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Acta Dermatovenerologica Croatica (ADC) aims to provide dermatovenerologists with up-to-date information on all aspects of the diagnosis and management of skin and venereal diseases. Accepted articles regularly include original scientific articles, short scientific communications, clinical articles, case reports, reviews, reports, news and correspondence. ADC is guided by a distinguished, international editorial board and encourages approach to continuing medical education for dermatovenerologists.

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Acta Dermatovenerol Croat 2004;12(2):71-76 ORIGINAL SCIENTIFIC ARTICLE

Serum Concentrations of Interleukin-2 Soluble Receptor (IL-2 sR) in Patients with Vitiligo: Relationship with Type and Extent of the Disease*

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*This study was awarded as one of the best poster presentations at the First Spring Symposium of EADV in Malta 2003.

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SUMMARY Vitiligo is a disorder of melanin pigmentation, affecting 1-2% of general population. The etiopathogenesis of the disease is not clear, but probably includes the interaction among nervous system, cytotoxic factors, and immune mechanisms. The aim of the study was to evaluate serum concentrations of interleukin-2 soluble receptor (IL-2 sR) in patients with vitiligo and assess a possible association between IL-2 sR and clinical type, extent, and duration of the disease. The study included 51 patients with vitiligo aged 7-70 years and 29 healthy controls aged 10-60 years. Serum concentrations of IL-2 sR were determined by ELISA method. The serum concentration of IL-2 sR in patients with vitiligo was lower than that in the control group (1,164.2± 357.2 vs. 1,301.6±404.1 pg/mL, respectively). Significantly decreased serum IL-2 sR concentrations were noticed in patients with non-dermatomal type A vitiligo (1,137.5±356.7 pg/mL), especially those suffering from type A1 (active lesions) of the disease (959.4±251.7 pg/mL), compared with both healthy controls and patients with type B (dermatomal) vitiligo (1,105.2±346.5 pg/mL). In patients with more extensive vitiliginous changes of the skin, IL-2 sR serum concentrations were significantly lower. Moreover, patients with type A1 vitiligo had significantly decreased serum IL-2 sR concentrations compared with type A2 (non-active lesions) patients. There was no difference in serum IL-2 sR concentrations between patients with dermatomal distribution of vitiligo and control group. Serum IL-2 sR concentrations in patients with vitiligo were decreased and depended on the activity and intensity of the disease process.

KEY WORDS adult; child; pigmentation disorders; receptors, interleukin-2; vitiligo

INTRODUCTION

Vitiligo is one of disorders of melanin pigmentation in which depigmented patches of various shapes and sizes appear in the skin as a result of the destruction of melanocytes. It is an acquired disease, affecting 1-2% of general population (1,2).

Considering etiopathogenesis of vitiligo, there are three hypotheses, each holding different factors responsible for the disease: nervous system, cytotoxic factors, and immune pathomechanism (1). In immune processes, including autoimmune mecha-

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nisms, a basic role is played by cytokines, secreted by activated cells. They influence other cells *via* specific membrane receptors, some of which have soluble forms, known as soluble receptors (sR). Interleukin-2 soluble receptor (IL-2 sR) is one of them, and it is thought to be very important parameter of immune mechanism of various pathological processes (3-19). The changes in serum IL-2 sR concentrations were found in many diseases, such as psoriasis (14-16), atopic dermatitis (17,18), multiple sclerosis (12, 13), rheumatoid arthritis (10), connective tissue diseases (3,5-7), cutaneous T cell lymphoma (19) and other malignant neoplasms (4,8,9,11). In some of these diseases, serum IL-2 sR concentration correlated with activity and intensity of the disease (e.g. positive relationship between IL-2 sR concentration and PASI in psoriasis), and may be used as a prognostic factor (9,15-17,19).

The literature data on IL-2 sR in patients with vitiligo are very limited and their results are controversial. Therefore, the aim of our study was to evaluate serum concentrations of IL-2 sR in vitiligo patients and assess the relation between IL-2 sR and clinical type, extent, and duration of vitiligo and patient sex.

PATIENTS AND METHODS

Patients

The study included 51 vitiligo patients aged 7-70 years (mean±SD age, 29±18 years). There were 23 male patients aged between 10 and 70 years (mean age, 30±19 years) and 28 female patients aged 7-67 years (mean age, 28±17 years). The control group consisted of 29 generally healthy people aged between 10 and 60 years (mean age, 29±16 years). There were 15 male patients aged between 10 and 60 years (mean age, 36±25 years) and 14 female patients aged 11-43 years (mean age, 21±12 years).

The patients who had received any treatment within previous 2 months were excluded from the study, as well as patients with any infections or diseases based on the immune pathomechanism, which could influence serum concentrations of IL-2 sR.

According to the clinical type of vitiligo, patients were divided into those with type A (with non-dermatomal distribution of vitiliginous skin) and those with type B (dermatomally distributed) of vitiligo. Type A was further divided into active (type A1) and inactive (type A2). Patients with type A1 were those who noticed a new depigmented lesion or enlargement of previous lesions during the three months prior to the study, whereas patients with type A2 were those with no changes in the skin lesions during the mentioned period.

Methods

Sera were separated from heparinized venous blood and stored at -75°C until analysis. All sera were measured at the same time. Serum concentrations of IL-2 sR were measured by an enzyme-linked immunosorbent assay (ELISA) technique, using Quantikine Human IL-2 sR Immunoassay (R&D Systems, Minneapolis, MN, USA).

In short, a monoclonal antibody specific for IL-2 sR was pre-coated onto a microplate. Standards, samples, and conjugate (a polyclonal antibody against IL-2 sR conjugated to horseradish peroxidase) were pipetted into the wells and any IL-2 sR present was sandwiched by the immobilized antibody and the enzyme-linked polyclonal antibody specific for IL-2 sR (conjugate). Following a wash to remove any unbound substances and/or antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of IL-2 sR bound. The color development was stopped and the intensity of the color was measured at 450 nm with a photometer ELISA STAT FAX 2100 (Awareness Technology Inc., Palm City, FL, USA). Serum concentrations of IL-2 sR were taken from a standard curve, obtained by diluted standard solution. The minimum detectable dose of IL-2 sR was determined as less than 10 pg/mL.

Extent of the vitiligo process was estimated on the basis of the following indexes: 1) the area of depigmented skin (vitiliginous area); 2) percentage of the vitiliginous area in relation to body surface area of each patient (percentage of vitiliginous area); and 3) proportion of the vitiliginous area to body mass index (BMI ratio).

The area of vitiliginous skin was measured for each patient by drawing contours of all depigmented patches on the transparent A4 foils, which then were scanned by Mustek Scan 6000 P scanner.

Following the scanning process, all areas were calculated by using Windows NT system, as well as Corel DRAW 9 and Auto Cad R14 software.

Body surface area of each vitiligo patient was calculated according to Mosteller formula (20), by calculation of square root of the value obtained from the following formula: *body height (cm) x body weight (kg) / 3600.* BMI was calculated as *BMI = body weight (kg) / body height (m)*^{2}.

Statistical Analysis

Data were analyzed with Mann-Whitney's U-test. The confidence level was set at =0.05. Statistica 5.1. software package (StatSoft Inc., Tulsa, OK, USA) and Microsoft Excel 7.0 for Windows were used for all statistical analyses.

RESULTS

Serum IL-2 sR concentrations were decreased in vitiligo patients in comparison with those in the control group (1,164.2±357.2 vs 1,301.6±404.1 pg/mL, respectively), but not significantly (p=0.059; Table 1). Significant decrease of the serum IL-2 sR concentrations was found in patients with type A vitiligo (1,137.5±356.7 pg/mL) compared with the control group (p=0.04) and type B vitiligo patients (p=0.04). Serum concentrations of IL-2 sR in patients with type B vitiligo did not significantly differ from those found in controls.

Serum concentrations of IL-2 sR were significantly lower in patients with active A1 type vitiligo (959.4±251.7 pg/mL) than in patients with inactive type A2 of the disease (1,252.1±370.7 pg/mL, p=0.004) and controls (1,301.6±406.1 pg/mL, p<0.001).

Analysis of IL-2 sR in adults and children in the control group revealed significantly lower IL-2 sR concentrations (p=0.007) in adults (1,127.9±224.8 pg/mL) than in children (1,547.7±483.4 pg/mL). Among adults, the decrease in IL-2 sR concentration was statistically significant only in the group of patients with active A1 type of vitiligo (884.6±300.0 pg/mL) in comparison to the control group (p<0.001; Table 2). In the group of examined children with vitiligo, the IL-2 sR serum concentrations were significantly lower in all type A patients, as well as in A1 and A2 type separately, than in control chil-

Table 1. Serum concentrations (mean±SD) of interleukin-2 soluble receptor (IL-2 sR) in patients with vitiligo divided according to clinical types of the disease in comparison with the control group

		Patients with vitiligo				
	Control (n=29)	all (n=51)	type A* (n=46)	type A1† (n=18)	type A2 (n=28)	type B (n=5)
IL-2 sR (pg/mL)	1,301.6±406.1	1,164.2±357.2	1,137.5±356.7	959.4±251.7	1,252.1±370.7	1,409.4±282.5
p‡		0.059	0.040	<0.001	0.4	0.3

*Type A vs type B, p=0.04.
†Type A1 vs type A2, p=0.004.
‡Difference vs. control.

Table 2. Serum concentrations (mean±SD) of interleukin-2 soluble receptor (IL-2 sR) in adult patients and children with vitiligo suffering from type A (A1-active and A2-inactive) form of the disease compared with their age-group matched controls

		Patients with vitiligo		
Age group	Control	type A	type A1*	type A2
Adult (n):	17	31	12	19
IL-2 sR (pg/mL)	1,127.9±224.8	1,124.0±349.9	884.6±201.0	1,275.1±342.1
p†		0.5	0.003	0.08
Children (n):	12	15	6	9
IL-2 sR (pg/mL)	1,547.7±483.4	1,165.6±381.4	1,108.8±293.7	1,203.5±443.3
p†		0.01	0.03	0.055

*Type A1 vs type A2, p<0.001 for adults and p=0.400 for children.
†Difference vs. controls.

dren (p=0.01, p=0.03, and p=0.05, respectively; Table 2).

In patients with large vitiliginous area (>200 cm^2), mean concentration of IL-2 sR was significantly lower than in the control group (p=0.04; Table 3). Patients with a small vitiliginous area (<200 cm^2) had less reduced IL-2 sR concentration than the control group or patients with large area of vitiliginous skin. Only in patients with a high percentage of vitiliginous area (>1%) was serum concentration of IL-2 sR significantly lower (1,105.2±346.5 pg/mL) than in the control group (p=0.03). The same relationship was observed in the group of patients with high (>7.0) BMI ratio (1,102.7±352.2 pg/mL) compared with the controls (p=0.03; Table 3).

Table 3. Serum concentrations (mean±SD) of interleukin-2 soluble receptor (IL-2 sR) in patients with different extent of vitiligo lesions in comparison with the control group

Lesion extent	IL-2 sR (pg/mL)	p*
Vitiliginous area:		
small (<200 cm2)	1,213.2±369.3	0.30
large (>200 cm2)	1,123.89±348.37	0.04
p†	0.20	
Percentage vitiliginous area:		
small (<1%)	1,255.6±362.9	0.40
large (>1%)	1,105.2±346.5	0.03
p†	0.08	
BMI ratio:		
small (<7.0)	1,252.0±354.1	0.40
large (>7.0)	1,102.7±352.2	0.03
p†	0.08	

*Difference vs. control (1,301.6±406.1 pg/mL).
†Small vs. large.

Patients with the disease lasting not longer than 1.5 year had significantly lower concentration of IL-2 sR (1,032.1±272.0 pg/mL) than that in the control group (p=0.04). No other relationships between serum concentration of IL-2 sR and disease duration were noted.

Serum IL-2 sR concentrations in female patients with vitiligo (1,111.8±314.4 pg/mL) were significantly decreased compared with those in the control group (p=0.03). This phenomenon was not observed in male patients (1,228.0±401.2 pg/mL, p=0.30).

DISCUSSION

Although the etiopathogenesis of vitiligo has not been fully understood, most researchers think that it is connected with immune processes. It has been reported that various autoimmune diseases often coexist with vitiligo, and various autoantibodies against different tissues, including those directly against melanocytes, have been noticed in vitiligo patients (21-25). They obviously have influence on the destruction of melanocytes, although it is not clear whether it is humoral immunity that plays the basic role in the disease or is it the secondary process due to the primary role of cell immunity. Van den Wijngaard *et al* (26) reported increased number of CD4+ lymphocytes at marginal skin of active vitiligo. Wolkentein *et al* (27) described induction of vitiligo in 4 out of 25 patients treated with IL-2 alone for metastatic melanoma. These studies suggested that vitiligo could be caused by an autoimmune response of cytotoxic T lymphocytes against melanocyte antigens. As IL-2 sR plays the key role in both cell and humoral immunity, our aim was to detect possible changes in IL-2 sR serum concentrations in vitiligo patients and association between these concentrations and various clinical parameters of the disease.

A limited number of studies on the problem were conducted by Honda *et al* (28) among Japanese, Yeo *et al* (29) among Koreans, and Caixia (30) among Chinese patients. None of the authors observed statistically significant differences between serum concentrations of IL-2 sR in patients with type B vitiligo and those in healthy controls (28-30). Our findings were similar. However, our observations on serum concentrations of IL-2 sR in patients with type A vitiligo were in contrast to previous findings (28-30). We found significantly decreased serum concentrations of IL-2 sR in vitiligo type A patients, compared with the control group and type B vitiligo patients. As we thought that there could be differences in IL-2 sR serum concentrations depending on the activity of the disease, we divided patients with type A vitiligo into those with active type A1 and inactive type A2 of the disease. We found that IL-2 sR serum concentrations were significantly decreased in patients with active A1 type vitiligo in comparison with not only healthy controls, but also with inactive type A2 of the disease. The observed differences between our and other stud-

ies may have resulted from differences in patient population, as our study was the first one performed among Caucasian subjects.

Like Gotoh *et al* (31) and Zola *et al* (32), we also noticed significantly lower IL-2 sR concentration in healthy adults than in healthy children. It seems that it is very important to have adequately age-matched control and patient groups. In our study, mean age of vitiligo patients was almost equal to that of the healthy controls. Previous investigators, however, did not seem concerned with this problem (28-30). To the best of our knowledge, our study is the first to have shown the significantly lower serum concentration of IL-2 sR only in patients with larger areas of vitiliginous lesions. Detailed studies in the relationship between serum IL-2 sR concentrations and surface of depigmented skin, however, have not been performed yet. We also noticed significantly decreased serum concentration of IL-2 sR in patients with a short disease duration. These results seem to confirm the relationship between serum concentrations of IL-2 sR and the activity of vitiligo (shortly lasting or extensive vitiligo may frequently be assumed as an active disease).

The question remains why the serum concentrations of IL-2 sR in our vitiligo patients were lower than those in healthy people, whereas in immune diseases, such as psoriasis (14-16), atopic dermatitis (17,18), cutaneous T cell lymphoma (19), and other malignant neoplasms (4,8,9,11), these concentrations were significantly increased. One of the explanations may be different character of vitiligo process than that in the above mentioned diseases. Vitiligo, in contrast to other diseases, could be regarded as a degenerative one, as there is evident lack of pigment in the skin. Moreover, Mozzanica *et al* (33) found the decreased number of CD4+ and CD8+ cells in the blood of vitiligo patients in the active stage of the disease. Recently, it has been reported that total number of activated lymphocytes was decreased in the circulation of vitiligo patients (34). As these cells may constitute source of IL-2 sR, we could speculate that their reduction may contribute to the observed decrease in the serum concentration of IL-2 sR. Gunduz *et al* (34) also noticed decreased serum concentration of IL-2 sR in vitiligo patients, but the difference from healthy controls did not reach statistical significance.

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Acta Dermatovenerol Croat 2004;12(2):77-83 CLINICAL ARTICLE

Fungal Infections of Urogenital System

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SUMMARY We analyzed the frequency of isolation of individual fungal species in the samples of urine, vaginal and cervical swabs from 107 patients (72% women and 28% men) aged 16-82 years, who were treated in primary care for cystitis, vulvovaginitis, and cervicitis. The samples were analyzed at the Microbiological Laboratory of Zagreb Institute of Public Health, Croatia, between September 1, 2002 and June 31, 2003. Eight species of yeast were isolated from the samples. *Candida (C.) albicans*, *C. glabrata,* and *C. krusei* were the most common isolates, with the frequency of 61.7%, 10.3% and 8.4%, respectively. Other species (*C. guilliermondii*, *C. famata*, *C. tropicalis*, *C. parapsilosis,* and *C. kefyr*) were less frequently isolated, between 0.9% and 7.5%. In women, the frequency of isolation of *C. albicans* species from urine samples ranged from 83.3% to 30.8%, and from uterine cervical swabs from 85.7% to 50%, showing a decreasing trend with patients' age. The frequency of *C. albicans* isolates from vaginal swabs was equal in younger (<30 years) and older women (51 years), but twice less frequent in middle-aged women. In men, the frequency of *C. albicans* species isolated from urine samples decreased with age from 100% to 52.4%. In the 107 analyzed samples with positive fungal culture, yeast isolations were significantly more frequent than pure culture (93.5%) and had a larger number of colony counts (57%). This suggested that these yeast species might have a pathogenic role in the causation of urogenital system infections.

KEY WORDS *Candida*; candidiasis; cervicitis; cystitis; mycoses; urinary tract infections; vulvovaginitis

INTRODUCTION

The incidence of fungal infections of the urogenital system is steadily rising (1). Yeasts of the *Candida* genus are the most common causative agents of these infections, whereas *Saccharomyces* and *Trichosporon* genera are much less frequent (1-5). Yeasts are most often isolated from urine samples of patients suffering from some urinary tract anomaly (reflux, obstruction) or diabetes mellitus, and from individuals with catheterized urinary bladder (1,6). *Candida (C.) albicans* accounts for the largest number of fungal isolates from urine samples, whereas isolations of the species *C. glabrata*, *C. kefyr*, *C. tropicalis*, and *C. guilliermondii* are less common (1-3,6,7).

Infection of genital mucus with yeasts is one of the most common clinical manifestations of candidiasis and the most common fungal infection of the genitalia in women of fertile age (1-3,6,7). It also infects male genitalia, but to a lesser extent (6). The incidence of vulvovaginal candidiasis, which

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amounts to 5-20%, is higher in women aged below 30 years, women in the third trimester of pregnancy, female diabetics, women taking oral contraceptives and broad-spectrum antibiotics, and women with HIV-infection (1-3,6-13). Although *C. albicans* is still the most common isolate from vaginal swabs of women with vulvovaginal candidiasis, an increase in the frequency of isolation of other yeast species has been observed in the last 10 years (1-12). Non-*Candida albicans* spp. (*C. glabrata*, *C. kefyr*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*) cause about 10-16% of all cases of vulvovaginitis (1-12). Symptomatic vulvovaginitis caused by these yeast species cannot be distinguished from those caused by *C. albicans* in terms of clinical features, but they can be recognized by their different response to therapy (1). *Saccharomyces cerevisiae* species may colonize vaginal mucus, whereas *Trichosporon beigelii* species can be isolated from the skin of the genital region (1-7).

Typical symptoms of vulvovaginal candidiasis include pruritus and vaginal discharge. Other symptoms may include vaginal soreness, vulvar burning and erythema, dyspareunia, and dysuria. *Candida* vaginitis is associated with a normal vaginal pH of 4.5 (1-3,7-12).

Although uterine cervical infection may be combined with vulvovaginitis, it may also develop in the absence of such an infection. Its occurrence is more common in women taking oral contraceptives, with erythema and epithelial erosions as the main signs of cervicitis, as well as in the presence of mucopurulent exudate. Several species of bacteria (e.g. *Neisseria gonorrhoeae* or *Chlamydia trachomatis*) and viruses (e.g. herpes simplex, human papillomavirus, adenoviruses, or cytomegalovirus) are more frequent causative agents of cervical infections than yeasts (1-3,6,7). Cervicitis is often asymptomatic, but in some cases it may be accompanied by abnormal vaginal discharge and vaginal bleeding (1). Yeast infections of female genital tract have been 4.6 times (14.4%) as common as trichomoniasis (3.13%), and the presence of the mixed fungal-protozoan infection has been shown in a very small number of women (0.6%) (8,10). The main site of trichomonadal parasitizing is vaginal mucus; the main sites of yeast parasitizing are vaginal mucus, uterine cervix, and other anatomical sites of the genital tract (8,10). Studies of the protective effect of cervical mucus in pregnant women have shown the localization and spread of infection (cervicitis, endocervicitis, and colpitis) to be primarily dependent on local immunity disorder (local immunodeficiency), and considerably less on the etiological agent of infection (9).

Mucus of the gastrointestinal tract is considered the most common source of the yeasts colonizing and/or infecting the mucus of urogenital tract. *C. albicans* may be transmitted sexually (1-3,6,7,11). Many studies have shown that less than 40% of infected women had acquired vaginal candidiasis through sexual contact (11,14). Because *C. albicans* species is a commensal yeast in human mucus (it has been isolated from vaginal smears of 45--50% asymptomatic women), it is understandable that the primary source of *Candida* infection is endogenous (1-3,6,7,11,14).

The first stage in the pathogenesis of fungal urogenital infection is the adherence of yeast blastoconidia to the epithelial cells of urogenital system (colonization). Adhering of yeast cells depends not only on the surface to which the yeast adheres (vaginal mucus or various prosthetic materials, e.g. urinary catheters), but also on yeast cell properties, such as surface hydrophobicity, growth conditions, hormonal influences, and immunologic status of the macro-organism (1-3,6,7). Surface molecules of yeast cells that make adherence to the epithelial cells of the mucus possible are called adhesions, and by chemical composition, they are mannans, mannoproteins, and chitin. The receptors of these molecules on epithelial cells are glucosamine, fucosyl, fibronectin, and arginine-glycine-asparaginine (2,6,7).

The ability to transform the (monocellular) yeast form into (multicellular) mycelium form and the production of hydrolytic enzymes (most important of which are aspartyl protease and phospholipases) are the factors facilitating tissue penetration and tissue invasion by *Candida*. Through the morphogenesis of blastoconidia into hyphae, which excrete large amounts of phospholipases, this form is made more invasive and helps the evasion of phagocytosis by macrophages. Immunomodulational action of fungal surface components, especially mannans, leads to the immunosuppression of response to fungal invasion (2,3,6,7). The rapid phenotyping

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ability of a variation of *C. albicans* facilitates the accommodation of this yeast to changed conditions in the macro-organism, which is why it easily evades the infected individual's immune response and the action of antimycotics (2,3,6,7).

MATERIAL AND METHODS

The urine, vaginal and cervical swabs samples were collected from 107 patients (72% women and 28% men) aged 16-82 years, who were treated in primary care for cystitis, vulvovaginitis, and cervicitis. The samples were analyzed with standard microbiological diagnostic methods at the Microbiological Laboratory of Zagreb Institute of Public Health, Croatia, between September 1, 2002 and June 31, 2003.

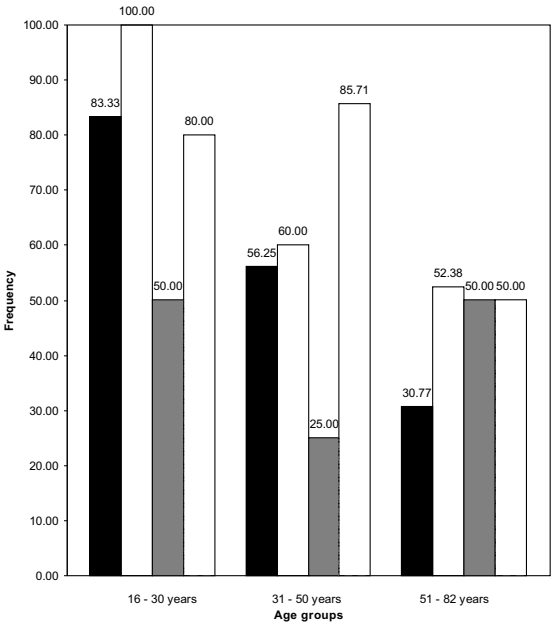

Figure 1. Frequency of isolation of *Candida albicans* species from urine samples and genital swabs by patient age and sex. Closed bars – urine specimens from women; open bars – urine specimens from men; grey bars – vaginal swab; striped bars – uterine cervical swab.

Inoculation of urine samples on blood agar and McConkey's agar was performed within 2 h of sampling, followed by incubation at 35 C for 24 h and incubation at 25 C for the next 24 h (optimal temperature for yeast conidiogenesis). The agars we inoculated with swabs of female genital tracts were the blood, Sabouraud's agar (incubation at 35 C for 24 h and than at 25 C for the next 24 h) and Thayer Martin agar (incubation under microaerophilic conditions at 35 C for 48 h) (2,3,6,7,11).

For the identification of *C. albicans* species (Fig. 1) we used the germination test, whereas for other yeast species we used their biochemical properties (by means of assimilation test and sugar fermentation) and the appearance of growth on maize agar at the Mycology Laboratory of the Croatian National Institute of Public Health (2,3,6,7,15).

RESULTS

Eight yeast species belonging to the genus *Candida* were isolated from both female and male urine samples. The most common isolate in these samples was *C. albicans* (61.5%), followed in order of frequency by *C. krusei*, *C. kefyr, C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. famata*, and *C. guilliermondii* (Table 1). From vaginal swabs we isolated three different species of yeasts. *C. glabrata* species had the highest isolation frequency (50%), *C. albicans* species closely followed (40%), and *C.*

Table 1. Yeast species and their frequency in 107 samples of urogenital system with positive fungal culture

	Samples (n,%)			
Yeast species isolated	urine	vaginal swab	uterine cervical swab	total
Candida (C.) albicans	51 (61.5)	4 (40.0)	11 (78.6)	66 (61.7)
C. glabrata	5 (6.0)	5 (50.0)	1 (7.1)	11 (10.3)
C. krusei	8 (9.6)		1 (7.1)	9 (8.4)
C. kefyr	7 (8.4)		1 (7.1)	8 (7.5)
C. parapsilosis	7 (8.4)			7 (6.5)
C. tropicalis	2 (2.4)	1 (10.0)		3 (2.8)
C. famata	2 (2.4)			2 (1.9)
C. guilliermondii	1 (1.2)			1 (0.9)
Total	83 (100.0)	10 (100.0)	14 (100.0)	107 (100.0)

tropicalis had the lowest frequency (10%). Out of the four yeast species isolated from cervical swabs, *C. albicans* species had the highest percentage of isolation (78.6%), whereas the percentages for other species (*C. krusei, C. kefyr*, and *C. glabrata*) were equal – 7.1% each (Table 1).

The incidence of *C. albicans* species isolations from urine samples was the highest in younger women (16-30 years) and men (83.3% and 100%, respectively), decreasing in both middle-aged (31-50 years) women and men (56.3% and 60%, respectively), and reaching a low in older-age group (51-82 years) of women and men (30.8% and 52.4%, respectively) (Table 2, Fig. 1).

In contrast, the frequency of other *Candida* isolates from urine samples showed increasing incidence with age in both sexes. With exception of *C. glabrata,* the percentage of non-*albicans* species of the genus *Candida* was the lowest in the urines from women and men aged up to 30 years, higher in middle-aged women and men, and the highest in older-age women and men (Table 2, Fig. 2).

C. glabrata species was absent from all urine samples obtained from younger-age women and men. The incidence of isolation of this yeast from urine samples slightly increased with age in women, reaching 6.3% in the middle-aged and 7.7% in elderly women. In middle-aged men, *C. glabrata* species isolates made up 20% of all yeast isolates from urine samples and 9.5% of isolates in elderly men (Table 2, Fig. 3).

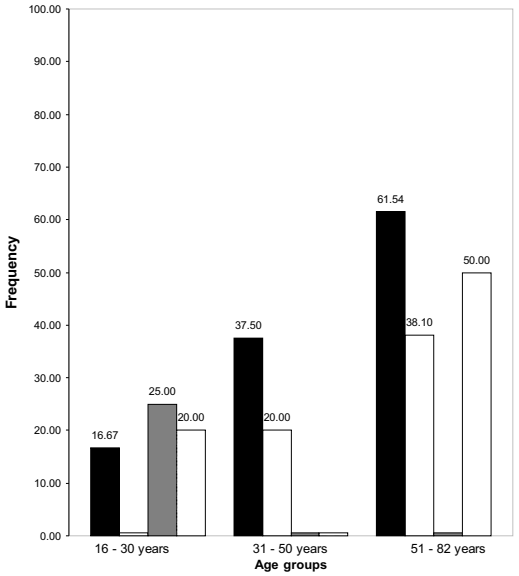

Figure 2. Frequency of isolation of species other than *Candida albicans* from urine samples and genital swabs by patient age and sex. Closed bars – urine specimens from women; open bars – urine specimens from men; grey bars – vaginal swab; striped bars – uterine cervical swab.

Table 2. The frequency of individual yeast species isolations from urine samples in relation to the patient age and sex*

	Age groups (n, %)						
	16-30 years		31-50 years		51-82 years		
Yeast species	F	M	F	M	F	M	total
Candida albicans	20 (83.3)	4 (100.0)	9 (56.3)	3 (60.0)	4 (30.8)	11 (52.4)	51 (61.5)
Non - *C. albicans* species	4 (16.7)		6 (37.5)	1 (20.0)	8 (61.5)	8 (38.1)	27 (32.5)
Candida glabrata			1 (6.2)	1 (20.0)	1 (7.7)	2 (9.5)	5 (6.0)
Total	24 (100.0)	4 (100.0)	16 (100.0)	5 (100.0)	13 (100.0)	21 (100.0)	83 (100.0)

*Abbreviations: F – female; M – male.

Table 3. Frequency of isolation of individual species of yeast from vaginal and uterine cervical swabs from women of different age

	Age groups (n, %)						
	16-30 years		31-50 years		51-82 years		
Yeast species	vaginal swab	uterine cervical swab	vaginal swab	uterine cervical swab	vaginal swab	uterine cervical swab	total
Candida albicans	2 (50.0)	4 (80.0)	1 (25.0)	6 (85.7)	1 (50.0)	1 (50.0)	15 (62.5)
Non-*Candida albicans* species	1 (25.0)	1 (20.0)				1 (50.0)	3 (12.5)
Candida glabrata	1 (25.0)		3 (75.0)	1 (14.3)	1 (50.0)		6 (25.0)
Total	4 (100.0)	5 (100.0)	4 (100.0)	7 (100.0)	2 (100.0)	2 (100.0)	24 (100.0)

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Table 4. Frequency of pure and mixed fungal-bacterial culture from 107 urine samples and genital swabs

	Samples (n, %)			
Yeast isolations	urine	vaginal swab	uterine cervical swab	total
Pure fungal culture	80 (96.4)	7 (70.0)	13 (92.9)	100 (93.5)
Mixed fungal-bacterial culture	3 (3.6)	3 (30.0)	1 (7.1)	7 (6.5)
Total	83 (100.0)	10 (100.0)	14 (100.0)	107 (100.0)

Table 5. Qualitative analysis of fungal isolations from 107 urine samples and genital swabs by number of colony forming units (CFU) grown per solid medium

	No. of patients (%)				
CFU (per 1 mL)	urine	CFU (per sample)	vaginal swab	uterine cervical swab	total
10^3	38 (45.8)	10^2	3 (30.0)	5 (35.7)	46 (43.0)
10^4	45 (54.2)	10^2	7 (70.0)	9 (64.3)	61 (57.0)
Total	83 (100.0)	Total	10 (100.0)	14 (100.0)	107 (100.0)

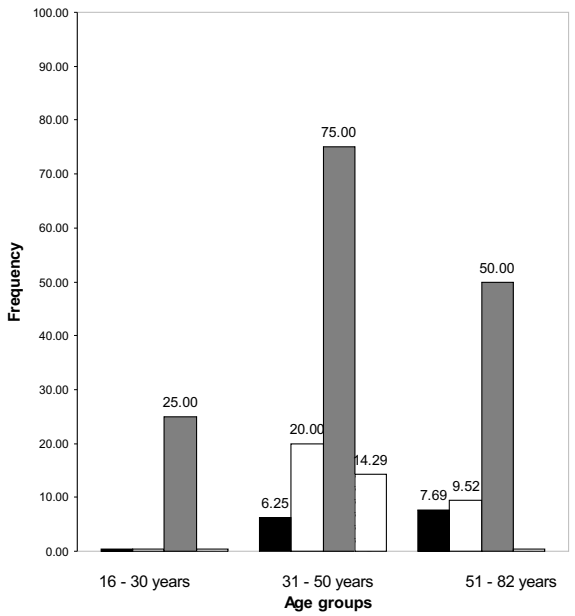

Figure 3. Frequency of *Candida glabrata* species isolations from urine samples and genital swabs by patient sex and age. Closed bars – urine specimens from women; open bars – urine specimens from men; grey bars – vaginal swab; striped bars – uterine cervical swab.

In vaginal swabs from middle-aged and elderly women, *C. albicans* isolates amounted to a half of all fungal isolates. In these isolates, middle-aged women accounted for one fourth of all isolates (Table 3, Fig. 1). Non-*Candida albicans* species were isolated only from vaginal swabs of women aged below 30 years (25%), and in older-aged women; such species accounted for 50% of all fungal isolates (Table 3, Fig. 2). The incidence of isolation of the *C. glabrata* was the highest in the vaginal swabs from middle-aged women (75%) and the lowest in women aged below 30 years (25%), with the incidence in elderly women in-between (50%) (Table 3 and Fig. 3).

Isolation frequency for cervical swabs of the *C. albicans* species was very high in younger (80%) and middle-aged women (85.7%), and lower in older-age women (50%) (Table 3, Fig. 1). Non-*Candida albicans* species made up one-fifth of all isolates from cervical swabs of younger-age women, with no such isolates from cervical swabs of middle-aged women. In samples from elderly women, they constituted one-half of all fungal isolates (Table 3, Fig. 2). We could isolate *C. glabrata* only from the cervical swabs of middle-aged women and found the frequency of isolation of this species to be very low (14.3%) (Table 3 and Fig. 3).

We isolated yeasts from urogenital samples in pure culture (93.5%) more frequently than in mixed fungal-bacterial culture (from 6.5% of positive samples), cultures with gram-positive cocci (enterococci and beta-haemolytic streptococci group B), or gram-negative species of the *Enterobacteriaceae* family (species *E. coli* and *Klebsiella* spp.). Mixed cultures most frequently grew on vaginal swabs (30%), cervical swabs (7.1%), and urine samples (3.6%) (Table 4).

Quantitative analysis of fungal cultures showed yeast isolations (from 57% of all samples) to be more common in a large number of colony counts.

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The colony count 10^2 grew on solid medium from 70% of vaginal swabs and 64.3% of cervical swabs. Also a significant amount of colony forming units (10^4) grew from 54.2% of urine samples (Table 5).

DISCUSSION

The frequency of individual yeast species isolation differs by urogenital sample type, patient age and sex. Although very extensive, the research into fungal urogenital infections is often targeted at certain population groups, e.g. hospitalized patients. Yeasts were isolated from urine samples from 1.4% of hospitalized adult patients in India, 9.3% hospitalized patients in UK, 20% of children and 25.3% of adult lying patients in USA, and from 60% of inpatients older than 63 years in Finland (6). According to the literature, it seems that *C. albicans* was the most frequently isolated species in hospitalized patients (1-3,6,7), followed by *C. glabrata* (2,3,6,7,13), and *C. krusei* species from the urine samples of hospitalized patients (2,3,6,7). Our findings on the frequency of isolation of individual yeast species correspond with the positive urine samples from primary care patients diagnosed with cystitis. During the 9-month-period of research a total of 29,997 urine specimen from men and women was received in the Laboratory of Microbiology, and yeasts were isolated from 83 samples (0.3%). In these samples, *C. albicans* was the most frequent isolate of fungal species, *C. krusei* ranked second, and isolations of other non-*C. albicans* and *C. glabrata* were less common. Our findings on the distribution of isolates of individual yeast species from urine samples of primary care patients with cystitis are in agreement with the results of other authors investigating the frequency of fungal isolates in urine samples from the inpatients having the clinical picture of cystitis (1-3,5-7).

Vulvovaginal candidiasis accounts for one-third of all vulvovaginal mucus infections (1-3,6-11). In their lifetime, about 75% of women experience at least one episode of vulvovaginal candidiasis, and 40-50% will have two or more episodes (1,8-13). A small percentage of women (up to 5%) experience recurrent vulvovaginal candidiasis (1). Risk factors for recurrent vulvovaginal candidiasis include uncontrolled diabetes mellitus, immunosuppression, and corticosteroid use (6,7,16). In some women, episodes occur after repeated topical or systemic antibacterial therapy. Many women suffering from recurrent vulvovaginal candidiasis have no apparent predisposing conditions (1-3,6,7,11). Our results showed that *C. albicans* was the most common fungal isolate from both younger (<30 years) and older (51 years) women, whereas *C. glabrata* species was most common in middle-aged women with the clinical picture of vulvovaginitis. In the past few years, many authors obtaind similar results showing increased frequency of isolation of other non-*C. albicans* yeast species from vaginal swabs (1-13). Isolates of the *C. albicans* species today still make up 35-96% of all fungal isolates from vaginal swabs of symptomatic women. Nevertheless, the work published so far suggests that *C. glabrata* or other non-*C. albicans* species, e.g., *C. krusei*, *C. kefyr*, *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii* are the cause of 1.1-33.3% of all vulvovaginitis cases (1-3,6-11).

Increased frequency of fungal urogenital infections caused by non-*C. albicans* species may be explained by the fact that these species are either less sensitive or have a primary, secondary, or cross-resistance to azole preparations (miconazole, ketoconazole, fluconazole, and itraconazole) (1-11,13,16).

Growth in pure culture and quantification of fungal isolates are important parameters for the interpretation of mycological findings (2,3,6,7,11,15). Our results indicating a high frequency of yeast isolations from urogenital samples in pure culture and in a large number of colonies could help in determining the clinical importance of isolates as pathogenic microorganisms and causes of urogenital infection.

CONCLUSION

Our findings show that *C. albicans* is the most common fungal isolate from cervical swabs of younger and middle-aged women. In elderly women, the isolation frequency of *C. albicans* and other non-*C. albicans* species is the same. Despite it being isolated in 14.3% of middle-aged women, *C. glabrata* is not a major causative agent of female cervicitis in any age group. Effective treatment of fungal urogenital infections depends on the results of mycological investigation serving to establish the pres-

ence and pathogenicity of fungal isolates and their sensitivity to antimycotics.

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Acta Dermatovenerol Croat 2004;12(2):84-91 CLINICAL ARTICLE

Flattening of Atrophic Acne Scars by Using Tretinoin by Iontophoresis

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SUMMARY Atrophic scars are a frequent consequence of acne, with a negative esthetical and psychological influence. Treatment of atrophic acne scars includes different invasive methods. In our study, we used a noninvasive method with local application of 0.05% tretinoin gel by iontophoresis. In patients with a tendency towards exacerbation, we performed mild peeling with 5% trichloroacetic acid (TCA) solution 3-4 times during the treatment. Twenty-minute treatments were applied on 38 patients, 29 women and 9 men, during 3.5 months on average. Median age of patients was 21 years (range, 16-29). Clinical assessment included an assessment of scars, pore size, skin moisture, vascularization, and skin firmness and elasticity. As confirmed by photographs taken before and after therapy, the treatment proved to be clinically effective in decreasing acne scars and persistence of effects. Flattening of acne scars was observed in 79% of the patients. The results depended on duration of scars persistence as well as on a the type of scars. The best results were achieved with younger scars as well as with superficial and ice pick scars. Side effects involved a very mild retinoid dermatitis and more often acne exacerbation. The therapy was clinically effective and the patients accepted the treatment very easily. Local therapy of acne scars with tretinoin by iontophoresis can in some cases successfully replace invasive techniques, and could also be combined with those techniques.

KEY WORDS acne scars; tretinoin; iontophoresis

INTRODUCTION

Atrophic acne scars often remain after acne, having negative esthetical and psychological impact on the patient (1). In 90% cases, facial acne scars are the leading cause of facial scars, and in the remaining 10% the scars remain after viral infections, traumatic, and iatrogenic events (2). Although type and size of the scars do not always correspond with the extent of the inflammation, they appear only after inflammatory acne. What precisely controls the development of scarring, its extent and type, is not known (3,4).

Common treatments of atrophic acne scars include various invasive techniques that require several days for patient recovery and have various unwanted side effects (5-10).

We used a non-invasive method of local 0.05% tretinoin gel application by iontophoresis. Tretinoin creams have long been used successfully for skin recovery in cases of solar degeneration (11-14) and as a therapy for other dermatological diseases with altered keratinization (15). It is well known that

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tretinoin stimulates mitotic rate of keratinocytes, increases epidermal thickness, stimulates collagen formation, widens superficial skin capillaries, and improves elastic fiber properties (11,16-19).

Iontophoresis is supposed to overcome stratum corneum barrier and to increase drug concentration in all skin layers (5,6,20,21). Our goal was to examine the efficiency of this non-invasive method for atrophic acne scars treatment.

PATIENTS AND METHODS

Patients

The study included 38 patients aged between 16 and 29 years with atrophic acne scars of different type and age (Table 1). The patients received treatment with local 0.05% tretinoin gel application by iontophoresis.

Table 1. Characteristics of 38 patients with acne and acne scars

Characteristic	No. of patients
Men/women	9/29
Age (years, median, range)	21 (16-29)
Acne type:	
acne papulopustulosis	2
acne pustulosis	16
acne nodulocystica	20
Scar type:	
superficial	10
macula atrophic	10
depressed fibrotic	7
ice pick	11
Scar age (years):	
0-1	9
1-2	9
2-4	13
4-10	7

Methods

Tretinoin gel was applied on the face after initial face cleaning with 70% alcohol. Twenty-minute treatments with 6-mA electric current were performed twice a week over 3.5 months on average. Patients that showed tendency towards acne exacerbation had mild peeling treatment with 5% trichloroacetic acid, 3-4 times over the course of the treatment.

Clinical effects were evaluated according to the following scale: score 1 – without change, score 2 – minimal, score 3 – visible, and score 4 – considerable improvement.

Skin properties such as scars depth, color, vascularization, pores size, and skin firmness and elasticity were clinically estimated. Skin moisture, sebum, and pH values were measured with combined equipment Sebumeter SM 810 + Corneometer CM 820 + Skin-pH-Meter pH 900 (Courage + Khazaka Electronic, Cologne, Germany). Treated parts of the skin were photographed before and after the treatment.

RESULTS

Retinoid Reaction and Acne Exacerbation

We expected that an average treatment duration of three and a half months would discourage patients, but it did not. Most patients showed high compliance during the whole treatment and readiness to continue it if eventually needed. This was especially noticeable for female patients who perceived twice-a-week treatments as taking care of their personal appearance. The majority of patients was more pleased with final result than the therapist herself.

Retinoid reaction in the form of mild erythema appeared in 5 out of 38 patients and lasted one to two days, whereas repeated exacerbation of existing acne appeared in 23 patients (Table 2). There was appearance of papules and papulopustules in patients who previously did not have acne at all.

Scars Flattening

Considerable improvement with respect to scars flattening was achieved in 10 out of 38 patients, visible improvement in 15, minimal but still noticeable by both the patient and the therapist in 5 patients, whereas no scar flattening was the result of the therapy in 8 patients (Table 2; Figs. 1-3).

Relationship between results and scar type was varying. Scars with small diameter (superficial and

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Table 2. Skin reactions to and result of local 0.05% tretinoin gel application by iontophoresis

Type of reaction/result	Onset	No. of patients (N=38)
Retinoid dermatitis:		
	immediately	1
	after 2 weeks	3
	after 8 weeks	1
	without dermatitis	33
Acne exacerbation:		
	after 2 weeks	10
	after 4 weeks	9
	after 8 weeks	4
	no exacerbation	15
Scar flattening:		
	none (score 1)	8
	minimum (score 2)	5
	visible(score 3)	15
	considerable (score 4)	10

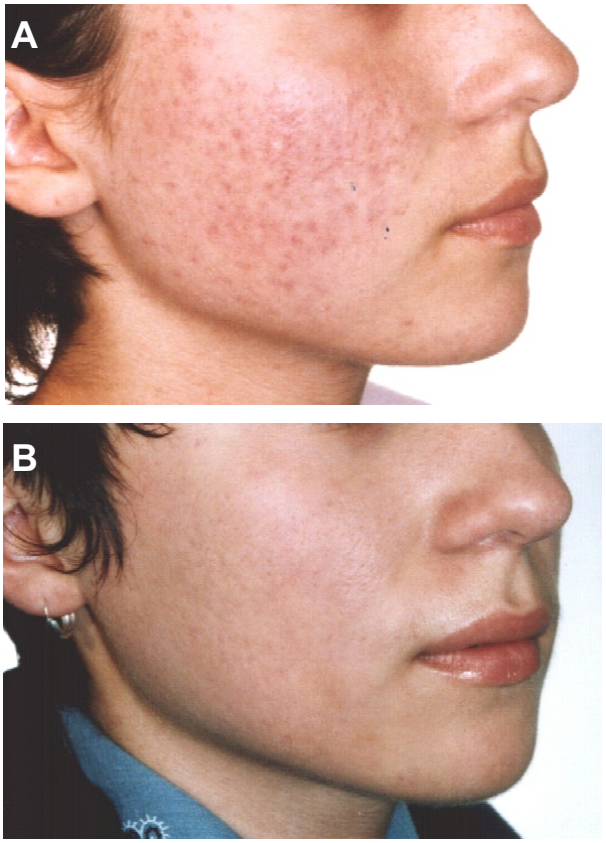

Figure 1. Patient XX before (**A**) and after (**B**) the treatment with 0.05% tretinoin gel application by iontophoresis.

ice pick) showed best results, whereas bigger scars (macula atrophic and depressed-fibrotic) did not react as well to the therapy. The worst results were obtained in patients 3with depressed fibrotic scars (Fig. 4).

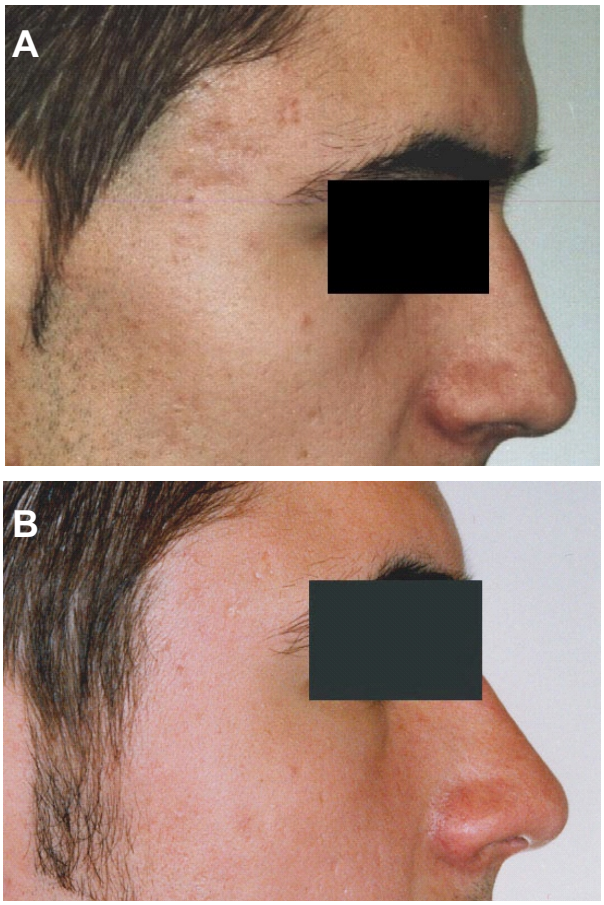

Figure 2. Patient YY before (**A**) and after (**B**) the treatment with 0.05% tretinoin gel application by iontophoresis.

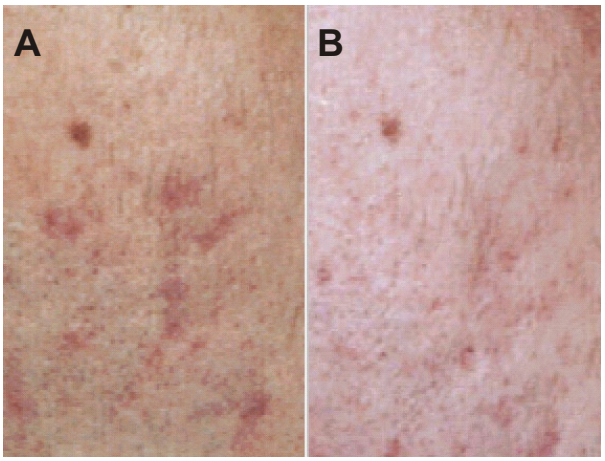

Figure 3. Patient XY before (**A**) and after (**B**) the treatment with 0.05% tretinoin gel application by iontophoresis. Detail of the right cheek.

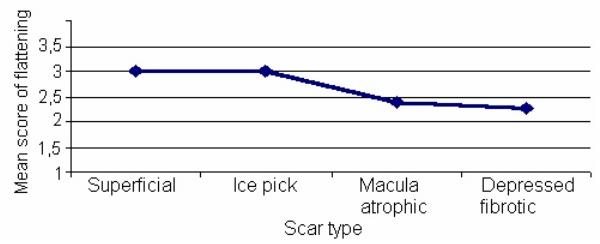

Figure 4. Improvement of different types of scars.

There was also a correlation between results and scars age, with older scars being more difficult to flatten. Improvement of the treatment result was getting worse as the age of scars was increasing (Fig. 5).

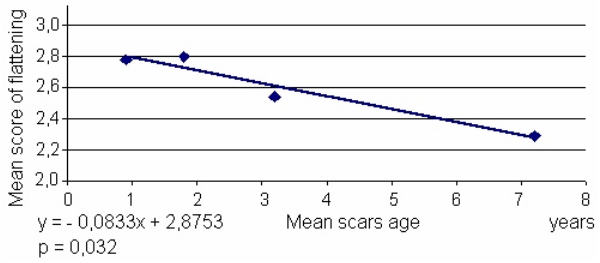

Figure 5. Scars flattening versus scars age.

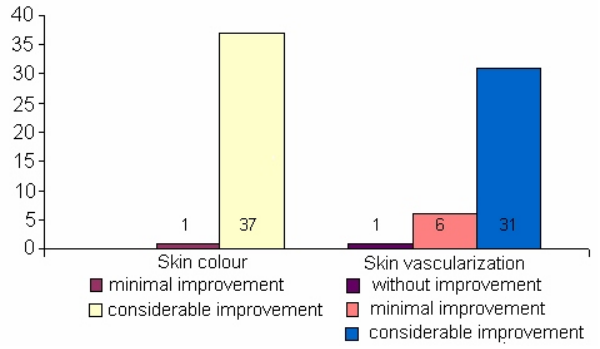

Figure 6. Improvement of skin color and vascularization.

Clinical Evaluation of Skin Properties

Treatment improved all clinically estimated skin properties (Fig. 6). Skin color was improved in all patients. Pigmentation was lightened and in most cases it become similar to the surrounding area. Skin vascularization was also improved in all patients. Skin got healthier look and more natural color. Pores also got smaller in almost all patients (Fig. 7).

Mechanical skin characteristics were also improved by the treatment. Skin firmness improvements consisted of increased firmness of scar tissue of atrophic macula and ice pick scars. With superficial scars, tissue consistency slightly improved, but even before the treatment those properties were not very adverse. Indurated scar tissue in depressed fibrotic scars became relaxed (Fig. 8). Skin elasticity improved in almost all patients, both of the scar tissue and of treated skin between scars (Fig. 8).

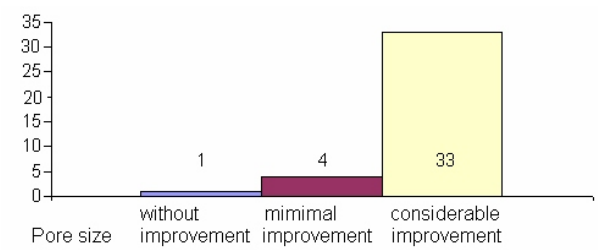

Figure 7. Improvement of pore size.

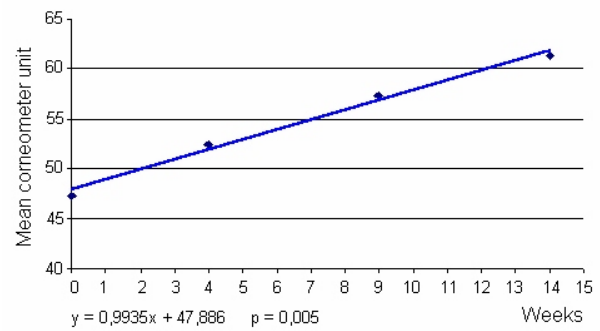

Figure 8. Skin firmness and elasticity.

Parameters of Skin's Hydrolipidic Film

At the beginning of the treatment cheek skin moisture was low. Significant (p=0.005) improvement of skin moisture in the treated areas probably resulted from the skin reparation after tretinoin treatment (Fig. 9). Amount of sebum fell after one-third of the therapy duration, and then restored to

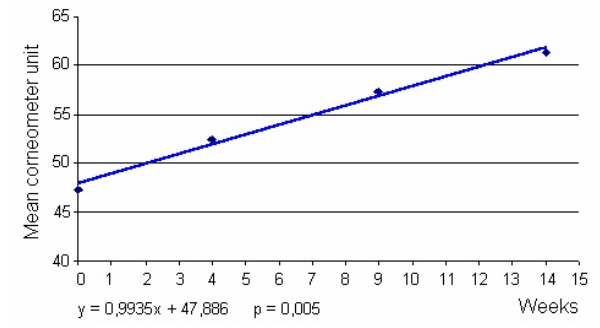

Figure 9. Skin moisture changes during treatment.

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the initial level, at which it remained until the end of the treatment (Fig. 10). Skin pH value was measured both before and after every treatment during the whole therapy. The treatment caused increased acidity for approximately 1,8 units. During the therapy, pH value did not change (Fig. 11).

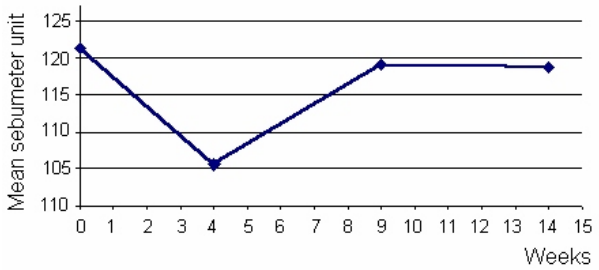

Figure 10. Sebum changes during treatment.

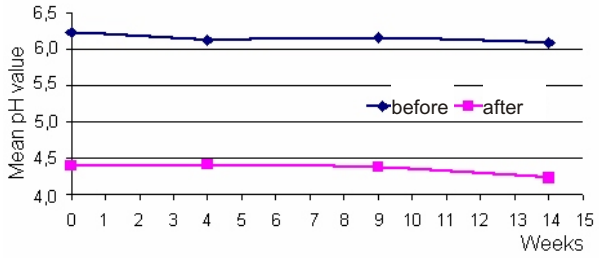

Figure 11. Mean pH values during therapy, measured both before and after the treatment with 0.05% tretinoin gel application by iontophoresis.

DISCUSSION

Scars Flattening

The local effect of tretinoin on acne and photoaging is well known. The beneficial effect on striae distensae, which is histologically and by the nature equivalent to the atrophic scars, has also been described (22). Tretinoin cream and tretinoin solution have a beneficial effect on acne scars as well (23,24).

The loss of collagen tissue and degradation of elastic tissue present in photoaging can be, to some extent, compared with results of histological examination of atrophic scars and striae distensae. Tretinoin stimulatory effect on the production of collagen and the improvement of elastic tissue's properties can explain its positive effect in such conditions. In the epidermis, it acts as an accelerator of the mitotic activity of keratinocytes, which results in the excretion of existing and prevention the formation of new comedones. A deposition of mucinous substances in the voids between stratum corneum and stratum spinosum cells takes place during the initial weeks of the treatment. However, the histologic condition in the epidermis returns after 3-4 months of treatment to the pretreatment stage while the clinical appearance of the skin keeps improving; this implies that the dermis is the final goal of the therapy (16,25,26). An improved compactness of the stratum corneum contributes to the flattening of the skin.

Local application of retinoids is limited by the barrier function of the skin. Iontophoresis is a method known to specialists in physical medicine; in dermatology, it is used to cure hyperhidrosis. The use of galvanic current considerably increases the concentration of a drug in the skin (27). The electric potential used in iontophoresis can change the permeability of the skin, causing changes in the distribution of lipids, proteins, and water molecules in stratum corneum. Transport of the drug in ionic form takes place along the sweat glands and discrete pores in the skin. These pores are created by the flow of electric current in the process of iontophoresis and one of the premises for the mechanism of their making is a so-called "flip-flop" model of the gate. According to this theory, the electric potential in the stratum corneum causes reorientation of polypeptide molecules of alpha-helix keratin into a parallel ordering. Pores are created between nonadjacent keratin helixes as the result of molecular reorientation and repulsion of nonadjacent dipoles (21,28,29).

Although a precise mechanism of the tretinoin action at molecular level is not explained, the cause of atrophic scars correction is proliferation of collagen and the consequential increment of the thickness of dermis layer. The drug results in an increased concentration of type III collagen (6).

It is assumed that the stimulation collagen synthesis by local application of tretinoin is achieved through at least two mechanisms: first, the increment of messenger ribonucleic acid (mRNA) for collagen type I and II; and second, the stimulation of the fibroblasts to induce tissue inhibitor of matrix metalloproteinases (MMPs), which inhibits collagenase, i.e. prevents the destruction of collagen (30-32). MMPs activity is of outmost importance during the reparation of the damaged tissue. MMPs

function is regulated by the protein transcription factor, the so-called activator protein-1 (AP-1). The improved activity of transcription factor AP-1 leads to the increased MMPs activity and to augmented destruction of collagen (16,32). Because of that it is assumed that tretinoin treatment can prevent formation of scars (33).

We achieved complete or partial flattening of scars in 79% of the cases. Schmidt *et al* (5,6) quoted in two studies that scars flattening was achieved in 93% and 94% of the cases by application of tretinoin 0.025% gel by iontophoresis. The degree of scars flattening was not defined.

Fernandes (34) treated two patients affected by varicella scars with tretinoin 0.05% gel. One of them had 4 months, the other 40 years old scars. Better results were achieved in a patient with younger scars that disappeared almost completely after 24 treatments. The older scars were less visible and shallower (34).

Retinoid Reaction and Acne Exacerbation

During the therapy, retinoid reaction occurred as a mild erythema in only 5 patients and lasted only 1-2 days. All patients had a mild desquamation in the form of a minimal pityriasiform peeling. This was not an estimated retinoid reaction. Schmidt *et al* (5,6) did not provide any information on retinoid reaction in their patients.

There is a large number of studies on the retinoid reaction of different tretinoin formulations and alternative A vitamin analogues, first adapalene and tazarotene recently, at local application without iontophoresis. These studies compared tolerance and effectiveness of these drugs and found that the treatment caused a retinoid reaction in about 30% of the cases (35,36).

We had acne exacerbation in a high percentage of patients. It is well known that, during the first weeks of retinoid application without iontophoresis, an aggravation of existing acne lesions can happen. Due to an increased concentration of drugs in the skin when iontophoresis is applied, the process can be accelerated, as it was in our study. We solved the problem by a mild peeling with 5% trichloroacetic acid. Schmidt *et al* (5,6) did not mention this phenomenon.

Skin's Properties

Our study has demonstrated that the tretinoin therapy improves vascularization, decreases skin pigmentation, reduces pore size, improves skin elasticity and firmness. Those results correspond with findings of other studies (5,6,11,34).

Hydrolipidic Film of the Skin

The moisture of the skin on the treated spots significantly increased and approached the normal values during therapy. We assumed that the low moisture of the skin on the cheeks before the treatment was the consequence of the skin damage caused by scars. Improvement of the skin moisture at these spots probably occurred as the consequence of skin tissue healing due the tretinoin treatment.

Fernandes (34) concluded that the treatment of damaged skin by iontophoresis led to increments of glycosaminoglycans and other natural factors that improve the skin moisture.

Sebum measurements proved that tretinoin treatment does not influence these values, which is in compliance with various authors findings (37-39).

The pH-values measured during the treatment proved that our patients had a less acid skin than normal. This can be explained by a regular use of the toilette before treatment (pH-value of Sarajevo tap water is approximately 8, soap used approximately 9,5) as well as by the skin damage caused due to acne.

CONCLUSION

Topical therapy of atrophic acne scars with retinoids by iontophoresis improved scars in most patients. This therapy also improved vascularization, decreased skin pigmentation, reduced pore size, and improved skin elasticity and firmness.

This therapy was well accepted by patients, it improved their psychological state, was easy to go through, and increased the interest in continuation and/or repetition of its application. This technique could be interesting as a complementary method to invasive techniques for acne scars reparation.

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Acta Dermatovenerol Croat 2004;12(2):92-95 CASE REPORT

A Child with *Bartonella Henselae* Osteomyelitis of the Right Humerus

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SUMMARY We present a case of a 22-month-old child with swollen upper part of the right arm and osteolytic lesion of the right humerus, which resembled a neoplastic process. Epidemiological history revealed no scratch marks on the skin or cutaneous papule or pustule. Presumptive diagnosis of hematogenous osteomyelitis was established, but treatment with fusidic acid was unsuccessful. Histological examination of the bioptic specimen of the soft tissue swelling showed a lymph node morphology, with numerous granulomas with central stellate necrosis. Indirect immunofluorescence assay for *Bartonella henselae* yielded positive results. New treatment included 15 days of trimetoprime and sulfamethoxazole, followed by azithromycin for 5 days. Four months later, swelling resolved and osteolytic lesion almost completely healed with formation of surrounding sclerosis. In conclusion, cat-scratch disease without positive epidemiological history and primary cutaneous papule or pustule may be a serious diagnostic problem, but can be solved by serological and histological examination.

KEY WORDS *Bartonella henselae;* cat-scratch disease; child; fluorescent antibody technique, indirect; osteomyelitis

INTRODUCTION

Cat-scratch disease (CSD) is a benign self-limited disease characterized by primary cutaneous papule or pustule and distal regional lymphadenopathy occurring after a cat scratch or bite. In some cases, primary cutaneous changes cannot be seen. *Bartonella henselae* is the primary agent causing cat-scratch disease (1). The majority of affected patients have a history of exposure to cats, whereas in some cases the disease develops after a dog scratch (2). Cat-scratch disease occurs primarily in children and young adults, ie, 55% of patients are younger than 18 years (3). Uncomplicated cat-scratch disease is most often manifested by regional lymphadenopathy, which usually resolves spontaneously within 2-6 months without the need for antibiotic therapy. Antibiotic therapy, however, can be considered for patients with severe form of cat-scratch disease, which is seen in up to 25% of cases (4,5). This form includes osteomyelitis, ocular involvement, encephalopathy, granulomatous hepatitis, hepatosplenic infection, endocarditis or intra-abdominal lymphadenitis (5). Osteomyelitis usually presents as osteolytic lesion and can be caused by lymphogenous or hematogenous spread of the bacteria or by infection progressing *per continuitatem*. Clinical presentation of cat-scratch

disease in the form of lymphadenopathy and osteolytic bone lesion without a skin lesion can raise the suspicion of neoplasm (6). In such cases, it is of particular importance to perform less invasive diagnostic procedures, such as lymph node needle biopsy, to avoid major surgeries.

CASE REPORT

A 22-month-old female child with swelling of the right shoulder was admitted to the Department of Pediatrics, Split University Hospital. She was the third child of healthy non-consanguineous parents. Her medical history was unremarkable, but epidemiological history revealed that she frequently played with her kitten. On admission she was febrile up to 37.2°C and appeared as a mildly ill child. Lymph nodes of approximately 1 cm in diameter were palpable on the right side of the neck. A subcutaneous painful swelling of 3.5 cm in diameter was found on the tip of the right upper arm. Physical examination of cardiac and pulmonary function showed no clinically significant abnormalities. Hepatomegaly and splenomegaly were not noted. Scratch marks were not found on the skin. Laboratory tests revealed the Westergren erythrocyte sedimentation rate of 16 mm/h. The peripheral leukocyte count was 7.4×10^9 cells/L (2% eosinophils, 36% segmented neutrophils, 0% band forms, 58% lymphocytes, and 4% monocytes). Hemoglobin concentration was 11.7 g/dL and the platelet count was $263x10^9$ platelets/L. There were 52% of T-lymphocytes and 10% of B-lymphocytes. Blood chemistry values (creatinine, blood urea nitrogen, total protein, albumen, globulin, immunoglobulin, amylase, serum aspartate aminotransferase, alanine aminotransferase, bilirubin, creatinine phosphokinase, glucose, calcium, and alkaline phosphatase) were in the ranges of reference values. C-reactive protein concentration was 31.5 mg/L (normal, <5 mg/L). Results of routine urinalysis were unremarkable. Initial ultrasound of abdominal organs revealed no enlargement of liver, spleen, or intra-abdominal lymph nodes. The results of multiple blood and urine bacterial cultures were negative. X-ray of the right humerus showed a destructive, osteolytic lesion in the epiphysial area that expanded into the metaphysial area (Fig. 1). Treatment with fusidic acid was started (18 mg/kg/day) and discontinued after 3 weeks because there were no changes in clinical condition of the child. On day 10 of hospitalization, bioptic samples of the soft tissue swelling of the right shoulder and bone fragments from the osteolytic metaphysial lesion of the humerus were taken. Histological examination of the soft tissue revealed lymph-node morphology, with numerous granulomas with central stellate necrosis. Warthin-Starry silver stains were negative for microorganisms. Gigantocellular granulomas without necrosis were found in the specimen of the metaphysial osteolytic lesion. No malignant changes were identified. Bacterial cultures of lymph nodes and bone were negative. Indirect immunofluorescence assay (IFA) for *Bartonella henselae* yielded positive results. Concentration of IgM and IgG were 1:64 and 1:128, respectively. IFA for *Bartonella quintana* was IgM-negative. Agglutination test for *Francisella tularensis* was also negative, as well as enzyme-linked immunosorbent assays (ELISA) for Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus (HIV), and *Toxoplasma gondii*. Tuberculin skin test result was also negative. The child was discharged from the hospital after 25 days. Upon discharge from the Department of Pediatrics, outpatient treatment was continued by a specialist in infectious diseases. New treatment included 15 days of oral suspension of trimetoprime (10 mg/kg) and sulfamethoxazole (50 mg/kg/day), followed by azithromycin for 5 days (20 mg/kg on day 1, and 10 mg/kg on days 2-5). After this antibiotic regimen, notable reduction of the swelling and slow resolution of the osteolytic lesion ensued. Three months

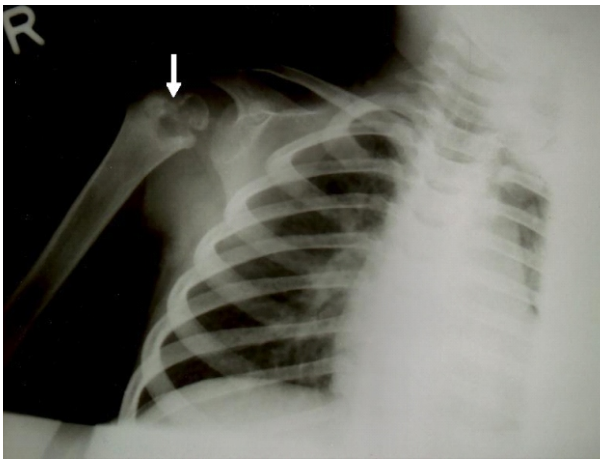

Figure 1. X-ray in a 22-month-old child with cat-scratch disease revealed destructive osteolytic lesion (arrow) of 2 ×1.5 cm in diameter in the epiphysial area, expanding into the metaphysial area of right humerus.

later X-ray examination showed considerable reduction of the osteolytic lesion. Six months after etiological diagnosis had been established, X-ray imaging revealed almost complete healing of the osteolytic lesion with formation of surrounding sclerosis.

DISCUSSION

This is the first case report of cat-scratch disease with osteomyelitis in Croatia. Osteomyelitis is a rare complication of cat-scratch disease (2), and in case of a lack of notable symptoms of infectious disease, physicians usually suspect malignant processes (7). Literature review showed no case report of cat-scratch disease with osteomyelitis in children younger than our patient (8). Because of the painful swelling on the right upper arm and young age of the child, presumptive diagnosis of hematogenous osteomyelitis was established. Since the treatment with fusidic acid was unsuccessful, further diagnostic tests were carried out. Although methicillin is usually the antibiotic of first choice, treatment with fusidic acid was administrated because causative agent of osteomyelitis, among others, could be methicillin resistant *Staphylococcus aureus*. Since neoplasm was suspected, a biopsy was performed and a small sample of the swollen tissue, a lymph node, and piece of bone was obtained. Histological findings, along with X-ray and history of exposure to the cat, suggested the diagnosis of cat-scratch disease. IFA is today used for establishing the diagnosis of cat-scratch disease, with sensitivity of 84% and specificity of 96% (9). Histopathologic examination by light microscopy may be used to confirm the diagnosis (1). The histopathologic findings of affected lymph nodes from patients with cat-scratch disease include stellate granulomas, microabscesses and follicular hyperplasia (2). Although *Bartonella henselae* may be identified by Warthin-Starry silver staining (1), in our patient the diagnosis of cat-scratch disease was based on epidemiological data (cats or kittens in the family), histological findings, and indirect immunofluorescence assay. Although there are reports of hematogenous spread of *Bartonella henselae* from the lymph node to the bone (10), because of their contiguity, we believe that bone involvement in our patient could have been the result of direct non-hematogenous expansion. Osteolytic lesion of the right humerus was observed by radiographic examinations, which revealed slow resolution of the osteolytic lesion. There are no prospective controlled clinical trials on the effectiveness of antimicrobial therapy for disseminated cat-scratch disease, but there are reports that recommend trimethoprime-sulfamethoxazole, ciprofloxacin or azithromycin in complicated cat-scratch disease (11).

We cannot be certain in the efficiency of the applied antimicrobial treatment because cat-scratch disease usually resolves without antimicrobial therapy (12), even in cases with atypical manifestations. Our case suggests that cat-scratch disease in its typical and atypical form should be considered even at a relatively young age of the patient. Meticulous epidemiological history is of essential importance (skin scratch, papule or pustule) even in very small children. Serological examinations should be performed as early as possible, and histological findings should support the diagnosis of cat-scratch disease.

Acknowledgment

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Acta Dermatovenerol Croat 2004;12(2):96-98 CASE REPORT

Traumatic Purpuric Penile Ulcer

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SUMMARY A 33-year-old man presented with a history of a penile ulcer of four days' duration. He was immediately treated with 2.4 million IU of benzathine penicillin IM and tested for syphilis, but subsequently proved non-reactive. He was already receiving doxycycline BID from another physician for this nonspecific genital ulcer and minocycline for acne. Past medical history revealed periodic flares of hidradenitis suppurativa, limited to the buttocks and inguinal region; acne, involving the face and back; and angiokeratoma of the scrotum. He had no known drug allergies, nor had he admitted to taking any other medicaments. Examination revealed an 8x15 mm irregularly shaped, shallow, tender ulcer over a larger purpuric base and involving part of the corona and the distal portion of the shaft. No inguinal adenopathy or buboes could be found on palpation. The patient was placed on a cream containing 3% iodochlorhydroxyquin 1% hydrocortisone. By the end of two weeks of therapy, the lesion had healed with no residual discoloration. Subsequently, the patient revealed that he masturbated daily, rubbing his penis back and forth on the bed sheet while lying prone on the bed. Eleven months later, he consulted us again for a new purpuric penile ulcer, because he had begun to masturabate in his usual fashion. The ulcer healed within several days, and there were no sequellae.

KEY WORDS masturbation; penis; ulcer

INTRODUCTION

Masturbation is defined as erotic stimulation involving the genital organs, commonly resulting in orgasm. It is achieved by manual or other bodily friction, exclusive of sexual intercourse. Instrumental manipulation, sexual fantasies, or various combinations can augment the process (1). There are over 300 slang terms in the English language for male masturbation, according to an Internet search, while only four synonyms appear regularly in the medical literature (self-pollution, Onansism, seminal pollution, and self-abuse) Some of these terms are euphemistic, others diminutive, and still others plain silly.

The Bible, in Genesis 38:9. implies that Onan's sin was his practice of coitus interruptus and not masturbation. He spilled his seed on the ground rather than inseminate his older brother's childless widow. The eldest son of such a union would inherit his dead brother's name, as well as his birthright. As the eldest surviving brother, he then would not inherit his father's fortune.

Prior to 1700, there is little evidence in Western medical literature that masturbation causes disease. By the early part of the 18th century, both lay and medical publications began to ascribe a variety

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of physical and especially mental illnesses to self-pollution. Masturbation, thereafter, created health consequences. Hardly a disease was not caused by so-called self-abuse. If an adolescent or young man indulged in such activity, he would undoubtedly be afflicted with acne, hair loss, epilepsy, prostate enlargement, premature ejaculation, semen leakage, sterility, alcoholism, tuberculosis, impaired memory and concentration, hypochondriasis, insomnia, anxiety, agoraphobia, paranoia, homicidal insanity, masturbation insanity, and dementia, to mention only a few of the dread illnesses he could inflict upon himself. With the changes in medical thought brought about by the First World War, physicians, and subsequently the laity, recognized that masturbation was the symptom of an unrelated disease and not the cause of a multitude of illnesses, both organic and psychiatric (2,3).

CASE REPORT

A 33-year-old man presented with a history of a penile ulcer of four days' duration. Due to concern over several sexually transmitted diseases, his family physician immediately treated him with 2.4 million IU of benzathine penicillin IM and drew a serologic test for syphilis that subsequently proved to be non-reactive. He was already receiving doxycycline BID from another physician for this nonspecific genital ulcer and minocycline for acne, unbeknown to the various physicians involved. Past medical history revealed periodic flares of hidradenitis suppurativa, limited to the buttocks and inguinal region; acne, involving the face and back; and angiokeratoma of the scrotum. He had no known drug allergies, nor had he admitted to taking any other medicaments. Examination revealed an 8x15 mm irregularly shaped, shallow, tender ulcer over a larger purpuric base and involving part of the corona and the distal portion of the shaft (Fig. 1). No inguinal adenopathy or buboes could be found on palpation.

We made a provisional diagnosis of a traumatic ulcer or possibly, even a fixed drug eruption. The patient was placed on a cream containing 3% iodochlorhydroxyquin 1% hydrocortisone. By the end of two weeks of therapy, the lesion had healed with no residual discoloration.

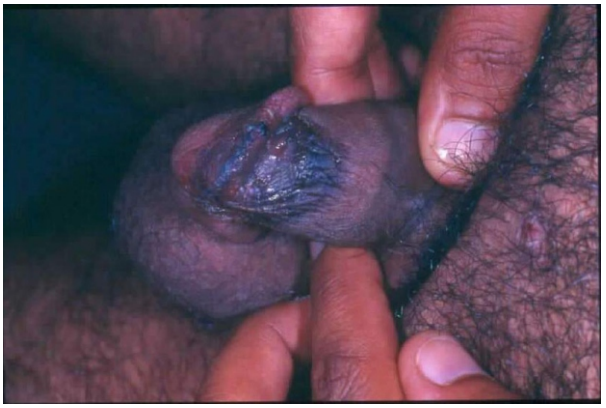

Figure 1. An 8x15 mm irregularly shaped, shallow, tender ulcer over a larger purpuric base and involving part of the corona and the distal portion of the shaft.

Subsequently, the patient revealed that he masturbated daily in the belief that this genital manipulation would be beneficial to his prostate. His form of masturbation involved first obtaining an erection and then rubbing his penis back and forth on the bed sheet while lying prone on the bed.

Eleven months later, he consulted us again for a new purpuric penile ulcer. Although he had frequent heterosexual intercourse during this period, he had begun to masturbate in his usual fashion. The ulcer healed within several days, and there were no sequellae.

DISCUSSION

The differential diagnosis could include any of the sexually transmitted diseases that have ulcerations, such as syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, and herpes progenitalis. While a syphilitic chancre would be painless and indurated, our patient had none of these manifestations. The non-reactive serology, in reality, was not helpful but did exclude previous syphilitic disease. The morphology of the lesion also helped to exclude chancroid, as there were no ragged or painful edges. Similarly, lymphogranuloma venereum and granuloma inguinale were eliminated from the differential diagnosis, as there were no palpable lymph nodes. While a herpetic infection could be a good diagnostic possibility, there was no history of recurrent penile lesions. A fixed drug eruption was suggested due to the purpura but was dismissed from the diagnostic considerations, as the ulcer healed on both occasions, despite con-

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tinuation of several medicaments; moreover, he has continued to take a multitude of similar agents, including tetracycline, doxycycline, and minocycline with no unwanted side effects. The ulcer most likely healed by itself, with little, if any, assistance from the prescribed therapy.

Masturbation can cause or aggravate a number of dermatologic and urologic conditions, ranging from penile edema (4) to eczema (5) and non-venereal sclerosing lymphangitis (6) to penile fracture (7), as well as strangulation of the penis (7). Not surprisingly, foreign bodies, introduced to the genitalia during excessive manipulation, have been extracted from the penile shaft and the lower portion of the urinary tract (7).

To our knowledge, this is the first reported instance of penile ulceration, with accompanying purpura, associated with masturbation. Although most penile ulcers are associated with sexually transmitted diseases, masturbation should be considered in the differential diagnosis.

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Acta Dermatovenerol Croat 2004;12(2):99-105 REVIEW

Erythromelalgia

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SUMMARY Erythromelalgia is a rare poorly understood clinical condition characterized by intense burning pain, pronounced erythema, and increased skin temperature. Although there are many classifications of the disease, it can basically be divided into primary, which begins spontaneously at any age, and secondary, which is associated with myeloproliferative disorders-related thrombocythemia, polycythemia, collagen-vascular diseases, diabetes mellitus, peripheral neuropathy, autoimmune and infectious diseases, and use of certain medicaments. A wide variety of etiological conditions can cause erythromelalgia, all having a single common pathogenetic mechanism – microvascular arteriovenous shunting. The disease is characterized by severe pain associated with redness and hotness in extremities. The diagnosis is based on the medical history and clinical findings. The most useful oral medications for erythromelalgia seem to be aspirin, propranolol, clonazepam, cyproheptadine, drugs inhibiting serotonin re-uptake (venlafaxine and sertraline), tricyclic antidepressants (amitriptyline, imipramine), anticonvulsants (gabapentin), calcium antagonists (nifedipine, diltiazem), and prostaglandins (micoprostol). Erythromelalgia is usually chronic, sometimes progressive, and disabling disease, which can greatly affect the quality of life. Some patients have stable disease and get better, or even experience full resolution of the disease, with time. This review article presents the etiological basis, diagnostics, and therapy of erythromelalgia.

KEY WORDS erythromelalgia; disease classification; pathogenesis; treatment

INTRODUCTION

Erythromelalgia is a rare, poorly understood clinical condition characterized with intense burning pain, marked erythema, and increased skin temperature (1). Some authors believe that it is not a separate entity but a pathophysiological response of the skin microcirculation (2). The name of the disease, first described by Mitchell in 1878, was derived from three Greek words – *erythros* (red), *melos* (extremities), and *algos* (pain) (3-5). The incidence in Norwegian population has been estimated at 2.5-3.3 per million inhabitants per year (6,7), and the disease affects twice as many women as men (2).

CLASSIFICATION

Drenth (8) and Michiels (9) divided erythromelalgia according to the patient's responsiveness to aspirin into three categories: platelet-mediated and aspirin-sensitive, primary erythermalgia, and secondary erythermalgia (8). Erythromelalgia seemed

to differ from erythermalgia (10). According to Drenth and Michiels (11), erythromelalgia was restricted to thrombocythemia of various myeloproliferative disorders. Primary erythermalgia was a mysterious congenital disorder, whereas secondary erythermalgia was associated with a detectable underlying disorder, or appears as a side effect of vasoactive drugs (11).

Kurzrock and Cohen (12) discerned between erythromelalgia of adult onset with idiopathic and secondary forms, and erythromelalgia of early onset, which was idiopathic and occurred in childhood. They classified erythromelalgia as primary or idiopathic if there was no accompanying disease, or as autosomal-dominant hereditary disorder with symptoms beginning in childhood (13). Secondary erythromelalgia was most commonly associated with myeloproliferative disorders-related thrombocythemia, polycythemia, collagen-vascular diseases, diabetes mellitus, peripheral neuropathy, autoimmune and infectious diseases, and use of certain medicaments (Table 1). Blech (14) recognized three subtypes of erythromelalgia: one associated with thrombocytosis/hyperviscosity, the other accompanying microvascular ischemia (vasoconstrictive), and the third a vasodilatory erythromelalgia. Mark *et al* (15) proposed a new classification based on the etiology and clinical findings. They divided erythromelalgia into a syndrome and a phenomenon, with each of them having an acute and chronic from. “Syndrome” was used when symptoms were restricted to the feet and legs, and appeared in childhood or adolescence. “Phenomenon” was used for all other cases. It could be primary or secondary. Primary phenomenon was a condition when erythromelalgia was idiopathic, and secondary when it was associated with primary disease, such as hematological, metabolic, connective tissue, musculoskeletal, infective, or drug-induced disease, or caused by paraneoplastic symptoms. The term “acute” was used when symptoms reached maximum strength within 1 month from the onset (15).

Table 1. Disorders associated with erythromelagia (adopted from ref. 1)

Hematologic disorders:	Infectious diseases:
polycythemia, trombocythemia	AIDS
leukemia (particularly chronic myeloid leukemia)	recurrent bacterial infections
hereditary spherocytosis	viral infections
pernicious anemia	syphilis
thrombotic trombocytopenic purpura	Musculoskeletal disorders:
Cardiovascular disorders:	sciatica
atherosclerosis	carpal tunnel syndrome
hypertension	back tunnel syndrome, peritendinitis
venous insufficiency	neck and other trauma
Embolic disease:	Neurological disorders:
cholesterol crystal emboli syndrome	neuropathies
Metabolic disorders:	multiple sclerosis
diabetes mellitus, types 1&2	spinal cord disease, sciatica
hypercholesterolemia	Drug induced:
gout	iodide contrast injection
familial nephritis	oral medications (nifedipine, felodipine, nicardipine, bromocriptine, norephedrine, pergolide, and ticlopidine)
Connective tissue disorders:	vaccines: influenza, hepatitis
rheumatoid arthritis	Other conditions:
systemic lupus erythematosus	cancer (abdominal, colon, thymoma, or astrocytoma)
mixed connective tissue disorder	frostbite
Sjogren's syndrome	conversion disorder
vasculitis	mercury poisoning

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PATHOGENESIS

A wide variety of etiological factors can cause erythromelalgia. However, there is only one common pathogenetic mechanism suspected – microvascular arteriovenous shunting (16). The pathology underlying erythromelalgia seems to involve reduced nutritive blood flow coupled with arteriovenous shunting. According to shunting hypothesis, symptoms are caused by tissue hypoxia, induced by misdistribution of skin microvascular blood flow, with increased thermoregulatory flow and inadequate nutritive perfusion (15). The skin temperature of patients with erythromelalgia, when not flaring, is lower then that of control subjects (17). This suggests a subclinical vasoconstriction during the day with subsequent reactive hyperemia at night. In erythromelalgia, some precapillary sphincters may be constricted while the arteriovenous shunts are open, creating an imbalance of increased total perfusion, yet deficient nutritive perfusion (2,18). The result is the coexistence of hypoxia and hyperemia of the skin. The products of tissue hypoxia trigger increased local blood flow, worsening the redness, warmth, and pain. Kazemi *et al* (19) performed sympathetic skin response test and concluded that sympathetic peripheral fibers (C fibers) were involved in erythromelalgia. Activated C fibers could cause vasodilatation.

Secondary erythromelalgia may result from humoral factors released from platelets of ischemic tissues or from C-fiber injury in some cases of neuropathy, whereas primary erythromelalgia could be due to a mutation of capsaicin receptor (13). Davis *et al* (20) confirmed that, in addition to other forms of neuropathy, majority of their 67 patients with erythromelalgia had small-fiber neuropathy. Their analysis of autonomic and neurophysiologic studies revealed two major findings. First, most patients with EM had evidence of postganglionic sudomotor failure, often with severe deficit. The finding of peripheral, especially distal, denervation suggested the presence of a distal small fiber neuropathy (20). Second, evidence from nerve conduction studies and electromyography of large fiber neuropathy was relatively common (20).

Although erythromelalgia is a heterogeneous condition, neuropathy and vasculopathy are the most likely mechanisms of causation (16-20). Vasculopathy and neuropathy coexist because vasculopathy with hypoxia may cause neuropathy (20).

CLINICAL PICTURE

Soles, toes, palms, feet, or fingertips are red, warm, and painful. Lower extremities are affected more frequently than upper extremities (Figs. 1 and 2). Most patients experience erythromelalgia in the feet, although severe erythromelalgia may spread up the legs or arms. There are intensive sensations of burning, itching, and prickling. The swelling is usually bilateral, although it may be unilateral, especially in secondary cases (1). All those symptoms are provoked or worsened by exercising, in some cases even by walking. Exposure to heat worsens the condition immediately, whereas cooling of the affected feet brings relief. The duration of attacks may last from a few minutes to several days. Relief of pain with ice water immersion is so common that is almost pathognomonic (1). The severity and fre-

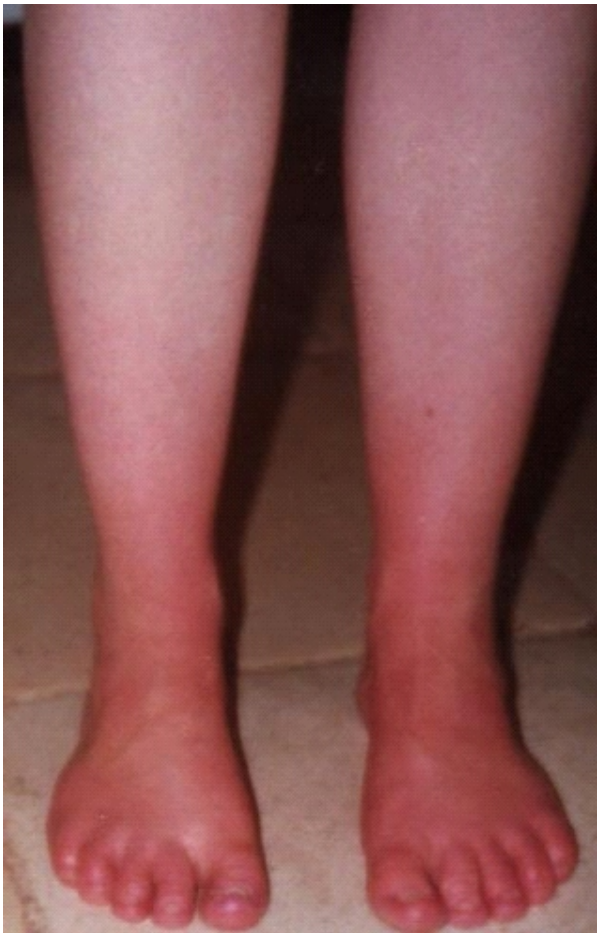

Figure 1. Swelling of the distal limbs during an episode of erythromelalgia.

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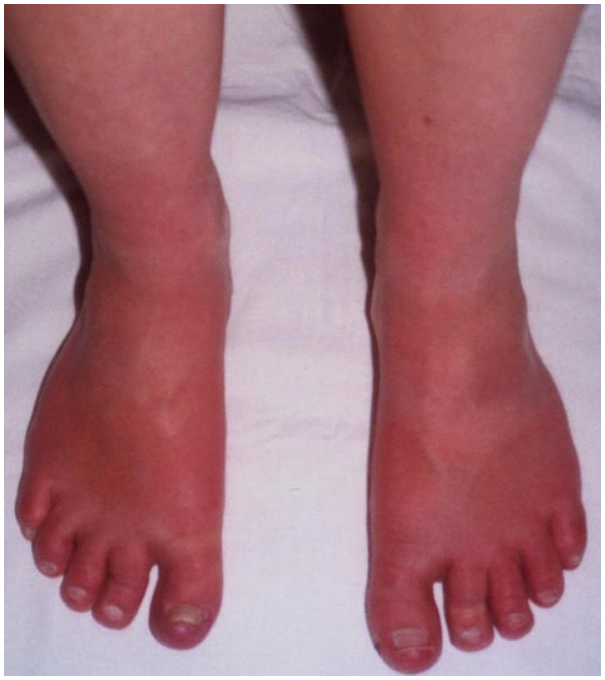

Figure 2. Swelling of the distal limbs during an episode of erythromelalgia (detail).

quency of attacks of erythromelalgia in some patients are increased during the summer (15).

Many patients have prodromal symptoms of burning, pain, heat intolerance, or flushing for many months or years before the appearance of characteristic vasomotor symptoms.

Secondary tissue damage and reactive hyperemia may be the result of the excessive cooling (15). Digital necrosis or skin ulceration with secondary infection can lead to amputation. If erythromelalgia is left untreated, it may progress to painful acrocyanosis and even peripheral gangrene (21). In some patients, spontaneous ulcerous with slow healing and gangrene on the extremities may lead to amputation (15). However, once reversed, remissions may last months, years, or indefinitely (1).

DIAGNOSIS

As no objective laboratory criteria are available, the diagnosis is based on the medical history and clinical findings (15). Patients with erythromelalgia can usually provide good description of their symptoms, from which a tentative diagnosis may be made. Typically, symptoms appear late in the day. The patient can present with normal skin during the regular daytime examination. If doubt remains, immersion of an affected area into hot water for 10-30 minutes sometimes (but not always) provokes flaring. Diagnostic criteria differ from author to author. Davis *et al* (22) used 3 inclusion criteria – red, hot, and burning extremities. Thompson *et al* (23) proposed 5 criteria – burning extremity pain, pain aggravated by warming, pain relieved by cooling, erythema of affected skin, and increased temperature of the affected skin.

Electromyographic studies are usually normal. Termography reveals increased skin temperatures in the affected area, but it is not necessary to establish diagnosis. X-ray of the feet and hands usually provides no specific findings.

DIFFERENTIAL DIAGNOSIS

The diagnosis of erythromelalgia is easily made on the basis of well-taken medical history and classical findings. Differential diagnosis includes Raynaud syndrome, “burning feet syndrome” (24), reflex sympathetic dystrophy (complex regional pain syndrome) (1), neuropathy, cellulitis, and frostbite. Menopausal symptoms and medication reactions may produce flushing or sensation of intense heat without profound, localized redness and pain characteristic of erythromelalgia (1).

Raynaud syndrome is often mistaken for erythromelalgia, but it is usually triggered by cold and has cyclic progression with initial vasospasm. Burning feet syndrome (24) has similar symptoms, but different physical findings – there is no local tenderness of affected parts, the overlying skin and blood vessels are normal in most patients, whereas in some cases there may be accompanying erythema of the feet with warm skin, as in erythromelalgia. Reflex sympathetic dystrophy occurs under different circumstances (at the site of an injury); it is a permanent chronic condition characterized by severe burning pain, pathological changes in bone and skin, excessive sweating, tissue swelling, and extreme sensitivity to touch.

TREATMENT

There is no consistently effective treatment for erythromelalgia. The reasons could be low prevalence of the disease and no characteristic laboratory findings. The management of erythromelalgia

is difficult and frequently involves multidisciplinary approach. Various treatment modalities have been tested, but none proved successful, especially for the idiopathic type (Table 2). There is still no treatment of primary erythromelalgia, whereas the treatment of the secondary form consists of cessation of causative drugs or treatment of the underlying disorders (4).

Table 2. Treatment modalities

Cooling or elevating the extremity	
Aspirin	
Nonsteroidal anti-inflammatory drugs (ibuprophen, indomethacin, nabumetone, neproxen, or sulindac)	
Chemotherapy (in myeloproliferative disorders)	
Phlebotomy (in patients with polycythemia vera)	
Surgery (in rare complication of gangrene)	
surgical sympathectomy	
Platelet inhibition (in patients with thrombocytosis)	
Propranol	No randomized trials of therapy
Epinephrine	
Biofeedback	
Sodium nitroprusside	
Methysergide	
Antihistamines (cyproheptadine hydrochloride, diphenhydramine, trimeprazine, cimetidine)	
Tricylic antidepressants	
Anticonvulsants	
Local anesthetics (lidocaine topical) + lidocaine intravenously	
Capsaicin cream	
Clonidine	
Opioids and -2-agonists	
Carbamazepine	
Ketamine intravenous	
Gabapentin	
Methysergide (serotonin reuptake inhibitor)	
Venlafaxine 37.5 mg twice daily (serotonin reuptake inhibitor)	
Clonazepam	
Amitriptyline	
Doxepine	
Prednisone	
Cyproheptadine	
Magnesium	
Pentazocine	
Mexiletine	
Buprenorphine	
Pizotifen	

The symptoms are relived upon cooling. Pain relief by cooling could be explained by the lowered metabolic rate and consequently reduced need for oxygen supply (15).

The most useful oral medications for erythromelalgia seem to be aspirin, propranolol, clonazepam, cyproheptadine, drugs inhibiting serotonin reuptake (venlafaxine and sertraline), tricyclic antidepressants (amitriptyline, imipramine), anticonvulsants (gabapentin), calcium antagonists (nifedipine, diltiazem), and prostaglandins (micoprostol) (1).

Smith and Allen (25) were the first to discover that a single dose of aspirin produces remarkable relief of burning pain, persisting for few days. Aspirin is effective in patients with thrombocythemia and polycythemia, although most cases are very resistant to treatment (1). Aspirin is usually a treatment of choice, as it inhibits prostaglandin synthesis and thus prevents formation of platelet-aggregation tromboxane A2.

Successful treatment was achieved with nitroprusside infusions in children and adolescents, and prostaglandin and lidocaine infusions, 10% topical capsaicin, or bilateral sympathectomies in adults. Nitroprusside is a valuable alternative for erythromelalgia resistant to aspirin therapy, especially in children (26). It seems that capsaicin accentuates the release of substance P from sensory nerves fibers and, after repeated applications, depletes the neurons of substance P (27). The underlying mechanism of pain in erythromelalgia is still unclear, but it could involve substance P (28).

Uchida *et al* (29) administered intravenously a low dose of ketamine, a drug considered to be effective for intractable pain, and pain subsided significantly and become completely controllable in combination with oral medications. Treatment of erythromelalgia can also be achieved with high-dose oral magnesium (30). Administration of intrathecal opioid and an 2-agonist can be effective in the treatment of the pain (31).

Sano *et al* (32) treated a 28-year-old woman with cyclosporine. Initial dose was 5 mg/kg/day, and over three-month period dose was reduced to 3 mg/kg/day, because the erythema and pain in her legs gradually subsided. Eighteen months later, the redness of her feet, calves, and thighs subsided, the surface temperature normalized and the quality

of her life remarkably improved (32). The exact mechanism of action of cyclosporine in amelioration of erythromelalgia is not known (32). It may reduce nitric oxide production in vascular smooth-muscle and endothelial cells through down-regulation of inducible nitric oxide synthase (33), and stimulate endothelin-1 production (34), resulting in an amelioration of the microvascular abnormalities. Surgical sympathectomy has been attempted, with different results (35,36). In addition, it is important to educate patients how to avoid episodes of erythromelalgia, relieve discomfort during the episodes, and control secondary and underlying factors.

PROGNOSIS

Although spontaneous remissions do occur, erythromelalgia may last for many years and be complicated by attempts to relieve the pain. The disease usually has a chronic, sometimes progressive and disabling course. The onset of erythromelalgia may be gradual, with some cases remaining mild and unchanged for decades, or it can begin acutely, spreading quickly and disabling the patient within weeks.

CONCLUSION

Erythromelalgia is usually a chronic, sometimes progressive and disabling disease, which can strongly affect the quality of life. Despite recent progress in understanding and treating erythromelalgia, this vascular disorder remains a painful and life-altering condition for many patients. No single therapy has been consistently effective. The outcome is difficult to predict and depends on the primary condition. In some patients, the disease is stable and improves with time, or even fully resolves.

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WELCOME ADDRESS

On behalf of the Organizing and Scientific Committee of the International Symposium "Update on atopic eczema/dermatitis syndrome" and Croatian Dermatovenerological Society Board, I cordially welcome you to Cavtat/Dubrovnik. I wish you a successful scientific meeting and hope you find the social program equally enjoyable.

I am very happy and proud to have the opportunity to organize this International Symposium. Here are again our friends and world known professors from 16 countries, such as Prof. Ring, Prof. Marks, Prof. Nakayama, Prof. Kapp, Prof. Stingl, Prof. Wolf, Prof. Turjanmaa, Prof. Ruzicka, and Prof. Berardesca.

I am also pleased to welcome Prof. Luger, who is for the first time in Croatia, as well as Prof. Seidenari, Prof. Deleuran, Prof. Fedenko, Prof. Diepgen, Prof. Wüttrich, Prof. Zuberbier, Prof. Werfel, Prof. Sziepetowski, Prof. Darsow, Prof. Vena, Prof. Silny, and Prof. Kaznacheeva.

We are especially proud to welcome Prof. Marks, President of the International League of Dermatology, Prof. Stingl, Secretary General, and Prof. Ring as EADV elected president.

We have prepared 55 oral presentations, 5 satellite symposia with 11 oral presentations, two sponsored lectures, and 11 posters.

We regret that Prof. Trevisan (Italy), Dr. Lawrence (USA), Prof. Girolomoni, Prof. Bieber, Secretary of Dermatology Section of EAACI, Prof. Therstrun-Pederson were not able to participate this Symposium.

We also want to thank our sponsors and exhibitors on help they provided with the organization of this meeting.

The last meeting at Plitvice in 2003 offered many up-to-date presentations on the main topic. I hope that this year's Symposium on atopic eczema/dermatitis syndrome, which is a great scientific trigger, is going to be equally interesting, informative, and exciting.

The Symposium is attended by participants from 16 different countries all over the world.

My obligation, as a new Board member of the Section Dermatology of the European Academy of Allergology and Clinical Immunology, is to fulfill the promise I made in Paris on June 7, 2003, and organize the meeting on atopic dermatitis under the sponsorship of EAACI.

Cavtat venue of the Symposium, near Dubrovnik, which is unique world central heritage under the auspices of UNESCO, gives your opportunity to fulfill your scientific and social expectations.

Prof. Jasna Lipozenčić, MD, PhD

Under the auspices of the Croatian Academy of Medical Sciences

International Symposium

UPDATE ON ATOPIC ECZEMA/DERMATITIS SYNDROME

organized by

Section Dermatology of the European Academy of Allergology and Clinical Immunology (EAACI)

and

Croatian Dermatovenerological Society of the Croatian Medical Association

sponsored by

International League of Dermatological Societies

Hotel Croatia, Cavtat/Dubrovnik, Croatia

April 25-28, 2004

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FINAL PROGRAM

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Target Audience

Dermatologists, pediatricians, allergists, and immunologist are invited along with their colleagues from basic and clinical research and pharmaceutical industry to participate in the discussion forum on early diagnosis of atopic eczema/dermatitis syndrome, with an emphasis on future developments in diagnosis, management, and prevention of the syndrome.

Atopic eczema/dermatitis syndrome strategies: The relevance of the diagnosis of allergy in atopic dermatitis and research in the pediatric allergy/dermatology will be presented by prominent lecturers.

Dear Colleagues,

Section Dermatology of the European Academy of Allergology and Clinical Immunology (EAACI) in cooperation with Croatian Dermatovenerological Society of the Croatian Medical Association and sponsored by the International League of Dermatological Societies organizes an International Symposium on Update on Atopic Eczema/Dermatitis Syndrome, which will take place in Cavtat. The city of Cavtat, near Dubrovnik, is the pearl of south Adriatic coast, considered one of the sunniest places in Europe, with unique world cultural heritage under the auspices of UNESCO. The scientific program of the Symposium is well balanced and tailored to the current needs of clinicians and basic scientists with a special interest in atopic eczema/dermatitis syndrome.

SCIENTIFIC PROGRAM

1. PROGRESSION IN ATOPIC DERMATITIS
2. CURRENT APPROACH TO ALLERGY
3. IMMUNOLOGIC BASIS OF ATOPIC DERMATITIS
4. DIAGNOSTIC STATE OF THE ART IN ATOPIC DERMATITIS
5. PREVENTION OF TRIGGER FACTORS IN ATOPIC DERMATITIS
6. ATOPIC DERMATITIS AND OTHER DISEASES
7. MANAGEMENT OF ATOPIC DERMATITIS
8. NEW DRUGS FOR ATOPIC DERMATITIS
9. POSTERS

Active participants:

Australia

Austria

Bosnia and Herzegovina

Croatia

Denmark

Finland

France

Germany

Italy

Israel

Japan

Poland

Russia

Slovenia

Switzerland

United States of America

BOOK OF ABSTRACTS

ORAL PRESENTATIONS

PLENARY LECTURES

O 1

THE ATOPIC ECZEMA/DERMATITIS SYNDROME: CLASSIFICATION, NATURAL COURSE AND IMMUNOLOGIC DIFFERENCES BETWEEN THE IgE-ASSOCIATED ("EXTRINSIC") AND THE NON-IgE-ASSOCIATED ("INTRINSIC") TYPE

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According to the Position Paper from the European Academy of Allergology and Clinical Immunology (EAACI) nomenclature task force,* the term "atopic eczema/dermatitis syndrome" (AEDS) should be used as the "umbrella" term to cover the different subtypes of atopic dermatitis (AD). The new nomenclature underlines the fact that AD is not one, single disease but rather an aggregation of several diseases with certain clinical characteristics in common. The so-called "intrinsic" type of AD (now nonallergic or non-IgE-associated AEDS) fulfils the most commonly used diagnostic criteria for AD. These patients have no associated respiratory diseases, such as bronchial asthma (BA) or allergic rhinitis (AR), show normal total serum IgE levels, no specific IgE, and negative skin prick tests to aeroallergens or foods. Immunologic differences between the IgE-associated type of AD and the non-IgE-associated type can be found in cell and cytokine pattern in peripheral blood and in the affected skin, and also by phenotyping characterization of epidermal dendritic cells. The current explanation of this distinction is based on differences in genetics and/or environmental conditions (micro milieu). However, the non-IgE-associated type may switch to an IgE-associated one during the course of the time what underlines the necessity of repeated allergological investigations over the years. However, children suffering from a non-IgE-associated type rarely develop BA and AR, contrary to the early atopic children with AEDS. The question arises whether we are missing some relevant allergens or antigens in the non-IgE-associated type, such as *Coprinus comatus* (shaggy cap) or *Malassezia furfur*/*sympodialis*. On the other side, by performing the so-called "atopy patch tests" with aeroallergens, foods and *Malassezia furfur*, a subgroup of non-IgE-associated type with a T-cell-associated AEDS in the absence of a IgE sensitization could be identified. Another pathogenetic factor, which has been demonstrated in both types of AEDS, is IgE antibody reactivity against human proteins. The sensitization against Hom S 1 has been suspected as the basis of the nonallergic AEDS. But this may rather support the hypothesis that autoantigens, such as Hom S 1, may play a part in the chronicity of AEDS in the absence of actual exogenous allergens. In the future, all the subgroups of AEDS should be better defined, as by an extensive allergological work-up by in vitro and in vivo testing, including atopy patch tests to aeroallergens, foods and fungi, and by deep immunologic investigations, mainly in skin biopsies.

*Allergy 2001;56:813-24.

O 2

IgE – A PATHOGENETIC FACTOR IN ATOPIC DERMATITIS?

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Abstract not received.

O 3

IgE vs NON-IgE-RELATED ATOPIC ECZEMA

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Atopic eczema (AE) is the most common non-contagious inflammatory skin disease in childhood and also increasingly more prevalent in the adult population. The role of allergy in atopic eczema has been debated in the past; now there is clear-cut evidence that aeroallergens and food allergens play a distinct and clinically relevant role in at least a subpopulation of AE patients, as can be shown by the atopy patch test and oral provocation tests. Compared with skin prick test and radio-allergo-sorbent test (RAST,) the atopy patch test has a lower sensitivity but much higher specificity (60-90%). In skin biopsies the high-affinity IgE receptor (Fc R1) can be demonstrated on epidermal Langerhans cells and especially in AE lesional skin, as well as in some patients with negative skin prick test and RAST. In an epidemiological study among school children, it has been shown that increased pollen counts are significantly associated with eczema flares during summer months in sensitized children. A certain number of patients with clinically typical AE does not show positive on skin prick test or RAST; this patient group has been called, according to B. Wüthrich, the “intrinsic type” of AE, in analogy with the common distinction “extrinsic vs. intrinsic” in bronchial asthma. “Intrinsic” is a negative definition reached only by exclusion. Maybe we are looking for too few or for the wrong allergens in screening for IgE antibodies. It may also be possible that under the “intrinsic” variant, individuals with allergy are hidden, and either IgE antibodies are only bound locally in the skin or other effector mechanisms (T cells or eosinophils) play the critical role. Recently, a task force “nomenclature” of the World Allergy Organization has proposed new definitions in the classification of allergic diseases, restricting “atopy” to IgE-associated diseases. In this terminology, an “intrinsic” variant can no longer be called “atopic”. Therefore, a new classification for eczematous/dermatitic skin diseases has been proposed, whereby the term “eczema” is to replace the term “atopic eczema” or “atopic dermatitis”. Only the IgE-associated variants of eczema should be called “atopic eczema”, while the “intrinsic” variants are then classified under “non-atopic eczema”. The controversies in terminology reflect different opinions on pathophysiology and arise from the lack of solid knowledge regarding the complex pathophysiology of AE. We hope that by future research, especially into the pathomechanisms of the “intrinsic” variant, a better understanding will be possible, giving rise not only to a more logical classification, but also to new strategies for diagnosis, therapy, and prevention of this disease.

O 4

STUDIES ON THE FREQUENCY AND CAUSATION OF ATOPIC ECZEMA IN AUSTRALIA

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Recent papers describing the frequency of atopic dermatitis indicate that the prevalence of the disease in the community may be increasing. Population-based sampling surveys by dermatologists using standardized criteria are the gold standard for determining the true frequency in the community. A series of population-based studies were carried out in the State of Victoria, Australia, aiming to determine the true prevalence of atopic dermatitis. They looked at the frequency of atopic dermatitis in (1)

Maternal and Child Health Centers for children up to the age of four; (2) Primary Schools with children aged 4-12 years; (3) Secondary Schools with children aged 12-18 years; and (4) community-based survey of adults aged 18 and over. Data collected included whether the participants knew if they had the disease; what treatment, if any, they had received; and who prescribed it. A longitudinal study was also done on the 12-month incidence of atopic dermatitis in three separate cohorts of newborn babies (Caucasians, ethnic Chinese and ethnic Vietnamese) in Australia. The results showed that the prevalence of atopic dermatitis was most common in the first 12 months, deceasing in frequency with increasing age, but still being present well into adulthood in a small proportion of the community. Parents of children with minimal to mild atopic dermatitis were frequently not aware that the child had some abnormality in their skin. In the longitudinal study on the three cohorts of infants, 21% of Caucasians, 44% of Chinese and 17% of Vietnamese infants developed atopic dermatitis in the first 12 months. Parents of Chinese and Caucasian infants had similar socio-economic and housing conditions compared with the parents of Vietnamese infants, who tended to be of low socio-economic status with communal housing and lack of plush pile carpeting. The high incidence of atopic dermatitis in Chinese compared with Caucasian infants may reflect genetic differences between the two populations, whereas the difference in incidence between the Chinese and Vietnamese infants possibly reflects more the environmental contribution to disease expression.

1. PROGRESSION IN ATOPIC DERMATITIS

O 5

T CELLS IN ATOPIC DERMATITIS

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The evidence for T-cells participating in the inflammatory process in atopic dermatitis (AD) is increasing. We demonstrated that adults with moderate AD have double the number of T-cells in the skin compared with their number in the peripheral blood compartment. There was also a significant reduction in the telomere length of both CD4+ and CD8+ T-cells in blood, as well as skin-homing T cells. These results pointed towards an increased turnover in the peripheral T-cell system. Treatments, which specifically inhibit T-cells, are very effective in controlling AD, supporting the view that T cells play a central role in the pathogenesis of AD. Many chemotactic factors are produced in the skin in patients with AD, inducing an invasion of T-cells into the skin. We shoed that T-cells in AD skin expressed the chemokine receptors CCR4 and CCR10 and that their ligands, TARC and CTACK, were unregulated. We observed three subpopulations of T helper cells in AD: CCR4+/CCR10+, CCR4+/CCR10^{-}, and CCR4-/CCR10+. This showed the heterogeneity of the T-cell population in the skin and supported the hypothesis that TARC and CTACK were independent lymphocyte-attracting chemokines in AD. We further showed that intradermal injection of TARC into the skin of BALB/c mice did result in accumulation of CD4+ lymphocytes in the skin. Observations indicated that CCR4+ cells were predominantly of the Th2 type, whereas the CCR10+ cells were of both Th1 and Th2 subset. TARC could enhance tumor necrotizing factor- -induced CTACK production, which suggested a possible mechanism for the shift from a Th2 response in the acute phase in AD to a mixed Th1/Th2 response in the more chronic phase of AD.

O 6

KERATINOCYTES CONTRIBUTE TO INFLAMMATORY CIRCUITS IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) results from a complex interplay of environmental, genetic, immunologic, and biochemical factors. Relevant to the amplification and persistence of inflammatory and immune responses in AD skin is the contribution of keratinocytes, which can be induced to secrete pro-inflam-

matory mediators in response to triggering factors including the epidermal barrier perturbation characterizing this disease and the potent cytokines released by infiltrating leukocytes. Moreover, keratinocytes from AD patients synthesize exaggerated amounts of mediators involved in the enhanced recruitment as well as sustained survival and activation of dendritic cells and T cells. A dysregulated cytokine production by epithelial cells can be primarily involved in the pathophysiology of atopic diseases. The biochemical mechanisms underlying excessive production of pro-inflammatory mediators by epithelial cells are probably multiple. Experimental evidence indicates that AD keratinocytes have a constitutive dysregulated activity of specific constituents of AP-1 complex, critically implicated in the control of the expression of numerous inflammatory genes, suggesting the existence of genetically predetermined mechanisms targeting atopic inflammation to the skin. Data obtained by microarray technology are currently under investigation aiming to detect relevant keratinocyte-specific molecular markers of the disease. Inasmuch as epithelial cells are an easily accessible target for therapeutic intervention, studies on the mechanisms that regulate expression of inflammatory genes in epithelial cells may ultimately afford new strategies for the control of AD.

O 7

THE ROLE OF DENDRITIC CELLS IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) presents as a chronic relapsing skin disease with a characteristic phenotype and typically distributed skin lesions, which often make the diagnosis of AD very simple and clear-cut. In contrast, the underlying pathophysiological and genetic mechanisms leading to the manifestation of AD are often not obvious. Challenged by this puzzle, scientific approaches of the last years have made considerable progress in gaining insights into the complexity of the mechanisms causing AD. This biphasic inflammatory skin disease is characterized by an initial phase predominated by Th2 cytokines and a second and more chronic eczematous phase dominated by Th1. Two different dendritic cell subtypes bearing high-affinity receptors for IgE (Fc RI) have been identified in the skin of AD patients: Fc RI-high Langerhans cells (LC) and Fc RI high-inflammatory dendritic epidermal cells (IDEC). These two dendritic cell subtypes are supposed to contribute distinctly to the biphasic nature and the outcome of T-cell responses in AD. In contrast, plasmacytoid dendritic cells, which have only recently been shown to bear the high-affinity receptor for IgE and which play an important role in the defense against viral infections, are nearly absent from the skin lesions of AD patients. At present, it seems that different IgE-receptor bearing dendritic cell subtypes in the blood and the skin of AD patients play a pivotal role in the complex pathophysiological network of AD.

O 8

ATOPIC DERMATITIS CAN BE ALLERGIC – BUT WHEN?

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Abstract not received.

O 9

THE NATURAL HISTORY OF ATOPIC DERMATITIS

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Abstract not received.

O 10

INTRINSIC VS. EXTRINSIC ATOPIC DERMATITIS: DIAGNOSTIC DEFINITION AND PREVALENCE

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Our aim was to investigate the criteria for classification of patients with atopic dermatitis (AD) into those with the “intrinsic” vs. “extrinsic” form, and to evaluate the frequency of these to AD types among our population. We performed skin prick tests in 282 patients with AD, 117 men and 165 women. The skin prick tests consisted of 2 panels of allergens: panel 1 with 11 and panel 2 with 66 substances, respectively. We observed 171 (61%) positive responses to panel 1 and 189 (67%) positive reactions to panel 2, implying that a different percentage of our patients could be considered affected by an extrinsic form of AD, according to the number of tested allergens. Moreover, atopy patch tests with 20 aeroallergens and food allergens were performed in all patients. Among the subjects with negative prick tests (93 “intrinsic” cases), 68 reacted to atopy patch tests indicating a delayed “extrinsic” influence of allergens on the dermatitis. Thus, only 9% of our study population could be classified as affected by intrinsic AD on the basis of skin test results. Finally, in 18 subjects from the intrinsic group, we performed oral challenges with peanut, cow’s milk, and egg, and observed a positive response to egg in a single case – it was one more patient that shifted from the intrinsic to the extrinsic group. In conclusion, our data proved that the prevalence of the intrinsic form of AD might be overestimated in case of inadequate diagnostic work-up.

O 11

THE ROLE OF MAST CELLS IN ATOPIC DERMATITIS

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For a long time mast cells have been suspected to play a role in atopic dermatitis (AD), especially because approximately 80% of AD patients show increased concentrations of IgE and specific sensitization against food or aeroallergens. However, their role has been disputed since older antihistamines showed little effect on pruritus and skin symptoms of eczema in AD patients. Research then focused on the role of T-cells in the pathogenesis of AD, and in the last years cross-links between T-cells and mast cells have been described. It has been shown that mast cells produce a number of pro-inflammatory cytokines, such as interleukin-13, in skin lesions of AD patients. The secretion of these non-histamine mediators can be induced not only by IgE-cross-linking, but also by other non-IgE dependent stimuli. Furthermore, the close interaction of mast cells and sensory nerves has been described repeatedly. In conclusion, new evidence suggests that mast cells may play a significant role as one of the pathogenetic factors in atopic eczema.

2. CURRENT APPROACH TO ALLERGY

O 12

NEUROIMMUNOLOGICAL INTERACTIONS IN ATOPIC DERMATITIS – EVIDENCE FOR NEURODERMATITIS?

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Many recent studies have investigated immunological interactions in atopic dermatitis or neurodermitis. Influx of activated CD4+ T-lymphocytes and eosinophils and an increase in antigen-presenting Langerhans-cells, which are important players of inflammatory actions, represent a hallmark of lesional skin in atopic dermatitis (AD). Unlike in other allergic diseases, cytokines of the Th2 as well as Th1 type are important mediators in AD. In acute AD lesions, Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13 may be detected, whereas chronic lesions are characterized by a Th1-cytokine pattern, such as interferon (IFN)- . It is not clear whether the interaction of peripheral nerves and immune cells plays a role in AD. Characteristic features and strong pruritus in the acute and chronic recurrent inflammatory skin lesions strongly suggests that, from the clinical point of view, the activation of the nervous system must contribute to the pathophysiology of AD. The term “neurodermitis”, defined by Brocq at the end of the 19th century, had already suggested a neurogenic reason behind the pathophysiology of AD. Indeed,

the density of nerve fibers in subacute AD, as well as lichenified and prurigo lesions, are significantly more pronounced than in uninvolved skin. Moreover, the nerve diameter in AD patients is higher than in non-atopics. Bulging of axons with many mitochondria and a loss of their surrounding sheath of Schwann cells suggests that free nerve endings in skin lesions of AD are in an active state of excitation. Nerve hypertrophy and hyperplasia is frequently observed in AD, which is why the function of the neurotrophin nerve growth factor (NGF) is of special interest, as basal keratinocyte-derived NGF induces hypertrophy of peripheral nerve fibres. AD patients often suffer from severe pruritus. The modulators of pruritus are neuropeptides, such as substance P and a subpopulation of receptive nerve endings of unmyelinated C-fibers. NGF induces substance P production in sensory neurons. The role of peripheral nerves in local inflammation in AD is emphasized by the detection of neuropeptide-positive nerve fibers, containing substance P and calcitonine gene-related peptide (CGRP), especially in skin lesions. Recently, a decreased electronical perception threshold of skin nerves has been shown in AD patients. This hyper-responsiveness could very likely be the explanation for the nerve hyper-reactivity to unspecific stimuli, such as wool clothes, resulting in the development of pruritus and inflammation. Psychological stress, scratching, and unspecific stimuli often result in an aggravation of AD. As peripheral nerves, neurotrophins, and neuropeptides are increased in AD skin lesions, it is very likely that these neuronal components modulate local inflammation.

To summarize, neuronal aspects gain increasing interest and importance in the regulatory mechanisms of AD and present a broad spectrum of new therapeutic as well as investigative options. We, therefore, think that the old term “neurodermitis” characterizes better this chronic inflammatory skin disease.

O 13

NEW DEVELOPMENTS IN ATOPIC ECZEMA

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Atopic eczema affects approximately 10-20% of children and 1% of adults in western Europe. The disease is multifactorial and requires an integrated therapeutic approach taking into account its complex pathophysiology. New insights have been gained into the mechanism of inflammation in atopic dermatitis. The recruitment of leukocytes into the dermis is brought about by the interaction of chemokines and their corresponding receptors. Drugs aiming at chemotactic mechanisms are being developed. Major steps forward have been made in the therapeutic sector. UVA-1 irradiation has shown beneficial in severe cases. For intermediate to severe form of disease, topical tacrolimus can be regarded as a breakthrough. Another breakthrough has been achieved in the field of hand eczema, which was a major therapeutic problem. A controlled double-blind study showed allitretinoin (9-cis-retinoid acid) to be highly effective.

O 14

THE SIGNIFICANCE OF *MALASSEZIA FURFUR* IN ETIOPATHOGENESIS OF ATOPIC DERMATITIS

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The aim of the study was to determine the significance of *M. furfur* in the etiopathogenesis of atopic dermatitis. The study included 50 patients with atopic dermatitis, 50 patients with seborrheic dermatitis, and 30 healthy examinees as a control group. Immediate and contact hypersensitivity were determined by skin prick test and patch test, which were carried out with water-soluble extract of *M. furfur* (protein concentration of 5 mg/mL). Skin prick test to *M. furfur* was positive in 27 of 50 patients with atopic dermatitis, 9 of 50 patients with seborrheic dermatitis, and only in a single healthy control subject. Positive reactions to patch test to *M. furfur* were observed in 7 patients with atopic dermatitis. and in none of the patients with seborrheic dermatitis and healthy controls. Positive reactions to skin prick test were significantly more frequent among the patients with atopic dermatitis than in the other

two groups of examinees ($p<0.05$); the same result was obtained for patch test results. Present study proved the role of immediate and contact hypersensitivity to *M. furfur* in exacerbation of atopic dermatitis, but not in seborrheic dermatitis.

O 15

ANTIGEN-SPECIFICITIES AND FUNCTIONS OF T-LYMPHOCYTES IN ATOPIC ECZEMA/DERMATITIS SYNDROME

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T-lymphocytes represent the majority of skin-infiltrating cells in atopic eczema/dermatitis syndrome (AEDS). When stained with immunohistochemical techniques, the mononuclear infiltrate in lesional skin of AEDS is similar to that of allergic contact dermatitis, ie, a T-cell mediated allergic skin disease characterized by a delayed type hypersensitivity reaction. CD4+ T-helper cells dominate the cellular infiltrate and many intralesional T-cells show signs of activation, as defined by the membrane expression of interleukin (IL)-2R and HLA-DR molecules. Patients suffering from this disease also have increased concentrations of activated circulating T-cells, L-selectin, and the secretory IL-2R, which are the markers of lymphocyte activation and correlate with disease severity. The number of CD4+ cells is increased, and CD8+ suppressor/cytotoxic lymphocytes are decreased in peripheral blood. However, psychological stress has recently been shown to lead to significantly higher increases in the number of circulating CD8+ T-lymphocytes in AD patients compared to healthy controls. The majority of allergen-specific T cells derived from skin lesions that had been provoked in patients with AEDS by epicutaneous application of inhalant allergens was found to produce predominantly Th2 cytokines, such as IL-4 or IL-5. This was considered to be a specific feature reflecting immune dysregulation in AD. However, polarized type 2 cytokine pattern is confined to atopy patch test sites since allergen-specific T cells in the blood and in chronic skin lesions of the same patients secrete both type 1 and type 2 cytokines. The type 1 cytokine interferon (IFN)- seems to be particularly important for the perpetuation of the cutaneous inflammatory reaction. Besides inhalant allergens, foods are well-established trigger factors of atopic dermatitis. The studies into the role of food-specific T-lymphocytes in these patients have shown that, by use of limiting dilution cultures, allergen-specific T cells represent only a minority (1-5%) of infiltrating T-cells in lesional skin. Therefore, other factors leading to the activation of T-cells at the site of inflammation (e.g., autoallergens, bacterial cell wall components, and exotoxines) are probably involved in the pathogenesis of AEDS.

O 16

MECHANISM OF ATOPIC ECZEMA

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Abstract not received.

O 17

MICROMORPHOLOGY OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) has a typical spectrum of acute, subacute, and chronic phases as seen in some other spongiotic (eczematous) processes. Biopsy, although rarely used to diagnose atopic dermatitis, may yield findings indistinguishable from those seen in nummular dermatitis and allergic contact dermatitis. The diagnosis of atopic dermatitis is made on the basis of a constellation of major and minor clinical features. Depending on the clinical stage of the disease, either acute spongiotic dermatitis or more chronic process with reactive epidermal changes may be found. Acute lesions show spongiosis, some spongiotic vesiculation, moderate intracellular edema, exocytosis of lymphocytes, a perivascular infiltrate of lymphocytes and macro-

phages around vessels of the superficial plexus, and occasional eosinophils. Subacute lesions show irregular acanthosis with possible epidermal psoriasiform hyperplasia. With increasing chronicity of the lesions, the changes of rubbing and scratching become more and the spongiosis less obvious. Chronic lesions show hyperkeratosis, moderate to pronounced psoriasiform hyperplasia, and variable but usually only mild spongiosis. Mast cells are now significantly increased in the superficial perivascular infiltrate. Small vessels appear prominent due to an increase in their number and thickening of their walls. Langerhans cells are increased in both the epidermis and dermis. With further lichenification of the lesions, there is prominent hyperkeratosis and some vertical streaking of collagen in the papillary dermis – the changes recognized as lichen simplex chronicus.

O 18

THE ROLE OF AIRBORNE ALLERGENS IN THE ETIOPATHOGENESIS OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic and recurrent inflammatory skin disease, which affects approximately 12-24% of the European population. In about 10-20% of all cases moderate or severe clinical course is observed. Up to 60% of patients with AD may develop atopic respiratory disorders. During the past 40 years, we have seen a pronounced increase in the prevalence of AD in the industrialized world and research has largely been directed towards the etiology of this disease. Genetic involvement is an important factor that may predispose certain individuals to develop AD. However, more data on the role of genetics in the development of AD is required. Patients with AD show an aberrant T-cell-mediated immune response, increased serum IgE concentrations, and eosinophilia. Th2-related cytokines release predominates in the acute phase and Th1 lymphocytes predominate in the chronic phase of the disease. The early onset of AD suggests that the environment may be of importance for the development of the disease. It seems that particularly exposure to the early environment, defined as the time from conception of the child until the end of the third year of life, may be crucial. Factors in our environment that may influence the development and expression of AD are related to allergen exposure – airborne and dietary allergens, infections, vaccinations, and others. Allergen exposure results in an increased risk of sensitization and in approximately 50-80% of patients with AD, we are able to prove an IgE-mediated type of allergy. Airborne allergy seems to be of special importance in cases of AD patients, both adults and children. Such patients tend to present with severe course of the disease and atopic involvement of respiratory system. Airborne allergens, such as house dust mites, plant pollen allergens, moulds, animal-derived allergens, and bacterial allergens, may be responsible for exacerbations of skin inflammation in AD patients on the basis of an IgE-mediated contact reaction. Our knowledge on this phenomenon is still incomplete, but from the clinical point of view, we should diagnose patients with AD towards airborne IgE-mediated allergy in order to propose the most appropriate type of treatment.

O 19

SERUM EOSINOPHIL CATIONIC PROTEIN (ECP) IN CHILDREN WITH ATOPIC DERMATITIS

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Eosinophil cationic protein (ECP) is a cytotoxic agent secreted by activated eosinophils during allergic and inflammatory processes. The main aim of the study was to determine the existence of correlation between ECP serum concentrations and clinical severity (evaluated by SCORAD index), as well as some relevant laboratory findings, in children with atopic dermatitis (AD). The study included 70 children, 49 with AD aged 3-36 months, and 21

non-atopic children of the same age as a control group. Detailed history, serum ECP concentrations (UniCAP FEIA), relative and absolute eosinophil counts, and total serum IgE antibodies were determined in both groups. The skin involvement was measured by SCORAD index in AD children. The calculated SCORAD index was between 16 and 83. IgE antibodies, relative and absolute eosinophil counts showed a wider range of values and a significantly higher median ($p<0.001$) in the AD patients than in the control group. The serum ECP median concentration in children with AD was 16.2 mg/L compared with 5.92 mg/L in the control group. The correlation of SCORAD index and serum ECP concentrations was negative, weak ($r= -0.0655$), and not significant. The same was found for the correlation between serum ECP and intensity of skin changes ($r= -0.0952$) and subjective symptoms ($r= -0.0451$). For serum ECP and extent of skin lesions the correlation was positive ($r=0.0795$), but not significantly. Although the correlation between ECP and SCORAD index was not determined in this study, this laboratory findings as well as IgE antibodies and eosinophils should be monitored as they represent an additional, non-invasive laboratory method in the evaluation, follow up, and treatment of patients with AD.

O 20

OVERVIEW ON ETIOPATHOGENESIS OF ATOPIC DERMATITIS

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In the etiopathogenesis of atopic dermatitis (AD), there are well known interactions among genetic, environmental, skin barrier, pharmacologic, stress and other emotional problems and immune factors. Genetic determinate of expression of AD, as a pure or mixed with concomitant respiratory or intestine allergy, depends on genetic susceptibility. Although many genes are involved in the development of allergic diseases, there has been a particular interest in chromosome 5q31-33, 3q21, 1q21, and 17q25. Immunologic abnormalities of type I and type IV reactions have been described in patients with AD. Immunologic triggers are aeroallergens, food allergens, microbial products, autoallergens, and contact allergens. The immunologic reactions determinate many features of AD, as well as a cell-mediated or delayed hypersensitivity. Type 2 (TH2) and type 1 (TH1) cytokines contribute to the pathogenesis of skin inflammation in AD. The currently accepted model proposes a predominant TH2 cytokine milieu in the initiating stages or acute lesions of AD and a mixed TH1 and TH2 pattern in chronic lesions. This biphasic pattern of T-cell activation has been demonstrated after epicutaneous application of aeroallergens by use of patch test. The increased expression of interleukin (IL)-4 mRNA and protein, IL-5, and IL-13 may be observed after 24 hours, when IL-4 expression starts to decline to basic levels. In contrast, interferon (IFN)- mRNA is strongly expressed at the 48-72 h time points, as development of TH1 lymphocytes is mediated by the cytokines IFN- and IL-2. Type 2 cytokines are thought to account for coincident eosinophilia and increase IgE production and attraction of macrophages, which in turn produce IL-12, a known activator of the TH1-type immune response suppressing IgE production. Thus, AD skin contains an increased number of IgE-bearing Langerhans cells (LC) that, *via* the high-affinity IgE receptor FC R1, are thought to bind allergens, as a major IgE-binding receptor. LC play an important role in cutaneous allergens presentation to TH2 cells *via* major histocompatibility molecules. These bridgings trigger a cascade of immunologic events. In chronic AD lesions, LC are present in increased numbers and have increased amounts of IgE bound to high-affinity surface receptors. LC have been shown to be hyperstimulatory to helper T cells and can activate helper T cells to the TH2 phenotype in the initiating phase of the disease. Degranulation of eosinophils occurs in the dermis with the release of toxic proteins like major basic proteins and could account for the inflammation. Mast cells are increased in number and produce mediators other than histamine that induce pruritus, and may have an effect on IFN- expression. Mast cell cytokines IL-4 and IL-13 are important for IgE production and mast cell chymase may induce eosinophil infiltration into AD lesional skin. The production of prosta-

glandin E2 (PGE2) by peripheral monocytes is increased. Prostaglandin E2 (PGE2) has at least two potential roles in the initiation of AD: it reduces IFN- production from T helper cells thereby favoring the initial predominantly TH2 immune response, and it directly enhances IgE production by B lymphocytes by increased secretion of IL-4, IL-5, and IL-13. As many lesions of AD result from scratching, it is tempting to speculate that immune perturbations in genetically predisposed individuals provoke the release of local pruritogens and keratinocyte-derived cytokines further exacerbating the previously described immune response.

3. IMMUNOLOGIC BASIS OF ATOPIC DERMATITIS

O 21

ASSOCIATION BETWEEN CD30 EXPRESSION AND ATOPIC DERMATITIS

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Atopic dermatitis (AD) is chronic pruritic inflammatory skin disorder characterized by several clinical, immunological, and biochemical alterations. Atopic dermatitis lesions contain TH2-like cells and TH2 cytokines. It has been recently reported that CD30, a 120 kDa membrane-bound glycoprotein, is an activation marker of T-cell clones, showing a TH2-related cytokine pattern of production. We investigated the presence of CD30+ cells in the lesional skin of patients with atopic dermatitis. We obtained 30 biopsy specimens (3-4 mm punch biopsy) from various skin regions of 15 AD patients (7 women and 7 men) and 15 volunteers with healthy skin (12 women and 3 men). The immunohistochemistry peroxidase-antiperoxidase method with monoclonal antibodies was used for the analysis of paraffin-embedded skin section biopsies, as well as anti-CD30, anti CD45RO^{+}, anti-CD3, and anti-CD4 dilutions of antibody sera (Multi-link Swine anti Goat-Mouse-Rabbit Immunoglobulins biotimylated, Dako, Copenhagen, Denmark). The number of CD30+ cells in the whole biopsy tissue (x40) was counted. A semiquantative grading was used. In all biopsy specimens from patients with acute AD, most infiltrating cells were CD3+ T cells. In AD patients, high CD30 expression was observed in a remarkable proportion of infiltrating cells. In the healthy skin specimens, CD30+ cells were very rare. Average CD30 expression was significantly higher in AD patients than in controls ($p<0.01$). Our analysis of CD30, CD3, and CD4 cells in skin biopsies of AD patients showed that CD30 expression in AD might be helpful in histologic differentiation of AD, which is in accordance with the results obtained by Caproni *et al* (1997) and Dumner *et al* (1998). The results suggested a specific regulatory function of CD30+ T cells in acute atopic dermatitis lesions.

O 22

FINE MAPPING OF ATOD2 IN ITALIAN NUCLEAR FAMILIES

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by itchy inflamed skin associated with cutaneous erythema, and severe pruritus. The disease mainly starts in early childhood. AD is a multifactorial disease triggered by both genetic and environmental risk factors and twin studies indicate that the genetic contribution is substantial. Many genome-wide linkage studies mapped a number of susceptibility regions on chromosomes 1q21 (ATOD2), 3q21 (ATOD1), 5q31-q33

(ATOD6), 13q12-q14 (ATOD5), 17q25 (ATOD4), and 20p (ATOD3). Three of these loci (1q21, 17q25, and 20p) are closely coincident with psoriasis susceptibility loci, although AD is quite distinct from psoriasis and rarely the two diseases occur together in the same patient. AD is likely to be influenced by the same genes that regulate the dermal responses to environmental factors (like in psoriasis), independently from atopic mechanisms. In the last years, we mapped the psoriasis susceptibility locus (PSORS4) on chromosome 1q21 and performed a fine mapping of PSORS4. In this study, we adopted the linkage disequilibrium approach to narrow the PSORS4 locus to refine the ATOD2 susceptibility locus. We recruited 100 nuclear families originating from different Italian regions and genotyped them for markers previously shown to have linkage disequilibrium to psoriasis. Since preliminary results do not seem to show close overlap between PSORS4 and ATOD2 fine-mapped susceptibility locus, we believe that the clinic homogeneity of AD could lead to a better refinement of the common susceptibility locus on chromosome 1q21. In fact, it should be kept in mind that recent studies have shown five overlapping chromosomal regions (1q21, 2q33, 5q31.1–q33.1, and 6p21, 11q13) that co-localize with disease loci for the following diseases: diabetes, asthma, atopic dermatitis, osteoporosis, and inflammatory bowel disease (IBD). It is most likely that the same predisposing genes are involved in different complex diseases. Finally, we are focusing on association studies approach based on maps of single nucleotide polymorphisms to achieve the exact localization of ATOD2.

O 23

IMMUNOLOGIC FACTORS INVOLVED IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) is an allergic skin disorder that occurs in individuals with atopy. It is characterized by immune disorders of humoral (type I) and cellular (type IV) immunity. The aim of this study was to evaluate immunologic factors in the blood and the skin lesions of AD patients, and to analyze the presence of inflammatory cell-surface markers in the blood and skin biopsies. The parameters for monitoring 40 patients with AD included results of cutaneous tests: prick and intradermal test to inhalatory and nutritive allergens, scratch test to preservatives and additives, and epicutaneous (patch) test to contact allergens; values of total IgE, serum immunoglobulins (IgG, IgA, and IgM), and different cell markers in the sera (CD3, CD4, CD8, CD20, CD21, CD23, and HLA-DR). We also analyzed the presence of inflammatory cell-surface markers (CD3, CD4, CD8, CD20, CD20, CD1a, CD23, CD29, CD45Ro, and interferon [IFN]- + markers) in the biopsies of skin lesions from 10 AD patients and 5 healthy controls by immunohistochemical analysis (method of avidin-biotin immunoperoxidase). The results showed mostly positive skin tests (prick 9/13, intradermal 24/27, scratch 23/40, and patch 26/40 tests), high values of IgE (29/40), and mainly normal values of IgG, IgA, IgM in AD patients. In contrast to healthy controls, AD patients had significantly increased CD23+cells and significantly decreased CD21+ cells ($p<0.05$). Statistically significant difference was found between the two groups for higher expression of CD3, CD21, CD23 in the blood, for CD3, CD4, CD8, CD29, CD45Ro, IFN- + markers in the skin, and intraepidermal CD23+ and intradermal CD1a+ cells in AD patients. We concluded that different immunological factors in the peripheral blood and within the skin in AD patients might be mediated by both type I and IV hypersensitivity mechanisms.

4. DIAGNOSTIC STATE OF THE ART IN ATOPIC DERMATITIS

O 24

DIAGNOSIS OF FOOD ALLERGY IN INFANTS WITH ATOPIC ECZEMA USING SKIN PRICK AND PATCH TESTS

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The prevalence of atopic diseases is increasing worldwide. Food allergies are the earliest manifestation of atopy. Atopic eczema affects about 18% of infants in the first two years of life and the main cause is allergy to multiple foods. A strong association has been shown between atopic eczema and IgE-mediated allergy to milk, egg or peanut, but more than two-thirds of patients intolerant to food proteins show no evidence of IgE sensitization to the relevant food protein. Recently, patch testing with proteins has been found to be helpful in diagnosing food allergy in cases where skin prick tests (SPT) and estimation of specific IgE-antibodies have failed. The methodology of atopy patch testing (APT) with foods has not been standardized, and contradictory results have been reported. In contrast to the more standardized APT methodology with aeroallergens, the sensitivities and specificities of food allergens can easily be estimated with food challenge tests. In multi-allergic children, performing APTs in addition to the SPTs and estimation of specific IgE antibodies gives more information for planning of a sufficiently wide elimination diet to get the skin and gastrointestinal tract free of symptoms in order to perform the challenge test, which remains the only reliable test for food allergy. Hill *et al* (1999) introduced the term "multiple food protein intolerance of infancy", whereby more than two-thirds of their patients intolerant to food proteins showed no evidence of IgE sensitization to the relevant food protein. Therefore, new diagnostic methods are needed since challenging with every suspected food is not possible. At the Tampere University Hospital, all infants under two years with atopic eczema and/or gastrointestinal disorders are tested on a regular basis with SPT for milk, egg, soy, pea, fish, natural rubber latex, potato, banana, hazelnut, mustard, cat, birch pollen, wheat, barley, rye, oat, rice, corn, millet, and buckwheat. The APT includes milk powder, lyophilized egg white, soy flour, wheat, barley, rye, and oat with microcristalline cellulose as a negative control. Total IgE and the major specific IgE-antibodies are also estimated. Open oral challenge tests of one-week duration are routinely made with milk and wheat. Small cohorts analyzed from our material (Majamaa *et al*; 1998, 1999) give a sensitivity of 14% and a specificity of 98% for milk SPT and 44% and 71% for APT, respectively. In infants positive to wheat challenge the corresponding numbers with SPT were 23% and 100%, with APT 86% and 35%. Niggemann (2000) and Strömberg (2002) obtained better results for sensitivity and specificity using APTs for children with atopic eczema. Boissieu et al (2003) also reported similar results for studying cow's milk allergy with digestive symptoms. Testing with prick-prick method for sensitization of fruit and vegetables has given much relevant information in small children. Testing with raw potato gave positive SPT results in 15 out of 146 children under two years in 2000, and in 12 out of 123 in 2001 at our clinic. Eczema symptoms were induced by cooked potato in some these children (Majamaa *et al*, 2001). It can be concluded that in children with atopic eczema and/or gastrointestinal symptoms, allergy to foods is the most common cause of skin symptoms, allergy to animal dander being the next most common cause. Development of new and better skin and serum tests and their routine use in infancy can be recommended to detect all possible allergens.

O 25

RESULTS OF PATCH AND PRICK TESTING WITH EGG, MILK AND PEANUT IN PATIENTS WITH ATOPIC DERMATITIS

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At the Department of Dermatology in Modena, 251 patients with atopic dermatitis (AD) of mean age of 12.6 years underwent patch and prick testing with egg, cow's milk, and peanut. Positive responses to atopy patch tests were observed in 24%, 11%, and 19% of cases, respectively, whereas skin prick tests proved positive in 10%, 7%, and 12% of patients. Relevance of positive skin tests responses was assessed by repeated open challenges with egg, cow's milk, and peanut, performed in 112, 103, and 132 cases, respectively. Sensitivity values of atopy patch tests for the identification of food allergic AD patients ranged from 44% (milk) to 75% (peanut), whereas specificity values varied between 82% (egg) and 91% (milk). As regards skin prick tests, sensitivity ranged from 19% (milk) to 48% (egg) and specificity from 90% (peanut) to 95% (both egg and milk). Whereas specificity fig-

ures were high for both prick and patch tests, we observed lower sensitivity for the former. From our findings it is advisable to include atopy patch tests with food allergens in the routine diagnostic work-up of AD patients.

O 26

USE OF CAST-ELISA TEST IN THE DIAGNOSIS OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a common, inflammatory skin disease that affects 10-20% of the population and usually represents the first manifestation of atopy in infancy. Although allergen-specific T-cells and immunoglobulin E (IgE) are involved in the clinical responses to inhalant and food allergens, identification of relevant allergens is usually very difficult. In this study, we investigated the diagnostic potential of cellular antigen stimulation test (CAST) in the diagnosis of hypersensitivity reactions to inhalant and food allergens in patients with atopic dermatitis. CAST-ELISA is based on the determination of sulfidoleukotrienes release by reactive cells after allergen-specific stimulation in vitro. We studied 28 patients with allergic reaction to the following allergens: Der p, Der f, grass polen, milk, egg, and soya. We found that 18 out of 26 patients had positive CAST to Der p, 10 out of 16 patients to Der f, 7 out of 7 to grass pollen, 4 out of 17 to milk, 7 out of 18 to egg, and 4 out of 10 to soya. Our data showed that CAST-ELISA was more sensitive for diagnosing hypersensitivity reaction to inhalant than to food allergens in patients with AD.

O 27

HYPERSENSITIVITY TO FOOD ADDITIVES IN ADULT ATOPIC PATIENTS

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Additives and preservatives added to food can be broadly separated according to their functions, but a single additive may perform a variety of defined functions. There are thousands of agents that are added to food we consume. These include preservatives, stabilizers, conditioners, colorings, flavorings, sweeteners, antioxidants, and many others. Food diaries and avoidance of some problem foods may be helpful in avoiding exacerbation of allergic reaction. Only a small number of them can induce a wide range of adverse reactions in sensitive individuals (patients with atopy, urticaria, angioedema, rhinoconjunctivitis, asthma, or gastrointestinal disturbances), as well as in some "healthy" individuals. Our study included 123 adult (48 men and 75 women) patients with atopic dermatitis. Scratch test to food additives and preservatives was performed in all 123 patients. Food preservatives and additives panel included acetylsalicylic acid, sodium benzoate, potassium metabisulfite, tartrazine, citric acid, sodium glutamate, and glutaraldehyde. Fifty-three (43%) patients had positive skin tests to at least one compound, whereas 70 (57%) were negative. Most patients were positive to sodium benzoate (24%), acetylsalicylic acid (20%), and glutaraldehyde (14%), whereas a smaller percentage of patients were positive to potassium metabisulfite (11%), citric acid (7%), and sodium glutamate (7%). The lowest percentage of patients was sensitive to tartrazine (3%). Nowadays, the sensitivity to food additives is becoming more and more frequent. We suggest testing of all suspected individuals, particularly patients with atopy to food additives and preservatives.

5. PREVENTION OF TRIGGER FACTORS IN ATOPIC DERMATITIS

O 28

PUBLIC AND PROFESSIONAL EDUCATION PROGRAMS ON ATOPIC DERMATITIS: A COMMUNITY-BASED APPROACH TO PREVENTION AND REDUCTION OF SEVERITY

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Community-based epidemiological studies on the frequency of atopic dermatitis reveal that the disease frequency is high, particularly in young children, in Australia. Studies reporting on the morbidity and cost of having atopic dermatitis in Australia also show that the disease causes considerable morbidity and considerable cost to those affected and those responsible for their care. There is a lack of understanding of the disease amongst the community and particularly on what simple measures might be of value in prevention of the disease, particularly at a time when the disease expression is mild. For these reasons, community-based education programs were developed in Australia for the professionals seeing young children. These include a program for Maternal and Child Health Nurses staffing baby health centers for children from birth up to the age of four; a Primary Schools Education Program for teachers of children aged 4-12 years; and a Secondary Schools Education Program for the teachers of children aged 12-18 years. Each of these Programs includes a component on atopic dermatitis; the nature of the disease; what may precipitate it; how it may be prevented; and how it may be treated, if treatment is necessary. An Education Program for community pharmacists was also developed and tested in the community pharmacy setting. It gave similar details of the nature of atopic dermatitis; the treatment that might be required; and the preventive approach that may be of value in those affected. Previous studies have shown that parents of children with atopic dermatitis and patients with atopic dermatitis frequently seek advice from pharmacists in Australia. A user-specific website has been developed to accompany the education programs with Information Sheets for the professionals as well as for the community. These have been translated into 12 community languages, apart from English, including Croatian. Preliminary analysis indicates that the education programs have been taken up well and are being used appropriately in the community setting.

O 29

QUALITY OF LIFE AND PSYCHOLOGICAL SYMPTOMS IN PATIENTS WITH ATOPIC DERMATITIS

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Atopic dermatitis (AD), with its variable clinical presentations and course, constitutes a syndrome composed of an identifiable group of signs and symptoms that represents the dermatological manifestation of atopic diathesis. It is characterized by severe pruritus and a chronically relapsing course. AD is one of the most frequent skin disorders with a presumed psychosomatic factor. The disease does not only cause physical discomfort, but can also have impairing consequences for the personal and social life and daily functioning of the patients. AD may have significant effects on quality of life, interfering with sleep, school activities, behavior, sports participation, vacations, and interactions with other children. It is a chronic disease, visible to others, and often causes embarrassment and discomfort. Family relationships may be compromised; parents often become frustrated by their child's discomfort caused by itching, the chronic course of the disease, and the lack of sleep. Adolescents and adults with AD report changes in self-image and social relationships, financial concerns, and occupational difficulties. The psychological impact of the disease on the patients leading to depression, anxiety, and frustration may cause reduced itch threshold and increase the inherent irritability of the skin, thus contributing to the pathogenesis of the disease. Psychological factors play a role in triggering and maintaining the lesions, while emotional aspects also have a strong impact on the itch-scratch cycle. Itching can be assumed as the central aspect associated with AD and provokes extreme psychological distress. Itch perception and consecutive scratching behavior are modulated by psychological factors. Patients with AD consider pruritus as the symptom that affects their health-related quality of life the most. Neuropeptides may be involved in the pathogenesis of AD. Proteases, kinins, prostaglandins, neuropeptides, acetylcholine, cytokines, and opioids can cause itch. Psychological stress also has been implicated as a major contributor to disease development. Stressful events often have been observed to occur before an AD exacerbation.

IMPACT OF AIRBORNE POLLEN, TEMPERATURE, AND HUMIDITY ON SEVERITY OF ATOPIC DERMATITIS IN CHILDREN

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Abstract not received.

O 33

ITCHING IN ATOPIC DERMATITIS

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Itching is defined as an unpleasant primary cutaneous sensation, which leads to extensive scratching. It is the major symptom in patients suffering from atopic dermatitis (AD). Itch is an important diagnostic feature of AD, included almost in all scoring methods for severity assessment of this disease. Itch is not only a symptom of AD, the itch-scratch cycle usually aggravates skin lesions in these patients. Although itching is present in every AD individual, the clinical features of this symptom have rarely been studied and the pathogenesis is not completely understood. Therefore, the aim of this talk is to give an overview of clinical and pathogenetical aspects of pruritus in AD patients. In more than three-quarters of AD patients itching appears on daily basis. It seems to be most common during night, causing sleep disturbances, and least frequent in the morning. The intensity of itching varies among patients; it is a very bothersome symptom resulting in lowering quality of life and even depression. The intensity of pruritus in AD is usually lower than in psoriatic patients. All the skin areas could be involved, lower extremities being the most commonly affected. The pathogenesis is probably multifactorial with several factors involved with documented or hypothesized roles. Histamine does not seem to play a major role. In recent literature, attention has been given to the increased release of several neuropeptides (substance P and CGRP), opioid peptides, disturbances in tryptase and chymase activity, and cytokines (IL-2). Moreover, xerosis, decreased itch threshold, prolonged itch duration to pruritogenic stimuli, as well as multiple triggering factors with stress could be of great importance in the pathogenesis of itch in AD. In conclusion, although many studies have been performed on itch in AD, further research is required to clarify the exact pathomechanism of this symptom in AD individuals.

O 34

TREATMENT OF PRURITUS IN ATOPIC DERMATITIS

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Atopic dermatitis is a common chronic inflammatory itching skin disease. The onset of atopic dermatitis frequently occurs in childhood and can have a broad impact on patient socioeconomic status. Its etiopathogenesis is still not completely understood, but genetic, environmental, humoral, and cellular immunologic factors have a role. Pruritus is one of the major diagnostic criteria of the disease. It is an unpleasant and frustrating cutaneous symptom, provoking the desire to scratch oneself. The intensity is hard to measure but can be distressing and lead to sleep disturbance. The cellular and molecular bases of pruritus in atopic dermatitis are not clear. Besides an increase in some inflammatory cells, mast cells are also present in the skin. Relevant mediators of pruritus are histamine, proteases, neurotransmitters (substance P and CGRP), cytokines, and arachidonic derivates (prostaglandin E2 and leukotriens). Soaps, detergents, or low humidity can exacerbate pruritus in atopic dermatitis. The treatment of this troublesome symptom may sometimes present a challenge for the physician. The treatment can be topical (emollients, corticosteroids, doxepin cream, or capsaicin) and systemic based on the use of sedating antihistamines, immunomodulators (cyclosporin A), phototherapy, or photochemotherapy. The patients must

avoid excessive bathing, wool fabrics, and alcoholic beverages.

6. ATOPIC DERMATITIS AND OTHER DISEASES

O 35

ATOPIC DERMATITIS – RESEMBLANCE WITH OTHER COMMON FACE SKIN CHANGES

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Atopic dermatitis on the face is chronic pruritic, clinically variable skin disease associated with atopy. Clinical features vary greatly with age of the patient. In young patients, the changes are quite acute with erythema, blisters, and crusts, whereas later on more chronic changes, including lichenification, are predominating. A single constant symptom seems to be pruritus, resulting in patients rubbing and scratching their skin. Patients with atopic dermatitis on the face have very dry skin, which is often made worse by almost any cleansing agent or even just frequent exposure to water. In adult patients, skin changes appear mostly with red patches on eyelids, forehead, perioral region, nape, and neck, often following emotional stress. The skin acquires a gray-yellow color making the patient appear older and sadder. Postinflammatory hyperpigmentation is common, most frequently seen around the eyes, and often associated with increased skin folds. While atopic dermatitis in infancy and childhood is not a diagnostic problem, it can be in older patients. Atopic dermatitis can resemble other common facial skin changes, such as seborrheic dermatitis, contact dermatitis (allergic or irritant), nummular eczema, psoriasis vulgaris, ichthyoses, and cutaneous T-cell lymphoma. Atopic dermatitis can also be complicated with bacterial (*Staphyloccocus aureus*), viral, and fungal (*Pityrosporum ovale*) infections that can modify a clinical picture. One of the serious problems is the abuse or uncontrolled application of topical steroids. In example, one of our patients, a 55-year-old woman with atopic dermatitis from early infancy, applied topical steroids for 25 years on her face. Lately she has developed heavy contact allergic dermatitis with positive patch test on several allergens.

O 36

IS THERE ANY ASSOCIATION BETWEEN PSORIASIS AND ATOPIC DISORDES?

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The aim of this study was to determine the prevalence of allergic disorders, especially atopic dermatitis (AD), among patients with psoriasis vulgaris (PV). In Osijek University Hospital, Department of Dermatology, we examined 60 patients aged 0-90 years of both sexes who were diagnosed with psoriasis vulgaris. The patients with atopic disorders were selected on the basis of medical history, questionnaire on psoriasis vulgaris and atopic dermatitis, total IgE serum concentrations, and skin allergic tests. The results will be presented in the tables.

O 37

ATOPIC DERMATITIS AND EARLY ASTHMA ONSET

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The aim of study was to research influence of atopic dermatitis (AD) on asthma (A) onset in childhood. The study included 400 asthmatic children aged 1-18 years (mean±SD, 8.7±4.0) visiting outpatient clinic from 1998 to 1999. Boys comprised 63% of the patients. The first attack of airway obstruction was at the mean age of 3.6±3.4 years, with mean annual frequency of 7.0±6.6. Examinees were divided in four groups according to the association of asthma with AD and allergic rhinitis (AR): 1) only A; 2) A + AD; 3) A + AR; and 4) A + AR + AD. The groups did not significantly differ according to

the sex, family history of allergic disorders, breast feeding, annual frequency of attacks, and allergic parameters. But in the momentary age ($p<0.001$) and age of the first attack ($p<0.001$), the groups significantly differed (Kruskal-Wallis analysis of variances; explicitly by Mann-Whitney's test). The group A + AD was the youngest group (6.7±3.8 years) and significantly differed from groups A + AR and A + AR + AD. The A + AD group was the youngest (2.1±2.3 years) at the time of the first attack of airway obstruction, significantly younger specially from groups A + AR and A + AR + AD. In the same group, 80% of patients had the first attack at age under 3 years. Many factors, particularly atopic dermatitis, have a strong influence on early asthma onset in childhood. In group A + AD patients, atopic dermatitis appeared 1.5-2 years before the first symptoms of asthma. Thus, we may infer that early detection and treatment of atopic dermatitis can delay development of serious disease as asthma.

7. MANAGEMENT OF ATOPIC DERMATITIS

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TREATMENT STRATEGIES TO CONTROL THE ITCH

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Itch is a crucial diagnostic feature of atopic eczema (AE) and has substantial impact on the quality of life of patients. Itch perception has many psychophysiologic aspects. Instruments for qualitative and quantitative registration of these central nervous factors and evaluation of therapeutic measures are still under development. The itch receptors, recently confirmed by human microneurographic studies, are free endings of thin, unmyelinated, slow-conducting C-fibers in the upper skin. Apart from histamine, mast cell tryptase and to date unknown itch mediators are likely to play a role in pruritic diseases like AE. Studies using the "Eppendorf Itch Questionnaire" and skin reflex experiments showed that itch can only partially be quantified by a single scale. Clinical itch, but also itch in atopy patch test models in patients with AE needs an increasing number of descriptors with higher intensities. This can be interpreted as central nervous system (CNS) processing of a nociceptive sensation. A complex pattern of cortical activation after experimental histamine stimuli was observed in a H215O PET correlation study in healthy volunteers, involving motor areas, insula, and cingulate. These results suggest to combine topical treatment of clinical itch (unspecific and specific, physical and antiinflammatory) with therapies targeting the CNS and other systemic therapies. Improving the course of AE is based on a combination of appropriate topical antiinflammatory, antibacterial and rehydrating treatment, and patient education ("patient management"). The use of topical corticosteroids with improved risk/benefit ratio has led to significantly better results in the long-term management of AE. A new group of antiinflammatory and thus antipruritic drugs for topical use are calcineurin-inhibitors like tacrolimus and pimecrolimus. They inhibit the T cell-dependent inflammatory response and the release of cytokines without causing skin atrophy or obvious systemic side effects. Immunomodulatory approaches including allergen-specific immunotherapy, anti-IgE, soluble interleukin-4 receptor, and antibodies to adhesion molecules or costimulatory molecules may prove effective. In addition, new antagonists and synthesis inhibitors of mediators of inflammation will be developed in the future. New models to measure itch may be useful for the research on therapeutic strategies against pruritus in AE.

O 39

ALCLOMETHASON DIPROPIONATE IN THE TREATMENT OF ATOPIC DERMATITIS

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Topical corticosteroids still represent the first line treatment for patients with atopic dermatitis. Although their application carries the risk of local and systemic side effects, if prescribed by physicians fa-

miliar with these drugs, this kind of treatment can be highly effective as well as safe. Factors that need to be considered when prescribing topical corticosteroids are potency and base of corticosteroid preparation, age of the patient, site of the lesions, extent of eczema, and method of application. It is extremely important that the physician is familiar with a few preparations from each potency category. Some prefer to prescribe a short burst of a potent steroid followed by a steroid-free period. The majority, including us at the Department, start the treatment with a more potent preparation, taper down the potency as the eczema subsides, and use prolonged, if necessary continuous, treatment with a less-potent corticosteroids preparation, usually mild or moderate, to control the disease. Since the majority of patients with atopic dermatitis need prolonged treatment with corticosteroids to achieve good disease control and avoid side effects, a mild corticosteroid preparation, especially when treating children or sensitive body areas, is mandatory. Alclometasone dipropionate is a mild non-fluorinated synthetic corticosteroid that comes in a 0.05% cream and ointment. Many clinical trials in children and adults with atopic dermatitis treated with alclometasone cream and ointment showed better results than those obtained with hydrocortisone 1.0%, hydrocortisone butirate 0.1%, or desonide 0.05%. Comparable results to clobethasone butirate 0.05% were observed, as well as equal safety results compared with treatment with the mentioned corticosteroids. Untoward effects, including burning, itching, stinging, irritation or redness, occurred in only 4% of adult patients and 6% of treated children. In healthy adult volunteers treated with 30 g of alclometasone dipropionate cream 0.05% twice a day to 80% of the body BID for three weeks and under occlusion (plastic body suits) during 12 h, no clinically significant suppression of hypothalamus-pituitary gland-adrenal glad (HPA) axis was observed. Alclometasone has been present on the Croatian market as Afloderm cream and ointment (0.05%) for 15 years and prescribed to children and adults with atopic dermatitis. In addition to the original cream or ointment (Afloderm), it can also be used in a diluted form, i.e. 20%, 40%, or 60% of the original cream in an emollient (Belobaza). In our treatment of patients with atopic dermatitis, the concentration depends on the intensity of the eczema, site and extend of skin lesions, and age of the patient. The patients and parents are given detailed instruction regarding corticosteroids application and are asked to write a diary concerning any application of corticosteroids, body site on which it was applied, and the exact amount of preparations used since the last visit to our clinic. In the majority of patients, the disease can be controlled as mentioned. Rarely, a short application of a more potent corticosteroid is necessary.

Even in prolonged application of alclometasone in patients with atopic dermatitis, including infants, we did not observe either local or systemic side effects. Therefore, we consider alclometasone to be effective and safe in treatment of atopic dermatitis patients, even in children.

O 40

MODERN ASPECTS OF TOPIC STEROID THERAPY IN ATOPIC DERMATITIS

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In the last years, there has been a constant and significant increase in the number of children with the allergic diseases. The share of atopic dermatitis (AD) in pediatric allergic pathology is 50-75%. One of the standard principles in the treatment of AD is application of topic steroids. In this study, the effects of application of alclometasone (Afloderm cream; Belupo, Pharmaceuticals & Cosmetics Inc.) in patients with AD were assessed. There were two groups of patients: 35 children aged between 6 months and 3 years with “infantile form” of AD (group 1), and 27 children aged 4-7 years with “children’s form” of AD (group 2). Forty-four out of a total of 62 patients had a widespread disease, and 18 patients had the limited form of skin process. Afloderm was applied 2 times per day over 7-14 days. Significant positive dynamics of skin cellular inflammatory parameters was observed during the therapy in all children, with a pronounced decrease in the relative quantity of neutrophils and eosinophils in “skin window” samples. Clinical efficiency of Afloderm was estimated by using SCORAD index; there was a positive dynamics in all patients. The mean SCORAD index decreased in group 1 from 40.0±6.8 before the treatment to

12.2 ±1.4 after two-week treatment, and from 39.6±8.2 to 10.9±1.5 in group 2. There were no side effects, either systemic or topic, and Afloderm was well tolerated by all children.

O 41

EVALUATION OF A NEW SEQUENTIAL TREATMENT WITH FUSIDIC ACID IN ATOPIC DERMATITIS PATIENTS

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Microbial factors, especially *Staphylococcus aureus*, play an important role in the pathogenesis of atopic dermatitis (AD). Skin colonization with *S. aureus* is notably increased in AD patients and may trigger and/or exacerbate the inflammatory network of AD. An open study was carried out to evaluate the activity of an antibiotic-glucocorticoid treatment in mild to moderate AD. For this purpose, patients suffering from localized forms of AD, with signs suggestive of potential microbial superinfections were selected. Treatment consisted of two sequential phases as follows: application of fusidic acid associated with betamethasone valerate (phase 1), followed by the use of a combination of fusidic acid and hydrocortisone acetate (phase 2). Clinical evaluations were performed at baseline, after phase 1, and after phase 2. The severity of skin signs and pruritus was assessed using a 4-point semiquantitative scale. A total of 178 patients were enrolled; of these, 42 were adults, 87 were 3-15 years old, and 49 aged <3 years. At the baseline, AD was mild in 67% of cases and moderate in 33%. Treatment was well tolerated. A relevant and progressive improvement of AD signs and symptoms was noted throughout the treatment period: AD severity was notably reduced after phase 1 and further improved after the subsequent phase. The patients' or parents' opinion on efficacy and acceptability of the treatment was positive in most cases.

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WHAT`S NEW IN MANAGEMENT OF ATOPIC DERMATITIS?

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Atopic dermatitis (AD) is a chronic, relapsing skin disease. Complex interactions among genetic, environmental, skin barrier, pharmacologic, and immunologic factors contribute to the pathogenesis of AD. Therapy includes identification and elimination of triggering factors, topical therapy (cutaneous hydration, topical corticoid treatment, or topical immunomodulators), and systemic therapy. Systemic corticosteroids are most often used to control flares and should be reserved primarily for control of acute exacerbations. Cyclosporine, a macrolide agent, is an effective treatment for AD in adults as well as children. It is prescribed on a short-term basis for recalcitrant cases of AD, with a typically rapid, 2-3 week response that includes pronounced reduction of pruritus, and reduction of lichenification and extent and activity of dermatitis after 6-8 weeks of therapy. Relapses usually occur after treatment, and post treatment disease often does not return to baseline levels. Long-term treatment is also possible. Azathioprine, a purine analog that rapidly converts in vivo to 6-mercaptopurine, has a slow onset of action of about 4-6 weeks. In many reports there is evidence of a consistent, significant, and persistent reduction in disease activity sustained for at least 3-6 months. Methotrexate is safer on a long-term basis and its consideration as a steroid-sparing agent is justified. Phototherapy should be added to the methotrexate treatment regimen once the drug has effectively controlled the level of inflammation.

Mycophenolate mofetil is useful in dermatologic inflammatory diseases, but it is not approved for the treatment of AD yet. Recent studies showed notable improvement in subjects with moderate to severe AD. Interferon was applied in several open-label studies for AD and the statistically significant improvement was seen in excoriations/erosions and erythema in patients with the lowest serum IgE levels and blood eosinophil percentage at

the beginning of the study. Intravenous immunoglobulin (IVIG), phosphodiesterase inhibitors, thalidomide, and leukotriene inhibitors have been used in AD but their effectiveness has not been confirmed. Data from experimental studies provide structure, mechanism of action, efficacy, optimal doses, safety, clinical guidelines, costs, and perspective of broad use of these drugs and insight into possible future treatment methods.

O 43

MANAGEMENT OF ATOPIC DERMATITIS IN INFANCY

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Atopic dermatitis (AD) is a chronic, inflammatory, itchy dermatosis with high prevalence. It is characterized by onset at early age, with first symptoms appearing in infancy in over 50% of cases. Initial assessment of patients must include detailed medical history, determination of disease severity and potential trigger factors. The goal of treatment is to control the symptoms, not cure of the disease. The treatment consists of adjuvant basic therapy, identification and avoidance of trigger factors, and anti-inflammatory measures. Adjuvant basic therapy includes bathing and regular application of moisturizers, which reduce clinical signs of dryness, scaling, and roughness; decrease itch and tightness; improve skin barrier function; give the skin protection from environmental irritants and allergens; and represent a steroid sparing alternative. Environmental provocative factors, such as low humidity, exposure to hard water, house dust mites, mould, cat and dog dander, cigarette smoke or skin contact with wool, synthetics, and certain food should be avoided. Data on prevalence of food allergy in children with AD are quite variable (from 15-30%). Clinically relevant adverse reaction to food should be considered in patients with moderate to severe AD. In general, the more severe the clinical picture and the younger the patient, the more likely the symptoms of AD be aggravated by food hypersensitivity. To prove food allergy skin prick tests (SPT), determination of specific IgE antibodies and atopy patch tests (APT) with food are performed. Sensitivity of skin prick tests are excellent in determining food allergy and negative predictive accuracy is excellent (>95%). Specificity and positive predictive accuracy, though, are poor (<40%). Thus, a positive skin prick test cannot be considered a proof of food allergy but a negative test virtually rules out allergy to the tested antigen. The results of allergen-specific IgE antibody values determination are comparable to those of skin prick tests. Quantitative measurement of allergen-specific IgE may represent a step forward in the diagnosis of food allergy in AD. “Decision points” for the levels of specific IgE to some food have been determined as providing >95% confidence that the patient has food hypersensitivity. APT may be a valuable additional tool in diagnosing food hypersensitivity. Standardization of this test is still in progress. Positive results of previously mentioned tests are usually only suggestive of clinically relevant food hypersensitivity. Although double-blind placebo-controlled food challenge test (DBPCFC) is considered to be the gold standard for diagnosis of food allergy, it is time consuming and can be replaced with opened food challenge test in infants. When the diagnosis of food allergy is determined adequate diet is introduced. Evaluation of nutritional status is extremely important because inadequate diets can lead to malnutrition in infants.

Topical corticosteroids (CS) are still first-line therapy for AD. Low to mid-potency topical CS, applied twice a day, are usually efficient in control of inflammation. Once the activity of the disease is controlled, the goal is intermittent application or withdrawal of CS. The least potent preparation that adequately controls the disease should be selected. Potential local and systemic side effects limit their use and frequently lead to undertreatment, especially in infants and children. Use of topical immunomodulatory agents, such as tacrolimus and pimecrolimus, are still not approved for use in children less than two years of age. Oral antihistamines may diminish pruritus and facilitate sleep, especially those with sedative effect, given at bedtime. Topical and oral antibiotics can be helpful in cases with clinical evidence of *Staphylococcus aureus* infection.

O 44

THE LIGHT IN ATOPIC DERMATITIS TREATMENT

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Atopic dermatitis (AD) is a common, multifactorial, chronic, and often relapsing inflammatory skin disease. Current treatment of severe AD consists almost exclusively of topical and systemic corticosteroid therapy. Because long-term corticosteroid therapy is known to cause a variety of side effects, attempts have been made to develop alternative treatments, such as phototherapy with UVB (narrowband) and UVA irradiation. We investigated the application of UV light in the treatment of AD in childhood and treated 21 children (7 boys and 14 girls, mean age 11.5 years) with AD with a combination of UVA and UVB (UVAB) irradiation three or five times a week. All of them had active disease involving at least 40% of the body area despite the use of topical steroids, emollients, antihistamines, and antibiotics. Ten of these children had a positive family history of atopy. Six patients had coexisting hay fever. The entire body was irradiated including the face. The cumulative dose of UVB ranged from 1.3 to 10.4 J/cm^2 (mean dose, 6.14 J/cm^2). The cumulative dose of UVA ranged from 27.5 J/cm^2 to 182 J/cm^2. The number of treatments ranged from 9 to 47 (median, 18). Excellent response to UVAB therapy that resulted in almost complete disappearance of eczema and pruritus was observed in 9 out of 21 children. Good and moderate response to UVAB therapy was recorded in 5 and 7 patients, respectively. Only minor side effects, such as mild erythema, were observed in four patients. Based on these results, we concluded that UVA and UVB (UVAB) is a useful and acceptable method for treating mild to moderate atopic dermatitis in the childhood.

O 45

RUSSIAN EXPERIENCE OF MANAGING OF ATOPIC DERMATITIS PATIENTS

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There is no unified position paper on atopic dermatitis (AD) in the world. Mutual understanding between the physicians becomes rather complicated because of discrepancy between different schools about AD. Russian National AD Position Paper was created in 2002. It consists of 4 documents, Guidelines for Practitioners, Atlas of Topical Treatment and Skin Care, Diet therapy, and Pharmaceutical Guide, which unite coordinated positions about the subject of allergologists, dermatologists, and pediatricians. The managing protocol of AD patients proposed in the Paper was based on the world and national experience in management of AD patients. Our report will be devoted to Russian experience of managing of AD patients.

O 46

STAPHYLOCOCCUS AUREUS ALLERGOVACCINE FOR DIAGNOSTICS AND TREATMENT OF ATOPIC DERMATITIS

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Our aim was to investigate the efficacy of allergovaccination with *S. aureus* allergen in atopic dermatitis patients sensitized to S. aureus and suffering from recurrent pyoderma. For this purpose, we used allergovaccine prepared from *S. aureus* taken from the skin of AD patients. The vaccine was prepared with a method of culturing microorganisms on cellophane film. The patient group consisted of 22 patients suffering from AD complicated with recurrent pyoderma. In 76.6% of AD patients, specific IgE to *S. aureus* was detected by using UniCap 100 system, Pharmacia Upjohn. Skin testing with *S. aureus* allergovaccine (scarification and intradermal tests) was carried out in 22 AD patients with remission of AD pyoderma. In 9 of these patients, the test was positive. Specific immunotherapy with *S. aureus* allergovaccine was performed in 4 patients. The control group consisted of 6 pa-

tients with chronic furunculosis without atopy. Allergovaccine prepared from *S. aureus* can be used for diagnostics of *S. aureus* sensitization in AD patients suffering from recurrent pyoderma and for the treatment of these patients.

O 47

BASE CREAMS FOR THE PREVENTION AND TREATMENT OF ATOPIC DERMATITIS

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According to general considerations on corneotherapy (Albert M. Kligman), the chemical composition and the physical structure of base creams play a major role in supporting the homoeostasis of the skin. From the physiological point of view, membrane-forming ingredients should be preferred due to the compounds and the bilayer structure of intercellular lipids of the horny layer. Typical membrane forming agents are ceramides, cholesterol, fatty acids, and phospholipids. Among phospholipids, phosphatidylcholine (PC) is the most important starting material, because of its availability and different behavior with regard to chemically bonded fatty acids. Native PC is a source of linoleic acid. It fluidizes the skin barrier and supports ceramide I formation, whereas hydrogenated PC (PC-H) shows strong barrier protection activities. In this respect, definite ratios of PC and PC-H together with materials influencing the skin hydration and skin roughness are of special interest for preventing and treating atopic skin. Because of the lamellar structure of such systems, they behave like the skin, i.e., they are able to take up hydrophilic and lipophilic active agents at room temperature, an important prerequisite for their use as base creams. As a result, the base creams enable a modular system in combination with simple skin analysis procedures like corneometry, sebumetry, and tewametry. Furthermore there is no break between medical treatment and cosmetic prevention. Experiences from practice have shown a high efficacy and tolerance with regard to atopic dermatitis.

O 48

SOPHISTICATED SCIENTIFIC SKIN DIAGNOSTIC METHOD – CK ELECTRONIC GmbH – COLOGNE

M. Rogić

Abstract not received.

8. NEW DRUGS FOR ATOPIC DERMATITIS

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EFFICACY AND SAFETY CHALLENGE WITH PIMECROLIMUS

LECTURE SPONSORED BY NOVARTIS

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Abstract not received.

O 50

TACROLIMUS 0.1% IN THE TREATMENT OF ATOPIC ECZEMA: OUR EXPERIENCE

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Atopic eczema (AE) is a chronically relapsing inflammatory skin disease, which seriously affects the life quality. Tacrolimus is a non-steroid calcineurin inhibitor originally developed as a systemic immunosuppressant to be used in organ transplantation. We treated 9 patients (4 men and 5 women; mean age, 36.5 years) affected with moderate to severe AE with tacrolimus 0.1% according to the standard method of administration. After the evaluation of first results, we treated other 8 patients (4 men and 4 women; mean age, 28 years). Only 4 out of the first 12 patients completed the study, with only 2 of them showing good clearance of the skin. All 8 patients from the second group finished the treatment, with 6 of them showing a good response to the treatment with tacrolimus. These data point to

the necessity to give complete and exhaustive information to the patients to obtain their full compliance and consequently good results. In our experience, the main problem for this treatment seems to be, besides the relatively high cost, skin burning and pruritus, which were present in more than 70% of our patients, particularly at the beginning of the treatment.

O 51

COMBINED THERAPY IN ATOPIC DERMATITIS

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Topical corticosteroids have been the mainstay of treatment for atopic dermatitis. They induce several changes in skin metabolism leading to reduction of inflammation and itching, and can be used both in acute and chronic phases of the disease. Their mechanism of action is broad and complex, affecting several pathways of inflammation and gene expression. Due to their potential side effects and depending upon the clinical severity of the disease, their use should be limited to the acute phases and then replaced by other treatments. Low to mild potency steroids should be preferred. Pulse steroid therapy in association with emollients or barrier restructuring creams has been shown to be effective, safe and useful in keeping disease-free chronic patients. In particular, physiologic mixtures containing free fatty acids, cholesterol, and ceramides can penetrate a disrupted stratum corneum, reach the nucleated epidermal cell layers, and be incorporated into nascent lamellar bilayers of the stratum corneum interstices. Clinical trials of ceramide-dominant barrier repair moisturizer has been shown to reduce the severity scoring of atopic dermatitis, normalizing TEWL and improving stratum corneum integrity. Combination therapy of mild corticosteroids with physiologic lipid mixtures can be a useful, low cost, and safe approach to the long-term management of atopic dermatitis.

O 52

TOPICAL TACROLIMUS IS MORE EFFECTIVE THAN PIMECROLIMUS IN PEDIATRIC ATOPIC DERMATITIS PATIENTS

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The safety and efficacy of tacrolimus ointment and pimecrolimus cream in the treatment of pediatric patients with atopic dermatitis (AD) was compared in a multi-center randomized, 6-week investigator-blinded study. Patients (2-15 years of age) with moderate to severe AD were randomized 1:1 to apply either tacrolimus ointment 0.1% or pimecrolimus cream 1% twice daily as monotherapy. Other AD treatments were prohibited during the wash-out period and study. Study visits occurred at baseline and at weeks 1, 3, and 6/end of study (EOS). Efficacy parameters included the Eczema Area and Severity Index (EASI), Investigator's Global Atopic Dermatitis Assessment (IGADA), percent body surface area (% BSA) affected, and patient assessment of itch. Safety was evaluated based on adverse events (AEs) reported throughout the study, both cutaneous AEs and systemic AEs (study drug-related). A total of 198 patients were considered evaluable for safety and 193 evaluable for efficacy. Tacrolimus ointment showed statistically significant superiority to pimecrolimus cream by the change from baseline in EASI (-79.9% vs. -68.2%, respectively; $p<0.001$), the percent of patients clear or almost clear by IGADA at week 6/EOS (38.3% vs. 20.2%, respectively; $p=0.006$), change from baseline in % BSA affected (-17.0% vs. -10.0%, respectively; $p<0.001$), and change from baseline in patient assessment of itch (-3.4 vs. -1.7, respectively; $p=0.001$). Adverse events occurred at less than an 11% incidence rate for any individual event. There were no statistically significant differences with regard to any AEs, including skin burning/stinging ($p=0.49$) between the two treatment groups. The results showed that tacrolimus ointment was more effective than pimecrolimus cream with a similar safety profile in pediatric patients with atopic dermatitis.

O 53

SPECIFIC IMMUNOTHERAPY IN THE TREATMENT OF PATIENTS WITH ATOPIC DERMATITIS: RESULTS OF DOUBLE BLIND PLACEBO CONTROLLED TRIAL

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We investigated 20 patients (15 women and 5 men) with atopic dermatitis (AD), aged 5-40 years, who were allergic to house dust mites or grass pollen allergens. Specific immunotherapy was performed with Novo Helisen Depot allergy vaccines of appropriate composition for the time period of 12 months. Placebo for our trial was supplied by ALLERGOPHARMA pharmaceutical company. In the treatment of 14 patients allergic to house dust mites, Novo Helisen Depot allergy vaccines composed of *Dermatophagoides pteronyssinus* 50% and *Dermatophagoides farinae* 50% were used (7 patients – an active vaccine; 7 patients – placebo). Six patients allergic to grass pollen allergen were treated with vaccines composed of 100% grass pollen extract (3 patients – an active vaccine; 3 patients – placebo). After 12 months of treatment, we observed a significant improvement of the clinical status of patients (based on W-AZS index) treated with an active vaccine ($p<0.01$). We also recorded a significant difference in the severity of AD between an active and placebo group after one year of therapy ($p<0.01$). In our trial, we monitored selected allergological (t IgE, as IgE, ECP) and immunological parameters (IL-4, IL-5, IL-10, sIL-2R, and IFN-). Our conclusion was that specific immunotherapy may be an effective method in the treatment of selected patients with atopic dermatitis and airborne allergy.

O 54

SPECIFIC IMMUNOTHERAPY IN ATOPIC DERMATITIS PATIENTS – OUR EXPERIENCE

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Specific immunotherapy (hyposensitization) is in practice based on repeated subcutaneous or oral (swallow or sublingual) application of specific allergens (with increasing concentrations). These allergens responsible for the allergic symptoms in patients are used in immunotherapy until the maintained dose is obtained or symptoms disappear. The mechanism of immunotherapy in atopic dermatitis patients is based on decreasing of specific IgE antibodies, and at the same time increasing of specific IgG. We have diagnosed patients with pure atopic dermatitis (AD) and mixture AD patients with concomitant allergy: allergic rhinitis (AR), allergic rhinoconjunctivitis (AC), and allergic bronchitis (AB). All patients were sensitive to inhalant allergens proved by in vivo and in vitro tests. During 1985-2003 period, there were 218 atopic patients in the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center, Croatia, included in the study. All patients were skin prick tested (SPT) according the European standard and White paper 2003. Patients with immunodeficiency, immunoinflammatory disease, malignancies, nonstabile asthma, cardiovascular manifestations, children under five years, elderly patients, and during pregnancy and lactation were excluded from the study. The allergens used for hyposensitization were Dermatophagoides pteronyssinus, house dust mite (together or separately), pollen mixtures (grass, tree, weed), or single allergens (Cocksfoot, Timothy, Meadow grass; Birch, Hazel tree; and Ragweed, Mugwort weed) from Institute of Immunology Zagreb; Bencard; Allergopharm; or Stallergeéns. For the control of usefulness of specific immunotherapy, values of immunoglobulins, especially total and specific IgE values were used. For total and specific IgE determination, we used Pharmacia UniCAP system FEIA and Upjohn reagents (kU/l). The concentration of the specific IgE (Pharmacia UniCAP system FEIA, and Upjohn reagents) were 0-6 kUA/L. All 218 atopic patients (AP) underwent specific subcutaneous immunotherapy for two or three years. In four of AR patients, good results were also achieved with sublingual immunotherapy. Three of 218 patients

had adverse reaction (worsening of the atopic disease) during one year of immunotherapy, and withdrew from the study. All 215 atopic patients showed improvement. Improvement was also observed in high percentage of other patients: 77 patients with AD, 29 with combination of AD and AR, 13 with AC and 12 with AB. After 19 years of experience with specific immunotherapy in the Allergy Clinic, we had very good results in atopic patients by using inhalant allergens. The best results were obtained by using grass pollen mixture, house dust mite (*Dermatophagoides pteronyssinus*), and Ragweed (*Ambrosia elatior*) as allergens. The highest level of improvement was seen in patients with AD+AR or AD+AC. Specific immunotherapy in our 215 patients was successful according to clinical findings and specific IgE results. Patients who have coexistence of AD with mucosal disease had better outcome then the patients with pure AD.

O 55

NAPHTHALAN IN THE TREATMENT OF PATIENTS WITH ATOPIC DERMATITIS

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In the last two years we have made several preliminary reports about our experience in the treatment of atopic dermatitis with naphthalan. As our research is coming to an end, we report the results of the therapy of 20 patients. We believe that this report is even more valuable since the follow-up is now 2 years. In this research, we included 20 patients aged 15 and above, who had different clinical forms of atopic dermatitis. Naphthalan therapy was applied for 3 weeks. During that period the patients were taking baths in tubs containing Naphthalan oil (temperature 34-38°C) once a day for 12-14 minutes. This is a common therapy applied in the last 15 years in the treatment of patients with psoriasis and rheumatic diseases. Besides naphthalan baths, patients were also applying neutral cream and oral antihistamines. The methods for the evaluation of the therapeutic results were as follows: 1) evaluation of skin changes; a) photographs taken before and after the 3-week therapy; b) SCORAD index (European expert group for atopic dermatitis) before and after the 3-week therapy; 2) skin biopsy before and after the 3-week therapy; 3) total IgE; and 4) hematological and biochemical tests before and after the 3-week therapy. On the basis of our results, we may say that naphthalan was a good treatment option in 70% of patients with atopic dermatitis. This kind of therapy can be recommended in the treatment of atopic dermatitis for adults as well as for children. The efficiency of the naphtalan therapy was evaluated according to SCORAD index (75.4 before and 30.9 after the 3-week therapy), photo documentation (remission of skin changes after the 3-week therapy) and histopathological examination (significant reduction of inflammatory infiltration as well as the reduction of acanthosis in about 2/3 of the examined patients). Standard laboratory analysis did not detect pathologic changes, which could perhaps suggest nephrotoxicity, hematotoxicitiy or hepatotoxicity of the naphthalan oil.

POSTERS

P 1

REPRODUCIBILITY OF ATOPY PATCH TESTS WITH AEROALLERGENS AND FOOD ALLERGENS

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Atopy patch tests (APT) are believed to be a useful diagnostic procedure for atopic dermatitis (AD), but their reproducibility has been poorly investigated so far. We investigated APT reproducibility by simultaneously applying two identical patch test series in 85 patients with AD. The test substances included two commercially available materials (Dermatophagoides mix 20% pet. and Alternaria alternata 2.4% pet.) and 8 self-made food preparations containing egg yolk, egg white, peanut, cow's milk, soy milk, wheat flour, rice, and corn meal. The percentage of agreement in the frequency of positive reactions ranged from 87% (corn

meal) to 100% (soymilk). The reproducibility was satisfactory for Dermatophagoides mix, Alternaria alternata, egg yolk, soymilk, and peanuts. In conclusion, for commercial allergens, such as Dermatophagoides and Alternaria alternata, the agreement rate was similar to that of standard patch tests and, therefore, APTs may be considered sufficiently reproducible to be used as a diagnostic testing procedure. As regards food materials, such as egg yolk, peanuts, and soymilk, APT reproducibility may be acceptable. For other foodstuffs, preparation methods should be improved.

P 2

ATOPY PATCH TESTS WITH DERMATOPHAGOIDES IN PATIENTS WITHOUT ATOPIC DERMATITIS

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So far the issue of atopy patch tests (APTs) in subjects not affected by atopic dermatitis (AD) has been investigated by few authors with contrasting results. Our aim was to evaluate the frequency and intensity of APT responses to mite allergens in non-AD subjects in comparison with AD patients. APTs were performed employing a mix of *Dermatophagoides pteronyssinus/farinae* on 75 non-AD subjects, including 33 patients with allergic rhinitis and/or asthma and 42 healthy volunteers, and on 210 AD patients. Positive reactions were observed in 23% of non-AD subjects and in 49% of AD ones. The former showed responses of lower intensity (mean score, 1.4) than AD patients (mean score, 2.1). The frequency of positive reactions to house dust mites proved significantly higher in the subgroup of healthy volunteers than in patients with respiratory symptoms. In conclusion, positive responses to APTs with Dermatophagoides are observable also in subjects not affected by AD, but their frequency and intensity are significantly lower in comparison with AD patients. Delayed skin reactivity in non-AD subjects may be regarded as equivalent to the finding of specific IgE by skin prick tests or RAST in healthy subjects, indicating an atopic state. For the occurrence of both, the induction and the elicitation of the dermatitis other skin factors should probably be concomitant.

P 3

A CASE OF ATOPIC DERMATITIS WITH SUDDEN APPEARANCE OF ACQUIRED ICHTHYOSIS

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Acquired ichthyosis is not inherited and occurs for the first time in adulthood. It is usually associated with some general systemic disease, such as cancer (lung, breast, ovary, or cervix), Hodgkin disease, lymphoma, sarcoidosis, Kaposi sarcoma, leprosy. thyroid disease, nutrition disorders, or HIV infection. It may be provoked by certain medications, such as nicotinic acid, hydroxyurea, cholesterol-lowering agent, or cimetidine clofazimine. A 25-year-old men without past history of skin disease was admitted to our Department. Two months before, he had noticed hyperkeratosis on his palms and soles with a painful fissuring. Gradually, his skin became dry with symmetrical lamellar hyperpigmented scaling, particularly on the extensor surfaces of the extremities and lateral aspects of his trunk and back. At birth his skin appeared normal. Nobody in the family had a history of atopic diseases. Laboratory results revealed low concentration of vitamin A in the serum and increased concentration of IgE. Histological findings confirmed vulgar ichthyosis associated with atopic dermatitis. Prick test was positive to many inhalant allergens. Internal diseases and malignancies were excluded. He was treated with local keratolitics and neutral ointments together with vitamin A preparations. Acquired ichthyosis is extremely rare. When it is suddenly presented in adult patient, associated internal diseases should be excluded. Recognition of these conditions is necessary to institute therapy that will reduce discomfort experienced by affected individuals.

P 4

PREVENTIVE MEASUREMENT OF pH – VALUE IN COSMETIC DERMATITIS PATIENTS

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Alpha hydroxy acids (AHAs) diminish the cohesion between corneocytes at lower stratum corneum layers, affecting the intercellular ionic bonds. According to E. J. Van Scott, the weakening of cohesive intercellular forces happens via three mechanisms: hydratization; reduction of electronegative ionic groups in the cells; and changing the medium between charges leading to the dissolution of desmosomes in the intercellular space. There are visible effects of AHA-based cosmetic products on rejuvenation of skin after long-term usage. For cosmetic products based on AHA, the pH value of the skin is one of the most important indicators of their efficacy, since their mechanism of action has a direct influence on the pH changes at the epidermal cell level. We measured skin pH changes in 120 subjects aged between 19 and 66 years. The pH was determined on the left and right cheek before the application of a cream, and then at 30 minutes, 4 weeks, and 8 weeks after application of a particular cream. Skin pH was determined by electrometric method in vivo by using a digital pH meter equipped with a Mettler "single pore flat" electrode. Measurement points were cheek skin surface of 9 cm^2 near the left and right ear. Environmental conditions were also measured: temperature as 21°C, and humidity 42% on the first, 60% on second, and 50% on third and fourth measurements. Subject response was 42% (50 out of 120 patients). The placebo cream (A) and AHA cream with pH 2.9 (B), pH 3.6 (C), and pH 5.1 (D) were tested. Skin pH for the cream A (pH 6.1) changed on average from 0.29 to -0.18 and -0,15; for the cream B (pH 2.9) it changed from –0.36 to .0.11, and from -0.32 to -0.46. For the cream C (pH 3.6), pH changed from -0.12 to -0.20 and from -0.18 to –0.23, and for the cream D (pH 5.1) from 0.26 to 0.33, and 0.30 to –0.07. More acid AHA-based creams with lower pH of about three causes a greater decrease in skin pH than the creams with pH higher than five, which do not contain AHAs in the form of free acid, or at all. The improvement of the method of electrometric measurement of skin surface pH in vivo will allow a quick control of the skin pH decrease, which is the basic prerequisite for the efficacy of AHA-based cosmetic products. It is to be expected that further research into all the mechanisms of action of AHAs will help towards establishing these substances as more than a "passing fashion", i.e., as a true contribution of our decade to the cosmetics of 21st century.

P 5

CONCOMITANT RESPIRATORY ALLERGY IN ATOPIC DERMATTIS

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Previous studies showed that similar mechanisms were involved in the pathogenesis of atopic diseases, e.g., allergic bronchial asthma (AB), allergic rhinoconjunctivitis (AR), and atopic dermatitis (AD). Because of the similarity of these diseases, there is a proposal to distinguish three main subtypes of AD: "mixed" type with concomitant respiratory allergy (RA); "extrinsic" type of "pure" AD without RA, but with allergen-specific IgE; and "intrinsic" type without immediate type sensitizations. The aim of this study was to determine the difference of test results between patients with "pure" AD (n=10) and "mixed" AD (n=20). There were 30 AD patients investigated. The parameters for monitoring were personal and family history; cutaneous tests (skin prick, scratch, and patch test), and values of total serum immunoglobulin E according standard European procedures. The results showed the age of onset was mostly in the early infancy – 15 patients had developed the disease while under the age of 2: 2 out of 10 developed "pure" AD, and 13 out of 20 developed "mixed" AD. Twenty patients had a history of RA ("mixed" AD), whereas the remaining 10 had "pure" AD. Seventeen AD patients also had one allergic disease, whereas 3 had two conditions (AR and AB). Family history was positive for atopy

in 22 AD patients (14 patients in a first-degree relative). Twenty-four patients had positive prick tests: 9 out of 10 with “pure” AD, and 15 out of 20 with “mixed” AD, mostly to house dust (n=20). Scratch test reactions were positive in 4 out of 10 patients with “pure” AD and 12 out of 20 with “mixed” AD. Patch test reactions were positive in 5 patients with “pure” AD and in 15 with “mixed” AD. Twenty-one out of 30 AD patients had higher serum IgE (7 out of 10 with “pure” AD and 14 out of 20 with “mixed” AD), but the concentrations were similar in both “pure” and “mixed” AD. No statistically significant differences between the “pure” and “mixed” AD patient groups were found, except for the age at onset, which was lower in the group of patients with concomitant RA. The results of our study did not show any differences in the allergologic parameters and the status between AD patients with concomitant RA and those without RA.

P 6

CLINICAL SAFETY OF PHOTOTHERAPY AND PIMECROLIMUS CREAM IN ATOPIC DERMATITIS PATIENTS

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We investigated the safety of phototherapy (280-400 nm) and pimecrolimus cream 1% (Elidel) in atopic dermatitis patients. The patient group consisted of 36 patients (13 male and 23 female) aged 7-25 years, who suffered from AD. Patients were randomized 1:1 to receive either pimecrolimus cream 1% or phototherapy. Pimecrolimus cream was applied daily until the signs and symptoms were resolved. Phototherapy was used during 20 days according to the standard method, i.e., 2 courses with 3-month interval in-between. The patients treated with pimecrolimus cream did not develop pyoderma or herpes simplex during the observation. In the group of patients undergoing phototherapy, there were 3 cases of pyoderma and one case of herpes simplex during the observation period. Long application of Elidel cream and phototherapy are the methods of treating mild and moderate atopic dermatitis. In this study, Elidel cream application proved to be safer then phototherapy.

P 7

CELL-MEDIATED AUTOIMMUNE REACTIONS BETWEEN SKIN-HOMING T-LYMFOCYTES AND PERIPHERAL MONONUCLEAR BLOOD CELLS IN PATIENTS WITH ATOPIC DERMATITIS

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Atopic Dermatitis (AD) is dominated by skin-homing T-lymphocytes. The aim of this study was to describe if there are any interactions in an autologous system between T-lymphocytes in the skin and peripheral mononuclear blood cells (PBMC), i.e. signs of a cell-mediated autoimmune reaction. T cell lines were obtained from skin biopsies from clinically affected skin of patients with AD. The biopsies were placed in RPMI-1640 with 10% human AB serum, interleukin (IL)-2 and IL-4, but without feeder cells, antigen, or mitogen. After four weeks, T-cell lines (9-12 cell doublings) were present in the media. Four weeks after the biopsies were taken and placed in growth medium, 30 mL heparinized blood was drawn from the patients. PBMC was isolated with Lymphoprep®. T-lymphocytes from the culture or PBMCs were treated with Mitomycin-C and mixed in 96-well plates with non-Mitomycin-C treated culture T-lymphocytes or PBMC. After 6 days, a proliferation assay was performed by using Cell Titer 96® Aqueous One Solution Cell Proliferation Assay, recording absorbance of 490 nm, and a 96-well plate reader. PBMC mixed with Mitomycin-C treated culture T-lymphocytes showed a significant higher proliferation rate than PBMC without culture T-lymphocytes. The results of the study indicated there was an interaction between PBMC and T-lymphocytes from the skin. This interaction could be an autoimmune reaction, a finding that may open up new perspectives for the understanding and the treatment of AD.

P 8

SERUM IgE LEVELS IN CONTACT ALLERGIC DERMATITIS

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Several correlations have been found between serum IgE concentrations and clinical findings in atopic dermatitis (AD). Observations suggest that the pathogenic increase in IgE may not be causal. Increased serum IgE concentrations are not unique to AD and have been documented in patients with contact allergic dermatitis (CAD), dyshidrotic eczema, and psoriasis. Most results suggest that increased serum IgE concentration is a secondary phenomenon rather than a primary causative factor. The aim of this study was to determine IgE serum concentrations in CAD, according to his duration. Patients (N=122) with CAD were divided into three groups according to the duration of the disease: ill for one year, 1-5 years, and over 5 years. In all of them the diagnosis of CAD was confirmed with clinical findings and epicutaneous patch test. Serum concentrations of IgE (single radial immunodiffusion) were determined and compared with 58 control values in healthy persons. No correlation between duration of CAD and serum IgE concentrations was found. Serum concentrations of IgE in patients with CAD and healthy persons were not significantly different.

P 9

CONTACT HYPERSENSITIVITY IN ATOPIC DERMATITIS PATIENTS

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Atopic dermatitis (AD) is a common chronic inflammatory disease, which generally begins in early infancy. Symptoms include rashes, vesicles, and itches, and are characteristic of the disease. Family history of atopy has confirmed that AD must have a distinct genetic background. Studies support the concept of an immunological basis of humoral and cell-mediated immune dysfunctions. An equal or higher occurrence of contact allergy in atopic than in nonatopic subjects has been reported. During 1998-2003 period, patch testing was carried out in 65 patients (31% men and 69% omen). Twenty-six patients, 7 men and 19 women, showed positive reactions to one or more allergens. Allergic reactions were more frequently found in women. The most common allergen was nickel (33%), followed by cobalt (11%), fragrance mix (11%), Hg pp albi (8%), and other allergens (36%). The association between nickel allergy and cobalt allergy was significant. We have concluded that contact hypersensitivity is not rare in patients with atopic dermatitis.

P 10

SPECIFIC IMMUNOTHERAPY OF ATOPIC DISEASES-OUR EXPIRIENCES

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Atopy is a very complex disease often accompanied by high IgE concentrations, respiratory, and cutaneous disorders. Genetic factor has an important role in appearance of the disease. We show our experience with hyposensibilization in atopic diseases. From January 1996 to January 2004, we treated 160 patients with atopic disorders: allergic rhinitis (n=60), atopic dermatitis (n=20), urticaria and edema angioneuroticum (n=36), asthma bronchiale (n=32), and allergic conjunctivitis (n=12). We used standardized solution of allergens made by Immunological Institute in Zagreb, Croatia, and treated patients by standard scheme, although sometimes it was necessary to adapt the dose individually. Patients for immunotherapy were chosen upon the results of intradermal testing and concentrations of total IgE. The most frequent allergens for immunotherapy were mixed house dust mite, dermatophagoides, mixed pollens, single pollens, plumage, animal dander, and pelt. Degree of success in specific immunotherapy was estimated according to the patient's judgment of subjective problems by using a 5-level scale (5 – very severe, 4 – severe, 3 – moderate, 2 – mild, and 1 – very mild).

The best results were achieved in patients suffering from atopic rhinitis, followed by those with asthma bronchiale and atopic dermatitis. During applications of specific immunotherapy, we noted four cases of generalized urticaria with bronchial obstruction and two cases of urticaria with abdominal colic.

P 11

ARE INCREASED CONCENTRATIONS OF EOSINOPHIL CATIONIC PROTEIN IN THE SERA OF CHILDREN WITH ATOPIC DERMATITIS MARKERS OF INFLAMMATION SEVERITY?

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Eosinophilia accompanies different inflammatory disorders, including atopic dermatitis. Eosinophils and their toxic products can be found in the inflamed tissue, e.g. in asthma, where their granule products seem to be major causes of the destruction of epithelial cells in the airways. In activated eosinophils, eosinophil cationic protein (ECP) is present in increased concentrations. The aim of our study was to determine the ECP values in children with atopic dermatitis and compare them with SCORAD index. Fluoroenzymeimmunoassay Uni-CAP ECP system (Pharmacia Diagnostics, Uppsala, Sweden) was used for in vitro quantitative measurement of ECP in the sera of 80 children with atopic dermatitis aged 1-7 years. Uni-CAP ECP assay was performed on a Uni-CAP 100 instrument (2.5 mL or 4 mL vacutainer hemogard and SST glass tube). The assay is based on the reaction between anti-ECP, covalently coupled to ImmunoCAP, and the ECP in the serum. After washing, enzyme-labeled antibodies against ECP are added to form a complex. After incubation, unbound enzyme-anti-ECP is washed out and the bound complex incubated with a developing agent. The fluorescence of the eluate measured after the reaction stops is transformed into the concentration by use of a calibration curve. Referent values of ECP concentration in the sera were 20 g/L, and SCORAD index was calculated according to the ETAC study group. ECP concentrations in the sera of 80 children ranged between 3.25 g/L and 75.70 g/L. Increased ECP concentrations were found in the sera of 25 children (range, 20.8-75.7 g/L; average, 35.7 g/L). The highest ECP concentrations were measured in children with SCORAD index between 55 and 60. Increased ECP concentrations in the sera of 25 children with atopic dermatitis indicated the presence of activated eosinophils. Determination of released ECP may a way to monitor diseases involving eosinophil activation and the effect of therapy.

Marko Polo´s Diary

Stella Fatović-Ferenčić, ESHDV representative of Croatia

On Art Nouveau esthetics, and on the relation of medicine and art – Reflections on the "Secession in Croatia" exhibition in the Museum of Arts and Crafts, Zagreb

This time I did not board an airplane, cross the borders, and leave the country. Yet, the visit to the Museum of Arts and Crafts made me equally excited, and left me with the extraordinary feeling of a travel throughout time, a fascination with beauty, which only the relation with art can create. The exhibition of *Secession in Croatia* in the Museum of Arts and Crafts in Zagreb was an impressive and extraordinary event, where more than a thousand art objects were displayed, representing one of the most exuberant and innovative of all styles. Since 1897, when a group of artists in Vienna gave birth to another form of modernism in the visual arts by formulating the *Secession,* the influence of this new esthetics of design quickly spread throughout the Europe (under different names, such as *Modern Style, Jugendstil, Liberty Style, Stile Floreal, Art Nouveau,* or *El modernismo*.), including Croatia. The diversity of paintings, sculptures, graphically designed objects, architecture, ceramics and glass, as well as furniture, fashion, theater, music, and all other aspects of life depicted an impressive landscape of harmony, admiration, and nostalgia. Every segment of the exhibition was represented by the most prominent masterpieces from Croatian artists, paitners Vlaho Bukovac, Bela Čikoš Sesija, Mirko Rački, Robert Frangeš, and Ivan Meštrović, and architects Rudolf Lubynski, Aladar Barany, Ignjat Fischer, Dionis Sunko, and many others. The lifestyle of that period was also presented through objects imported from other cities of *Jugendstil*, e.g., the furniture of Joseph Hoffmann and glass objects of Louis Comfort Tiffany, showing in detail the awareness that every single artist had for the "total work of art". The field of history of medicine, pharmacy, and dentistry was presented by the architecture of the prominent figures of the period, such as Feller, Pečić, and the dentist Rado. Eugen Viktor Feller (1871-1936) was known for his famous specialty production *Elsa fluid.* At the turn of the 19th century, he became one of the most successful pharmacists and entrepreneurs of the period, whose investments in Zagreb's architecture were significant. Among the most popular buildings of that time was the four-story *Elsa fluid house* erected in 1905/6 at the corner of Jurišićeva Street and Jelačić square. This edifice represented a design of the most prominent and productive architectural bureau of the period. A huge bottle paced in the south-west part where the two main fronts of the building meet represented *Elsa fluid* and became a trademark of the first big pharmaceutical company in Zagreb. Evidently, *Secession* was deeply present in all aspect of human life, and medicine was not an exception. Suffice it to remember Gustav Klimt (1862-1918), one of the founders of *Art Nouveau* in Vienna and his three allegorical images completed for the large amphitheatre at the University of Vienna. Among those paintings was also *Medicine,* first presented in 1901, which showed the power-

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lessness of the art of healing instead of the triumph of medicine. The issue was so severely criticized and attacked that it even reached the parliament. Withdrawing from work for the university, Klimt believed that no person or institution had the right to limit the artistic freedom or ruin the artistic integrity. This stimulated him further to form *The Vienna Secession*.

The connection between art and medicine is getting stronger and recently, it has been introduced even into medical curricula throughout the world by efforts of medical humanities. Filled with beauty my eyes had seen in the Museum, I thought that perhaps the best way to educate ourselves and learn about the world around us, including medicine, is through visual arts. This could create a sense for beauty and ability to focus on elements we usually oversee in the mass of data, as well as enable us to preserve and recall them and give them importance and space in our lives, for our own and our patients' benefit.

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The world is waiting, sretan vam put !

stella@hazu.hr

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UPCOMING MEDICAL MEETINGS

Continuing Medical Education Course

UPDATE ON PSORIASIS

Zagreb, Croatia

October 15-16, 2004

The Continuing Medical Education Course is organized by the Chair of Dermatovenerology, Zagreb University School of Medicine and Department of Dermatology and Venerology, under the auspices of **Academy of Medical Sciences of Croatia**.

Known experts in the field of psoriasis will also participate in the Course.

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Prof. Jasna Lipozenčić, MD, PhD

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Book Review

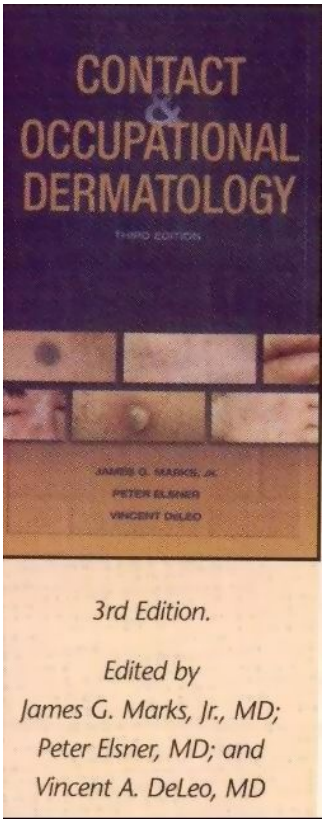
Marks JG Jr, Elsner P, DeLeo VA, editors. Contact and Occupational Dermatology. 3rd edition. St. Louis (MO): Mosby; 2002. Pages: 431. Price: US$134.80 ISBN: 0323014739

The third edition of Contact and Occupational Dermatology, edited by Drs. Marks, Elsner, and DeLeo, is intended for clinicians to use it in their everyday practice when evaluating patients with occupational dermatoses. Practicing dermatologists and physicians may only benefit from brief, informative, user-friendly text, free of unnecessary details and excessive (and often daunting) chemical nomenclature, which they can read with ease. This book is just what they are looking for. The classification of occupations has different levels. This book provides practitioners and occupational health care professionals with a scientific tool to develop codes of practice in exposure to chemical agents.

The book begins with an overview of the traditional pathophysiology, mechanisms, and immunology of contact allergic and/or irritant dermatitis, and continues with evaluation; history taking; examination; patch test technique; reading, recording, and interpreting the results; and instructions for patients how to avoid allergens.

Various allergens are presented in Chapters 5-11. Tables that accompany allergen descriptions are easy-to-consult and very practical. Dermatologists performing a patch test know how difficult it is to find a detailed protocol for photo-patch testing, and one can find it in this book. Besides clarifying protocols and tables, there is a useful and easy-to-read guide to patch testing and patient care.

The condensed chapters on occupational skin diseases cover most of the relevant professions/occupations and summarize a great deal of information in an accessible and well-organized way. The reader can only benefit from a generous number of tables, figures, and illustrations, highlighted information of special importance, and a list of accepted norms regarding occupational dermatology.



For practicing general dermatologists who daily see in their office more than a few patients with contact dermatitis, this book is an excellent choice as it would not only enrich the library of every dermatologist but also find its use in everyday practice.

Aida Pašić, MD, PhD

Prof. Jasna Lipozenčić, MD, PhD

ANNOUNCEMENTS

Update on Atopic Eczema/Dermatitis Syndrome, Cavtat, Croatia, April 25-28, 2004. Contact: Prof. Jasna Lipozenčić, Department of Dermatology and Venerology, Zagreb University Hospital Center, Šalata 4, 10000 Zagreb; Contact: *jasna.lipozencic@zg.tel.hr*; *www.cybermed.hr/4dermkh*

Second EADV International Spring Symposium, Budapest, Hungary, April 29-May 1, 2004. Contact: *info@eadvbudapest2004.com*; *www.eadvbudapest2004.com*

66th Annual Meeting of the Society for Investigative Dermatology, St. Louis, Missouri, May 4-7, 2004. Contact: Kate Rader, Meetings Manager, 820 W. Superior Avenue, 7th Floor, Cleveland; *Krader@sidnet.org*

31st Annual Joint Meeting of Society for Cutaneous Ultrastructure Research and European Society for Dermatopathology, Rome, Italy, May 6-8, 2004. Contact: *www.prex.it/congressi/scur/index.html*

9th International Congress of Dermatology, Beijing, China, May 19-22, 2004. Contact: ICD2004 Secretariat, International Department, Chinese Medical Association, 42 Dongsi Xidajie, Beijing 100710, China; *ICD2004@chinamed.com.cn*; *www.chinamed.com.cn/dermatology*

International Symposium "Frontiers in Allergy and Autoimmunity", Mainz, Germany, May 21-22, 2004. Contact: *Anja.Oberlaender@uni-mainz.de*

15th Ljudevit Jurak International Symposium on Comparative Pathology; Main Topic: Head & Neck Pathology, Zagreb, Croatia, June 4-5, 2003. Contact: *www.kbsm.hr/jurak/symposium.htm*

Slovak Dermatovenerological Congress with International Participation, Bratislava, Slovak Republic, June 4-6, 2004. Contact: *scretarysma@ba.telecom.sk*

7th Congress of the European Society of Contact Dermatitis, Copenhagen, Denmark, June 6-8, 2004. Contact: Organizing Secretariat PREXY S.r.l. – Vialle Monza 20125 Milano, Italy; *Liss@ics.dk*; *congressi@prex.it*, *www.iscd2004.info*

23rd Congress of the European Academy of Allergology and Clinical Immunology, Amsterdam, Netherlands, June 12-16, 2004. Contact: Dept. Allergology, University Hospital Rotterdam, dr. Molewaterplein 40, NL-3015 GD Rotterdam, The Netherlands; *degroot@algo.azr.nl*; *www.congrex.com/eaaci2004*

28th Annual Meeting of the Israel Society of Dermatology and Venereology, Eilat, Israel, June 16-17, 2004. Contact: Prof. Sarah Brenner, The Tel Aviv Sourasky Medical Center, Weizman Street, Tel Aviv 64239, Israel; tel: 972 3 6974287; fax: 972 3 6974810

10th Congress of the European Confederation of Medical Mycology, June 17-20, 2004, Warsaw, Poland. Contact: Congress Care, Muntelbolwerk 1, P.O. Box 440, 5201 AK's-Hertogenbosch, The Netherlands; *info@congresscare.com* , *www.congresscare.com*

4th European Congress of Aestetic Medicine and 6th Congress of the Swiss Society of Aestetic Medicine, Zurich, Switzerland, June 25-26, 2004. Contact: Pro Services Consulting - Patricia Lafitte, Chemin des Baules 14, CH - 1268 Begnins - Switzerland; *patricialafitte@deckpoint.ch*; *www.ssme.ch*

10th World Congress of Pediatric Dermatology, Rome, Italy, July 7-10, 2004. Contact: Triumph Congressi, Via Lucilio, 60, 00136 Rome, Italy; *dermo@gruppotriumph.it*; *www.gruppotriumph.it*

19th Continuing Medical Education Course for Practical Dermatology and Venerology, Munich, Germany, July 25-30, 2004. Contact: *www.fortbildungswoche.de*

American Academy of Dermatology, Academy '04, New York, USA, July 28-August 1, 2004. Contact: American Academy of Dermatology, Department of Meetings & Conventions, 930 E Woodfield Road, Schaumburg, IL 60173; fax: 847 330 1090

7th Dresden Symposium on Autoantibodies, Dresden, Germany, September 1-4, 2004. Contact: *k_conrad@rcs.urz.tu-dresden.de*

3rd Congress of the Dermatovenerologists, Struga, Macedonia, September 15-18, 2004.; Contact: Congress Secretariat, Clinic of Dermatovenerology, Vodnjanska 17, 91000 Skopje, Macedonia; *makderm@unet.com.mk*

Allergie Kongress 2004, Aachen, Germany, September 15-19, 2004. Contact: *Gerhard.Schultze-Werninghaus@ruhr-uni-bochum.de*; *www.allergie-kongress-2004.de*

1st Croatian Congress of Psychodermatology, Cavtat, Croatia, September 23-26, 2004. Contact: Prof. Mirna Šitum, Department of Dermatology and Venerology, Sestre milosrdnice University Hospital, Vinogradska 29, 10000 Zagreb, Croatia; *msitum@kbsm.hr*

7th International Congress of Dermatology, Teheran, Iran, September 29-October 2, 2004. Contact: i*nfo@iranderm.org*; *www.iranderm.org*

25th Annual Meeting of the International Society of Dermatologic Surgery, Barcelona, Spain, October 6-9, 2004. Contact: *isds2004@mccann.es*; *www.isds2004.com*

Update on Psoriasis, Continuing Medical Education Course organized by Chair of Dermatovenerology of the Zagreb University School of Medicine, Šalata 4, 10000 Zagreb, Croatia, October 15-16, 2004. Contact: Prof. Jasna Lipozenčić, Šalata 4, 10000 Zagreb, Croatia. Phone/Fax: +385-1-4920-014; *jasna.lipozencic@zg.tel.hr*

Psoriasis 2004, European Congress on Psoriasis, Paris, France, October 21-24, 2004. Contact: *www.pso2004.com*; *pso2004@mci-group.com*

Therapeutic Innovations in Dermatology and Dermatocosmetology, Bangkok, Thailand, October 23-25, 2004. Contact: *thadapiaru.@thaicosderm.org*

4th International Congress on Autoimmunity, Budapest, Hungary, November 3-7, 2004. Contact: fax:0041 22 732 2850; phone 0041 22 908 0488

Italian-Croatian Meeting on Psoriasis and 1st Meeting on the Lyme Disease, Grado, Italy, November 12-13, 2004.

13th Congress of the European Academy of Dermatology and Venereology, Florence, Italy, November 17-21, 2004. Contact: *president@eadv2004.org*; *registration@eadv2004.org*; *www.eadv2004.org*

10th World Congress on Cancers of the Skin, Vienna, Austria, March 19-23, 2005. Contact: Elfriede Pomp, Department of Dermatology, University of Vienna, Vienna General Hospital, Waehringer Guertel 18-20, A-1090 Vienna; *info@wccs.at*; *www.wccs.at*

Spring Symposium of the European Academy of Dermatology and Venerology, Sofia, Bulgaria, 2005. Contact: Bulgarian Dermatological Society; *dermven@bg.com*

8th Congress of the European Society for Pediatric Dermatology, Budapest, Hungary, May 5-7, 2005. Contact: *www.convention.hu*; *www.espd2005.com*

World Allergy Congress – 19th International Congress of Allergology and Clinical Immunology and 24th Congress of the European Academy of Allergology and Clinical Immunology, Munich, Ger-

many, June 26-July 1, 2005. Contact: *wac2005@congrex.se www.congrex.com/wac2005*

16th Biennal Meeting of the International Society for Sexually Transmitted Diseases Research (ISSTDR), Amsterdam, Netherlands, July 10-13, 2005. Contact: *isstdr@aidsfonds.nl; www.isstdr.org*

4thIACD (International Academy of Cosmetic Dermatology) World Congress, Paris, France, July 3-5, 2005. Contact: *iacd2005@mci-group.com*; *www.iacd-paris2005.com*

6th World Congress on Melanoma, Vancouver, B.C., Canada, September 2-9, 2005. Contact: Venue West Conference Services Ltd., Vancouver, B.C., Canada; *congress@venuewest.com*

15th World Congress of the International Union of Phlebology, Rio de Janeiro, October 2-7, 2005; Contact: RIO UIP 2005 - Secretary, Rua Santa Clara, 494 - Sorocaba - 108030-421 SP - Brasil; *inspemoc@dglnet.com.br*; *angelo.scuderi@flebologiabrasil.com.br*; *www.flebologiabrasil.com.be*

6th Dermatology and Dermatopathology Meeting of the Turkish Society of Dermatopathology, Istanbul, Turkey, October 7, 2004. Contact: Rana Yavuzer Anadolu, M.D., Ankara Uni Koza sok. 114-86, 00670 Ankara Turkey; *ranaadolu@hotmail.com*

14th Congress of the European Academy of Dermatology and Venereology, London, October 12-15, 2005. Contact: Prof. Martin Black, Congress President; British Association of Dermatologists Conference Services; *eadv@bad.org.uk*; *president@eadv2005.org*; *www.eadv2005.org*

21st World Congress of Dermatology, Buenos Aires, Argentina, October 1-5, 2007. Contact: *info@dermato2007.org*

INSTRUCTIONS TO AUTHORS

ACTA DERMATOVENEROLOGICA CROATICA (ADC) is a quarterly peer-reviewed journal, indexed in Index Medicus/MEDLINE and Excerpta Medica/EMBASE. It publishes original scientific articles, short scientific communications, clinical articles, case reports, reviews, reports, news and comments, and announcements in the fields of dermatology and venerology.

General Guidelines

Type the complete manuscript double-spaced, on one side of A4 bond paper, with a left side margin of at least 4 cm.

The manuscripts should not exceed 12-15 typed pages in case of original scientific papers, and 6-8 pages in case of short communications, clinical articles, case reports, and reviews.

The manuscripts should be written in English. The authors are responsible for ensuring that the English used is suitable for publication. All material is assumed to be submitted exclusively to this journal.

All manuscripts are subject to peer review.

Preparation of Manuscripts for Submission

Title Page

The title page should carry (a) the title of the paper, which should be concise but informative; (b) full name of each author, with institutional affiliation; (c) name(s) of department(s) and institution(s) to which the work should be attributed; (d) name and address (with telephone and fax numbers as well as the e-mail adress) of the author to whom requests for reprints should be addressed; (f) source(s) of support in the form of grants, equipment, drugs, or all of these; and (g) a short running head of not more than 40 characters (count letters and spaces) at the foot of the title page.

Second Page

The second page should carry a summary of not more than 250 words, followed by three to six key words from the Medical Subject Headings (MeSH) list of Index Medicus.

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The text of observational and experimental is usually, but not necessarily, divided into sections with the headings Introduction, Material (Patients) and Methods, Results, and Discussion. Long articles may need subheadings within some sections to clarify their contents, especially Results and Discussion sections. Other types of articles, such as case reports, reviews, and editorials, are likely to need other format.

Abbreviated terms should be written in full the first time they are used in the text, with abbreviation in parentheses.

Underline the words that must be printed in italic.

References should be identified in the text by arabic numerals in parentheses, and be numbered and listed consecutively at the end of the manuscript in the order in which they are first cited in the text.

Indicate in the text where the illustrations (figures and tables) should be inserted.

Tables and figures should be provided each on a separate sheet of paper after the references. Descriptive legends to figures should be typed double-spaced on a separate sheet of paper, whereas figures should be submitted in an envelope, with the number, the name of the (first) author, and title of the manuscript on the back: each table should be typed on a separate sheet of paper, numbered in the order in which they are first cited in the text, with a title and descriptive legend. Terms used in tables should not be abbreviated.

Ethics

When reporting experiments on human subjects, indicate whether the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Helsinki Declaration from 1975 as revised in 1983. Do not use patients, names, initials or hospital numbers, especially any illustrative material.

Statistics

Describe statistical methods and provide enough data to enable a knowledgeable reader to assess the reported results him or herself. Please state the statistical package (version, manufacturer) used for statistical analysis.

Acknowledgements

Please specify: (a) contributions that need acknowledging but do not justify authorship, such as general support by a departmental chairman; (b) acknowledgements of technical help; (c) acknowledgements of financial and material support, specifying the nature of support; (d) financial relationship that may be a source of conflict of in-

terest. Technical help should be acknowledged in a separate paragraph as well as other contributions.

References

References should be typed double-spaced on a separate sheet of paper. The Vancouver style, proposed by the International Committee of Medical Journal Editors, is used (Engl J Med 1991,324:421-8, BMJ 1991,302:338-41, or *www.icmje.org*) . Examples of correct forms of references are given below:

Journal article

Standard journal article (list all authors, but if their number exceeds six, give six followed by *et al*.)

You CH, Lee KY, Chey RY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1989;79:311-4.

Chapter in a book

Weinstein L, Swartz MN. Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editors. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

Article not in English

Massone L, Borghi S, Pestarino A, Piccini R, Gambini C. Localisations palmaires purpuriques de la dermatite herpetiforme. Ann Dermatol Venerol 1987;114:1545-7.

Conference paper

Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB, Kaye SV, editors. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (MI):Lewis, 1985:69-78.

Disertation

Youssef NM. School adjustment of children with congenital heart diseases (dissertation). Pittsburgh (PA): University of Pittsburgh; 1988.

Submission of Manuscripts

Manuscripts should be printed on paper and submitted in triplicate, with one copy on a floppy disk, and sent to:

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Manuscripts on Disks

Floppy disks should be 3.5-inch (1.44 MB) IBM formatted and labeled with the name of the author.

The file, the word-processing program, and version used should be indicated by pen. MS-Word for Windows is preferred, although manuscripts prepared using any other IBM-compatible word-processor are acceptable.

Avoid complex formatting; the text will be styled according to the ADC design specifications. Do not use bold, capitalized text, or a running head. Do not use footnotes or endnotes. Submit the text, tables, and illustrations as separate files. For tables, always use table editor function; ensure that each data point is contained within a unique cell, i.e. do not use carriage returns within cells. For illustrations, the preferred formats are TIFF of 300 dpi resolution, although any format in general use is acceptable provided it is not application-specific. If MS Excell is used for charts make sure to enclose original Excell file.

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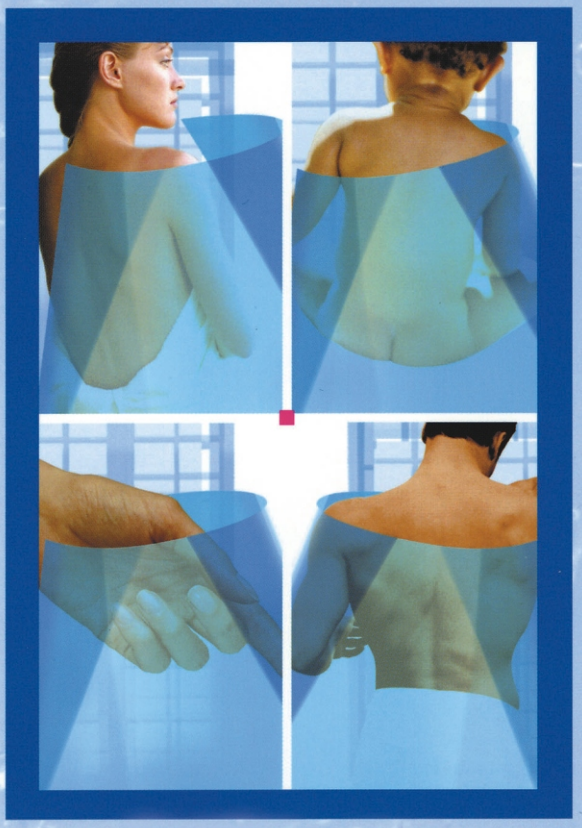

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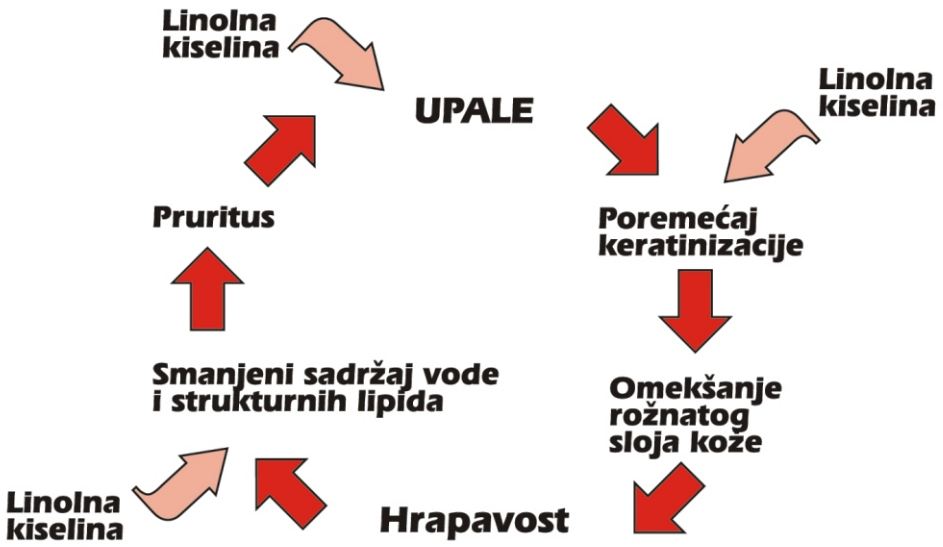

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New Genetic Research: Technologies That Help Your Hair

(NAPS)—People spend billions of dollars each year on dyes, shampoos and styling products in an attempt to get the kind of hair they weren't born with—and the science community is listening seriously.

Now, using genetic research, hair care technology is going where dyes and shampoos have never gone before to improve hair health and appearance.

Recently, scientists, researchers, and hair care clinicians from all over the world gathered at the Oxford Hair Foundation (OHF) conference in London to report on breakthrough discoveries that could mean the end of bad hair days forever.

"Our hair characteristics are genetically pre-determined," said Procter & Gamble Beauty scientist Lauren Thaman Hodges. "Understanding genetics and biology and the role DNA plays in dictating the color, condition and health of hair gives us a powerful tool to further explore the science of altering our physical appearance."

Detailing breakthroughs in hair biology, leading global hair researchers discussed how genetic research is radically changing hair care technology.

No more gray days

What if you could go blonde for a day or stop the gray permanently without using dyes? These options are increasingly rooted in reality. As a person ages, individual hairs turn gray or white when their cells stop producing natural color. Current research is working to identify the biological "on/off" switch so graying can be stopped or even reversed.

Although this is successful in lab experiments, consumers will have to wait for products like these until scientists determine how to consistently pinpoint only hair cells to safely restore pigmentation.

New discoveries bring back healthy hair and color

No matter what we do, hair seems to lose its color and shine over the years, and P&G Beauty scientists think they know why—and how to reverse the trend. They have discovered "EDDS," a molecule dubbed the "copper blocker." Tap water contains copper, which over time can leave hair dry and

brittle. EDDS, which helps prevent copper damage, is being used in some of the company's hair care products to enhance color and condition by limiting the damage caused by copper.

Scientists have also identified a method of replacing three essential amino acids in the hair that are lost through everyday wear and tear. Amino acids are the building blocks of protein, from which hair is created. "A real advancement would be a daily shampoo that replenishes those lost amino acids," says Dr. John Gray, an OHF board director.

Genes shed light on flaky problem

Dandruff affects at least 45 percent of the world's population at some time in their lives and is the most widespread condition apart from the common cold. Fungi have been the suspected cause for more than 100 years, but no one was able to pinpoint the specific triggers for dandruff. Recently, P&G scientists changed that, identifying the exact microorganism that causes dandruff by tracking its genetic code.

Discovering the species responsible for dandruff has opened the doors for genetic studies of other fungal diseases that may be fatal to those with suppressed immune systems. The research and techniques used may also lead to finding magic bullet cures for other fungal disorders.

As Chris Gummer, a P&G scientist explains, these better technologies can give people more than just healthier hair. "It will give people freedom of choice over their hair."

For more information, visit www.oxfordhairfoundation.org.


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