**Eosinophilic dermatosis of hematologic malignancy:
Correlation of molecular characteristics of skin lesions and extracutaneous
manifestations of hematologic malignancy**

Short running title:

Neoplastic cells in eosinophilic dermatosis

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/cup.13389](https://doi.org/10.1111/cup.13389)

Key words

Eosinophilic dermatosis of hematologic malignancy, insect bite-like reaction, chronic lymphocytic leukemia, molecular genetics, immunoglobulin gene rearrangement

Abstract

Background

Skin diseases are frequent in patients with chronic lymphocytic leukemia and other hematological neoplasias. Eosinophilic dermatosis of hematologic malignancy (ED) has long been considered a nonspecific cutaneous reaction pattern. Recently neoplastic cells have been shown to be present in ED thus challenging the classification as a nonspecific dermatosis.

Methods

We report 5 patients with ED in association with chronic lymphocytic leukemia (CLL). We further investigated the presence of neoplastic B-cells in the skin infiltrate by immunohistochemistry and immunoglobulin heavy chain rearrangement and compared these to extracutaneous manifestations of CLL.

Results

The phenotype of the lymphocytic infiltrate was predominately CD3+ (range: 60-90%). CD20+ and CD79a+ lymphocytes were less frequent accounting for up to 15% (range: absent – 15%). CD23+ lymphocytes represented up to 20% (range: absent – 20%) of the infiltrate. The analysis of the immunoglobulin heavy chain rearrangement in the skin specimens showed clonal rearrangements in 4/5 patients and in 3 of these 4 patients clones were identical to extracutaneous CLL manifestations.

Conclusion

Our data show that neoplastic B-cells are very frequently found in ED when systematically evaluated. This findings support the hypothesis that leukemic cells play a pathogenetic role in eosinophilic dermatosis of hematologic malignancy.

Key words

Eosinophilic dermatosis of hematologic malignancy, insect bite-like reaction, chronic lymphocytic leukemia, molecular genetics, immunoglobulin gene rearrangement

Introduction

Skin diseases are frequently reported in patients with chronic lymphocytic leukemia (CLL) and other hematological neoplasias.¹⁻³ Approximately 8 % of all patients with CLL develop specific leukemic skin infiltrates, while a greater proportion of patients (25-50%) suffer from nonspecific or paraneoplastic dermatoses without evidence of leukemic cells in the infiltrate.²⁻⁵ Eosinophilic dermatosis of hematologic malignancy (ED, synonym: insect bite-like reaction) has long been considered a rare and unique variant of a nonspecific cutaneous reaction pattern.^{6,7} It was assumed that the underlying hematological disease causes an altered immune response and an increased secretion of TH2-cytokines (IL4 and IL5) stimulating the development of eosinophilic skin infiltrates.⁸⁻¹⁰ Eosinophilic dermatosis in hematologic malignancy was first described by Weed in 1965.¹¹ Diagnostic criteria were defined by Byrd et al.⁶ and are based on clinical (insect bite-like eruption, refractory to standard therapies) and histological (superficial and deep lymphohistiocytic infiltrate with abundant eosinophils) criteria as well as on the patients' medical history (underlying hematological disease, exclusion of other reasons for tissue eosinophilia). Until 2012 approximately 40-50 cases of ED had been published in case reports and small case series.¹² In 2012 Bairey et al.¹³ reported 48 patients with ED in a cohort of CLL patients in a retrospective study of the Israeli

Chronic Lymphocytic Leukemia Study Group. According to this study the prevalence of ED in CLL patients is 6-7%, which is in line with other reports.^{3,11} Thus ED occurs more often than previously assumed.^{6,14}

Mitteldorf et al. detected neoplastic CLL-cells within the skin infiltrate in a patient with ED by fluorescence in situ hybridization (FISH) analysis and demonstrated clonal immunoglobulin heavy chain (IgH) rearrangement identical to the one found in peripheral blood.¹⁰ These findings questioned the previous classification of ED as a nonspecific cutaneous reaction pattern. Earlier studies used different methods (southern blot analysis or IgH-PCR) to detect neoplastic cells in the infiltrate and found monoclonal B-cell populations indicating malignant infiltrates in various amounts (Table 1).^{8,15-17} In 2018 the French Study Group on Cutaneous Lymphomas added another 37 patients with ED.¹⁸ In a subset of reported patients the authors compared the molecular data of skin specimens with the molecular data of the same patients' peripheral blood samples and found monoclonal IgH rearrangements in 71.4% of patients (Table 1), which were identical to the neoplastic clone in the blood in 92.1% of cases.¹⁸

As more detailed data on the presence of CLL-infiltrates in ED are available now, it is worth evaluating if ED is a specific or nonspecific cutaneous reaction pattern. Thus we report another 5 patients with ED in association with CLL. We further investigated the presence of neoplastic B-cells in the skin infiltrate and compared these to extracutaneous manifestations of CLL and discuss our data in the context of literature.

Material/patients and methods

Patients

From 2009 to 2015 5 patients with characteristic itchy papular or vesiculobullous eruptions suggestive of insect bites were referred to our Department of Dermatology. In all patients, the diagnosis of chronic lymphocytic leukemia (CLL) was based on peripheral blood findings and/or on either bone marrow trephine biopsy, lymph-node biopsy or tonsillectomy specimen. The clinical and histologic findings of one of these patients (patient No. 1) has previously been published as a case report („Clinical Quiz“), but the additional molecular workup reported here has not been published.⁹

Laboratory methods

In total, 8 skin biopsies were performed in all patients for routine diagnostic workup. Sections of the formalin fixed paraffin-embedded tissue were stained with hematoxylin-eosin (HE). For immunohistochemistry the slides were deparaffinized and stained for CD3 (Ventana, CONFIRM, anti-CD3, Clone 2GV6), CD20 (Ventana, CONFIRM anti-CD20, Clone L26), CD23 (DAKO, FLEX, Clone DAK-CD23, RTU) and CD79a (DAKO, FLEX, Clone JBC117, RTU) with a Ventana BenchMark Ultra (CD3 and CD20) or a DAKO Autostainer Link 48 according to the manufacturers' instructions. Sections were counterstained with hematoxylin and mounted.

In order to detect malignant/monoclonal B-cells in the infiltrate the immunoglobulin heavy chain rearrangement was analyzed, using the Biomed2-protocol¹⁹, in all skin biopsies and extracutaneous CLL-infiltrates of the included patients. The analyses were performed in duplicates in order to differentiate between clonal and pseudoclonal amplification patterns.

Institutional Review Board Approval

The study protocol was approved by the ethics committee of the University of Freiburg, Germany (Vote No.: 321/17).

Results

Clinical data

Patients were 4 females and 1 male aged 60 to 83 years at the time of diagnosis of ED. All patients showed characteristic clinical features of ED with itchy papular, urticarial and/or vesiculobullous eruptions (Figure 1A-C). Insect bites were not reported by the patients and there was no seasonal occurrence pattern evident nor were close relatives affected by the eruption. In 4 of 5 patients the diagnosis of CLL had already been established since years (mean 127.8 months, range 35-282 months). The mean duration of reported ED symptoms was 27.3 months (range 3-62 months) until diagnosis of ED was established. Only in one patient the diagnosis of ED preceded the diagnosis of CLL by one month, as the typical histopathology of ED triggered subsequent hematological work-up, which revealed CLL. At the time ED was diagnosed, 3 of 5 patients showed marked leukocytosis with lymphocytosis. 3 of them were without CLL-specific therapy at that time. 4/5 patients had previously received therapy with rituximab and 2 patients had ongoing systemic therapy for CLL and were treated

with chlorambucil (patient 1 and 3). In all patients ED did not improve under therapy of CLL. Peripheral blood eosinophilia was absent in all patients. Extracutaneous samples of CLL-infiltrates were available from all patients at the Department of Pathology and could be used to compare molecular genetic findings of CLL with skin infiltrates. Patients' characteristics are listed in Table 2.

Histopathology

The histologic findings are summarized in Table 3. Lymphohistiocytic infiltrates and superficial and deep dermal eosinophil-rich infiltrates were present in all patients (Figure 2A), the density of the infiltrate varied among cases. Lymphoid nodular infiltrates were present in two specimens from the same patient. Eosinophilic spongiosis was present in 50% (Figure 2B) and intra- as well as subepidermal blister formation in 62.5% of skin biopsies. Eosinophilic panniculitis (Figure 2C) was evident in 72.5% of cases with assessable subcutaneous fat. Flame figures with eosinophils and focal collagen disruption were found in only 2 specimens (25%).

Immunohistochemistry

Immunohistochemistry (Table 4) showed the phenotype of the lymphocytic infiltrate to be predominately CD3-positive (60 - 90%) (Figure 3A). CD20+ and CD79a+ lymphocytes were seen less frequently accounting for up to 15% (range: absent - 15%) (Figure 3B/D). CD23+ lymphocytes presented up to 20% (range: single cells - 20%) of the lymphocytic infiltrate (Figure 3C).

Molecular genetic findings in skin specimens

The analysis of the immunoglobulin heavy chain rearrangement in the skin specimens showed clonal rearrangements in 4/5 patients. The pattern in one patient's biopsy was pseudoclonal with various non-reproducible peaks.

Comparison of molecular genetic findings in extracutaneous CLL-Infiltrates and skin specimens

In direct comparison with the extracutaneous CLL manifestations, which all showed clonally rearranged immunoglobulin heavy chains, 1/5 patient's skin biopsy displayed an identical

clone as the CLL (Table 5, patient 5, Figure 3E/F). This argues for the presence of CLL-cells in the skin biopsy. Two more patients (Table 5, patients 1 and 2) showed identical or very similar clonal peaks in at least one of the three analyzed frameworks, thus being suspicious for at least a minor CLL component in the skin. Patients 3 and 4 did not show identical clonal peaks in skin and extracutaneous CLL manifestation. In comparison with the immunohistochemical findings a CLL-identical/suspicious monoclonal rearrangement of the immunoglobulin heavy chain could be detected only in the skin specimens with more than 10% CD23+ lymphocytes.

Discussion

Eosinophilic dermatosis of hematologic malignancy (ED) has long been defined as a nonspecific cutaneous eruption in patients with hematologic malignancies, in contrast to specific cutaneous lesions, referred to as leukemia cutis.^{2,3,20} ED is most often associated with CLL^{12,18}, but has also been reported in patients with other hematologic neoplasias mainly of B-cell origin.^{8,12,16,18} As observed in our cases, diagnosis of underlying hematologic disease usually precedes the onset ED and accurate diagnosis of ED is commonly delayed by months resulting in impaired quality of life.¹³ Recently Visseaux et al.¹⁸ have proposed a new denomination of ED as „T-cell papulosis associated with B-cell malignancy“ (TCB-BCM). This term eliminates the reference to eosinophilic infiltration as a hallmark of the diagnostic criteria established by Byrd et al.⁶ Interestingly, the authors¹⁸ included about 25% of patients where eosinophils were absent and thus diagnosis of ED would have been missed by the established histological diagnostic criteria.⁶ To evaluate, whether these cases of TCB-BCM without eosinophils can be included in one category with cases with eosinophils, it is necessary to investigate if neoplastic B-cells can be detected in a similar proportion of these cases as in the classic ED cases. In order to ensure optimal comparability with the literature we adhered to the classic definition of ED. Thus eosinophils were a histological hallmark in all skin specimens and additional histological features with eosinophilic spongiosis (50%), intra- and subepidermal blister formation (62.5%), superficial and deep lymphohistiocytic infiltrate (100%), flame figures (25%) and eosinophilic panniculitis (71.5%) were consistent with reported criteria of ED in literature.^{15,16}

While the exact pathogenesis of ED is still unknown at least two hypotheses have been delineated: 1) leukemic cells are causative for ED and 2) leukemic cells are bystanders due to altered adhesion molecules thus leading to dermal tropism.¹⁰

There is clinical evidence that the underlying malignancy is causative for ED because this reaction has not been described in nonmalignant conditions and healthy people.¹⁰ Interestingly, „exaggerated insect bite reactions“ have been described in patients positive for human immunodeficiency virus (HIV)²¹⁻²³ and patients with congenital agammaglobulinemia²⁴ and have therefore been linked to the first description of Weed who coined this term in CLL patients.¹¹ Having a closer look at these publications²¹⁻²⁴, however, in all these patients „true“ insect bites are reported which clearly differentiates these conditions from ED. In contrast in ED insect bites are no longer thought to be causative.^{13,18} Concomitantly, insect bites were reported in none of our patients. Moreover, a causal relation to the underlying hematological malignancy is clinically implied by the cases in which therapy of underlying disease led to resolution of skin lesions.^{6,8} On the other hand a lack of association between skin changes and the course of the hematological disorder has also been described in the literature.^{8,13} In two of our cases the patients were under therapy for their CLL while presenting with ED. Moreover these patients had normal leukocyte and lymphocyte counts and in one of these patients (patient 2) we were still able to detect a CLL-identical monoclonal IgH rearrangement in the skin lesion. Thus minimal residual disease seems to be sufficient to trigger ED eruptions despite ongoing therapy.

The skin infiltrate in ED is typically composed of a predominantly T-lymphocytic infiltrate with eosinophils.^{7,8,10,15,16,18} B-lymphocytes are usually admixed with a variable amount ranging from 0-70%.¹⁰ Using morphological features and appropriate immunohistochemical stainings, clusters of tumor B cells have been detected in 47% of biopsy samples by Visseaux and coworkers¹⁸. Mitteldorf et al. also found CD20+ B-lymphocytes with coexpression of CD5 and CD23, the characteristic phenotype of CLL-cells.¹⁰ Accordingly all our cases showed a T-cell-rich phenotype. Analysis with pan-B-cell markers (CD20, CD79a) revealed up to 15% (range 0-15%) B-cells and up to 20% of the lymphocytes (range 0- 20%) expressed CD23, being in line with CLL-cells.

In light of our results and other recent findings^{10,18} the definition of ED as a nonspecific reaction pattern must be questioned. Mitteldorf et al.¹⁰ were the first who identified CLL-cells in skin specimens of an ED patient using IgH-PCR and FISH analysis and speculated on a pathogenic role of leukemic cells in ED. In previous reports molecular diagnostics were infrequently performed in ED. Moreover the findings were inconsistent and verified IgH monoclonality in skin specimens varied from 0-100%.^{8,15-17} None of these early reports compared the findings with extracutaneous CLL manifestations.^{8,15-17} Summarizing the results from our literature review and including our cases, in 20/37 (54.1%) monoclonal IgH gene arrangement could be identified in ED (Table 1). If only recent publications^{10,17,18} and our cases were included, all of them using the standardized BIOMED2 protocol¹⁹, the proportion of verified monoclonal IgH gene rearrangements increased to 80% (16/20 cases). Additionally, in 86.6% (13/15 cases) of these cases the IgH rearrangement was identical to the one detected in extracutaneous CLL manifestations. These striking results support the hypothesis of a direct pathogenic role of tumor B-cells in ED. The tumor B-cells are mostly of low number and not detectable by conventional histology. Using appropriate immunohistochemical staining (e.g. CD20, CD79a, CD 5, CD23)^{25,26}, however, in about 50% of ED cases clusters of CLL-infiltrates can be detected.^{10,18} The more specific diagnostic work-up is performed the higher is the proportion of cases with proven CLL-infiltrates (conventional histology 0% -> immunohistochemistry 47%¹⁸ -> IgH PCR 80%^{10,17,18,our case series} -> FISH analysis 100%¹⁰ (in cases with cytogenetic aberrations)). Due to the small number of neoplastic cells in the infiltrates of ED it might be difficult to detect the aberrant cells in the in situ hybridization of tissue sections, however. The few cases without proven CLL-cells in the skin lesions might be explained with a too low sensitivity of the detection methods. Thus, further investigation, e. g. a next generation sequencing clonality assay, which is able to detect single cells of a specific clone, is warranted.

In conclusion, our data show that neoplastic B-cells are very frequently found in ED when systematically evaluated. This findings support the hypothesis that leukemic cells play a pathogenic role in eosinophilic dermatosis of hematologic malignancy.

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Figure legends

Figure 1:

Typical clinical findings of ED with urticarial papules (A), bulla on urticarial ground (B) and eroded bulla (C)

Figure 2:

Histopathology features with superficial and deep eosinophil-rich infiltrate (A, HE, original magnification x25), eosinophilic spongiosis (B, HE, original magnification x100) and eosinophilic panniculitis (C, HE, original magnification x100)

Figure 3:

Immunohistochemical (original magnification x200) and molecular findings of the lymphocyte population in the skin biopsy of patient 5 in comparison to molecular findings in the patient's CLL:

Immunohistochemistry of the skin biopsy (panels A-D) shows a predominance of T-lymphocytes (A, CD3), rather few admixed B-lymphocytes (B, CD20 and D, CD79a) and a significant population of CD23+ B-cells (C, CD23).

In concordance with the abnormal CD23-positive lymphocytes in the skin biopsy, the analyses of the immunoglobulin heavy chain (panels E and F) in the skin biopsy (E) and in bone marrow infiltrates of the CLL (F) show an identical rearrangement in all three frameworks, which could be detected in duplicates (not shown), thus confirming neoplastic CLL-cells in the skin.

Tables

Table 1: Summary of previous and current reports of eosinophilic dermatosis in hematologic malignancy reporting molecular genetic findings

Table 2: Patients characteristics and clinical data on hematologic malignancy and eosinophilic dermatosis

Table 3: Histopathological characteristics in 8 cutaneous specimens from 5 patients with eosinophilic dermatosis of hematologic malignancy

Table 4: Immunohistochemistry results in 8 cutaneous lesions from 5 patients with eosinophilic dermatosis of hematologic malignancy

Table 5: Immunoglobulin heavy chain rearrangements in skin specimens and extracutaneous CLL manifestations

Tables

Table 1: Summary of previous and current reports of eosinophilic dermatosis in hematologic malignancy reporting molecular genetic findings

Author (year)	Method	Monoclonality detected in skin specimen	Comparison to extracutaneous CLL manifestation
Davis et al. (1998) ⁴	IgH-Southern blot	4/6 (66%)	No
Barzilai et al. (1999) ⁶	IgH-PCR	1/8 (12.5%)	No
Khamaysi et al. (2005) ¹³	IgH-PCR	0/2 (0%)	No
Ulmer et al. (2007) ¹⁰	IgH-PCR	1/1 (100%)	No
Mitteldorf et al. (2012) ⁶	IgH-PCR FISH analysis	1/1 (100%)	Yes (identical to blood clone)
Visseaux et al. (2018) ¹²	IgH-PCR	10/14 (71.4%)	Yes (identical to blood clone in 9/10 cases)
Meiss et al. (Patients reported in this manuscript)	IgH-PCR	4/5 (80%)	Yes (3/4 identical to clone of extra-cutaneous CLL samples)
		Total: 20/37 (54.1%)	Total: 13/15 (86.6%)

Table 2: Patients characteristics and clinical data on hematologic malignancy and eosinophilic dermatosis

No.	Sex/ Age [y]	Hematologic disease/ date of diagnosis [m/y]	Date of clinical onset of eosinophilic dermatosis [m/y]	Date of diagnosis of eosinophilic dermatosis [m/y]	Number of skin biopsies	Leukocyte count [10 ³ /μl]/ relative lymphocyte proportion [%]	Peri- pheral blood eosino- philia	Type of extra- cutaneous tissue with CLL- manifestation
1	F/60	CLL 02/1989	02/2012	08/2012	2	22.0/77.5*	no	bone marrow
2	M/72	CLL 08/2006	06/2009	08/2012	2	9.8/15.9 [#]	no	lymph node
3	F/83	CLL 11/2009	07/2012	10/2012	1	6.2 /31.0*	no	tonsil
4	F/74	CLL 09/2009	UNK	08/2009	2	15.6/73.0 [#]	no	peripheral blood
5	F/72	CLL 07/2005	07/2010	09/2015	1	58.7/68.0 [#]	no	bone marrow

No., patient number; y, year; m, month, F, female; M, male; CLL, chronic lymphocytic leukemia; UNK, unknown; * patients with ongoing therapy of CLL; [#] patients without recent therapy for CLL.

Table 3: Histopathological characteristics in 8 cutaneous specimens from 5 patients with eosinophilic dermatosis of hematologic malignancy

No.	Lympho-histio-cytic infiltrate	Eosinophils in the upper und deep dermis	Lymphoid nodules	Eosinophilic spongiosis	Eosinophilic panniculitis	Intra-/sub-epidermal bullae	Flame figures
1a	+	+	-	+	+	+	-
1b	+	+	-	-	+	+	-
2a	+	+	-	+	+	+	-
2b	+	+	-	-	-	-	-
3	+	+	-	-	+	-	+
4a	+	+	+	-	+	-	-
4b	+	+	+	+	n.d.	+	-
5	+	+	-	+	+	+	+
Total [%]	100	100	25	50	71.5	62.5	25

No., patient number; a/b used if more than 1 specimen was available from the same patient; n.d., not done because subcutaneous fat was not assessable.

Table 4: Immunohistochemistry results in 8 cutaneous lesions from 5 patients with eosinophilic dermatosis of hematologic malignancy

No.	CD 3	CD20	CD79a	CD 23
1a	60-80%	5%	10%	10%
1b	80%	10%	15%	15%
2a	90%	< 2%	single cells	10%
2b	90%	10%	10%	10%
3	> 90%	< 2%	single cells	<5%
4a	90%	negative	n.a.	n.a.
4b	> 90 %	< 1 %	single cells	only dendritic cells
5	90 %	< 2%	single cells	20%

No., patient number; a/b used if more than 1 specimen was available from the same patient; n.a., not applicable because stainings not performed due to lack of sufficient material

Table 5: Immunoglobulin heavy chain rearrangements in skin specimens and extracutaneous CLL manifestations.

No.	Skin specimens			Extracutaneous CLL tissue		
	FR1	FR2	FR3	FR1	FR2	FR3
1	n.e.	283	143	318 343 354	261 281 287	132 142 148
2	n.e.	283*	136	n.e.	278	126 136
3	n.e.	pc	pc	323	261	112 122
4	341*	pc	144	351	286	153
5	329*	261	128	329	261	118 127

Table shows the clonal peaks, which were detectable in duplicates. Skin and extracutaneous tissue of patient 5 shows identical B-cell clones, indicating a presence of CLL-cells in the skin. Specimens (highlighted in green) of patients 1 and 2 display partly identical/very similar B-cell clones, suspicious of some CLL-cells in the skin (highlighted in yellow). Patients 3 and 4 show no identical clones in skin and extracutaneous tissue (highlighted in red). No., patient number; n.e., analysis not evaluable; *, only one of the duplicates evaluable; CLL, chronic lymphocytic leukemia; FR, frame work region; pc, pseudoclonal.

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Key words

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Figure 1:
Typical clinical findings of ED with urticarial papules (A), bulla on urticarial ground (B) and eroded bulla (C)

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Figure 2:
Histopathology features with superficial and deep eosinophil-rich infiltrate (A, HE, original magnification x25), eosinophilic spongiosis (B, HE, original magnification x100) and eosinophilic panniculitis (C, HE, original magnification x100)

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