

# Contact Allergy to Special and Standard Allergens in Patients with Venous Ulcers

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## ABSTRACT

*The aim of the study was to determine the prevalence of contact sensitivity in patients with leg ulcers, and possible difference in the rate of contact hypersensitivity to standard series of allergens used in patch testing, and to particular topical agents used in local therapy of leg ulcers in special series, patients with and without atopy. The study included 60 patients, 45 female and 15 male, aged 37–85 (mean 68.37 female and 51.13 male), 30 of them with and 30 without allergic contact dermatitis (ACD) of the leg (control group). The mean duration of leg ulceration was 5.62 years. The two groups of patients underwent testing to standard series allergens and target series allergens including mupirocin, bepanthene, silver sulfadiazine, chloramphenicol + clostridiopeptidase, betamethasone dipropionate, hydrocortisone + oxytetracycline, momethasone, alginate, hydrocolloid, lanolin, pyrogallol, Vaseline, permanganate, Rivanol, povidone-iodine, gentamicin, i.e. local agents most frequently used by the patients. Contact allergic hypersensitivity to standard series allergens was demonstrated in 25 patients with a total of 49 positive reactions and a mean of 1.6 reactions per patient. Positive reactions were most commonly recorded to balsam of Peru, fragrance mix and neomycin sulfate. There were 12 positive reactions to target series allergens, mean 0.4 reactions per patient. Forty-five positive reactions, mean 0.1 reactions per patient, were recorded in the control group. Positive reactions were most commonly demonstrated to corticosteroid ointments, lanolin and bepanthene. Study results did not confirm a statistically significantly higher rate of sensitization to particular topical agents frequently used in the treatment of patients with venous ulcers. Patch testing to standard and special series allergens should be performed in case of prolonged leg ulcer epithelization.*

**Key words:** contact allergy, topical agents, venous leg ulcer

## Introduction

Allergic contact dermatitis (ACD) is inflammatory dermatosis of allergic genesis that occurs consequentially to low molecular mass substances (haptens, contact allergens)<sup>1,2</sup>. The main pathogenetic mechanism is delayed sensitivity (type IV reaction according to Coombs and Gell) demonstrated by skin patch test<sup>3</sup>. The pathogenesis of ACD is predominated by cellular immune reaction, which implies a number of factors from the allergen through the skin lymphoid system to cellular infiltrate consisting of Langerhans cells, T lymphocytes, B lymphocytes, keratinocytes, macrophages and basophil leukocytes<sup>4</sup>. It is estimated that more than six million chemicals are present in the environment, some 3000 of which may act as contact sensitizers. Almost every inorganic

substance may induce delayed allergic inflammatory reaction, i.e. dermatitis<sup>5</sup>.

The diagnosis of ACD is based on clinical picture and positive patch test findings. The cause of allergic contact dermatitis can frequently be suspected by analysis of the distribution and appearance of lesions in combination with patient history data. However, in most cases these are inadequate to determine the etiologic agent, which requires additional functional testing to verify the existence of sensitization. Patch test is a gold standard diagnostic procedure used for long time in dermatology and allergology to relate contact hypersensitivity, introduced as the patch test method by Jadassohn and Bloch in

1895. Positive allergic test reaction includes pruritus, erythema, papules, infiltrate, and occasionally vesicles at the site of contact with the respective allergen. The reaction may persist for several days. Initial reading is done at 48 h, second at 72 h and third at 96 h, according to International Contact Dermatitis Research group. Some substances such as neomycin and corticosteroids may produce positive reaction only after 4–6 days, thus the last reading is done after 7 days<sup>5,6</sup>. In patients with ACD demonstrated by patch test, appropriate therapy results in a considerably better improvement of skin lesions than in those without patch test allergen identification<sup>7</sup>.

## Patients and Methods

Study population included a randomized sample of 60 inpatients with venous leg ulcer, 30 with ACD and 30 without signs of contact allergy on the skin around leg ulcers. Based to questionnaire all 60 patients atopy history, underwent bacteriologic and mycologic analysis of the ulcer and surrounding skin, performed according to standard principles, and patch testing to standard and special series of allergens. Patch testing was performed according to the International Contact Dermatitis Research Group (ICDRG) recommendations using a standard series of contact allergens and target series allergens. The standard series allergens (n=24) at nontoxic concentrations of up to 2% in Vaseline or water included the following: potassium bichromate, cobalt chloride, nickel sulfate, formaldehyde, paraphenylenediamine, balsam of Peru, epoxy resins, colophony, white mercury precipitate, Anestasin (benzocaine), carba compounds, mercapto compounds, black gum mix (PPD mix), fragrance mix, thiuram compounds, coal tars, parabene mix, neomycin sulfate, Quaternium-15, thimerosal, Vim (cleaning agent), Ariel and Faks (laundry detergents), and Čarli (dish-washing liquid). Our own special series allergens contained 16 agents and drugs used by the patients in the treatment of their leg ulcers (according to patient history data): mupirocin, bepanthene, silver sulfadiazine, chloramphenicol + clostridiopeptidase, betamethasone dipropionate, hydrocortisone + oxytetracycline, mometasone, alginate, hydrocolloid, lanolin, pyrogallol, Vaseline, permanganate, Rivanol, povidone-iodine, and gentamycin. The special series allergens were prepared at concentrations recommended by ICDRG. Patch testing was performed on the patient back skin, with reading at 48, 72 and 96 h according to ICDRG criteria.

The  $\chi^2$ -test and Mann-Whitney U test were used on statistical analysis.

## Results

The study included 60 patients (45 female and 15 male), 30 with ACD and 30 free from signs of allergic sensitization on their legs, aged 37–85, mean age 64.06 (68.37 female and 51.13 male). Leg ulcer duration of up to one year was most commonly reported (n=27), how-

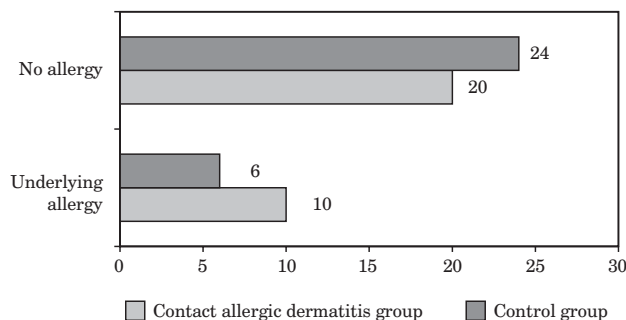


Fig. 1. Prevalence of atopy in 60 patients according to questionnaire.

ever, five patients reported on 30-year persistence. Underlying allergy and atopy were reported by 24 ACD patients and 20 non-ACD patients (control group) (Fig. 1).

In the group of ACD patients, *Pseudomonas aeruginosa* was the most commonly isolated agent (n=13), followed by *Streptococcus epidermidis* (n=12). In the control group, *Staphylococcus aureus* was the most commonly isolated agent. Native and culture mycological findings of leg ulcer swabs were negative in all 60 patients. Mycological examination of the skin surrounding leg ulcer was also negative in all 60 patients. Results of patch testing to standard series allergens in 30 ACD and 30 non-ACD patients are presented in Table 1. In the group of 30 ACD patients, the most common standard series allergens were balsam of Peru, fragrance mix and neomycin sulfate (n=8 each), followed by parabene mix (n=7) and coal tars (n=4). In the control group, the most common standard series allergens were neomycin sulfate (n=6), balsam of Peru (n=4) and parabene mix (n=4).  $\chi^2$ -test for correlation between contact sensitization and standard series allergens yielded a statistically significant difference for fragrance mix at the 5% level of significance. Special series allergens identified in ACD and non-ACD patients are shown in Table 2. In the group of 30 ACD patients, the following target series allergens were identified: hydrocortisone + oxytetracycline, lanolin, Rivanol, betamethasone dipropionate and bepanthene (n=2 each), followed by povidone-iodine and alginates (n=1 each). In the control group, the following target series allergens were identified: lanolin (n=2), betamethasone dipropionate, bepanthene and mometasone (n=1 each).  $\chi^2$ -test for correlation between contact sensitization and target series allergens showed no statistically significant difference for any of the allergens tested.

In the study group as a whole (N=60), neomycin sulfate, fragrance mix, balsam of Peru, parabene mix and coal tars were the most common allergens, all five being common sensitizing agents found in topical medicinal products. According to study results, lanolin showed a comparable frequency in both ACD and control groups of patients, whereas the remaining four allergens yielded significant between-group differences. The distribution of allergens across all 60 patients is presented in Table 3.

**TABLE 1**  
PATCH TEST RESULTS IN 60 PATIENTS TESTED TO STANDARD SERIES ALLERGENS

No.	Allergen	Concentration (%)		No. of positive tests reactions		
				ACD	Control group	Total (%)
1	Potassium dichromate	0.5	% pet.	1	0	1.6
2	Cobalt chloride	1.0	% pet.	0	0	0
3	Nickel sulfate	5.0	% pet.	1	0	1.6
4	Formaldehyde	1.0	% aq	0	1	1.6
5	Paraphenylenediamine	0.5	% pet.	1	1	3.33
6	Balsam of Peru	25.0	% pet.	8	4	20
7	Epoxy resin	1.0	% pet.	1	0	1.6
8	Colophony	20.0	% pet.	2	0	3.33
9	White mercury precipitate	10.0	% pet.	1	1	3.33
10	Anestasin (benzocaine)	5.0	% pet.	1	0	1.6
11	Carba mix	3.0	% pet.	1	2	5
12	Mercapto mix	2.0	% pet.	0	1	1.6
13	PPD-mix	0.6	% pet.	2	1	3
14	Fragrance mix	8.0	% pet.	8	2	16
15	Thiuram mix	1.0	% pet.	2	2	5
16	Coal tars	12.0	% pet.	4	2	10
17	Parabene mix	15.0	% pet.	7	4	18.3
18	Neomycin sulfate	20.0	% pet.	8	6	23.3
19	Quaternium-15	1.0	% pet.	0	0	0
20	Thimerosal	0.1	% pet.	0	1	1.6
21	Vim (cleaner)	2.0	% pet.	0	0	0
22	Ariel detergent	2.0	% pet.	0	0	0
23	Čarli detergent	2.0	% pet.	0	0	0
24	Faks detergent	2.0	% pet.	1	1	1.6

ACD – allergic contact dermatitis group, pet. – petrolatum, aq – water

Six ACD patients and 17 control patients showed negative patch test reaction. One patient from either group showed positive reaction to six allergens without possible angry back reaction.

Patch test results according to the duration of leg ulcer are shown in Table 4. Leg ulcers persisting for more than one year were recorded in as many as 28 patients, in 16 of them for 2 to 5 years. Comparison of the rate of contact sensitization between leg ulcer patients with and without a history of allergic diseases by  $\chi^2$ -test yielded no statistically significant difference ( $\chi^2=1.36$ ;  $p=0.2429$ ). The rate of positive patch test results was significantly higher in patients with dermatitis affecting the skin around leg ulcer than in those without such perilesional skin changes.

Contact hypersensitivity to medicament ingredients and bases for topical agents was quite common in our leg ulcer patients. Positive reaction was most frequently observed to neomycin, fragrance mix and balsam of Peru of the standard series, and to corticosteroid ointments in combination with antibiotics, and lanolin of the target series allergens.

In our patient sample, the rate of sensitization was comparable to that reported elsewhere. It was more frequent in patients with longstanding or recurrent leg ulcers. The study confirmed the polyvalent sensitization, i.e. hypersensitivity to multiple substances, to be quite common in patients with persistent leg ulcers, in those with inflammation of the skin around the ulcer as well as in some patients free from such lesions.

## Discussion

Contact allergic dermatitis has for years been known to occur in patients with venous ulcers and hypostatic dermatitis, the issue being investigated in numerous studies. Besides contact dermatitis, the area around the ulcer may also be involved by bacterial or fungal colonization. In the present study, the bacterial agents *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and others were isolated in both ACD and non-ADC patient groups. Most authors report on gram-negative microorganisms and streptococci as the most common wound swab isolates<sup>8-10</sup>, which is consis-

**TABLE 2**  
PATCH TEST RESULTS IN 60 PATIENTS TESTED TO SPECIAL SERIES OF ALLERGENS

No.	Allergen	Concentration	No. of positive reactions		
			ACD	Control group	Total (%)
1	Mupirocin	Conc	0	0	0
2	Bepanthene	Conc	2	1	5
3	Silver sulfadiazine	2% pet.	0	0	0
4	Chloramphenicol+clostridiopeptidase	5% pet.	0	0	0
5	Betamethasone dipropionate	Conc	2	1	5
6	Hydrocortisone+oxytetracycline	1% pet.	2	0	3.33
7	Mometasone	Conc	0	1	1.6
8	Alginate dressing	Conc	1	0	1.6
9	Hydrocolloid	Conc	0	0	0
10	Lanolin	20% pet.	2	2	6.66
11	Pyrogallol	1% pet.	0	0	0
12	Vaseline	Conc	0	0	0
13	Permanganate	1% aq	0	0	0
14	Rivanol	2% aq	2	0	3.33
15	Povidone-iodine	2% aq	1	0	1.66
16	Gentamicin	Conc	0	0	0

ACD – allergic contact dermatitis group, pet. – petrolatum, aq – water

**TABLE 3**  
DISTRIBUTION OF MONO- AND POLYSENSITIZATION IN  
ACD AND CONTROL GROUP OF PATIENTS

Patch test positive reactions	CAD patients (n=30)	Control group (n=30)
All negative	6	17
One positive	6	6
Two positive	8	2
Three positive	6	2
Four positive	0	1
Five positive	3	0
Six positive	1	1
Eight positive	0	1

tent with our results. Fungal agents were excluded in and around the wound, thus ruling out the possible effect of dermatophytes and yeasts in inflammatory reaction in both ACD and non-ACD patient groups.

In the ACD group, 25/30 patients showed single or multiple positive patch test reactions. Patch testing to standard series allergens produced a total of 49 positive reactions in ACD patients, mean 1.6% *per* patient. Positive reactions were most frequently recorded to balsam of Peru, fragrance mix, and neomycin sulfate. The rate of sensitization to standard series allergens was 76%. These results are comparable to those reported in the literature<sup>11-17</sup>. In the control group of patients free from signs of contact sensitization (non-ACD group) there were 29 positive patch test reactions, most frequently to neomycin sulfate.

**TABLE 4**  
DISTRIBUTION OF POSITIVE PATCH TEST RESULTS IN 60 STUDY PATIENTS ACCORDING TO DURATION OF LEG ULCER

Leg ulcer duration(yrs)	No. of patients	Total number of positive patch test results									
		0	1	2	3	4	5	6	7	8	
<1	28	16	4	3	1	1	0	2	0	1	
2-5	16	7	3	1	3	0	1	1	0	0	
6-10	7	0	2	3	0	0	2	0	0	0	
11-15	2	0	1	0	1	0	0	0	0	0	
16-20	1	0	0	1	0	0	0	0	0	0	
21-25	3	0	0	2	1	0	0	0	0	0	
25-30	3	0	2	0	1	0	0	0	0	0	
31-35	1	0	0	0	1	0	0	0	0	0	

Patch testing to special series allergens including topical agents (ointments, creams, dressings) produced 12 (3%) positive reactions in ACD patients, mean 0.4% *per patient*. In the control group of patients free from signs of contact sensitization (non-ACD), there were 5 positive patch test reactions, mean 0.1% *per patient*. Positive patch test reactions were most commonly recorded to corticosteroid ointments (betamethasone dipropionate, hydrocortisone, oxytetracycline), lanolin, and bepantene. Allergic reactions to these agents are frequently observed in practice. We also included hydrocolloids and alginates, relatively new agents, reported to have been used by our patients over a period preceding the study. Special series allergens included local corticosteroid agents, which have recently been extensively investigated<sup>18–23</sup>.

Antibiotics intended for local application frequently cause contact hypersensitivity, as preceding in a number of studies<sup>11–14</sup>. Our patients were tested for neomycin, mupirocin, and oxytetracycline as a constituent of Geokorton ointment frequently applied around leg ulcers. Hypersensitivity to an antibiotic or corticosteroid in a combined product is difficult to assess, pointing to the importance of using monocomponent products on testing. In most studies, neomycin sensitization in patients with leg ulcer and stasis dermatitis ranges between 3% and 19%<sup>11–14</sup>, whereas in our study it was 2.6%. Although antibiotics have long been known to frequently induce sensitization, they are still quite commonly and abundantly used in the management of venous ulcers, being unjustifiably applied over the wound or on the adjacent skin. While cross-reactivity between aminoglycoside antibiotics is well known, it was not demonstrated in the present study. We recorded positive reaction to neomycin but not to geomycin in the form of gentamycin ointment. Target series allergens included mupirocin, which is considered to very infrequently lead to sensitization. It was confirmed in this study, as none of the study patients exhibited sensitization to mupirocin.

The use of antiseptic dressings such as povidone-iodine can successfully reduce bacterial colonization, eliminate malodor, and clean the wound at a low risk of sensitization<sup>24–27</sup>. According to Mochida *et al.*, ACD to iodine products is extremely rare, so an irritative or systemic reaction should rather be expected<sup>26</sup>.

Our patients showed quite a high rate of positive reactions to fragrance mix. When ACD due to drugs or cosmetic products is suspected, patients should primarily be tested to fragrance mix. Numerous components of fragrance products may act as potent sensitizers, thus their application over inflamed and irritated skin should be avoided<sup>27</sup>. Fragrances are frequently used in officinal and magistral products for local application. Balsam of Peru is another common cause of contact allergic dermatitis. The rate of sensitization to balsam of Peru varies; however, most authors agree it is a common allergen with a rising tendency of positive reactions. Balsam of Peru is an ingredient found in many products for topical therapy of wounds, burns or pruritus as well as in sunscreens. In patients with leg ulcer, the rate of sensitization to balsam of Peru is threefold that recorded in other patients<sup>28–30</sup>.

According to many authors, hypersensitivity to betamethasone dipropionate is very common. Hydrocortisone is a common contact allergen.

Lepoittevin *et al.* report on their observation that many patients are allergic to multiple local corticosteroids. They have adopted and recommended classification into four groups, concluding that allergic reactions to tixocortol pivalate and hydrocortisone are definitely associated, whereas reactions to butesonide strongly correlate with acetonide group and ester group<sup>31</sup>. Burden and Beck estimate the prevalence of corticosteroid hypersensitivity at 4%–9% and believe that this type of allergy frequently remains unrecognized, therefore suggesting the test to corticosteroid agents to be included in routine patch testing<sup>32</sup>. On testing with corticosteroids, however, numerous reactions emerging from local product basis may pose a serious problem<sup>33</sup>.

In special series allergens, we recorded two (6.66%) positive reactions to lanolin, a common ingredient in topical medicinal and cosmetic products. According to literature reports, the rate of sensitization to lanolin ranges from 1.7% to 30%<sup>34</sup>. Negative reactions to hydrocolloid products and alginates observed in both ACD and non-ACD groups of patients could be explained by the relatively short and not very frequent use of these products in our patients. The reason for our negative results to dressings probably lies in considerably shorter application than recommended for particular agents<sup>35</sup>.

The standard series allergens used in the study included thimerosal, a mercury chromium derivative, frequently used as a preservative in various topical medicinal and cosmetic products. Mercury chromium used to be frequently utilized in the treatment of ulcer<sup>36,37</sup>. None of our study patients showed positive reaction to thimerosal.

The results of the present study revealed that contact sensitization associated with venous ulcer treatment presented a considerable problem in our patients as well. Early patch testing allowed for timely identification of allergens and thus to avoid contact with particular allergens, thereby hopefully contributing to successful cure. Highly indifferent products and those of known basis free from preservatives should be locally applied on ulcers, thus reducing the rate of ACD, which causes additional discomfort to the patient. Awareness and avoidance of thus identified allergens along with early diagnosis of contact allergic sensitization offer a successful therapeutic option for leg ulcers.

## Conclusion

During the treatment of leg ulcers, the possible existence of contact sensitization should be considered, appropriate testing indicated, and potential allergens identified on time, as it is a precondition for successful cure of leg ulcer. The products known to cause frequent sensitization and the bases containing lanolin should be avoided. Topical products free from fragrance mixture should be used.

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## KONTAKTNA ALERGIJA NA SPECIJALNE I STANDARDNE ALERGENE KOD PACIJENATA S VENOZNIM ČIREVIMA

### SAŽETAK

Cilj je studije bio odrediti učestalost kontaktne preosjetljivosti u bolesnika s vrijedom na potkoljenicama. Ustanoviti razliku kontaktne senzibilizaciju na alergene epikutane standardne serije u odnosu na moguće alergene iz lokalne terapije koja je primjenjivana u bolesnika. U istraživanje je uključeno 60 bolesnika, 45 žene i 15 muškaraca u dobi 37 do 85 godina (prosječna do za žene je 68.37 i za muškarce 51.13 godina). Kontaktni alergijski dermatitis (KAD) je imalo 30 bolesnika na potkoljenicama, a 30 je bilo bez KAD-a (kontrolna skupina). Prosječno trajanje vrijeda bilo 5.62 godine. Svi su bolesnici testirani na alergene standardne serije kao i ciljane serije alergena koju sačinjava: mupirocin, bepanten, srebrni sulfadiazin, kloramfenikol + klostridiopeptidaza, betametazon dipropionat, hidrokortizon + oksitetraciklin, mometazon, alginat, hidrokoloid, lanolin, pirogalol, vazelin, hipermangan, rivanol, povidon jodid, gentamicin, koji su bolesnici upotrebljavali pri liječenju vrijeda. KAD na alergene standardne serije utvrđen je u 25 bolesnika s ukupno 49 pozitivne reakcije i prosječno 1.6 reakcija po bolesniku. Najčešći alergeni bili su: Peruvijanski balzam, smjesa mirisa i neomicin sulfat. Utvrđeno je 12 pozitivnih reakcija na alergene ciljane serije s prosječno po bolesniku 0.4 pozitivne reakcije. U kontrolnoj skupini bilo je 45 pozitivnih reakcija s prosječno 0.1 reakcija po bolesniku. Najčešći alergeni bili su kortikosteroidne masti, lanolin i bepanten. Ovo istraživanje nije potvrdilo statistički značajno veći postotak preosjetljivosti na primijenjene tvari koje su korištene u liječenju bolesnika s vrijedom na potkoljenicama. Epikutano testiranje na standardnu i ciljanu seriju alergena treba primijeniti u slučajevima kada je spora epitelizacija vrijeda.