Patch test reactions through the lens of dermoscopy. Further insights, particularly on weak allergic reactions

Running head: Dermoscopy of patch test reactions

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Abstract

Background: Distinguishing weak allergic from irritant patch test reactions may be difficult.

Objectives: To describe the dermoscopic features of both allergic, especially weak allergic, and irritant patch test reactions and to assess the suitability of dermoscopy in supporting differential diagnosis.

Methods: Consecutive adult outpatients patch tested during a 12-month period, who developed any skin reaction, were eligible for this observational, cross-sectional study. Healthy volunteers were patch tested with sodium lauryl sulfate as irritant controls. At the 72-hour reading, patch test reactions were recorded both with a digital camera and a digital dermoscopic system. For each reaction, clinical and dermoscopic variables were separately assessed, scored and then compared.

Results: Erythema, vesiculation and vessels were constant dermoscopic features of allergic reactions (n=173). In 46 weak (+) allergic reactions, dermoscopy showed: i) erythema (100%), ii) dense polymorphic vessels (100%), iii) whitish vesicles (78.3%). Scores of vesicles and dotted vessels were significantly higher in weak allergic than in irritant reactions. Vesicles were identified as the chief dermoscopic parameter for correctly distinguishing weak allergic from irritant reactions.

Conclusions: Dermoscopy can improve accuracy in the differential diagnosis between weak allergic and irritant patch test reactions.

Key words: dermoscopy, patch test, differential diagnosis, weak allergic reactions, irritant reactions

1. Introduction

In a previous study, we described for the first time the main dermoscopic features of patch test reactions.[1] We found that dermoscopic patterns of allergic and irritant patch test reactions differ significantly. Highly sensitive and/or specific dermoscopic features may support differential diagnosis. In particular, allergic reactions exhibit quite intense erythema, almost constant presence of whitish, soap bubble-like vesicles, and rich pleomorphic vascularization. Moreover, orange-yellowish patchy areas and crusts have been shown to be highly specific for allergic reactions. Consistent with these findings, dermoscopy could be a useful tool in the clinical setting in order to better differentiate between these two conditions.

However, some issues remain unanswered, despite the promising results of that pilot study. First, the value of dermoscopy in weak positive allergic patch test reactions is of interest. Moreover, the correlation between clinical and dermoscopic features has not been specifically addressed in the initial study. Furthermore, no data on irritant reactions induced by a well-established chemical irritant were available. The latter may serve as a comparison with respect to both allergic and "real

life" irritant reactions. With the aim of focusing these issues, the previous study population has been further assessed.

2. Materials and Methods

In this observational, cross-sectional study, all consecutive adult outpatients (≥18 years) patch tested at the Allergy Unit of the Department of Dermatology of Ferrara during a 6-month period for suspected allergic contact dermatitis were screened for inclusion. The patients included during this second phase of the study were added to the previously enrolled population.[1] In order to assess the dermoscopic features of induced irritant reactions, healthy volunteers were patch tested with sodium lauryl sulfate (SLS), which has been used extensively as the positive control for skin irritation tests.

The principles outlined in the Helsinki Declaration of 1975, as revised in 1983, were followed for all the patients included. The present research was carried out on completely de-identified data. As all patients who attend our University Hospital give their written consent for the use of their data in anonymized form for scientific research, approval by the institutional review board was not required for the present study.

2.1 Patients

Patients were patch tested with the Società Italiana di Dermatologia Allergologica Professionale ed Ambientale (SIDAPA) baseline series (Lofarma, Milano, Italy). On the basis of the history and/or clinical features, patch tests with additional series or with the patients' own products were

performed. Test allergens were applied to the upper back of patients for 48 hours, using patch test chambers (Van der Bend Brielle, The Netherlands and Finn Chambers on Scanpor). Test sites were evaluated after 2 and 3 days. Clinical assessment and scoring (+ weak positive reaction, ++ strong positive reaction, +++ extreme positive reaction) was performed by an experienced dermatologist (M.C.), in accordance with the ESCD guidelines.[2] All patients with any allergic and/or irritant patch test reaction were eligible for the study. Patients were excluded only in the case of no patch test reactions or doubtful ones. Relevance of reactions was indifferent to the aims of the study.

Eleven healthy volunteers consented to be patch tested with SLS (99% purity) 2.5% aq. All were physicians working in our Department. Occlusive patch testing with SLS was performed for 48 hours, using the same patch test chambers of the study population, on the volar side of the left forearm in accordance with the guidelines on SLS testing.[3] An SLS concentration of 2.5% was chosen to ensure a strong irritant reaction after 48 hour application.[4] Test sites were evaluated after 72 hours.

2.2 Study assessments

At the day 3 reading, the patch test reactions were documented both with a digital camera (Nikon D7200 reflex camera, Nikon USA, Melville, New York) and a digital dermoscopic system (Vidix Dermascope 7, Medici Medical, Castelfranco Emilia, Italy). All patch test reactions were documented, regardless of the sensitizing or irritating agent. In the case of multiple reactions in the same patient, all those considered undoubtedly allergic or irritant were included in the analysis.

For all the clinical pictures, the same dermatologist who assessed and graded the patch test reactions compiled a file concerning the main clinical features. The following clinical parameters were

addressed: erythema, oedema, vesicles, pustules, poral reaction. These were scored as present or absent without grading. Separately, two dermatologists (A.B., G.T.), unaware of the clinical diagnosis, assessed in consensus the dermoscopic images of the same reactions. The irritant reactions to SLS were assessed in a blinded fashion by the same dermatologists as well. A selection of both vascular and non-vascular dermoscopic variables was included in the evaluation process. Each dermoscopic variable was arbitrarily graded according to a 4-point scale (0–3, where 0 represents absent or normal characteristic and 3 represents most present or abnormal extent). The results of clinical and dermoscopic assessments were recorded in two separate databases for the subsequent comparative evaluation.

Three main analyses were carried out. First, we performed a comparison of the scores of each dermoscopic variable between allergic reactions and irritant reactions and between weak (+) allergic reactions and irritant reactions. Dermoscopic variables of allergic reactions were compared with those of irritant reactions from both the study population and the healthy volunteers patch tested with SLS. The purpose was to detect the main dermoscopic differences between patch test reactions of different aetiology, ie weak allergic and irritant reactions. Dermoscopic features of irritant reactions observed and acquired among the patients were compared with those of positive irritant controls (2.5% SLS). Second, an assessment of sensitivity, specificity, positive predictive value, negative predictive value, accuracy and odds ratio of each clinical and dermoscopic variable for the correct diagnosis of both allergic and weak allergic reaction was carried out. This was assessed in order to quantify the weight of each variable in correctly orienting the diagnosis. Third, we compared the scores of each addressed dermoscopic variable among weak (+), strong (++) and

extreme (+++) allergic reactions. This latter comparison aimed to assess the level of correspondence between clinical and dermoscopic scores.

2.3 Statistics

Continuous variables were presented as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Comparisons between groups were made using the t-test for quantitative variables, while Pearson's chi-squared test or Fisher's exact test were used for qualitative variables. *P*-values obtained from the t-test were corrected with Bonferroni's method for multiple comparisons. Analysis of variance (ANOVA) was used for determining the existence of differences among the weak (+), strong (++) and extreme (+++) reactions' dermoscopic mean values. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy and odds ratio were calculated for each dermoscopic parameter, comparing the whole allergic reactions with irritant reactions (both those from the study population and those from the healthy volunteers patch tested with SLS) and weak (+) allergic reactions with irritant reactions (as above). All statistical analyses were performed using Stata version 13, setting the significant level α to 0.05.

3. Results

One hundred and thirty-nine patients were included, 102 females (73.4%) and 37 males (26.6%), with an overall 173 allergic reactions (in 109 patients) and 54 irritant reactions (in 46 patients). Online supplemental Table 1 shows the patch test reactions in detail, including 11 irritant reactions induced by 2.5% SLS (positive irritant controls).

3.1 Clinical features of patch test reactions

From a clinical point of view, all allergic reactions presented erythema and almost all oedema (99.4%); vesiculation was observed in about 73% of all allergic reactions, whereas it was absent in weak allergic reactions. Erythema was the most common clinical feature in irritant reactions (90.7%), followed by "poral reactions" (20.4%), selectively in cobalt patch testing, as previously described,[5,6] and pustules (13%). Clinical features of irritant reactions induced by SLS in healthy volunteers did not significantly differ from those observed in the study patients, with the exception of the poral pattern and pustules which were not found among the former.

3.2 Dermoscopic features of patch test reactions

Table 2 summarizes and compares the dermoscopic features of allergic (both all allergic and just weak allergic, i.e. +, reactions) and irritant patch test reactions (both irritant reactions captured from patients and those induced by SLS in healthy volunteers). Dermoscopy of allergic reactions as a whole, regardless of their clinical scores, was similar to what has already been described.[1] Considering selectively the weak allergic reactions, dermoscopic examination demonstrated vesicles in 36 (78.3%) cases, while they are not observable by the naked eye (OR= 323, 95% CI: 18.3 to 5701, $P<0.001$). Mean scores of patients' irritant reactions did not differ from those of positive irritant controls. On the other hand, vesicles and orange-yellowish areas and crusts, although quite uncommon in irritant reactions from the study patients, were not observed at all in irritant reactions to SLS.

Sensitivity, specificity, positive and negative predictive values, accuracy and odds ratios of the addressed dermoscopic variables for the diagnosis of allergic reactions and weak allergic reactions are summarized in Table 3 and Table 4. In particular, allergic reactions were compared with patients' irritant reactions in Table 3 and with irritant reactions to SLS in Table 4. Considering the weak allergic reactions alone, vesicles and vessels were the dermoscopic variables more significantly associated with an allergic aetiology of the reactions than with an irritant one. Erythema, vesicles and vessels had high sensitivity for the correct differential diagnosis of allergic reactions whereas vesicles, orange-yellowish patchy areas and crusts, pustules and petechial spots had high specificity. Accuracy, which is the overall probability that a weak allergic reaction is correctly classified in the presence of a given parameter, was particularly high for vesicles and, to a lesser extent, orange-yellowish crusts and dotted vessels. The results were similar considering separately the irritant reactions from the study patients and the positive controls induced by SLS, with some minor differences.

Table 5 shows a comparison of mean values of the dermoscopic parameters addressed between allergic reactions of different intensity, namely extreme (+++), strong (++) and weak (+) positive reactions. Mean values of all dermoscopic parameters, except vessels, significantly differ between reactions with different clinical scores. Mean dermoscopic scores of vesicles and dotted vessels significantly differ in all comparisons.

4. Discussion

Correct interpretation of patch tests in terms of distinguishing allergic from irritant reactions is of major importance, but can be challenging. Histological examination may not provide a reliable

improvement in distinguishing allergic from irritant reactions;[7,8] moreover, it is an invasive method difficult to apply in the clinical setting. Some non-invasive tests have been applied in an attempt to improve diagnostic specificity, including reflectance confocal microscopy,[9-12] ultrasound,[13] conventional[14] as well as high-definition optical coherence tomography.[15,16] So far, only one previous paper has reported the use of dermoscopy in the assessment of reactions to patch tests.[1] This is rather surprising, as the dermatoscope, unlike other diagnostic devices, is a universal tool, very familiar to dermatologists and available in almost any outpatient setting.

The main objective of the present study was to extend the available evidence, in particular describing the dermoscopic patterns of weak allergic reactions in order to assess whether dermoscopy could be useful in improving the differential diagnosis between allergic and irritant patch test reactions. Investigating the relationshipt between clinical and dermoscopic assessments of the intensity of reactions was a further aim.

Our results confirmed that patch test reactions exhibit characteristic dermoscopic patterns, which significantly differ according to their allergic or irritant nature, as already found (Figure 1).[1] With specific reference to weak (+) allergic reactions, the most noteworthy finding was that they exhibited typical whitish vesicles in a considerable share of cases (about 78%). Mean scores for vesicles were significantly higher when compared with irritant reactions (Table 2). The mean score for erythema was higher than that of irritant reactions detected among the study patients but did not significantly differ from mean score of irritant reaction to SLS. This may be due to the fact that SLS concentration of 2.5% usually induces a strong irritant reaction. Thus, intensity of erythema does

not seem to allow to differentiate between weak allergic reactions and strong irritant ones. Overall, mean scores for vessels did not significantly differ from those of irritant reactions. However, considering selectively dotted vessels, their mean value was significantly higher when compared with the irritant positive controls. Furthermore, vessels were a highly sensitive parameter for the correct differential diagnosis and their negative predictive value was 100%. This means that a weak allergic reaction is very unlikely when vessels are absent at dermoscopic examination.

Taken together, these findings indicate that dermoscopy greatly enhances the visualization of the inflammatory process, which leads to both erythema and spongiosis, also in weak forms of allergic reactions. Spongiosis, in turn, results in the formation and exudation of vesicles, which can be perceived at dermoscopic assessment even when they are not visible to the naked eye. Size and sharpness of vesicles at dermoscopy may depend on both entity and depth of fluid accumulation in the epidermis. As spongiosis can be focally or evenly distributed along the length of the epidermis, vesicles may appear at dermoscopic examination as thick or sparse, with a diffuse, clustered or isolated arrangement.

Vesicles were the main dermoscopic parameter for correctly distinguishing allergic from irritant nature in weak positive reactions (Table 3 and Table 4). Erythema appeared to be a sensitive dermoscopic parameter for correct diagnosis in weak allergic reactions. Dotted vessels, in terms of both prevalence and scores, resulted highly associated with weak allergic reactions in comparison with irritant ones. This is in line with the inflammatory nature of the former. In fact, vessels dotted in shape are typically described in inflammatory skin disorders, like psoriasis and eczema.[17,18]

Orange-yellowish patchy areas and crusts were observed in just 15% of weak allergic reactions. They are the dermoscopic expression of an acute exudation, which causes ruptures at the epidermal

surface, as previously reported in different types of eczema.[17,18] The detection of orange-yellowish crusting in a minority of allergic reactions, unlike what is usually found at dermoscopy in acute eczema in which it is an almost constant finding, is probably due to the patch test reading made at an early stage of inflammatory reaction. In fact, 3 days from contact with the sensitizing allergen only a part of vesicles and spongiotic elements had gone through exudation. The fact that in weak allergic reactions this parameter is less present than in allergic reactions as a whole (31.2%) may be due to a less intense spongiotic response compared to stronger reactions. In keeping with this, mean values of orange-yellowish patchy areas and crusts were significantly lower in weak reactions in comparison with extreme reactions (Table 5). Orange-yellowish elements were very specific for allergic reactions and had a high specificity and positive predictive value, even in weak cases.

Overall, there was a strong relation between clinical and dermoscopic scoring (Table 5). In particular, the dermoscopic scores of vesicles and dotted vessels, the main dermoscopic hallmarks of inflammation in patch test reactions, were significantly different among extreme, strong and weak positive reactions. This suggests that dermoscopy may also be a suitable tool for properly scoring allergic reactions, especially when clinical assessment is not definitive in this regard.

Some comments shall address dermoscopic features of irritant reactions. Although vesicle formation, due to spongiotic phenomena or irritant cytolysis, may also be present in irritant reactions and is visible at histologically[7,8] and in high-definition optical coherence tomography[15], with dermoscopic observation vesiculation is a sporadic finding in this kind of reaction. No significant differences were found in mean scores between irritant reactions in patients and irritant controls exposed to SLS. This seems to confirm that the irritant reactions included were likely irritant in nature.

It should be pointed out that the 'poral pattern' was an almost constant dermoscopic finding in irritant reactions to cobalt, as previously observed.[1,19]

Our study has several limitations. First, only cases that could be clinically defined as allergic or irritant beyond any reasonable doubt were included; therefore, it is not possible to state with certainty that our findings can also be extended to cases with doubtful reactions. On the other hand, the inclusion of cases of allergic or irritant reactions that are certain allows the described dermoscopic findings to be considered fitting with each of these two different forms of patch test reactions. In the analysis of sensitivity and specificity of the observed dermoscopic features, we did not include a comparison with dermoscopic images of normal skin. Both clinical and dermoscopic features were captured at the day 3 reading, thus they are not representative of either early or late phases of the reactions. Since the reactions were not histologically examined, a correlation between dermoscopic features and histological changes cannot be addressed. Finally, our study results have not been stratified for patients' age, sex and sensitizing agent.

In conclusion, our results indicate that dermoscopy could be a support in distinguishing weak allergic and irritant patch test reactions. In fact, weak allergic reactions present highly sensitive and/or specific dermoscopic characteristics. Most of all, finding vesicles and dotted vessels at dermoscopic observation indicates, with high likelihood, an allergic aetiology of the patch test reaction. Moreover, dermoscopy provides a strong basis for scoring allergic reactions by several variables, namely vesicles, dotted vessels, erythema and orange-yellowish patchy areas and crusts.

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Table 2. Dermoscopic findings in i) allergic reactions as a whole, ii) weak (+) allergic reactions, iii) irritant reactions and iv), irritant reactions to sodium lauryl sulfate, with comparison of mean dermoscopic scores.

Dermoscopic features		**Allergic reactions, total 173** n. (%) **mean value** [min-max] *(SD)*	**Irritant reactions, total 54** n. (%) **mean value** [min-max] *(SD)*	**Weak allergic reactions, total 46** n. (%) **mean value** [min-max] *(SD)*	**Irritant reactions to SLS, total 11** n. (%) **mean value** [min-max] *(SD)*	***P*** **allergic *versus* irritant reactions and *versus* irritant reactions to SLS (in grey)** mean value	***P*** **weak allergic *versus* irritant reactions and *versus* irritant reactions to SLS (in grey)** mean value	***P*** **irritant reactions *versus* irritant reactions to SLS** mean value
Non-vascular	Erythema	172 (99.42%) **1.95** [0-3] *(0.69)*	51 (94.44%) **1.24** [0-2] *(0.54)*	46 (100%) **1.61** [0-3] *(0.61)*	11 (100%) **1.45** [0-2] *(0.50)*	**<0.001** **0.02**	**0.002** 0.45	0.119
	White soap bubble-like vesicles:*							
	Any vesicle	169 (97.69%) **0.87** [0-3] *(0.91)*	3 (5.56%) **0.04** [0-2] *(0.22)*	36 (78.26%) **0.36** [0-3] *(0.59)*	0 (0%) **0** [0] *(0)*	**<0.001** **<0.001**	**<0.001** **<0.001**	0.168
	Clustered	85 (49.13%) **0.87** [0-3] *(1.03)*	2 (3.70%) **0.04** [0-1] *(0.19)*	10 (21.74%) **0.33** [0-3] *(0.69)*	0 (0%) **0** [0] *(0)*	**<0.001** **0.006**	**0.004** 0.131	0.219
	Isolated	141 (81.50%) **1.18** [0-3] *(0.81)*	3 (5.56%) **0.07** [0-2] *(0.32)*	23 (50.00%) **0.52** [0-2] *(0.54)*	0 (0%) **0** [0] *(0)*	**<0.001** **<0.001**	**<0.001** **0.002**	0.23
	Follicular	72 (41.62%) **0.57** [0-3] *(0.78)*	0 (0%) **0** [0] *(0)*	9 (19.57%) **0.22** [0-2] *(0.46)*	0 (0%) **0** [0] *(0)*	**<0.001** **<0.001**	**<0.001** 0.13	0.5
	Orange-yellowish areas and crusts	54 (31.21%) **0.55** [0-3] *(0.93)*	3 (5.56%) **0.07** [0-2] *(0.32)*	7 (15.22%) **0.28** [0-2] *(0.74)*	0 (0%) **0** [0] *(0)*	**<0.001** **0.05**	0.07 0.11	0.23
	Poral pattern	0 (0%)	11 (20.37%)	0 (0%)	0 (0%)	**<0.001**	**0.001**	0.56

		0 [0] *(0)*	**0.46** [0-3] *(0.94)*	**0** [0] *(0)*	**0** [0] *(0)*	***n.a.***	***n.a.***	
	Pustules	27 (15.61%) **0.21** [0-3] *(0.55)*	7 (12.96%) **0.17** [0-2] *(0.46)*	1 (2.17%) **0.02** [0-2] *(0.15)*	0 (0%) **0** [0] *(0)*	0.57 0.20	**0.046** 0.63	0.12
Vascular	Any vessel	170 (98.26%) **0.81** [0-3] *(0.91)*	46 (85.19%) **0.65** [0-3] *(0.90)*	46 (100%) **0.73** [0-3] *(0.92)*	46 (86.79%) **0.42** [0-2] *(0.70)*	**0.043** **0.016**	0.43 0.08	0.09
	Dotted vessels*	144 (83.24%) **1.32** [0-3] *(0.89)*	27 (50.00%) **0.67** [0-3] *(0.82)*	31 (67.39%) **0.83** [0-3] *(0.70)*	27 (50.94%) **0.27** [0-1] *(0.45)*	**<0.001** **<0.001**	0.15 **0.017**	0.07
	Linear vessels*	113 (65.32%) **0.94** [0-3] *(0.87)*	38 (70.37%) **1.20** [0-3] *(1.00)*	33 (71.74%) **1.28** [0-3] *(1.06)*	37 (69.81%) **1.00** [0-2] *(0.85)*	0.06 0.84	0.71 0.42	0.27
	Glomerular vessels*	25 (14.451%) **0.18** [0-3] *(0.52)*	3 (5.56%) **0.07** [0-2] *(0.32)*	2 (4.35%) **0.09** [0-3] *(0.46)*	0 (0%) **0** [0] *(0)*	0.14 0.24	0.87 0.54	0.23
	Petechial spots	3 (1.73%) **0.03** [0-2] *(0.26)*	1 (1.85%) **0.04** [0-2] *(0.27)*	0 (0%) **0** [0] *(0)*	0 (0%) **0** [0] *(0)*	0.95 0.66	0.36 ***n.a.***	0.33

*Vesicles could be arranged with different distribution in the same patients and vessels with different morphology could co-exist; SLS, sodium lauryl sulfate; *n.a.*, not applicable. Significant values in bold.

Table 3. Sensitivity, specificity, positive and negative predictive values, accuracy and odds ratio of the dermoscopic and clinical criteria for the correct diagnosis of allergic (white lines) and weak (+) allergic (grey lines) *versus* irritant patch test reactions.

Dermoscopic features		Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[b]	Odds ratio (95% CI)	Accuracy[c]
non-vascular features	Erythema	99.4%	5. 6%	77.1%	75%	10.1 (1.03 to 99.4)	77.1%
		100%	5. 6%	47.4%	100%	6.32 (0.32 to 125)	49%
	White soap bubble-like vesicles						
	Any vesicle	94.2%	94.4 %	98.2%	83.6%	277 (73.4 to 1045)	94.3%

		78.3%	94.4%	92.3%	83.6%	61.2 (15.7 to 238)	87%
	Clustered	49.1%	96%	97.7%	37.1%	26.1 (6.16 to 110)	60.4%
		21.7%	96 %	83.3%	59.1%	7.50 (1.55 to 36.3)	62%
	Isolated	80.4%	94.4 %	97.9%	60%	69.5 (20.5 to 236)	83.7%
		50%	94.4 %	88.5%	68.9 %	17.0 (4.63 to 62.4)	74%
	Follicular	41.6%	100%	100%	34.8%	77.9 (4.73 to 1281)	55.5%
		19.6%	100%	100%	59.3%	27.6 (1.56 to 489)	63%
	Orange areas / crusts	31.2%	94.4 %	94.7%	30 %	8.10 (2.42 to 27.2)	46.3%
		15.2%	94.4 %	70%	56.7 %	2.54 (0.62 to 10.4)	58%
	Poral pattern	0%	79.6%	0%	19.9%	0.011 (0.0006 to 0.19)	18.9%
		0%	79.6%	0%	48.3%	0.041 (0.002 to 0.71)	43%
	Pustules	15.6%	87%	79.4%	24.4%	1.24 (0.508 to 3.04)	32.6%
		2.1%	87%	12.5%	50.5%	0.15 (0.018 to 1.26)	47.5%
vascular features	Any vessel	97.7%	14.8%	78.6%	66.7 %	7.35 (2.12 to 25.5)	78%
		100%	14.8 %	50%	100%	17.0 (0.95 to 303)	54%
	Dotted vessels	83.2%	50%	84.2%	48.2%	4.97 (2.55 to 9.67)	75.3%
		67.4%	50%	53.5%	64.3%	2.07 (0.91 to 4.67)	58%
	Linear vessels	65.3%	29.6%	74.8%	21.1%	0.79 (0.41 to 1.54)	56.8%
		71.7%	29.6%	46.5%	55.2%	1.07 (0.45 to 2.55)	49%
	Glomerular vessels	14.5%	94.4 %	89.3%	25.6%	2.87 (0.83 to 9.91)	33.5%
		4.4%	94.4 %	40%	53.7 %	0.77 (0.12 to 4.84)	53%
	Petechial spots	1.7%	100%	100%	23.8%	2.24 (0.11 to 44.0)	24.8%
		0%	98.2 %	0%	53.5%	0.38 (0.015 to 9.64)	53%
Clinical features		**Sensitivity**	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Odds ratio (95% CI)**	**Accuracy**
Erythema		100%	9.3%	77.9%	100 %	38.56 (2.10 to 709)	78.4%
		100%	9.3%	48.4%	100 %	10.3 (0.56 to 192)	51%
Oedema		99.4%	94.4 %	98.3%	98.1%	2924 (297 to 28721)	98.2%
		100%	94.4 %	93.9%	100%	1368 (68.8 to 27202)	97%
Vesicles		74%	100%	100%	54.6%	307 (18.6 to 5087)	80.2%
		0%	100%	***n.a.***	54%	1.17 (0.023 to 60.2)	54%

Pustules	12.1%	87%	75%	23.6%	0.93 (0.37 to 2.32)	29.9%
	2.2%	87%	12.5%	51.1%	0.15 (0.018 to 1.26)	48%
Poral reaction	0%	79.6%	0%	19.9%	0.011 (0.0006 to 0.19)	18.9%
	0%	79.6%	0%	48.3%	0.041 (0.002 to 0.71)	43%

[a] positive predictive value is the probability that an allergic reaction is present when the test is positive, [b] negative predictive value is the probability that an allergic reaction is not present when the test is negative, [c] accuracy is the overall probability that an allergic reaction will be correctly classified ; *n.a.*, not applicable. Significant values in bold.

Table 4. Sensitivity, specificity, positive and negative predictive values, accuracy and odds ratio of the dermoscopic and clinical criteria for the correct diagnosis of allergic (white lines) and weak (+) allergic (grey lines) *versus* irritant patch test reactions induced by SLS.

Dermoscopic features		Sensitivity	Specificity	Positive predictive value[a]	Negative predictive value[b]	Odds ratio (95% CI)	Accuracy[c]
non-vascular features	Erythema	99.42%	0%	93.99%	0%	5.00 (0.1927 to 129.7089), p=0.332	93.48%
		100%	0%	80.70%	***n.a.***	7.4158 (0.3736 to 147.1876), p=0.189	80.70%
	White soap bubble-like vesicles						
	Any vesicle	94.22%	100%	100%	52.38%	358.1429 (19.7177 to 6505.1422), ***p*<0.001**	94.57%
		78.26%	100%	100%	52.38%	79.9524 (4.3403 to 1472.7868), ***p*=0.003**	82.46%
	Clustered	49.13%	100%	100%	11.11%	22.2203 (1.2891 to 383.0019), ***p*=0.03**	52.17%
		21.74%	100 %	100%	23.40%	6.6164 (0.3592 to 121.8801), p=0.20	36.84%
	Isolated	80.35%	100 %	100%	24.44%	93.0000 (5.3479 to 1617.2709), ***p*=0.002**	81.52%
		50%	100 %	100%	32.35%	23.0000 (1.2800 to 413.2805), ***p*=0.03**	59.65%
	Follicular	41.62%	100%	100%	9.82%	16.4286 (0.9527 to 283.2960), ***p*=0.05**	45.11%
		19.57%	100%	100%	22.92%	65.4776 (3.8313 to 1119.0183), ***p*=0.003**	35.09%
	Orange areas / crusts	31.21%	100%	100%	8.46%	10.4895 (0.6070 to 181.2578), p=0.106	35.33%
		15.22%	100%	100%	22.00%	4.3671 (0.2315 to 82.3703), p=0.325	31.58%
	Poral pattern	0%	100%	***n.a.***	5.98%	0.0663 (0.0013 to 3.4950), p=0.180	5.98%
		0%	100%	***n.a.***	19.30 %	0.2473 (0.0047 to 13.1403), p=0.491	19.30%
	Pustules	15.61%	100%	100%	7.01%	4.3174 (0.2471 to 75.4356), p=0.316	20.65%
		2.17%	100%	100%	19.64%	0.7582 (0.0290 to 19.8585), p=0.868	21.05%
vascular features	Any vessel	97.69%	9.09 %	94.41%	20 %	4.2250 (0.4312 to 41.4016), p=0.216	92.39%
		100%	9.09 %	82.14%	100%	13.2857 (0.5050 to 349.5568), p=0.121	82.46%
	Dotted vessels	83.24%	72.73 %	97.96%	21.62%	13.2414 (3.3127 to 52.9286), ***p*<0.001**	82.61%

		67.39%	72.73%	91.18%	34.78%	5.5111 (1.2759 to 23.8051), ***p*=0.02**	68.42%
	Linear vessels	65.32%	36.36%	94.17%	6.25%	1.0762 (0.3029 to 3.8236), *p*=0.91	63.59%
		67.92%	30.19 %	49.32%	48.48 %	1.1786 (0.3007 to 4.6192), *p*=0.81	49.06%
	Glomerular vessels	14.45%	100%	100%	6.92%	3.9495 (0.2256 to 69.1352), *p*=0.35	19.57%
		9.43%	94.34 %	62.50%	51.02 %	1.2921 (0.0579 to 28.8228), *p*=0.87	51.89%
	Petechial spots	1.73%	100%	100%	6.08%	0.4721 (0.0230 to 9.7020), *p*=0.63	7.61%
		0%	100%	***n.a.***	19,30%	0.2473 (0.0047 to 13.1403), *p*=0.49	19.30%
Clinical features		**Sensitivity**	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Odds ratio (95% CI)**	**Accuracy**
Erythema		100%	0%	94.02%	***n.a.***	15.0870 (0.2861 to 795.5257), *p*=0.18	94.02%
		100%	0%	80.7%	***n.a.***	4.0435 (0.0761 to 214.8406), *p*=0.49	80.7%
Oedema		99.42%	100%	100%	91.67%	2645 (101.9591 to 68615.9926), ***p*<0.001**	99.46%
		100%	100%	100%	100%	2139 (40.2578 to 113650.6525), ***p*<0.001**	100%
Vesicles		73.41%	100%	100%	19.3%	63.0645 (3.6431 to 1091.6802), ***p*=0.004**	75%
		0%	100%	***n.a.***	19.3%	0.2473 (0.0047 to 13.1403), *p*=0.49	19.3%
Pustules		12.14%	100%	100%	6.75%	3.2426 (0.1844 to 57.0331), *p*=0.421	17.39%
		2.13%	100%	100%	19.3%	0.7582 (0.0290 to 19.8585), *p*=0.868	20.69%
Poral reaction		0%	100%	***n.a.***	5.98%	0.0663 (0.0013 to 3.4950), *p*= 0.179	5.98%
		0%	100%	***n.a.***	19.30%	0.2473 (0.0047 to 13.1403), *p*=0.49	19.30%

[a] positive predictive value is the probability that an allergic reaction is present when the test is positive, [b] negative predictive value is the probability that an allergic reaction is not present when the test is negative, [c] accuracy is the overall probability that an allergic reaction will be correctly classified; *n.a.*, not applicable. Significant values in bold.

Dermoscopic features	Weak allergic	Strong allergic	Extreme allergic	*P*	*P**	*P**	*p**

Table 5. Comparison of dermoscopic mean scores among weak (+), strong (++) and extreme (+++) positive reactions.

***** Bonferroni adjusted *P*-values. Significant values in bold

	reactions, n. 53 mean value (SD)	**reactions, n. 63** mean value (SD)	**reactions, n. 46** mean value (SD)	**ANOVA**	**weak *versus* strong allergic reactions**	**weak *versus* extreme allergic reactions**	**strong *versus* extreme allergic reactions**
Erythema	1.61 (0.61)	1.89 (0.64)	2.42 (0.58)	**<0.001**	0.053	**<0.001**	***P*<0.001**
Vesicles (any)	0.36 (0.59)	0.92 (0-87)	1.30 (1.01)	**<0.001**	**<0.001**	**<0.001**	***p*<0.001**
Orange-yellowish areas and crusts	0.28 (0.74)	0.44 (0.77)	1.02 (1.18)	**<0.001**	0.81	**0.002**	***p*=0.007**
Vessels (any)	0.73 (0.92)	0.78 (0.86)	0.96 (0.96)	0.093	0.99	0.15	*p*=0.209
Dotted vessels	0.83 (0.70)	1.33 (0.86)	1.8 (0.83)	**<0.001**	**0.003**	**<0.001**	***p*=0.011**

Figure Legend

Figure 1. (a1-3) Representative dermoscopic images of three extreme (+++) patch test allergic reactions to hydroxyethyl methacrylate 2%, disperse dyes mix 6.6% and nickel sulfate 5%, respectively. Numerous whitish soap bubbles-like vesicles, varying in size, both isolated and distributed in clusters, are present over an erythematous background. In a1 and, mostly, in a2, the vesicles tend to coalesce forming larger, irregular figures. Dense vessels, mainly dotted in shape, are observable in a1 and a3. **(b1-3)** Characteristic dermoscopic images of three weak (+) patch test allergic reactions to nickel sulfate 5% (b1), neomycin sulfate 20% (b2) and nickel sulfate 5% (b3). In all these reactions erythema is clearly visible but is less intense than in extreme reactions. Vesicles are fewer in number, less sharply bordered compared with extreme reactions and mainly arranged in isolated elements. In b3, it is possible to observe orange crusts. **(c1-3)** Dermoscopic images of three patch test irritant reactions (c1, cocamidopropyl betaine 1%, c2 cobalt chloride 1%, c3 nickel sulfate 5%). Erythema and vessels are less featured than in allergic reactions whereas vesicles are not present. In the irritant reaction to cobalt (c2) a typical “poral pattern” is observed. A pustule developed in the reaction to nickel (c3). **(d1-3)** Representative dermoscopic images of irritant reactions to sodium lauryl sulfate 2.5%. Erythema and linear vessels are visible. No further dermoscopic variables are present; (original magnification x10 for all images).