

Relevance of Positive Patch-Test Reactions to Fragrance Mix

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Background: Fragrances are an important cause of allergic contact dermatitis. We presume that the traditional fragrance mix (FM) detects 70 to 80% of fragrance-allergic patients. FM has an irritant potential. Weak positive reactions may have a greater chance of being irrelevant than strong reactions.

Objective: To improve the appraisal of FM patch-test reactions, we studied the relevance of reactions of different strength. We also studied the predictive value of the following on the relevance of the initial FM patch-test results: patch-test results of a repeated FM test; results of patch tests with balsam of Peru, colophony, and ingredients of the mix; and (history of) atopic dermatitis.

Methods: One hundred thirty-eight patients who had doubtful positive (?) or positive (+ to +++) reactions were included in the study. We determined relevance by history taking, location and course of the dermatitis, and additional patch testing. Patients were retested with FM and with each ingredient separately.

Results: The relevance of reactions to FM increases with the strength of the reactions. Predictors of relevance are the results of retesting with FM, the results of tests with the ingredients, and a history and/or present symptoms of atopic dermatitis.

Conclusion: Retesting with FM and its ingredients may add to the benefit of patch testing.

FRAGRANCES are an important cause of allergic contact dermatitis and are generally recognized as one of the five most common causes of contact sensitization.¹ Worldwide studies have reported prevalences of positive reactions to fragrances ranging from 5 to 14%.^{2–4} The studies show a rising trend, which may be the result of an increased use of fragrance-containing cosmetics and toiletries. About 3,000 different fragrance materials of synthetic and natural origin are used in perfumery. The

traditional fragrance mix (FM), containing eight fragrances and sorbitan sesquileate, is used as a screening tool for fragrance sensitivity in the European Standard series. We presume that testing with FM detects 70 to 80% of patients with a fragrance allergy.¹ Studies on the specificity of FM are not available. Because of the relatively low rate of detection, an additional mix as screening tool is introduced.⁵

Altered skin care, changed cleansing habits, and an increased incidence of (subclinical) atopic dermatitis may change the vulnerability to irritant skin reactions. An increase in false-positive (irritant) patch-test reactions may contribute to this rising trend.^{6,7} FM is not the sole indicator for fragrance allergy; balsam of Peru and colophony are additional indicator substances.^{8,9}

New markers for fragrance hypersensitivity have been searched for and tested; examples are hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall, IFF Global Headquarters, New York, NY), ylang ylang oil, lemongrass oil, jasmine, and *Narcissus*. These materials have been claimed as potential ingredients for the FM, but only Lyrall proved to be a good candidate, at least in Europe.^{2,10} The addition of Lyrall into FM possibly increases sensitivity but may do so at the expense of specificity. Today, Lyrall is included in a new FM added to the European and North American standard series.^{5,10}

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Because of the marginal irritant potential of FM, weak positive reactions, in particular, may not be regarded as proof of contact sensitization (low specificity of the test). This means that weak positive reactions should be evaluated more carefully for relevance to the particular skin disease. A more complete picture of the factors relating to the relevance of positive FM test results helps the clinician to evaluate (weak) positive patch-test reactions more precisely. Which reactions are likely to be irritant and are therefore likely to be irrelevant? Which patient-related factors may help to differentiate between irritant, possibly not relevant reactions, and (weakly) positive possibly relevant reactions? We attempt to delineate these factors in this study.

Candidate factors were as follows: (1) strength of the reaction (weak reactions may be irritant and therefore more likely to be not relevant), (2) atopic dermatitis or history of atopic dermatitis (disturbed barrier function in subclinical atopic dermatitis may influence the relevance of contact reactions¹¹), (3) results of patch tests with balsam of Peru and colophony (positive reactions to other markers for fragrance allergy may be associated with a higher probability of having a relevant reaction), (4) results of repeated testing with FM (repeated positive reactions to FM may be associated with a higher probability of having a relevant reaction), and (5) results of tests with the ingredients of FM (a positive reaction to one or more ingredients may be associated with a higher probability of having a relevant reaction).

We collected a group of patients with (weakly) positive reactions to FM from among consecutively tested patients. Then we assessed the relevance of the (weakly) positive reactions, as described under "Materials and Methods." Two groups were formed, one of patients in which relevance was unlikely (indicated further in the text as "not relevant") and one of patients in which relevance was likely (indicated further in the text as "relevant"). The groups were used to study the influence of factors possibly predicting relevance. The following questions were raised:

- Were patients with strong reactions more likely to be in the group of patients with relevant reactions?
- Were patients more likely to be in the group of patients with relevant reactions if they also reacted to balsam of Peru or colophony?
- Were patients more likely to be in one or the other group if they had (or previously had) atopic dermatitis?
- Were patients more likely to be in the group of patients with relevant reactions if the result of repetitive testing with FM was positive?

- Were patients more likely to be in the group of patients with relevant reactions if the results of patch tests with one or more ingredients (sorbitan sesquioleate excluded) were positive?

Materials and Methods

One hundred thirty-seven patients who were routinely tested with the European Standard series and additional series and/or their own skin care products and toiletries (if indicated) and who had consecutive doubtful positive (?+) or positive (1+ to +++) reactions to the FM in the European Standard series were entered in the study. We recorded the patch-test reactions according to International Contact Dermatitis Research Group (ICDRG) guidelines.¹² Patients with an initial irritant reaction were not included. We determined the present relevance of the patch-test reactions by (1) the patient's experience with an eczematous reaction or worsening of a preexisting dermatitis after contact with fragrances or fragrance-containing products, (2) the correspondence of the area where the suspect product was applied with the localization of the dermatitis, and (3) the results of patch tests with the patient's own fragrance-containing cosmetics and toiletries. We were unable to identify fragrances in these products. We categorized the relevance of the patch-test reactions as none, possible, probable, or definite, according to the criteria of Rietschel and Fowler.¹³ If all criteria for relevance were concordant, the reaction was classified as none or definite. If not all three criteria were concordant, it was up to the investigator to assess the relevance as possible or probable. For the calculation (see statistics), reactions classified as not relevant or possibly relevant were taken together as not relevant, and reactions classified as probable and definite were considered as relevant.

We then repeated the patch tests with FM and a battery of the nine FM ingredients at specified concentrations in petrolatum (Table 1). We studied the predictive value of (1) the results of the repeated FM test; (2) the results of patch tests with the FM ingredients (sorbitan sesquioleate excluded), balsam of Peru, and colophony; and (3) atopic dermatitis (or a history of atopic dermatitis) on the relevance of the initial FM patch-test results.

The study was carried out at departments of dermatology in hospitals both in the Netherlands and in Belgium. The patients were retested as soon as possible (4 weeks at the earliest) after the initial patch tests. Retesting was performed at the same hospital where initial testing was

Table 1. Number of Positive Reactions to Fragrance Mix Ingredients and Percentages of Patients with Positive Results in Initial and Repeated Fragrance Mix Tests

<i>Test Compound*</i>	<i>No. of Positive Reactions</i>	<i>Initial Test % (n = 138)</i>	<i>Repeated Test % (n = 121)</i>
Cinnamyl alcohol 5%	2	1.4	1.7
Cinnamal 2%	21	15.2	17.4
Eugenol 5%	15	10.9	12.4
Amyl cinnamal 5%	8	5.8	6.6
Hydroxycitronellal 5%	14	10.1	11.8
Geraniol 5%	20	14.5	16.5
Isoeugenol 5%	19	13.8	15.7
<i>Evernia prunastri</i> (oak moss) 5%	12	8.7	9.9
Sorbitan sesquioleate 20%	16	11.6	13.2

*In petrolatum.

carried out, and the same investigator read the results. We used Van der Bend (Van der Bend BV, Brielle, the Netherlands) or Hayes (HAL Allergy B.V., Haarlem, the Netherlands) test chambers, affixed with Fixomull Stretch (Beiersdorf AG, Hamburg, Germany) tape. Test material was removed after 2 days, and the results were read on day 2 (D2) and on day 3 (D3).

We used the chi-square statistic to test the relationship between the strength of the positive reaction and the percentage of relevant reactions. The predictors were related to the relevance of the first FM test in a two-by-two contingency table. The strength of the relation was quantified by the sensitivity, specificity, positive predictive value, and negative predictive value. In the calculation, “not relevant” or “possibly relevant” was grouped together as not relevant, and “probably relevant” and “definitely relevant” were classed as relevant. *Statistical Package for Social Sciences* (SPSS) (SPSS Inc., Chicago, IL) software (release 11.0.1, November 2001) was used for analysis.

Results

The initial test with FM, read at D3, showed a ?+ reaction in 40 (29%) of the patients, a + reaction in 42 (30.4%) of the patients, a ++ reaction in 28 patients (20.3%), and a +++ reaction in 28 patients (20.3%). In the group of 138 patients reacting to FM, 46 patients (33.3%) had a history of atopic dermatitis and/or mild signs of atopic dermatitis, 47 (34.1%) had a positive reaction to balsam of Peru, and 14 (11.1%) had a positive reaction to colophony. The percentages of relevant ?+, +, ++, and +++ reactions were 25.0%, 40.5%, 71.4%, and 75.0%, respectively. Thus, the

percentage of relevant reactions increased significantly with the strength of the skin reaction ($p < .001$). The percentage of all relevant (weak) positive reactions combined was 49.3%. On retesting, FM yielded positive (+ to +++) patch-test reactions at D2 in 110 patients (79.7%) and at D3 in 121 patients (87.7%). In 17 (12.3%) of the patients, the retesting result at D3 was negative. All these patients had an initial ?+ reaction, and in this group of patients with negative results on repeated tests, we did not observe positive reactions to FM ingredients.

In the group of patients who had a ?+ reaction in the initial FM test, 11 of 40 showed a positive reaction to the ingredients. Of the patients who had a +, ++, or +++ reaction, 32 of 42, 28 of 28, and 28 of 28, respectively, showed positive reactions to the ingredients. In total, 99 patients (81.8%) showed a positive reaction to one or more ingredients. Table 1 shows the numbers of positive reactions to the battery of the nine FM ingredients, which are only partly in line with those found in other studies.^{14–16} Remarkably, 16 patients reacted to sorbitan sesquioleate. One patient had angry back and reacted to all the compounds.

Table 2 is a two-by-two contingency table showing the relationship between each predictor and the relevance of the first FM test, as quantified by sensitivity, specificity, positive predictive value, and negative predictive value. The odds of a patient with a positive result on initial and repeated FM patch testing having a relevant patch test are at least 20 times as great as the odds of a patient's having a positive initial test and a negative repeated test. The odds of a patient with a positive test reaction to the FM ingredients having a relevant patch test as compared to a

Table 2. Accuracy of (Combined) Testing in Detecting Relevance

<i>Prognostic Variable</i>	<i>Se (%)</i>	<i>Sp (%)</i>	<i>PPV (%)</i>	<i>NPV (%)</i>	<i>DOR, 95% CI</i>
Positive retest, D2	93	33	57	82	6.2, 2.2–17.4
Positive retest, D3	100	24	56	100	> 20
Reaction to FM ingredient(s)	91	47	63	85	9.2, 3.5–24.1
Balsam of Peru	38	70	55	54	1.4, 0.7–2.9
Colophony	7	87	36	49	0.5, 0.2–1.7
Atopic dermatitis	41	74	61	57	2.0, 1.0–4.2

CI = confidence interval; D2 = day 2; D3 = day 3; DOR = diagnostic odds ratio; FM = fragrance mix; NPV = negative predictive value; PPV = positive predictive value; Se = sensitivity; Sp = specificity.

patient with a positive initial FM test and a negative test reaction to the ingredients are more than six times as great.

Discussion

The ICDRG recommends a reaction scoring system of from + to +++. Questionable reactions are recorded by a question mark, (?+) and irritant reactions are recorded as “IR.” The paradigm is that + to +++ reactions are identified as positive reactions, indicating contact sensitization.¹³ However, a patch test is a bioassay, and there are no absolutely distinctive morphologic criteria to distinguish contact allergy from an irritant reaction. The validity of the patch test is generally regarded as adequate if the test is done according to the guidelines. This study reveals clinical relevance of ?+, +, ++, and +++ reactions to FM respectively in 25%, 40.5%, 71.4%, and 75% of the patients.

We conclude that the validity of the FM patch-test procedure varies with the strength of the test reaction. A positive reaction on retesting to FM or to one of its ingredients adds significantly to the probability of a relevant test. A positive test reaction to balsam of Peru or colophony does not predict relevance of the positive FM reactions. A patient's having atopic dermatitis may have a

slight predictive value for the relevance of a positive FM reaction (Table 3).

The accuracy of the patch test as proof of sensitization cannot always be determined precisely since reliable confirmation of contact sensitization by an in vivo or in vitro test is not possible. The patch test cannot always determine or rule out contact sensitization beyond any doubt.

However, the clinician is interested in the relevance of the findings in relation to the skin complaints. Does the test accurately predict the presence or absence of contact reactions, and are the findings relevant to the skin disease? The relevance of the test can be assessed on clinical grounds (history, physical examination, testing with the patient's own cosmetics, and follow-up).

The percentages of relevant reactions in this study and in other studies differ.^{17,18} Differences in definition of the level of relevance vary and may be responsible for this. Our findings and data published elsewhere show a limited relevance of the FM patch-test outcome.¹⁸ This study indicates that the reactions to sorbitan sesquioleate in the mix, which may be irritant in nature, are partly responsible for this.¹⁹ This study also shows that retesting with FM and testing with its individual ingredients help predict relevance.

Table 3. Relevance and Number of Patients with (Weak) Positive Patch-Test Reactions to Fragrance Mix

<i>Initial Patch-Test Reaction</i>	<i>Relevance</i>			
	<i>Patients with Positive Initial Test Result (n = 138)</i>	<i>Patients Also with Positive Repeated Test Result (n = 121)</i>	<i>Patients Also Positive to Ingredient(s) (n = 99)</i>	<i>Patients Also with Atopic Dermatitis (n = 46)</i>
?+	25.0% (40)	43.5% (23)	54.5% (11)	12.5% (8)
+	40.5% (42)	40.5% (42)	46.9% (32)	70.0% (10)
++	71.4% (28)	71.4% (28)	71.4% (28)	71.4% (14)
+++	75.0% (28)	75.0% (28)	75.0% (28)	60.9% (14)

?+ = doubtful positive.

The percentage of FM-positive patients who react to one or more ingredients is higher in our study than the percentages reported by some previous studies.²⁰ The relatively high test concentration of the FM ingredients used in our study may be partly responsible for this difference.²¹

Which patients should be retested with FM and its ingredients? The low FM sensitivity in an unselected population (as estimated in the literature¹) and the low specificity of the FM patch-test procedure (as estimated in this study) make the FM patch test less useful than is desirable. This study shows that retesting a patient who has a weak positive reaction to FM aids substantially in predicting relevance.

This study was done to investigate predisposing factors for relevance of (weakly) positive FM patch-test reactions in an unselected population. It was not the aim of this study to determine the relevance of the reactions in each individual case beyond any doubt.

In a selected population of patients with a high suspicion of having a contact allergy to a fragrance, testing with a more extensive tray of fragrance ingredients²² can obtain more elaborate information on sensitization to fragrance materials. This information can be matched with patient's consumer products. Patch testing and (if appropriate) user tests can be done with culprit consumer products to obtain accurate information on the relevance of contact sensitization to a particular fragrance. Such a procedure was, however, not the aim of this study.

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