

Histomorphology and Immunophenotype of Eczematous Skin Lesions Revisited—Skin Biopsies Are Not Reliable in Differentiating Allergic Contact Dermatitis, Irritant Contact Dermatitis, and Atopic Dermatitis

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Abstract: Lesions of allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), and atopic dermatitis (AD) share similar clinical features and thus, their diagnosis can be challenging. The aim of this study was to reassess histopathology and immunophenotyping properties to distinguish between ACD, ICD, and AD. Charts of patients with eczema, who had undergone complete routine diagnostic workup (skin biopsies, patch tests, skin prick tests, and respectively or serum IgE levels), were reviewed. Thirty-five skin biopsy specimens of 28 patients (mean age 64 ± 15 years; ♀ = 13 ♂ = 15) with clear diagnosis of ACD (n = 15), ICD (n = 6), or AD (n = 14) were analyzed. Histomorphological and immunohistochemical (CD3, CD4, CD8, CD11c, CD34, CD123, S100, and IL-17) parameters were evaluated using Kruskal–Wallis test, Wilcoxon test, Fisher exact test, and decision tree analysis. Eosinophils were statistically significant ($P = 0.0184$), more often observed in AD than in ACD or ICD. No other statistically significant differences were found with regard to epidermal patterns, patterns of dermal infiltrates, or immunophenotyping. Using predictive modeling approaches, dermal eosinophils were found to be associated with AD, necrotic epidermal keratinocytes with ICD, and a focal type of parakeratosis with ACD. As an additional finding, pseudo-Pautrier microabscesses, which were present in the skin of 2 AD and 2 ACD patients, contained myeloid dendritic cells (CD11c+). Differentiation of ACD, ICD, and AD should be based on clinical features and results of allergy tests. Histopathology does not reliably differentiate between ACD, ICD, and AD, but helps to exclude psoriasis, tinea, or T-cell lymphoma.

Key Words: eosinophils, necrotic keratinocytes, myeloid dendritic cell, histopathology, immunophenotype

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INTRODUCTION

Allergic contact dermatitis (ACD), irritant contact dermatitis (ICD), and atopic dermatitis (AD) are frequent subtypes of eczematous skin diseases. Despite their pathogenetic differences they share, clinical features such as itch, erythema, and skin barrier disruption as well as histopathological properties such as spongiosis and superficial perivascular infiltrates predominated by lymphocytes.^{1,2}

ACD is caused by a delayed-type hypersensitivity response to low-molecular weight allergens that come into close contact with the skin.^{3,4} Innate and adaptive immune responses are involved in the pathogenesis of ACD. Sensitization to contact allergens results from uptake by antigen-presenting cells in the skin followed by the priming of allergen-specific CD4⁺ and CD8⁺ T cells in the draining lymph nodes.³ Present understanding of the mechanisms of ACD derives mainly from mouse models demonstrating the dominating proinflammatory effector role of cytotoxic CD8⁺ T cells. The CD4⁺ T-cell compartment comprises cell populations with immunomodulatory properties known as regulatory T cells.^{5,6} New insights on molecular and cellular pathomechanisms in ACD have been obtained recently: The roles of natural killer T cells, natural killer cells, mast cells, and neutrophils were characterized. As it turns out, the latter plays a pivotal role in the sensitization and elicitation phase of contact hypersensitivity.^{4,7}

Chemicals with proinflammatory impact significantly induce ICD. This results in epidermal damage and subsequent activation of the innate immune system. Inflammation in ICD exhibits characteristics of a nonspecific inflammatory reaction, ie, the hyperproduction of proinflammatory cytokines and chemokines, the presence of a polymorphic inflammatory infiltrate, and the necrosis of epidermal keratinocytes. Thus, it has been suggested that ICD and ACD can be differentiated by the presence (in ACD) or absence (in ICD) of antigen-specific effector T cells and the presence (in ICD) of necrotic keratinocytes (NKs) in eczematous lesions.^{2,8}

Cutaneous inflammation in AD results from an impaired epidermal barrier function and complex interactions of various cell types, among them being CD4⁺ and CD8⁺ T cells, dendritic cells (DCs), and keratinocytes.⁹ Genetic susceptibility and environmental factors (ie, allergens, irritants) are involved in the pathogenesis of AD.^{9,10} Previous studies showed that acute AD is largely a Th2-driven process, whereas in the chronic

phase, a transition occurs to a mixed Th1/Th2 profile.^{11,12} In addition, CD8⁺ lymphocytes can be recruited in the early phases of the skin reaction, and may contribute to the induction of keratinocyte apoptosis along with tissue damage.^{13,14}

The clinical differentiation of eczematous diseases can pose major challenges. Apart from allergy tests, skin biopsies are often performed in conventional diagnostic routines. We aimed at reassessing the diagnostic value of histopathology and immunophenotyping in the differentiation of ACD, AD, and ICD. We therefore analyzed histomorphological features along with using a broad panel of monoclonal antibodies directed to surface and intracellular antigens.

MATERIALS AND METHODS

Data Assessment

Records of 1601 patients (at least 18 years of age) who had undergone routine diagnostic procedures due to eczematous skin lesions between January 1, 2009 and December 31, 2014 were reviewed. Patients had received patch tests with the German standard series according to recommendations of the German Contact Dermatitis Research Group (DKG), and with further allergens according to their medical history. Furthermore, skin prick tests with a panel of common inhalant allergens and/or determination of total IgE levels and specific IgE antibodies to common inhalant allergens in the serum were performed. Skin biopsies had been taken from lesional skin before treatment to exclude noneczematous skin diseases (eg, cutaneous T-cell lymphoma, psoriasis, and tinea). A senior dermatologist and allergologist (KB) had performed all patch test readings and established the final diagnosis (Table 1). Data of patients who could be clearly attributed to ACD, ICD, or AD due to clinical examination and allergy test results were included in the analysis. Skin biopsies of these patients were used for histomorphological studies and immunophenotyping (ACD, n = 15; ICD, n = 6; and AD, n = 14). As required, patients had given informed consent for the analysis of their skin biopsy specimen for scientific purposes at the time of the biopsy.

TABLE 1. Diagnostic Criteria of ACD, ICD, and AD

Disease	Clinical Criteria
ACD (n = 15)	Clinically relevant sensitization to contact allergen(s) No immediate type sensitization to inhalant allergens Normal total serum IgE level Improvement of eczema after avoidance of contact allergen(s)
ICD (n = 6)	Negative allergy tests, history of skin contact to irritants
AD (n = 14)	Clinically relevant sensitization to inhalant allergens, i.e., allergic rhinitis and respectively or allergic asthma, elevated total serum IgE level, negative patch test to contact allergens

Thirty-five biopsies of 28 patients were evaluated.

Histopathology

Biopsy specimens were fixed in 4% neutral buffered formalin, processed routinely, and subsequently embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) stain and reviewed independently by a board-certified dermatopathologist (AB) and a resident dermatologist (VGF) under blinded conditions regarding the results of the allergy tests. Epidermal and dermal morphology were assessed for all features (present/absent) given in Table 2A. Wherever applicable, the intensity of variables was scored semiquantitatively (Table 2B). Any discrepancies were reassessed to yield an ultimate consensus.

Immunohistochemistry

Immunohistochemical staining of formalin-fixed paraffin-embedded tissue was performed with anti-CD3, anti-CD4, anti-CD8, anti-CD34, anti-S-100 (all DakoCytomation, Glostrup, Denmark), anti-CD11c (Abcam, Cambridge, United Kingdom), anti-CD123 (eBioscience, Frankfurt am Main, Germany), and anti-IL-17 (R&D Systems, Abingdon, United Kingdom) according to the manufacturer's instructions (see **Table 1, Supplemental Digital Content 1**, <http://links.lww.com/AJDP/A56>). The Dako Real Detection System with alkaline phosphatase/RED-conjugated antirabbit, antimouse antibody (K5005, biotinylated linked antibody and ready-to-use streptavidin conjugated with alkaline phosphatase) from DakoCytomation Denmark A/S (Glostrup, Denmark) was used for all antibodies except anti-IL-17. Here, staining was performed using the secondary "Multi-Link" polyclonal swine, antigoat, antimouse, antirabbit antibody (E0453, biotinylated-linked antibody between a primary antibody and avidin- or streptavidin-labeled enzymes or other markers) from DakoCytomation Denmark A/S (Glostrup, Denmark).

Immunohistochemically positive cells were assessed semiquantitatively in the epidermis (0 = 0 cells, 1 = 1–10 cells, 2 = 11–20 cells, and 3 ≥ 20 cells) and superficial perivascular, deep perivascular, and interstitial dermis (0 = 0 cells, 1 = 1–20 cells, 2 = 21–100 cells, and 3 ≥ 100 cells). Staining with CD34 was evaluated as being either fully expressed or reduced.

Statistics

Data were evaluated using Microsoft Excel software (version 14.0.0; Microsoft Corporation, Redmond, WA) and the R Project for Statistical Computing software for Mac (version 3.1 and R-package C50; The R Foundation for Statistical Computing, Vienna, Austria). Variables are shown in terms of absolute and relative frequencies. For ordinal variables, we applied the nonparametric Kruskal–Wallis test (1-way analysis of variance) as global test to identify variables differentiating between ACD, ICD, and AD. The Kruskal–Wallis test assesses significant differences of a continuous dependent variable in relation to a grouping independent variable (ie, 3 diseases in our study). Afterward, the pairwise Wilcoxon rank-sum test is required to identify which disease it is that the variable differentiates. Nominal variables were analyzed using Fisher exact test. A *P* value of <0.05 was considered statistically significant.

TABLE 2. Assessment of Epidermal and Dermal Alterations in ACD, ICD, and AD

A: Qualitative Variables		Assessment Score	
Spongiosis			Absent/present
Type of spongiosis: focal			
Type of spongiosis: broad			
Acanthosis			
Type of acanthosis: regular			
Type of acanthosis: irregular			
Hyperkeratosis			
Type of hyperkeratosis: basket woven			
Type of hyperkeratosis: compact			
Parakeratosis			
Type of parakeratosis: focal			
Type of parakeratosis: broad			
Crust			
Hypergranulosis			
Type of hypergranulosis: focal			
Type of hypergranulosis: broad			
Hypogranulosis			
Type of hypogranulosis: focal			
Type of hypogranulosis: broad			
Erosion			
NK			
NK in the lower epidermis			
NK in the mid epidermis			
NK in the upper epidermis			
Intraepidermal lymphocytes			
Intraepidermal eosinophils			
Intraepidermal neutrophils			
Bacterial lawn			
Dermal infiltrate			
Superficial dermal infiltrate			
Deep dermal infiltrate			
Perivascular dermal infiltrate			
Interstitial dermal infiltrate			
Nodular dermal infiltrate			
Diffuse dermal infiltrate			
Lymphocytes in the dermal infiltrate			
Eosinophils in the dermal infiltrate			
Histiocytes in the dermal infiltrate			
Neutrophils in the dermal infiltrate			
Plasma cells in the dermal infiltrate			
Erythrocyte extravasates in the dermis			
Edema in the papillary dermis			
Dermal fibrosis			
Vertical bundles of collagen			
B: Semi-quantitative Variables			
Amount of spongiosis	1 = widened intercellular spaces	2 = beginning separation of keratinocytes	3 = frank vesiculation

TABLE 2. (Continued) Assessment of Epidermal and Dermal Alterations in ACD, ICD, and AD

B: Semi-quantitative Variables		Assessment Score		
Amount of acanthosis		1 = mild	2 = moderate	3 = strong
Amount of NKs	1 = 1–10 cells 2 = 11–20 cells 3 ≥20 cells			
Amount of intraepidermal lymphocytes				
Amount of intraepidermal eosinophils				
Amount of intraepidermal neutrophils				
Amount of dermal infiltrate	1 = 1–20 cells 2 = 21–100 cells 3 ≥100 cells			
Amount of superficial dermal infiltrate				
Amount of deep dermal infiltrate				
Amount of lymphocytes in the dermal infiltrate				
Amount of eosinophils in the dermal infiltrate				
Amount of histiocytes in the dermal infiltrate				
Amount of neutrophils in the dermal infiltrate				
Amount of plasma cells in the dermal infiltrate				
Amount of erythrocyte extravasates in the dermal infiltrate				
Amount of edema in the papillary dermis		1 = mild	2 = moderate	3 = strong
Amount of vertical bundles of collagen				
Parameters were evaluated by the absence/presence of histomorphological patterns and semiquantitative scores (epidermal infiltrate: 0 = 0 cells, 1 = 1–10 cells, 2 = 11–20 cells, and 3 ≥20 cells; dermal infiltrate: 0 = 0 cells, 1 = 1–20 cells, 2 = 21–100 cells, and 3 ≥100 cells).				

Subsequently, a decision tree algorithm was applied using a heuristic by the name of recursive partitioning. This approach uses a given set of variables and splits it automatically such that the most relevant variables lead to a specific diagnosis. Beginning with the entire data set, the algorithm chooses a variable that, according to relative frequencies, is the most predictive of the target class, ie, ACD, ICD, or AD. The algorithm continues to divide-and-conquer the data set, each time choosing the best variable and forming a new decision branch until a stop criterion is reached. This might occur if all (or nearly all) of the variables have the same class, or if there are no remaining features to distinguish among diseases. We chose an exploratory analysis with clinically relevant variables selected by an expert allergologist (KB) and an expert histopathologist (AB).

RESULTS

A total of 35 skin biopsy specimens of 28 patients (mean age 64 ± 15 years; ♀ = 13 ♂ = 15) who could be

clearly attributed to ACD (n = 15), ICD (n = 6), or AD (n = 14) were used for further analysis.

Histomorphological Analysis and Immunophenotyping of Eczema Subtypes

Table 3 shows the comparison of ACD, ICD, and AD according to certain (1) nominal distributed and (2) semiquantitatively scored variables. In general, ACD, ICD, and AD showed similar epidermal and dermal changes. Only eosinophils in the dermal infiltrate were significantly more often present ($P = 0.0184$, Fisher exact test) in AD (86% of the skin sections, n = 12/14) than in ACD (40%, n = 6/15) or ICD (33%, n = 2/6). Immunophenotyping did not identify any significant difference between the groups. Trends (defined as $P < 0.5$) could, however, be established (Table 3).

In lesions of ACD, parakeratosis was often focally distributed, and bacteria were encountered more commonly in the cornified layer as compared to ICD or AD (Fig. 1A). In addition, the presence of a deep dermal infiltrate and the absence of an edema of the papillary dermis were more

TABLE 3. Statistical Analysis of Variables Showing Trends ($P < 0.5$) Toward ACD, ICD, or AD

A: Qualitative Variables	Evaluated Slides (n = Absolute Numbers)			Percentages, %						Fisher Exact Test
	ACD, n	ICD, n	AD, n	ACD		ICD		AD		P
				Absent	Present	Absent	Present	Absent	Present	
Hyperkeratosis	15	6	14	20	80	50	50	21	79	0.4532
Type of parakeratosis: focal	13	6	10	46	54	67	33	80	20	0.2614
Type of parakeratosis: broad	13	6	10	54	46	33	67	20	80	0.2614
Type of hypergranulosis: focal	4	2	6	50	50	0	100	67	33	0.4805
Type of hypergranulosis: broad	4	2	6	50	50	100	0	33	67	0.4805
Hypogranulosis	15	6	14	27	73	17	83	50	50	0.3217
NKs	15	6	14	40	60	33	67	64	36	0.3572
Bacterial lawn	15	6	14	40	60	83	17	64	36	0.1633
Deep dermal infiltrate	15	6	14	40	60	83	17	57	43	0.2159
Eosinophils in the dermal infiltrate	15	6	14	60	40	67	33	14	86	0.0184*
Neutrophils in the dermal infiltrate	15	6	14	33	67	17	83	57	43	0.1992
Edema in the papillary dermis	15	6	14	53	47	17	83	29	71	0.2433
Fibrosis	15	6	14	80	20	50	50	71	29	0.4234
CD34 regular staining	15	6	13	73	27	50	50	38	62	0.1834
CD34 reduced staining	15	6	13	27	73	50	50	62	38	0.1834

B: Semi-quantitative Variables	Evaluated Slides (n = Absolute Numbers)			Semiquantitative Scores												Kruskal-Wallis Test
	ACD, n	ICD, n	AD, n	ACD				ICD				AD				P
				0	1	2	3	0	1	2	3	0	1	2	3	
CD3 (SPVD)	15	5	13	0	0	40	60	0	0	40	60	0	0	62	38	0.4919
CD3 (DPVD)	15	5	13	67	26	7	0	80	20	0	0	38	54	8	0	0.2029
CD3 (interstitial dermis)	15	5	13	46	27	27	0	60	40	0	0	62	38	0	0	0.3991
CD4 (DPVD)	15	6	12	67	26	7	0	67	33	0	0	33	59	8	0	0.2103
CD8 (SPVD)	15	6	13	0	40	53	7	0	33	33	33	0	69	31	0	0.1192
S100 (SPVD)	13	5	13	0	69	31	0	0	40	60	0	8	69	23	0	0.2832
S100 (DPVD)	13	5	13	77	23	0	0	80	20	0	0	31	69	0	0	0.0361

*Statistically significant parameter.

Semiquantitative scores: epidermal infiltrate: 0 = 0 cells, 1 = 1–10 cells, 2 = 11–20 cells, and 3 ≥ 20 cells; dermal infiltrate: 0 = 0 cells, 1 = 1–20 cells, 2 = 21–100 cells, and 3 ≥ 100 cells.

DPVD, deep perivascular dermis; SPVD, superficial perivascular dermis.

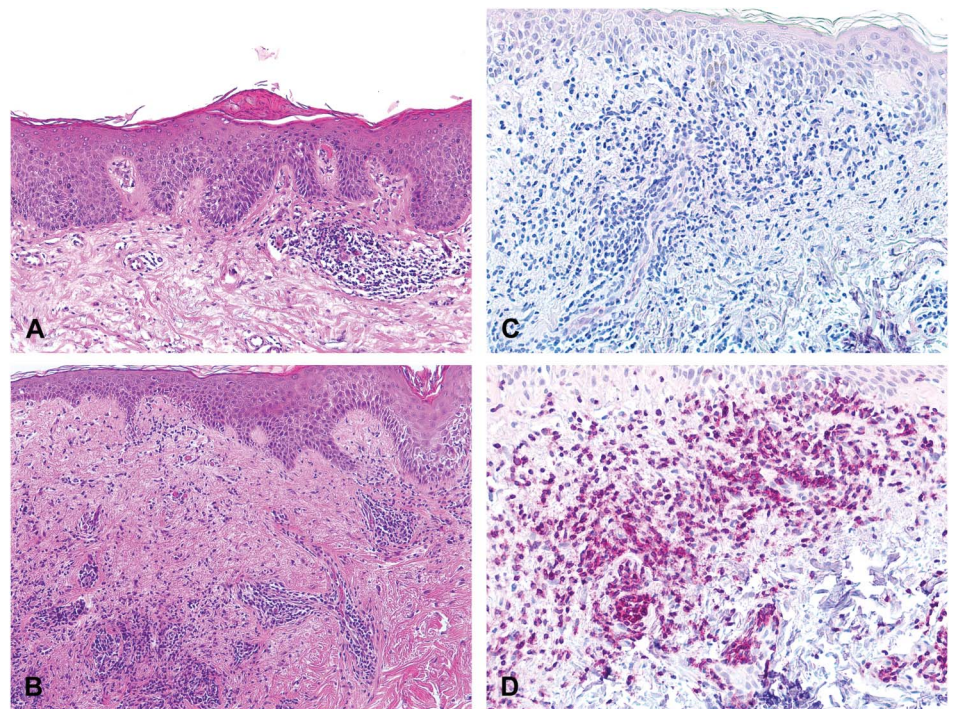


FIGURE 1. Histomorphological and immunohistochemical trends in ACD. Focally distributed parakeratosis (A), a deep dermal infiltrate without edema of the papillary dermis (B), a reduced CD34 expression (C), and CD3⁺ cells in the interstitial dermis.

frequently seen in ACD (Fig. 1B). Immunohistochemically, CD34 expression was commonly reduced (Fig. 1C) and, in the interstitial dermis, cells stained more often CD3⁺ (Fig. 1D) in ACD than in ICD or AD (Table 3).

In ICD, hyperkeratosis was not as often seen, but hypogranulosis was more commonly seen than in ACD or AD. If, however, hypergranulosis was encountered, it was only

focally distributed (Fig. 2A). NKs were only slightly more often present than in ACD (Fig. 2B). In the dermis, neutrophils were regularly part of the infiltrate (Fig. 2C), and fibrosis was less frequently seen as compared to ACD or AD. More frequently than in the other studied diseases (Table 3), immunohistochemistry identified S100⁺ cells as part of the deep perivascular dermal infiltrate (Fig. 2D).

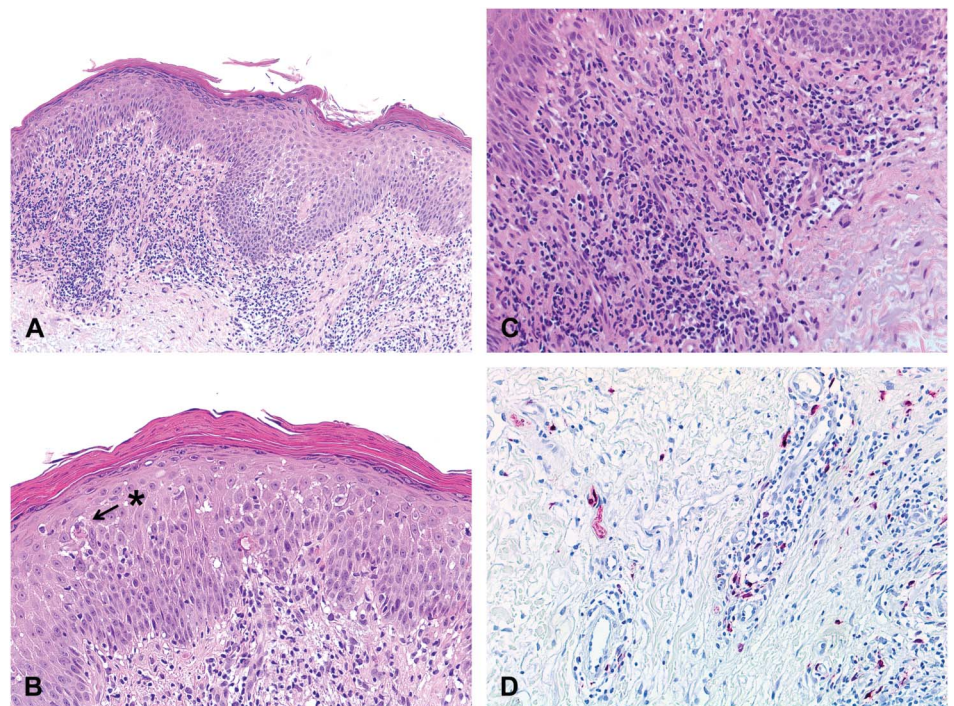


FIGURE 2. Histomorphological and immunohistochemical trends in ICD. Focally distributed hypergranulosis besides regularly encountered hypogranulosis (A). NKs [B, indicated by arrow (*)] in the epidermis and neutrophils in the dermal infiltrate (C). S100⁺ cells in the deep perivascular dermal infiltrate (D).

A broad parakeratosis and a broad hypergranulosis were more commonly found in AD than in ACD or ICD (Fig. 3A). NKs were, however, less frequently seen in the epidermis of AD. In the dermis, an infiltrate housing eosinophils (Fig. 3B) proved to be—as already mentioned—a statistically significant property of AD ($P = 0.0184$). Neutrophils in the dermal infiltrate were found considerably less than in contact dermatoses. In immunophenotyping, expression of CD3 (Fig. 3C) and CD4 (Fig. 3D) in the superficial and expression of CD3 (Fig. 3C), CD8 (Fig. 3E), and S100 (Fig. 3F) in the deep perivascular infiltrate were seen more frequently in AD as compared to ACD and ICD. CD34 expression was not reduced in AD (Table 3).

In 2 of the AD (Figs. 4A–C) and 2 of the ACD (Figs. 4D–F) cases, the so-called “spongiotic simulators of Pautrier microabscesses” were encountered. Cells forming those collections expressed CD11c+ rather than S100, indicating a myeloid DC (mDC) lineage.

Predictive Modeling Approaches Based on Decision Tree Analysis

Decision tree analysis was used as a decision support model. Multiple decision tree models were calculated with

emphasis on epidermal, dermal, histomorphological, or immunohistochemical parameters. The following features were evident in all calculated models: eosinophils in the dermal infiltrate were associated with AD, NKs in the epidermis with ICD, and focal parakeratosis with ACD. As it turns out, it was rather histomorphology than immunophenotyping which allowed differentiating between the diseases. The most promising immunohistochemical parameters in decision tree analysis were the quantity of CD8+ cells in the epidermis or deep perivascular dermis, of CD11c+ cells in the epidermis, and of IL17+ cells in the superficial perivascular dermis. CD8+ cells in the deep perivascular dermis often indicated ACD. Adding those parameters to a histomorphological decision tree analysis, unfortunately, gained no additional insight (data not shown). The most efficient model is depicted in Figure 5.

DISCUSSION

This study investigated histomorphological features and immunophenotype of ACD, ICD, and AD to identify parameters that allow their reliable distinction in a biopsy

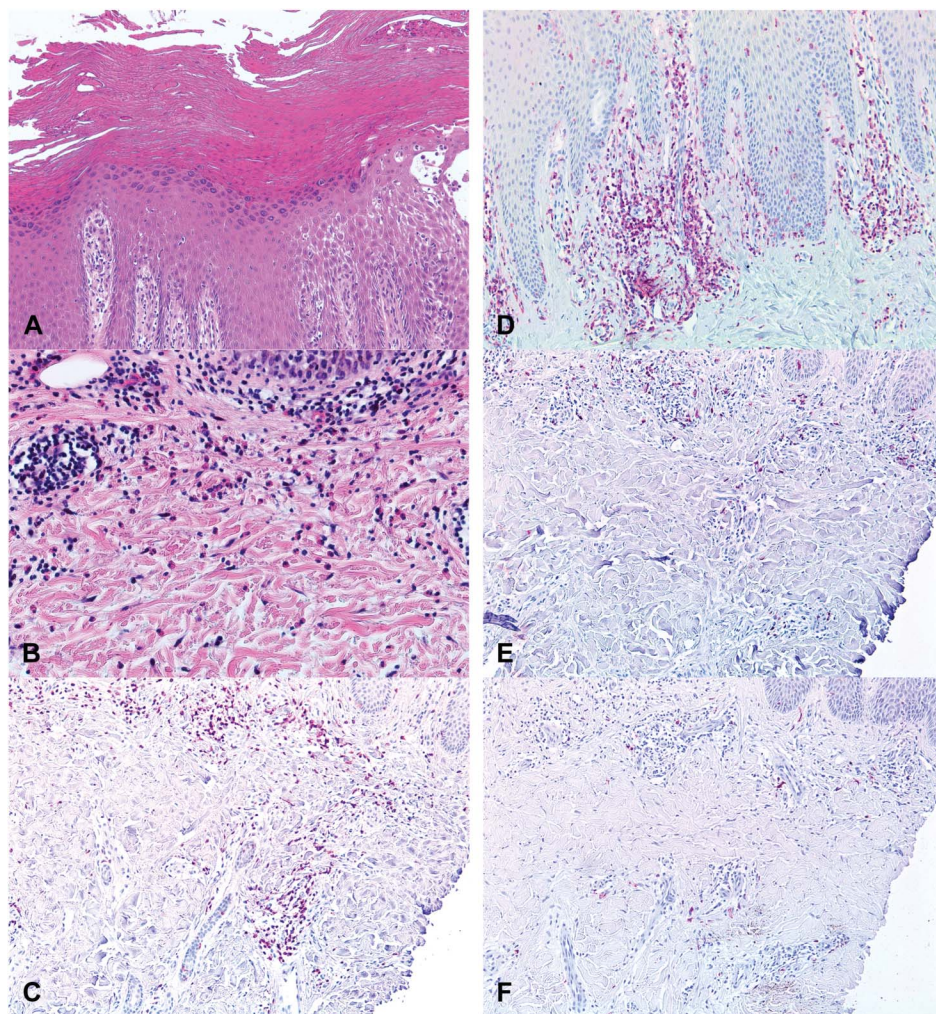


FIGURE 3. Histomorphological and immunohistochemical characteristics and trends in AD. A broad parakeratosis and a broad hypergranulosis (A), a dermal infiltrate housing eosinophils (B). CD3+ in the superficial and deep perivascular dermis (C), CD4+ cells in the superficial infiltrate (D), and CD8+ (E) and S100+ cells (F) in the deep perivascular dermis.

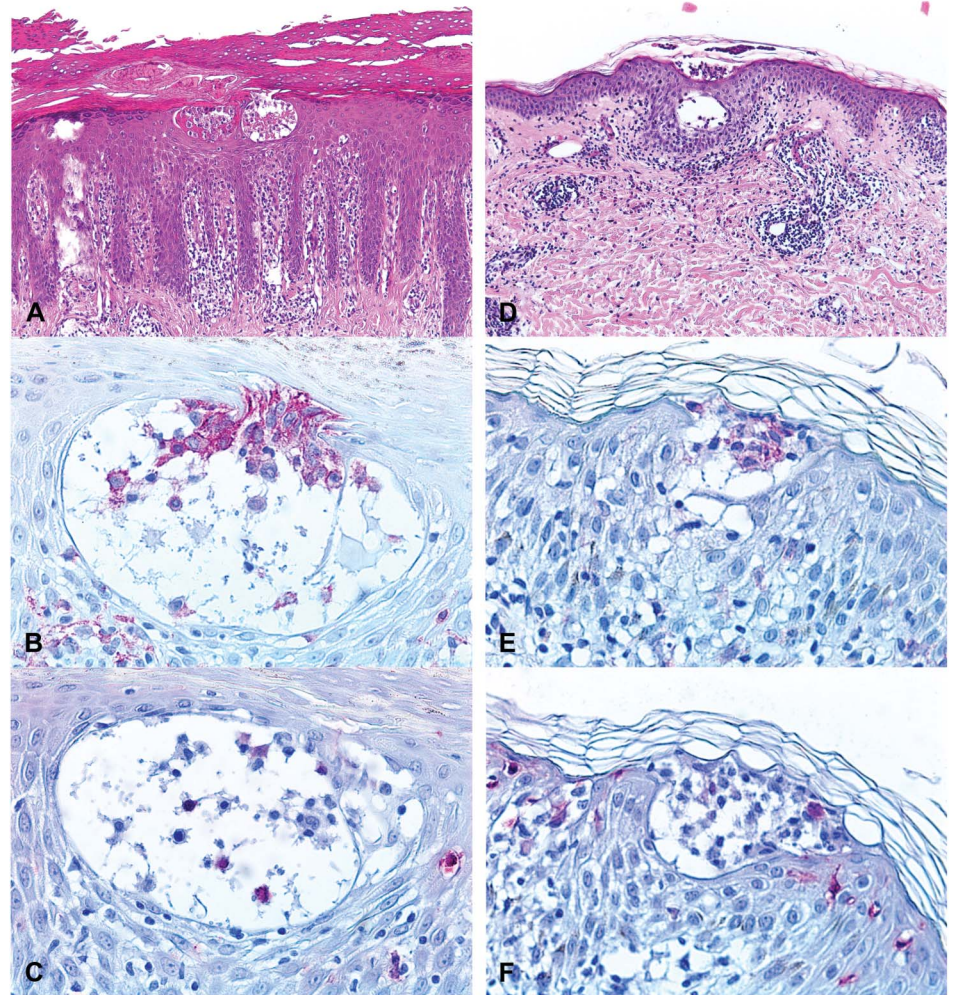


FIGURE 4. So-called “spongiotic simulators of Pautrier microabscesses” in lesions of ACD (A–C) and AD (D–F), staining CD11c+ (B and E) and S100+ (C and F), respectively.

specimen. Using lesional skin of patients who could be clearly attributed to ACD, ICD, or AD, we attempted to reassess the histomorphological and immunohistochemical criteria for their diagnosis. To go beyond studies using biopsies from patch test areas or animal models,^{15–19} we conducted our study in a realistic setting of lesional human skin.

We provided evidence that only eosinophils in the dermal infiltrate of AD and none of the other investigated morphological or immunohistochemical parameters reached statistical significance in lesional skin biopsies of the studied diseases. Eosinophils in blood and skin are often elevated²⁰ in and are conjectured to be associated with allergic conditions, in particular with certain drug reactions.²¹ The presence of eosinophils, especially in the form of eosinophilic spongiosis, has been reported to be an important histopathologic feature in ACD.²² Kempf et al²³ suggest that a superficial perivascular lymphocytic infiltrate rich in eosinophils is relevant both in ACD and AD. In contrast, Rosa et al²⁴ report that there are no differences in the number of eosinophils in the skin of patch test–positive individuals and those who showed no reaction in allergic patch tests. Our results, however, endorse that eosinophils in the dermal infiltrate are an important clue to AD. This is supported by Simon

et al,²⁵ who hypothesized eosinophils to be the prominent phenotype of AD. In the epidermis, eosinophils are not relevant for diagnosis.

The cutaneous inflammation in ICD is considered to be a nonspecific reaction with necrosis of the epidermal cells, directly induced by chemical toxicity.⁸ Our results corroborate previous findings,^{2,8,23} stating that the presence of NKs in the epidermis can provide valuable hints at the diagnosis of ICD. We calculated only slightly higher frequencies for NKs in ICD than in ACD. This might be due to the overall low number of ICD cases in our study. As soon as NKs are used as a variable in the decision tree analysis (see **Table** and **Figures, Supplemental Digital Content 2–5**, <http://links.lww.com/AJDP/A56>), however, they reveal themselves as important information regarding the diagnosis of ICD.

It has been mentioned previously that late stages of ICD can present themselves histologically identical to ACD.^{2,26,27} According to Kempf et al,²³ parakeratosis can be seen in stages of contact dermatitis and AD. We found that parakeratosis may be present in all these entities, but the pattern of focal parakeratosis is more frequently seen in ACD. Parakeratosis is a mode of keratinization characterized by the

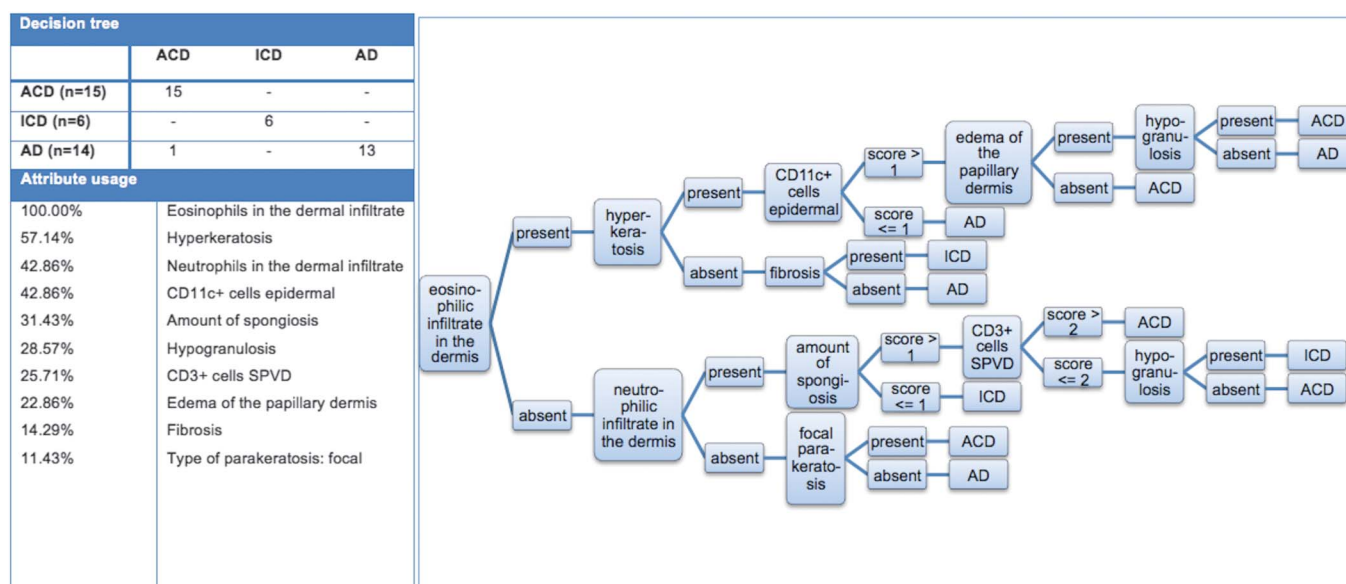


FIGURE 5. Decision tree: Variables showing tendencies ($P < 0.5$) toward ACD, ICD, or AD. Attribute usage indicates how often (in percentages) a variable was used to diagnose ACD, ICD, or AD. One sample was classified wrong; the error rate is 2.9%. DPVD, deep perivascular dermis; SPVD, superficial perivascular dermis.

retention of nuclei in the stratum corneum. It is associated with the thinning or loss of the granular layer, and is usually seen in diseases of increased cell turnover.²³

The pattern of spongiotic dermatitis, as seen in ACD, ICD, and AD, is perhaps best known for its potential to simulate cutaneous T-cell lymphoma. Histologically, the finding of nonlymphoid intraepidermal cell collections (the so-called “pseudo-Pautrier microabscesses,” “spongiotic simulators of Pautrier’s microabscesses,” or “Langerhans cell collections”) tends to support the diagnosis of spongiotic dermatitis. These collections are distinguishable by their vase shape in the epidermis.²⁸ Previous studies found them to be positive for CD1a, CD36, CD11b, S100, CD68, and variably for CD83. They lack CD14, similar to mature Langerhans cells.²⁹ As a result, it has been suggested that these cells are an intermediate stage between monocytes and mature Langerhans cells, and represent a morphologic expression of the recruitment of DC precursors during immune-mediated skin disorders.³⁰ Our study shows that CD11c+ mDCs dominate the pseudo-Pautrier microabscesses. DCs are professional antigen-presenting cells that orchestrate the decision-making process for the polarization of the immune response toward a Th1 or Th2 profile.^{30,31} At least 2 DC subsets have been identified³²: CD11c+ mDCs and CD123+ plasmacytoid DCs. We observed CD11c+ cells mostly in the papillary dermis of ACD. In epidermal collections, however, they were found equally in ACD and AD. Therefore, we support the finding of Rosa et al²⁴ that the presence of pseudo-Pautrier microabscesses might be of use in evaluating spongiotic dermatides. Our immunohistochemical results still suggest that those collections mainly consist of mDCs, not Langerhans cells. Studies with higher case numbers are needed to analyze whether those CD11c+ collections can really be used as a diagnostic clue to ACD or AD.

Because IL-17 has pleiotropic effects on tissue and several immune cells, and is an important mediator in tissue inflammation,^{33,34} we intended to investigate its role in ACD, ICD, and AD. A role for IL-17 in allergic skin diseases is consistent with the observation that IL-17-deficient mice show reduced contact and delayed-type hypersensitivity responses on sensitization and challenge with the corresponding allergen.³⁴ These mice also have an increased skin-barrier dysfunction.³⁵ We could, however, not prove a relevant expression of IL-17 in any of the studied diseases.

Just one of the parameters investigated in our study reached statistical significance. We hence decided to use predictive modeling approaches to obtain a descriptive model of the relationship between a given set of the investigated variables. The constructed trees can be consulted to classify cases where the diagnosis is not obvious. Data can be classified without bias because it is not possible to assign different priorities to the variables or to start the decision tree analysis with a preselected variable, while it is still possible to restrict the set of variables. The decision tree algorithm has been applied in many previous studies, ranging from safety validation of decision trees for hepatocellular carcinoma³⁶ to validating a decision tree for serious infection among acutely ill children.³⁷ In addition, Ture et al³⁸ analyzed several decision tree methods (CHAID, CART, QUEST, C4.5, and ID3) together with Kaplan–Meier estimates to investigate their predictive power of recurrence-free survival in patients with breast cancer. In summary, decision trees are considered to be a powerful tool for classification and are easy to interpret. In our study, simple models with few variables showed a higher error rate than complex decision trees with multiple variables. Extensive models, however, proved to be complicated and tend to illogical parameter repetition, ie, when a set of variables repeatedly appears within the decision tree indicating different

diseases. To sum up, mainly histomorphological parameters, notably epidermis-related variables, but not immunophenotyping were helpful to correctly classify the biopsy specimens. The most promising decision tree is depicted in Figure 5.

Strengths and Limitations

Some of the previous limitations in the identification of histomorphological and immunohistochemical features of ACD, ICD, and AD were overcome in this study using lesional human skin gained in a nonartificial setting. The patient collective was well characterized, and a clearly attributed diagnosis was established. Mixed phenotypes (ie, atopy combined with ACD or ACD combined with irritation) were excluded. This allows for an authentic histological analysis of the skin samples as encountered in routine practice. The small number of samples, especially of ICD, is a limitation to this study. To overcome this limitation, we also analyzed ACD against AD and contact dermatoses (ACD and ICD) against AD. No further information could be gathered through it (data not shown). Studies with larger sample numbers are needed to delineate if further statistically significant parameters can eventually be identified. One major limitation of this study is that patients with characteristic skin lesions are rarely biopsied in routine practice. Those patients, who are biopsied after all, often show secondary changes that obscure the diagnosis, for example, pronounced acanthosis due to extensive scratching. In most cases, biopsies are taken in clinically challenging cases of eczematous skin lesions to rule out other, partly malignant skin conditions. Still, our results derive from routine specimens and, thus, represent everyday clinical practice.

CONCLUSIONS

This study shows that a specific distinction of ACD, ICD, and AD remains difficult if it is solely based on histomorphology. Even a broad panel of immunohistochemical markers does not offer additional information. Although certain trends could be identified, just one variable proved to be statistically significant in the diagnosis of AD (eosinophils in the dermal infiltrate). We, therefore, emphasize that the main value of skin biopsies in the setting of eczematous skin lesions is to rule out other skin conditions such as psoriasis, tinea, or cutaneous T-cell lymphoma. The differentiation between ACD, ICD, and AD is a question to be answered mainly by the clinician/allergologist, and not by the dermatopathologist.

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