

ORIGINAL ARTICLE

Unexpected positive patch test reactions to sesquiterpene lactones in patients sensitized to the glucose sensor FreeStyle Libre

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Abstract

Background: Most diabetic patients sensitized to FreeStyle Libre react to isobornyl acrylate (IBOA), with a considerable number of them also showing unexpected positive patch test reactions to sesquiterpene lactone (SL) mix (SLM) tested in the baseline series.

Objectives: To compile patch test results of subjects affected, and provide potential explanations for this association.

Patients and Methods: Fifty-three Freestyle Libre-allergic patients were patch tested with IBOA and/or SLM, and several were also patch tested with the components of SLM. Chromatographic analyses were performed on the glucose sensor, IBOA, and the components of SLM.

Results: Thirty-three patients reacted positively to the components of SLM, and 11 of 27 patients reacted positively to alantolactone, in particular. Gas chromatography-mass spectrometry (GC-MS) analyses did not detect these chemicals in the different parts of the glucose sensor, or in IBOA.

Conclusion: Significant co-sensitizations between SLs on the one hand and the glucose sensor FreeStyle Libre and/or isobornyl acrylate on the other hand exist, without evidence of the presence of SLs via GC-MS analysis. Cross-reactions between them seem improbable. As a possible hypothesis, a common precursor for both, such as camphene, may exist.

KEYWORDS

allergic contact dermatitis, chemical analysis, diabetes mellitus, FreeStyle Libre, GC-MS, glucose sensor, isobornyl acrylate, medical device, sesquiterpene lactones, simultaneous reactions

1 | INTRODUCTION

Isobornyl acrylate (IBOA) (CAS no. 5888-33-5) has recently been identified as the major culprit allergen in diabetic patients sensitized to the glucose sensor Freestyle Libre (Abbott Diabetes Care, Witney, UK)¹

and similar medical devices.^{2,3} As previously reported,¹ numerous patients allergic to Freestyle Libre also reacted positively to sesquiterpene lactone (SL) mix (SLM) (Chemotechnique Diagnostics, Vellinge, Sweden), which is included in the baseline series, and contains equimolar concentrations of alantolactone (0.033%; CAS no. 546-43-0), costunolide

(0.033%; CAS no. 553-21-9), and dehydrocostus lactone (0.033%; CAS no. 477-43-0).

This study sought to investigate the simultaneous patch test reactions to IBOA/FreeStyle Libre and SLM in three contact allergy departments in Belgium. Several hypotheses were additionally explored by means of patch tests and chemical analyses, the latter performed at the Department of Occupational and Environmental Dermatology in Malmö, in order to explain this association.

2 | PATIENTS AND METHODS

2.1 | Patients

All patients included in this study suffered from diabetes mellitus type I, and had presented with allergic contact dermatitis (ACD) caused by the glucose sensor FreeStyle Libre (Abbott Diabetes Care). The results of patch tests performed between January 2016 and June 2018, in which contact allergy to this glucose sensor, IBOA or both had been confirmed, were analysed. Fifty-three cases from three Belgian university hospitals were involved, namely, 12, 16 and 25 patients from the dermatology departments of UZ Leuven, University Hospital Antwerp (UZA), and Brussels (Cliniques universitaires Saint-Luc), respectively. Among the 53 patients tested, there were more females than males (33 vs 20, or 62.3% vs 37.7%, respectively); the median age was 37 years (range: 4-76 years). Both the study and the data collection and analysis were conducted following protocol approval from the Institutional Ethical Committee, namely the Commission d'Ethique Biomédicale Hospitalo-Facultaire de l'Université Catholique de Louvain.

2.2 | Patch tests

Fifty-three patients were included in this study, all with ACD caused by the glucose sensor FreeStyle Libre, confirmed by positive patch test reactions to the adhesive part of it, to IBOA, or to both. In the respective centres, patch tests were performed with the European baseline series (Chemotechnique Diagnostics and/or Allergeaze, SmartPractice, Phoenix, Arizona), including SLM 0.1% pet. and Compositae mix 2.5% pet. Twenty-seven of the 53 patients were tested with pieces of the adhesive part of the glucose sensor FreeStyle Libre, and 52 of them were tested with IBOA, purchased from Sigma-Aldrich (Steinheim, Germany) and diluted 0.1% in pet. by the hospital pharmacy (St Luc); an in-house preparation of IBOA 0.1% pet. made with the raw material obtained from Kowa Europe (Düsseldorf, Germany) was used in UZA and UZ Leuven.

With regard to the number of patients who were patch tested with the individual lactones, 14 were tested with costunolide and dehydrocostus lactone, purchased from Sigma-Aldrich and prepared by the hospital pharmacy (0.1% pet.); 27 were tested with allantolactone (0.1% pet.); 23 were tested with α -methylene- γ -butyrolactone (0.01% pet.); and 25 were tested with parthenolide (0.1% pet.). All of the above chemicals were obtained from Chemotechnique Diagnostics.

Patch tests were applied on the upper back and occluded for 48 hours with IQ Ultra test chambers from Chemotechnique Diagnostics in Brussels, and with Allergeaze patch test chambers (SmartPractice, Calgary, Canada) in Antwerp and Leuven. Patch tests were fixed with Fixomull stretch (BSN Medical, Hamburg, Germany) in Antwerp and Brussels, and with Mefix (Mölnlycke, Göteborg, Sweden) in Leuven. Readings were performed on day (D) 2 and D4, according to European Society of Contact Dermatitis criteria.⁴

2.3 | Thin-layer chromatography patch tests

The following extracts were prepared for thin-layer chromatography (TLC) patch testing:

- Adhesive patches removed from two FreeStyle Libre sensors were extracted in 2.5 mL of ethanol for 12 hours, after which the extract was evaporated to a volume of 0.5 mL.
- The plastic covers from two sensors (with the patches removed) were cut into small pieces and extracted in 10 mL of acetone for 12 hours; this was then evaporated to a volume of 1 mL.
- Five hundred milligrams of an SLM pet. test preparation from Chemotechnique Diagnostics was dissolved in 5 mL of heptane. The heptane solution was then extracted three times with 5 mL of methanol. The methanol phases were merged and were then evaporated to a volume of ~2 mL. Thereafter, the methanol phase was washed twice with 5 mL of heptane in order to remove pet. residues, and evaporated to a volume of 300 μ L.

In addition to the above-mentioned extracts, TLC patch testing was also performed with 0.1% IBOA and 0.1% costunolide in acetone and ethanol, respectively.

TLC was performed on TLC Silica gel 60 F254 plastic sheets (Merck, Darmstadt, Germany). For each solution, several applications were made approx. 2.5 cm from each other on a line marked 2 cm from the bottom of the TLC sheet. The volumes applied on each spot were 20 μ L for the extract of the adhesive patch, 30 μ L for the extract of the sensor, 15 μ L for IBOA, 30 μ L for the SLM extract, and 15 μ L for costunolide. The samples were eluted with a mobile phase consisting of 70% (vol/vol) heptane (Merck) and 30% (vol/vol) ethyl acetate (VWR International, Fontenay-sous-Bois, France). TLC sheets were investigated under ultraviolet (UV) light at 254 and 366 nm. The visualized spots were marked with a pencil. Thereafter, the TLC sheets were cut into strips (one strip for each application) to be used for patch testing, and the positions of the spots were marked on the plastic backing of the TLC strips with a marker pen. One strip of each chromatogram was used as a template for reading of the test, and at least one strip was left to be used for chemical investigations of areas giving positive test reactions.⁵ Overall, five patients were tested with TLC strips of the adhesive patch and the sensor. Only one of these patients was tested with TLC strips of IBOA, SLM extract, and costunolide.

2.4 | Chemical analysis

Chemical analyses were performed with gas chromatography-mass spectrometry (GC-MS) at the Malmö department.¹ All extracts used for TLC patch testing were analysed as such. When the areas of the TLC strips of the adhesive patch and the sensor were analysed, the silica gel was scraped off from these areas and was then extracted in a small volume of methanol (~200 µL). The extracts were then filtered and analysed with GC-MS. In addition, analyses were performed on an extract of the circuit board from a FreeStyle Libre sensor. The circuit board was cut into small pieces, and these were extracted in ~2 mL of acetone for 4 hours. These extracts were filtered and concentrated to a volume of ~0.2 mL. Dilutions of IBOA in acetone and of alantolactone, costunolide and dehydrocostus lactone in ethanol were used as reference standards. The detection limits were estimated to 0.01 mg/mL for IBOA, 0.03 mg/mL for alantolactone, 0.3 mg/mL for costunolide, and 0.1 mg/mL for dehydrocostus lactone.

3 | RESULTS

3.1 | Patients and patch tests

All 52 patients tested with IBOA reacted positively, and, of 27 patients tested with the pieces of adhesive of the Freestyle Libre sensor, 22 reacted positively. Of 53 patients, 33 (62.3%; 10 males and 23 females), with a mean age of 39 years, reacted positively to SLM; 14 of these were also tested with costunolide and dehydrocostus lactone, and 27 with alantolactone: three reacted positively to costunolide, one to dehydrocostus lactone, and 11 (40.7%) to alantolactone. Eleven patients (20.8%) of 53 tested also reacted positively to Compositae mix, whereas only one patient reacted to parthenolide (tested in 25 cases). Only one of the 11 patients tested with α -methylene- γ -butyrolactone reacted positively. All data, including patch test results and demographic data, are summarized in Table 1.

3.2 | TLC patch tests

The positive reactions obtained with the TLC strips are summarized in Table 2 and Figure 1. All five patients tested with the TLC strips reacted positively. Concerning the FreeStyle Libre sensor, they all reacted positively to an area that was not visible with the eye, or observed under UV light. Concerning the more diffuse reaction in patient 5, the centre of the reaction was at the same location as in the reactions in the other cases. Likewise, three patients reacted positively to areas of the TLC strips of the FreeStyle Libre patch, which were also not visible. Both TLC areas had the same Rf value (~0.6). One patient was tested with three TLC strips containing costunolide, SLM extract, and IBOA. She reacted to one spot of the costunolide thin-layer chromatogram and one spot of the SLM thin-layer chromatogram, with the same Rf values (~0.4). On the IBOA thin-layer chromatogram, this patient showed a positive reaction to an area with

the same Rf value (~0.6) as the areas with positive reactions observed for the TLC strips containing the FreeStyle Libre sensor and patch extracts.

3.3 | Chemical analysis

Chromatograms of costunolide, alantolactone, dehydrocostus lactone, IBOA, the FreeStyle Libre sensor extract and the patch extract are shown in Supporting Information Figure S1. No alantolactone, costunolide or dehydrocostus lactone could be detected in the extracts of the different parts of the FreeStyle Libre sensor. Likewise, comparison of the retention time and mass spectra of GC-MS peaks did not show any alantolactone, costunolide or dehydrocostus lactone in IBOA (analysed at a concentration of up to 2% in acetone) used for patch testing. Conversely, no IBOA was found in the SLM extract or in the alantolactone, costunolide and dehydrocostus lactone solutions. All of these compounds were analysed at concentrations up to 2% in ethanol.

The TLC spots giving positive reactions for both the patch and the sensor of Freestyle Libre were shown to contain IBOA (Supporting Information Figure S2). The positive area of the thin-layer chromatogram of IBOA contained IBOA, and no other substance was observed in these extracts. The positive area of the costunolide thin-layer chromatogram contained costunolide, and the positive area of the SLM extract thin-layer chromatogram contained alantolactone, costunolide, and dehydrocostus lactone, all of which also had similar Rf values when eluted individually on a TLC sheet.

4 | DISCUSSION

SLs constitute a group of terpenoids that are present in several plant families, including the Lauraceae, Magnoliaceae, and Compositae, the last of which is the largest family. In most cases of ACD, sensitization from SLs has been caused by cosmetics, pharmaceutical products, herbal medicines, flower handling, or consumption of SL-containing plants.⁶ Some SLs are characterized by an α -methylene- γ -butyrolactone ring (Figure 2). Such structures and, notably, the α,β -unsaturated carbonyl chemical functional group have been shown to be good electrophiles that undergo covalent binding with nucleophilic residues of skin proteins via 1,4-addition or Michael addition, which is responsible for their sensitizing potential.^{7,8}

SLM was developed for patch testing, and is estimated to detect 60% to 70% of all Compositae contact allergies⁹; it is therefore included in the European baseline series.¹⁰ Interestingly, within a country, significant differences between the percentages of simultaneous reactions to SLM and Compositae mix can be observed.¹¹ The prevalence of sensitization to SLs (based on the SLM patch tests) observed in European patch test clinics ranges from 0.1% to 2.0%,^{6,12} and it was estimated to be 0.1% in the European general population.¹³

Among the 53 patients tested for ACD caused by the glucose sensor FreeStyle Libre, IBOA, or both, more than half (62.3%)

TABLE 1 Demographic data and patch tests results of 53 patients reacting positively to isobornyl acrylate (IBOA) or FreeStyle Libre

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of) adhesive		SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
						Libre D2/D4	FreeStyle D2/D4							
1	LEU	2017	59	M	+/+	NT	NT	+/+	-/-	NT	NT	NT	-	MA series: EA -/?+
2	LEU	2017	46	F	++/++	NT	NT	+/+	-/-	NT	NT	NT	MP +/+ Ni ?+/+ Limonene -/+ Isoeugenol +/+	MA series: EA +/+
3	LEU	2017	33	M	++/+	NT	NT	++/+	-/-	NT	NT	NT	-/-	MA series: negative
4	LEU	2017	56	F	++/+++	+++	+++	+/+	?/-	NT	NT	NT	MP ?+/- Ni ?+/-	MA series: EA +/+ TGDA +/+
5	LEU	2017	58	F	++/++	NT	NT	+/+	-/-	NT	NT	NT	Colophonium +/?+ Ni +/+ MP +/+	MA series: negative
6	LEU	2017	42	F	-/+	NT	NT	-/?+	-/-	NT	NT	NT	Ni +/+ Co +/+	MA series: EA -/+
7	LEU	2018	34	F	++/++	NT	NT	+/+	?/-	NT	NT	NT	-	MA series: EA +/+
8	LEU	2017	21	F	+/+	NT	NT	?/-	-/-	NT	NT	NT	-	MA series: EA +/+
9	LEU	2017	46	F	+/++	NT	NT	-/?+	-/-	NT	NT	NT	MP +/+++ Ni +/+++ FMI -/+	MA series: EA +/++
10	LEU	2017	62	M	+/+	NT	NT	NT	-/-	NT	NT	NT	NT	MA series: EA ?+/?+
11	LEU	2018	42	M	++/+++	NT	NT	+/+	+/+	NT	NT	NT	Ni -/?+ Colophonium -/+ Isopropanol -/+	MA series: EA ?+/?+
12	LEU	2018	14	F	+/++	NT	NT	+/+	?+/+	NT	NT	NT	Quaternium 15 +/+ Cetearyl alcohol ?+/+	MA series: EA +/+
13	UZA	2016	9	M	++/++	NT	NT	NT	NT	NT	NT	NT	NT	MA series: HPA +/+

(Continues)

TABLE 1 (Continued)

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of adhesive of FreeStyle Libre D2/D4)	SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
14	UZA	2017	14	M	-/+	NT	NT	NT	NT	NT	NT	NT	
15	UZA	2017	49	F	++/++	NT	++/+++	-/+	NT	NT	NT	PPD ?/+ Ni +/++ Neomycin -/+ FMI -/?+ MCI/MI -/+	MA series: TGDMA +++/+ BIS-GMA -/?+ HDA +/++ THFMA ++/++ TGDA +++/+++ EA -/+ HPA +/- BDA ++/++ DGDA ++/++ TPGDA -/+
16	UZA	2017	16	F	++/++	+/+	++/+	-/+	NT	NT	NT	MP -/+ Colophonium +/- CAPB ?/+ HG ?/+ PG ?/+ DMAPA ?/+ Limonene ?/+ TDM -/?+	MA series: TGDMA ?/+/?+
17	UZA	2017	24	F	++/++	NT	++/+	+/+	++/++	NT	NT	MP ?/+ PG ?/+ SSO ?/+	MA series: EA +/?++ Plants: <i>Chrysanthemum</i> <i>cinerariaefolium</i> ?/+? <i>Tanacetum vulgare</i> +/?+
18	UZA	2017	9	F	+/+	NT	NT	NT	-/-	NT	NT	NT	MA series: DGDA -/?+ BDMA -/?+
19	UZA	2017	15	F	++/++	NT	++/++	+/+	+/+	NT	NT	Neomycin ?/- Thiuram mix +/- Ni ?/+? PG ?/+	MA series: BMA ?/+ BDA ?/+ BA ?/+
20	UZA	2017	11	M	+++/>+++	NT	++/++	+/+	+/+	NT	NT	Neomycin -/?+ Cr ?/+? MP +/- Lanolin alcohol +/- Mercapto mix ++/++	MA series: EA +/- EHA +/- BA +/?+

(Continues)

TABLE 1 (Continued)

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of adhesive of FreeStyle Libre D2/D4)	SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
21	UZA	2017	11	F	+/+	NT	-/-	-/-	-/-	NT	NT	MCI/MI +++ MBT ++/+++ Amerchol L101 +/?+ MI ++/++ BIT +/+ OIT ?+/?+ PG -/IRR Thiuram mix -/?+ Colophonium -/?+	
22	UZA	2018	64	M	++/++	NT	-/-	-/-	NT	NT	NT	MCI/MI -/+ Benzoic acid +/+ Bronopol -/?+ DU ?+/?+ IU ?+/- Linalool +/+ OIT ?+/?+ BIT +/+ SSO ?+/- DPG ?+/?+	MA series: negative MA series: negative
23	UZA	2018	13	M	+/+	NT	NT	NT	-/-	NT	NT	NT	MA series: negative
24	UZA	2018	64	F	++/++	NT	-/+	-/-	?+/+	NT	NT	Co +/+ Paraben mix ?+/- Ni ?+/?+ MCI/MI -/+	MA series: negative
25	UZA	2018	24	M	++/++	NT	+/+	-/-	+/+	NT	NT	Mercapto mix +/? Paraben mix +/IRR Bronopol +/IRR Parthenolide +/+ Limonene +/+ Linalool +/+	MA series: negative
26	UZA	2018	5	M	++/++	NT	-/-	-/-	+/-	NT	NT	FMI +/-	

(Continues)

TABLE 1 (Continued)

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of adhesive of FreeStyle Libre D2/D4)	SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/Compositae) D2/D4	Additional patch tests D2/D4
27	UZA	2018	40	F	+/++	NT	+/+	-/-	?+/-	NT	NT	Ni -/?+ DPG +/-	MA series: negative
28	UZA	2018	17	F	?+/++	NT	-/+	-/-	-/-	NT	NT	Ni -/?+ Limonene -/?+ CAPB ?+/+	MA series: MMA -/?+ DGDA -/?+
29	UCL	2016	9	M	NT	+/+	-/-	-/-	NT	NT	NT	-/-	MA series: Hexamethylene diisocyanate ++/++ P&G: Abitol ++/++ Hydroquinone ++/++
30	UCL	2017	12	F	+++ /+++	+/+	+++ /+++	-/-	NT	NT	NT	MP -/+ TP +/+ FMI +/+ PG +/+ BIT +/?+	P&G: Epoxy resin +/+ Cycloaliphatic +/+
31	UCL	2017	41	F	++/++	+/+	-/-	-/-	NT	NT	NT	Ni ++/++	MA series: negative P&G: negative
32	UCL	2017	44	F	+++ /+++	-/-	-/-	-/-	NT	NT	NT	-/-	MA series: negative P&G: negative
33	UCL	2017	52	F	++/++	+++ /+++	+/+	-/-	NT	NT	NT	Ni ++/++	MA series: negative P&G: negative
34	UCL	2017	41	F	++/++	-/-	+/+	-/-	NT	NT	NT	FMI +/+	MA series: negative P&G: negative
35	UCL	2017	12	M	+/++	+/+	+/+	-/-	NT	NT	NT	-/-	MA series: (Continues)

TABLE 1 (Continued)

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of) adhesive of FreeStyle Libre D2/D4	SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
36	UCL	2017	49	M	++/+	+/+	-/-	-/-	NT	NT	NT	-/-	MA series: negative P&G: negative
37	UCL	2017	4	F	+/+	+/+	-/-	-/-	-/+	NT	NT	-/-	MA series: negative P&G: negative
38	UCL	2017	39	F	+/+	?+/-	+/+	-/-	NT	NT	NT	Co ?+/+ Ni +++/++	MA series: negative P&G: negative
39	UCL	2017	65	F	+/++	+/++	+/+	-/-	-/-	NT	NT	-/-	MA series: negative P&G: negative
40	UCL	2017	13	F	+/+	-/+	-/-	-/-	-/-	-/-	+/-	-/-	MA series: HPMA -/+ Plants: <i>Tanacetum</i> <i>vulgare</i> extract +/-
41	UCL	2017	26	F	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	MA series: negative P&G: negative Plants: negative
42	UCL	2018	33	M	++/++	+/++	-/+	-/-	-/-	-/-	-/-	Cr -/+	MA series: negative P&G: negative Plants: negative
43	UCL	2018	52	F	+/++	+/++	++/++	+/+	+/++	+/+	-/-	Resorcinol monobenzoate +/- Hydroquinone -/+ Propolis -/+ MP -/+	
44	UCL	2018	47	F	+/+	+/+	-/-	-/-	-/-	-/-	-/-	Colophonium -/+	MA series:

(Continues)

TABLE 1 (Continued)

Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of) adhesive of FreeStyle Libre D2/D4	SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
45	UCL	2018	62	F	+/+	+/+	-/-	-/-	-/-	-/-	-/-	Ni +/+ Sodium metabisulfite +/+	BA -/+ EA +/++ HEA -/+ HDA +/+ TGDA +++/+++ TGDMA +++/+++ P&G: Abitol -/+ Plants: negative
46	UCL	2018	76	F	+/+	+/+	-/+	-/-	-/-	-/-	-/-	Co -/+ Ni +/++	MA series: negative P&G: negative Plants: negative
47	UCL	2018	42	M	+++ /++	+++	+++ /++	-/-	+++ /++	-/-	-/-	MA series: negative P&G: Abitol -/+ Plants: Alantolactone +++ /++	MA series: negative P&G: negative Plants: negative
48	UCL	2018	63	F	++ /++	+++	+++ /++	-/+	+/+	+++	-/-	MP -/+	MA series: negative P&G: negative Plants: Alantolactone +/+
49	UCL	2018	47	M	++ /++	+++	+++	-/-	-/-	-/+	-/-	Lanolin alcohol +/+ DPG +/+ TDM -/+	P&G: Abitol -/+ Plants: negative
50	UCL	2018	51	M	++ /++	-/-	-/-	-/-	-/-	-/-	-/-	Colophonium -/+ Ni -/+	MA series: EA -/+

(Continues)

TABLE 1 (Continued)

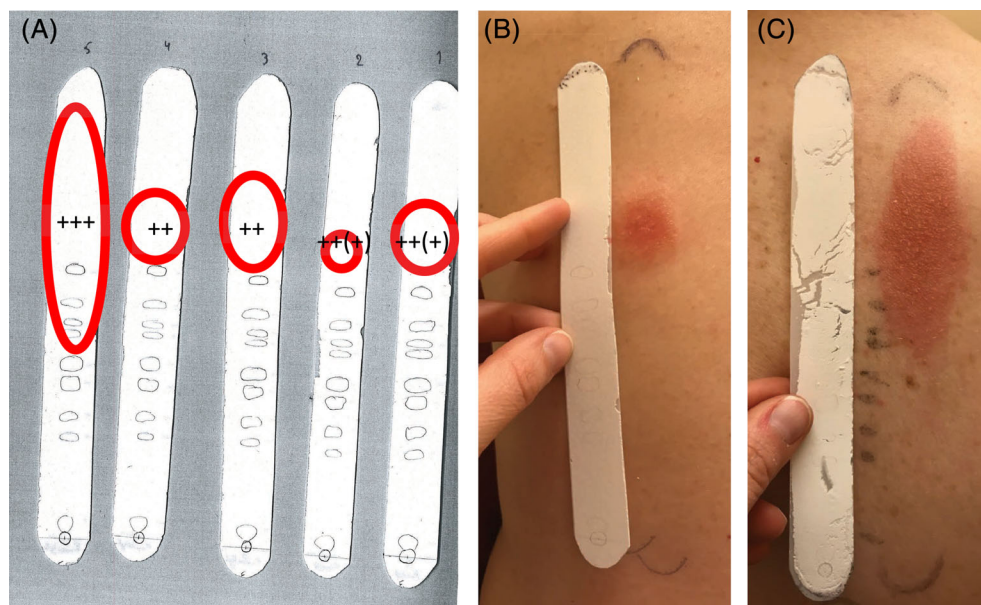
Patient	Centre	Year	Age (y)	Sex	IBOA D2/D4	(Pieces of) adhesive		SLM D2/D4	Compositae mix D2/D4	Alantolactone D2/D4	Costunolide D2/D4	Dehydrocostus D2/D4	European baseline series (except SLM/ Compositae) D2/D4	Additional patch tests D2/D4
						Libre	D2/D4							
51	UCL	2018	58	M	+/++	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	P&G: negative Plants: negative
													Sodium metabisulfite +/+ Limonene -/? +	
52	UCL	2018	55	F	+/+	-/+	-/+	-/-	-/-	-/-	-/-	-/-	Ni -/+	MA series: negative P&G: negative Plants: negative
53	UCL	2018	66	M	++/++	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	MA series: negative P&G: negative Plants: negative

Abbreviations: BA, butyl acrylate; BDA, butanediol diacrylate; BDMA, butanediol dimethacrylate; BIS-GMA, bisphenol A glycerolate dimethacrylate; BIT, benzisothiazolinone; BMA, butyl methacrylate; CAPB, cocamidopropyl betaine; Co, cobalt; Cr, chromium; DGDA, diethylene glycol diacrylate; DMAPA, dimethylaminopropylamine; DPG, diphenylguanidine; DU, diazolidinylurea; EA, ethylacrylate; EHA, ethylhexyl acrylate; F, female; FMI, fragrance mix I; HDA, hexanediol diacrylate; HEA, hydroxyethyl acrylate; HG, hexylene glycol; HPA, hydroxypropyl methacrylate; HPMA, hydroxypropyl methacrylate; IRR, irritant reaction; IU, imidazolidinylurea; LEU, Dermatology, University Hospitals KU Leuven; M, male; MA, methacrylate; MBT, mercapto benzothiazole; MCI, methylchloroisothiazolinone; MI, methylisothiazolinone; MMA, methyl methacrylate; MP, *Myroxylon perëirae*; Ni, nickel; OIT, octylisothiazolinone; P&G, plastic and glues series; NT, not tested; PG, propylene glycol; PPD, *p*-phenylenediamine; PTBP-FR, *p*-tert-butylphenol-formaldehyde; SLM, sesquiterpene lactone mix; SSO, sorbitan sesquioleate, TDM, textile dye mix, TGDA, triethyleneglycol diacrylate; TGDMA, triethyleneglycol dimethacrylate; TP, tixocortol pivalate; TPGDA, tri(propylene glycol) diacrylate; THFMA, tetrahydrofurfuryl methacrylate; UCL, Dermatology, Cliniques Universitaires Saint-Luc; UZA, University Hospital Antwerp; -, negative; ?+, doubtful; + to +++, positive.

TABLE 2 Tests results obtained with thin-layer chromatography (TLC) strips, with FreeStyle Libre patch and sensor patch test results correlating with isobornyl acrylate (IBOA) and sesquiterpene lactone mix (SLM) patch test results

Patient	Age (y)	Sex	TLC FreeStyle patch D2/D4	TLC FreeStyle sensor D2/D4	IBOA 0.1%	SLM
No. 1 (31)	45	F	–/–	Area 1 +++/++(+)	+++ /+++	–/–
No. 2 (32)	47	F	Area 1 +/–	Area 1 +/++(+)	+++ /+++	–/–
No. 3 (37)	4	F	Area 1 ++/++	Area 1 ++/++	+ /+	–/–
No. 4 (34)	41	F	–/–	Area 1 ++/++	++ /++	+ /+
No. 5 (39)	65	F	Area 1 +/+++	Area 1 +/+++	+ /++	+ /+

Abbreviations: D, day; F, female.

**FIGURE 1** (A), Day (D) 4 reading of positive reactions observed with thin-layer chromatography strips (FreeStyle Libre sensor) in the five patients tested. (B), Positive reaction (D4, +) in patient 1. (C), Positive reaction (D4, +++) in patient 5

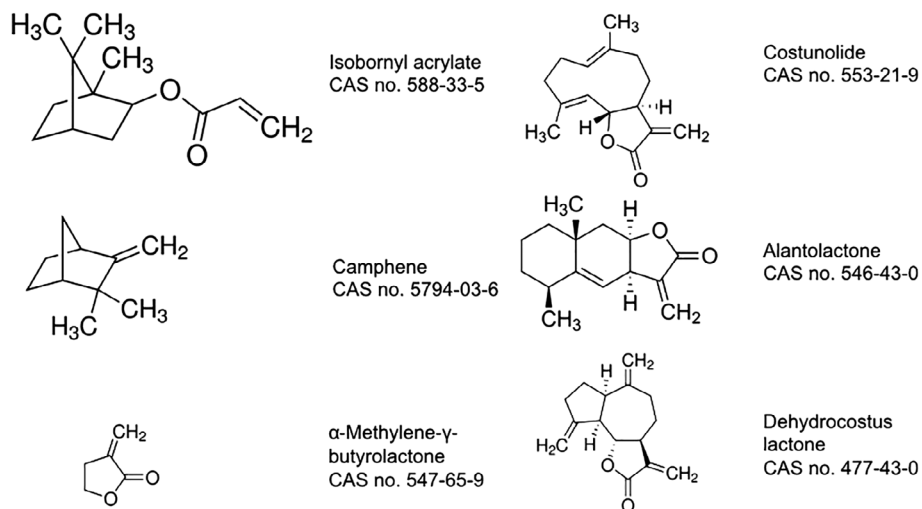
showed sensitization to SLs, which accounts for the increased prevalence of sensitization observed in the three contact allergy units involved in this study. The prevalences of sensitization to SLs from 2014 to 2017 in these clinics, summarized in Table 3, have indeed shown a relative increase in recent years. For example, at the Cliniques universitaires Saint-Luc in Brussels, the rates of sensitization to SLs in patients attending the contact allergy unit were 0.19% in 2015, 0.50% in 2016, and 1.66% in 2017, the year in which contact allergy problems caused by glucose sensors started to appear. Over the period analysed, from January 2016 to June 2018, 3902 patients were tested for SLM in the three universities. The rate of sensitization to SLM during this period was 1.23%. With exclusion of patients tested with and sensitized to IBOA, the rate decreases to 0.72%. However, the exact mechanism or mechanisms of this co-sensitization remain unclear.

4.1 | Cross-reactions between SLs and IBOA

Cross-reactivity between chemical compounds has to be considered when a close relationship between sensitization frequencies is observed. IBOA, and alantolactone, costunolide, and dehydrocostus,

the three lactones present in SLM, share the same chemically reactive functional group. Indeed, these molecules contain a carbonyl functional group (double bond between a carbon and an oxygen atom), and are able to induce a Michael addition; this reaction represents the addition of a nucleophile to an α,β -unsaturated carbonyl compound, acting as a Michael acceptor with a skin protein, or an amino acid responsible for the protein activity, such as cysteine.⁷ However, during the elicitation phase, two factors are crucial, that is, the function of the chemical groups and the three-dimensional structure. The spatial structure of IBOA and that of the three SLM derivatives are completely different, so they are not likely to activate the same T cell receptor; therefore, cross-reactivity appears to be quite improbable. The retesting procedure model, as proposed by Rustemeyer et al,¹⁴ which is a test technique that is based on skin hyper-reactivity resulting from local cutaneous residual T cells with memory of a specific allergen, could provide evidence for distinguishing cross-reactivity from co-sensitization.

Cross-reactions between different SLs are possible because of structural similarities of the α -methylene- γ -butyrolactone ring,¹⁵ which, according to a study by Stampf et al, constitute a prerequisite for cross-sensitization between them, although apparently not being sufficient

FIGURE 2 Molecular structures of different chemical molecules**TABLE 3** Percentage of patients sensitized of sesquiterpene lactone mix from the different Belgian universities from 2014 to 2017

University	Years	%
UZA	2014	0
	2015	0
	2016	0.43
	2017	1.02
UCL	2014	0.38
	2015	0.19
	2016	0.50
	2017	1.66
LEU	2014	0.21
	2015	0.90
	2016	0.72
	2017	0.78

Abbreviations: LEU, University Hospitals KU Leuven; UCL, Cliniques universitaires Saint-Luc in Brussels; UZA, University Hospital Antwerp.

for sensitization. Cross-reactions between alantolactone and costunolide have been described, but so have cross-reactions between alantolactone and two types of spirolactone, containing an α -methylene- γ -butyrolactone ring, which can be synthesized from camphene.^{15,16} However, no cross-reaction was observed between these lactones and α -methylene- γ -butyrolactone which was tested separately. The structural homology of α -methylene- γ -butyrolactone appears not to be able to activate the same lymphocytes as those activated by these other lactones. Similar results have been obtained in animal tests.¹⁷ In the present study, two patients showed co-sensitization between alantolactone and costunolide, but only one co-reacted to both SLM and α -methylene- γ -butyrolactone. Further investigations are thus necessary to investigate possible cross-reactivity between the three lactones in SLM and IBOA, considering camphene as the starting molecule that is necessary for the synthesis of these molecules.

Finally, enzymatic, but also non-enzymatic, reactions, such as autoxidation on air exposure, could result in modifications of the chemical IBOA structure that could, in turn, induce the formation of a new metabolite¹⁸ that is able to cross-react with SLs.

4.2 | Presence of SLs in the glucose sensor

Another potential explanation for the present surprising association is co-sensitization resulting from contact with SLs present in the glucose sensor. Despite our multiple and different requests, no information about the exact composition of the sensors could be obtained from the manufacturers. Analyses with GC-MS were therefore performed to investigate the composition of the sensor, through which the presence of IBOA was previously highlighted.¹ Further analyses were performed, comparing the retention times and mass spectra of the SLM components with the different peaks observed in the chromatograms of the extracts of the different parts of the glucose sensor (ie, adhesives part, plastic part, or circuit board). However, these analyses did not detect SLM components. Nevertheless, it cannot be completely ruled out that small amounts of alantolactone, costunolide, and dehydrocostus lactone, or other SLs, are present in the sensor. The chromatographic method used is not optimal for all SLs, and costunolide gave a very broad peak, which would make its identification in small amounts in a complex mixture, such as the investigated extracts, more difficult.

Furthermore, constituents in the extracts of the FreeStyle Libre sensor and patch were separated on TLC strips. These strips were used for patch testing to investigate whether the patients reacted to one or several areas on the strip. Indeed, the presence of more than one positive spot would confirm the presence of several simultaneous allergens. However, all patients reacted to the same area, that is, corresponding to IBOA in the TLC strip of the adhesive patch of FreeStyle Libre, which was confirmed with GC-MS analysis. The results support the absence of SLM components in the sensors. Therefore, a positive patch test reaction to SLM seems more likely to represent a cross-reaction than co-sensitization resulting from the presence of

SLs in the sensor. However, it can never be completely excluded that small amounts of SLs might be present in the sensor, but not in sufficient quantity to cause a reaction.

The presence of plant materials in medical devices has already been reported, for example, concerning ACD caused by self-adhesive electrocardiography electrodes in a young girl.¹⁹ In this case, positive patch test reactions to Compositae mix, but not to SLM components, were noted; chromatographic analysis confirmed the presence of herbal extracts in the adhesive part of the electrodes (not specified). In the case of glucose sensors, additional analyses might be required to completely rule out the presence of related SLs or other plant components.

4.3 | IBOA impurities

Foti et al reported IBOA to be an impurity of alkyl glucosides, and perhaps the sensitizing culprit in them.²⁰ Therefore, the presence of IBOA impurities in the SLM sample and, conversely, also of SL impurities in the IBOA sample, was explored. According to GC-MS analyses, comparing the retention times and mass spectra of both the SLM components and IBOA, the latter was not present as an impurity, although, again, we cannot exclude the possibility of small amounts being present. Several peaks were detected by means of GC-MS in IBOA used for patch testing and purchased from Sigma-Aldrich, probably corresponding to impurities. However, the retention times of these different peaks did not match with those of the SLM components; the exact nature of them was not identified, but most appeared to have an isobornyl group in their structure. It should be noted that recently, at the Antwerp department, additional chemical analyses failed to confirm IBOA as an impurity in alkyl glucosides.²¹

Sigma-Aldrich purchased IBOA from Arkema (Colombes, France), who informed us that it was obtained by means of a chemical reaction between acrylic acid and camphene, a bicyclic monoterpene. Although the reaction product was purified by distillation, camphene residues at <1% weight could still be present. Note that camphene can also be found in SL-containing plants,²² and as an ingredient, at concentrations ranging from 0.1% to 1%, in UV curing adhesives used for medical devices, that is, Loctite AA3926 LC MED (Henkel, Düsseldorf, Germany).²³

4.4 | Previous sensitization to SLs via antidiabetic treatments

For many years, medical researchers have attempted to develop new, pharmacologically active agents from natural plant sources. Several SLs have been used in animal experiments in order to prevent or to control diabetic complications. However, the already known molecules do not contain the unsaturated methylene group, which is a prerequisite for a Michael addition and for the allergenic properties. The patients included in the current study had not previously participated in a study protocol focusing on these drug types, and insulin or other typical drugs used for diabetic patients do not contain SLs. Moreover, chemical analyses to formally exclude the presence of SLs in the different medications taken by diabetic patients, especially different insulin types, have not yet been undertaken. These hypotheses must

be further explored in order to better understand this surprising co-sensitization, and to exclude reactions between IBOA and other constituents of the UV curing adhesive in the sensor.

5 | CONCLUSION

Concomitant sensitization to SLM, as present in the baseline series, has been observed in diabetic patients reacting to the glucose sensor Free-style Libre and IBOA, or to both; however, the precise mechanism or mechanisms remain elusive. The presence of the three components of the mix, that is, alantolactone, costunolide, and dehydrocostus lactone, in the glucose sensor, as well as impurities in the patch test materials, or in the sensor, could not be shown with GC-MS analyses. Notwithstanding their very similar chemical functional groups, cross-reactions between the three SLs and IBOA seem unlikely, owing to their different spatial structures. Therefore, at present, co-sensitization, rather than cross-reactivity, remains the most likely explanation, and the retesting model might enable us to effectively rule out cross-reactivity. The presence of a common precursor for IBOA and lactones, such as camphene, might account for the simultaneous sensitizations observed. These hypotheses, however, should be the subject of further investigations.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Herman A, Mowitz M, Aerts O, et al. Unexpected positive patch test reactions to sesquiterpene lactones in patients sensitized to the glucose sensor FreeStyle Libre. *Contact Dermatitis*. 2019;81:354-367. <https://doi.org/10.1111/cod.13330>

Fig 2. Total ion chromatograms of costunolide, alantolactone, dehydrocostus lactone (each 0.1% in ethanol) and isobornyl acrylate (IBOA, 0.1% in acetone) as well as separate acetone extracts of the FreeStyle Libre sensor and the adhesive patch removed from the sensor. IBOA was observed in both extracts. No traces of the sesquiterpene lactones were observed in the extracts or in the IBOA sample. No traces of IBOA were observed in the sesquiterpene lactone samples.

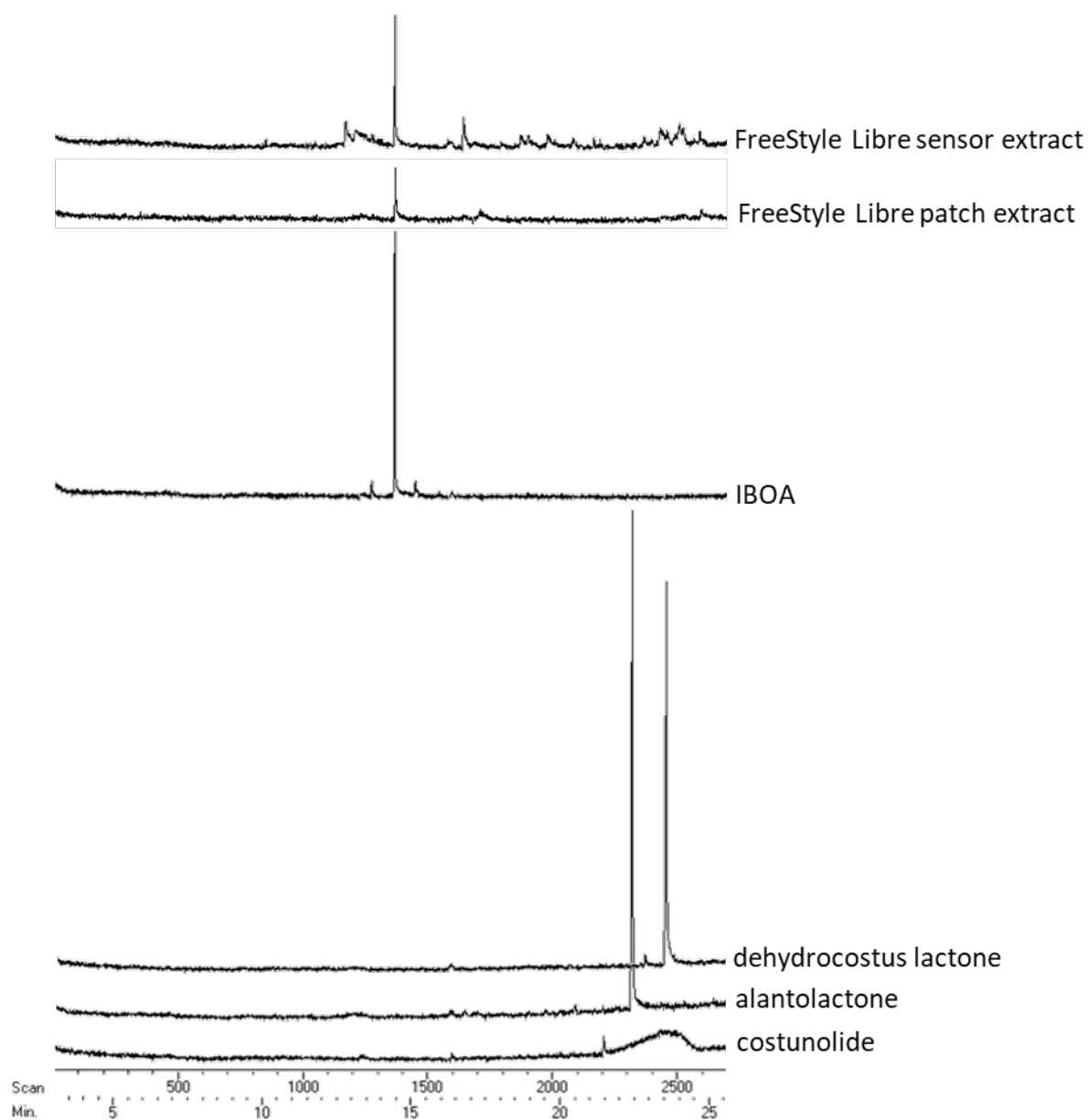


Fig. 3. a) Total ion chromatogram of an extract made from material scraped off from the area giving positive reactions in patients tested with thin-layer chromatograms of the FreeStyle Libre sensor, b) mass spectrum of the peak at 14.5 minutes, and c) mass spectrum of an isobornyl acrylate reference sample.

