

## INVESTIGATIVE REPORT

## The Prevalence of *Malassezia* Yeasts in Patients with Atopic Dermatitis, Seborrhoeic Dermatitis and Healthy Controls

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Cultures for *Malassezia* yeasts were taken from both normal-looking skin and lesional skin in 124 patients with atopic dermatitis, 16 patients with seborrhoeic dermatitis and from normal skin of 31 healthy controls. Positive *Malassezia* growth was found in fewer patients with atopic dermatitis (56%) than in patients with seborrhoeic dermatitis (88%) or in healthy controls (84%,  $p < 0.01$ ). In the patients with atopic dermatitis, fewer positive cultures were found in lesional (28%) than in non-lesional skin (44%,  $p < 0.05$ ), while positive cultures were found in 75% of both lesional and non-lesional skin of patients with seborrhoeic dermatitis (not significant). *M. sympodialis* dominated in patients with atopic dermatitis (46%) and in healthy controls (69%). In patients with seborrhoeic dermatitis both *M. sympodialis* and *M. obtusa* were cultured in 43%. A *Malassezia* species extract mixture would increase the possibility of detecting IgE sensitization to *Malassezia* in patients with atopic dermatitis. **Key words:** prevalence; *Malassezia* yeasts; atopic dermatitis; seborrhoeic dermatitis.

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Molecular biology using rRNA sequence analysis and nDNA comparisons, as well as guanine/cytosine (GC) genomic ratios in extracted DNA, has clearly divided the genus *Malassezia* into seven different species (1, 2). Today this genus consists of *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. slooffiae*, *M. restricta* and *M. obtusa*. Recently, a new member of the genus *Malassezia* has been isolated from the skin of patients with atopic dermatitis (AD), *M. dermatis* (3). This species has only been identified using ribosomal DNA sequence analysis, but not with a cultivation identification system. Just recently there has been a report of yet another member of the genus *Malassezia* named *M. japonica* (4). The *Malassezia*

species are lipid-dependent for growth, except for *M. pachydermatis*.

The *Malassezia* species can be identified using various physical, chemical and metabolic characteristics (1, 2). We have recently developed a modification of the previous identification systems, by which seven different *Malassezia* species can easily be identified (5). The advantage of this system is that it is easier, faster and excludes the Tween reaction, which is often difficult to read. The *Malassezia* yeasts were isolated using the medium described by Leeming & Notman (6). This medium contains, among other ingredients, ox bile, glycerol, glycerol monostearate, Tween 60 and full-fat cow's milk. Compared with other culture media, this was the most effective for isolation of the *Malassezia* yeasts (7).

The *Malassezia* yeasts are members of the normal human cutaneous flora (8–10). However, there is great variation regarding presence and density in various skin locations (11, 12), in children compared with adults (7, 13) and in normal skin compared with diseased skin (14, 15). The *Malassezia* yeasts are also associated with several skin diseases such as pityriasis (tinea) versicolor, *Malassezia* folliculitis, seborrhoeic dermatitis (SD), AD and some forms of confluent and reticulate papillomatosis (Gougerot-Carteaud) (9, 13, 16). They may even be involved in systemic diseases (13, 17).

Only a few culture studies have been made using the new classification of the *Malassezia* yeasts (18–22). However, it is important to look at the distribution of the various *Malassezia* species not only on normal skin but also on the skin of patients with *Malassezia*-associated skin diseases.

The aim of the present study was to isolate the various *Malassezia* yeasts from patients with AD and SD and from healthy controls (HC). The relationship between a positive *Malassezia* culture, the severity of AD, positive reactions to skin prick test (SPT) and atopy patch test (APT) to *M. sympodialis* was investigated. In patients with AD and SD positive in *Malassezia* culture, serum IgE reactivity against the seven *Malassezia* species was tested.

## MATERIALS AND METHODS

### Subjects

Adult patients from three different centres in Sweden (Gothenburg, Lund and Stockholm) were included in the study and cultured for *Malassezia* yeast (Table I): 124 with AD, 16 with SD and 31 HC. The study was part of a multicentre study (24), where patients with AD and SD, and also HC, were tested for SPT and APT reactivity to a crude *M. sympodialis* extract, prepared from strain no. 42132, American Type Culture Collection (ATCC), as described previously (25). Serum IgE to *M. sympodialis* extract was analysed using commercially available ImmunoCAP™ (m70, prepared from ATCC strain no 42132, Pharmacia Diagnostics, Uppsala, Sweden). The study was approved by the regional Ethics Committees.

### Isolation and culture of Malassezia

The patients and HCs were instructed not to shower or use emollients on the day of investigation. Use of systemic glucocorticoids, systemic antifungal treatment or ultraviolet therapy was not allowed for 2 months prior to the investigation. Topical antifungal treatment was not allowed for 1 month before and topical corticosteroids were not allowed on the test sites for 1 week before the study. The *Malassezia* yeasts were isolated from the skin with contact plates containing a modified Leeming-Notman's agar medium (6). Standard regular milk containing 3% fat was used, and all the milk was from the same dairy. This medium was also used for maintenance of culture of the *Malassezia* yeasts. Samples were taken from normal-looking skin of the upper back, and for the patients with AD and SD they were also taken from lesional skin, mainly from the upper trunk. The culture plates were incubated at 37°C for 6 days. All *Malassezia* strains grow well on Leeming-Notman's agar at 37°C. The relative humidity in the incubator was 85% and all culture plates were incubated in plastic bags. This increases the humidity and diminishes the risk of the medium drying out.

### Identification of the Malassezia yeasts

A physiological typing system was used, as previously described in detail (5) (Fig. 1). The various *Malassezia* species were identified and the number of colonies was counted, giving a semi-quantitative number of organisms. The highest number of organisms that it was possible to count was 100. Actively growing cultures obtained after one successful transfer on fresh Leeming-Notman's agar medium and then incubated for 48 h were used for identification.

**Growth on regular Sabouraud agar.** The first identification step was to incubate the culture onto Sabouraud agar without any lipid supplement and then incubate the cultures at 37°C for 6 days.

**Catalase reaction.** The catalase reaction was performed by application of a drop of hydrogen peroxide 10% onto a culture smear on a glass slide. The production of gas bubbles indicated a positive reaction.

**Cremophor EL.** The ability of the various *Malassezia* species to grow on a simple agar medium with the addition of PEG-35 castor oil (Cremophor EL, Sigma, Stockholm, Sweden) as the only lipid source was tested using a modification of the method described by Mayser & Gross (26).

We used Sabouraud agar instead of the medium described. Eight mm wells were made with a punch biopsy and the wells

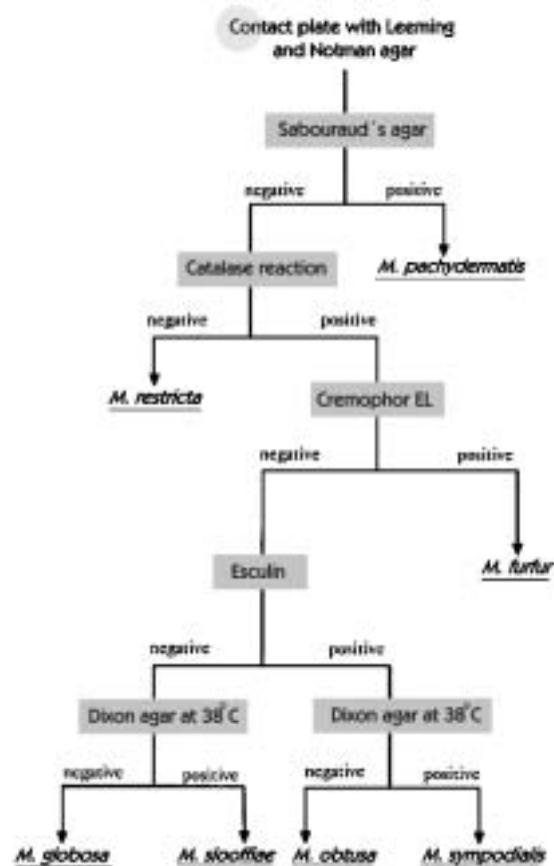


Fig. 1. Typing of *Malassezia* species.

were then filled with 100 µl of Cremophor EL. The plates were incubated for 10 days at 37°C and assessed for growth around the wells (5).

**Splitting of esculin.** The β-glucosidase activity of the various *Malassezia* species was investigated using esculin agar tubes (5). A loop from an actively growing yeast colony was inoculated deep into the agar tube and incubated for 5 days at 32°C. The splitting of esculin to esculetin and glucose is demonstrated by a darkening of the agar medium with precipitation of soluble ferric salts incorporated in the agar.

**Growth at 38°C.** The ability of the various *Malassezia* species to grow on a modified Dixon agar medium at 38°C was studied. We used a modification of the technique described by Guého et al. (1). A sample from actively growing cultures on Leeming-Notman agar was transferred to Dixon agar and incubated at 38°C for 7 days, after which the ability to grow was investigated.

The advantage of using Leeming-Notman agar for isolation and maintenance is that growth is much faster than in Dixon agar, and with this typing system a final species identification can be made within 3 weeks (5).

### Serum IgE antibodies to the various Malassezia species

In addition to analyses of IgE antibodies to *M. sympodialis* (Table I), sera from 62 patients with AD and 12 patients with SD who had positive cultures for *Malassezia* were analysed for the presence of IgE antibodies to the other *Malassezia* species

Table I. Characterization of subjects with atopic dermatitis (AD) and seborrhoeic dermatitis (SD) and healthy controls (HC)

	Gender	Age in years	SCORAD <sup>a</sup>	Head and	Total serum IgE <sup>b</sup>	SPT+	m70+ <sup>c</sup>	APT+	
<i>n</i>	F/M ( <i>n</i> )	median (range)	median (range)	neck AD <i>n</i> (%)	(kU/l) median (range)	<i>M. sympodialis</i> extract <i>n</i> (%)	>0.35 kU/l <i>n</i> (%)	<i>M. sympodialis</i> extract <i>n</i> (%)	
AD	124	82/42	30 (18–59)	35 (8–86)	88 (74) <sup>d</sup>	310 <sup>e</sup> (2–24000)	57 (47) <sup>e</sup>	54 (44) <sup>e</sup>	48 (40) <sup>f</sup>
SD	16	6/10	33 (18–73)	NA	NA	11 (2–45)	0	0	1 (6)
HC	31	29/2	42 (21–64)	NA	NA	25 (4–230)	0	0	0

NA, not applicable; SPT, skin prick test; APT, atopy patch test.

<sup>a</sup>Assessed using SCORAD (23). <sup>b</sup>ImmunoCAP<sup>TM</sup> (Pharmacia Diagnostics), reference range 2–122 kU/l. <sup>c</sup>Serum IgE antibodies to *M. sympodialis* extract (m70), ImmunoCAP<sup>TM</sup> (Pharmacia Diagnostics). <sup>d</sup>Recorded in 119 AD patients. <sup>e</sup>Investigated in all but one AD patient. <sup>f</sup>Investigated in 121 AD patients.

(see Table V). Yeast extracts were prepared from the following cultures from CBS (Centraalbureau voor Schimmelcultures, Delft, the Netherlands): *M. obtusa* 7876, *M. globosa* 7966, *M. slooffiae* 7956, *M. furfur* 7019, *M. restricta* 7877 and *M. pachydermatis* 1879 as described previously (27). The extracts were covalently coupled to the cellulose solid phase (ImmunoCAP<sup>TM</sup>) and tested in the Pharmacia CAP system<sup>TM</sup> according to the manufacturer's instructions. A value of  $\geq 0.35$  KU/l was considered positive.

#### Statistical analysis

The differences in the numbers of the various *Malassezia* species cultured from subjects with AD and SD and HC were compared using the  $\chi^2$  test. The differences in numbers of the various *Malassezia* species cultured from lesional and non-lesional skin in patients with AD and SD were compared using Fisher's exact test. In patients with AD the differences in SCORAD above or below 40, head and neck location of AD, positive SPT reaction, serum IgE antibodies to *M. sympodialis* extract (m70) and positive APT reaction were compared with a positive or negative *Malassezia* culture using Fisher's exact test. A difference between groups was considered statistically significant when  $p < 0.05$ .

Table II. Number of individuals with positive *Malassezia* cultures

	AD <sup>a,b</sup>	SD <sup>a,b</sup>	HC	<i>p</i> -value <sup>c</sup>
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
<i>M. sympodialis</i>	32 (46)	6 (43)	18 (69)	0.11
<i>M. obtusa</i>	21 (30)	6 (43)	4 (15)	0.15
<i>M. globosa</i>	19 (28)	5 (36)	3 (12)	0.16
<i>M. slooffiae</i>	5 (7)	2 (14)	1 (4)	0.48
<i>M. furfur</i>	3 (4)	1 (7)	0	–
<i>M. restricta</i>	2 (3)	0	0	–
<i>M. pachydermatis</i>	0	0	0	–
Total <i>Malassezia</i> culture pos. pat.	69 (56) <sup>d</sup>	14 (88) <sup>e</sup>	26 (84) <sup>f</sup>	0.002

AD, atopic dermatitis; SD, seborrhoeic dermatitis; HC, healthy controls.

<sup>a</sup>In lesional and/or non-lesional skin.

<sup>b</sup>One patient may have more than one *Malassezia* species.

<sup>c</sup> $\chi^2$  test. Level of significance =  $p < 0.05$ .

<sup>d</sup>Percentage of 124 AD patients.

<sup>e</sup>Percentage of 16 SD patients.

<sup>f</sup>Percentage of 31 HC.

## RESULTS

### Malassezia cultures

Fewer cultures were positive for *Malassezia* growth in patients with AD (56%) than in patients with SD (88%) or in HC (84%,  $p < 0.01$ ) (Table II). In 18 individuals (11%), more than one *Malassezia* species was cultured. *M. sympodialis* was the most frequent species found in patients with AD and in HC. In patients with SD *M. sympodialis* and *M. obtusa* were the most frequently found species, cultured at the same frequency. However, there was no statistically significant difference between AD, SD or HC in terms of the prevalence of the various *Malassezia* species found in culture-positive subjects (Table II).

In the patients with AD, fewer positive cultures were found in lesional (28%) than non-lesional skin (44%,  $p < 0.05$ ), while positive cultures were found in 75% of both lesional and non-lesional skin of patients with SD ( $p = 1.00$ , Table III). For lesional skin in patients with AD, *M. globosa* was found in 40% of the culture-positive patients and in non-lesional skin *M. sympodialis* was found in 45%. In patients with SD both *M. sympodialis* and *M. obtusa* were found at 33% each in the culture-positive patients from lesional skin, and in non-lesional skin *M. globosa* was found in 42% (Table III). However, no statistically significant differences were found between lesional and non-lesional skin except for *M. globosa* in patients with AD ( $p = 0.04$ ).

In 12 patients with AD (10%), more than one *Malassezia* species was cultured. In 11 of these patients two different species were cultured, and in one patient three different species were found. In three patients the two different species were cultured from either lesional (one patient) or non-lesional (two patients) skin. The species found together on lesional skin were *M. sympodialis* and *M. globosa*. On non-lesional skin *M. sympodialis* and *M. slooffiae* were found in one patient and *M. obtusa* and *M. furfur* in the other. In all the other patients, one species was found in lesional skin and the other in non-lesional skin.

In six patients with SD (38%) more than one *Malassezia* species was cultured. In one patient

Table III. Distribution of the various *Malassezia* species on lesional and non-lesional skin in patients with atopic dermatitis and seborrhoeic dermatitis

	Atopic dermatitis			Seborrhoeic dermatitis		
	Lesional <sup>a</sup> n (%)	Non-lesional <sup>a</sup> n (%)	Lesional vs non-lesional p-value <sup>b</sup>	Lesional <sup>a</sup> n (%)	Non-lesional <sup>a</sup> n (%)	Lesional vs non-lesional p-value <sup>b</sup>
<i>M. sympodialis</i>	11 (31)	25 (45)	0.27	4 (33)	4 (33)	1.00
<i>M. obtusa</i>	6 (17)	17 (31)	0.22	4 (33)	4 (33)	1.00
<i>M. globosa</i>	14 (40)	10 (18)	0.04	1 (8)	5 (42)	0.16
<i>M. slooffiae</i>	4 (11)	1 (2)	0.15	2 (17)	0	0.48
<i>M. furfur</i>	0	3 (5)	0.44	1 (8)	0	0.50
<i>M. restricta</i>	1 (3)	1 (2)	1.00	0	0	1.00
Total <i>Malassezia</i> culture positive patients	35 (28) <sup>c</sup>	55 (44) <sup>c</sup>	0.012	12 (75) <sup>d</sup>	12 (75) <sup>d</sup>	1.00

<sup>a</sup>A patient may have more than one *Malassezia* species and a patient can be in both the lesional and non-lesional groups.

<sup>b</sup>Fisher's exact test. Level of significance  $p < 0.05$ .

<sup>c</sup>Percentage of 124 AD patients.

<sup>d</sup>Percentage of 16 SD patients.

both *M. sympodialis* and *M. globosa* were found in non-lesional skin. Otherwise, one species was found in lesional skin and the other in non-lesional skin. In HC, only one species was cultured from each subject.

#### The number of colonies from lesional and non-lesional skin

The number of individuals with positive cultures was too low and the variations in the number of colonies were too high for any meaningful statistical consideration. In patients with AD, *M. obtusa* colonies were found in a median number of 19 (range 1–100) in lesional skin and in 31 (range 1–100) in non-lesional skin. *M. globosa* was found in a median number of one colony (range 1–9) in lesional skin, and in non-lesional skin a median number of three colonies (range 1–100) were found. *M. sympodialis* was, as mentioned above, the most commonly cultured species in patients with AD. However, the median number of colonies was only four (range 1–100) in lesional skin and 12 (range 1–100) in non-lesional skin. The other three lipophilic *Malassezia* species were only cultured on one to four occasions.

In patients with SD, *M. sympodialis* colonies were found in a median number of 22 colonies (range 2–100). The corresponding number for *M. globosa* was 25 (only one patient) and for *M. obtusa* the median number was 4 (range 1–44) in lesional skin. In non-lesional skin *M. globosa* was cultured in a median number of 73 colonies (range 3–100), *M. sympodialis* in a median number of 53 colonies (range 1–100) and *M. obtusa* in a median number of 16 colonies (range 1–33).

In HC, *M. sympodialis* colonies were cultured in a median number of 23 (range 1–100), *M. globosa* in a

median number of 11 (range 1–19) and *M. obtusa* in a median number of 9 (range 1–40).

#### Positive *Malassezia* culture in relation to the severity of AD, SPT+, m70+ and APT+

In patients with severe AD and SCORAD >40 *Malassezia* cultures were positive in 29 of 50 patients (58%) compared with 40 of 74 patients (54%) of patients with AD with SCORAD ≤40 (Table IV) ( $p=0.80$ ). The *Malassezia* species most commonly cultured from patients with AD with SCORAD >40 were: *M. sympodialis* in 13 of 29 patients (45%), *M. globosa* in 9 patients (31%) and *M. obtusa* in 6 patients (21%). The *Malassezia* species most commonly cultured

Table IV. Patients with atopic dermatitis (AD) as characterized by: eczema severity index (SCORAD), head and neck location, skin prick test (SPT), serum IgE antibodies to *M. sympodialis* extract (m70) and atopy patch test (APT), in relation to a positive or negative *Malassezia* culture

	Malassezia culture		p-value <sup>a</sup>
	Positive n=69 (%)	Negative n=55 (%)	
SCORAD ≤40	40 (54)	34 (46)	0.80
SCORAD >40	29 (58)	21 (42)	
With head and neck location	47 (53)	41 (46)	0.39
Without head and neck location	20 (65)	11 (35)	
SPT + <sup>b</sup>	23 (40)	34 (60)	0.002
m70+	21 (39)	33 (61)	0.001
APT + <sup>b</sup>	21 (44)	27 (56)	0.058

<sup>a</sup>Fisher's exact test. Level of significance= $p < 0.05$ .

<sup>b</sup>To *M. sympodialis* extract.

from patients with AD with SCORAD  $\leq 40$  were: *M. sympodialis* in 22 of 40 patients (55%), *M. obtusa* in 14 patients (35%) and *M. globosa* in 8 patients (20%).

Eighty-eight patients had AD located in the head and neck region and 47 of these patients (53%) had a positive *Malassezia* culture (Table IV). There was no statistically significant difference between these patients and patients with no head and neck eczema ( $p=0.39$ ).

Twenty-three of 57 patients with AD (40%) with positive SPT reactions to *M. sympodialis* extract had positive *Malassezia* cultures. Twenty-one of 54 patients with AD (39%) with IgE antibodies to *M. sympodialis* extract (m70) had positive *Malassezia* cultures. Twenty-one of 48 patients with AD (44%) with positive APT to *M. sympodialis* extract had positive *Malassezia* cultures. There was a lower number of patients with AD with positive SPT reactions ( $p=0.002$ ) or with serum IgE antibodies to *M. sympodialis* (m70+) ( $p=0.001$ ) who were *Malassezia* culture-positive than negative (Table IV).

#### IgE antibodies to the various *Malassezia* species

Sera from 62 culture-positive patients with AD were analysed. Twenty-one (34%) had IgE antibodies to *M. sympodialis* extract (m70) and all of them had serum IgE to *M. obtusa*, *M. globosa* and *M. restricta* (Table V). Among the 41 patients with m70 negative reactivity, 16 patients (39%) had serum IgE antibodies to one or more of the other *Malassezia* species.

We found no association between the *Malassezia* species cultured from the individual patients and their serum IgE antibodies to the various species (data not shown). Eighteen patients with AD had serum IgE antibodies to all tested *Malassezia* species in combination with a positive culture for one or two (in three of the patients) *Malassezia* species. In 25 of the culture-positive patients with AD no serum IgE antibodies to *Malassezia* were found. None of the 12 culture-positive patients with SD who were investigated had IgE antibodies to any of the *Malassezia* species.

## DISCUSSION

According to earlier reports, *M. sympodialis*, *M. globosa* and *M. furfur* were the most commonly isolated *Malassezia* species in HC and in diseases such as AD and SD (18–22). However, there were differences with respect to the species most commonly isolated, not only between normal healthy individuals and patients with various skin diseases but also as regards results from different countries. In our study and in the study from Canada (20), *M. sympodialis* was the dominant species in healthy controls. In patients with AD, the dominant species in lesional skin was *M. globosa* in our study, *M. furfur* in Japan (22) and *M. sympodialis* in Canada (20). This difference may be attributable to the sampling and culture techniques as well as to geographical differences.

In a previous study from our department the prevalence of the *Malassezia* yeasts was significantly higher in healthy individuals in the age range of 29–31 compared with the age range of 39–41 years (28). The median ages of our groups were 30 years for patients with AD, 33 years for patients with SD and 42 years for HC. Despite this the numbers of the *Malassezia* yeasts were still significantly lower in the AD group compared with the HC group. This is further proof for our observation that patients with AD harbour a lower number of *Malassezia* yeasts compared not only with patients with SD but also with HC. One reason may be the reduced amount of lipids in the skin of patients with AD compared to HC (29) and patients with SD (30). In the present study the number of yeast cells in patients with AD was lower in lesional skin compared with non-lesional skin. Cultures from lesional skin in patients with AD were, in approximately 25% of subjects, taken from the arm or leg, locations known to harbour *Malassezia* less often than, for example, the trunk, neck and forehead (11). Another explanation may be the anti-fungal activity of mediators and/or inflammatory cells present in lesions.

*Malassezia* may be an important allergen and trigger factor in AD, especially AD located in the head and

Table V. IgE antibodies to different *Malassezia* species in sera from culture-positive patients with atopic dermatitis (AD)

	Number (%) of patients	Number (%) of patients with AD positive to					
		<i>M. obtusa</i> CBS 7876 <sup>b</sup>	<i>M. globosa</i> CBS 7966 <sup>b</sup>	<i>M. slooffiae</i> <sup>c</sup> CBS 7956 <sup>b</sup>	<i>M. furfur</i> <sup>c</sup> CBS 7019 <sup>b</sup>	<i>M. restricta</i> CBS 7877 <sup>b</sup>	<i>M. pachydermatis</i> <sup>d</sup> CBS 1879 <sup>b</sup>
m70 <sup>a</sup>							
m70+	21 (34)	21 (100)	21 (100)	17 (85)	19 (95)	21 (100)	19 (95)
m70–	41 (66)	8 (20)	13 (32)	3 (8)	5 (13)	8 (20)	5 (12)
Total no. of patients	62	29 (47)	34 (54)	20 (33)	24 (40)	29 (47)	24 (39)

<sup>a</sup>Serum IgE antibodies to *M. sympodialis* extract (m70, allergen source *M. sympodialis* (ATCC 42132), ImmunoCAP<sup>TM</sup> (Pharmacia Diagnostics), reference range  $<0.35$  KU/l.

<sup>b</sup>The allergen sources are strains obtained from Centraalbureau voor Schimmelcultures (CBS), Delft, the Netherlands.

<sup>c</sup>Investigated in 60 patients.

<sup>d</sup>Investigated in 61 patients.

neck region (3, 8, 16). In a recent study, we described the presence of positive SPT and APT reactions to an extract and to recombinant allergens from *M. sympodialis* in a high proportion of patients with AD, even in those without head and neck distribution (24). However, in this study the numbers of positive *Malassezia* cultures were not higher in patients with head and neck location of AD compared to other patients with AD. The same was found in those with severe AD or SCORAD >40 as compared with those with SCORAD ≤40 and those with APT+ reactions to *M. sympodialis* extract. The role of the *Malassezia* yeasts as a trigger factor in AD is probably due to an allergic reaction. Therefore the mere presence of the *Malassezia* on the skin, and not the number of yeast cells, is the important factor.

*Malassezia* species contain both species-specific and common *Malassezia* allergenic components reacting with IgE antibodies (27). In the present study serum IgE antibodies to *M. obtusa*, *M. globosa* and *M. restricta* were also present in 100% of those *Malassezia* culture-positive patients with AD with IgE antibodies to *M. sympodialis* (m70+). In agreement with previous findings, we found that patients who are negative for m70 may have IgE antibodies to other *Malassezia* species (27). Therefore, if only m70 is included in the test panel, there is a risk that IgE reactivity to other *Malassezia* species will not be detected. There was no clear relation between the results of culture and the results of IgE reactivity to the different *Malassezia* species. In several patients one *Malassezia* species was cultured, but no IgE antibodies to this species were found. However, IgE antibodies to other *Malassezia* species were often present. There could be several reasons for this. The patient may be or may have been in contact with species other than the species cultured, on other parts of the body and/or at an earlier time. Reactions to several species may be due to cross-reactivity to common *Malassezia* allergens. Therefore a mixture of several *Malassezia* species would increase the possibility of detecting IgE sensitization to *Malassezia*.

Patients with AD with positive SPT or IgE antibodies to *M. sympodialis* extract (m70+) were less often positive in the *Malassezia* cultures. The difference in the proportion of SPT- or m70-positive patients with AD between the *Malassezia* culture-positive and -negative groups is difficult to explain. However, cultures were only taken at one location from lesional and non-lesional skin, and only once. Therefore, cultures could be positive at a different location or time.

In conclusion, we found fewer individuals with positive *Malassezia* culture in the AD group than in the SD group, or among the HCs. The species cultured from the AD patients' skin differed between normal and diseased skin, but knowledge about which *Malassezia* species was present on the skin in AD was only found to be of minor importance, because many patients had IgE antibodies to

several *Malassezia* species. To increase the possibility of detecting IgE reactivity to *Malassezia* in patients with AD, a mixture of allergens from different *Malassezia* species could be introduced into the test panel.

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## REFERENCES

- Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 1996; 69: 337–355.
- Guillot J, Guého E, Lesourd M, Midgley G, Chévrier G, Dupont B. Identification of *Malassezia* species – a practical approach. *Med Mycol* 1996; 6: 103–110.
- Sugita T, Takashima M, Shinoda T, Suto H, Unno T, Tsuboi R, et al. New yeast species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. *J Clin Microbiol* 2002; 40: 1363–1367.
- Sugita T, Takashima M, Kodama M, Tsuboi R, Nishikawa A. Description of a new yeast species, *Malassezia japonica*, and its detection in patients with atopic dermatitis and healthy subjects. *J Clin Microbiol* 2003; 41: 4695–4699.
- Faergemann J. Atopic dermatitis and fungi. *Clin Microb Rev* 2002; 15: 545–563.
- Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987; 25: 2017–2019.
- Bergbrant IM, Broberg A. *Pityrosporum ovale* culture from the forehead of healthy children. *Acta Derm Venereol* 1994; 74: 260–261.
- Faergemann J. Lipophilic yeasts in skin disease. *Sem Dermatol* 1985; 4: 173–184.
- Faergemann J. *Pityrosporum* species as a cause of allergy and infection. *Allergy* 1999; 54: 413–419.
- Gordon MA. The lipophilic mycoflora of the skin. *Mycologia* 1951; 43: 524–534.
- Faergemann J, Aly R, Maibach HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. *Acta Derm Venereol* 1983; 63: 346–348.
- Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria in human skin. *J Appl Bacteriol* 1989; 67: 47–52.
- Faergemann J, Fredriksson T. Age incidence of *Pityrosporum orbiculare* on human skin. *Acta Derm Venereol* 1980; 60: 531–533.
- Faergemann J. The use of contact plates for quantitative culture of *Pityrosporum orbiculare*. *Mykosen* 1987; 30: 298–304.
- Gordon MA. Lipophilic yeastlike organisms associated with tinea versicolor. *J Invest Dermatol* 1951; 17: 267–272.
- Scheynius A, Johansson C, Buentke E, Zargari A, Tengvall Linder M. Atopic eczema/dermatitis syndrome and *Malassezia*. *Int Arch Allergy Immunol* 2002; 127: 161–169.

17. Redline RW, Dahms BB. *Malassezia* pulmonary vasculitis in an infant on long-term intralipid therapy. *N Engl J Med* 1981; 305: 1395–1398.
18. Erchiga VC, Martos AO, Casaño AV, Erchiga AC, Fajardo FS. *Malassezia globosa* as the causative agent of pityriasis versicolor. *Br J Dermatol* 2000; 143: 799–803.
19. Gupta AK, Kohli Y, Faergemann J, Summerbell RC. Epidemiology of *Malassezia* yeasts associated with pityriasis versicolor in Ontario, Canada. *Med Mycol* 2001; 39: 199–206.
20. Gupta AK, Kohli Y, Summerbell RC, Faergemann J. Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol* 2001; 39: 243–251.
21. Kim SC, Kim HU. The distribution of *Malassezia* species on the normal human skin according to body region. *Kor J Med Mycol* 2000; 5: 120–128.
22. Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000; 38: 337–341.
23. Stalder JF, Taïeb A, Atherton DJ, Bieber T, et al. Severity Scoring of Atopic Dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; 186: 23–31.
24. Johansson C, Sandström MH, Bartosik J, Särnhult T, Christiansen J, Zargari A, et al. Atopy patch test reactions to *Malassezia* allergens differentiate subgroups of atopic dermatitis patients. *Br J Dermatol* 2003; 148: 479–488.
25. Zargari A, Härfast B, Johansson S, Johansson SGO, Scheynius A. Identification of allergen components of the opportunistic yeast *Pityrosporum orbiculare* by monoclonal antibodies. *Allergy* 1994; 49: 50–56.
26. Mayser P, Gross A. IgE antibodies to *Malassezia furfur*, *M. sympodialis* and *Pityrosporum orbiculare* in patients with atopic dermatitis, seborrhoeic eczema or pityriasis versicolor, and identification of respective allergens. *Acta Derm Venereol* 2000; 80: 357–361.
27. Zargari A, Midgley G, Bäck O, Johansson SGO, Scheynius A. IgE-reactivity to seven *Malassezia* species. *Allergy* 2003; 58: 306–311.
28. Bergbrant IM, Faergemann J. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. *Acta Derm Venereol* 1988; 68: 537–540.
29. Schäfer L, Kragballe K. Abnormalities in epidermal lipid metabolism in patients with atopic dermatitis. *J Invest Dermatol* 1991; 96: 10–15.
30. Bergbrant IM, Faergemann J. Seborrhoeic dermatitis and *Pityrosporum ovale*: a cultural and immunological study. *Acta Derm Venereol* 1989; 69: 332–335.