In 1908, Janeway and Mosenthal described a Jewish girl who had episodic abdominal pain and fever. Additional cases were subsequently described, yet almost half a century elapsed till Siegal and later Heller et al. recognized this disorder first as benign paroxysmal peritonitis and latter as familial Mediterranean fever (FMF). Most of the patients present with recurrent self-limiting short (6 to 96 h) attacks of fever and serositis (peritonitis, pleuritis, or arthritis). Sometimes the sole presentation may be recurrent episodes of fever. Skin lesions include a distinctive erysipelas-like lesion mainly on the lower leg present in 7% to 40% of patients. The chief complication of the disease is deposition of AA amyloidosis, which is found mostly in the kidneys but the gastrointestinal tract, liver, and spleen and eventually the heart, testes, and thyroid can also be affected. The prevalence of amyloidosis varies according to the population. However, in general about 1/4 to 60% of cases develop or even present with renal amyloidosis that may lead to renal insufficiency, which is the most frequent cause of death.

Colchicine, which inhibits neutrophil migration, reduces the frequency of the attacks and the occurrence of amyloidosis and, thus, changes the prognosis. More than 75% of the treated patients have complete response.

What is familial Mediterranean fever?

FMF is the most frequent member of an expanding group of hereditary disorders known as familial autoinflammatory syndromes. They are characterized by seemingly unprovoked inflammation in the absence of high-titre autoantibodies or antigen-specific T cells. The classical clinical manifestations are recurrent episodes of fever and inflammation. Other syndromes included in the familial autoinflammatory syndromes are TNF-receptor associated periodic syndrome (TRAPS) and hyper-IgD and periodic fever syndrome (HIDS).

About 90% of patients with FMF have their first attack before the age of 20 years. Abdominal pain of 24-48 h duration occurs in the vast majority (95%) of patients. Some patients have only mild abdominal pain without peritonitis, but the majority present with an acute abdomen. The only manifestation in 75% of patients, is monoarthritis involving the knee or ankle.
etc. In 30% of patients, chest pain due to unilateral pleuritis have been reported; however, overt pericarditis is rare and occurs in less than 1%. Young (<20 years) male patients may present with acute scrotal swelling and tenderness.

Familial Mediterranean fever - cutaneous manifestations

Although erysipelas-like skin lesions on the shins or feet, is considered to be a pathognomonic clinical finding for the disease, other skin findings can be found. Among the most common nonspecific cutaneous manifestations are: angioneurotic edema, recurrent oral ulcers, various vasculitides: including Henoch-Schönlein purpura, nonspecific purpura and vasculitic nodules. Less commonly described skin manifestations include: urticaria, pyodermia, bullous skin lesions and erythema nodosum.

Histologic examination of the erysipelas-like lesion reveals slight edema in the superficial dermis. Sparse perivascular infiltrate consisting of lymphocytes, neutrophils, histiocytes and nuclear dust. There is a blurring of capillary walls, but no clear vasculitis. In direct immunofluorescence assay (DIF) C3 deposits are found in the capillary walls.

How common is it

FMF is an autosomal recessive disease. It affects more than 10,000 patients worldwide, predominantly prevalent in the Mediterranean basin (Sephardic Jews, Arabs, Turks, Armenians, Greeks and Italians). Prevalence is estimated to be as high as 1 in 700 in Israel and 1 in 1400 in Turkey.

Interestingly, carrier rates of 1:3.5 to 1:4.7 have been reported for 4 mutations (M680I, M694V, V726A and E148Q) in Ashkenazi and Iraqi Jews, Moroccans and Muslim Arabs. Aksentijevich et al. found a mutant allele frequency of 1:5 of known FMF mutations among 200 American Ashkenazi Jewish individuals undergoing genetic screening for other diseases. The carrier rate in Turkey has also been estimated at 1:5.

Laboratory analyses

Unfortunately, there is no specific biologic marker of FMF. Nonspecific findings include a transient elevation in white-cell count, erythrocyte sedimentation rate and increases in inflammatory mediators, such as serum amyloid A, fibrinogen, and C-reactive protein, during febrile attacks. Proteinuria (>0.5 g of protein per 24 h) in patients with FMF is a firm indication of amyloidosis.

In addition, patients lack a specific protease, normally present in serosal fluids, that can inactivate both interleukin-8 and the chemotactic complement factor 5a inhibitor, but this protease can be detected only in research settings.

Pathogenesis, genetic features and new horizons

The pathogenesis of FMF is still not completely understood. However, the absence of the C5a/IL-8 inhibitor allows IL-8 and C5a to accumulate, inducing a massive neutrophil chemotaxis that results in an inflammatory reaction.

Important information was gained after FMF was mapped to the short arm of chromosome 16 near the α-globin gene, and, 5 years later, 2 unrelated groups cloned the gene designated MEFV. In consequence of the cloning of the responsible gene, the protein (pyrin, or marenostrin) encoded by MEFV was detected. MEFV is predominantly expressed in myeloid cells, and its expression is up-regulated during myeloid differentiation. Interferon-γ and tumor necrosis factor are also effective stimuli for expression of the gene. The precise function of pyrin is unclear. It is mainly expressed in the cytoplasm of mature neutrophils and monocytes and is thought to suppress neutrophil mediated inflammation. Thus, the mutant forms of pyrin are nonfunctional and probably interrupt the normal pyrin-mediated feedback loop leading to uninhibited escalation of the inflammatory response following “trivial” stimuli.

Moreover, in light of the cloning of the responsible MEFV gene, the study of the molecular biology of pyrin has opened new horizons for molecular immunology. The evidence is gathering that pyrin is an interacting element in a signaling network that connects the cytoskeleton, inflammation, and apoptosis. Of particular interest is the growing number of inflammatory syndromes, such as Crohn’s disease, Bechet’s disease, which have all been described in increased incidence in MEFV carriers. Thus, it is possible that
mutations in the MEFV gene act as modifiers of a common inflammatory pathway.

About 40 different mutations have been described until now in the MEFV gene, most of which are clustered in one exon—exon 10, although just 4 or 5 mutations account for the majority of FMF cases. The mutations prevalence varies according to the population tested. 

The M694V mutation is associated with a more severe phenotype than the V726A mutation and homozygosity for M694V carries a higher risk of amyloidosis. 

The frequency of the susceptibility gene varies widely; it is very high among Armenians (ratio of persons with the gene to those without it, 1:7) and Sephardic Jews (1:5 to 1:16).

**Treatment**

Colchicine has been the first-line treatment for patients with FMF since 1972. Its efficacy has been established in 2 controlled clinical trials. Colchicine prevents febrile attacks in 60% of patients and significantly reduces the number of attacks in another 20% to 30%. Even if colchicine therapy does not prevent febrile attacks, it still prevents amyloidosis. The lack of efficacy in the minority (5% to 10%) of patients may be due in part to noncompliance.

Sometimes, diarrhea or abdominal pain may develop, but are mostly controlled by dose reduction. Rarely, myopathy, neuropathy and leukopenia may occur mainly in patients with renal or liver impairment. Seldom, colchicine may affect male fertility by inducing oligospermia or azoospermia. However, colchicine does not seem to affect female fertility, frequency of miscarriage or teratogenicity; despite opposing results from *in vitro* studies. In fact, female fertility and outcome of pregnancy have improved in FMF patients using colchicine, due to a decreased incidence of peritoneal adhesions and of acute attacks which cause miscarriage and/or early delivery.

In Israel, pregnant female FMF patients on colchicine are offered amniocentesis with karyotyping. However, there is no strong evidence that suggests that colchicine use throughout pregnancy carries a substantial teratogenic or mutagenic risk when used at recommended doses. Breast-feeding was favourable. In children, long-term colchicine use has been shown to be safe and without a negative effect on growth; rather, the cessation of FMF attacks and return to health will improve growth and development.

Colchicine does not stop an established attack, and diclofenac can be used for pain control. The discontinuation of colchicines may produce an attack within a few days, therefore, compliance is essential.

Other agents such, as interferon-α, have been suggested but seem to have limited efficacy; on the contrary, prazosin may be useful.

**Prognosis**

The prognosis for patients with FMF depends mainly on the presence or absence of AA amyloidosis. Before the introduction of colchicines prophylaxis, amyloidosis occurred in 60% of affected patients who were over 40 years of age, and it was the main cause of death in such patients. Since the introduction of colchicines prophylaxis, the incidence of amyloidosis in FMF has dropped dramatically. Without amyloidosis life expectancy is normal.

**Diagnostic approach**

It is important to review the medical history carefully in patients with recurrent febrile attacks. It is crucial to examine the patient during an attack; as between attacks the examination is usually normal. Patients with periodic fever that persists for more than 2 years probably do not have infections or malignant disorders. Thus, attacks with a predictable course and a similar set of symptoms, along with a family history of such attacks, may suggest the presence of a non-infectious form of autoinflammatory syndrome. Therefore, the diagnosis of FMF is a bedside diagnosis. Furthermore, for diagnostic purposes, a set of clinical criteria was designed and validated by Livneh et al. (Table I). A molecular diagnosis of FMF is possible, but there are some limitations. First, the genetic screening is usually made for the 5 most frequent mutations (M694V, V726A, V680I, E148Q, and V694I). In addition, although FMF is an autosomal recessive disease, the MEFV mutations occur on both alleles in only about 70% of typical cases.
only one mutation or none can be detected, even after sequencing. There is also evidence of nonpenetrance (i.e., 2 mutant MEFV alleles in the absence of clinical disease). Therefore, molecular testing can be used as a confirmatory test in cases in which there is a high clinical index of suspicion. It is important to emphasize that regardless of the molecular test results, based on the clinical diagnosis, treatment with colchicine should be advised in symptomatic cases.

What is the message to take home?

The case report described by Mourellou et al. is a typical clinical story for a patient with FMF. Recurrent (several times per year, monthly or bimonthly), typical attacks consisting of short (48-96 h) episodes of fever (38-40°C) and erysipelas-like exanthema of the left lumbar and gluteal area which were sometimes associated with orchiepiedimitis, abdominal pain and arthritis.

A delay in FMF diagnosis is common. There is often a delay of 5-10 years from the onset of symptoms before the correct diagnosis is made. The major factors contributing to this delay were found to be patient’s neglect of symptoms and physician’s unawareness of FMF. Even in a region, where FMF can be encountered frequently, at least 11 FMF patients could be detected in a group of 59 children who had been given the diagnosis functional abdominal pain per exclusion. Indeed, the 30 years delay in diagnosing this patient is probably due to neglect in synthesizing all the symptoms, which are typical for FMF. The lesson that we as dermatologists must keep in mind is that seemingly simple dermatologic lesions should always be viewed in context with the patient’s general medical status. Other signs and symptoms should always be evaluated.

Reports on FMF from Greece are relatively sparse. According to Konstantopoulos et al., this is probably due to underdiagnosis or misdiagnosis. They tested 62 patients who were diagnosed with FMF, by rapid molecular tests. Within the Greek patients (representing the vast majority in the country), the more prevalent mutations were E148Q and M694V, accounting for 14% and 48%, respectively, of all mutations that they identified. It is interesting to note a relatively high prevalence of the E148Q especially since this mutation seems to produce very mild disease or, in some cases, no symptoms at all. In fact, there is still argument as to whether the E148Q change is truly a mutation or simply a polymorphism, thus its role in the development of FMF remains inconclusive. They conclude that the demonstration of a similar spectrum of variation of MEFV mutations within FMF patients in Greece, indicates that the disease, although not universal, is a not uncommon genetic disease in the whole Mediterranean basin, including Greece.

Thus, this demonstrative case report, is a fine reminder that recurrent periodic episodes of a cellulitis and erysipelas-like syndrome accompanied by serositis should alert physicians to the possibility of FMF, which should be included in the differential diagnosis of such cases, especially in a Mediterranean country.

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FAMILIAL MEDITERRANEAN FEVER

DAVIDOVICI

45. Tunca M, Tankurt E, Akbaylar Akpinar H, Akar S, Hizli N, Gonen O.


Amitriptyline as therapeutic and not symptomatic approach in the treatment of *prurigo nodularis*

A pilot study

**Aim.** *Prurigo nodularis* (PN) is a chronic skin disease of unknown etiology characterized by an intense pruritus, which affects the patients’ life quality. The aim of this study was to investigate whether amitriptyline, a well investigated tricyclic antidepressant with a strong antipruritic effect based on a high binding affinity for the histamine $H_1$ receptor, could represent a novel therapeutic approach.

**Methods.** In this open, uncontrolled pilot study, we investigated the efficacy and safety of amitriptyline, administered orally, in the treatment of PN. Patients were treated with an initial dosage of 60 mg for 3 weeks, followed by a dose reduction to 30 mg for 2 weeks and 10 mg for 1 week.

**Results.** Of a total of 17 patients with PN, response was achieved in 82.4% of patients. Side effects were generally mild and included mostly reduced concentration during the day, but no patient discontinued the treatment. Disease relapse occurred in 5 patients within 7 months, but reintroduction of amitriptyline lead again to immediate response.

**Conclusions.** The significant remission achieved in our patients may designate amitriptyline as a novel therapeutic approach of PN.

**Key words:** *Prurigo nodularis*, diagnosis - *Prurigo nodularis*, drug therapy - Amitriptyline.
investigating the therapeutic effect and value of amitriptyline used as a first line agent for the treatment of PN itself and not of the associated psychiatric symptoms.

Materials and methods

In this study, a total of 17 patients with PN, seen at our outpatients clinic from October 2003 to January 2005, were included. In Table I the patients demographics and medical history are listed. There was a marked female predominance with 14 women and only 3 men. Median age was 72.8 years (range from 44 years to 87 years). The medical records included clinically non relevant hepatitis C infection in 4 patients, as well as the regular use of drugs for the treatment of arterial hypertension, hypercholesterinemia and thyroid gland dysfunction. In 2 cases a history of depressive episodes requiring medication was reported. In no patient, clinical, laboratory and/or anamnestic data were suggestive for atopy.

The diagnosis of PN was based on the typical clinical features, but in 5 patients additional histopathologic examination was performed. The time between onset of disease and first visit at our clinic varied from 3 to 27 months, with a mean time of disease duration of 9.6 months. In all patients, previous treatment including various topical steroids, emollients and systemic antihistamines was ineffective and/or resulted in only short-time response. Although in patient 10 and 16 topical capsaicin lead to a complete remission of PN, the patients refused the treatment at relapse due to the displeasing application. Patient 10 received additionally systemic steroids with initial response, but the skin lesions re-appeared shortly after treatment discontinuation. In all 17 patients the key distressing symptom of PN was the intense pruritus that caused, in most cases, insomnia and consequently loss of concentration (Table I).

Based on the well known antipruritic effect, but also taking into consideration the sedating action of amitriptyline, we designed an empiric, low dose protocol with an initial dosage of 30 mg per os, twice daily for 3 weeks, followed by a gradually dose reduction to 30 mg per os for 2 weeks and finally 10 mg per os for 1 week. After 6 weeks the treatment was stopped.

Before treatment start all patients were informed about the treatment protocol and eventual side effects of amitriptyline and/or symptoms of intolerance. Each patient was visited after the first 3 weeks of treat-

Table I. — Patients demographics, medical history as well as duration of disease, dosage of amitriptyline and time of treatment. Response was defined as a significant reduction of skin manifestations and symptoms of pruritus after the first 3 weeks of treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Medical records</th>
<th>Duration of disease (in months)</th>
<th>Associated psychiatric symptoms</th>
<th>Initial dosage of amitriptyline</th>
<th>Time of treatment (in weeks)</th>
<th>Response</th>
<th>1 year follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 55 F TGD 6</td>
<td>40 mg</td>
<td>6 + -</td>
<td>3</td>
<td>60 mg</td>
<td>8 + relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 80 F AH 24</td>
<td>8 Loss of concentration</td>
<td>40 mg</td>
<td>6 + -</td>
<td>20 mg</td>
<td>10 - relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 58 F HCV 8</td>
<td>60 mg</td>
<td>6 + -</td>
<td>12</td>
<td>60 mg</td>
<td>6 + -</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4 46 F TGD, D 3</td>
<td>60 mg</td>
<td>6 + -</td>
<td>66 F HCV 18</td>
<td>20 mg</td>
<td>10 - relapse</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5 68 F AH, HCV 6</td>
<td>60 mg</td>
<td>6 + -</td>
<td>87 M 27 OCD 8</td>
<td>8 mg</td>
<td>10 + -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 75 F AH, HCV 12</td>
<td>60 mg</td>
<td>6 + -</td>
<td>56 F 7</td>
<td>60 mg</td>
<td>6 + -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 72 M 5</td>
<td>60 mg</td>
<td>8 - relapse</td>
<td>73 M AH 14 Loss of concentration 40 mg</td>
<td>6 + -</td>
<td>54 F 3 Loss of concentration 40 mg</td>
<td>6 + -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 72 F AH, HCV 10 Loss of concentration 40 mg</td>
<td>6 + -</td>
<td>44 F HC 3</td>
<td>6 + -</td>
<td>62 F AH 3</td>
<td>6 + -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 44 F HC 3</td>
<td>60 mg</td>
<td>4 + -</td>
<td>74 F 10 Loss of concentration 60 mg</td>
<td>6 + relapse</td>
<td>55 F D 4 Loss of concentration 40 mg</td>
<td>8 - relapse</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: F: Female; M: Male; TGD: Thyroid gland dysfunction; AH: Arterial hypertension; HCV: clinically non-relevant Hepatitis C virus infection; D: Depression; OCD: Obsessive compulsive depression
ment in order to ensure treatment response and/or the absence of eventual serious side effects, requiring dosage adaptation or treatment discontinuation. When a good tolerability and efficacy of the treatment was confirmed, treatment was continued according to the protocol. At the sixth week of treatment, each patient was again examined before treatment was finally stopped.

In a few patients, we modified the protocol and adjusted the initial dosage for the following reasons (Table I).

In patients 1, 3, 11–14 and 17, the initial dosage of amitriptyline was adjusted to 20 mg per os, twice daily due to underweight in patient 1 and a reduced concentration during the day life in patients 3, 11–14 and 17.

In patient 7 we chose a modified protocol due to the patient’s anxiety of possible side effects of the treatment. In patient 8 we reduced the initial dosage significantly due the advanced age (87 years).

Prolongation of treatment protocol was adjusted according to the clinical response and performed in 5 patients.

In one patient, the response to amitriptyline was significant enough to merit therapy discontinuation already 4 weeks after therapy start.

**Results**

Of a total of 17 patients with PN, response was achieved in 82.4% of patients. Side effects were generally mild and included mostly reduced concentration during the day, but no patient discontinued the treatment. Disease relapse occurred in 5 patients within 7 months, but reinitiation of amitriptyline lead again to immediate response.

**Discussion and conclusions**

PN is a chronic pruriginous skin disease of unknown etiology. Its leading clinical symptom is the intense pruritus resulting often into extensive skin excoriation and reduced patient’s quality of life. Although pruritus per se is a nonspecific clinical symptom, PN is considered to represent a distinctive disease. However, PN may also be associated with other internal, neurologic, psychiatric and oncologic disorders, which may induce and/or aggravate pruritus. The response to various treatments including corticosteroids, capsaicin, oral antihistamines, photochemotherapy, cyclosporine and thalidomide is often unsatisfactory and limited by a number of side effects.

Because the intensity of the chronic pruritus has a profound impact on the patients lives, resulting in the eventual development of serious psychiatric disorders including depression with suicidal thoughts and obsessive-compulsive behaviors (such as the chronic picking of the skin), further treatment recommendations include accompanying psychopharmacological drugs.

Amitriptyline is a well investigated tertiary amine
tricyclic antidepressant with sedative and antipruritic effects. Although the mechanism of action is not fully understood, it does not act primarily by stimulation of the central nervous system, but it is believed that the most important effect is the decreased reuptake of norepinephrine and serotonin in adrenergic and serotonergic neurons. This action may potentiate or prolong neuronal activity since reuptake of these biogenic amines is important physiologically in terminating transmitting activity. Since amitriptyline possesses a strong anticholinergic activity, caution should be considered in patients with cardiac diseases, prostate hyperplasia, urinary retention, respiratory diseases, gastrointestinal diseases or glaucoma, to name just a few.\(^5\) Amitriptyline is frequently used in anaesthesiology, neurology and psychiatry to treat depression and/or chronic neuropathic pain. By contrast to its unknown mechanism of sedation and analgesia, its antipruritic effect is well recognized and caused by a high binding affinity to the H\(_1\) receptors. The advantages of amitriptyline are also known in dermatology, where it is generally used in the symptomatic treatment of various neuropathic and skin diseases such as postherpetic neuralgia, notalgia paraesthetica, vulvodynia, atopic dermatitis, chronic urticaria, eczema but also PN.\(^7\)

However, in PN the value of amitriptyline is merely supplementary to relief symptoms and in combination with other treatments, but not to treat the disease \textit{per se}.\(^2,4\) Accordingly, there is little known about a therapeutic effect of amitriptyline in the treatment of PN. Interestingly, Alfadley \textit{et al.}\(^8\) reported on a patient with PN successfully treated with thalidomide, who was concomitantly treated with amitriptyline because of a major depression. Although the treatment with thalidomide was discontinued due to its side effects, the patient remained relapse free, but the authors did not clarify if the treatment with amitriptyline was continued or not. In another study, one patient with PN of the scalp, in a series of 11 women with scalp dysesthesia, achieved long-term remission of her symptoms after treatment with amitriptyline.\(^9\)

In our study we observed a significant response of PN to amitriptyline used as therapeutic agent in more than 80% of patients, characterized by the rapid and long-time remission of the clinical manifestations with their associated symptoms. Although 5 patients relapsed within 7 months, the reintroduction of amitriptyline achieved again a rapid remission. Since our study protocol was based on empiric and personal observations, we chose a much lower dosage than usually reported. Side effects were limited to reduced concentration during day activities or dry mouth, but none of our patients discontinued the therapy. However, because of the side effects, we reduce the initial dosage of 60 mg/day to 40 mg/day in 5 patients, of whom only one required treatment longer than 6 weeks. We, therefore, suggest an initial dosage of amitriptyline between 40 mg and 60 mg/day.

One explanation for the therapeutic effects of amitriptyline in our patients might be given by the action as a sedating antidepressant, which might decrease the restless activity to continuously scratch the skin.\(^4\) On the other hand, amitriptyline may relieve the itching sensation by its antihistaminic function due to affinity to the histamine H\(_1\) receptors.\(^3,5\)

A further hypothesis for the antipruritic value of amitriptyline in the treatment of PN is based on recent insights in the neurocutaneous biology of pain and pruritus. It has been postulated that both sensations, pruritus and pain, seem to be strongly connected and induced by nociceptive, unmyelinated type C neurons.\(^9,10\) Hence, histamine stimulation can induce pain instead of pruritus in patients with complex regional pain syndrome and postherpetic neuralgia. By contrast, painful stimuli may evoke itching sensations instead of pain in patients with chronic pruritus by means of the so-called central sensitization for itch.\(^10,11\) As a consequence, one could assume that amitriptyline, known in the treatment of neuropathic pain, may act on the same nerve fibres, resulting in an antipruriginous effect in patients with chronic pruritus.

Other substances have been recently discovered in the pathogenesis of pruritus and pain, among them, serotonin. Hence, the antipruritic effect of amitriptyline might be related to its serotonin re-uptake blockade at the peripheral nociceptive C-fibres, although previous studies did not show significant antipruritic effects of serotonin receptor antagonists in renal itch.\(^12,13\)

It must be underlined that we did not investigate the neuropathophysiological changes in our patients and, therefore, the given explanations needs to be considered hypothetical.
However, the significant remission achieved in the majority of our patients may designate amitriptyline as a future alternative in the therapeutic approach of PN.

Riassunto

Amitriptilina come approccio terapeutico e non sintomatico nel trattamento della prurigo nodulare: uno studio pilota

Obiettivo. La prurigo nodulare (PN) è una malattia cronica, a eziologia sconosciuta, caratterizzata da un prurito di intensità tale da compromettere la qualità di vita del paziente. Lo scopo di questo studio era verificare se l’amitriptilina, noto antidepressivo triciclico dotato di notevole efficacia contro il prurito e di un’elevata affinità di legame per il recettore H1 dell’istamina, possa rappresentare un nuovo approccio terapeutico per la PN.

Metodi. In questo studio sono stati verificati l’efficacia e gli eventuali rischi dell’amitriptilina, somministrata per via orale, come trattamento della PN. I pazienti sono stati trattati con una dose iniziale di 60 mg/die per 3 settimane, con riduzione a 30 mg/die per 2 settimane e, infine, a 10 mg/die per una settimana.

Risultati. Su 17 pazienti con PN si è ottenuta una risposta positiva pari all’82,4%. Non sono stati riscontrati effetti collaterali di notevole entità e questi, se presenti, erano principalmente caratterizzati da una diminuita capacità di concentrazione. Nessun paziente ha, tuttavia, dovuto sospendere il trattamento. Recidiva di malattia si è presentata in 5 pazienti entro 7 mesi dalla sospensione del farmaco, ma si è ottenuta una remissione immediata mediante tempestiva reintroduzione del farmaco.

Conclusioni. La significativa remissione ottenuta nei nostri pazienti ci consente di ipotizzare che l’amitriptilina possa costituire un nuovo ed efficace approccio terapeutico in pazienti affetti da PN.

Parole chiave: Prurigo nodulare, diagnosi - Prurigo nodulare, terapia farmacologica - Amitriptilina.

References

Dermatological and instrumental evaluation of the activity of a new dermocosmetic product adjuvant in the treatment of acne

A. SPARAVIGNA 1, M. SETARO 1, S. SORMANI 2, M. BERGAMASCHI 2

Aim. Although the pathogenesis of acne is not completely clarified, there exists a general agreement in considering this skin disorder as the result of a complicated interplay of multiple factors, such as ductal epidermal hyperproliferation, excess sebum, local inflammation, and microbial colonization with the presence of Propionibacterium acnes, each of these factors contributing in a different way to the development of acne. During the last 20 years, the number of topical and systemic drugs for the treatment of acne has been largely increased by the discovery and introduction of new drugs or further developments of already available therapeutic agents. This includes topical antibiotics, retinoids, topical antimicrobials, along with benzoyl peroxide and azelaic acid. Cosmetic preparations are also widely used for the treatment of acne, whose cosmetic formulation and the selected products meet the guidelines for sensitive skin. This paper describes the results obtained in a group of healthy volunteers affected by mild to moderate degree of acne who have been treated for 4 weeks with a cosmetic preparation, named Acne cream, that contains neolignans extracted from the roots of Krameria, lauric acid and escin-β-sitosterol complex, a formulation intended as a coadjuvant for the treatment of acne.

Methods. Thirty-two healthy volunteers, 20 females and 12 male aged 13 to 45 years (average 22 years) who did not receive specific treatment for one month before the beginning of the study were enrolled. All the patients had clinically diagnosed mild to moderate degree of acne according to the classification reported by Cunliffe and Shuster and gave their informed consent.

Results. The results obtained in this study show that the continuous application of this preparation is able to reduce the increase of sebum in the investigated areas to a significant extent, possibly by controlling its outpouring to the cutaneous surface. Moreover, the treatment did reduce the presence of Propionobacterium acnes, Streptococcus pyogenes and Staphylococcus aureus in the same skin areas. These results showed a significant diminution of the major clinical signs associated to acne pathology, including erythema, scaling and the number of papule and pustules. Significant attenuation of subjective symptoms, such as itch and pain have also been observed.

Conclusions. The overall results coupled with an excellent tolerability and the subjective statement of a significant clinical efficacy and good cosmetic acceptability indicate that Acne cream could be considered a valid coadjuvant for the treatment of acne.

KEY WORDS: Acne, drug therapy - Anti-androgens - Escin beta - Sitosterol - Cosmetics.

Acne is a very common skin disease1 whose pathogenesis is not completely clarified yet, although the relevance of some factors for the development of this disorder is largely accepted. At present, there is, in fact, a general consensus in considering acne as the result of a complicated interplay of multiple factors that contribute to its development, including: ductal epidermal hyperproliferation, excess sebum, local inflam-
information, and microbial colonization with the presence of Propionibacterium acnes. The cutaneous alterations that take place with acne, as well as with dermatitis, eczema and other disorders, leave the skin vulnerable to external insults, partly as a result of varying levels of barrier dysfunction. But even though progresses have been made in the knowledge of acne pathology, much remains unknown in the understanding of the pathogenesis of this disorder. As a matter of fact factors like the nature of the initial stimulus for follicular hyperproliferation, the fact that some people develop acne and others do not despite similar serum hormone levels or comparable bacterial colonization and the reason for difference in the severity of acne in given patients, still remain unanswered questions.

The first line treatment for these cases is generally via the topical route, whereas systemic medication is indicated when higher severity grades with small nodes or scarring occur. During the last 20 years, the number of topical and systemic drugs for the treatment of acne has been increased including newly discovered agents and further developments of already available agents or galenic formulation have improved efficacy or local tolerance.

Topical agents are now the mainstay of maintenance therapy and several topical agents are available that affect at least one of the main pathogenetic factors responsible for the development of acne, i.e. hyperseborrhoea, hyperkeratosis, microbial colonisation and/or inflammatory and immunological reactions. Ad hoc studies have also shown that topical antiacne agents are well tolerated and, possibly due to their limited transdermal uptake, other significant safety concerns have not arisen so far.

Antibiotics, such as clindamycin, erythromycin and tetracyclines remain the most common prescribed agent for the treatment of acne, but improper use of antibiotics in the dermatological setting needs to be evaluated to prevent the increasing prevalence of antibiotic resistance. So it is now recommended that topical antibiotics should be used less often than in the past and only for short periods to avoid the development of resistances and to prevent possible side-effects. To this end, it has been suggested to combine those agents with topical retinoids, benzoyl peroxide or with azelaic acid to enhance the efficacy and slow down the development of resistance.

Topical retinoids, such as tretinoin, its isomer isotretinoin, and retinoid analogs, such as adapalene and tazarotene, has been shown to normalize ductal hyperproliferation, to greatly diminish sebum production and sebocyte terminal differentiation, to decrease the number of Propionibacterium acnes organisms thereby halting the progression to inflammatory lesions. Despite its therapeutic success the exact mechanism of isotretinoin remains still unknown. Azelaic acid is a naturally occurring acid whose antiacne activities are due to its antimicrobial action on Propionibacterium acnes, and to its inhibiting activity on comedo formation, based on the decrease of hyperkeratinization. Benzoyl peroxide has moderate to strong antibacterial effect based on its potent oxidizing properties, and does not induce bacterial resistance. In a recent study the efficacy of benzoyl peroxide against Propionibacterium acnes has been compared with that of clindamycin in 2 groups of subjects who underwent application of benzoyl peroxide or clindamycin twice a day for 14 days. The results of quantitative sampling of Propionibacterium acnes after 3, 7 and 14 days of treatment did show that the treatment with benzoyl peroxide formulation is able to reduce the follicular population of Propionibacterium acnes more rapidly and to a greater extent than did topical clindamycin benzoyl peroxide.

It has been shown that androgen hormones play a crucial role in the pathogenesis of acne in men, women, children and adults, and this finding led to the rationale consideration that inhibition of androgen expres-
sion would constitute a rationale for the therapy of acne. Subsequent studies proved that antiandrogens provide a valuable alternative to standard therapy especially in selected women.

The hormonal treatment is mainly directed to the reduction of circulating androgen levels and to androgen receptor blockade (spironolactone, cyproterone acetate, and flutamide). More recently, studies have pointed to compounds that may influence the metabolism of androgen precursors to active androgens within pilosebaceous units, and among them those endowed with 5-α-reductase inhibiting action, whose benefit has already been recognized in androgenetic alopecia in men.

In general, topical antiacne agents are well tolerated and, as it would be expected from their limited transdermal uptake, other significant safety concerns have not so far arisen. Apart from the retinoids, which can be associated with the risk of embryotoxicity/teratogenicity limiting their safety of use in pregnancy, and clindamycin, that might cause pseudomembranous colitis, information on the systemic pharmacokinetics of other topical agents is not readily available.

Cosmetics are also widely used for the treatment of acne, under the strict indication that they must be noncomedogenic, nonacnegenic, nonirritating and hypoallergenic: this requires a basic understanding of cosmetic formulation and the selection of products that meet guidelines for sensitive skin.

This paper describes the results obtained in a group of healthy volunteers affected by mild to moderate degree of acne who have been treated for 4 weeks with a cosmetic preparation, named Acne cream, that contains neolignans extracted from the roots of Krameria, lauric acid and escin-β-sitosterol complex, a formulation intended as a coadjuvant for the treatment of acne.

Previous studies have shown that neolignans are highly effective against Gram positive bacteria, such as Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis and, in particular, Propionibacterium acnes. The antioxidant and cytoprotective potential of Krameria triandra extract, containing 15% of neolignans, has been evaluated in different cell models, rat erythrocytes and human keratinocytes cell lines, exposed to chemical and physical (UVB radiation) free radical inducers. The results obtained in that study clearly indicate the potential for the use of the neolignans contained in Rhatany extracts as topical antioxidants and radical scavengers against skin photodamage.

As already reported, antiandrogens are able to block or lessen acne-promoting effects of androgenic hormones, as they reduce sebum production and hyperseborrhoea thereby improving acne in some patients. Lauric acid is a n-dodecanoic fatty acid extracted from Laurus nobilis whose antiandrogenic activity is mediated through its influence on the metabolism of androgen precursors in the pilo-sebaceous unit, i.e. via its ability to inhibit both type 1- and type 2 - 5-α-reductase activity.

Figure 2.—Typical photographic example of the effect of the repeated treatment with Acne cream (volunteer 1: female, 17 years old). A) Before treatment. B) End of treatment.
Escin-β-sitosterol is a patented complex of escin with β-sitosterol and phospholipids and has been demonstrated to exert topical anti-inflammatory action. Escin is extracted from the seeds of *Aesculus hippocastanum* and is widely used as an antioedema and vasoprotecting agent. Escin is the active principle contained in pharmaceutical preparations like Reparil and Essaven developed for local treatment of venous and microcirculatory alterations, such as varicose veins, chronic venous insufficiency, bruises and swelling secondary to contusions and blunt injuries. β-sitosterol is a vegetable sterol which is known to exert a hypocholesterolemic action, as it lowers plasma concentration of low density lipoprotein (LDL), but it has no effect on very low-density lipoprotein (VLDL). Moreover, results from a recent study have shown that β-sitosterol, similar to the other phitosterol components, stigmasterol, and campesterol, exerts antioxidant effects of against lipid peroxidation, and that, similar to phitosterol, it chemically acts as an antioxidant and a mild radical scavenger, and physically as a stabilizer in the cell membranes.

### Materials and methods

Investigations have been carried out in 32 healthy volunteers, 20 females and 12 males aged 13 to 45 years (average 22 years), who did not receive specific treatment for one month before the beginning of the study. All the enrolled subjects had clinically diagnosed mild to moderate degree of acne on the face according to the classification reported by Cunliffe and Shuster and gave their informed consent. Exclusion criteria were: pregnancy or lactation, participation to similar studies in the previous month, presence of skin disorders in the areas of treatment, presence of other systemic pathologies, like diabetes, liver and renal insufficiency and heart failure. Subjects who were administered drugs, like aspirin or other NSAIDs, systemic corticosteroids during 3 months preceding the present study were also excluded.

The objective of the study was the clinical evaluation, using noninvasive instrumental methods, of the therapeutic activity of a cosmetic formulation Acne cream (supplied by Farmaka S.r.l., Como, Italy) over a period of 4 weeks treatment. The study has been performed according to an open study protocol, and the cosmetic preparation under investigation was self-administered 2 times per day, 1 at morning and 1 at night, possibly at the same time every day, for 4 consecutive weeks. The topical treatment was applied to the face, to the left and right side of the suprascapular region of the back, according to a previously defined random scheme. In the course of the study, each sub-
Subject underwent medical examination by a dermatologist before and at the end of treatments.

The degree of acne at the different experimental times has been analyzed as follows:

1) Clinical evaluation: the clinical criteria for the evaluation of acne refers to symptoms and cutaneous signs such as erythema, desquamation, number of open and closed comedons, papule, pustule, dyschromias, burning, itching and pain;

2) Instrumental evaluation: the following techniques have been utilized:

   — Photographic evaluation: photographs of the areas of acne have been taken before and at the end of treatments in each subject participating in the study;
   
   — Follicular biopsy for the assessment of the comedolytic activity: samples of the cutaneous surface under investigation have been taken by stripping of 5 x 5 cm areas before and at the end of treatments. With this procedure the follicular biopsy is the cast of the follicular infundibulum with included hair and keratinic material. In the acneic cutis the keratinic material can be increased forming microcomedons up to the formation of clinically evident comedons. The density and the total area of the comedons have been submitted to statistical analysis using the parametric Student’s t-test or non parametric tests.
   
   — Sebutape (Cu-Derm Corp. USA20, 21): sebum drops rising from the cutaneous surface were collected by applying special supports to the skin for 60 min. The sebum spots are then submitted to computerized morphometric analysis for the evaluation of the following parameters: ratio between the surface covered by sebum and the total surface of the specimen, mean of the parameters of the sebum spots, i.e.: area, diameter, major and minor axes of the spots. The image analysis has been carried out on samples of the same area of the cutis, by a stereomicroscope (12x) connected with an analogical video camera.22 Specific software allowed the calculation of the number and mean value of the cutaneous area with comedons (area ≥ 0.016 mm²). For each subject the biopsies taken...
before and at the end of treatments have been analyzed using this method;
— subjective clinical data: at the end of the trial each subject was requested to express a judgment on the characteristics of the product used, including color, flavor, consistency, smearing, freshness, absorption, effect on the skin and residuals of the product, tolerability and efficacy of the treatment under investigation.

**Results**

**Clinical assessment**

The repeated treatment with Acne cream for 4 weeks in the subjects included in the present trial has shown that the severity of acne was clearly decreased in 38% of the treated subjects. Figure 1 shows, in fact, that the percentage of subjects with higher degree of acne, scored 0.75-1 according to the Cunliffe photographic scale,\(^9\) did decrease, while those scored 0.25 did increase at the end of treatments with Acne cream, indicating an improvement of their condition.

Erythema associated with the acneic pathology was significantly improved in 86% of subjects, at the end of the 4 weeks treatment (control vs end treatment \(P=0:\) Wilcoxon test), while only 14% did not show amelioration of their condition. Erythema did not worsen in any of the subjects participating in the study (Figures 2, 3).

Scaling significantly improved in 76% of the treat-
ed subjects (control vs end treatment $P=0$: Wilcoxon test) and remained unaltered in 24% of them. Again in none of the subjects scaling got worse.

Papule and pustule were significantly decreased in 41% and 52% of subjects respectively (control vs end treatment $P<0.01$: Wilcoxon test), as indicated by the pattern of the relative clinical scores.

At the end of treatments, closed and open comedons were significantly decreased in 45% (control vs end treatment $P<0.001$: Wilcoxon test) and 48% of subjects (control vs end treatment $P=0.01$: Wilcoxon test). Figure 4 shows in addition that the percentage of subjects with higher scores relative to open comedons, scores 2 and 3 (moderate and severe), were markedly reduced, while those with lower scores did increase at the end of treatments with Acne cream.

Concerning the subjective symptoms, the clinical scores for hitch associated with acne were significantly reduced in 38% of the subjects participating into the trial (control vs end treatment $P<0.06$: Wilcoxon test) (Figure 5). Burning and pain were not significantly modified at the end of treatments. No sign of worsening was detected in the subjects treated with Acne cream.

Figure 9.—Typical pictures of the sebum drops rising from the cutaneous surface after 60 min application of the special supports to the skin. The specimens have been obtained in 2 subjects (volunteer 2, female, 14 years old, volunteer 14, male, 14 years old) before (A, B) and at the end of the repeated treatment with Acne cream (C, D).
Instrumental assessment

Three subjects, volunteers number 5, 15 and 18, have been excluded by statistical analysis due to technical problems with the biopsies made at the end of treatments.

Comedolytic efficacy evaluated by follicular biopsy

The experimental evaluation of the comedolytic activity of Acne cream using follicular biopsy before and after treatments has shown a significant reduction of the total (-64%: \(P<0.001\)) and average (-44%: \(P<0.001\)) skin area affected by macrocomedons, that was paralleled by a 54% reduction (\(P<0.001\)) of the density of macrocomedons per unit of skin surface (Figures 6, 7).

On the contrary, the repeated treatment with Acne cream did not introduced significant variations in the observed parameters related to microcomedons, i.e. total and average areas and density. This result seems to rule out the possibility of a comedogenic activity of the investigated preparation (Figure 8).

Efficacy on sebum regulation assessed by Sebutape

The results of the computerized image analysis relating to the morphometric study of the sebum drops collected with the aid of Sebutape technique clearly show that the repeated application of Acne cream in the subjects included in the present investigation did produce a significant 11% diminution (\(P<0.01\)) in the parameters related to the average dimensions of the sebum spots, such as mean of the perimeters and mean of the major axes of the spots (Figure 9).

As it concerns the means of the minor axes and the ratio between the specimen’s surface covered by sebum and the total surface area recorded at the end of the treatments with Acne cream did not differ significantly from those recorded before the beginning of the treatments. Data reported in Table I show, in fact, that the mean values regarding the mentioned parameters recorded at the end of treatments were similar to those recorded before treatments were started.

This result clearly indicate that the Acne cream does not modify the amount of sebum produced by the skin, as it is requested for a cosmetic product, but rather it is able to control its outpouring to the cutaneous surface.

Cosmetic acceptability

The analysis of the data concerning cosmetic adequacy, tolerability and subjective efficacy of Acne cream emerging from the questionnaire given to the volunteers at the end of the treatments, have shown that the acceptability of this cosmetic preparation has been generally good (\(\chi^2 r: P<0.\)). As it concerns the efficacy of the treatment, this has been considered good by 45% of the subjects participating into the study and very good by 10% of them. In addition, Acne cream was, in general, well tolerated as tolerability has been reported to be good to very good in 90% of the subjects.

Discussion and conclusions

Acne is a family of disorders that vary to a great extent in its pathogenetic and clinical manifestations and, for these reasons, treatment therapeutic options may vary greatly with the stage and intensity of the disease.\(^2\) No single agent has yet been developed that addresses the different processes which participate in the etiopathogenesis of this disorder and the therapy consists of combination regimens which usually includes agents that counteract bacterial colonization, reduces plugging (hyperkeratinization) of the pilosebacous follicles, and compounds that have efficacy on the inflammatory reaction.\(^2\) Cosmetic preparations are also widely used for the treatment of acne, provided they are noncomedogenic, nonacnegenic, non-irritating and hypoallergenic.\(^1\)

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Table I.—Results of the computerized analysis of sebutapes.

<table>
<thead>
<tr>
<th></th>
<th>Sum of areas mm(^2)</th>
<th>Mean of perimeters mm(^2)</th>
<th>Mean of major axes mm</th>
<th>Mean of minor axes mm</th>
<th>Surface of the specimens sebum area/total surface</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Final</td>
<td>Control Final</td>
<td>Control Final</td>
<td>Control Final</td>
<td>Control Final</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>27.59±15.81 22.80±12.47</td>
<td>0.66±0.17 0.58±0.15</td>
<td>0.28±0.07 0.25±0.06</td>
<td>0.08±0.03 0.08±0.02</td>
<td>0.74±0.02 0.74±0.04</td>
</tr>
<tr>
<td>SEM</td>
<td>3.04 2.40</td>
<td>0.03 0.03</td>
<td>0.01 0.01</td>
<td>0.00 0.00</td>
<td>0.00 0.01</td>
</tr>
</tbody>
</table>
Acne cream, a cosmetic preparation that contains neolignans extracted from the roots of *Krameria*, lauric acid and escin-β-sitosterol complex and is intended as a coadjuvant for the treatment of acne, has been shown to fulfil the mentioned requirements in a variety of preclinical tests. Moreover, a preliminary efficacy study has been carried out in 20 subjects, male and female aged 18 to 35 years, affected by *acne vulgaris*, who applied the cosmetic formulation twice a day for 30 days. The results of that study have shown that the continuous application of the preparation did decrease to a significant extent the production of sebum in the investigated areas, *i.e.* forehead, cheek and chin, while placebo did not. Moreover, it reduced the presence of *Propionobacterium acnes*, *Streptococcus pyogenes* and *Staphylococcus aureus* in the cheeks-chin areas. Moreover, the formulation appeared to be endowed with an excellent tolerability throughout the experimental period.

The present study did confirm the clinical efficacy and tolerability of this cosmetic preparation. The results obtained in the group of healthy volunteers affected by mild to moderate degree of acne has shown, in fact, that the use of Acne cream significantly influences the investigated processes involved in acne pathology. In fact, Acne cream did reduce to a significant extent sebum production, and the data obtained using sebulae technique clearly indicated in addition that this preparation does not modify the amount of sebum produced by the skin, but rather it seems to act through the control of its outpouring to the cutaneous surface.

Our results have shown in addition that Acne cream had a significant comedolytic activity on macrocomeds. Moreover, the evidence that the treatment had no influence on microcomedons seems to rule out the possibility of a comedogenic activity of the investigated preparation.

These results were paralleled by significant diminutions in the major clinical signs associated to acne pathology, including erythema, scaling, and the presence of papule and pustules, and by a significant attenuation of subjective symptoms, such as itch and pain.

The overall results obtained in the present investigation were confirmed by the positive clinical evaluation made by the clinician at the end of the study, who reported that the treatment with Acne cream produced a global improvement of acne pathology in 76% of subjects included in the study. These results, coupled with the subjective statement of a significant clinical efficacy, good cosmetic acceptability and of a good to optimal tolerability, indicate that the cosmetic preparation Acne cream containing a definite association of neolignans extracted from the roots of *Krameria*, lauric acid and escin-β-sitosterol complex could be considered as a valid coadjuvant for the treatment of acne.

**Riassunto**

Valutazione dermatologica e strumentale dell’attività di un nuovo prodotto dermocosmetico adiuvante per il trattamento dell’acne

**Obiettivo.** Sebbene la patogenesi dell’acne non sia stata completamente chiarita, esiste un accordo generale nel considerare questo disordine cutaneo come il risultato di un’interazione complessa di diversi fattori, quali l’iperproliferazione epidermica, l’eccesso di sebo, l’inflammazione locale e la colonizzazione batterica con la presenza di *Propionibacterium acnes*; ognuno di questi fattori contribuisce in misura diversa allo sviluppo dell’acne. Nel corso degli ultimi 20 anni, il numero di farmaci per uso topico e sistemico per il trattamento dell’acne è enormemente aumentato dalla scoperta e dall’introduzione di nuove molecole o dagli ulteriori sviluppi di agenti terapeutici già disponibili. Essi comprendono gli antibiotici per uso topico, i retinoidi per uso topico, gli antimicrobici, il benzil perossido e l’acido ascorbico. Per il trattamento dell’acne vengono anche ampiamente utilizzate preparazioni cosmetiche, le cui formulazioni e i prodotti scelti soddisfano le linee guida relative alla sensibilità della cute. Questo lavoro descrive i risultati ottenuti in un gruppo di volontari sani affetti da acne di grado da lieve a moderato, che sono stati trattati per 4 settimane con una preparazione cosmetica, denominata Acne cream, che contiene neolignans estratti dalle radici della *Krameria*, acido laurico e complesso escin-beta-sitosterolo. Questa formulazione è stata intesa come coadiuvante per il trattamento dell’acne.

**Metodi.** Sono stati arruolati nello studio 32 volontari sani, 20 di sesso femminile, 12 di sesso maschile, età compresa tra 13 e 45 anni (età media 22 anni) che nel mese precedente l’inizio dello studio non si erano sottoposti a trattamento specifico per l’acne. Tutti i pazienti avevano una diagnosi clinica di acne di grado lieve o moderato, secondo la classificazione utilizzata da Cunliffe e Shuster, e tutti hanno fornito un consenso informato.

**Risultati.** I risultati ottenuti in questo studio evidenziano che l’applicazione continua di questa preparazione è in grado di ridurre in modo significativo l’aumento del sebo nelle aree cutanee studiate, probabilmente controllando la sua fuoriuscita dai pori verso la superficie cutanea. Inoltre, il trattamento ha ridotto la presenza di *Propionobacterium acnes*, *Streptococcus pyogenes* e *Staphylococcus aureus* nelle stesse aree cutanee. Questi risultati hanno dimostrato...
una diminuzione significativa dei segni clinici maggiori associati alla patologia acneica, quali l’eritema, la desquamazione e il numero di papule e di pustole. Sono anche stati osservati una diminuzione significativa di sintomi soggettivi quali il prurito e il dolore.

Conclusioni. In generale, i risultati, accoppiati ad un’eccellente tollerabilità e alla sensazione soggettiva di una significativa efficacia clinica e di una buona accettabilità cosmetica, indicano che Acne cream potrebbe essere considerato un valido coadiuvante per il trattamento dell’acne.

Parole chiave: Acne, terapia farmacologica - Farmaci anti-acne - Cosmetica.

References

Quantitative and qualitative evaluation of the effects of three cosmetic products containing pre-ceramides in restoring the skin barrier

L. CELLENO 1, S. BUSTACCHINI 2, S. REGGIO 2

Aim. Ceramides are synthesised from precursors, glycosphingolipids or pre-ceramides, and play a major role in maintenance and restoration of the skin barrier integrity. The aim of the conducted test is the evaluation of the cosmetic effect of 3 products containing pre-ceramides (face, body and hand treatment) for the restoration of the skin barrier physiological role in subjects presenting with a condition of dry skin and/or desquamation of face or body or presenting with hands affected by contact dermatitis.

Methods. Thirty volunteer subjects (10 for each formulation), aged 25-64 years, have been included in a controlled test comparing the cosmetic formulations containing pre-ceramides with a similar product (without pre-ceramides). Both active and control products have been used twice daily for 2 weeks. Instrumental evaluation of skin condition (with evaporimeter and corneometer) and evaluation of cosmetic effects and tolerability have been performed before the enrollment (t0), after 2 h since the first application, after 1 week of treatment usage (t7) and at the end of the test (t14).

Results. The products containing pre-ceramides, when compared with the control product, have rapidly induced a significant decrease in transepidermal water loss (P<0.003), a significant increase of skin hydration levels (P<0.001), a significant improvement in skin conditions with reduction of dryness and desquamation on face and body (P<0.0001) and a decrease of number of hand fissures (P<0.04).

Conclusions. The products containing pre-ceramides have shown a superior cosmetic effect, versus the control product, in improving the skin hydration and restoring the physiological role of skin barrier.

KEY WORDS: Skin barrier - Skin hydration - Glycosphingolipids - Ceramides.
ty acids, they form the highly ordered intercellular lipid lamellae. Ceramides are known to be synthesised from precursors, glycosphingolipids or pre-ceramides, and together with other polar lipids are secreted into the extracellular space of the stratum corneum by lamellar bodies and are subsequently processed by a set of colocalized lysosomal lipid hydrolases (β-glucocerebrosidases) into a more hydrophobic mixture, enriched in ceramides which play a major role in maintenance and restoration of the skin barrier integrity.

The exposure to various stressors, including pollutants, ultraviolet (UV) rays, chemical products, irritants and aggressive cleansing products, may alter the normal structure of stratum corneum, of the epidermal barrier function and of the skin surface lipids, particularly ceramides.

There is also evidence that skin disorders, such as skin xerosis, atopic dermatitis and others, also show a decreased barrier function associated with a disturbance of the lamellar structure of the stratum corneum lipids and alteration of the ceramide level. The aim of the conducted test is the evaluation of the cosmetic effects of 3 products containing pre-ceramides, for the restoration of the skin barrier physiological role in subjects presenting with a condition of dry skin and/or desquamation of face or body, or presenting with hands affected by contact dermatitis. The test has been performed in controlled conditions using instrumental methods (evaporimetry and corneometry) and dermatological examinations. In particular, separate tests have been carried out on 3 different formulations for face, body and hand treatment of high-quality and high-technology cosmetic products (CeramIDI Regeneration®, IDI Farmaceutici s.r.l., Pomezia, Rome, Italy), containing natural glycosphingolipids or pre-ceramides (derived through a biotech process from sunflower lecithin), together with other functional principles (moisturizers, emollients, film formers, cholesterol) to support the restoration of skin barrier physiological role.

**Materials and methods**

Three separate tests have been conducted on the 3 different cosmetic products containing pre-ceramides (pre-ceramides face formulation, pre-ceramides body formulation, pre-ceramides hand formulation), in controlled conditions and in comparison with a control product not containing pre-ceramides. Thirty volunteer female subjects have been enrolled (10 volunteers for each formulation), aged between 20 and 65 years. In detail, pre-ceramides face formulation and pre-ceramides body formulation have been evaluated in subjects presenting with a condition of dry skin and/or desquamation of face or the body (arm region) related to alteration of skin barrier physiological role (including, for example, atopic dermatitis or psoriasis in remission phase). The pre-ceramides hand formulation has been evaluated in subjects presenting with hands affected by contact dermatitis.

Further selection criteria have been used:

- Inclusion criteria:
  - good general health conditions;
  - absence of concomitant topical or systemic pharmaceutical treatments;
  - avoidance of UV exposure for tested skin area;
  - absence of concomitant usage of moisturizers or products for the treatment of contact dermatitis;
  - no changes in current life style;
  - no participations in other tests during the last 3 months.

- Exclusion criteria:
  - pregnancy or lactation;
  - any condition different from what stated in inclusion criteria.

All selected subjects gave their written informed consent to participation and have been enrolled and followed up for 2 weeks. They were directed to apply, twice daily, the tested pre-ceramides formulations on the right side of face, on the right arm and on the right hand, while the control product have been applied on the left side of face, on the left arm and on the left hand.

Scientific standards, controlled test designs and objective methods have been adopted in evaluations carried out under dermatologist control.

At inclusion (t0), 2 h after the first product application (t2h), after 1 week (t7) and 2 weeks of treatment (t14, end of the test period), each subject has been evaluated with objective methodologies for hydration of the stratum corneum and transepidermal water loss (TEWL). Dermatologic and subjective evaluations of cosmetic effects and tolerability were also included. The pre-ceramides hand formulation has been evaluated at t0, t7 e t14.

Objective instrumental testing at different times
QUANTITATIVE AND QUALITATIVE EVALUATION OF THE EFFECTS OF THREE COSMETIC PRODUCTS CONTAINING PRE-CERAMIDES CELLENO

included evaporimetry, to evaluate the skin barrier function and TEWL (g/m²/h), using Evaporimeter Ep-1 (Servo Med). Pre-ceramides face formulation and pre-ceramides body formulation were also evaluated through corneometry (Corneometer® CM 825 - Courage + Khazaka electronic GmbH), to measure the level of hydration of the stratum corneum.

At the same observation times, the dermatologist also performed an objective evaluation of the treated areas in term of degree of desquamation and skin dryness for pre-ceramides face formulation and pre-ceramides body formulation and degree of desquamation and erythema and number of skin fissures for pre-ceramides hand formulation. The enrolled volunteers, in the same condition, were asked for subjective evaluations in term of overall skin conditions including dryness, desquamation, redness, itching and burning sensation.

The evaluations have been expressed as scores from 0 to 6, where 0=none; 2=mild; 4=moderate; 6=severe. The final overall skin conditions have been scored from 0 to 4, where 0=dry skin; 2=normal; 4=oily skin.

The volunteers were also asked to give their opinion regarding the sensory performance of the tested formulations in terms of odour (scored from 0 to 4, where 0=unpleasant; 2=quite pleasant; 4=pleasant), spreadability (scored from 0 to 4, where 0=difficult; 2=quite easy; 4=easy) and absorption (scored from 0 to 4, where 0=slow; 2=normal; 4=fast).

Finally, the products were also tested in terms of local tolerability and occurrence of adverse events.

### Statistical analysis

The statistical analysis has been carried out using “Statgraphics” Version 5.1. Collected data have been evaluated with descriptive statistics (mean, standard deviation, range). The 2 treatment groups have been compared with multifactor analysis of variance (MANOVA), using subjects, treatments and time as classification factors. A post-hoc analysis with multiple range test (MRT) have been performed to identify possible single observation times which would show statistically significant differences between treatments.

### Results

The overall test results for the 3 different products, pre-ceramides face formulation, pre-ceramides body formulation and pre-ceramides hand formulation, are synthetically reported in Tables I, II e III, respectively. The results obtained with the objective instrumental evaluations (evaporimetry and corneometry) for pre-ceramides face formulation, pre-ceramides body formulation and pre-ceramides hand formulation are shown in Figures 1, 2 e 3, respectively.
Pre-ceramides face formulation and pre-ceramides body formulation, in comparison with the control product, have shown a progressive and statistically significant overall improvement for all tested parameters (Tables I e II). Significant differences versus the control product have been also detected since the first week of treatment (t7).

Pre-ceramides face formulation induced a significant decrease in TEWL (-36.6% versus -30.3% obtained with the control product; P=0.003) and a significant increase of hydration of stratum corneum (+38.6% versus +22.7%; P<0.0001) (Figure 1). The comparisons with the control product also resulted in a significant decrease of skin dryness (-74.5% versus -36.2%; P<0.0001), of desquamation (-86.8% versus -46.7%; P<0.0001), and a significant reduction of scores for subjective parameters: redness (-63.2% versus -47.4%; P=0.0123), skin dryness (-84.4% versus -42.2%; P<0.0001), desquamation (-88.6% versus -45.7%; P<0.0001), itching (-88% versus -48%; P=0.001), burning sensation (-88.9% versus -44.4%).

Similar differences have been also observed for pre-ceramides body formulation with a significant decrease in TEWL (-36.6% versus -30.3% obtained with the control product; P=0.0006) and a significant increase of hydration of stratum corneum (+40.7% versus +24.4%; P<0.0001) (Figure 2). The comparisons with the control product also resulted in a significant decrease of skin dryness (-67.3% versus -38.5%; P<0.0001), of desquamation (-77.1% versus -48.6%; P<0.0001), and a significant reduction of scores for subjective parameters: redness (-64.3% versus -50%; P=0.0013), skin dryness (-88.9% versus -50.5%; P=0.0001), itching (-88% versus -48%; P=0.001), burning sensation (-88.9% versus -44.4%).

### Table II.—Pre-ceramides body formulation. Synthesis of results (mean values).

<table>
<thead>
<tr>
<th></th>
<th>t0</th>
<th>t2h</th>
<th>t7</th>
<th>t14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-ceramides</td>
<td>Control</td>
<td>Pre-ceramides</td>
<td>Control</td>
</tr>
<tr>
<td>Corneometry</td>
<td>35.60</td>
<td>36.10</td>
<td>43.50</td>
<td>40.70</td>
</tr>
<tr>
<td>TEWL (g/m²/h)</td>
<td>10.83</td>
<td>10.82</td>
<td>9.44</td>
<td>9.70</td>
</tr>
<tr>
<td>Objective evaluations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin dryness*</td>
<td>5.20</td>
<td>5.20</td>
<td>3.40</td>
<td>4.10</td>
</tr>
<tr>
<td>Desquamation*</td>
<td>3.50</td>
<td>3.50</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Subjective evaluations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness*</td>
<td>1.40</td>
<td>1.40</td>
<td>1.20</td>
<td>1.30</td>
</tr>
<tr>
<td>Skin dryness*</td>
<td>4.80</td>
<td>4.80</td>
<td>3</td>
<td>3.80</td>
</tr>
<tr>
<td>Desquamation*</td>
<td>3.80</td>
<td>3.80</td>
<td>2.40</td>
<td>3.10</td>
</tr>
<tr>
<td>Itching*</td>
<td>2.50</td>
<td>2.50</td>
<td>1.50</td>
<td>2</td>
</tr>
<tr>
<td>Burning sensation*</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Overall skin condition**</td>
<td>0.70</td>
<td>0.50</td>
<td>1.50</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*) Scores from 0 to 6, where 0=none; 2=mild; 4=moderate; 6=severe. **) Scores from 0 to 4, where 0=dry skin; 2=normal; 4=ooily skin.

### Table III.—Pre-ceramides hand formulation. Synthesis of results (mean values).

<table>
<thead>
<tr>
<th></th>
<th>t0</th>
<th>t7</th>
<th>t14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-ceramides</td>
<td>Control</td>
<td>Pre-ceramides</td>
</tr>
<tr>
<td>TEWL (g/m²/h)</td>
<td>17.17</td>
<td>17.28</td>
<td>13.07</td>
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<tr>
<td>Objective evaluations</td>
<td></td>
<td></td>
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<tr>
<td>Erythema*</td>
<td>3.30</td>
<td>3.30</td>
<td>2.10</td>
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<tr>
<td>Desquamation*</td>
<td>4.10</td>
<td>4</td>
<td>2.70</td>
</tr>
<tr>
<td>Fissures*</td>
<td>3.10</td>
<td>2.90</td>
<td>1.80</td>
</tr>
<tr>
<td>Subjective evaluations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness*</td>
<td>2.60</td>
<td>2.60</td>
<td>1.60</td>
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<tr>
<td>Skin dryness*</td>
<td>4.30</td>
<td>4.20</td>
<td>2.20</td>
</tr>
<tr>
<td>Itching*</td>
<td>3.20</td>
<td>3.10</td>
<td>1.50</td>
</tr>
<tr>
<td>Burning sensation*</td>
<td>2.60</td>
<td>2.40</td>
<td>1.30</td>
</tr>
</tbody>
</table>

*) Scores from 0 to 6, where 0=none; 2=mild; 4=moderate; 6=severe. **) Scores from 0 to 4, where 0=dry skin; 2=normal; 4=ooily skin.
QUANTITATIVE AND QUALITATIVE EVALUATION OF THE EFFECTS OF THREE COSMETIC PRODUCTS CONTAINING PRE-CERAMIDES

celleno

dryness (-70.8% versus -43.8%; P<0.0001), desquamation (-81.6% versus -55.3%; P=0.0001), itching (76% versus -52%; P=0.0358). Pre-ceramides hand formulation has shown a significant and progressive decrease of TEWL (-39.6% versus -31% with the control product; P=0.0007) (Figure 3) and a significant reduction of the number of skin fissures (-61.3% versus -51.7%; P=0.04). This formulation showed also a better trend, versus the control product, in terms of improvement of desquamation and skin dryness, as evaluated by the dermatologist, and for dryness, redness, itching and burning sensation, as judged by the participants (Table III).

A noticeable improvement of the overall skin conditions at the end of the treatment period, in comparison with the initial status of the skin, has been underlined both by the dermatologist and by volunteers.

A positive evaluation of the sensory performance of the tested products has been given by the participants: the 3 formulations have a pleasant odour, an easy spreadability and were absorbed quite rapidly. No adverse events or issues for local tolerability have been reported for all the 3 tested cosmetic products.

**Discussion and conclusions**

Many skin disorders, including skin dryness and desquamation, are characterized by alterations of the skin barrier properties and ability to maintain an adequate hydration level of the outer layer of the epidermis: the *stratum corneum*.

The barrier function of the *stratum corneum* is provided by patterned lipid lamellae localized to the extracellular spaces between corneocytes. Cholesterol, free fatty acids and particularly ceramides form the main constituents of the intercellular lipid lamellae in *stratum corneum* and are responsible for and regulate the skin barrier function.
Ceramides are synthesised from precursors, glycosphingolipids or pre-ceramides, and play a major role in maintenance and restoration of the skin barrier integrity.

Scope of the present test was the evaluation of the cosmetic effect of 3 products containing pre-ceramides (face, body and hand formulations) for the restoration of the skin barrier physiological role in subjects presenting with a condition of dry skin and/or desquamation of face or body or presenting with hands affected by contact dermatitis.

Data obtained in the different conditions of use have shown that the effects of pre-ceramides formulations, applied for 2 weeks in 30 subjects with alteration of the skin barrier function, were superior to the effects associated with the use of the control product. Indeed, the analysis of data obtained with instrumental evaluations (evaporimetry and corneometry) showed a progressive and statistically significant overall improvement of TEWL and of hydration of the stratum corneum, in comparison with the results obtained with the control product. These evidences have been confirmed by the significant improvement of desquamation and skin dryness during the treatment with pre-ceramides face formulation and pre-ceramides body formulation, and the reduction of the number of skin fissures with pre-ceramides hand formulation. Both investigator dermatologist and volunteer participants also reported a significant subjective improvement of overall skin conditions, versus the basal conditions and in comparison with the control product, in terms of improvement of skin dryness, redness, itching and burning sensation.

The superior cosmetic activity of the tested face, body and hand formulations may be related to their content of ceramide precursors, or pre-ceramides (functional ingredient now available from sunflower lecithin through a biotech process of extraction), with hydrating and moisturizing properties and able to induce functional changes of human skin. A minor improvement of skin hydration was also showed by the control product, which does not contain pre-ceramides but included a range of moisturizing agents and lipids with a supporting role in maintaining epidermis integrity.

In conclusion, the products containing pre-ceramides have shown a superior cosmetic effect, versus the control product and through innovative and objective testing methodologies, in maintaining and restoring the physiological role of skin barrier and improving the skin hydration. These effects may be related to an increase production of lipids, particularly ceramides, included in the lipid lamellae surrounding corneocytes in the stratum corneum.

**Riassunto**

Valutazione quantitativa e qualitativa degli effetti di ripristino della barriera cutanea di tre formulazioni cosmetiche in crema con pre-ceramidi

**Obiettivo.** Le ceramidi, sintetizzate de novo a partire da gli-cosfingolipidi o pre-ceramidi, hanno un ruolo primario nel mantenimento dell’integrità della barriera cutanea. Scopo della ricerca è la valutazione dell’effetto cosmetico di 3 formulazioni in crema, per il viso, per il corpo e per le mani, contenenti pre-ceramidi, nel ripristino della fisiologica funzionalità della barriera cutanea, in soggetti con secchezza e/o desquamazione cutanea sul viso o nell’avambraccio e dermatite irritativa da contatto alle mani.

**Metodi.** È stato condotto un test controllato verso un prodotto di confronto, su 30 soggetti volontari (10 per ciascuna formulazione), tra i 25 e i 64 anni, trattati con le formulazioni con pre-ceramidi o la crema base 2 volte al giorno per 2 settimane. Prima dell’inizio del test (t0), dopo 2 h dalla prima applicazione (t2h), dopo 1 settimana di trattamento (t7) e al termine del test (t14) sono state condotte valutazioni fisiologiche cutanee (evaporimetrica e corneometrica) e valutazioni sugli effetti cosmetici e di tollerabilità locale.

**Risultati.** Le 3 formulazioni di crema con pre-ceramidi, in confronto alla crema base, hanno rapidamente indotto una significativa diminuzione della perdita d’acqua transepidermica (P<0,003), un significativo aumento della quantità d’acqua presente nello strato corneo (P<0,001), un significativo miglioramento della secchezza e della desquamazione della cute del viso e del corpo (P<0,0001) e delle fissurazioni nelle mani (P<0,04).

**Conclusioni.** Le 3 formulazioni di crema con pre-ceramidi hanno dimostrato un effetto superiore, rispetto alla crema base, nel migliorare l’idratazione e nel ripristinare la fisiologica funzionalità della barriera cutanea.

**Parole chiave:** Barriera cutanea - Idratazione cutanea - Glicosfingolipidi - Ceramidi.

**References**

Psoriasis and heart. Something new under the sun

A. PIETRZAK 1, K. JANOWSKI 2, J. LOPATYNSKI 3, G. CHODOROWSKA 1, A. IGNATOWICZ 3, S. STEUDEN 2
A. WITCZAK 3, D. KRASOWSKA 1, T. M. LOTTI 4

The article reviews classical and current literature concerning relationships between psoriasis and heart. Numerous epidemiological studies point to an increased risk of heart conditions in psoriasis patients. Various risk factors for cardiac pathologies were also observed to be increased in psoriasis patients. Studies applying echocardiographic and electrocardiographic examinations showed higher frequency of cardiac abnormalities in patients with psoriasis than in control groups. Possible mechanisms responsible for an increased prevalence of cardiac pathologies in psoriasis are discussed including lipid metabolism disturbances, systemic effects of altered patterns of proinflammatory cytokines, increased cardiac pathologies due to other conditions comorbid with psoriasis and side effects of systemic treatments for psoriasis.

Key Words: Psoriasis - Skin - Heart.

Psoriasis is defined as a chronic inflammatory skin disease.1, 2 In recent years more and more papers have been published2-5 suggesting that psoriasis should be regarded as a disease which affects not only the skin, but also other organs and systems. One of them is heart and the cardiovascular system. Associations between psoriasis and pathologies of the cardiovascular system have repeatedly been reported, although research in this area is still in the initial phase.2, 3, 6-8

Epidemiology of cardiac pathologies in psoriasis

Prevalence of cardiovascular disease and cardiovascular risk factors in psoriasis

Most epidemiological studies demonstrated an increased prevalence of cardiovascular diseases (CVD) in patients with psoriasis. Increased incidence of occlusive vascular diseases and higher prevalence of CVD was observed in patients with psoriasis as compared to patients with other cutaneous diseases in well controlled studies.2 Moreover, a higher rate of ischemic heart disease, hypertension, hypercholesterolemia and hypertriglyceridemia were reported in hospitalised psoriasis patients when compared to patients hospitalised for other dermatological conditions.2 A statistically significant association between psoriasis and hospitalisation for hypertension in males and hospi-
talisation for myocardial infarction in females was also reported.\(^2\) In patients hospitalised for psoriasis an increased prevalence of hypertension and heart failure was observed. Hospitalisation for psoriasis was also found to be associated with mortality due to cardiovascular causes: the standardized mortality ratio among outpatients with psoriasis was 0.94, whereas among patients hospitalised at least once for psoriasis it was increased by 50%.\(^2\)

Robinson \textit{et al.}\(^6\) carried out an extensive analysis of risk factors for comorbidity in psoriasis, using two large national US claims databases. A total of 54 455 patients with psoriasis were identified in the databases and included into the analysis. Patients with psoriasis were found to have a higher risk for atherosclerosis, hypertension and type 2 diabetes mellitus than nonpsoriatic controls. In another large-scale epidemiological study, Neumann \textit{et al.}\(^8\) evaluated cardiovascular risk factors in 131 560 patients with psoriasis identified in the General Practice Research Database in the United Kingdom. In comparison to nonpsoriatic controls, patients with psoriasis had a higher risk for diabetes, hypertension and hyperlipidemia and were also more likely to be current smokers. Gelfand \textit{et al.}\(^7\) used the same General Practice Research Database in the United Kingdom to evaluate the risk for myocardial infarction among patients with mild and severe psoriasis. They found that both groups of patients with psoriasis had a higher risk for developing myocardial infarction in comparison to controls, although the risk ratio was higher in the group with severe psoriasis. These authors also observed a modifying effect of age on the risk of myocardial infarction, with younger patients being at a greater relative risk. In a large-scale epidemiological study, Crown \textit{et al.}\(^9\) found a significantly increased incidence of diabetes and hypertension in patients with psoriasis in comparison to nonpsoriatic patients. Diabetes occurred in 13.2% of psoriasis patients as compared to 9.6% of nonpsoriatic patients and hypertension was present in 35% of psoriasis patients as compared to 29.1% of nonpsoriatic patients. Systemic conditions comorbid with psoriasis were also found to be predictors of annual hospitalisation risk.

Several studies found an association between psoriasis and obesity – a known cardiovascular risk factor.\(^2\)-\(^10\) In a case-control study, patients with new onset psoriasis were more likely to be obese than patients with other skin problems. This association persisted even when controlling for other potentially confounding variables.\(^10\) A positive association was also found between psoriasis onset and body mass index (BMI).\(^10\)

In patients with psoriasis and in their first-degree relatives, increased incidence of diabetes – another cardiovascular risk factor – was also observed.\(^11\) An increased prevalence of diabetes mellitus in psoriatic patients was reported in a study that included 40 000 subjects.\(^2\),\(^11\),\(^12\) The prevalence of psoriasis among patients with diabetes mellitus was also found to be increased. Ucak \textit{et al.}\(^12\) and Romano \textit{et al.}\(^13\) found that the prevalence of psoriasis in diabetic patients was 9%, which was higher than in the general population.

Several studies provided evidence for the relationship between psoriatic arthritis (PA) and CVD. Reed \textit{et al.}\(^14\) observed an increased rate of myocardial infarction, pulmonary emboli and pulmonary infarcts as a cause of mortality in patients with PA. Other authors sporadically reported cardiac abnormalities (e.g. aortic regurgitation) co-occurring with PA.\(^15\)

Although most studies confirmed an increased prevalence of CVD and cardiovascular risk factors in psoriasis, some studies, however, failed to find such associations. Neumann \textit{et al.}\(^2\) quoted also studies which found no evidence of increased cardiovascular mortality in these patients as compared to the expected rates in the general population. Further epidemiological studies are needed to explain these controversies.

### Cardiomyopathies in psoriasis

There are single reports on co-occurrence of cardiomyopathy, psoriasis and cardiac muscle diseases.\(^16\),\(^17\) Wojtyna and Enseleit\(^15\) reported a rare case of a complete heart block after topical treatment for psoriasis. The substance applied into the skin was a homemade lotion of \textit{Nerium oleander} blooms and leaves preboiled with water. Although cases of poisoning with \textit{Nerium oleander} had been known earlier, this was the first report in which the intoxication-induced cardiac failure resulted accidentally from the transdermal application of the substance for psoriasis. Jha \textit{et al.}\(^18\) reported a case of 75-year-old woman suffering from erythrodermic psoriasis who developed a cardiogenic shock. The patient exhibited persistent severe vasodilation after coronary artery bypass surgery, which was caused by a flare-up of erythro-
dermic psoriasis previously controlled by treatment with methotrexat. Vasodilatation disappeared after treatment was re instituted. Overall, however, the co-occurrence of cardiomyopathies and psoriasis seems coincidental.

**Echocardiographic assessment of the cardiac function in psoriasis and psoriatic arthritis**

Echocardiography is a gold standard method in the evaluation of the heart function in different disorders. Being easily available and precise, echocardiography enables detailed evaluation of numerous parameters of the heart muscle function and valvular structure and function. Application of this methods makes it possible to perform many measurements as following:

- size of the heart and large vessels as well as left ventricle mass;
- systolic function of the left ventricle, including the global systolic function with its parameter ejection fraction (EF) and regional wall motion abnormalities;
- diastolic left ventricle function, including parameters of mitral inflow (E to A ratio, deceleration time, DT, isovolumetric relaxation time, IVRT) and pulmonary vein inflow;
- valvular disorders, including structural and functional abnormalities, especially stenoses and regurgitations;
- pericardial disease, including pericardial effusion, tumours, calcification;
- noninvasive calculation of maximal pulmonary artery systolic pressure (PASP);
- qualification and quantification of congenital heart disease;
- pathological intracardiac masses, including thrombi, tumours and vegetations.

More advanced techniques like tissue Doppler imaging enable to evaluate regional velocities of heart muscle. Basing on these measurements, it is possible to assess systolic function (S’ wave as the velocity of the given region during right ventricle or left ventricle systole) or diastolic one (E’ to A’ ratio, where E’ reflects the velocity of early filling, A’-late filling of the given region of any ventricle).

Wojas-Pelc et al.\(^\text{15}\) reported results of the echocardiographic examination carried out in 3 groups: 28 healthy volunteers, 15 patients with *psoriasis vulgaris* and 7 patients with PA. The authors conducted one- and two-dimensional echocardiography and cardiac blood flow examinations with continuous, pulse and colour Doppler’s method. The patients with PA more frequently exhibited aortic insufficiency, dilation of the ascending aorta and systolic left dimension. The dilation of the ascending aorta correlated significantly with the frequency of the aortic wave regurgitation, the duration of psoriasis and patients’ age.

Markuszewski et al.\(^\text{19}\) conducted echocardiography in 89 patients with psoriasis, 63 of whom were men and 26 women, with age ranging from 31 to 55. The mean duration of psoriasis was 10 years. The parameters of the systolic and diastolic functions of the left ventricle did not differ significantly in psoriasis and healthy controls groups. However, mitral insufficiency was observed significantly more frequently in patients with psoriasis than in healthy controls (52 vs 4). Aortic insufficiency also occurred more frequently in psoriasis patients than in controls (10 vs 1). The authors also observed a correlation between the frequency of mitral incompetence and the duration of psoriasis.

Saricaoglu et al.\(^\text{1}\) demonstrated more frequent co-occurrence of PA and changes in echocardiography. They examined 21 patients with PA and 16 healthy volunteers matched for age and sex by means of echocardiography using Doppler’s method. The results showed that the left ventricle end-diastolic and end-systolic diameters in the patients with PA were significantly different from those in the control group. No difference was found in EF and the mitral early and late (E/A) diastolic filling wave ratios. The occurrence of diastolic dysfunction was associated to the presence of arthropathy and the duration of psoriasis. In patients with PA, the mean left ventricle diastolic diameter was 52.76 mm, whereas in the control group it was 46.87 mm. The diastolic dysfunction (mitral valve E/A ratio) was found in 11 of the 21 patients. Systolic dysfunction (EF <55%) occurred in 3 patients. The analyses carried out to control sex effects showed that sex had no influence on the parameters in either group.

**Electrocardiographic assessment of the cardiac function in psoriasis**

Abnormalities in the neurovegetative system were observed in psoriasis in some cases evidenced by ECG examinations. Psoriasis patients were frequently found to show changes in the neurovegetative system, which was confirmed by means of various methods. In particular, Huriez et al.\(^\text{20}\) applying physiological and bio-
Mechanisms accounting for the associations between psoriasis and cardiac function

The mechanisms responsible for the associations between psoriasis and increased rates of various cardiac conditions reported in epidemiological studies are still subject to ongoing research. It is generally believed that these associations may be accounted for by diverse mechanisms involving the inflammatory processes observed in psoriasis, abnormalities in microcirculation, abnormalities in lipid or carbohydrates metabolism, oxidative stress, other conditions co-occurring with psoriasis and side-effects of treatments used in psoriasis. Some of these mechanisms will now be discussed.

Lipid metabolism disturbances in psoriasis

In psoriasis, abnormalities in lipid homeostasis occur on various levels, not only in the blood plasma but also in the psoriatic epidermis. Patients with psoriasis were observed to have abnormal lipid profiles more frequently than appropriately matched subjects from control groups. Psoriasis is itself accompanied by numerous lipid phenomena related to everyday loss of cholesterol from the surface of the permanently desquamating skin, damage to the plasmatic membranes, altered cellular adherence, and widening of the extracellular space. The dysregulation of the mutual adhesion of cells in the epidermis probably results from the abnormalities of lipid metabolism in this layer. Recent studies reported associations between lipids and the dendritic cell (DC) function, whose role in the pathogenesis of psoriasis is well documented. An example of such associations is production of anti-inflammatory oxidized fatty acids and proinflammatory lysophosphatidylcholine (LPC) in the course of LDL oxidation. Oxidized LDL (oxLDL) provides informative molecules such as LPC which stimulate the generation of mature DC from differentiating monocytes. LPC affects the functions of peroxisome proliferators-activated receptors (PPARs). The stimulation of DC maturation by LPC is associated with complete inhibition of PPARγ activation and increased activity of an unspecified nuclear receptor binding the DNA sequence which modifies the response to factors activating peroxisome proliferation. Oxidized fatty acids generated during LDL oxidation are natural ligands for PPARγ and inhibit maturation of DC induced by oxLDL and LPC. Therefore, the relative amount of oxidized fatty acids and LPC affects the immunological significance of oxLDL for DC functions, partially by regulation of the transmission of the signal through PPAR.

Other types of oxidized lipids identified in oxLDL show anti-inflammatory effects. These lipids are products of the peroxidation of linoleic and arachidonic acids which occur in the majority of human LDL fatty acids. LDL oxidation leads to transformation of linoleic acid and arachidonic acid into hydroperoxy derivatives which are then converted to hydroxyoctadecadienoic acid (HODE) and hydroxyeicosatetraenoic acid (HETE), respectively. OxLDL and modified phospholipids such as LPC produced during acute phase response (APR) can inform the immune system about the presence of a dangerous situation. They also favour the development of adaptive immunity by stimulation of mature DC generation. Campanati et al. demonstrated increased serum levels of anticardiolipin antibodies in psoriasis patients as compared to healthy controls. The interaction between anticardiolipin antibodies and oxLDL, whose increased levels were also reported in psoriasis, may promote the pathological processes characteristic for the initial stage of atheroma formation and inflammation within arterial vessels. Autoantibodies against double-stranded DNA (dsDNA) in patients with PA were also found. So far, the role of autoantibodies against dsDNA has been implicated in pathogenesis of psoriasis and systemic lupus erythematosus although their functions are still not fully understood.

There are only few studies concerning serum phospholipid concentrations in psoriasis and their results are inconclusive. Some authors observed decreased serum concentrations of total phospholipids and individual phospholipid fractions, such as phosphatidy-
loethanolidin and lecitin, and the lecithin/cholesterol index, whereas other researchers found increased concentrations of certain serum phospholipid fractions.\textsuperscript{26} Decreased levels of polyunsaturated phospholipid fatty acids – linoleic, docosatetraenoic, docosapentaenoic and docosahexaenoic acids were observed in patients with psoriasis.\textsuperscript{26} Investigation concerning concentrations of phospholipid fatty acids showed increased levels of palmitic (16:0), palmitoleic (16:1 \textgreek{c} 7) and dihomo-\textgreek{g}–linoleic (20:3 \textgreek{c} 6) acids. Although the function of phosphatidylcholine was started to be investigated years ago, it is only now that it was suggested to be significant for DCs.\textsuperscript{26} It would be most interesting to simultaneously observe the lipid and immune changes occurring in the blood and epidermis.

Abnormal phospholipid metabolism was also demonstrated both within psoriatic lesions and the lesion-free skin.\textsuperscript{26} Psoriasis was suggested to involve a decrease in the rate of fatty acid metabolism, particularly in phospholipid molecules containing phosphatidylcholine.\textsuperscript{26} Epidermis in psoriatic lesions, in areas adjacent to the lesions and healthy epidermis showed moderate but statistically significant changes with respect to some phospholipids.\textsuperscript{26} Ansidei et al.\textsuperscript{27} observed a significant increase in phospholipid concentrations and decrease in phospholipid arachidonic acid in psoriatic lesions.

**Proinflammatory cytokines, cardiac conditions and psoriasis**

Numerous immunological studies indicate pathology in the patterns of cytokines and their receptors within the psoriatic epidermis and in the peripheral blood. On the other hand, recent reports\textsuperscript{28} provide data suggesting associations between certain cytokines and CVD. Tiret et al.\textsuperscript{28} found that baseline IL-18 levels were predictive of mortality due to cardiovascular causes over 4 years of follow-up. Variations in the IL-18 gene were related to differences in levels of circulating IL-18 and to clinical outcomes in patients with CVD. It is of interest that the same interleukin has also been implicated in the pathogenesis of psoriasis. Increased levels of IL-18 were reported both in the blood and epidermis cells of psoriatic patients.\textsuperscript{29, 30} It is probable that proinflammatory effects of IL-18 and other cytokines influence simultaneously the skin and other target organs, such as coronary arteries. Therefore, the abnormal expression of cytokines observed in psoriasis may also play a causal role in CVD.

In a study carried out at our centre,\textsuperscript{22} we found evidence for the associations between selected indices of the inflammatory process in psoriasis and known biochemical cardiovascular risk factors. Thirty-four men with psoriasis and 26 healthy men took part in the study. Patients and controls were particularly matched for BMI, age, and alcohol and tobacco use. Serum lipid, lipoproteins, autoantibodies against oxidized LDL (AuAb-oxLDL) and total peroxide concentrations were measured as the potential factors known to play a role in CVD. Additionally, peripheral blood DC and plasma IL-18 levels were measured as indices of the immune response in psoriasis. Patients with psoriasis were also assessed on measures of psoriasis severity (PASI, percentage of the affected skin, duration of the disease and duration of the current relapse).

We found no differences between psoriasis patients and healthy controls in the total serum cholesterol level. However, patients with psoriasis had significantly lower levels of HDL cholesterol and significantly increased levels of lipoprotein A-I, AuAb-oxLDL and total peroxide concentration in comparison to the control group. Blood DC subset count was significantly decreased and plasma IL-18 level was significantly increased in patients with psoriasis as compared to healthy controls, which is a finding indicative of an increased inflammatory process in psoriasis. In the group of patients, the assessed potential risk factors for CVD were found to correlate significantly both with indices of the immune response in psoriasis, such as DC counts and IL-18 level, and with clinical characteristics of psoriasis, such as the PASI score, percentage of lesional skin and duration of the disease. These findings suggest a disturbed pattern of cholesterol fractions, lipoprotein A-I, AuAb-oxLDL and the total peroxide concentration in patients with psoriasis. Their correlations with both immunological parameters and clinical features of psoriasis suggest that psoriatic inflammatory processes can be pathogenetically linked to lipid metabolism abnormalities playing a role in CVD.

**Cardiac pathologies related to other comorbidity in psoriasis**

Some cardiac conditions may be related to comorbidities in psoriasis, such as diabetes mellitus. It is
Side-effects of treatments for psoriasis

Some treatments used for psoriasis carry an increased risk of cardiovascular complications. The presence of lipid pathologies comorbid with psoriasis, both occurring prior to the onset of psoriasis and independent of psoriasis may increase the cardiac risks especially when a systemic treatment is introduced. This problem is particularly important in treatment with etretinate and acitretin.

Recent introduction of biological therapies for psoriasis also raised the question of their relationship to cardiovascular risks. Anti-TNF therapies (infliximab and etanercept) may contribute to the onset or worsening of pre-existing heart-failure symptoms in some patients with psoriasis. A temporal association was observed between occurrence of the cardiac symptoms and introduction of these drugs. In younger patients improvement in cardiac symptoms was also observed after withdrawal of anti-TNF treatment. British Association of Dermatologists guidelines advise careful assessment of psoriasis patients with heart diseases prior to treatment with anti-TNF therapies and withdrawal of this treatment in case of occurrence or worsening of cardiac symptoms.

Conclusions

Most studies review in this article provide evidence for an increased risk of cardiac pathologies in the population of psoriasis patients. The mechanisms accounting for the associations between psoriasis and heart abnormalities are diverse and still not completely clarified. Psoriatics seem to be especially susceptible to lipid metabolism disturbances, and thereby to dyslipidemia with changes in serum lipid composition resembling those in CVD. Overall, the evidence gathered so far on the relationships between psoriasis and heart, in clinical practice warrants more careful screening of psoriasis patients for potential adverse effects psoriasis can exert on the heart function.

References

Some genotypic and environmental conditions of the host in cutaneous malignant melanoma

M. R. BONGIORNO, G. PISTONE, M. ARICÒ

The factors involving the development of melanoma vary according to the phenotype and environment conditions experienced by the host. MC1R variant alleles that are associated with increased melanoma risk are likely to sensitize melanocytes to DNA damage by ultraviolet (UV) exposure, and the interactions between UV radiation and the genome induce melanoma. In fact, the non-functional MC1R cells have a very slow rate of cyclobutane pyrimidine dimers removal. The most significant mutations induced by UV in the skin occur in the CDKN2A gene being the major target of UV, that encodes 2 distinct proteins cell cycle regulators: p16INK4A and p14ARF. Inactivation of both tumor suppressors results in escape from cell cycle arrest because of disruption of the G1/S restriction point. The outcome is premature cell cycle progression and incomplete repair of DNA damage that leads to genomic instability and mutations. In fact, the signaling pathways of UV in melanocytes reveal the complex interrelationship between the pathways that regulate survival, proliferation and melanogenesis.

KEY WORDS: Environment - Ultraviolet rays - Melanoma.

The development of a melanoma generally requires many steps, each governed by multiple factors, which are dependent on the genetic constitution of the individual, on his or her environment and way of life.

The skin is a highly dynamic and protective organ and is exposed daily to ultraviolet radiation (UVR) from sun, occupational light sources, and phototherapy systems.

According to the Commission Internationale de l’Eclairage, 1987, UVR is divided into 3 regions: UV-A (320-400 nm), UV-B (290-320 nm with a peak at 305 nm) and UV-C (200-90 nm with a peak at 254 nm).

The net effects of UVR are dependent upon the epidermal thickness, the concentration, and distribution of chromophores, UVR absorbing biomolecules, including melanin, DNA, amino acids, caroten and urocanic acids. In the skin, melanin is an important chromophore and acts as a filter by absorbing the UVB, UVA, and visible spectrum.

The ability to adjust the melanization of epidermal cells after sunlight exposure reflects the individual’s melanogenic potential, which led to the concept of facultative and constitutive skin color.

Constitutive skin color designates a genetically determined level of cutaneous melanin, in the absence of acquired exogenous or endogenous influences. Facultative pigmentation, on the other hand, designates an induced level of increased epidermal melanin content as a result of solar radiation, hormones or other environmental factors.

The significance of human cutaneous pigmentation...
lies in its protective role against sun-induced DNA damage and photocarcinogenesis. Eumelanin fulfils photoprotective functions in the skin by directly absorbing both UV photons and the reactive oxygen species generated by the interaction of UVR with membrane lipids or other cellular components. Inversely, pheomelanin may contribute to skin carcinogenesis by producing free radicals in response to UVR.

Variations in human skin pigmentation are the result of mixed melanogenesis. Total melanin content and the relative amounts of eumelanin, the black-brown pigment, and pheomelanin, the red-yellow pigment, synthesized by human epidermal and follicular melanocytes are important determinants of skin and hair color, respectively. Red hair has a low ratio of eumelanin to pheomelanin, and black hair has a high ratio of eumelanin to pheomelanin, whereas blond hair contains little of either class of melanin. These relatively small differences in total melanin content apparently account for up to a 100-fold difference in UVR sensitivity, as judged by erythemal responses. Epidemiologic studies have identified certain phenotypic factors consistently associated with increased risk for the development of malignant melanoma. These factors include blue, green, or gray eyes; blond or red hair; light complexion; propensity to sunburn, and a number of severe sunburn episodes during youth.

The genetic control of human pigmentation is complex. It is likely that a relatively small set of genes contribute most of the variation in pigmentation phenotypes seen in human populations, and that they do this by regulating the level of synthesis, chemical composition, packaging and distribution of melanin. Human pigmentation, however, is regulated by more than 120 genes, and among them MC1R is the only gene in which variations can explain differences in normal pigmentation in humans.

The MC1R gene, located on 16q24, encodes a G protein coupled receptor with 7 transmembrane domains expressed on many cell types, including melanocytes. The melanocortin-1 receptor (MC1R) functions as a primary regulator of eumelanin synthesis in mammalian melanocytes and has been found to express high-affinity receptors for the α-melanocyte-stimulating-hormone (α-MSH) ligand, a cleavage product of pro-opiomelanocortin prohormone (POMC), which is a physiologic agent that controls pigmentation in mammals by inducing melanocyte differentiation and melanin production.

Its binding to the MC1R activates adenylate cyclase, increases intracellular cyclic adenosine monophosphate (cAMP) production, and then leads to activation of protein kinase A (PKA), which in turn leads to increased transcription of microphthalmia transcription factor (MITF) essential for the activation of the eumelanogenic pathway.

MITF expression leads to increased transcription of a range of genes (including genes encoding tyrosinase and tyrosinase-related protein 1 and 2) involved in melanocyte proliferation and, ultimately, in the control of the relative and absolute amounts of eumelanin and pheomelanin. Regulation of melanogenesis also depends on many paracrine factors, such as agouti signalling protein, a 132-amino acid protein, produced by the dermal papillae cell. In fact, the MITF expression induction can be inhibited by the agouti signal protein that acts as a competitive inhibitor of α-MSH binding to MC1R causing a switch from brown/black eumelanin to red/yellow pheomelanin.

However, the eumelanin/pheomelanin ratio may differ markedly in individuals so that individuals with a similar appearance may vary widely in the amount and type of pigment within the skin and this ratio may influence the minimal erythemal dose or UV sensitivity.

MC1R is remarkably polymorphic in whites, with more than 30 allelic variants, with amino acid substitutions within the coding region. The human MC1R variants can lead to a change in ligand binding, a change in function, or loss of function of the MC1 receptor.

Loss of function variants may lead to reduced ability to stimulate the production of intracellular cAMP and underlines the diverse range of human pigmentation phenotypes and skin phototypes. Among the variants of the MC1R, Arg151Cys, Arg160Trp, and Asp294His are some of the mutations mainly associated with fairer skin type, red-hair phenotype and other traits such as nevus density and reduced ability to tan that are considered to be at high risk for skin cancer. Categorization of skin- and hair-color phenotypes is based on broad divisions that, by their generalized
nature, cannot be applied as an accurate predictor of the outcome of any individual’s lifetime sun exposure and resultant skin-cancer rate.

It is, thus, necessary to seek a more direct and quantitative genotypic assessment of risk. The extent to which nevi are precursor lesions, a marker of sun exposition and/or a marker of a genetic defect that is associated with the development of melanoma remains uncertain. An example that illustrates the uncertain connection between typical nevi and malignant melanoma risk is that girls with Turner syndrome have a significantly increased number of nevi without an apparent increase in malignant melanoma risk.

However, the MC1R genotype does not exert any effect on CMM risk through the intermediate variable of mole count, especially in the pale-skinned phenotype in which its effect is greatest. This group, in fact, is associated with a decreased mole count.

This mechanism is a direct effect of pheomelanin and is consistent with the two-hit “divergent-pathway” model. In this model, epidermal melanocytes are predominantly initiated and transformed in early life following exposure to UVR, and the factors which then promote and drive tumor development vary according to phenotypic (presence of pheomelanin), and the environmental conditions experienced by the host. However, in the wavelength range of UVR, the photons of light are highly energetic and can initiate photochemical reactions in the biological molecules.

UVB and UVC irradiation can generate pyrimidine dimer photoproducts, particularly cyclobutane pyrimidine dimers (CPD) and 6,4-photoproducts (64PP), at a ratio that varies from 4:1 to 10:1. The phototoxic effect of UVA radiation is much lower than UVB, since DNA is not a chromophore for the long UVA wavelengths.

However, UVA can produce DNA damage indirectly through the generation of oxidative stress. Melanocytes with high eumelanin to pheomelanin ratios and MC1R loss-of-function are more sensitive to UVR-induced cytotoxicity, which suggests that the inability of melanocytes to respond to a-MSH reduces their defense mechanisms against UVR genotoxicity. In fact, the nonfunctional MC1R cells have a very slow rate of CPD removal, suggesting the importance of MC1R/α-MSH in DNA repair. However, the most significant mutations induced by UV in the skin occur in the CDKN2A gene being the major target of UV.

The CDKN2A region of chromosome 9p21 encodes 2 distinct proteins, translated in alternate reading frames (ARFs) from alternatively spliced transcripts. The alpha transcript, which comprises exons 1α, 2, and 3, encodes a low-molecular weight protein, p16INK4A. The smaller beta transcript, which comprises exons 1β and 2, encodes the alternative protein product, p14ARF.

P16INK4a is a cell cycle regulator that specifically inhibits the cyclin-dependent kinases CDK4 and CDK6 (CDK4/6) and consequently the cyclin D-dependent phosphorylation of the Rb, leading to reduced transcription of E2F responsive genes which promotes the G1-to-S phase transition of the cell cycle. When p16 is induced, the Rb protein is maintained in its nonphosphorylated (active) form, E2F is not activated and replication is halted. The relationship between p16 and melanoma has been explained by the observation that UV light can induce p16 expression in human skin, thereby implying a role for p16INK4a in the repair of UV-induced DNA damage. The ARF promoter is transcriptionally activated by E2F-1 and conversely repressed by p53. These interactions provide an autoregulatory feedback loop between p53, HDM2, a ubiquitin ligase, that targets p53 to destroy by proteasomes, and the ARF pathway to that of Rb.

This activates p21 which then inhibits the phosphorylation of Rb, leading to cell cycle arrest in both G1 and G2.

The ARF promoter is transcriptionally activated by E2F-1 and conversely repressed by p53. These interactions provide an autoregulatory feedback loop between p53, HDM2, and ARF and also link the ARF pathway to that of Rb.

Inactivation of both tumor suppressors results in escape from cell cycle arrest because of disruption of the G1/S restriction point. The outcome is premature cell cycle progression and incomplete repair of DNA damage that results in genomic instability and mutations.

These abnormalities in the genetic pathways within the melanocyte has been seen in “atypical or dysplastic nevi”, but the terms atypical or dysplastic nevi
remain controversial. As described by Clark in 1978 atypical nevi are larger by 5-6 mm, somewhat irregular in shape, and with mild colour variation, but these are the same features that are employed by dermatologists, worldwide, for clinical diagnosis of melanoma. In short, Clark’s criteria with regard to the clinical diagnosis of “dysplastic nevi” simply do not work because they do not allow for a differentiation between that particular kind of nevus and melanoma.

For these reasons the term “dysplastic nevi” must be used only for those lesions that have been shown histologically to contain dysplastic melanocytes, while these clinical and pathologic features not are always correlated.

All these findings suggest that the factors driving the development of melanoma vary according to the genotypic and environmental conditions experienced by the host. MC1R variant alleles that are associated with increased skin cancer risk are likely to sensitize melanocytes to DNA damage by UV exposure, and the interactions between UVR and the genome induce melanoma. In fact, the signaling pathways of UV in melanocytes reveal the complex interrelationship between the pathways that regulate survival, proliferation and melanogenesis.

Therefore, identifying genetic markers of the disease will allow for more precise screening of individuals with a high risk of melanoma.

Riassunto

Condizioni genotipiche e ambientali dell’ospite con melanoma cutaneo maligno

I fattori che guidano lo sviluppo del melanoma variano a seconda delle condizioni fenotipiche e ambientali dell’ospite. Le varianti all’eliche MC1R che sono associate a un rischio aumentato di melanoma probabilmente rendono il DNA dei melanociti più sensibile ai danni provocati dall’esposizione ai raggi UV e le interazioni tra la radiazione UV e il DNA inducono il melanoma. Infatti, le cellule MC1R non funzionali hanno un tasso molto basso di riparazione incompleta dei danni a carico del DNA che portano all’instabilità gnomica e alle mutazioni.

Infatti, la via attraverso la quale gli UV agiscono sui melanociti rivela la complessa interrelazione esistente tra le vie che regolano la sopravvivenza, la proliferazione e la melanogenesi.

Parole chiave: Fattori ambientali - Raggi ultravioletti - Melanoma.

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Experimental, epidemiologic and clinical studies in the last years underline the importance of longer ultraviolet (UV) in actinic keratoses. Most UV-induced lesions are repaired but mutations and immunological modifications can appear and modify the surveillance against UV-induced carcinogenic elements. Oncosuppressor genes (p53 and others) have a protective role and UV modifies the expression of some molecules (ligand CD05, TRIAL e TRIAL receptors) that preserve cell integrity and transformation. Numerous important photoprotection endogenous systems activated by radiation, become not sufficient after intense and prolonged UV-irradiation. Actinic keratoses can evolve into epitheliomas. Transformation is induced by cumulative solar radiation effects. Progression of actinic keratoses versus carcinoma is correlated with region 9 p21 deletion codifying for tumor suppressor p16 (INK4a). An initial step of actinic keratoses progression from dysplastic status versus carcinoma is the expression of metalloproteinase (MMP-1). UV-induced DNA damage activates repairing systems and MSH2 system. Recent studies demonstrate that feomelanin/eumelanin can represent a parameter as risk index for skin damage.

**Key Words:** Keratosis - Ultraviolet rays - Skin.

Actinic keratoses originate from a complex sequence of events beginning with ultraviolet (UV) light exposure. The prevalence is correlated to the age and to clinical aspects and signs of prolonged solar exposure, like elastosis and lentigo. To study the role of solar radiation it is necessary first of all to consider the interaction factors between solar radiation and skin.

**Solar radiation and skin: interactions and effects**

The solar radiation has a wide spectrum of radiations, but UV ones involve primarily the skin. UV radiations induce: 1) damage of DNA keratinocytes; 2) reduction of the capacity to repair skin modifications; 3) alteration of local immune reactivity. UV radiations present direct and indirect effects on the skin. They are adsorbed by endogenous photoreceptors (chromophores): coenzymes (NADH-NADPH, riboflavine, cytochromes), aromatic aminoacids as tryptophan, enzymatic proteins, DNA bases, urocanic acid. This indirect action is based on free radicals and reactive oxygen species (ROS, singlet oxygen, peroxide hydrogen, superoxide anion) production with reduction and damage of antioxidants systems, with lipids and proteins oxidation, and genic alterations.

Most of UV-induced lesions is repaired but mutations can result. Many damaged cells continue to grow and divide with irregular keratinization with the appearance...
of an epidermic stoned area, characterizing the keratoses clinically.

Experimental, epidemiological and clinical data during the last few years showed the importance of longer UV in the genesis of actinic keratoses as in skin degenerative processes. UVA radiation (320-400 nm) induces indirect DNA damage and mutations, as experimentally demonstrated, immunodepression, etc. UVA radiation is adsorbed by important cellular components (porphyrines, riboflavine etc.) and ROS formation by an oxygen-dependent photodynamic reaction. The importance of UVA radiation, on the other hand, emerges if it is considered that the curve of solar energy shows that UVA arrives to the earth surface with an intensity many times greater than shorter UV and that 95% of UV radiation is represented by UVA, that crosses glasses and penetrates deeply in the skin. Therefore, in the prolonged solar exposure, UVA alterations can be greater than those induced by UVB.

The UV radiation induces different immunological alterations with surveillance modification towards UV-induced cancer elements. The UV irradiation stimulates keratinocytes secretion, quite small in the normal skin, of cytokines and growth factors with action on immunocompetent cells; also of proopiomelanocortine (precursor of melanotropic, corticotropic and opioid peptides), of alpha-MSH and ACTH, that mediates immunomodulation.

UVB actives alpha-MSH receptors and proopiomelanocortine-derived peptides, influencing skin melanogenesis.

Other factors can influence immunosurveillance. Among these, urocanic acid (UCA), produced by histidine decarboxilation in the skin. After solar irradiation it is transformed in its isomer cis-UCA, especially in young and clear phototype subjects. Cis-UCA UV-induced immunodepression can be important in the activation of the cancerogenesis, even if cis-isomer duration in the skin is very short (approximately 2 weeks). Moreover, UV can induce arachidonic acid metabolites production. These are produced as a result of inflammatory processes, and they are able to inhibit both apoptosis and immunosurveillance, have a role in the conversion of procarcinogens to carcinogens and other mutagenic effects. These and other elements induced by irradiation could influence the initiation and the development of skin tumors (reversible phenomenon, followed by still reversible promotion and irreversible progression).

Control systems and defense of the skin

The oncosuppressor genes

About the pathogenetic aspects of actinic keratoses the repairing control systems have to be considered. The protection functions about human skin radiation are covered by oncosuppressor genes and other interacting cellular systems involving membrane and cytoplasmic molecular structures.

The genes involved in the repairing processes constitute, on the other hand, the target of UV radiation, that has some effects on the oncosuppressor genes function. The function of p53 gene is important. This gene codifies for a protein that repairs DNA damage and is UV radiation. The p53 mutations constitute premature events in the proliferation of the epidermocytes and in human skin UV-induced carcinogenesis. A recent study has demonstrated elevated incidence of p53 mutations in actinic keratoses (54%); mutations found in squamocelluar carcinoma in higher percentages (69%). It has to be mentioned that damaged skin already presents gene p53 mutations in 38.5% of cases, very greater than not sun-damaged skin (14%). In the protection systems another oncosuppressor gene would be involved: CDKN2.

CD 95, CD05 ligand, TRIAL and TRIAL receptors

In chronically sun-exposed keratinocytes expression of the membrane protein CD 95 (Fas) is increased, able to control sun-damage: UV-induced CD 95 (Fas) activation induces apoptosis, important mechanism of defense against carcinogenesis. Further irradiation provokes CD 95 alteration and, therefore, down-regulation of apoptosis. As attended, CD 95 (Fas) is frequently absent in actinic keratoses and it is only focally expressed in invasive carcinoma.

Among the alterations of repairing control systems it has been recently demonstrated that the UV radiation modifies the expression of some molecules (CD05 ligand,TRIAL and TRIAL receptors) keeping cellular integrity and preventing their transformation. The modification of expression of these molecules produces alteration and reduction of their function. The expression of these molecules is low or early absent in actinic keratoses and the down-regulation of these
Anti-oxidant enzymic and not enzymic systems

The photoprotective skin properties are assured also by other endogenous modalities: skin has numerous other efficient photoprotective interacting systems involving various molecular structures. UV radiation activates these endogenous protection systems.

Moreover, UV radiation activates important elements as P450 cytochrome, glutathione, catalase and superoxidodismutase, GSH-S-transferase, ceruloplasmine etc. These endogenous antioxidants, therefore, work like scavengers of free radicals. Photoprotection against UV, as recently demonstrated, is linked to increased expression of heat shock proteins (HSP): emoxigenase, HSP 72 and others that induce UV-induced damage cellular protection and prevention of cancerogenesis and photoaging.

However, these UV-activated protective systems become insufficient after intense and extended UV irradiation, and, therefore, actinic keratoses can be transformed in epitheliomas.

Solar keratoses and skin damage: transformation models

The solar exposure to which the population is subordinated in the normal life is sufficient to provoke skin damage in a meaningful percentage of subjects; and the transformation happens as a result of UV irradiation cumulative effects. Approximately 80% of the total UV exposure is accumulated until 20 years, and this cancer transformation, as demonstrated in clinical and epidemiological studies, is linked in squamocellular carcinomas to continue irradiation, while basocellular epitheliomas and cutaneous melanomas are linked to intense and intermittent irradiation. The progression of actinic keratoses to carcinoma is correlated with some recently evidenced aspects. The epithelioma transformation correlates with region 9 p21 deletion, that codifies for p16 tumor suppressor (INK4a). A premature event of the progression of actinic keratoses to carcinoma is the expression a metalloproteinase (MMP-1). UV-induced DNA damage also activates a repairing system named MSH2 (“mismatch repair protein”); this system expression has been found also in skin cancer and its activation indicates progression to carcinoma.

Eumelanin/phaeomelanin relationship as marker of carcinogenetic risk?

The importance of genetic factors in the development of actinic keratoses is known for a long time; as the prevalence of actinic keratoses in red hair subjects, with freckles and clear phototype; these characteristics indicates risk of possible photodamage.

It has been recently demonstrated that skin reactivity to UV radiation is correlated with the type and level of skin melamins: UV sensitivity is greater how higher is pheomelanine presence (colored melamins). The relationship between the different melanine types is photosensitivity index and could constitute the molecular basis of phototype, as hypotesized by Prota and subsequently confirmed.

To the different melanin types with different chemical-physical properties correspond similar photobiological properties. The relationship pheomelanin/eumelanin can constitute risk index of skin damage and melanoma. A pheomelanic marker (6-2-amino-2-carboxetyl-2-carboxy-4-hydroybenzotiazole) is high UV-sensitivity (MED) expression and, therefore, useful. In this prospective new pheomelanin markers (1,3-thiazole acid -2,4,5 – tricarboxylic and benzotiazole carboxylic acid) have been identified by microanalysis on hair.

Some conditions with pigmentation and/or repairing systems deficit present tendency to greater development of actinic keratoses: immunodeficiencies, organ transplants, albinism, xeroderma pigmentosum, verruciform epidermodysplasia etc.

In conclusion, the natural skin photoprotection is assured by the corneum layer thickness, melamine content and type of pigmentation, and also by numerous and efficient cellular protection systems. The irradiated skin protect itself with a “sos-like response”, enhancing the defense systems in case of subsequent sun-exposures. These systems can be opportunely integrated by topical systems as, for example, antioxidants (E vitamin etc.), enzymes (catalase etc.) and, as showed by different experimental data, also by anti-inflammatory drugs.

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travioletto più lungo nella genesi delle cheratosi attiniche. La maggior parte delle lesioni UV-indotte sono riparate, ma possono risultare mutazioni e una cascata di alterazioni immunologiche che modificano la sorveglianza verso elementi cancerogenetici UV indotti. Le funzioni di protezione sono assunte da geni oncosoppressori (p53 e altri) e l’ultravioletto modifica l’espressione di alcune molecole (ligando CD05,TRIAL e recettori TRIAL) che preservano l’integrità cellulare e ne prevenzano la trasformazione. Numerosi efficienti sistemi di fotoprotezione endogene attivati dalla radiazione danno rivelano tuttavia insufficienti dopo intensa e prolungata irradiazione ultravioletta. Le cheratosi solari possono trasformarsi in epiteliomi. La trasformazione avviene in seguito agli effetti cumulativi dell’irradiazione solare. La progressione delle cheratosi solari a carcinoma è in correlazione con la delezione della regione 9p21 che codifica per il tumore suppressor p16 (INK4a). Un evento precoce della progressione dello stato displastico di cheratosi attiniche verso carcinoma è l’espressione di una metalloproteinasi (MMP-1). Il danno del DNA indotto da UV solare attiva, oltre ad altri sistemi riparativi, anche il sistema MSH2. Da recenti ricerche risulta che il rapporto feomelanine/eumelanine può guidare la progressione dello stato displastico di cheratosi attiniche verso carcinoma. L’irradiazione ultravioletta in seguito agli effetti cumulativi dell’irradiazione solare è un evento di primo piano nella genesi delle cheratosi attiniche. Le cheratosi solari possono trasformarsi in epiteliomi. La trasformazione avviene in seguito agli effetti cumulativi dell’irradiazione solare. La progressione delle cheratosi solari a carcinoma è in correlazione con la delezione della regione 9p21 che codifica per il tumore suppressor p16 (INK4a). Un evento precoce della progressione dello stato displastico di cheratosi attiniche verso carcinoma è l’espressione di una metalloproteinasi (MMP-1). Il danno del DNA indotto da UV solare attiva, oltre ad altri sistemi riparativi, anche il sistema MSH2. Da recenti ricerche risulta che il rapporto feomelanine/eumelanine può costituire parametro indice di rischio per danno cutaneo. Parole chiave: Cheratosi solari - Radiazione ultravioletta - Cute.

References

A 45-year-old chemical worker was referred to the Intensive Care Unit of Policlinico San Matteo Hospital of Pavia, following cardiopulmonary arrest, after inhalation of high pressure cyanuric chloride. He has been successfully resuscitated at his workplace, and treated with 7.5 g i.v. hydroxocobalamin for suspected cyanide poisoning. A few hours after admission, he developed a diffuse skin rash with coalescing intense-pink macules and patches, particularly evident on his abdomen. The skin temperature was normal. Urine was fuchsia coloured. A cutaneous punch biopsy was done showing superficial and deep perivascular lymphocytic infiltrate, with no evidence of pigment deposition. Cutaneous discoloration gradually faded and disappeared completely within 5 days. Cyanide poisoning was then excluded by negative essays in blood, urine and gastric content. Skin biopsy was performed and showed superficial and deep perivascular lymphocytic infiltrate, with no evidence of pigment deposition (Figure 1), more evident on his abdomen. Skin temperature was normal and the urine colour had the same intense-pink hue.

Hydroxocobalamin has been shown to be a safe, rapid and effective cyanide antidote in experimental and clinical studies.1

Case report

A 45-year-old chemical worker was admitted to the Intensive Care Unit of Policlinico San Matteo of Pavia, following cardiopulmonary arrest. This was presumed to have occurred as a result of accidental inhalation of cyanuric chloride 30 min previously in the course of his employment in agrochemical manufacturing.

He was resuscitated (intubation, ventilation, cardiac massage, 3 mg epinephrine i.v. in 5 min) directly at the accident site. Because the patient had been exposed to cyanuric chloride, cyanide poisoning was considered to be the cause of his cardiac arrest, and therefore 7.5 g of hydroxocobalamin were infused intravenously. Hydroxocobalamin has been shown to be a safe, rapid and effective cyanide antidote in experimental and clinical studies.1 Few hours later dermatologic consultation was requested after the patient presented with a sudden onset intense-pink diffuse macular discoloration (Figure 1), more evident on his abdomen. Skin temperature was normal and the urine colour had the same intense-pink hue.

Skin biopsy was performed and showed superficial and deep perivascular lymphocytic infiltrate with no evidence of pigment deposition (Figure 2).

Cyanide ions were undetectable in the patient’s blood, urine and gastric content samples, thus excluding cyanide poisoning.

He was given methylprednisolone and chlorpheniramine for his cutaneous lesions which resolved completely by 5 days.
BASSI TRANSIENT PINK SKIN DISCOLORATION FOLLOWING HIGH DOSE INTRAVENOUS HYDROXOCOBALAMIN

Discussion

Cyanuric chloride ($\text{C}_3\text{Cl}_3\text{N}_3$) is a stable organic chemical, which is used as an intermediate for manufacturing agrochemicals, dyestuff, optical brighteners, tanning and softening agents and pharmaceuticals. Systemic effects of cyanide poisoning include neurotoxicity, cardiac failure, pulmonary oedema, haemorrhagic gastritis. Cutaneously, cyanide poisoning is manifested by cherry-red diffuse discoloration of the skin, due to increased oxygen saturation in venous blood. The highly stable chemical bond between cyanide and chlorine in the $\text{C}_3\text{Cl}_3\text{N}_3$ molecule ensures that cyanide release \textit{per se} only occurs rarely, so that cyanide intoxication from cyanuric chloride inhalation or ingestion is remote. The absence of detectable cyanide ions in the patient’s blood, urine and gastric contents permits the exclusion of cyanide poisoning in this case.

Hydroxocobalamin is a well-known antidote in cyanide intoxication. It reacts stochiometrically with cyanide in the range 50:1, according to molecular weight, so that 5 g (antidotal dose 5-10 g) bind nearly 40 µmol/L of cyanide. It detoxifies cyanide by removing an hydroxyl group (OH), in the liver it is metabolised as 5-deoxyadenosylcobalamin, and excreted in the urine as nontoxic cyanocobalamin.

It is possible to get indirect indication of cyanide’s presence dosing serum hydroxocobalamin and cyanocobalamin. These doses are commonly measured by virtue of differential spectrophotometry techniques (the two chemical species show in fact different peaks in absorbance spectra: hydroxocobalamin at 272, 351 and 525 nm; cyanocobalamin at 276, 359 and 544 nm). Unfortunately these techniques are not available in our hospital.

Debate exists on hydroxocobalamin half-life. According to Osterich \textit{et al.} there would be a rapid alpha phase (distributional) of 0.52 h and a slower beta phase of 2.64-5.4 h.

Hydroxocobalamin at therapeutic doses causes sporadic side effects, including acne, folliculitis, pruritus, bullous disease, urticaria, angioedema, and anaphylactic shock has been reported after injection of even low doses. It may also result in alteration in laboratory estimations for bilirubin, iron and creatinine, because of interference with their spectrometric evaluation according to hydroxocobalamin elimination half-life.

As other drugs containing vitamin B12, hydroxocobalamin presents an intense pink hue. A transient, unnatural pink muco-cutaneous and urinary discoloration is reported, although non examined in detail.
It is, therefore, probable that hydroxocobalamin had caused the transient pink rash in our patient.

**Conclusions**

We were unable to find any reports in the literature regarding the histological findings in rashes due to hydroxocobalamin. The absence of pigment deposition in our case may only reflect limitations of H and E staining or may as well proceed along with the transience of the lesions. No specific stain for vitamin B12 in the skin is available. The lymphocytic infiltrate within the dermis is nonspecific in our case.

In conclusion, a case of cutaneous pink discoloration following the administration of hydroxocobalamin is an antidote to presumed but unproven cyanide intoxication.

**References**

Dear Editor,

Two main needs supported the achievement of open-air schools at the beginning of the 20th century: one educational and the other of social/humanitarian content. A return to the nature was regarded as an antidote to the harms of urbanization and industrialization, such as tuberculosis, childhood frailty and unhealthy conditions of life. Following the European and North-American model, also in Italy open-air schools were built in Rome, Padua and Bologna. In Milan in 1919 started the execution of the school “Umberto di Savoia”: it would have been the largest in Italy and among the most important open-air schools in Europe. Built in the area of the ancient riding-track, this school gathered children in poor health coming from primary schools, and in particular those with parents affected with tuberculosis.

Among the health services provided by the school there was also a room for artificial heliotherapy, prescribed to the most delicate children during winter and offered up to the 60s in the same school. With warm weather the children underwent the so-called “baths of sunlight” in the garden of the school.

From 1967 to 1992, 3 siblings (2 females and 1 male; aged 43, 70 and 65 years) were first visited at our department: they were affected by multiple basal cell carcinomas with gradual onset on the face, the back, and hips, with a total number of lesions for each patient of 3, 16 and 13. In the family there was no other subject affected by epithelial neoplasms and the patients did not show any sign or symptom that could be attributed to a basal cell nevus syndrome. Among the 3 siblings, the eldest was a housewife and the other two had worked as employees. In the history, a fact had arisen our interest: in the 30s all three had attended the open-air school Umberto di Savoia, where they not only underwent the “baths of sunlight”, but also artificial phototherapy in winter, performed once in a week or every second week.

The use of phototherapy in the first decades of the 20th century in the treatment of tuberculosis is well documented, either as generalized or local irradiation, in order to treat skin localizations (Kromayer lamp) or pulmonary involvement (total body irradiation). For the latter it was hypothesized both an indirect favourable effect and a promoting action on skin immunity exerted by ultraviolet (UV) radiations. Besides, phototherapy was administered in order to prevent and treat rickets. To this aim open-air schools and those for rachitic children were provided with a room equipped with UV lamps. In those times quartz lamps with mercury vapour were mainly used. Among the phototherapy equipments, which were more often employed for the generalized irradiation, there was the Jesionek lamp; some of them arranged in a dedicated room allowed the so-called bath of UV light. The lamps, provided with large reflectors, were disposed along the walls: the patients walked in the centre of the room moving along concentric circles drawn on the floor, in order to maintain a settled distance from the UV source (no lesser than 60-70 cm) (Figure 1). The treatment began along the inner circle and, with the increase of the skin tolerance to the irradiation, it went on along the outer ones. During the treatment the children wore only pants and mask-shaped protective glasses.
The emission spectrum of quartz lamps is discontinuous, with peak bands at 254, 263, 297 and 366 nm, including also short UV rays (i.e. UVC, wavelength 100-280 nm), which are potentially carcinogenic and require to be filtered. In the first decades of the 20th century, the filters associated to those lamps were made of glass or uviol glass, a special glass characterized by UV transmission from 250 nm on, that was inadequate to cover the whole spectrum of more dangerous UV. We might hypothesize that the use of these equipments, endowed with a less than optimal filtration and utilized without a precise and reproducible dosimetry, could have had a role in promoting photaging skin damages and in the pathogenesis of multiple basal cell carcinomas in the 3 siblings.

Many recent reports have evaluated the relationship between phototherapy and photochemotherapy and onset of epithelial skin neoplasms and studies on the pathogenesis of basal cell carcinoma have attributed an important role to childhood sun exposure, but in the literature comparable data of the late side effects of artificial phototherapy performed to prevent rickets and tuberculosis in childhood are lacking. For this reason, since the increasing incidence of skin epithelial neoplasms, on one side due to the increment of the mean age of the population and on the other to the changes of ways of life, it would be interesting to investigate about a possible exposure to artificial sources of UV for the prevention or care of tuberculosis or rickets in childhood.

References

Dear Editor,

Orificial tuberculosis: a case with anal localization

E. PEZZAROSSA, E. DOMANESCHI
Unit of Dermatology
Azienda Istituti Ospitalieri di Cremona, Cremona, Italy

Dear Editor,

Orificial tuberculosis is an exceptional kind of cutaneous tuberculosis that can affect the oral cavity, lips, perioral and perianal area and genital mucosa. Lesions are caused by direct inoculation or by hematogenous and lymphatic diffusion. Usually autoinoculation is the cause, but, sometimes, it has an external origin. This pathology often involves immunodepressed patients and it seems a symptom of a systemic disease with an unfavorable prognosis. Mantoux test is usually negative, showing anergic condition instead. The association with malignancy and Evan’s Syndrome has been described. Clinically, at the very beginning, it seems a little nodule that ulcerates progressively, getting a destructive appearance with a deep pain.

Histology often shows an unspecific inflammatory report and it is rare to point out the acid-fast bacilli with specific stain. So, it is necessary to isolate the mycobacterium with the bacteriological exam. We report the case of a 80 year-old woman suffering from liver’s cirrhosis HCV+ and chronic heart failure admitted to a surgical department as a consequence of anal haemorrhage with pain, for a suspect anal carcinoma. We were consulted because the histological examination excluded this first clinical supposition. Dermatological examination revealed a painful, deep and sharply demarcated ulceration with a granulating base covered in part by a purulent matter (Figure 1). The case history, hardly reliable, did not supply irrefutable evidence about the time in which the ulcer occurred. The examination of the histological specimen showed the presence of soft tissues without epithelium and granulomatous infiltrate in the dermis consisting of epithelioid cells and a few Langhans giant cells. Focal areas of caseation necrosis were also present. A Ziehl-Neelsen stain did not show acid-fast bacilli (Figures 2, 3).

Laboratory findings confirmed a serious liver disease: GOT 187 U/L, GPT 78 U/L, GGT 553 U/L. alkaline phosphatase 698 U/L, haematic albumin 12 633 mg/dL, compatible with hepatic cirrhosis. Syphilis serology (VDRL, TPPA,FTA abs) was negative. A screening for a possible primitive tubercular infection (radiological examination: chest X-ray, direct intestinal X-ray, abdomen and pelvic ultrasound) ruled out tubercular infection active or healed.
Direct smears of sputum, wound, faeces and urine were negative for acid-fast bacilli.

At least Mycobacterium Tuberculosis was cultured from a skin biopsy specimen after 40 days on Lowenstein-Jansen medium (Figure 4).

A few days after cultural findings of the BK, the patient died for worsening of her general conditions and so we were not able to complete the intestinal study by colonoscopy and not even to begin a suitable antitubercular therapy.

Anal localization is reported in 0.7% of all the extra-pulmonary tuberculosis with a major occurrence in male sex.

In recent years, due to AIDS pandemia and the rising of drugs resistance in some mycobacterium stock, tuberculosis has recorded a new peak of impact. Anyway orificial tuberculosis hasn’t been observed in HIV patients.

Orificial tuberculosis is considered one of the endogenous kind of cutaneous TBC even if, sometimes, it is possible an external infection source and it is always indicative of scarce immunity.

In the clinical differential diagnosis a neoplasm, like a squamous cell carcinoma, was considered in primis; however, different kinds of ulcerative process, like Crohn disease or ulcerative colitis, a trauma, venereal diseases, pyoderma gangrenosum, sarcoidosis and deep mycoses were also considered.

The histological differential diagnosis between cutaneous tuberculosis and other granulomatous chronic diseases is

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**Figure 1.**—A deep and sharply demarcated ulceration with a purulent base.

**Figure 2.**—Aspecific granulomatous inflammation in the dermis (ematoxylin-eosin 40 X).

**Figure 3.**—Granulomatous infiltrate with epithelioid cells, Langhans giant cells and caseation necrosis (Alcian-Pas 100 X).

**Figure 4.**—Colonies of m. tuberculosis on Lowenstein-Jansen medium.
often difficult, especially when caseation necrosis is absent. Other infectious processes with a tubercular-like pattern are tertiary sifilis, deep mycoses and leishmaniosis.

In our report the diagnostic iter was concluded only with microbiological results. Polymerase-chain reaction (PCR) permits a faster typification of mycobacterium,\textsuperscript{5} but, due to high costs, it was not used. The exams to reveal possible endogenous source of tubercular infection could not be completed with a colonoscopy because of the worsening of the patient. So, we can’t exclude a primitive intestinal localization.

Nevertheless, the negativity of the microbiologic examinations for BK on expectoration, faeces and urine let us suspect an external infection.

We have considered this case interesting both because of his rarity and also for the risk of infectivity that an orificial tuberculosis not promptly diagnosed can present.

\section*{References}


Address reprint requests to: E. Pezzarossa, Piazza Roma 2, 26100 Cremona, Italy. E-mail: enricopez@libero.it