, (b) pattern of expression of CD1a by dendritic cells and (c) pattern of expression of S100 by dendritic cells.

Results: We found pseudolymphomatous folliculitis to have a significant increase in PD-1+ T cells compared with MALT lymphoma ($p < 0.0001$). The pattern of CD1a staining is also informative: MALT lymphoma is significantly more likely to demonstrate a peripheral concentration of CD1a+ dendritic cells around lymphoid nodules than pseudolymphomatous folliculitis ($p < 0.0003$) or CLH ($p < 0.05$). Pseudolymphomatous folliculitis demonstrates an interstitial distribution of CD1a+ cells more often than MALT lymphoma ($p < 0.04$). S100 staining was not a helpful discriminator.

Conclusions: Histopathologic factors including PD-1 and CD1a staining patterns may allow for more certainty in distinguishing lymphoid hyperplasia, including pseudolymphomatous folliculitis, from MALT lymphoma.

Keywords: CD1a, lymphoid hyperplasia, MALT lymphoma, PD-1, pseudolymphomatous folliculitis

Goyal A, Moore JB, Gimbel D, Carter JB, Kroshinsky D, Ferry JA, Harris NL, Duncan LM. PD-1, S-100 and CD1a expression in

Amrita Goyal, Johanna B. Moore, Devon Gimbel, Joi B. Carter, Daniela Kroshinsky, Judith A. Ferry, Nancy L. Harris and Lyn M. Duncan

Dermatopathology Unit, Massachusetts
General Hospital, Boston, MA, USA

Lyn McDivitt Duncan, MD
Dermatopathology Unit, WRN 825
Massachusetts General Hospital, 55 Fruit Street,
Boston, MA 02114, USA
Tel: +617 726 8890
Fax: +617 726 8711
e-mail: duncan@helix.mgh.harvard.edu

Accepted for publication October 12, 2014

pseudolymphomatous folliculitis, primary cutaneous marginal zone B-cell lymphoma (MALT lymphoma) and cutaneous lymphoid hyperplasia.

J Cutan Pathol 2014. © 2014 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Pseudolymphomatous folliculitis represents a variant of cutaneous lymphoid hyperplasia (CLH) that presents as a solitary, often violaceous, domed nodule on the face, scalp or upper trunk.^{1–12} This form of lymphocytic infiltrate was initially defined solely based on histopathology, classically presenting as a dense, patchy or diffuse, dermal lymphocytic infiltrate with characteristic ‘activation’ of folliculosebaceous units.^{1–4,9} Hair follicles in pseudolymphomatous folliculitis characteristically are hyperplastic, branching and irregularly shaped and include intra-follicular dendritic cells. Granulomas may also be present.^{1,4}

Widespread use of immunohistochemical staining has allowed further definition of pseudolymphomatous folliculitis as a distinct entity.^{13,14} In 1999, Arai et al. found that cutaneous biopsies of pseudolymphomatous folliculitis demonstrate increased numbers of S-100-positive or CD1a+ dendritic cells within hair follicle epithelium compared with other forms of CLH.² In conjunction with histopathologic findings, these immunohistochemical characteristics have become integral in diagnosing pseudolymphomatous folliculitis.^{1,2,5–8,10,12}

The primary challenge in diagnosing pseudolymphomatous folliculitis is that it may closely mimic lymphoid malignancies, both clinically and histopathologically. The most important neoplasm in the differential diagnosis for pseudolymphomatous folliculitis is primary cutaneous marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). The authors acknowledge that the term marginal zone lymphoma may be preferred for this tumor when it occurs in the skin;¹⁵ nevertheless, in this report, we have used the terminology in the latest World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues.¹⁶

The clinical presentation as an erythematous dome-shaped nodule in pseudolymphomatous folliculitis and MALT lymphoma can be indistinguishable; the diagnosis ultimately rests on histopathologic analysis. MALT lymphomas are histopathologically characterized by dermal nodules of lymphoid cells with a

proliferation of CD20+ marginal zone cells, plasma cells with monotypic expression of immunoglobulin light chains (in 70% of cases) and clonal immunoglobulin heavy chain rearrangement.^{1,15,16} Biopsies of MALT lymphomas and pseudolymphomatous folliculitis can be strikingly similar: both may present with hyperplasia of the folliculosebaceous units clonal T-cell receptor or IgH rearrangements, atypical lymphocytes and numerous admixed B and T cells.^{1,4,9,17}

Although MALT lymphomas are typically indolent and have little risk of extracutaneous involvement, a diagnosis of MALT lymphoma can result in more staging studies and careful follow-up.¹⁸ An incorrect diagnosis of pseudolymphomatous folliculitis may result in under-staging and under-treatment of a lymphoma, while an inappropriate diagnosis of MALT lymphoma may subject a patient to unnecessary testing, treatment and expense. Thus, differentiating pseudolymphomatous folliculitis and MALT lymphoma is of great importance to patient care and improved methods of distinguishing these entities based on histopathology and immunohistochemistry are needed.

In this study, we propose that differential expression patterns of programmed death-1 (PD-1), CD1a and S-100 in tissue samples may be helpful in distinguishing pseudolymphomatous folliculitis, MALT lymphoma and CLH. In order to assess the diagnostic value of immunohistochemical staining for PD-1, CD1a and S100, we examined staining patterns in 31 unique tissue samples: 12 biopsy samples from 9 patients with pseudolymphomatous folliculitis, 13 biopsies from 11 patients with MALT lymphoma, and 6 biopsies from 5 patients with CLH.

Materials and methods

Paraffin-embedded skin biopsies and/or glass slides from 9 patients with pseudolymphomatous folliculitis, 11 patients with MALT lymphoma and 5 patients with CLH were identified from the archives of the Dermatopathology Unit and the private consultation files of the authors. Multiple biopsy samples were available for each of three

of the patients with pseudolymphomatous folliculitis, one of the patients with MALT lymphoma and one of the patients with CLH, yielding a total of 11 tissue samples of pseudolymphomatous folliculitis, 13 of MALT lymphoma and 6 of CLH. All cases had been previously reviewed by expert dermatopathologists and hematopathologists. Cases were selected by searching the electronic pathology files. Initially, we sought to compare cases of pseudolymphomatous folliculitis to cases of MALT lymphoma occurring on the face. We found few cases of MALT lymphoma on the face and elected to include tumors from other sites as well. MALT lymphomas were diagnosed based on the standards set out in the most recent edition of the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.^{16,19} The presence of light chain restriction or clonal IgH rearrangement prohibited a diagnosis of pseudolymphomatous folliculitis. Cases of pseudolymphomatous folliculitis were identified histopathologically based on the presence of hyperplastic, irregular hair follicles containing S100+ and CD1a+ dendritic cells and a nodular to diffuse dermal mixed lymphocytic infiltrate lacking significant atypia. In all instances, cases were only excluded if tissue for further immunohistochemical studies or necessary slides were unavailable.

All patients diagnosed with MALT lymphoma and for whom follow-up data were available were staged as was considered clinically appropriate, including via physical examinations, blood counts and computed tomography. No patients showed any evidence of extracutaneous disease at the time of diagnosis. Clinical characteristics examined included sex, age, clinical diagnosis, solitary or multiple lesions, anatomic location, tumor size and duration of the lesion.

Sections from all biopsies were routinely stained with hematoxylin/eosin. Histopathologic features examined included lesion size, architectural pattern, lymphocytic infiltration of folliculosebaceous units, hair follicle morphology and irregularity (activation), grenz zone, presence and architecture of lymphoid follicles (defined as aggregates of B cells organized with a follicular dendritic cell meshwork, often including a germinal center), zones of plasma cells and marginal zone cells.

Initially performed for diagnostic work ups, CD3 and CD20 immunostains were available for all tissue samples. Other immunohistochemical stains varied from sample to sample and included reagents to detect CD2, CD4, CD5, CD7, CD8, CD10, CD21, CD30, CD68, CD79a,

Bcl2, Bcl6, Ki-67, kappa light chain and lambda light chain. Immunohistochemical staining was performed as previously described.²⁰ In most cases, T-cell receptor and immunoglobulin heavy chain rearrangement were assessed by polymerase chain reaction after DNA extraction from the paraffin tissue blocks per established techniques.²⁰ For the purpose of this investigation, immunohistochemical staining for PD-1 (Z0311, Dako, Carpinteria, CA, USA), CD1a (PA0235, Cell Marque, Rocklin, CA, USA) and S100 (315M-96, Cell Marque, Rocklin, CA, USA) immunostains were performed on formalin-fixed paraffin-embedded tissue sections.^{21,22} Control lymphoid tissue was present on the majority of slides; slides for which control tissue stained aberrantly were excluded or remade. Not all cases were stained for all markers owing to limitations in the specimen size and block availability.

PD-1+ cells in the context of CD3+ T cells were evaluated immunohistochemically in 7 biopsies of pseudolymphomatous folliculitis, 7 of MALT lymphoma and 6 of CLH. The percentage of cells expressing PD-1 was evaluated as previously described by Cetinozman et al.²³ We added the use of ImageJ technology to provide a more quantitative estimate as follows: four photos of representative areas of each slide were taken at ×60 magnification. The positive and negative cells in each photo were manually counted using ImageJ (Bethesda, MD), and the percentage of PD-1 positive cells averaged across the four images. The number of CD3+ cells was assessed similarly, and the ratio of PD-1 to CD3 positive cells was then calculated.

To identify dendritic cell distribution, immunohistochemical stains for S100 and CD1a were performed on 12 cases of pseudolymphomatous folliculitis, 13 cases of MALT lymphoma and 6 cases of CLH. The pattern of staining of S100 and CD1a was categorized as within the hair follicle epithelium (intra-follicular), interstitial throughout the dermis or peripheral about the lymphoid aggregates or excluded from reactive lymphoid follicles and scored on a scale of 0–3, 0 representing no staining and 3 indicating many dendritic cells with intense staining in the specified location. Statistical analysis was performed using Student's *t*-test, Fisher's exact test and 1-way analysis of variance test. *p*-Values <0.05 were considered significant.

Results

The cases examined included 12 biopsies from 9 patients with pseudolymphomatous

Table 1. Clinical features of patients with pseudolymphomatous folliculitis, MALT lymphoma and CLH

	Pseudolymphomatous folliculitis	MALT lymphoma	CLH
Number of patients	9	11	5
Age	17–69 years Mean: 52 years	38–84 years Mean: 65 years	36–77 years Mean: 48 years
M/F	8/1	5/6	2/3
Size of lesions	0.2–2 cm Mean: 1.03 cm	0.4–4 cm Mean: 1.16 cm	0.5–1.6 cm Mean: 1.15 cm
Duration of follow-up	2 months–9 years Mean: 2.8 years	3 months–15 years Mean: 5.5 years	5–9 years Mean: 7 years
Number of patients with multifocal lesions	1	1	1
Number of lesions biopsied	12	13	6
Lesion locations			
Head	12	1	1
Trunk/extremities	0	12	5

CLH, cutaneous lymphoid hyperplasia; F, female; M, male; MALT, mucosa-associated lymphoid tissue.

folliculitis, 13 biopsies from 11 with MALT lymphoma and 6 from 5 patients with CLH. There was no statistically significant difference in patient age or gender (Table 1). While all cases of pseudolymphomatous folliculitis occurred on the head and neck, MALT lymphoma and CLH were more likely to occur on the trunk or extremities ($p < 0.0001$ and $p < 0.01$, respectively).

While pseudolymphomatous folliculitis, MALT lymphoma and CLH were difficult to distinguish based on examination of the routinely stained sections alone, some characteristic features were observed. All cases of pseudolymphomatous folliculitis demonstrated hair follicles with irregularly shaped hyperplastic epithelial structures in the dermis (Fig. 1). Lymphoid follicles and zones

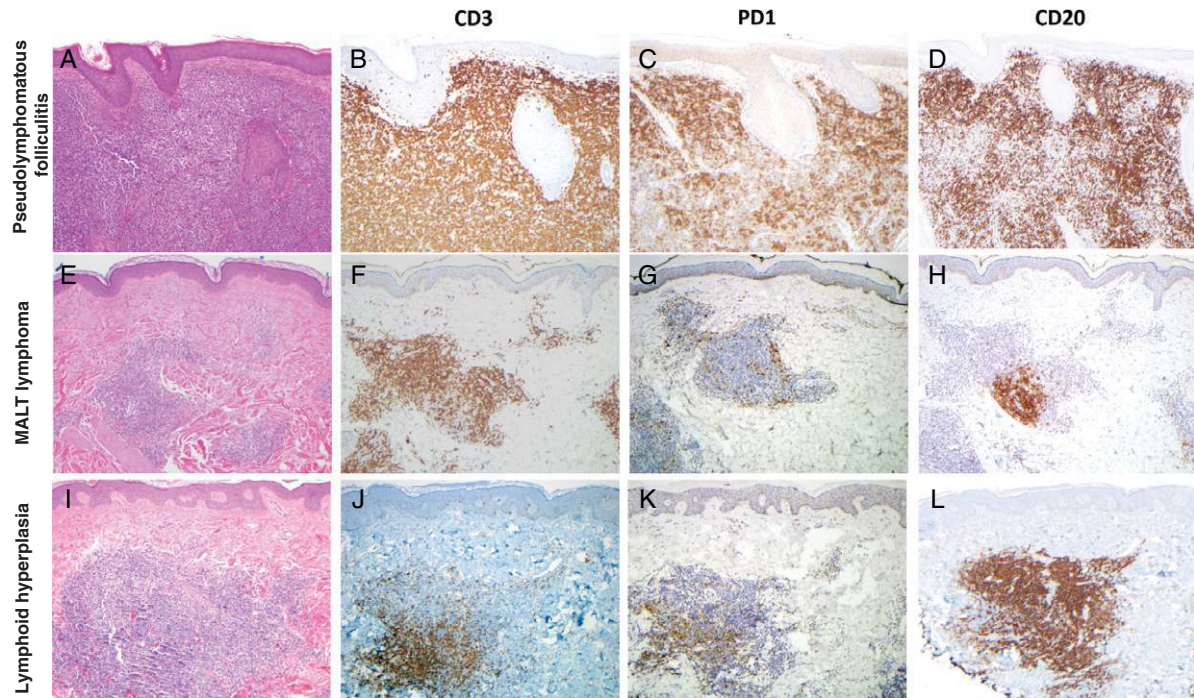


Fig. 1. CD3 and programmed death-1 (PD-1) in pseudolymphomatous folliculitis, mucosa-associated lymphoid tissue (MALT) lymphoma and cutaneous lymphoid hyperplasia (CLH). Pseudolymphomatous folliculitis, with a dense superficial and deep dermal lymphocytic infiltrate (A, hematoxylin/eosin, $\times 10$), numerous CD3+ T cells (B, CD3, $\times 10$) and many PD-1+ cells (C, PD-1, $\times 10$). MALT lymphoma with dense dermal nodules of marginal zone B cells and reactive T cells (D, hematoxylin/eosin, $\times 10$), reactive CD3+ T cells within the nodular proliferation (E, CD3, $\times 10$) and fewer than a third of the CD3+ T cells express PD-1 (F, PD-1, $\times 10$). CLH with a dense nodular mixed lymphohistiocytic infiltrate (G, hematoxylin/eosin, $\times 10$), a substantial population of CD3+ T cells (H, CD3, $\times 10$) and less than half of CD3+ T cells express PD-1 (I, PD-1, $\times 10$).

PD-1 and CD1a in lymphoma and pseudolymphoma

Table 2. PD-1 and CD3 staining in pseudolymphomatous folliculitis, MALT lymphoma and CLH

	N	%PD-1+ (range)	Reactive lymphocytes %CD3+ (range)	PD-1/CD3 (range)
Pseudolymphomatous folliculitis	7	54 (38–70)	69 (57–78)	78 (65–97)
MALT	6	16 (6–21)	67 (29–80)	23 (17–34)
CLH	6	24 (13–30)	61 (53–64)	40 (21–56)
		p < 0.0001	p = 0.49	p < 0.0001

The mean percentage of cells with small round nuclei positive for PD-1 and CD3 for each biopsy and percentage of CD3+ T cells expressing PD-1. CLH, cutaneous lymphoid hyperplasia; MALT, mucosa-associated lymphoid tissue; PD-1, programmed death-1.

*One-way analysis of variance for determining significant differences between the three groups.

of plasma cells were most commonly observed in MALT lymphoma, and light chain restriction (12/13 cases, 7 kappa, 5 lambda) was used as a diagnostic criterion in MALT lymphoma (and was not seen in pseudolymphomatous folliculitis or CLH). Neither hair follicle activation nor light chain restriction was seen in CLH.

PD-1 and CD3 staining

The lymphoid infiltrate in all cases was composed of numerous admixed CD3+ T and CD20+ B cells. In pseudolymphomatous folliculitis, CD3+ T cells comprised 57–78% of the infiltrate (mean 69%), whereas 38–70% (mean 54%) of the small round lymphocytes stained positively for PD-1 (Fig. 1). In MALT lymphoma, CD3+ T cells composed 29–80% of the infiltrate (mean 67%), with 6–21% (mean 16%) of the small round lymphocytes staining positively for PD-1. In CLH, CD3+ T cells accounted for 53–64% of the infiltrate (mean 61%), and 13–30% (mean 24%) of the small round lymphocytes stained positively for PD-1.

The proportion of reactive CD3+ T cells expressing PD-1, calculated as the PD-1/CD3 ratio,²³ ranged from 65 to 97% (mean 78%) in pseudolymphomatous folliculitis, to 17–34% (mean 23%) in MALT lymphoma and 21–56% (mean 40%) in CLH (Table 2).

S100 and CD1a staining

S100+ dendritic cells were present in the hair follicle epithelium in all cases with hair follicles present, including 12 cases of pseudolymphomatous folliculitis, 3 cases of MALT lymphoma and 2 cases of CLH. The distribution of S100+ dendritic cells in the dermis was examined. An interstitial pattern throughout the dermal infiltrate was observed in all 11 cases of pseudolymphomatous folliculitis, in 6 of 13 cases of MALT lymphoma and 5 of 6 cases of CLH. Localization of S100+ dendritic cells at the periphery of the

lymphoid nodules was not seen in pseudolymphomatous folliculitis (0/10 cases, 1 case was a small punch biopsy without the edge of a lymphoid nodule) or CLH (0/6) and was only seen in 1 case of MALT lymphoma (1/7 cases).

A prominent population of CD1a+ dendritic cells was observed in the hair follicle epithelium of all cases of pseudolymphomatous folliculitis (10/10 cases), in the 2 cases of MALT lymphoma with hair follicle epithelium and in both cases of CLH with hair follicles present (Fig. 2). CD1a+ dendritic cells were observed throughout the dermis in an interstitial pattern in most cases of pseudolymphomatous folliculitis (9/10), occasionally in MALT lymphoma (6/13 cases) and most cases of CLH (5/6 cases) (Table 3). CD1a+ dendritic cells were not observed localized to the periphery of lymphoid nodules in cases of pseudolymphomatous folliculitis with lymphoid nodules (0/7 cases), but were seen in the majority of cases of MALT lymphoma (11/13 cases), and occasionally seen in CLH (2/6 cases). In cases with reactive lymphoid follicles (most cases of MALT lymphoma and rare cases of CLH), the CD1a+ cells were absent in lymphoid follicles (Fig. 2F).

Discussion

In this study, we reviewed paraffin-embedded tissue samples from 9 patients with pseudolymphomatous folliculitis, 11 patients with MALT lymphoma and 5 patients with CLH. The clinical, histopathologic and immunohistochemical characteristics were reviewed with the goal of determining the utility of PD-1, S100 and CD1a in differentiating these three entities. We examined three major immunohistochemical features: (a) the proportion of T cells expressing PD-1, (b) the pattern of expression of CD1a and (c) the pattern of expression of S100. We found that the density of PD-1+ T cells and the distribution of CD1a+ dendritic cells were helpful in distinguishing these three entities.

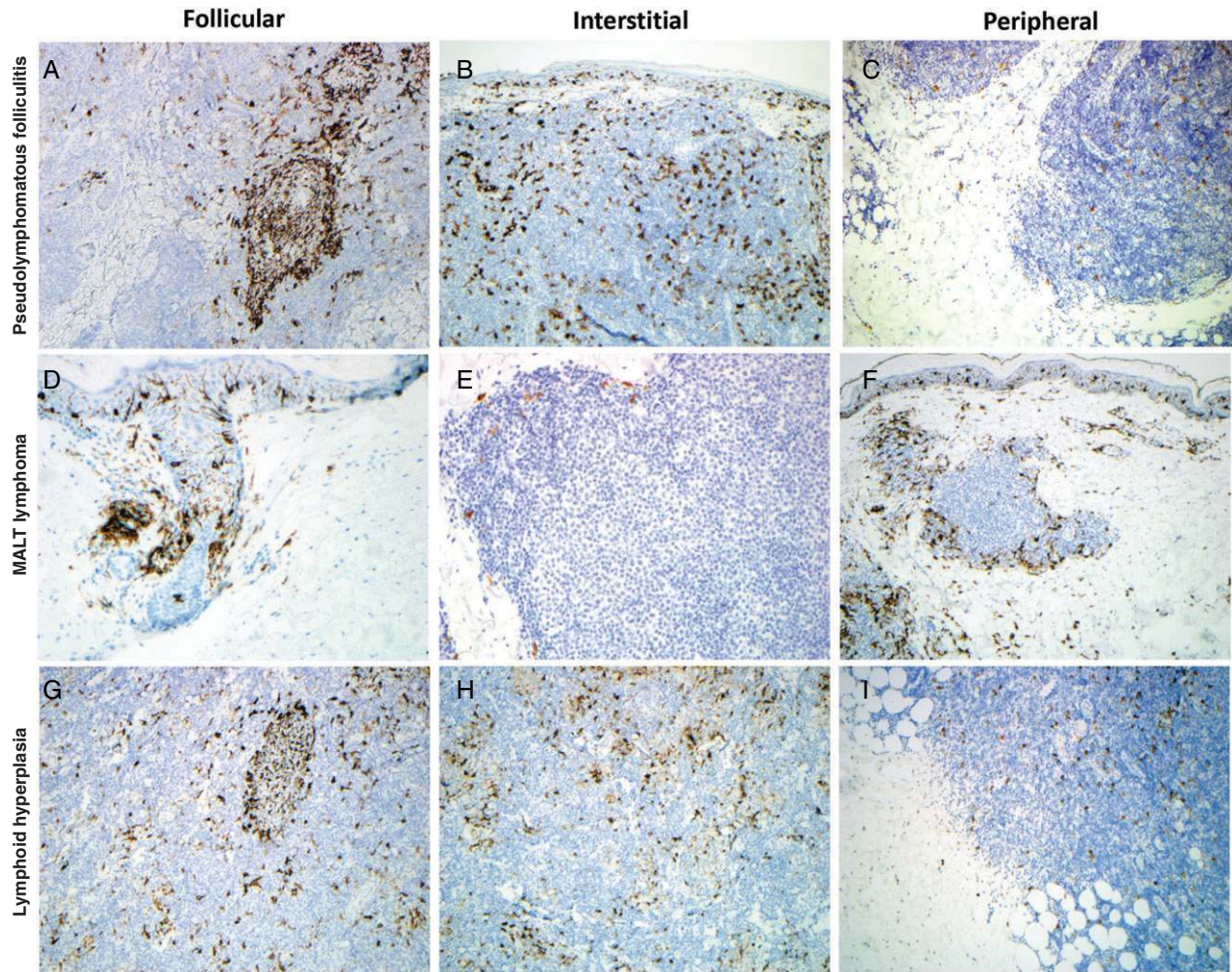


Fig. 2. CD1a in pseudolymphomatous folliculitis, mucosa-associated lymphoid tissue (MALT) lymphoma and cutaneous lymphoid hyperplasia (CLH). Immunohistochemical staining patterns for CD1a staining were classified as follicular (within the hair follicle epithelium), interstitial (within the lymphocytic infiltrate) or peripheral (around the periphery of lymphoid nodules or excluded from reactive lymphoid follicles). In pseudolymphomatous folliculitis, there are numerous CD1a+ dendritic cells are present within the hair follicle epithelium (A) and interspersed in the lymphocytic infiltrate (B). Peripheral staining is absent (C). MALT lymphoma with CD1a+ dendritic cells are present in the hair follicle epithelium (D). While interstitial CD1a+ dendritic cells are absent (E), there are numerous intensely-staining cells around the periphery of a lymphoid aggregate (F). CLH demonstrates numerous perifollicular (G) and interstitial (H) CD1a+ dendritic cells. Peripheral staining pattern is absent in CLH (I). (CD1a, $\times 10$).

Regarding patient characteristics in this study, there were no significant differences in patient age or gender. Of note, while all cases of pseudolymphomatous folliculitis were found on the head and neck, MALT lymphoma and CLH were more likely to present on the trunk or extremities ($p < 0.0001$ for pseudolymphomatous folliculitis vs. MALT lymphoma and $p < 0.01$ for pseudolymphomatous folliculitis vs. CLH).

Differentiating reactive cutaneous lymphoid proliferations (particularly pseudolymphomatous folliculitis and CLH) from MALT lymphoma is critical for appropriate patient care and can pose a significant histopathologic challenge. First, while lymphoid follicles, zones of plasma

cells and zones of neoplastic B cells are characteristically present in MALT lymphoma,⁹ cytologic atypia may be subtle and B cells may be outnumbered by T cells.^{24,25} Because numerous B cells are present in some cases of pseudolymphomatous folliculitis, the proportion of B cells and T cells cannot be relied upon to distinguish these two conditions.^{1,2,4} The identification of light chain restricted plasma cells (with immunohistochemistry or in situ hybridization) allows for the diagnosis of MALT lymphoma in over 70% of cases, and in the remaining 30% of cases clonal immunoglobulin heavy chain (IgH) rearrangement is detected by polymerase chain reaction. While detected in most cases of MALT

PD-1 and CD1a in lymphoma and pseudolymphoma

Table 3. CD1a and S100 in pseudolymphomatous folliculitis, MALT lymphoma and CLH

	CD1a			S100		
	Follicular	Interstitial	Peripheral	Follicular	Interstitial	Peripheral
Pseudo-lymphomatous folliculitis	11/11	10/11	0/8	11/11	11/11	0/10
MALT lymphoma	2/2	6/13	11/13	1/2	6/7	1/7
CLH	2/2	5/6	2/6	1/2	4/6	0/6
	NS	Pseudolymphomatous folliculitis > MALT*	MALT > pseudolymphomatous folliculitis** MALT > CLH***	NS	NS	NS

Pattern of distribution was defined as follicular (within the hair follicle epithelium), interstitial (scattered throughout the dermal infiltrate) and peripheral (around the periphery of lymphoid nodules, or excluded from lymphoid follicles). Listed are the number of cases that demonstrated positive staining in each distribution, over the total number of cases assessed. Pseudolymphomatous folliculitis is more likely to have CD1a+ dendritic cells in an interstitial pattern than is MALT lymphoma ($p < 0.04$). MALT lymphoma is more likely to demonstrate a peripheral pattern of CD1a+ dendritic cells than in pseudolymphomatous folliculitis ($p < 0.005$) or CLH ($p < 0.05$). CLH, cutaneous lymphoid hyperplasia; MALT, mucosa-associated lymphoid tissue; NS, not significant.

* $p < 0.04$.

** $p < 0.005$.

*** $p < 0.05$.

lymphoma, clonal IgH rearrangements of the TCR may be also rarely observed in pseudolymphomatous folliculitis and CLH. Additionally, T-cell receptor (TCR) rearrangements may be detected rarely in MALT lymphoma.^{1,4,17} In our cases of pseudolymphomatous folliculitis, 5 had TCR gene rearrangement testing (2 revealed clonal rearrangement, 3 were negative) and 3 patients had IgH gene rearrangement testing, all were negative.

The term pseudolymphomatous folliculitis was first coined by McNutt in 1986.²⁶ The entity was more formally established as a distinct subtype of CLH in 1999 by Arai et al., who distinguished it from other types of CLH because it was characterized by lymphocytic invasion into the epithelial lining of folliculosebaceous units, resulting in architectural distortion (but not destruction) of the hair follicle epithelium. Adnexal invasion was previously considered a histopathologic characteristic favoring cutaneous lymphoma over benign hyperplasia. Since then many authors have published case reports and small series, establishing that pseudolymphomatous folliculitis has distinct clinical and histopathological features including the presence of hyperplastic, irregularly shaped hair follicles in the setting of a benign lymphoid infiltrate distinctive from the changes observed in other forms of CLH.

Given the difficulties inherent in clinically and pathologically differentiating MALT lymphoma, pseudolymphomatous folliculitis and CLH, we sought new immunohistochemical tools to elucidate this differential diagnosis. In this study, we found that there is a significant difference

in expression of PD-1 by small round lymphocytes in pseudolymphomatous folliculitis, MALT lymphoma and CLH with mean expression of 54, 16 and 24% of lymphocytes, respectively. The proportion of lymphocytes expressing PD-1 is significantly higher in pseudolymphomatous folliculitis compared with MALT lymphoma ($p < 0.0001$), and likewise, higher in CLH than MALT lymphoma ($p = 0.03$). In contrast, we observed no significant difference in CD3+ T-cell density between the three groups ($p = 0.49$).

On evaluation of the proportion of CD3+ T cells expressing PD-1, we also found significant differences: our samples demonstrated a higher ratio of CD3+ cells expressing PD-1 in pseudolymphomatous folliculitis (mean 78%) than MALT lymphoma (mean 25%, $p < 0.0001$) or CLH (mean 40%, $p < 0.002$). The PD-1/CD3 ratio is also higher in CLH than in MALT lymphoma ($p < 0.01$). Our findings are in accordance with a recent study published by Cetinozoman et al., in which they found that 10% of all cells and 20% of T cells in MALT lymphomas express PD-1.²³ An additional important observation from our data is that in the cases of MALT lymphoma studied, CD1a+ dendritic cells tend to concentrate in areas of PD-1+ T cells.

Because of the significant overlapping features between CLH and pseudolymphomatous folliculitis, some consider pseudolymphomatous folliculitis to be a clinically distinct form of CLH. While these processes share the clinical presentation of an erythematous non-ulcerated nodule and the histopathological features of a dense

dermal lymphoid infiltrate occasionally with lymphoid follicles, there are some distinguishing features. Pseudolymphomatous folliculitis is usually localized to the face and occurs commonly in young men, histopathologically there is a distinctive hyperplasia of hair follicle epithelium. In this study, we also find that T cells more commonly express PD-1 (65–97%) in pseudolymphomatous folliculitis compared with CLH (21–57%). The distinct immunological mechanisms that result in these differences remains unclear, nevertheless, these findings support the distinction of pseudolymphomatous folliculitis from CLH.

PD-1, an inhibitory member of the CD28 family, is predominantly expressed on activated follicular helper T cells. This cell surface protein likely plays a role in peripheral tolerance. PD-1 over-expression has been observed in a variety of indolent T-cell lymphomas, including mycosis fungoides and primary cutaneous CD4+ small/medium-sized T-cell lymphoma (pcSMTCL).^{27–29} Of note, there is significant clinical and histopathologic overlap between pcSMTCL, CLH and pseudolymphomatous folliculitis; some European experts suggest that pcSMTCL actually belongs under the umbrella of pseudolymphoma, alongside pseudolymphomatous folliculitis and CLH.^{28,30} While it is thus unsurprising that PD-1 is also expressed in pseudolymphomatous folliculitis, the immunological role of PD-1 in this setting remains unclear. Further complicating the picture are reports of PD-1 expression in aggressive T-cell lymphomas including angioimmunoblastic T-cell lymphoma and adult T-cell leukemia/lymphoma.^{31,32}

Several recent papers have examined the expression patterns of PD-1 in cutaneous B-cell lymphomas. Cetinozoman et al. and Mitteldorf et al. have demonstrated that MALT lymphoma shows a greater density of PD-1+ T cells than in primary cutaneous follicle center lymphoma or cutaneous diffuse large B-cell lymphoma.^{23,33} Others have found that an increased density of PD-1+ T cells correlates with improved survival in systemic B-cell lymphomas.³⁴ Given our incomplete understanding of the varied functional roles of PD-1, it is difficult to speculate as to the significance of PD-1 expression in the pathogenesis of pseudolymphomatous folliculitis, CLH or cutaneous B-cell lymphomas. In our study, PD-1 expression was more commonly seen in the T cells of pseudolymphomatous folliculitis and CLH. Interestingly, absent from the lymphoid follicles in the cases, we studied were the PD-1 positive cells characteristically

observed in the lymphoid follicles of lymph nodes. All cases had tonsillar lymphoid tissue on the stained slide serving as a positive control. Further understanding of the role of PD-1+ cells in immune response to foreign antigens, such as in viral infection, may lend insight into the pathogenesis of pseudolymphomatous folliculitis.³⁵

Proliferation of CD1a-positive dendritic cells in the hair follicle epithelium has been reported as one of the defining characteristics of pseudolymphomatous folliculitis; we thus elected to study this immunohistochemical marker because of the diagnostic significance already ascribed to it in the literature.^{1,2} Although we found no notable difference in CD1a expression within the hair follicles in pseudolymphomatous folliculitis, MALT lymphoma or CLH, there was a striking difference in the distribution of CD1a+ cells in the dermal component of the infiltrate. Pseudolymphomatous folliculitis and CLH demonstrated CD1a staining of dendritic cells diffusely throughout the dermal infiltrate. In contrast, in MALT lymphomas, CD1a+ cells were concentrated around the periphery of the nodular aggregates of neoplastic B cells, absent from reactive lymphoid follicles, with few scattered throughout the dermis ($p < 0.005$ and < 0.05 vs. pseudolymphomatous folliculitis and CLH, respectively). This pattern is probably similar to the aggregates of CD123+ plasmacytoid dendritic cells previously reported by Kempf.³⁶ The diffuse pattern of CD1a staining in pseudolymphomatous folliculitis found in this study is consistent with CD1a staining patterns reported in other forms of CLH, also termed cutaneous pseudolymphomas. Our findings suggest that CD1a staining can be a valuable adjunct in identifying a cutaneous lymphoid infiltrate as a MALT lymphoma – a peripheral pattern supports the diagnosis of MALT lymphoma, while an interstitial pattern is more consistent with pseudolymphomatous folliculitis or CLH.

CD1a is a transmembrane cell-surface protein expressed by dendritic cells, immature T cells, activated monocytes and a subset of B cells.³⁷ In association with $\beta 2$ -microglobulin, CD1a presents glycolipid antigens to α/β T cells, allowing the T cells to detect changes in the lipid milieu that may be caused by infection, malignancy or inflammation.³⁸ CD1a+ dendritic cells are abundant in a variety of T-cell-rich lymphoproliferative disorders, both benign and malignant. These include reactive conditions such as atopic dermatitis, psoriasis and polymorphous light eruption. Schmutz

et al. hypothesize that the presence of CD1a+ dendritic cells in reactive conditions indicates active antigen presentation to T cells.³⁷

While the presence of CD1a+ dendritic cells has been reported in T-cell neoplasms including mycosis fungoides and lymphomatoid papulosis, several studies have shown depletion of CD1a+ dendritic cells cutaneous B-cell lymphomas.^{37,39} Given the purported role of CD1a+ dendritic cells in the presentation of antigens to T cells, dendritic cells may preferentially associate with T-cell-rich areas. This would be consistent with the observations in this study: CD1a+ dendritic cells are numerous throughout the T-cell-rich dermal infiltrate of CLH and pseudolymphomatous folliculitis, abundant in the T-cell-rich periphery of the dermal nodules of MALT lymphoma, but absent within the neoplastic proliferation of B cells, and absent in reactive lymphoid follicles. Pigozzi et al. suggest that the neoplastic B-cell proliferation may hinder recruitment of CD1a to T-cell-rich areas.³⁹ The restriction of CD1a staining to areas of high T-cell density in B-cell lymphomas has previously been reported.³⁷

Others have reported S100+ dendritic cells in the hair follicle epithelium as a major diagnostic criterion in diagnosis of pseudolymphomatous folliculitis.^{1,2} However, while we did find numerous intra-follicular S100+ dendritic cells in pseudolymphomatous folliculitis, our results indicate no statistically significant increase in intra-follicular S100+ dendritic cells between pseudolymphomatous folliculitis, MALT lymphoma and CLH. We also did not find any significant difference in the pattern of dermal distribution of S100+ cells. Although historically

S100 staining has been considered integral to identifying cases of pseudolymphomatous folliculitis, it may be less useful for discriminating between pseudolymphomatous folliculitis, from MALT lymphoma and CLH. The observation that the CD1a and S100 staining patterns differ, and that often more CD1a-positive cells were observed than S100+ cells, reflects that they are staining different populations of dermal dendritic cells.

In summary, our immunohistochemical evaluation found that cases of pseudolymphomatous folliculitis have a statistically significant increase in PD-1 expression by T lymphocytes compared with MALT lymphoma. We also found that although S100 staining was not helpful in differentiating these conditions, the pattern of CD1a staining was informative, with MALT lymphoma significantly more likely to have CD1a+ cells at the periphery of the dermal lymphoid infiltrate, with few CD1a+ cells in lymphoid follicles, whereas pseudolymphomatous folliculitis and CLH have CD1a+ dendritic cells scattered throughout the infiltrate. While the findings of this study may be limited by spectrum (sampling) bias, our results suggest that immunohistochemical resources may be helpful in distinguishing these dense dermal lymphocytic infiltrates. MALT lymphoma is most readily distinguished from pseudolymphomatous folliculitis and CLH by the presence of light chain restricted plasma cells or demonstration of IgH gene rearrangements. However in up to 30% of cases of MALT lymphoma, clonality is not detected. In these cases, PD-1 and CD1a may be effective additions to the diagnostic algorithm.

References

1. Arai E, Shimizu M, Hirose T. A review of 55 cases of cutaneous lymphoid hyperplasia: reassessment of the histopathologic findings leading to reclassification of 4 lesions as cutaneous marginal zone lymphoma and 19 as pseudolymphomatous folliculitis. *Hum Pathol* 2005; 36: 505.
2. Arai E, Okubo H, Tsuchida T, Kitamura K, Katayama I. Pseudolymphomatous folliculitis: a clinicopathologic study of 15 cases of cutaneous pseudolymphoma with follicular invasion. *Am J Surg Pathol* 1999; 23: 1313.
3. Kibbi A, Scrimenti R, Koenig R, Mihm M. A solitary nodule of the left cheek. *Arch Dermatol* 1988; 124: 1271.
4. Kazakov D, Belousova I. Hyperplasia of hair follicles and other adnexal structures in cutaneous lymphoproliferative disorders: a study of 53 cases, including so-called pseudolymphomatous folliculitis and overt lymphomas. *Am J Surg Pathol* 2008; 32: 1468.
5. Dargent JL, Debois J, Sass U, Theunis A, André J, Simonart T. Unusual T cell pseudolymphoma with features of so-called pseudolymphomatous folliculitis. *Dermatology* 2002; 204: 159.
6. Fujimura T, Hidaka T, Hashimoto A, Aiba S. Dermoscopy findings of pseudolymphomatous folliculitis. *Case Rep Dermatol* 2012; 4: 154.
7. Kwon EJ, Kristjansson AK, Meyerson HJ, et al. A case of recurrent pseudolymphomatous folliculitis: a mimic of cutaneous lymphoma. *J Am Acad Dermatol* 2009; 60: 994.
8. Lee M-W, Lee D-K, Choi J-H, Moon K-C, Koh J-K. Clinicopathologic study of cutaneous pseudolymphomas. *J Dermatol* 2005; 32: 594.
9. Baldassano MF, Bailey EM, Ferry JA, Harris NL, Duncan LM. Cutaneous lymphoid hyperplasia and cutaneous marginal zone lymphoma: comparison of morphologic and immunophenotypic features. *Am J Surg Pathol* 1999; 23: 88.
10. Nakamura M, Kabashima K, Tokura Y. Pseudolymphomatous folliculitis presenting with multiple nodules. *Eur J Dermatology* 2009; 19: 262.
11. Petersson F. Pseudolymphomatous folliculitis with marked lymphocytic folliculo- and focal epidermotropism—expanding the morphologic spectrum. *Am J Dermatopathol* 2011; 33: 323.
12. Gutte RM. Pseudolymphomatous folliculitis: a distinctive cutaneous lymphoid hyperplasia. *Indian J Dermatol* 2013; 58: 278.
13. Ploysangam T, Breneman DL, Mutasim DF. Cutaneous pseudolymphomas. *J Am Acad Dermatol* 1998; 38: 877.

14. Kakizaki A, Fujimura T, Numata I, Hashimoto A, Aiba S. Pseudolymphomatous folliculitis on the nose. *Case Rep Dermatol* 2012; 4: 27.
15. Servitje O, Gallardo F, Estrach T, Pujol RM, Blanco A. Clinical and laboratory investigations primary cutaneous marginal zone B-cell lymphoma: a clinical, histopathological, immunophenotypic and molecular genetic study of 22 cases. *Br J Dermatol* 2002; 147: 1147.
16. Isaacson P, Chott A, Nakamura S, Muller-Hermelink HK, Harris NL, Swerdlow SH. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO classification of tumors of haematopoietic and lymphoid tissues*, 4th ed. Lyon: International Agency for Research on Cancer, 2008; 214.
17. Nihal M, Mikkola D, Horvath N, et al. Cutaneous lymphoid hyperplasia: a lymphoproliferative continuum with lymphomatous potential. *Hum Pathol* 2003; 34: 617.
18. NCCN. Non-Hodgkin's Lymphomas (Version 1.2014). 2014. http://www.nccn.org/professionals/physician_gls/pdf/nhl.pdf [accessed on 27 February, 2014].
19. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood* 2005; 105: 3768.
20. de Leval L, Harris NL, Longtine J, Ferry JA, Duncan LM. Cutaneous B-cell lymphomas of follicular and marginal zone types: use of Bcl-6, CD10, Bcl-2, and CD21 in diagnosis and classification. *Am J Surg Pathol* 2001; 25: 732.
21. Krenács L, Tiszalvicz L, Krenács T, Boumsell L. Immunohistochemical detection of CD1A antigen in formalin-fixed and paraffin-embedded tissue sections with monoclonal antibody 010. *J Pathol* 1993; 171: 99.
22. Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Takeda I, Akagi T. Immunohistochemical localization and distribution of S-100 proteins in the human lymphoreticular system. *Am J Pathol* 1984; 116: 497.
23. Çetinözman F, Koens L, Jansen PM, Willemze R. Programmed death-1 expression in cutaneous B-cell lymphoma. *J Cutan Pathol* 2014; 41: 14.
24. Geyer JT, Ferry JA, Longtine JA, Flotte TJ, Harris NL, Zukerberg LR. Characteristics of cutaneous marginal zone lymphomas with marked plasmacytic differentiation and a T cell-rich background. *Am J Clin Pathol* 2010; 133: 59.
25. Bailey EM, Ferry JA, Harris NL, Mihm MC, Jacobson JO, Duncan LM. Marginal zone lymphoma (low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type) of skin and subcutaneous tissue: a study of 15 patients. *Am J Surg Pathol* 1996; 20: 1011.
26. McNutt NS. Cutaneous lymphohistiocytic infiltrates simulating malignant lymphoma. In Murphy G, Mihm M, eds. *Lymphoproliferative disorders of the skin*. Boston: Butterworths, 1986; 256.
27. Rodríguez Pinilla SM, Roncador G, Rodríguez-Peralto JL, et al. Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma expresses follicular T-cell markers. *Am J Surg Pathol* 2009; 33: 81.
28. Cetinozman F, Jansen PM, Willemze R. Expression of programmed death-1 in primary cutaneous CD4+positive small/medium-sized pleomorphic T-cell lymphoma, cutaneous pseudo-T-cell lymphoma, and other types of cutaneous T-cell lymphoma. *Am J Surg Pathol* 2012; 36: 109.
29. Wada DA, Wilcox RA, Harrington SM, Kwon ED, Ansell SM, Comfere NI. Programmed death 1 is expressed in cutaneous infiltrates of mycosis fungoides and Sézary syndrome. *Am J Hematol* 2011; 86: 325.
30. Beltraminelli H, Leinweber B, Kerl H, Cerroni L. Primary cutaneous CD4+ small-/medium-sized pleomorphic T-cell lymphoma: a cutaneous nodular proliferation of pleomorphic T lymphocytes of undetermined significance? A study of 136 cases. *Am J Dermatopathol* 2009; 31: 317.
31. Yu H, Shahsafaei A, Dorfman DM. Germinal-center T-helper-cell markers PD-1 and CXCL13 are both expressed by neoplastic cells in angioimmunoblastic T-cell lymphoma. *Am J Clin Pathol* 2009; 131: 33.
32. Kozako T, Yoshimitsu M, Fujiwara H, et al. PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients. *Leukemia* 2009; 23: 375.
33. Mitteldorf C, Bieri M, Wey N, et al. Expression of programmed death-1 (CD279) in primary cutaneous B-cell lymphomas with correlation to lymphoma entities and biological behaviour. *Br J Dermatol* 2013; 169: 1212.
34. Muenst S, Hoeller S, Willi N, Dirnhofera S, Tzankov A. Diagnostic and prognostic utility of PD-1 in B cell lymphomas. *Dis Markers* 2010; 29: 47.
35. Krishnan C, Warnke RA, Arber DA, Natkunam Y. PD-1 expression in T-cell lymphomas and reactive lymphoid entities: potential overlap in staining patterns between lymphoma and viral lymphadenitis. *Am J Surg Pathol* 2010; 34: 178.
36. Kutzner H, Kerl H, Pfaltz MC, Kempf W. CD123-positive plasmacytoid dendritic cells in primary cutaneous marginal zone B-cell lymphoma: diagnostic and pathogenetic implications. *Am J Surg Pathol* 2009; 33: 1307.
37. Schmuth M, Sidoroff A, Danner B, Topar G, Sepp NT. Reduced number of CD1a+ cells in cutaneous B-cell lymphoma. *Am J Clin Pathol* 2001; 116: 72.
38. De Jong A, Peña-Cruz V, Cheng T-Y, Clark RA, Van Rhijn I, Moody DB. CD1a-autoreactive T cells are a normal component of the human $\alpha\beta$ T cell repertoire. *Nat Immunol* 2010; 11: 1102.
39. Pigozzi B. Expression of the CD1a molecule in B-and T-lymphoproliferative skin conditions. *Oncol Rep* 2006; 15: 347.