



Cyclin D1 expression, cell proliferation, and clonal persistence characterize primary cutaneous CD4⁺ small or medium T-cell lymphoproliferative disorder

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Date of submission 5 July 2022

Accepted for publication 1 November 2022

Published online Article Accepted 7 November 2022

Oschlies I, Kock K, Wüseke T, Richter J, Koch K, Wehkamp U & Klapper W

(2023) *Histopathology* 82, 485–494. <https://doi.org/10.1111/his.14834>

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Aims: The aim was to gain insight into the biology of primary cutaneous CD4⁺ small/medium T-cell lymphoproliferative disorder (PCSM-LPD).

Methods: We describe the histopathological and clinical characteristics of 177 PCSM-LPD diagnosed at our consultation centre. We performed immunohistochemical multistaining in a subset of cases ($n = 46$) including PD1, Cyclin D1, and multiple markers of proliferation. We evaluated clonal T-cell-receptor-(TCR) rearrangements and used tissue microdissection to analyse TCR-clonality of PD1(+) cells.

Results: The cohort of $n = 177$ PCSM-LPD included 84 males and 93 females (median age 57, range 13–85). Clinical presentation was as a solitary nodule or plaque (head and neck > trunk > extremities). Most patients were treated by local excision or steroids (96%, 69/72); relapses occurred in 12/65 (18%) of patients with follow up. Histopathology revealed the

predominance of a nodular pattern (75%, 134/177) and frequent clustering of PD1(+) large cells (70%, 103/147). We detected Cyclin D1 and PD1 coexpression (>10% of PD1(+)-cells) in 26/46 (57%), which was not associated with *CCND1* breaks or amplifications. PD1(+)-cells in PCSM-LPDs showed a significantly higher expression of proliferation-associated proteins compared to PD1(–)-cells. A clonal TCR-rearrangement was present in 176/177 (99%), with a clonal persistence in 7/8 patients at relapse including distant sites. Tissue-microdissection revealed PD1 (+)-cells as the source of clonality, whilst PD1(–)-cells remained polyclonal.

Conclusion: PCSM-LPD is a clinically indolent, albeit neoplastic, disease driven by clonal expansion of PD1 (+)-cells. We demonstrate Cyclin D1-expression associated with accelerated proliferation as a surprising new biological feature of the disease.

Keywords: clonality, clusters, Cyclin D1, follicular T-helper cells, microdissection

Introduction

Primary cutaneous CD4⁺ small/medium T-cell lymphoproliferative disorder (PCSM-LPD) is a cutaneous disease that rarely spreads beyond skin.¹ Within the

skin, PCSM-LPD mostly present as a single nodule that can be treated successfully by excision or local therapy.^{2–5} The rather benign clinical presentation of PCSM-LPD led to a name change of the disease from “lymphoma” to “lymphoproliferative disorder” in the

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WHO classification 2016.¹ The diagnosis of PCSM-LPD by histological assessment can be challenging, as the lesions contain a fairly large number of B-cells that may mimic pseudolymphoma or even B-cell neoplasms.^{6–8} The biology of PCSM-LPD is poorly understood. Within lesions, eponymous CD4(+) T-cells are the most abundant cell population. Only a subset of these cells show a T-follicular helper cell (Tfh) phenotype and is arranged in clusters within the bulk of PD1 negative B- and T-cells.⁶ Clonal rearrangement of TCR has been demonstrated by bulk analysis of PCSM-LPD^{9,10}; however, it remains uncertain which T-cell population represents the clonal population. Since somatic mutations are rarely found in PCSM-LPD,⁵ the question of whether PCSM-LPD present an inflammatory or neoplastic disease seems to be unanswered.

We aimed to gain new insights into the underlying cellular biology of PCSM-LPDs by studying cell-cycle-associated markers expressed in the PD1(+)-cell population. Here we provide novel insights by demonstrating clonal persistence over time and distant localization reminiscent of neoplastic disease. In addition, we prove by laser-microdissection that the PD1 (+)-cells are the clonally expanded population.

Materials and methods

SELECTION OF CASES

In all, 177 cases of PCSM-LPD were identified in the files of the Hematopathology Section and Lymph Node Registry at the Department of Pathology University Hospital Schleswig-Holstein, Campus Kiel, Germany, with diagnosis between 2011–2020. All cases were reviewed and fulfilled the following criteria: (i) specimen submitted as single lesion without a previous history or concurrent clinical features of systemic or cutaneous lymphoma (e.g. mycosis fungoides), (ii) histopathology compatible with features of PCSM-LPD as previously described,^{1,6,9,10} (iii) evidence of TCR-clonality, and (iv) exclusion of a cutaneous B-cell lymphoma in cases rich in B-cells or plasma-cells by light chain immunohistochemistry and/or B-cell receptor (BCR)-clonality analysis. Clinical data were retrieved by direct contact with treating clinicians at the time of diagnosis retrospectively by a survey using a questionnaire. Due to the retrospective nature of the study, the clinical data differed in completeness (Table 1). *N* = 26 cases of pseudolymphomas lacking the diagnostic criteria of PCSM-LPD served as controls. These cases included nine cutaneous B-cell rich pseudo lymphomas and 17

cutaneous T-cell rich pseudo lymphomas (Table S2). In all cases, cutaneous lymphoma was excluded by the appropriate immunophenotypical or molecular testing, depending on the individual cases.

ETHICS APPROVAL STATEMENT

This study was conducted in accordance with the recommendations of the Ethics Advisory Committee of the Medical Faculty of the University of Kiel (review numbers D464/2017 and 507/2020).

IMMUNOHISTOCHEMISTRY AND MULTISTAINING

Immunohistochemistry and double-staining were performed using Histofine Simple Stain MAX (Medac, Chicago, IL, USA) and UltraVision LP Detection System AP Polymer (Thermo Fisher, Waltham, MA, USA) using antibodies as described in Table S1. The percentages of B-cells (CD20), T- cells (CD3), PD1(+)-cells (PD1), and Cyclin D1(+) lymphatic cells as well as the proliferation rate (Ki67) were assessed microscopically on whole-slide sections in 5% steps by visual inspection. Double staining of Cyclin D1 and PD1 was performed on 46 PCSM-LPDs and 26 pseudolymphomas. We also performed double-staining using various markers of the cell-cycle and proliferation (Ki67, Survivin, pH 3), which are positive in different phases of the cell cycle. Cyclin D1 mediates the G1-S-phase transition in the cell cycle. The assessed proliferation-associated proteins are expressed during different phases of the cell cycle. Ki67 is expressed in all cell cycle phases, except G0, and is detectable during the G1, S, G2, and M phases. Survivin has been reported to be restricted to the S, G2, and M phases, and pH 3 expression is limited to the mitotic phase (M). In order to understand whether the overexpression of cyclin D1 is associated with a dysregulated cell cycle, for example, a shortened G1 phase, we calculated the ratios between the percentage of Ki67-and survivin-and Ki67 and pH 3 positive cells as established previously.¹² Plasma cell content was interpreted in all cases with available kappa/lambda staining semiquantitatively by visual inspection as (i) scattered/few, (ii) intermediate, and (iii) increased with cluster formation. If not otherwise specified, double-staining was evaluated by visual inspection of 200 cells in each specimen by manual counting of positive cells.

MICRODISSECTION AND IMMUNOGENE REARRANGEMENTS

Bulk tumour DNA was analysed for clonal rearrangement of TCR using the BIOMED2 protocol.¹³

Table 1. Clinical presentation of PCSM-LPD

	PCSM-LPD cohort (<i>n</i> = 177)*	Band-like (<i>n</i> = 26)	Nodular (<i>n</i> = 134)	<i>P</i> -value (nodular versus band-like pattern)
Age (median, range)	57 (13–85)	54 (21–82)	59 (13–85)	0.81
Gender (m/f)	84/93	7/19	69/65	0.03
Number of lesions				
Single lesion	98/98 (100%)	14/14 (100%)	77/77 (100%)	n.e.
Multiple lesions	0/98 (0%)	0/14 (0%)	0/77 (0%)	n.e.
Localization				
Head/neck	97/172 (56%)	8/26 (31%)	81/129 (63%)	<0.01
Trunk	43/172 (25%)	12/26 (46%)	27/129 (21%)	0.01
Extremities	32/172 (19%)	6/26 (23%)	21/129 (16%)	0.40
Treatment				
Biopsy only	0/72 (0%)	0/11 (0%)	0/55 (0%)	n.e.
Excision	57/72 (79%)	7/11 (64%)	45/55 (82%)	0.23**
Topical steroids	12/72 (17%)	4/11 (36%)	7/55 (13%)	0.08**
Excision and radiation	1/72 (1%)	0/11 (0%)	1/55 (2%)	n.e.
Systemic cytostatic	0/72 (0%)	0/11 (0%)	0/55 (0%)	n.e.
Other	2/72 (3%)	0/11 (0%)	2/55 (4%)	n.e.
Outcome				
Complete response, no recurrence	53/65 (82%)	8/12 (67%)	44/50 (88%)	0.09
Recurrence	12/65 (18%)	4/12 (33%)	6/50 (12%)	0.09
Median follow-up in months (range)	8 (1–33)	10 (2–19)	7 (1–33)	n.e.

Data indicated as number of cases (*n*) and percentage (%). Group comparison was performed using Fisher's exact test or the Mann–Whitney Test (#) and significant *P*-values (*P* < 0.05) are shown in bold.

n.e., Not evaluable.

*Including *n* = 17 additional cases, which presented with a histopathology intermediate between nodular and band-like. These cases were excluded from the statistical analysis.

***P*-value was evaluated without the categories "biopsy only" and "systemic cytostatic," as no patient received that treatment. Data for median follow-up were available for 40 patients (5 band-like, 35 nodular pattern).

Tissue-microdissection was performed using MMI CellCut (Molecular Machines and Industries, Eching, Germany). We dissected 1.6 mm² of the PD1(+) and PD1(−) areas. DNA was extracted using a laser-microdissected tissue DNA Micro Kit (Qiagen, Hilden, Germany). We utilised the supplied protocol for laser-microdissected tissues, with minor modifications provided within the supplementary data.

In all, 5 µl DNA was used for the detection of TCR rearrangements in PD1(+) and PD1(−) areas using the BIOMED2 protocol.¹³

FLUORESCENCE IN SITU HYBRIDIZATION

Fluorescence *in situ* hybridization (FISH) for *Cyclin D1* (CCND1) breaks or copy number gains was performed as previously described.¹⁴

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism 7 for Windows (v. 7.02, San Diego, CA, USA).

Results

HISTOLOGICAL FEATURES, CELLULAR COMPOSITION, AND CLONALITY OF PCSM-LPD

All 177 cases fulfilled the diagnostic criteria of a PCSM-LPD^{9,11} with a dermal T-cell dominated nonepitheliotropic mixed cellularity infiltration consisting of small- to medium-sized lymphoid cells with variable numbers of admixed larger cells, histiocytes, B-cells, and plasma-cells. In our cohort the previously described histological patterns of infiltration were identified as nodular in 134/177 (75%) and band-like in 26/177 (15%). In 17/177 (10%), the histological pattern was intermediate or not assessable owing to tissue size and/or quality. The CD4:CD8 ratio was >5:1 in 95% (70/74) and >3:1 in 5% (4/74) of cases with available data. For 153/177 samples PD1 staining was available. The content of PD1(+) cells within the lesion was higher in patients with a band-like than in those with a nodular pattern, although the difference was not statistically significant (mean 23.6%, $n = 25$ and mean 18.6%, $n = 128$, respectively, $P = 0.07$, t -test). Clustering of PD1(+)-cells was observed by visual inspection in 103 of 147 cases evaluable for this feature, (70%) with 15/25 (60%) band-like, 85/113 (75%) nodular lesions, and 3/9 (33%) intermediate histological pattern ($P = 0.14$, Fisher's exact test).

We did not detect differences in B-cell content between band-like and nodular PCSM-LPD (mean 20.4%, and mean 23.7%, respectively, $P = 0.19$, t -test). In samples with Ki67 staining being available, the number of positive cells were 5–30% in 60/67 (90%), 31–45% in 6/67 (9%). Only 1/67 (1%) of samples showed 50% Ki-67-positive cells. Germinal centres were rare (3/134, 2%) and exclusively found in the nodular pattern. 64/108 (59%) of samples showed scattered/few, 27/108 (25%) intermediate, and 17/108 (16%) increased plasma cells. Plasma cells displayed polyclonal light chain expression in all cases analysed (108/108, 100%). In line with these findings and/or selection criteria, BCR-clonality was absent in all cases (56/56, 100%) and TCR-clonality was detected in all PCSM-LPD with evaluable results (176/177, 99%). Histopathological and molecular features of the 26 pseudolymphomas are provided in Table S2.

CLINICAL FEATURES OF PCSM-LPD

The disease occurred in virtually all age groups (median age 57, range 13–85) without major sex predilection and presented exclusively as single

lesions (98/98, 100%, Table 1). When detailed descriptions were available, the lesions were described as nodes (45/74, 61%), plaques (17/74, 23%), patches (6/74, 8%), or tumours (5/74, 7%). Only one case (1/74, 1%) with a diameter of 2.8 cm displayed ulceration. The largest diameter of the lesion ranged between 0.2 cm to 6.0 cm, and 44/58 (76%) cases presented with a lesion >1 cm. All lesions were described as solitary (85/85); no patient with available clinical data presented at initial diagnosis with multiple or disseminated lesions. A drug association was denied in 85/85 case with available clinical data. Most of the patients were treated with excision or topical steroids (69/72, 96%, Table 1). In 12/65 (18%) patients with available clinical data, the lesion reappeared. None of the patients with data on staging and follow-up developed extensive skin lesions, systemic disease, or skin lesions compatible with mycosis fungoides. Lesions with a nodular pattern were significantly more often localised in the head/neck region, whereas lesions with a band-like pattern occurred predominantly on the trunk ($P < 0.01$, $P < 0.01$, respectively, Fisher's exact test, Table 1), which is in line with observations of others.⁵

CYCLIN D1 EXPRESSION IN PD1(+) CELLS IS A TYPICAL FEATURE OF PCSM-LPD

Whilst nonlymphatic cells such as macrophages, endothelium, and other stromal cells physiologically might express Cyclin D1, this protein is not detectable by immunohistochemistry in reactive lymphatic cells.¹⁵ Nevertheless, Cyclin D1 expression in lymphatic cells is a typical feature of several lymphatic neoplasias.¹⁶ Interestingly we detected Cyclin D1-positive cells in PCSM-LPD by immunohistochemistry. The number and distribution of Cyclin D1(+)-cells were suggestive for lymphatic PD1(+)-cells to be the positive population (Figure 1). We confirmed coexpression of Cyclin D1 and PD1 using double-staining in 46 randomly selected cases of PCSM-LPDs. Of note, only a subset of PD1(+)-cells coexpressed Cyclin D1 and PD1(–) stromal cells coexpressing Cyclin D1 were detected too (Figure 2). By visual inspection, 57% (26/46) of PCSM-LPDs showed a Cyclin D1 expression in more than 10% of PD1(+)-cells (max. 35%), whereas the remaining samples (20/46, 43%) showed Cyclin D1 in <10% of PD1(+)-cells. In the samples with >10% of Cyclin D1(+) cells, by eyeballing the Cyclin D1 expression was analysed more in detail by counting Cyclin D1(+) cells among 200 PD1(+)-cells. Counting revealed Cyclin D1 coexpression in PD1(+)-cells in <10% in 20/46

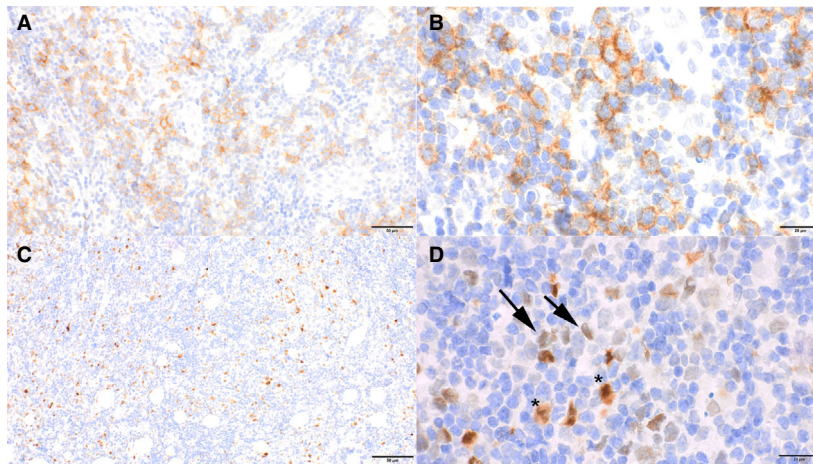


Figure 1. Representative images of an individual PCSM-LPD stained for PD1 (A,B) with formation of clusters. Staining for Cyclin D1 (C,D) with an overexpression pattern reminiscent of the PD1 staining. Arrows indicate expression of Cyclin D1 in lymphatic cells with slightly weaker nuclear staining in this case compared to histiocytic cells (asterisks) (original magnification 400× in A, C, 1000× in B, D).

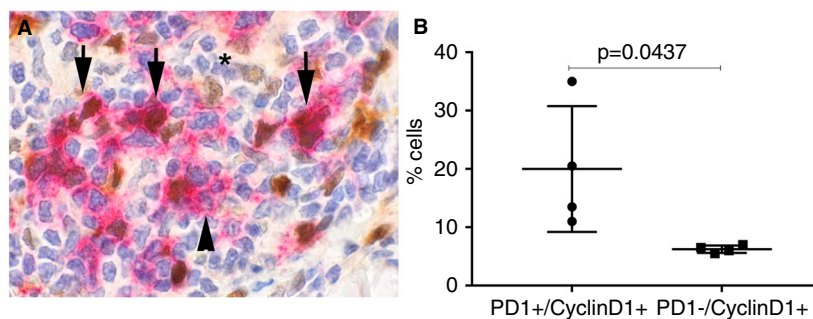


Figure 2. Cyclin D1 expression in PCSM-LPD. Immunohistochemistry double-staining for PD1 (red) and cyclin D1 (brown) in a representative example (A). Arrows point to PD1(+)- cells coexpressing Cyclin D1, arrowhead indicate PD1(+) cell without coexpression of Cyclin D1, and asterisk in the area of Cyclin D1(+) macrophages/stromal cells (original magnification 1000×). Percentage of PD1(+) and PD1(-)-cells coexpressing Cyclin D1 quantified by counting in five representative PCSM-LPD (B, *t*-test).

(43%), 10–15% in 11/46 (24%), 16–20% in 11/46 (24%), 21–25% in 3/46 (6%), and 35% in 1/46 (1%). There was no statistically significant difference between nodular and band-like patterns with regard to Cyclin D1 and PD1 expression status (data not shown). PD1(–) cells coexpressing Cyclin D1 was quantified in five cases in which all presented a higher percentage of PD1(+)/Cyclin D1(+) cells compared to PD1(–)/Cyclin D1(+) cells (Figure 2, $P = 0.04$). Only 2/26 (8%) of pseudolymphomas showed a coexpression of Cyclin D1 in PD1(+)-cells in >10% (10% and 14% Cyclin D1(+)-cells, respectively). Of note, one case of pseudolymphomas with PD1(+)/cyclin D1(+) cells was polyclonal in the TCR analysis of the bulk tissue; in the other case TCR analysis was inconclusive. Both cases had scattered small groups of PD1(+) cells but lacked obvious PD1 clustering

(additional data on clonality of pseudolymphomas is shown in Table S2).

To gain insight into the pathomechanism of Cyclin D1 expression, we performed fluorescence *in situ* hybridization (FISH) to identify chromosomal *CCND1* aberrations. Among 15 cases of PCSM-LPD expressing Cyclin D1 at high levels (>15% of PD1(+)-cells), neither breaks nor copy number gains of the *CCND1* gene locus were detected (data not shown).

PD1(+)-CELLS IN PCSM-LPD ARE THE MAIN PROLIFERATING CELL POPULATION

Cyclin D1 expression is considered a pathogenetic mechanism in B-cell lymphomas that drives cell proliferation.¹⁶ Although PD1(+)-cells are arranged in clusters within the PCSM-LPD, it is impossible to

determine the phenotype of these cells by conventional staining and visual inspection. Thus, we performed double-staining using various markers of the cell-cycle and proliferation (Ki67, Survivin, pH 3) which are positive in different phases of the cell cycle. Positivity was detected in PD1(+) and PD1(-)-cells (Figure 3). We quantified coexpression in PD1(+) and PD1(-)-cells by counting within five PCSM-LPDs. The coexpression of the proliferation markers appeared to be significantly higher in PD1(+) compared to PD1(-)-cells in the same lesion ($P < 0.01$, $P < 0.01$, and $P = 0.03$) for Ki67, pH 3, and Survivin respectively (Figure 3). A cell cycle dysregulation such as arrest in a certain phase of the cell cycle as previously described¹² was not detected (data not shown).

T-CELL CLONES CAN BE TRACKED OVER MULTIPLE BIOPSIES AND TIMEPOINTS

We identified eight patients for whom two biopsies from different timepoints and/or localization were available. All but one of these patients showed identical TCR fragment lengths in the biopsies of each individual patient, suggesting an identical T-cell clone

(Table 2). Thus, most lesions were clonally related within the corresponding patient. A clonal relationship was also observed for multiple biopsies occurring in the same area of the body in five patients, of which three were biopsied within ≤ 2 months, most likely representing second excision biopsies of the initial lesion, and were not interpreted as relapses (patients 3, 4, and 6, Table 2). However, two patients showed clonally related lesions in the same body area occurring subsequently at 36 and 24 months between the first and the second lesion, respectively (patients 2 and 7, Table 2). Moreover, in two patients lesions at distant body sites were clonally related (patients 1 and 5, Table 2), indicating that the clonal population in PCSM-LPD can disseminate within the skin over longer distances. Interestingly, in one of those patients (patient 5), the two lesions occurred with a time interval of 24 months.

PD1(+)-CELLS ARE THE CLONAL POPULATION IN PCSM-LPD

We selected two cases with well-developed PD1(+)-cell clusters applicable for further laser-microdissection

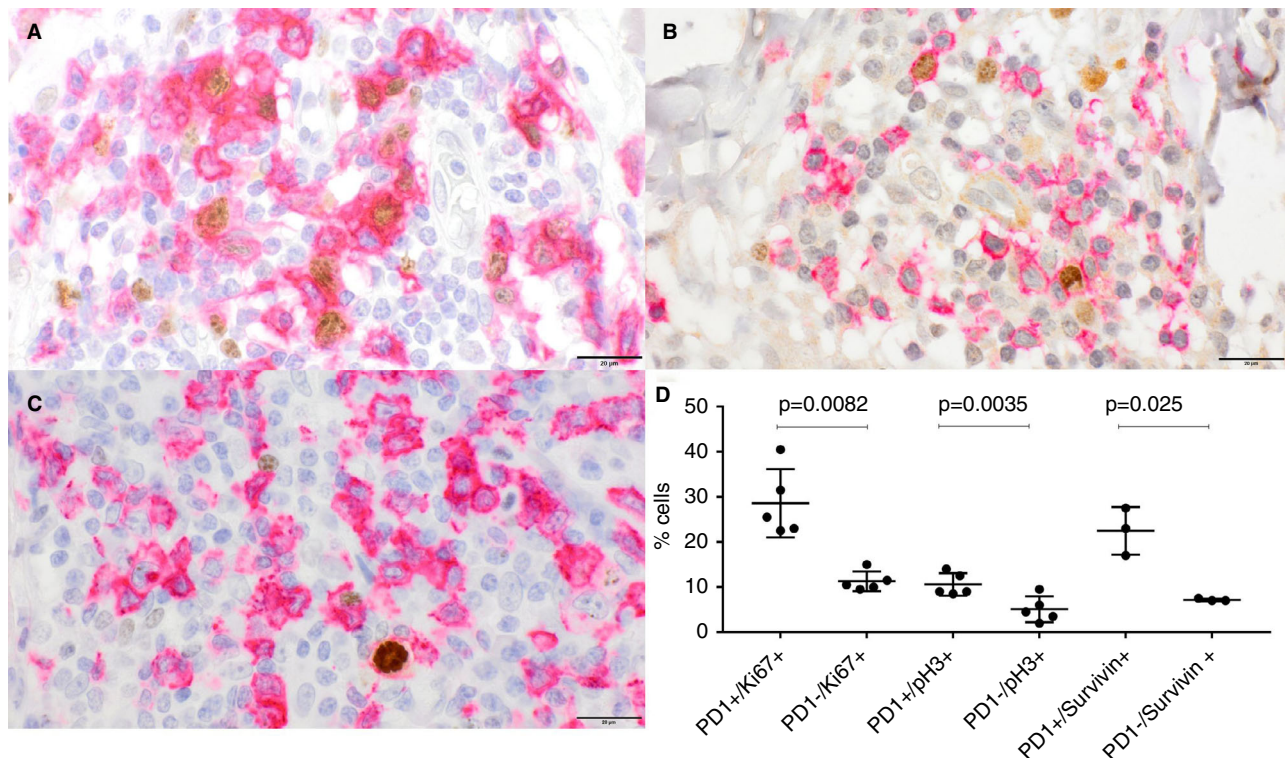


Figure 3. Expression of proliferation-associated antigens in PD1(+) and PD1(-)-cells within PCSM-LPD. Representative image of one case (A: PD1 [red] and Ki67 [brown], B: PD1 [red] and Survivin [brown], C: PD1 [red] and pH 3 [brown]). Original magnification 1000 \times , bar indicates 20 μ m. Quantification of expression of Ki67, pH 3, and survivin in PD1(+) and PD1(-) cells, P-values by *t*-test (D). [Color figure can be viewed at wileyonlinelibrary.com]

Table 2. Clinical characteristics, and clonal relatedness of PCSM-LPD with multiple biopsies.

Patient	Age	Gender	Clonal related	Same site	First localization	Second localization	Time interval
1	72	Male	Yes	No	Head	Trunk (back)	2 months
2	48	Female	No	Yes	Head	Head	36 months
3	77	Male	Yes	Yes	Groin	Groin	1month*
4	41	Female	Yes	Yes	Trunk (breast)	Trunk (breast)	<1 month*
5	63	Male	Yes	No	Trunk (sternal)	Trunk (back)	24 months
6	21	Female	Yes	Yes	Head	Head	2 months*
7	82	Male	Yes	Yes	Head	Head	28 months
8	60	Female	Yes	Yes	Head	Head	19 months

*The first and second biopsies of these cases probably arise from the same initial lesion.

analysis. We separated areas with PD1(+) clusters from adjacent PD1(−) areas. The monoclonal TCR-rearrangement detected in the bulk tissue of both cases, previously, was found in the PD1(+) but not in the PD1(−)-cell population. Figure 4 shows a representative example.

Discussion

To the best of our knowledge, this study presents the largest cohort of PCSM-LPD hitherto published. First, we confirmed the clinical features of the disease, which are increasingly being recognised by dermatologists and dermatopathologists. And on the other hand, we provide novel insights into the biology of PCSM-LPD as a benign but neoplastic cutaneous lymphatic disorder.

Our study confirms PCSM-LPD as a localised and benign skin confined disease with a risk of local relapse.⁵ We also confirmed previously reported histopathological features of this disease, such as the predominance of nodular over the less-frequent band-like growth pattern, high B-cell content despite the lack of germinal centres, and PD1(+)-cells arranged in clusters.⁵ The formation of clusters has previously been explained by an arrangement around centrally located B-cells, analogous to the formation of rosettes around Hodgkin–Reed–Sternberg cells.⁶ However, we failed to detect B-cells in the centre of the T-cell in most cases (data not shown). Our data suggest a different explanation for the formation of the clusters. As proliferating cells are more likely to be found in close proximity to each other, the clusters may simply be caused by cell proliferation. However, in contrast to previous reports, we do not believe that the presence

of clonal plasma cells and/or clonal immunoglobulin gene rearrangements in PCSM-LPD is a recurrent phenomenon.^{5,17} Primary cutaneous marginal zone lymphomas frequently harbour light-chain-restricted plasma cells and may present with abundant reactive CD4(+) and PD1(+)-cells.^{18–20} Whilst single cases with overlapping features of T-and B-cell lymphoproliferative disorders certainly exist, we cannot confirm this overlap to be a recurrent finding in our study. The discrepancies between our data and the published data with respect to B-cell clonality may be due to our inclusion criteria, as we systematically excluded marginal zone lymphomas by staining for light chains and/or additional BCR-clonality analysis.²¹

The tissue composition of PCSM-LPD is characterised by multiple cell types and their specific patterns or arrangements (e.g. PD1(+)-cells in clusters and B-cells without germinal centres). Our data suggest that with these histological features, the diagnosis can be established with certainty even in the absence of clinical information, since none of our cases required reclassification as aggressive cutaneous or systemic T-cell lymphoma after retrospective completion of clinical data. Our cohort and previously published cohorts suffer from a high number of patients for whom complete clinical data including follow-up is not available.²² The incompleteness of clinical data is due to the retrospective nature of our series. In addition, we assume that the loss of patients to follow-up probably reflects the high rate of cure after resection and the absence of association with other cutaneous T-cell lymphomas, explaining why patients no longer consult dermatologists after treatment. Consequently, in the current guidelines for cutaneous lymphomas, no specific recommendations

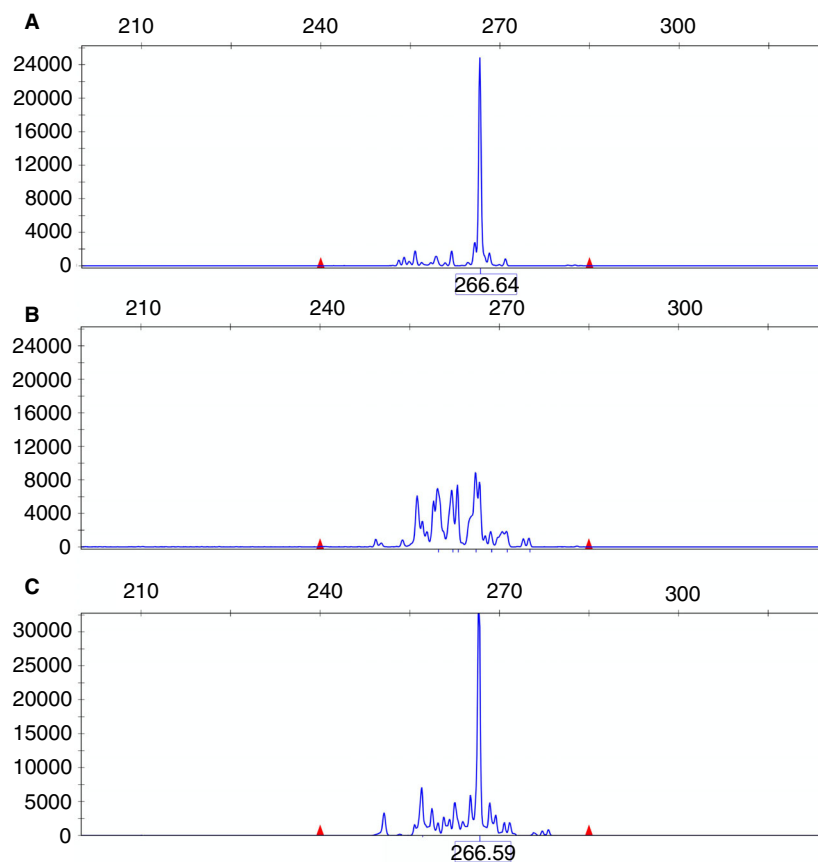


Figure 4. TCR analysis in a representative case of a PCSM-LPD as the bulk tissue (A) microdissected PD1(–)-cells (B) found immediately adjacent to microdissected PD1(+)-cells (C). The scale on top of each panel indicates fragment length in base pairs. [Color figure can be viewed at wileyonlinelibrary.com]

for follow-up of PCSM-LPDs are included.²³ However, for rare cutaneous lymphoproliferations, it is recommended to perform follow-up based on individual cases and clinical presentations.^{23,24}

Furthermore, we aimed to provide insights into the pathomechanisms of this disease. In our study we showed that overexpression of Cyclin D1 is a frequent finding in PCSM-LPD, a feature that has not been recognised previously. Cyclin D1 is involved in the G1-S-phase transition during the cell-cycle. Despite its role in proliferating cells, its expression level in lymphatic tissue is low, and it cannot be detected by immunohistochemistry under physiological conditions.¹⁶ To the best of our knowledge, it has not been reported in other cutaneous T-cell lymphomas. Similarly, cutaneous pseudo lymphomas rich in T-cells, which are the most relevant differential diagnosis of PCSM-LPD,²⁵ rarely displayed coexpression of Cyclin D1 in PD1(+)-cells, as shown in our study. The pathomechanism of Cyclin D1 activation remains uncertain at the current time. Since genetic aberrations of the *CCND1* gene were not detected, signals from the

microenvironment may drive the expression of Cyclin D1.

Aberrant Cyclin D1 expression in B-cell lymphomas and rare T-cell lymphomas due to *CCND1* gene translocations or unknown mechanisms are well described.^{16,26} Here we added another lymphatic disease to the group of Cyclin D1 expressing lymphoproliferations. However, the affected Cyclin D1 PD1(+) coexpressing cell-population represent a minority of cells within the lesions as compared with other B-cell lymphoma. In analogy to other lymphomas, we demonstrated a significantly higher proliferation of PD1(+)-cells compared to the abundant rest of the cells. Our finding of clonal persistence over time (primary biopsy and relapse) and at distant sites is not compatible with an inflammatory process. Moreover, we demonstrated that the PD1(+)-but not the PD1(–)-cells were clonal. Despite constituting a minority of cells within the lesions, PD1(+)-cells combine features of neoplastic cells, which are (i) clonality, (ii) Cyclin D1 overexpression, and (iii) accelerated cell proliferation. As these cells have previously been

demonstrated to show a rather complete phenotype of Tfh, we consider PCSM-LPD as a neoplastic Tfh disorder with benign behaviour.

In summary, the current findings confirm PCSM-LPD as a localised skin-confined and clinically benign condition. The disease nevertheless displays features of neoplasia with clonal expansion of PD1(+)-cells with clonal persistence in relapse biopsies and accelerated cell-cycle via aberrant expression of Cyclin D1.

Author contributions

I.O. and W.K. designed the study. I.O., K.S., T.W., J.R. and W.K. performed the research and analysed the data. U.W. provided clinical data, K.K. performed and analysed the fluorescence *in-situ* hybridization, I.O. and W.K. wrote the article; all authors read and approved the final version of the article. The authors thank Reina Zühlke, Dana Germer, Lorena Valles, and Charlotte Botz-von Drathen, Department of Pathology, University Hospital Schleswig-Holstein, for their excellent technical assistance. We are greatly indebted to the patients, families, and physicians who referred cases to Kiel.

Funding information

This work was supported in part by the KinderkrebsInitiative Buchholz, Holm-Seppensen (KKI).

Conflict of interest

The authors report no conflicts of interest.

Data availability statement

Data availability statement is provided upon request.

Acknowledgement

Open Access funding enabled and organized by Projekt DEAL.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Antibodies, dilutions and antigen retrieval used for immunohistochemistry.

Table S2. Characteristics of $n = 26$ pseudolymphomas (Site: head/neck = 1, trunk = 2, extremities = 3; Gender: male = m, Female = f; Number of lesions: single = 1, multiple = 2; Infiltration pattern: band-like = 1, nodular = 2; PD1 cluster: yes = 1, no = 0; GC: germinal centre; Plasma cells: few = 1, moderate = 2, increased = 3; clonality plasma cells: polyclonal = 1, monoclonal = 2; clonality TCR (T-cell receptor): poly = polyclonal; *In these cases a minimal predominant clone was observed in polyclonal background; **Scattered small groups of PD1+ cells were observed but no obvious clustering). NA, Not available.