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Clinical relevance of positive patch test reactions to lanolin: A ROAT study

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Abstract

Background: Lanolin is often included when patch testing for common contact allergens. The clinical relevance of a positive patch test reaction to lanolin markers is, however, still a subject for debate.

Objectives: To evaluate Amerchol L101 as a marker of lanolin allergy and investigate the clinical impact of lanolin-containing moisturizers on healthy and damaged skin using the repeated open application test (ROAT).

Methods: Twelve test subjects and 14 controls were patch tested with Amerchol L 101 and additional lanolin markers. Subsequently, a blinded ROAT was performed on the arms of the study participants for 4 weeks. Each participant applied a lanolin-free cream base and two different lanolin-containing test creams twice daily on one arm with intact skin and on the other arm with irritant dermatitis, induced by sodium lauryl sulfate (SLS).

Results: Eleven test subjects (92%) had positive patch test reactions to Amerchol L 101 when retested and one test subject (8%) had a doubtful reaction. None of the study participants had any skin reactions to the ROAT on intact skin and all participants healed during the ROAT on damaged skin.

Conclusions: Lanolin-containing emollients do not cause or worsen existing dermatitis when performing ROAT in volunteers patch test positive to Amerchol L101.

KEYWORDS

allergic contact dermatitis, Amerchol L101, CAS no. 8027-33-6, emollient, lanolin, patch testing, relevance, repeated open application test

1 | INTRODUCTION

In 1922, a German report described a patient who developed a "skin reaction" when using a cream containing 6% wool alcohol.[1] Because of many similar reports, lanolin was included in the early baseline series and has been there since.

Because of its emollient properties, lanolin is widely used in medicaments and skin care products, for example, for atopic/dry skin, wound healing, and in nipple care creams. Lanolin is also found in leather softeners and as a lubricant in ball bearings. Meanwhile, how to patch test for lanolin allergy has been much discussed, mainly due to the complexity of this product.[1,2]

Lanolin is derived from the secretions of the sebaceous glands of sheep and the composition may vary depending on the sheep's breed, geographic location, and methods of extraction, etc.[2] The product named "lanolin" is actually a mixture of substances[3] and much effort

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has been put into finding the potential allergens.[3-5] Most researchers state that the allergen resides in the alcoholic fraction,[3-5] but oxidation with the production of possible haptens has also been found to be of importance.[6]

While at the start lanolin may vary in composition, the refining and purifying processes may also differ and, therefore, the end product becomes difficult to define in detail. Thus, the question arises of whether the lanolin chosen for patch tests is representative of the lanolin derivatives encountered in products.

Over the years, manufacturers of lanolin-containing products have refined lanolin and claim the lanolin used today is free from sensitizers and, therefore, no longer a source of lanolin contact allergy.[7,8] Owing to this notion, the clinical relevance of a positive patch test reaction to lanolin has been questioned.[1,9]

At the Department of Occupational and Environmental Dermatology (DOED) in Malmö, lanolin contact allergy has traditionally been detected using a test preparation of Amerchol L101 "as is" (100%). Historically, Amerchol L101 50% pet. as well as lanolin alcohol 30% pet. have been tested in parallel with Amerchol L101 100% at the DOED. Amerchol L101 "as is" has regularly detected statistically more positive patch test reactions than Amerchol L101 50% pet. (3.0% vs 0.53%; $P < .0001$, two-sided Fisher's exact test) and lanolin alcohol 30% pet. (3.7% vs 0.64%; $P < .0001$, two-sided Fisher's exact test). In Malmö the prevalence figures for lanolin contact allergy (0.53% for Amerchol L101 50% pet. and 0.64% for lanolin alcohol 30% pet.) are slightly lower than figures recently published in European studies.[10-12] These studies report values of 1.52%–3.48% for Amerchol L101 50% pet. and 0.90%–2.38% for lanolin alcohol 30% pet. However, in our department, weak positive patch test reactions to lanolin test preparations are commonly observed, again raising the question of clinical relevance. Recent studies have, therefore, advocated the need for a repeated open application test (ROAT) to address the relevance issue.[11,13]

The aim of this study was to evaluate Amerchol L101 as a marker of lanolin allergy. Using ROAT, the objective was also to investigate whether lanolin or lanolin derivatives in commercially sold creams can elicit a skin reaction on healthy and damaged skin, respectively, in those testing positive for Amerchol L101. Comparison between patch testing with markers of lanolin allergy and additional lanolin derivatives was considered a secondary aim.

2 | METHODS

2.1 | Study design and procedure

The study was designed as an experimental, double-blind, randomized case–control study. Patch tests were performed with lanolin markers in different concentrations, lanolin-containing creams "as is", and their lanolin samples separately, followed by a ROAT with the lanolin samples in an without any active ingredients base. The ROAT was applied on the arms of the participants: one arm randomly allocated for the ROATs on intact skin, and on the other arm ROATs were performed on a sodium lauryl sulfate (SLS)-induced irritant dermatitis. For each individual, Latin square design was applied to randomize the skin area used for each moisturizer. The moisturizer was colour coded and the skin area was marked correspondingly to prevent mistakes during the study.

Two experienced dermatologists read the patch tests and ROATs, respectively. The positions of the patch tests with a dilution series on the back were randomized and blinded for the reading dermatologist. The dermatologist reading the ROATs was not aware of the patch test results. The ROAT was performed for a maximum of 4 weeks. The study design is presented in Figure 1.

The study was approved by the Regional Ethical Review Board in Lund, Sweden (Dnr. 2011/606). All the 26 participants gave written consent prior to inclusion.

2.2 | Study population

From our data register of consecutively patch tested dermatitis patients, 125 individuals (>18 years) were identified who had a positive reaction to Amerchol L101 100% between 2009 and 2013. In the identified group of 125 adults, 25 individuals had a ++ /+++ reaction. The aim was to include patients with the stronger reactions, therefore these 25 individuals were contacted first. Known or suspected pregnancy, oral corticosteroids, ongoing eczema on the arms or back, and phototherapy were exclusion criterias as well as topical corticosteroids used on the arms or back 2 weeks prior to or during the study period. Patients were contacted and 18 test subjects were included (14 with + reaction, four with a ++ reaction). Age- and sex-matched controls without contact allergy to Amerchol L101 and lanolin were enrolled from the same register. Twelve test subjects (five males, seven females, mean age 54 years) and 14 controls (seven males, seven females, mean age 46 years) completed the study. Six test subjects and two controls withdrew their consent before day (D) 0 and two controls only showed up on D0. Six of 12 test subjects had atopic manifestations compared to two of the 14 controls ($P = .09$; two-sided Fisher's exact test) (Table 1). All study participants were patch tested at our clinic prior to the present study with an extended Swedish baseline series comprising either 73 or 75 allergens, depending on time of investigation. The number of contact allergies for each study participant prior to the present study is presented in Table 1.

2.3 | Repeated open application test creams

The aim was to use lanolin derivatives that are actually used in products declared to contain lanolin. For this purpose, a company of Scandinavian origin and a German company producing skin care products were contacted and the lanolin used in their products was purchased. Three different lanolin products were tested (denoted as Lanolin A, B and C in Table 2). Since the aim was to investigate different lanolin derivatives, we chose to use the same cream base for all used creams. The cream base for a commonly used cream in Sweden containing lanolin was chosen. The cream base was used as negative contro; the

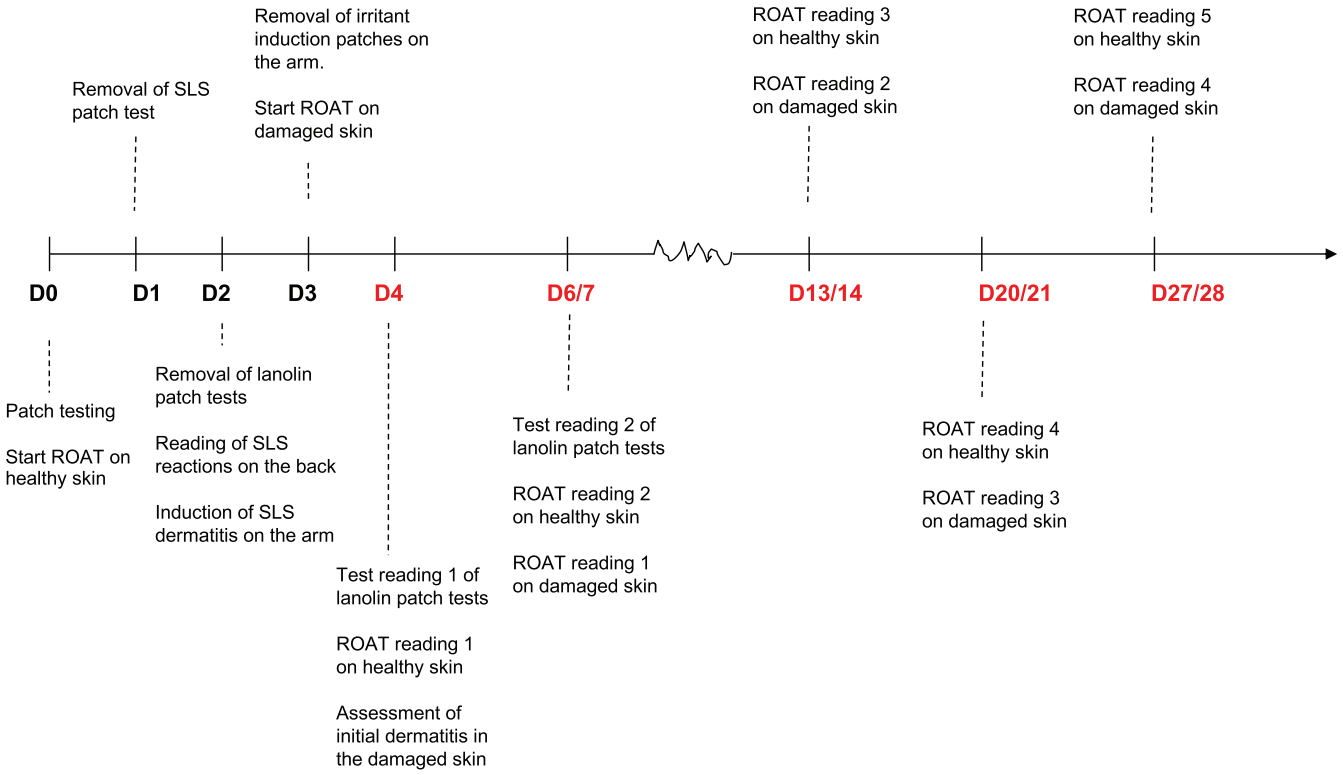

FIGURE 1 Flow chart illustrating study interventions and clinic visits on a timeline. Second patch test reading was performed on either day 6 or 7 and the participants were followed every 7th day for the following 21 days. ROAT, repeated open application test

cream with its lanolin derivative (Lanolin A) was evaluated in all participants and the cream base also served as a base for the other evaluated creams. The ingredients list for the cream base is presented in Box 1.

BOX 1 Cream base, ingredient list

Aqua, isopropyl Myristate, glycerin, Sorbitan stearate, White soft paraffin, Dimethicone, Cetyl alcohol, Polysorbate 60, Sorbic acid.

All three lanolin derivatives (Lanolin A, B and C) were used at 6% (w/w) in the cream base, according to the concentration of the commercially available product containing Lanolin A. The test creams were prepared at the laboratory of the DOED in Malmö and used in the ROAT (ie, cream A, B and C). For the ROAT, only two test creams together with the negative control (cream base) were evaluated in each participant because of the limited skin area available. Thus, cream A was analyzed in all participants, cream B was evaluated in 12 participants (seven test subjects and five controls), and cream C in 14 participants (six test subjects and eight controls) (Table 1).

2.4 | Patch test preparations

All participants were initially patch tested with a dilution series of Amerchol L101 (Chemotechnique Diagnostics, Vellinge, Sweden), two dilutions of lanolin alcohol (Chemotechnique Diagnostics) as well as a commercially available cream marketed to contain "pure lanolin" (named cream D in Table 2). Additionally, patch testing of the lanolin derivatives and the test creams for the subsequent ROAT study was performed "as is" as well as with dilution series (Table 2). The dilution series were prepared at the laboratory of the DOED in Malmö, by adding white vaseline (APL, Tamro, Sweden).

All patients were patch tested with Finn chambers (SmartPractice, Phoenix, Arizona) on Scanpor tape (Norgeplaster, Vennesla, Norway) and approximately 20 mg of each test preparation was applied as recommended by the ESCD.[14] The tests were applied on the upper back and removed after 48 hours and readings according to the criteria of the ESCD[15,16] were carried out on day (D) 4 and D6/7 (Figure 1).

All participants were also patch tested with dilutions of SLS purchased from Acros Organics (Geel, Belgium) in the concentrations 3.0%, 2.0%, 1.0%, 0.5%, and 0.25% aq, (w/v) to find a suitable concentration for induction of an irritant contact dermatitis for the subsequent ROAT[17] (Table 2). The amount of twenty-five μL of each SLS preparation was applied with a micropipette on a 10x10 mm filter paper (Munktell Filter, Grycksbo, Sweden) attached to the inner surface of a single 30x30 mm hydrocolloid dressing (Hydrocoll Extra

TABLE 1 Characteristics, patch test and repeated open application test results for the 26 study participants[a]

	Atopy			Results from patch-testing prior to study		Lowest SLS concentration mimic-king a + reaction (%)	Lowest concentration rendering a positive patch test/number of doubtful reactions (%/no)[b]					Patch test reactions		Time of healing[c]	
No.	D	R	A	No of CA	A L101[‡] 100% / at study date		AL101	Lanolin A	Lanolin B	Lanolin C	Lanolin alcohol	Cream base	Cream A/B/C	Cream base	Cream A/B/C
Test subjects															
1	No	Yes	No	1	++/+	3.0	100/1	*/*	*/*	*/*	*/*	-	−/−/−	T4	T2/T2/NT[§]
2	Yes	Yes	No	2	+/+	2.0	10/2	*/2	*/4	1/2	*/2	-	(+)/−/++	T3	T3/NT/T3
3	Yes	No	Yes	1	+/+	3.0	100/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T3/NT/T3
4	No	No	No	2	++/+	2.0	50/1	*/*	*/*	*/2	*/*	-	−/−/−	T4	T4/T2/NT
5	No	No	No	2	+/+	1.0	100/2	*/*	*/*	*/2	*/*	-	−/−/−	T3	T3/NT/T3
6	No	Yes	No	10	+/+	1.0	50/2	*/*	*/*	*/3	*/*	-	−/−/−	T3	T2/T3/NT
7	No	No	No	1	+/+	2.0	3/*	*/5	*/2	1/*	3/*	-	−/−/+	T3	T3/T3/NT
8	No	No	No	5	+/+	3.0	100/3	*/*	*/*	*/2	*/*	-	(+)/(+)/(+)	T3	T3/NT/T4
9	No	Yes	No	2	++/+	2.0	100/1	*/*	*/*	*/*	*/*	-	−/−/−	T2	T3/T3/NT
10	No	No	No	4	++/+	3.0	100/2	*/*	*/*	*/4	*/1	-	−/−/−	T3	NH/T3/NT
11	Yes	No	No	2	+/(+)	3.0	*/2	*/*	*/*	*/*	*/*	-	−/−/−	T3	T3/T3/NT
12	No	No	No	4	+/++	3.0	30/1	1/1	*/2	3/3	*/1	-	−/−/(+)	NH[¶]	NH/NT/T3
13[d]	No	No	No	2	−/+	3.0	100/1	*/*	*/*	*/*	*/*	-	−/−/−	T4	T4/NT/T3
Controls															
14	No	No	No	0	−/−	1.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T3	T3/NT/T3
15	No	No	No	0	−/−	2.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T2/T2/NT
16	No	No	No	0	−/−	3.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T2/T2/NT
17	No	No	No	1	−/−	3.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T2/NT/T2
18	No	No	No	0	−/−	3.0	*/*	*/*	*/*	*/3	*/*	-	−/−/−	T3	T2/NT/NV[e]
19	No	No	No	0	−/−	2.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T2/NT/T2
20	Yes	Yes	No	4	−/−	1.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T3	T3/NT/T1
21	No	No	No	2	−/(+)	3.0	*/1	*/*	*/*	*/*	*/*	-	−/−/−	T3	T3/NT/T3
22	No	No	No	0	−/−	3.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T2	T4/NT/T4
23	No	No	No	2	−/−	2.0	*/*	*/*	*/*	*/2	*/*	-	−/−/−	T2	T2/T2/NT
24	No	No	No	0	−/(+)	2.0	*/2	*/*	*/*	*/*	*/1	-	−/−/−	T2	T2/NT/T4
25	No	No	No	2	−/−	3.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T3	T2/T2/NT
26	Yes	No	No	0	−/−	3.0	*/*	*/*	*/*	*/*	*/*	-	−/−/−	T4	T2/T2/NT

Abbreviations: A, asthma; CA, contact allergy; D, atopic dermatitis; NH, not healed; NT, not tested; NV, not valid; R, rhinoconjuctivitis; ROAT, repeated open application test; SLS, sodium lauryl sulfate.

[a]All 26 participants had negative ROATs to the cream base and all creams containing lanolin (A, B, C) on intact skin.

[b]No such reaction = *.

[c]The first visit recording healing of damaged skin on ROAT application site. ROAT reading; T1 = Day 3/4, T2 = Day 10/11, T3 = Day 17/18, T4 = Day 24/25.

[d]Enrolled as a control subject.

[e]NH = Not healed, doubtful reaction remaining.

[f]NV= Not valid, due to the failure of inducing the initial dermatitis.

TABLE 2 Patch test preparations

Amerchol L101 100%	Lanolin product C 50%pet.
Amerchol L101 50% pet.	Lanolin product C 30% pet.
Amerchol L101 30% pet.	Lanolin product C 10% pet.
Amerchol L101 10% pet.	Lanolin product C 6% pet.
Amerchol L101 3% pet.	Lanolin product C 3% pet.
Lanolin product A "as is"	Lanolin product C 1% pet.
Lanolin product A 30% pet.	Lanolin alcohol 30% pet.
Lanolin product A 10% pet.	Lanolin alcohol 3% pet.
Lanolin product A 6% pet.	Cream base "as is"
Lanolin product A 3% pet.	Cream A "as is"
Lanolin product A 1% pet.	Cream B "as is"
Lanolin product B 50% pet.	Cream C "as is"
Lanolin product B 30% pet.	Cream D "as is"
Lanolin product B 10% pet.	SLS†: 3%, 2%, 0.5%, 0.25% aq.
Lanolin product B 6% pet.	
Lanolin product B 3% pet.	
Lanolin product B 1% pet.	

†Sodium Lauryl Sulfate.

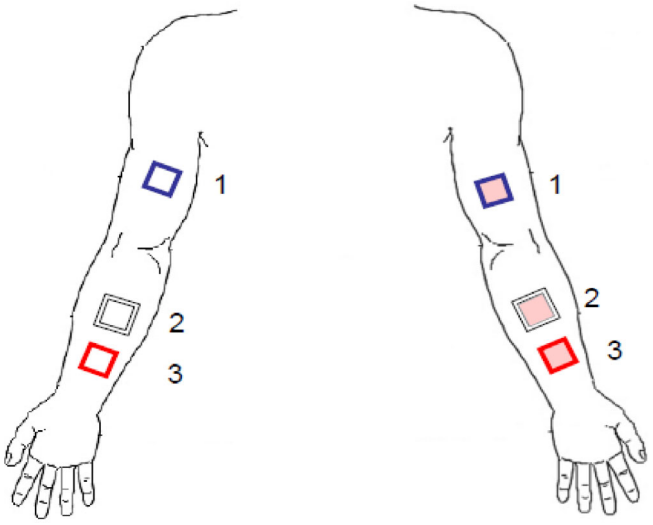

FIGURE 2 Three applications sites on each arm for the repeated open application test (ROAT), color coded and paired. Randomly chosen, one arm for ROAT on intact skin, one arm with sodium lauryl sulfate induced damaged skin

Thin, Hartmann, Germany). The dressings were applied using Scanpor tape on the back separated from the other patch tests, enabling removal by the patient after 24 hours. The volume was chosen because it soaked into the filter paper without leaking.[17] The SLS patch tests were read on D2 (Figure 1). Based on the SLS patch test results, the lowest concentration mimicking a + reaction (erythema and infiltration, according to ESCD criteria[18]) was chosen to provoke an irritant contact dermatitis for the ROAT on one arm, see below.

2.5 | Repeated open application test

The ROAT was performed on the volar aspect of the arms of the study participants. On D0 each participant had three areas the size 30x30 mm marked out by the dermatologist with a surgical pen, one above the arm fold (area 1) and two below (area 2 and 3) (Figure 2). On this arm a ROAT on intact skin was induced on D0. On the other arm, an irritant contact dermatitis was provoked. For this purpose, 200 μL of the selected concentration of SLS was applied on each one of the three pieces of 30x30 mm filter paper and attached to a hydrocolloid dressing to secure adherence and applied to the ventral aspects of the participants' arm. The participants removed the patches after 24 hours (D3), followed by the introduction of the ROAT on injured skin on D3 (Figure 1); the same instructions applied as described above for the ROAT on intact skin. In 13 participants (eight test subjects, five controls) the induced dermatitis areas were placed on the right arm and in 13 participants (five test subjects, eight controls) they were placed on the left arm.

Each participant received three color-coded (red, blue and white) syringes containing three different creams. Each cream was to be applied on either area 1, 2 or 3 on both arms. The individuals received disposable rulers and were instructed to apply a 7–8 mm long string of cream corresponding to approximately 2.0 mg/cm^2, three times daily onto the marked areas. To avoid contamination the participants were instructed to wash their hands carefully between each contact with the different syringes, or to use different fingertips on application. The instructions were repeated and followed up through weekly feedback at each clinic visit for the ROAT readings.

A ROAT protocol was used throughout the study to record the healing time and to register possible signs of induction of dermatitis or aggravating contact dermatitis, according to the criteria recommended by Johansen et al.[19] Healing was defined by the absence of erythema, infiltration, papules, and vesicles, while a doubtful reaction was defined as a dermatitis without morphological features fulfilling the criteria of a positive reaction.[19]

The fields of the ROAT were first inspected on D4 (Figure 1). At this point the impact of the application of creams for 4 days on healthy skin was assessed as well as the initial status of the injured skin. The ROATs were also read on D6/7 when the ROAT on intact skin had been performed for 6/7 days and on the injured skin for 3/4 days. After this the skin was controlled once every week for a maximum of 4 weeks. Regardless of healing of the application sites, the patients were asked to continue the ROAT for 4 weeks, since the aim was not only to decide time for healing, ie negative ROAT, but also to ensure that the cream applied would neither by irritation nor contact allergy delay healing or elicit a contact dermatitis.

2.6 | Statistical analysis

McNemar´s two-sided test was applied to compare healing at a given time between the areas treated with the different creams intraindividually, while Fisher´s two-sided exact test was applied to

compare healing between test subjects and controls. The mean healing time and the comparison of SLS concentrations used for inducing the dermatitis between different defined groups was compared by the Mann–Whitney *U* test for two independent variables and presented as median and interquartile range (IQR), while Wilcoxon's signed test was used for calculating the mean healing time for the different creams. A *P*-value < .05 was considered significant.

3 | RESULTS

3.1 | Patch tests

Of the test subjects, 11 out of 12 had a positive patch test reaction upon retesting with Amerchol L 101 at 100% concentration. In one test subject only a doubtful reaction was noted. All four test subjects who previously reacted with a ++ reaction had merely a + reaction upon retesting. One of the controls, a 71 year-old non-atopic male, had a + reaction to Amerchol L101 100% and thus was redefined as a test subject for the following analysis of the results (Table 1). With regard to patch testing of Amerchol L 101 50% pet., a positive reaction was found in five out of 13 test subjects and a doubtful reaction in seven test subjects (Table 1).

Only three of 13 test subjects had positive reactions to patch test preparations other than Amerchol, namely Lanolin product C, cream C, Lanolin product A, and lanolin alcohol (Chemotechnique Diagnostics) (Table 1). None of the test subjects reacted to the cream base, cream A or cream B when tested "as is", or to Lanolin product B. When comparing the controls and the test subjects it should, however, be noted that eight of 13 test subjects had doubtful reactions to test substances other than Amerchol L101, and doubtful reactions were much more common among test subjects (61 doubtful reactions) compared to controls (nine doubtful reactions), *P* < .001 (Table 1). None of the controls had positive patch test reactions to any test preparations apart from the expected irritant reactions to SLS. All 26 participants had negative patch test results to Lanolin product D.

3.2 | Experimental irritant dermatitis

The concentrations of SLS chosen for the elicitation of the irritant dermatitis were equally distributed among the Amerchol positive subjects and the controls (median SLS conc. 3.0%, IQR 1.0%; *P* > .99). No significant differences were observed with regard to atopic constitution (atopic: median SLS conc. 2.5%, IQR 1.75%; non-atopic: median SLS conc. 3.0%, IQR 1.0%; *P* = .68) or atopic dermatitis (AD: median SLS conc. 3.0%, IQR 1.5%; non-AD: median SLS conc. 3.0%, IQR 1.0%; *P* = .90). Intraindividual variation was observed regarding the intensity of the dermatitis provoked on three sites on the same arm, by the same SLS concentration. The varying intensity showed no relationship to arm or anatomic site in the study population.

3.3 | Repeated open application test

For all 26 participants there were no reactions found to either cream base or the creams containing lanolin (A, B, C) on intact skin during the ROAT period. All but one (77 of 78) application sites of the SLS-induced dermatitis presented with an infiltrated erythema at the initiation of the ROAT. On one of the controls, the occlusive treatment for the induction of the SLS dermatitis failed on the area to be treated with cream C and no reaction was observed on this area during the study. No papules or vesicles developed on any application sites during the ROAT and 75 of 78 application sites were healed at the last examination on D24/25. Three application sites in two test subjects

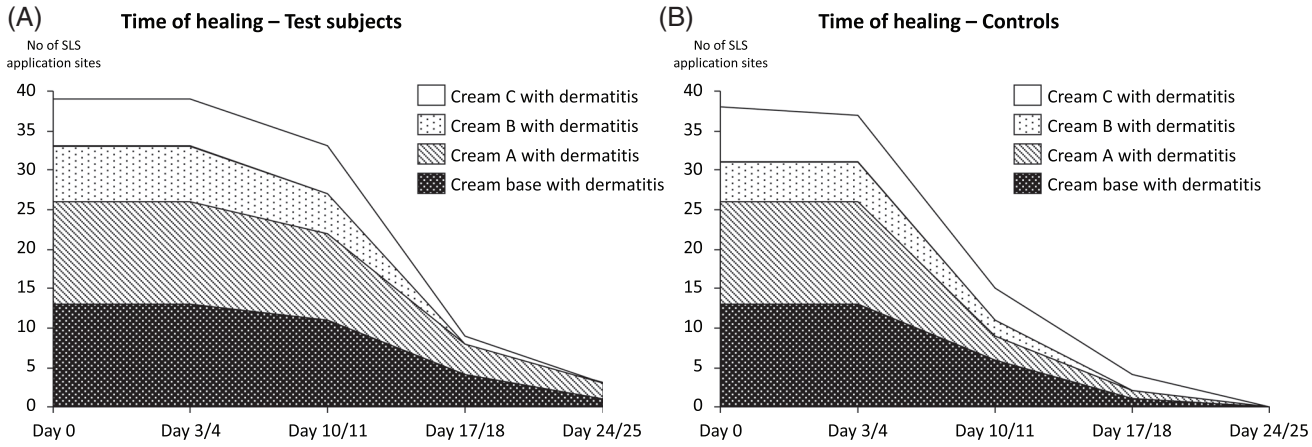

FIGURE 3 Illustrating the number of sodium lauryl sulphate (SLS) application sites with remaining dermatitis observed at each visit at the clinic. Day 0 corresponding to the removal of the SLS patches and the initiation of the repeated open application test on damaged skin (ie D3 in Figure 1). (**A**), number of application sites on the test subjects. At day 0: Cream base = 13, cream A = 13, cream B = 7, cream C = 6. (**B**), number of application sites on the controls. At day 0: Cream base = 13, cream A = 13, cream B = 5, cream C = 7 †. † one application site excluded due to the failure of inducing the initial dermatitis

(Table 1) presented with a remaining doubtful reaction, a spotty weak macular erythema, at D24/25. All three sites had severe dermatitis reactions at the initiation of the ROAT.

The number of test subjects and controls with ongoing dermatitis at each clinical examination are illustrated by Figure 3(A) (test subjects) and 3B (controls). The three application sites with remaining doubtful reactions on D24/25 all had a severe SLS-induced dermatitis but had markedly improved during the ROAT period; hence a clearance would have been expected had there been a 5th follow-up on D31/32. Therefore, these three application sites were included for the analysis comparing healing time. To avoid doubt regarding statistical significance due to assumptions, D24/25 was used as time of healing since calculating with a healing time on D31/32 would result in larger differences in the analysis.

A significant difference was observed between test subjects and controls regarding healing time, with the controls healing significantly faster on cream base (test subjects: median 17 days, IQR 7 days; controls: median 10 days, IQR 7 days; $P = .044$), on lanolin creams (cream A + B + C) (test subjects: median 17 days, IQR 0 days, controls: median 10 days, IQR 7 days; $P = .001$) and also on cream A separately (test subjects: median 17 days, IQR 7 days; controls: median 10 days, IQR 3.5 days; $P = .006$) (Figure 3(A and B). Regarding healing on lanolin creams (cream A + B + C) and lanolin cream A, the differences between the test subjects and the controls were already significant on the second ROAT reading, ie after applying creams for 10/11 days on damaged skin (cream A + B + C; 4/26 application sites on test subjects vs 18/25 application sites on controls, $P < .001$, cream A; 2/13 test subjects vs 10/13 controls, $P = .005$). This early significant difference was not observed with cream base (2/13 test subjects vs 7/13 controls; $P = .097$).

No significant differences or trends regarding the median healing time, or comparisons on healing at the time of ROAT readings were observed when comparing cream base to cream A or all three lanolin creams within the test subjects or control group (all P-values > .30). The ROAT outcome for the three test subjects with positive patch test reactions to Lanolin product A, Lanolin product C, and cream C is presented in Table 1.

4 | DISCUSSION

A secondary aim in this study was to compare patch testing with markers of lanolin allergy and additional lanolin derivatives. Patch testing with the commercially available markers simultaneously, namely Amerchol L101 "as is" and 50% pet., and lanolin alcohol 30% pet., has shown poor concordance,[12,13,20-22] which is also the case in the present study. Additionally, there seems to be no correlation between a positive reaction to the test substances used as markers of lanolin allergy and the lanolin derivatives that we could purchase for testing, ie, those that are actually used in leave-on products. This is consistent with other reported study results and the recommendation of testing with patients' own products in case of a clinical suspicion of lanolin contact allergy.[20-22]

The main aim was to find a possible indication of clinical relevance between patch test reaction and products used and, therefore, a ROAT was performed with three applications per day for a period of 4 weeks. Hauksson et al showed the value of performing ROAT on irritant dermatitis skin as they investigated the clinical relevance of low concentrations of formaldehyde and found a low exposure sufficient to worsen an ongoing dermatitis.[17] Lanolin-containing creams are often promoted as rich emollients especially suited for cracked, dry or eczematous skin. Presuming consumers apply lanolin-containing creams also on damaged skin, we found it relevant to perform a ROAT on irritant dermatitis skin as well to mimic consumers "true" exposure. The exposure frequency was standardized as three applications per day for all study participants based on the assumption that a consumer is more inclined to use emollients more frequently when experiencing dry or damaged skin.

A few ROAT studies have been performed on induced irritant dermatitis, with dermatitis being present at the starting point of the ROAT.[17,23] These studies had deterioration as the outcome measure. However, in the present study no deterioration was observed, only a difference in healing time. To the best of our knowledge, this is the first time that healing time has been used as an outcome measure in a ROAT study.

Regarding the study results, no positive ROAT reactions were provoked, either in the control or the test subject group. This assessment was based on the following observations: (i) no reactions observed with ROAT on either intact skin or SLS-induced dermatitis, (ii) none of the application sites developed papules or vesicles, (iii) the initial erythema and infiltration improved throughout the ROAT, and (iv) there was no significant difference in healing time between cream base and lanolin-containing creams in respective individuals among the test subjects. However, the test subjects healed significantly slower when applying lanolin creams (creams A + B + C) and cream A on injured skin compared to the control group ($P = .001$ and $P = .006$, respectively) and this was also the case when applying cream base on injured skin ($P = .044$).

Thus, our results showed a significant difference in healing time on cream base between test subjects and controls, and no significant difference in healing between applying cream base and lanolin creams in the test subject group. We found no obvious explanation to these observations.

Erfurt-Berge et al performed a register data study on patch test results in patients with chronic leg ulcers and found that 19% of the patients with positive patch test reactions to lanolin alcohol 30% pet. had concomitant reactions to cetearyl alcohol 20% pet.[24] This knowledge should have been taken into consideration when choosing the cream base for the current ROAT study, however, it was not available at the time our ROAT study was planned and performed. The cream base for the study was not chosen based on the ingredients list, aside from containing lanolin. The cream was chosen due to its wide consumption in Sweden, especially in children and on "sensitive" skin. The one test subject with a positive patch test reaction to lanolin alcohol healed on all three applications sites with induced irritant dermatitis on day 17/18 (Table 1) therefore, we do not believe that the cetearyl alcohol in the cream base is a confounder in the study results.

Positive patch test reactions to lanolin are often reported to be more common among atopic dermatitis patients.[12,25,26] The explanation has mainly been an impaired barrier function and a higher exposure to emollients, thus also lanolin-containing ones. The observation made in the present study cannot be explained by overrepresentation of either atopy or atopic dermatitis in the test group since the individuals with atopic constitution did not have slower healing time (AD: $P = .90$, atopy: $P = .68$) (Table.1).

SLS is a surfactant used in studies to induce dermatitis and measure skin irritancy.[18,27] An irritant skin reaction to low concentrations of SLS would imply a tendency of irritant skin reactions.[28] In the present study, however, there was no significant difference in the mean SLS concentration for eliciting a weak positive irritant reaction in test subjects versus controls. Some contact allergens, such as hexavalent chromium and parabens, have been reported to elicit irritant reactions more frequently.[29,30] This has also been proposed for lanolin[27] and Amerchol L101 may have irritant properties due to a high concentration of mineral oil.[9,31] All 26 participants had been patch tested prior to the present study (2009–2013), none showing reactions to either hexavalent chromium or parabens, which could have supported a possible tendency for irritancy.

The majority of reactions to lanolin preparations are + reactions and reactions stronger than ++ are rare.[12,27] This is consistent with the clinical experience at the DOED and is reflected in the present study results. It is reasonable to expect a faster or stronger elicitation of a contact allergic reaction in an individual with a strong (++/+++) patch test reaction as compared to a weak (+) reaction to the relevant allergen. In the present study, only four test subjects (30.8%) had a strong reaction to Amerchol L101 prior to the study and only one test subject (7.7%) presented with a strong reaction to Amerchol L101. The low percentage of test subjects with strong patch test reactions to the lanolin markers might be considered a study limitation. The evaluation of the ROAT outcome, and hence the clinical relevance, might be more convincing when performed on individuals with a strong contact allergy to lanolin. Also, performing the ROAT with all three lanolin-containing test creams on all study participants would have been ideal, but was not possible because of the limited skin area available. This limitation resulted, for example, in a test subject with a positive patch test reaction to lanolin C and test cream C not performing a ROAT with cream C due to of random selection (Table 1). Performing the study with a larger study population would also increase the statistical power of the study. However, considering the extensive patch testing and the numerous visits to the clinic and daily applications during a period of 4 weeks, we are grateful to all 26 study participants for their dedication and perseverance.

It is said to be difficult to read patch tests of lanolin-related test substances, and the reproducibility of the positive patch test reaction is low.[9,31] Studies have shown that in repetitive testing, weak + reactions to contact allergens can alternate with doubtful reactions to the same allergen.[32,33] Once a patient has a positive patch test reaction to a contact allergen, however, the patient is considered sensitized, despite a doubtful reaction upon retesting. Therefore, test subject 11 (Table 1) was considered sensitized to Amerchol L101 and was not excluded from the study.

In this study we could reproduce our results with regard to positive or negative reaction when patch testing with Amerchol L 101 "as is" except for a new +reaction in one control and a doubtful reaction in one test subject. However, the strength of the reaction was not reproducible in five of 12 previous Amerchol-positive individuals, which is also quite often the case for other test substances.[33,34] With regard to the morphology of the positive reactions, these fulfilled the criteria for an allergic reaction as defined by ESCD.[16]

From the results found in the present study with regard to patch testing with Amerchol as compared to the lanolin derivatives actually used in products tested here, there seems to be no correlation. When patch testing with the other lanolin substances, regardless of concentration, there were only three test subjects with positive reactions. There were, however, several test subjects that showed doubtful reactions. Two test subjects had positive patch test reactions to Lanolin product C and cream C. The test subject with a ++ reaction to cream C had a negative ROAT and also healed fast when applying cream C on SLS induced dermatitis, hence presenting no correlation between patch test and ROAT outcome for cream C. The other test subject was, unfortunately, not randomized to apply cream C (Table 1).

It is difficult to explain the discrepancy between patch test reactions with different lanolin markers, the ROAT results as well as finding of more doubtful reactions to the other lanolin markers and the delayed healing time per se, including for the cream base in the test subject group. A possible interpretation of our results is that Amerchol allergy is a false-positive, ie not a true sign of contact allergy, even though fulfilling the morphological criteria for a positive reaction. This has also been proposed because of the low reproducibility,[12,13,27] a higher prevalence of lanolin reactions in children,[35-37] and doubtful reactions to lanolin being common. If arguing in favour of lanolin not being a true contact allergen but a marker of unspecific reactivity in the skin, it appears the reactivity provoked by lanolin differs from the reactivity induced by SLS or the sensitivity observed in atopic skin.

Our results support the clinical observation that many patients found to have contact allergic to lanolin or Amerchol L101 seem to be able to use lanolin-containing emollients on healthy skin without eliciting a skin reaction. Regarding application on damaged skin, further studies are needed to assess whether the slower healing is a result of true contact allergy or something else.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Ada Uldahl: Conceptualization; data curation; formal analysis; investigation; writing-original draft; writing-review and editing. **Malin Engfeldt:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; writing-original draft; writing-review and editing. **Cecilia Svedman:** Conceptualization;

funding acquisition; investigation; methodology; resources; supervision; writing-original draft; writing-review and editing.

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