Dermatologic radiotherapy: when, why and how?

R. G. PANIZZON

For a long time, papers on dermatologic radiotherapy were practically inexistent. It is with great pleasure that we realize a renaissance of communications about this treatment, not only for lentigo maligna and lentigo maligna melanoma, for lymphomas but also for skin carcinomas as mentioned in the paper published in the February issue of the journal. The paper worked out by Percivalle et al. is a very good example to describe and to remind to the readers, especially the dermatologists, the excellence of this treatment modality.

When?

The paper by Percivalle et al. shows that there are especially elderly people who profit from radiotherapy. We realize that we practically have no negative answers from all these elderly irradiated patients. They confirm that they would repeat this treatment without hesitation.

There are medium sized tumours especially basal cell carcinomas, but also squamous cell carcinomas which represent an excellent indication since they show a good radiosensitivity and radioresponsiveness. Then the localization of the tumors in the face are a good indication, the functional and cosmetic results being excellent, especially as mentioned by the authors on the eyelids. Therefore, it is not surprising that the authors are able to demonstrate a cure-rate of 96.72%.

In addition, we have to stress that the department, in Milan, has a long time experience in this field, another important factor for this success rate.

Why?

Why do elderly patients especially appreciate radiotherapy? Because this treatment modality has the following advantages:

1) provokes no pain;
2) the patient may sit or lay down only for a couple of minutes;
3) it is done on an outpatient basis;
4) the functional and also cosmetic results are excellent;
5) important organs can be protected;
6) there is nearly no scarring.

How?

How is the treatment performed? One thing must clearly be communicated to the patients: radiotherapy can only be given in fractionated doses, this means in

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several sessions. A single session therapy is practically abandoned. On the other hand, it is not always necessary to irradiate on a daily basis, but there is the possibility of 2 or 3 sessions per week. We see no difficulty either by members of the family or of some organizations (e.g., Red Cross) helping for the transportation of these patients. It must also be said that the first session needs a little bit more time for the treatment planning and documentation (around 45 min), but the following irradiation sessions as mentioned above are lasting only for a couple of minutes. Radiation reactions also will appear but only at the end of the treatment and even if these reactions sometimes are oozing, they are not painful for the patient. Another possibility and advantage is that the radiotherapist has not to calculate with 1, 2 or 3 mm, but mostly can include a security margin of 5 mm or more, and still preserve the healthy tissue, this margin being an important advantage of this treatment. Moreover, after incomplete surgical removal which occurs in a good part of the patients, radiotherapy might be followed. We also want to stress that the rest of the body doesn’t irradiate and has no consequences for the patients. In addition, there will be no general hair loss and no intestinal (general) symptoms, and important organs such as the eyes can be protected. After a cancericidal dose there might be hair loss in the radiation field or loss of the eyelashes (if eyelids are irradiated) as described in this paper. In elderly patients a loss of the eyelashes is nearly not realized by other persons. An important advantage with irradiation of the eye structures is that there is never an obliteration of the lacrimal duct and there is nearly no leucoplaquia as a consequence. Therefore, it is not astonishing that the cosmetic result reported by Percivalle et al. is acceptable or good in 98.9%.

Can you offer a better treatment result especially after a mean follow-up of 5 years for your patients? Therefore: there is a renaissance of Dermatologic Radiotherapy!

References
Early knowledge of potential metastasis is a long existing wish of many physicians. Malignant melanoma is a frequent dermatological malignant tumor with a high potential of metastasis. A strong correlation exists between the metastatic potential and the tumor depth in the dermis, as measured by the Breslow method. However, even thin tumors (Breslow < 0.75 mm) will sometimes metastasize.

Knowledge of early metastasis is only of value if the impact of such a finding is of help for the patient. In the past, it has been proven that blind lymph node dissection will not lead to higher survival rates for melanoma patients. More recently the sentinel node technique is widely used for melanoma patients. Until now there is no clear evidence for the benefits of such a procedure. Medalie and Ackerman concludes in short, no surgeon, pathologist, or oncologist is a seer, diviner, or prophet when it comes to predicting accurately the outcome for a patient with metastasis of melanoma; the end could come in weeks, months, or decades. There is no justification, whatsoever, for the procedure, scientifically or practically, and for that reason it should be abandoned, without delay, now. However, there also seems to be some new data supporting some effectiveness from the sentinel node biopsy in melanoma patients.

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Rörsman was able to detect small amounts of melanin metabolites in urine as possible result of metastasing. This test has never entered the clinic. Of course, nowadays there is also the 18 F-fluorodeoxyglucose (FDG) PET scan to screen patients for metastasis, which are not found by conventional screening methods. Only in the case of small long nodules and brain metastasis conventional computer tomography (CT) and magnetic resonance imaging (MRI) are better evaluated. Nonetheless, FDG-PET is the modality of choice in patients with high risk for distant metastases, suspicious for distant metastases, known with distant metastases which can benefit from customized therapies if new lesions are discovered or treated lesions regress and at last patients at high risk for systemic relapse after aggressive medical therapy.

In the April issue of this journal, Ferrari et al. present their results about nm23 expression in primary melanoma and the prognosis for a 10 year disease-free interval. Their study population is very small for making strong evidence. They found that expression of nm23 protein was correlated with a disease-free interval of 5 years and even with 10 years. Consequence of this, they also found the same positive correlation with the survival.

What about the already in 1988 by Steeg et al. discovered nm23 gene?
Nucleoside diphosphate kinases (NDP kinases) are a family of highly conserved proteins in eukaryotes of which 8 different genes (nm23-H1 to nm23-H8) have been identified in humans. NDP kinases play a major role in cell metabolism since they transfer the terminal phosphate of a nucleoside diphosphate to a nucleoside diphosphate, thus equilibrating the NDP and NTP cellular pools independently of the nature of the purine or pyrimidine bases. The nm23 tumor metastasis suppressor gene was found to encode a protein identical to NDP kinase. Nm23-H1 and nm 23-H2 tumor suppressor activities have been identified in a number of human cancers. Studies supported the hypothesis that the nm23 protein may act as a metastasis suppressor gene. In cancer cell lines, in vitro, expression of nm23 reduces metastatic potential and cell motility. Although this effect has been extensively described, the molecular mechanism underlying the role of nm23 in cancer is poorly understood. A number of data indicate that nm23 is a multifunctional protein reportedly involved in a variety of cellular functions including differentiation, proliferation, and apoptosis. Nm23 expression in melanoma is supposed to be associated with a relative better prognosis.

Melanoma prognosis is based on histological criteria such as tumor thickness (measured by Breslow index), level of invasion (Clark’s level), presence of ulceration and number of mitoses per mm². However, these parameters do not provide a precise prognosis in all cases: thin melanomas may develop metastases and thick melanomas may remain focalized for many years. For these reasons, the search for other prognostic factors is still ongoing. Many molecules playing a part in the invasiveness and metastatic dissemination of melanoma have now been identified. Expression of these molecules has been investigated in primary melanoma and correlated with prognosis. An increase in the number of cells positive for Ki67 (detected by Mib1), cycline A, cycline D, p35, MMP-2, beta 1 and beta 3 integrins, osteonectin, the presence of an intense inflammatory infiltrate and capillary invasion are considered as factors of poor prognosis as well as a decrease in P16, p27, Melan A and nm23. The significance of CD44 modifications is still controversial. Only a small number of these different proteins have a prognostic value independent of tumor thickness. These results need to be confirmed on larger series of patients. Additional hope is given to new techniques such as the analysis of the genes implied in tumor progression by micro array technique in such a way as to provide a molecular map of each tumor.

Tumor suppressor genes encode for proteins whose normal function is to inhibit cell transformation and whose inactivation is advantageous for tumor cell growth and survival.

Tumor suppressor genes participate in a variety of critical and highly conserved cell functions, including regulation of the cell cycle and apoptosis, differentiation, surveillance of genomic integrity and repair of DNA errors, signal transduction, and cell adhesion. Tumor suppression functions can be separated into 2 major categories: gatekeepers and caretakers. Gatekeepers inhibit directly tumor growth. Inactivation of these genes contributes directly to tumor progression. In melanoma the nm23 protein could play a caretaker role. Functionally, this molecular finding can be of great importance for the patient. However, when a special marker will be of use in daily practice, it should be proven to add something new to the already known and easy to obtain prognostic parameters, like the Breslow thickness. It is more or less the same discussion as indicated above for the patient value, e.g. to increase lifespan as well as quality of life for the remaining lifespan, sentinel node biopsy and PET scan.

The major question will be if the positive expression of nm23 is also correlated to the Breslow thickness. If this is the case, there is no extra value of this on it self-interested finding. In case, especially in thin melanomas the Breslow thickness and nm23 expression are not correlated together regarding disease-free interval. A large trial should be done to put this nm23 expression test on the map.

Pacifico et al. Found a significant correlation as well between Clark levels as Breslow depth in a group of 200 melanoma patients. Nevertheless, the question is still open if nm23 negative patients with low Breslow depth are those patients who have a bad prognosis.

References
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Clinical relevance of antibiotic resistance in acne

F. R. OCHSENDORF

In the April issue of the journal, Bettoli et al. report the prevalence of *P. acnes*’ antibiotic resistance in Ferrara over a time period of more than 5 years. More than half of the *P. acnes* strains (55.9%) cultured from the skin of 1,579 acne patients were resistant to at least one antibiotic (erythromycin, clindamycin, tetracyclines or minocyclin). Possible methodological problems in the context with studies like this, such as unknown prior or present therapies of patients, method of surface sampling (in this study the whole face was sampled correctly), cultivation methods, focus on *P. acnes* and not as well *P. granulosum* or *P. avidum*, or the way of reporting the data have been nicely addressed elsewhere and will not be discussed here. Given the findings of a large number of resistant *P. acnes* to different antibiotics, this report poses several questions to the clinician: are the findings relevant 1) to other areas than Ferrara, 2) to the individual acne-patient, 3) the general population and 4) should these results influence actual treatment recommendations for acne?

**Local or general problems**

One could argue that the problem of resistance might only affect certain areas. Unfortunately, this is not the case. Though there are methodological discrepancies between different studies it is evident that resistant *P. acnes* strains can be found all over Europe and even worldwide since decades. The prevalence of erythromycin-resistance ranged from 41.5% in Northern Europe to 91% in Spain. Resistant *P. acnes* strains were also reported in other continents, such as Australia, Japan or the United States. Interestingly, the last study on propionibacterial resistance from the USA was published in 1983.

The regional differences can be attributed, at least partly, to prescribing habits of antibiotics. Although in acne patients prospective studies of this association are still lacking, this link can be deduced from the published reports. The lowest rates of erythromycin resistance were found in Australia where topical antibiotics were made available only recently. In Europe topical erythromycin and clindamycin were introduced in the mid-1970’s. The first resistant strains were reported in 1979. Later, the highest rate of antibiotic resistance was found in Spain where 84% of patients had been treated with antibiotics before in contrast to a rate of 51% in Hungary with only 18% prior antibiotic therapy. Furthermore, direct associations between prevalence of resistant bacteria and the use of certain antibiotics are well known from other species: asso-
associations of *Staph. aureus* resistance rates and use of oxacillin or an increase of streptococci-resistance with increasing use of erythromycin were reported. So the rate of resistance can be very much influenced by the use of antibiotics. Therefore the report of Bettoli et al. exemplarily highlights again a European and even worldwide problem: the high incidence of *P. acnes* strains resistant to different antibiotics. In Europe, every second patient with acne seems to be colonized by strains resistant to erythromycin and clindamycin.

**Relevance for the individual patient**

Although not generally investigated or monitored, a high incidence of antibiotic resistance of *P. acnes* must be expected everywhere. The question arises whether these findings are just “laboratory findings” or whether they are clinically relevant to the patient.

Already in 1989 Eady et al. reported an association of therapeutic failures during oral erythromycin therapy and the identification of resistant *P. acnes*. Recently, a connection of tetracycline resistance and impaired clinical efficacy to oral oxytetracycline and minocycline was reported. In 2002, Mills et al. saw no clinical effect of topical erythromycin 2% gel compared to its vehicle on acne lesions. Simonart and Draimax just reviewed the effects of topical antibiotic therapies since 1970. The authors found a linear decrease of the clinical effects over time in the studies using erythromycin. The effects of clindamycin were stable over time. The authors attributed the association of diminishing efficacy with time to the emergence of resistant strains of *P. acnes*. So, for the individual patient, resistance of *P. acnes* apparently is associated with therapeutic failure or at least reduced efficacy.

It has to be kept in mind that the role of *P. acnes* in acne can be looked at as “multiple isolated infections of the sebaceous follicles”. As not every follicle of the patient will harbour resistant *P. acnes*, the clinical response depends, among other factors, on the number of follicles which are colonized by resistant strains. As resistant organisms are not residing in all follicles, patients will not fail to respond completely but rather will show a reduced response in comparison with individuals with fully susceptible propionibacterial floras.

However, a differentiation is necessary here. Acne not responding to antibiotics should be named “antibiotic recalcitrant acne”. A therapeutic failure thus could either be due to antibiotic resistance of *P. acnes* or could have other causes. Bacterial resistance is certainly not the only reason for therapeutic failures in acne. For example, non-compliance has to be taken into account in 31-53% of acne patients (Kupfer J, personal communication). This, however, also drives the emergence of resistant strains during antibiotic therapy. Other factors, such as increased sebum-excretion rates diluting the concentration of the antibiotic, inadequate dose, follicular pH or route of administration (among several others) might impair the therapeutic outcome of antibacterials in the absence of any bacterial resistance.

The following factors bear an increased risk for the emergence of resistant *P. acnes* in individual patients: poor compliance, a history of multiple courses of antibiotics, especially sequential use of related compounds, antibiotic treatment times longer than 12 weeks, living with an antibiotic-treated patient with acne and treatment by an acne specialist. The latter dermatologists were more likely to be colonized with resistant strains than other dermatologists or primary care physicians.

**Relevance for the community**

Fortunately the resistance-mechanisms of *P. acnes* are different from those of other bacteria. If acne had been due to, for example, *staphylococci*, in which resistance emerges so rapidly via plasmids, acne could have never been treated with antibiotics. But *P. acnes* is a bacterium of low genetic adaptability. Resistance develops by mutational change which is transferred vertically. Therefore, antibiotics could be used for so long.

There is no evidence that *P. acnes* or *P. granulosum* cause acute infections. So, neither the patient nor the community are at risk of resistant propionibacterial infections. However, acne lesions are dominantly colonized by *P. acnes* and *S. epidermidis*. Both bacteria could be isolated simultaneously in about 50% of acne lesions. In Japan, more than 30% of *S. epidermidis* strains were resistant to erythromycin, clindamycin and roxithromycin. *S. epidermidis* can, in contrast to *P. acnes*, transfer resistance via plasmids to *S. aureus*. This has been demonstrated for gentamycin-resistance. Mills et al. reported a higher carriage rate and dissemination of erythromycin-resistant *S. aureus* from the nares during a 12 week course of topical erythromycin. So, the nares have to be regarded as a reservoir for both *P. acnes* and *S. aureus*. It is
very difficult to eradicate the organisms from this location.

In the latter study the rate of erythromycin-resistant coagulase-negative staphylococci increased from 37% to 88% during the treatment. This rate did not decrease after the end of antibiotic therapy. So antibiotic resistance can persist for a considerable time.

The authors found that resistant strains were transferred to untreated areas. Furthermore, it was shown that a sequential antibiotic therapy promoted the carriage rate of resistant *Staphylococci* on the skin of contacts. Although not definitely proven, a direct transfer of resistant organisms to persons with close contact is therefore very probable.

Other studies showed a 3-fold increase in the prevalence of *Strep. pyogenes* in acne-patients using antibiotics. This rate is as high as in patients with symptomatic pharyngitis. The carriage rate did not differ between topical or systemic therapy. The carriage of resistant bacteria on the skin of acne-patients treated with antibiotics was not associated with an increased rate of cutaneous infections. However, a recent study showed that patients, who had received antibiotic treatment for their acne, were more likely to develop an upper respiratory tract infection, but not a urinary tract infection, than those with acne who were not receiving such treatment.

A mere change of the antibiotic in cases of antibiotic-refractory acne is no solution. The lower incidence of tetracycline resistance in the actual and reported studies indicates that selectivity of oral tetracyclines is less than topical erythromycin or clindamycin as far as propionibacterial resistance is concerned. The history of antibiotic therapy shows, however, that the use of new antibiotics just postpones the problem to the future as any antibiotic use drives the emergence of resistant strains. Therefore, it appears more important to ensure that future patients can be offered antibiotic treatment, rather than focusing on the patient presenting today.

Antibiotic acne therapy thus poses selective pressure on different bacteria. Whether and to what extent antibiotic therapy for acne has contributed to the prevalence of resistant bacteria and resistant bacterial infections in the community is unknown.

**Consequences for acne therapy in daily practice**

The emergence and spread of resistant *P. acnes* can be limited and perhaps even reduced by wiser prescribing practices; antibiotics should not be used for mild acne. Topical retinoids should be the first line agents in acne therapy. Unfortunately topical retinoids are still underused in the treatment of acne.

If indicated, antibiotics should not be used as monotherapy. A recent prospective controlled study of Ozolins *et al.* demonstrated the superiority of topical antibiotic/benzoylperoxide (BPO) combinations. The authors investigated the effects of oxytetracycline (2×500 mg/d p.o.), minocycline (100 mg/d p.o.), BPO (5% topically twice daily), combination of erythromycin (3% topically once daily) and BPO (5% topically once daily) or a fixed combination of erythromycin 2%/BPO 5% (topically once daily) in 649 patients with mild to moderate inflammatory acne vulgaris of the face. They found that topical antimicrobial therapies performed at least as well as oral antibiotics in terms of clinical efficacy. BPO was the most cost-effective and minocycline the least cost-effective therapy for facial acne. The efficacy of all three topical regimens was not compromised by pre-existing propionibacterial resistance. The topical therapies, however, were associated with a stronger irritation of the skin, especially in BPO monotherapy.

Topical antibacterial therapies effectively reduce *P. acnes*, but only at the treated sites. Systemic agents, such as oral antibiotics, reduce the density of the propionibacterial population on all areas of the skin and even in the nares. BPO and isotretinoin reduce the propionibacterial population density by >99%. Periodic short wash-outs (effectiveness proven after only 2 days, recommended 3-5 days) with BPO during any antibiotic therapy reduces antibiotic resistant propionibacterial populations. However, this happens only at the treated areas and not the nares. All approaches do not completely eliminate the organisms. One can only hope that resistant strains are slower in re-colonizing the skin than the resistant ones.

So localized inflammatory acne can be treated topically. For widespread inflammatory acne systemic antibiotics are still indicated. As the identification of resistance patterns is, in practice, almost impossible, the treating physician has to think before prescribing multiple courses of antibiotics for acne. He should be aware of the resistance problem and combine any antibiotic treatment with BPO (continuous or peri-
In case of rapid recurrences, he has to consider alternative therapies, such as isotretinoin or antiandrogens.

Besides a correct indication of antibiotics, another possible approach could be to limit the spread of resistant organisms. The person-to-person transfer of pre-existing resistant strains appears to be the main route of transmission. So the acne-specialist has to become aware that he himself may transfer resistant strains between patients! Dermatologists have to recognize this and take appropriate methods of hygiene to avoid it.

If antibiotics are indicated, the patient should be informed that a good compliance is required in order to reduce the emergence of resistance. The patient should be clinically monitored every 6 weeks. Antibiotic therapy should be stopped as soon as doctor and patient agree that there is no further improvement. Finally antibiotics should not be used for maintenance therapy. Here alternatives, such a retinoids, BPO or azelaic acid, could be used. If these measures will be taken an effective further use of antibiotics for acne will still be possible. In the future other local approaches, such as blue-light or PDT, or systemic treatments, such as hormones or lipoxigenase-inhibitors, may overcome the resistance problems during acne therapy.

References

Adverse cutaneous drug reactions to cardiovascular drugs

S. BRENNER, J. MASHIAH

Azori et al. present the frequency, clinical pattern and course of cutaneous adverse reaction to cardiovascular drugs recorded from October 1999 to November 2004 in hospitalized patients and outpatients of the Dermatology Department of Cagliari University. Out of 409 cases of adverse cutaneous drug reactions (ADR), 35 (8.5%) were associated with the exclusive use of cardiovascular drugs. The algorithm adopted by the Collaborating Centre for International Drug Monitoring of the World Health Organization (WHO) was used to determine the level of probability of the association between the drug administration and the adverse reaction. Cases of multiple drug use were excluded when cardiovascular drugs were not the principle cause of the ADR, probably resulting in an understimation of the true incidence of ADRs. The culprit drugs were primarily ACE inhibitors, followed by hydrochlorothiazide + ACE inhibitors or angiotensin II antagonists, diuretics, beta-blockers, calcium channel blockers, antiarrhythmics, aggregation inhibitors, vasodilatators, and hypolipidemic agents. The clinical spectrum of the ADR included exanthemic reactions, urticaria, photosensitivity, pityriasis rosea-like eruptions, Stevens-Johnson syndrome, lichenoid dermatitis, TEN, vasculitis, erythroderma, and psoriasis.

The WHO defined ADR in 1972 as a “Response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function”. Edwards and Aronson proposed a modified definition: “An appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product”. These authors classified ADRs into 6 categories: a) dose related, including reactions related to dosage and formulation, toxic effects; b) non-dose-related or bizarre, including immunological and idiosyncratic reactions; c) dose-related and time-related reactions, related to the cumulative dose; d) time-related, delayed reactions such as teratogenesis and carcinogenesis; e) withdrawal reactions; f) unexpected failure of therapy.

ADRs are common but estimating their true incidence is nearly impossible due to inadequate reporting. In the United States the estimated rate of medication-related visits to office based physicians is 7.7 per 1 000 persons, but only 7% of them initiated the visit due to ADR. Recognizing ADRs requires several steps. One of the main problems is to clarify whether the patient is taking medication: people tend to forget tak-
ing occasional drugs, overlook over the counter and tradi-
tional drugs, and don’t consider long-term treatments
such as oral contraceptives as treatment. Once drug
intake has been established, it remains to decide whether
the reaction can be attributed to drug intake, and if so,
which drug is the culprit. This causality assessment of
reported ADRs is usually performed according to the
global introspection (GI) based on the World Health
Organization scale, as well as to other decisional algo-
rithms purported to be less subjective and imprecise
than the GI.3 The causality levels used by most algo-
rithms are certain, probable, possible, unlikely, condi-
tional/unclassified, unassessable/unclassifiable.

Among the useful causality assessment tools are
the temporal relation between the adverse reaction
and the drug intake, including the introduction or ces-
sation of the drug, and dosage changes. The time
elapsed between the introduction of the culprit drug and
the ADR varies from seconds, minutes, hours, days,
weeks, months, to 11 years.4 Another tool is the known
ADR pattern previously related to the culprit drug, a
reaction which is in some way specific. Challenge test
is informative but is potentially hazardous and
therefore of limited applicability. Other in vivo skin
tests include the prick test, patch test, and delayed
(tuberculin-type) test.

Several in vitro tests can help determine the offend-
ing drug but are limited by false negative results due
to an antigenic determinant that may include a binding
protein in addition to the drug, and/or an immunolog-
ic reaction arising from a metabolite of the drug rather
than the drug itself. Several in vitro tests are available
and are described below.5

**RAST (radioallergosorbent test)**

This test measures specific immunoglobulin E anti-
bodies in the serum. Immunosorbent composed of
allergen extract is reacted with serum, washed, react-
ed with radiolabeled anti-human IgE antibody, and
washed again. The labeled antibody is proportional
to the level of specific serum IgE antibodies to the
allergen. The test is limited to IgE antibody-mediated
reactions and to drugs in which the epitope has been
defined.

**Mast cell degranulation test**

This type I hypersensitivity reaction-related test
measures the histamine release from mast cells after
incubation with the suspected drug. The specificity
and sensitivity of the test have not been defined.

**Lymphocyte transformation test**

After incubating lymphocytes with and without the
suspected drug for a few days, 3H-thymidine is added
for a few hours. The ratio between thymidine uptake
with and without the drug represents the lymphocyte
proliferation. However, only minor proliferation is
measured, yielding inaccurate results.

**Diagnosis of reactions that involve immune complexes**

Based on the fact that immune complexes cause ele-
vated levels of lysosomal enzymes such as b-glu-
curonidase, the suspected drug and the patient’s leuko-
cyes are incubated and the levels of b-gluconuridase
levels are measured.

**Lymphocyte toxicity assay**

This assay attempts to identify defects in the detox-
ification of toxic metabolites in drugs and is therefore
useful in hypersensitivity syndrome. Lymphocytes of
the patient and the drug are incubated in the presence
of human-like microsome, which includes cytochrome
p450.

**Macrophage migratory inhibition factor test**

This test measures the lymphokines released from
sensitized T lymphocytes by the specific antigen. Its
expression of activity correlates with delayed hyper-
sensitivity and cellular immunity. The migration index
is the ratio between macrophage migration with and
without the suspected drug. A MIF test is considered
to be positive when the migration index is 0.80 or less.

**Interferon-gamma release test**

One of the most accurate and useful tests is the inter-
eron-gamma release test, demonstrated in a number
of studies to identify the offending drug.6 Several stud-
ies have demonstrated its potential to identify the
offending drug based on the release of IFN\(\gamma\) from
lymphocytes after exposure to one or more suspected
drugs.7 It is based on the ability of Th1 lymphocytes to
produce interleukin-2 and IFN\(\gamma\) when they are acti-
vated in delayed-type hypersensitivity reactions. In

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### Table I.—Cutaneous adverse drug reactions caused by cardiovascular drugs.

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<th>Drug Reaction</th>
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Table I continued
Adverse cutaneous drug reactions to cardiovascular drugs

Brenner

Cases of immediate-type hypersensitivity, Th2 lymphocytes are activated and produce IL-4, IL-5 and IL-10. The test is performed by incubating the patient’s lymphocytes with and without the suspected drugs. IFN-γ is collected from the supernatant and its level is measured by enzyme-linked immunosorbent assay. The test has a sensitivity of 54% and specificity of 92%.5

Cardiovascular diseases are one of the major medical concerns in the world today, especially in developed countries, accounting for the prevalence of cardiovascular drug usage. ADRs to these drugs were found to account for 9.3% of medication-related office-based physician visits in the United States. The cutaneous reactions were the most commonly reported primary symptom;2 while they may occur at any time during the course of the treatment, some require a specific duration of drug administration before they appear. Most cutaneous reactions are mild; indeed, some are undistinguishable with no clinical importance, but can become serious and potentially life threatening. It is therefore crucial to recognize and treat adverse drug reactions, either by reducing the dose or by replacing the offending drug, while monitoring the cardiovascular disease.

Treating, and even anticipating drug reactions, requires knowledge of the mechanisms underlying the ADR. ADRs are like any other malady. In most cases they are multifactorial, with several mechanisms involved in their pathogenesis. Pemphigus is an illustrative case in point. Pemphigus is a severe, chronic, bullous, antibody-mediated disease of the skin and mucous membranes, characterized by acute flares-ups and remissions, and is generally considered to stem from a genetic predisposition to the disease triggered and/or exacerbated by one or more exogenous factors. The acronym PEMPHIGUS was proposed to denote the many causes of the disease: Pesticides Malignancy Pharmaceuticals Hormones Infectious agents, Gastrointestinal, Ultraviolet radiation, and Stress.4 Numerous factors are involved in the propagation and behavior of ADRs in pemphigus.7 The genetic factor: there are genes that determine drug behavior, sensitivity, metabolism, and disposition, in addition to other genes
responsible for the enzymes propagating oxidation, hydroxylation, and the like. The gender factor: a higher ADR rate is observed in women than in men, due in part to hormonal influences, pharmacodynamic factors, and reporting bias. Pharmacological factors: ADRs can be provoked by drug dose, formulation, pharmacokinetics, pharmacodynamics, and drug interactions. Immunologic factor: immune-mediated ADRs can be caused by the formation of hapten, or a stimuli exerted by a drug or its metabolites that acts as a signal to the immune system. Predisposing illnesses: immuno-suppressed patients or patients with a neoplastic disease are at increased risk to develop ADR.

The range of dermatologic adverse reactions attributed to cardiovascular drugs is extensive and versatile. These ADRs are generally classified by the type of reaction or by drugs and they can cause (Table I). Classification of adverse drug reactions that take into account the mechanisms triggering and exacerbating the ADR can ameliorate the adverse outcome of drug treatment. Such a classification has been applied to the drugs triggering and exacerbating pemphigus. They are divided into 3 main groups according to their chemical structure: drugs containing a sulfhydryl (SH) radical (thiol drugs or SH drugs), phenols, and nonthiol nonphenol drugs. Both classifications concerning cardiovascular drugs are merged into one table (Table II).

Adverse drug reactions are a common problem capable of interfering with the treatment of every malady, especially a life-threatening one such as a cardiovascular disease. Knowledge of the possible ADRs of each medication, and the mechanisms involved in the triggering and exacerbating the adverse reaction could hasten the recognition and treatment of the reaction, and even avoid it.

References

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Chronic urticaria: recent advances in diagnosis and treatment

M. W. GREAVES 1, 2

Urticaria affects 15-30% of the population and in 1.0-1.5% it persists daily or almost daily for more than 6 weeks and is therefore conventionally deemed to be chronic. In this form it causes severe impairment of quality of life, comparable with that experienced by patients with triple coronary arterial disease.1

Patients with chronic urticaria (CU) are too often poorly served by dermatologists and allergists alike, especially with regard to accurate diagnosis and optimum treatment. Significant progress has recently been made on understanding of the immunopathology of CU, and the efficacy of H1 antihistamines, still the cornerstone of drug treatment, continues to improve. However, for the majority of CU patients the etiology remains enigmatic, and although H1 antihistamines are usually effective in controlling itch of CU, they are often less impressive in preventing or reducing the visible hive (wheal) - especially in chronic idiopathic urticaria (CIU). Presumably this is because, as a late-phase immunological reaction 2 the wheal in CIU results from actions of a range of mediators including eicosanoids, TH2 cytokines and proteases as well as histamine.

Diagnosis: sub-types of chronic urticaria

Does the patient have urticaria? Most of us would have little difficulty in diagnosis, but errors can some-

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times be made. Pitfalls include urticarial dermatitis 3 the rash of pre-pemphigoid, maculopapular drug eruptions, acute contact dermatitis and insect bite reactions.

A practical approach to the diagnosis of CU requires that the 3 main subtypes, physical urticaria, urticarial vasculitis and CIU be considered. Although accurate information is often very hard to elicit, the duration of individual wheals can be a useful diagnostic pointer. Short lived wheals (less than 1 h in duration) suggest a physical urticaria. An exception is delayed pressure urticaria in which wheals frequently last over 24 h. Wheals lasting more than 4-6 h but less than 24 h are characteristic of CIU, whereas wheals persisting over 24 h should prompt consideration of urticarial vasculitis.

Physical urticaria

Physical urticarias represent about 45% of CU. Characteristically individual wheals last less than 1 h except for delayed pressure urticaria. The commonest physical urticarias are symptomatic demographism and cholinergic urticaria. Delayed pressure urticaria frequently co-exists with CIU. If a physical urticaria turns
out to be the patient’s sole or at least predominant problem then, apart from confirming the diagnosis by appropriate physical urticaria challenge-testing, no further investigations are warranted, and symptomatic control using H1 antihistamines should be sought. The physical urticarias have recently been reviewed.4

**Urticarial vasculitis**

Urticarial vasculitis represents about 5% of all cases of CU, but is probably underdiagnosed. Individual wheals last more than 24 h and may stain the skin due to purpura. Itching is variable and pain or tenderness may predominate, and unlike CIU systemic symptoms (arthralgia, fatigue, fever) may occur. This diagnosis, which needs to be confirmed by histological examination of a skin biopsy, is important to make as a detailed search will need to be made for underlying causes including autoimmune connective tissue diseases, inflammatory bowel disease, viral hepatitis and paraproteinemia, as well as for evidence of involvement of other organs, especially the lungs and kidneys. Schnitzler’s syndrome (IgM kappa paraproteinemia, fever, bone pain and urticaria) may or may not show vasculitis on skin biopsy.

**Chronic idiopathic urticaria**

The late-phase reaction which characterizes the wheals of CIU is due to activation of dermal mast cells. Although a large number of naturally occurring immunological and non-immunological agents are known to be capable of activating dermal mast cells, only allergen-specific IgE reactions, anti-IgE and anti-FcεR1 autoantibodies and complement C5α are of established importance in urticaria. Dermal mast cells secrete preformed mediators including histamine (mainly the cause of pruritus), proteases, interleukin-1 and tumor necrosis factor-α. The cytokines cause increased expression of adhesion molecules by endothelium of post capillary venules. De novo synthesized mediators include leukotrienes, prostaglandins, cytokines and chemokines resulting in leukocyte recruitment including eosinophils which characterise the late-phase reaction. An MHC class II- dependent signaling pathway enables mast cells to behave as antigen presenting cells which by activating T cells are able to maintain the longevity of the wheals.5

Until recently CIU was truly “idiopathic”, but during the past decade evidence has emerged that at least some of these patients have an autoimmune disease manifesting itself as CU. This type of chronic urticaria is conventionally designated as chronic autoimmune urticaria (CAU).

**Immunopathology of chronic autoimmune urticaria**

**IgG autoantibodies in chronic autoimmune urticaria**

This topic has recently been reviewed.6, 7 Isolation of IgG high affinity IgE receptor autoantibody subclasses using protein G affinity chromatography in patients’ sera has shown that the majority of the histamine releasing activity core-localises predominantly with IgG subclasses IgG1 and IgG3 and similar results were also reported using immunoblotting. Significant amounts of non-functional (non-histamine releasing) IgG anti-FcεR1 immunoreactivity were found concurrently with histamine releasing IgG in all subclasses studied.

The precise binding sites for anti-FcεR1 autoantibodies have yet to be elucidated. The high affinity IgE receptor, FcεR1, consists of 4 peptide chains: intracellular γ1, γ2 and β chains and an α-chain with an extracellular portion which bears the binding site for IgE. Competitive inhibition studies using human recombinant α-chain of FcεR1 have established that the binding sites for IgG anti-FcεR1 autoantibodies are located on the external portion of the α-chain. Binding can occur on at least 2 domains in this region of the α-chain since inhibition experiments utilizing myeloma IgE have demonstrated blockade of some but not all of these autoantibodies, an effect which could be reversed by lactic acid stripping to remove basophil or mast cell-bound IgE. These autoantibodies cross-link and dimerise FcεR1 leading to mast cell or basophil activation and release of histamine and other mediators. Up to 5-10% of patients with CAU have autoantibodies with a specificity for IgE itself. These combine with and cross-link receptor-bound IgE and, by dimerising mast cell-bound IgE, evoke release of histamine. Removal of IgE from mast cells or basophils by lactic acid stripping renders these autoantibodies inactive.

Anti-FcεR1 and anti-IgE autoantibodies are functional, leading to mast cell and basophil activation and release of histamine and other pro-inflammatory mediators.


Role of complement

Complement depletion or inactivation diminishes histamine release evoked by anti-FcεR1 autoantibodies in vitro. Subsequent studies established the involvement of C5a and the classical complement activation pathway. This finding may also explain the otherwise puzzling observation that symptoms and signs consequent upon interaction between anti-FcεR1 autoantibodies and their antigenic targets on mast cell membranes are limited to the skin since pulmonary mast cells are devoid of C5a receptors. Activation of the classical complement pathway and formation of C5a is important not only to bring about dermal mast cell activation but also because C5a is a neutrophil and eosinophil chemoattractant contributing to the observed accumulation of these cells in lesional skin.

Diagnosis of chronic autoimmune urticaria

Clinical and histological features

Although there are minor clinical differences between CAU and CIU, none of these is sufficiently distinctive to be clinically useful. Patients with CAU have a significantly lower serum IgE than those with no autoantibodies. The significance of this finding is unclear. Histological examination of skin biopsies shows that although the infiltrate in patients with CAU is characterized by a more prominent granulocyte infiltrate than that of non-autoimmune patients, the frequency of other infiltrating cells was similar in both groups, although there was a slight increase in serum tryptase and cytokines in the CAU patients. These small differences are insufficient to assist in diagnosis.

Autologous serum skin test

In the autologous serum skin test (ASST), serum is obtained from the patient, preferably when the urticaria is in relapse. This is injected intradermally into the same patient's uninvolved skin and the injected skin examined for wheal formation 30 min later. Necessary controls include intradermal histamine and saline injections into adjacent skin. The test is positive if the diameter of the serum-induced wheal is at least 1.5 mm greater than the saline wheal. The test has a sensitivity of about 70% and specificity of about 80% if performed as described above. However, experience is required to obtain reproducible results. In a recent study we found that of 78 patients with CIU, 35% had a positive ASST. However, histamine-releasing activity against donor basophils was found in sera of only 25%. Presumably the "false" positive ASST's were due to the presence in serum of other wheal-inducing factors. The ASST is at best a crude screening test and should not be used as a specific test for circulating autoantibodies.

In vitro histamine release tests

Demonstration that the patient's serum will release histamine (or other mediator) from donor basophils or dermal mast cells remains the benchmark confirmatory test for autoimmune urticaria. Although human basophils are widely used, human mast cells (utilizing thin slices of foreskin or suspensions of partially purified dermal mast cells) are effective alternatives. A rat basophil cell line in which the basophils have been transfected with genes encoding for the α–, γ1- and γ2-chains of FcεR1 has also been used successfully. Utilising human donor basophils, sera from 78 CIU patients, 27 (35%) were positive indicative of the presence of functional anti-FcεR1 or anti-IgE autoantibodies, the corresponding figures using dermal mast cells in this study being 31 (40%). This test is now commercially available.

In addition, recent studies in 52 children have shown that approximately 45% of children with CIU had autoimmune urticaria as judged by basophil histamine release evoked by these patients' sera.

Initially it was hoped that the diagnosis of autoimmune urticaria could be confirmed using a simple and convenient immunoassay. However, numerous attempts to develop immunoassays which will measure serum levels of IgG anti-FcεR1 or anti-IgE have shown low specificity and poor correlation with in vitro serum histamine releasing and urticarial disease activity. Positive immunoreactivity has been found in several nonurticarial autoimmune diseases.

Recently there have been reports of the presence of innate anti-FcεR1 autoantibody activity in normal serum. These antibodies which are primarily IgM, show cross reactivity with tetanus toxoid and may be functional if basophils which have had IgE removed by lactic acid are used. It has been suggested that a state of "conditional autoimmunity" may exist in CU, dermal mast cell activation only occurring if these autoan...
tibodies encounter mast cells devoid of IgE. However, other investigators have been unable to neutralize anti-FcεR1α using tetanus toxoid or inhibit histamine release by this means and little or no evidence of histamine-releasing activity has been detected in healthy sera even though large numbers of samples have been screened. It seems reasonable, at present, to rely on demonstration of histamine release from donor basophils as the benchmark diagnostic test for autoimmune urticaria.

**General management of chronic urticaria**

**Diagnostic workup**

Patients with CU are frequently overinvestigated, and a recent study failed to show that increasing the number of laboratory tests performed increased the number of identified diagnoses. Guidelines for diagnosis have recently been formulated. The first step requires exclusion of physical urticarias by appropriate challenge testing. This includes stroking the skin firmly for symptomatic dermographism, exercise testing for cholinergic urticaria and ice cube challenge testing for cold urticaria. These and other procedures have recently been reviewed. Identification of physical urticarias is important as, once the diagnosis is confirmed, no further investigations are warranted, and response to avoidance of provoking stimuli coupled with H1 antihistamine treatment is all that is needed.

If physical urticarias can be excluded, consideration should be given to the possibility that the patient has urticarial vasculitis. Clinical features to look for have been described above, but the clinical picture may be unremarkable. In all cases the diagnosis must be confirmed by a skin biopsy, histological examination of which should reveal leucocytoclastic vasculitis. Direct immunofluorescence examination of the skin biopsy is unhelpful. If the diagnosis is confirmed, then underlying causes must be sought as previously outlined and investigation should include a search for evidence of associated systemic vasculitic disease. Patients with urticarial vasculitis associated with hypocomplementemia are more likely to have systemic involvement than those without.

A total white blood cell count and differential is worth doing to exclude parasitic infestation - especially in developing countries. Absence of a blood eosinophilia obviates the necessity for stool examinations for ova. There is a strong association between presence of thyroid autoantibodies and CU and these co-segregate with anti-FcεR1 autoantibodies. Most of these patients are euthyroid, but occasionally they are hyper- or hypothyroid. Claims have been made that correcting thyroid dysfunction, if present, or, in euthyroid patients with antithyroid autoantibodies, treatment with thyroxine, ameliorated the urticaria but the evidence is not convincing. However, the association between thyroid disease and CAU is sufficiently strong to prompt adding measurement of the plasma level of thyroid stimulating hormone level (as a screen for thyroid dysfunction) and thyroid autoantibodies to the routine workup. Search for foci of infection, food allergy and food pseudoallergy has little or no place in the routine investigation of CU.

**Treatment**

For all forms of CU basic lifestyle ground rules need to be observed. These include avoidance of stress, overtiredness, alcohol, NSAIDs, and tight fitting garments. Nocturnal pruritus can be reduced by tepid showering and keeping the ambient temperature of the bedroom cool. Cooling lotions such as calamine with 1% menthol are popular with patients.

**H1 antihistamines: pharmacological considerations**

The H1 receptor, cloned and sequenced by Yamashita et al. in 1991, is a G protein-coupled receptor with a molecular weight of 40 kDa. H1 antihistamines, now all believed to act as inverse agonists of histamine by down-regulating the constitutively activated form of the H1 receptor, remain the cornerstone of drug treatment of all types of CU except urticarial vasculitis in which they are usually ineffective alone. Their clinical pharmacology and correct usage in urticaria has recently been comprehensively reviewed. First introduced for the treatment of urticaria and other dermatoses in 1947, the first generation of H1 antihistamines were effective in relieving the itch of urticaria, but at significant cost due to sedation and atropine-like side effects such as bladder dysfunction, paralysis of accommodation and raised intraocular pressure, especially in the elderly. These and other unwanted effects were mainly due to the ease with which the early H1 antihistamines penetrated the blood-
Chronic urticaria: recent advances in diagnosis and treatment

Second generation H1 antihistamines

The advent in the 1980's of a new "second generation" of H1 antihistamines (represented initially by terfenadine and astemizole - later withdrawn due to cardiotoxicity - and later by loratidine and cetirizine) was a major advance. The new antihistamines caused little or no sedation in licensed dosage due to minimal penetration of the blood brain barrier as demonstrated by the use of 11C-doxepin in positron emission tomography studies, and their recognition by the P-glycoprotein efflux pump which is present in the endothelial cells of the cerebral vasculature. However, these second generation compounds did cause sedation in some patients if used in off-label dosages.

New low sedation H1 antihistamines

In an effort to fine-tune the therapeutic index of these antihistamines, improved versions have subsequently been licensed in the USA and Europe for the indication of urticaria - initially fexofenadine, and latterly levocetirizine (L-cetirizine) and descarboethoxyloratidine (desloratadine). These antihistamines, which are active metabolites (or, in the case of levocetirizine, the active R-enantiomer) of the parent compound cause sedation no greater than placebo even if prescribed in twice the recommended dosages or more as demonstrated by objective measures of cognitive function.

But, theoretical considerations aside, do these antihistamines show evidence-based improved efficacy in patients with urticaria?

Pruritus is the main symptom of most types of urticaria and its diurnal periodicity should be determined in every patient. Many patients find that itching is less troublesome during the working day when attention is distracted, onset of itching occurring characteristically when they are relaxing at home in the evening. This needs to be taken into account in timing the dose schedule of the antihistamines used. However, for many patients pruritus is troublesome day and night and for them a twice daily dosage of a low sedation H1 antihistamine is adequate.

How to get the best response with H1 antihistamines in CU

Pruritus is the main symptom of most types of urticaria and its diurnal periodicity should be determined in every patient. Many patients find that itching is less troublesome during the working day when attention is distracted, onset of itching occurring characteristically when they are relaxing at home in the evening. This needs to be taken into account in timing the dose schedule of the antihistamines used. However, for many patients pruritus is troublesome day and night and for them a twice daily dosage of a low sedation H1 antihistamine is adequate.

H2 antagonists

Cimetidine or ranitidine may occasionally be useful in patients with severe urticaria who experience gastric hyperacidity due to elevated histamine levels, and in patients receiving short courses of oral steroids during relapses, but otherwise have no place in contemporary management of CU.
Other treatments

Leukotriene antagonists

The value of adding a leukotriene antagonist such as montelukast 10 mg daily to H1 antihistamine treatment is controversial. A review of the literature suggests that, although there is no clear evidence of efficacy, addition of a leukotriene antagonist does appear to help some patients, especially those experiencing flare-ups due to aspirin and other NSAIDs. Adverse effects are rare but Churg-Strauss vasculopathy has been reported.

Systemic corticosteroids

Systemic corticosteroids are useful in patients experiencing severe sometimes predictable flare-ups which cannot be controlled by antihistamines. Brief tapering courses (e.g. 30 mg daily for 3 days; 20 mg daily for 3 days; 10 mg daily for 3 days; 5 mg daily for 3 days then cease) should be prescribed. European guidelines 10 discourage the use of systemic steroids as long-term treatment. Oral cyclosporine, a calcineurin inhibitor which inhibits T cell activation and which is proposed for the treatment of CAU (see below), is also effective in CIU but not in physical urticarias. I use it in selected CIU patients with severe QOL impairment who are poorly responsive to antihistamines.

Treatment of CAU: special considerations

Patients with proven CAU are initially treated in exactly the same way as patients with CIU. However, they usually have severe disease and frequently prove poorly responsive to antihistamines, even in off-label dosage.

Cyclosporin

Cyclosporin was shown to be effective in selected patients who were ASST positive in a double blind placebo controlled study. 17 The dose range used is 4-6 mg/kg/day in addition to antihistamines. Our practice is to offer a course of up to 3 months. Eighty per cent of patients experience total or partial remission. In these, withdrawal of cyclosporine is followed by sustained remission in about 1/3 of patients. One third of patients relapse but are responsive to regular dosages of H1 antihistamines. Although the remaining 1/3 of patients relapse to a severity approximating to that suffered before initiating cyclosporine, I have rarely experienced a rebound effect such as that frequently occurring after withdrawal of oral steroids. These patients can be offered further cyclosporine treatment. All patients receiving cyclosporine must be regularly monitored for renal function, serum cholesterol and triglycerides and blood pressure. Cyclosporin has also proved to be effective in non-autoimmune "ordinary" CIU. Other immunomodulators that have been reported effective in selected patients with CAU include intravenous immunoglobulin and plasmapheresis.

References

Chronic idiopathic urticaria (CIU) is a skin condition defined as itchy wheals on almost a daily basis for 6 weeks or more, were a specific cause has not been identified after full evaluation.1 Itch is the predominant symptom of urticaria and pain and burning sensations are more suggestive of urticaria vasculitis. CIU is a self-limited disease with a median duration of 2–4 years.

The etiopathogenesis of CIU is considered to be autoimmune in up to 50% of cases. These patients have detectable anti-FcER1 or anti-IgE auto-antibodies. A strong association with thyroid autoimmunity has been observed and confirmed in a recent large scale study.2 CIU can cause significant disability. The quality of life of CIU patients has been studied in 142 outpatients, who experienced an impairment similar to patients with coronary artery disease.3 The patients had significant sleep disturbances, diminished energy, social isolation, and difficulties in relation to work, home activities and social life. Another smaller scale study of 21 patients in Italy has shown that patients with chronic urticaria had lower quality of life scores compared to patients with respiratory allergies and both were significantly lower than healthy subjects.4

Although the cause of CIU may remain elusive, there are number of factors that worsen and aggravate its main symptom of itch. These include ambient heat and sweating, drugs such as NSAIDs and aspirin, alcoholic beverages, and food additives containing salicylates and aromatic compounds. Emotional stress can cause flairs of CIU. Itch intensity in CIU has been related to stress; however, it is significantly less in comparison with other chronic pruritic dermatoses such as atopic eczema and psoriasis.5 The clinical presentation of itch in chronic urticaria is unique in comparison with other types of itch, since there are usually no excoriations or scratch marks. Most patients rub their skin rather than scratching. The areas where wheals occur coincide with areas of itch and are mainly located in the arms, back, and waistband in sites of pressure. There is a significant diurnal variation in itch of CIU; patients report more itch in the evening and at night and 64% of patients reported being awaken by their itch.5

H1-antihistamines are the first-line treatment for all patients with CIU.6 Short or long-term relief of itching was achieved in 92% of patients with CIU.5 Low-sedation antihistamines such as loratadine, cetirizine, desloratadine, fexofandane have been shown to be effective in reducing daytime symptoms of chronic urticaria.

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In this issue, Seidenari et al. report a large multi-center study performed in Italy on the effect of desloratadine 5 mg on quality of life of 255 patients aged 18-79 years with moderate to severe CIU using the dermatology life quality index (DLQI), a well-validated tool. After 28 days of treatment, desloratadine improved the quality of life of the CIU patients and their itch intensity. One of the limitations of this study, as identified by the authors is its design as an open-label study. A placebo arm would have significantly improved the scientific quality of this large scale study. However, the large study population participating in this multicenter study suggests that desloratadine reduces symptoms of itch and hives and has beneficial effects on the quality of life of CIU patients. Moreover, a recent multicenter study in Belgium assessed the effect of desloratadine 5 mg on quality of life in 121 patients with CIU for 42 days in an open-label fashion. Similar results were reported to the Italian study and 77% of patients had a clinically significant change. The improvement in clinical signs of itch and hives correlated to change in DLQI scores. Monroe et al. performed the only placebo-controlled study on the effect of desloratadine 5 mg on sleep, itch and severity of CIU in 226 patients from several centers in the US and demonstrated significant improvement. However, the authors did not evaluate quality of life using validated QOL tools.

Desloratadine is the major orally active metabolite of loratadine. It is metabolized by glucuronidation in the cytochrome P450 system. Desloratadine has a half-life of 19-34 h and is excreted in the urine and feces. It is considered a safe drug and has low risk of adverse cardiovascular effects, and no reported ECG changes, which have been noted previously with astemizole and terfenadine. No known interactions have been reported between desloratadine and other drugs that affect cytochrome P450. There is no data on safety of this drug in pregnancy, and therefore it is best to avoid using desloratadine and most other antihistamines in pregnancy.

The efficacy of non-sedating antihistamines in CIU has been well-documented; however, the sedating antihistamines still have a significant role in controlling CIU. As Seidenari et al. pointed out, patients with CIU have significant sleep disturbance. The classical, sedating H1-antihistamines are frequently required to manage CIU, especially in those patients who report sleep disturbance. Hydroxyzine at 25-50 mg t.i.d. seems to be the most effective sedating H1-blocker in reducing hives and itch at night time. Doxepin is a potent oral antihistamine, but has significant sedating and anticholinergic effects and many patients do not tolerate it. Combination of both sedating and non-sedating H1-blockers is commonly used and recommended both in Europe as well as in the USA. Increasing the dose of non-sedating antihistamines higher than recommended by manufacturers to twice daily is common practice in the USA. Alternating between different types of antihistamines is also helpful in reducing the symptoms of CIU and itch. A significant number of patients do not respond to oral antihistamines alone.

Other treatments for CIU include oral corticosteroids. Short courses of high-dose oral corticosteroids are helpful for antihistamine-resistant CIU. Several regimens have been used. In the USA, there is tendency to use higher doses tapered down gradually from 40 mg in a 3 week taper. Other treatments that seem effective include ciclosporine A 3-5 mg/kg, which has been shown to be effective in severe cases of autoimmune CIU. During treatment of CIU, antihistamines should be continuously administered. Recently, oral tacrolimus has been shown to be an effective treatment for severe cases of CIU in low doses ranging between 0.05-0.2 mg/kg/day. Intravenous gammaglobulins in severe cases of CIU have also been reported in case series of autoimmune CIU with circulating anti-FcER1 or anti-IgE auto-antibodies. Other treatments commonly used include hydroxychloroquine 400 mg/day, as well as colchicine and dapsone; however, there are no controlled studies to provide evidence for the efficacy of these regimens.

In conclusion, antihistamines have an important role in reducing CIU symptoms; however, a significant proportion of patients do not respond to these treatments alone. Other treatments for this disabling symptom have been utilized but not subjected to controlled studies. Therefore, these treatments should be further evaluated through double-blind, controlled studies.
References

Sequential treatment of severe recalcitrant psoriasis with infliximab and cyclosporine

W. STERRY

The use of biologicals in the treatment of psoriasis has changed dramatically our options particularly in patients with severe psoriasis. Many patients benefit in situations were conventional treatments have failed, are contraindicated or have produced side effects.

Two aspects, however, remain open at present: first, biologicals are expensive (although the overall benefit regarding not only costs for the drug, but also lower frequency of hospital admission, days of sick leave and even loss of working capability need to be calculated) and thus reimbursement remains a matter of daily discussion with health insurances, and second, the combination of biologicals with conventional systemic drugs, UV therapies and with other biologicals.

The approach to the second question will be mainly investigator initiated, since the companies producing biologicals will at present not initiate such trials. Thus, large and randomized clinical studies will not be possible for each of the potential combinations, be it parallel or sequential combinations. Rather, small pilot studies seem feasible to allow testing hypothesis of beneficial action of certain combinations.

One aspect that has come with the advent of biologicals and the concept of long term control of psoriasis is the distinction between induction and maintenance treatments in psoriasis. Once patients with severe psoriasis have responded to an intensive induction treatment, other therapeutic options may come into place to maintain the response. However, induction is not always easy to achieve in patients with highly active and severe psoriasis.

Here comes the study conducted by G. A. Vena et al. into the field. They speculated that patients that have shown be recalcitrant to conventional treatments including cyclosporine might become sensitive to cyclosporine after induction treatment with just 2 courses of infliximab. In their small but highly relevant study they found that 10 out of 10 patients responded well to infliximab at week 2, and that this response could be maintained in 9 of 10 patients.

What are the conclusions from this study? First, it shows that nonresponsiveness of psoriasis to conventional systemic treatment can be overcome by short courses of infliximab, with subsequent responsiveness to the previous treatment. Second, it demonstrates that our armamentarium of antipsoriatic drugs may become more efficient by combing different classes of systemic drugs. Third, and possibly most important, it shows that the dermatological community will be able to contribute independently from pharmaceutical companies to the development of new treatment strategies. Of course, there are many

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possible combinations that are worth to be tested, but G. A. Vena and his group have demonstrated how to proceed to gain scientific information within this relevant therapeutic area for those patients with psoriasis that are extremely difficult to treat. For example, in Germany, where fumarates are used for systemic treatment of psoriasis, exacerbations are managed by using etanercept for 4-8 months; formal studies are lacking so far.

However, a great deal of clinical experience and meticulous monitoring of such patients are mandatory. Two recent publications have summarized our current knowledge, mainly based on single case reports, in combining biologics with non-biologics. Such case reports include the development of malignant lymphoma in a patient with cyclosporine and infliximab; most interestingly, the lymphoma went into complete remission 5 months after the treatment had been stopped. Moreover, cases with rare or opportunistic infections have been published under a variety of combination treatments. Among the infections reported were mollusca contagiosa of the eyebrows, Nocardia, Cryptococcus, Listeria, and septicaemia. Some of the infections were fatal.

In summary, after the introduction of biologics in the treatment of psoriasis, we have started to combine them with other treatment options. The study of the possible combinations may bring further therapeutic benefit to our patients, but needs to be brought forward under controlled clinical trials.

References
Desloratadine 5 mg once daily improves quality of life in chronic idiopathic urticaria

Aim. Desloratadine (DL) is an effective treatment for chronic idiopathic urticaria (CIU) symptoms, but there is little information about its impact on patients’ perceived general well-being. The primary objective was to measure the effect of DL on the quality of life (QoL) of CIU patients, using the Dermatology Life Quality Index (DLQI). Secondary objectives were: evaluation of the effects of DL on pruritus and number of hives, sleep and daily activities, overall CIU condition, and assessment of therapeutic response. Safety and tolerability of DL were also evaluated.

Methods. Two-hundred and fifty-five patients, aged 18-79 years and with moderate to severe CIU, were treated with DL 5 mg once daily for 28 days. Patients recorded signs and symptoms scores daily, and completed the DLQI questionnaire at baseline and on treatment days 7, 14, 21 and 28. Patients and investigators jointly evaluated overall CIU condition and therapeutic response at days 14 and 28.

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Results. After 28 days of treatment, DL significantly reduced mean overall DLQI score from 9.4 (±5.4) at baseline to 3 (±4.1) (–66.1%; P<0.001), with no significant differences between moderate and severe patients. Improvement in QoL was significant (P<0.001) at all time points and for all DLQI domains. All signs and symptoms scores were significantly correlated with DLQI score.

Conclusion. Treatment with DL 5 mg for 4 weeks significantly improves symptoms and QoL in patients with CIU, irrespective of disease severity, and with a rapid onset of action.

Key words: Antihistamines - Chronic idiopathic urticaria - Dermatology life quality index (DLQI) - Desloratadine - Quality of life.

Urticaria is a very common disorder, with chronic idiopathic urticaria (CIU) being the form most frequently encountered. CIU is defined as a six-week or longer history of widespread itchy wheals or hives in the absence of a detectable allergic, physical or environmental cause.1 If not adequately treated, CIU patients report problems with daily activities, social interactions, emotions, mobility and sleep. Symptoms and signs of CIU can be distressing and debilitating, and CIU has been shown to affect patients’ quality of life (QoL) even more than other severe skin diseases, such as acne or psoriasis.2,4 Some authors found the impact on QoL reported by CIU patients to be comparable to that of older patients with ischaemic heart disease,5 and greater than that of patients with respiratory allergy.6

Over the past decade, information on QoL in dermatology has become an important additional measure to assess treatment efficacy, second only to clinical symptoms evaluation, especially in chronic and recurrent diseases in which the clinical manifestations can lead to significant decrements in emotional well-being, social functioning and sleep habits. Specific tools for QoL assessment allow the evaluation of a patient’s subjective perception of the effects of treatment on his/her life and the identification of the items (physical, social or psychological) that most influence improvement.7 Thus, the more information that is collected about the degree of improvement in QoL, the patient’s subjective perception of the effects of treatment on his/her life and the identification of the items (physical, social or psychological) that most influence improvement.7 Thus, the more information that is collected about the degree of improvement in QoL, the more precise can be the selection of the most effective therapy for a specific group of patients.

As the symptoms and signs of CIU are primarily mediated by histamine, antihistamines are the first-line therapy in CIU.2 Desloratadine (DL) is a nonse-dating antihistamine with potent peripheral H1-receptor blockade. This agent is indicated for the treatment of allergic rhinitis and CIU in the United States and in most countries in Europe, Asia and South America.8 DL was proven to be effective in the treatment of CIU in 2 large, multicentre, double-blind, placebo-controlled studies. Administered at a dose of 5 mg orally once daily (o.d.),9, 10 it significantly reduced pruritus, total symptom score, number of hives and interference with sleep and daily activities, and improved the overall therapeutic response and global CIU status. No QoL questionnaires were used in these studies, hence data regarding the effects of DL treatment on the QoL of chronic urticaria patients are lacking.

The important implications of the psychosocial aspects of CIU for the optimal management of patients have prompted us to evaluate the actual impact of DL on the QoL of CIU patients, using the Dermatology Life Quality Index (DLQI), a dermatology-specific questionnaire that has been used extensively for different conditions over the past decade.3,4,11-13 Recently, the clinical meaning of the DLQI score and of its change have been further clarified.14, 15 This multicentre, noncontrolled clinical study was designed as an exploratory assessment of the effect of 5 mg o.d. DL on the QoL of patients with moderate to severe CIU, as measured by the DLQI. Secondary objectives were evaluation of the effects of DL on pruritus and number of hives, and on sleep and daily activities, as well as assessment of the improvement in overall CIU condition and the therapeutic response. Safety and tolerability were also assessed.

Materials and methods

Patients

After giving written informed consent, male and female patients aged ≥18 years, and with a history of CIU (defined as at least 6 weeks of pruritus and hives, with hives lasting <24 h and occurring at least 2 days per week) in the absence of identifiable causal factors at clinical history and physical examination, were considered for admission to the run-in phase if they presented with a pruritus score ≥2 and a hive score ≥1 within 12 h prior to the consent visit. After an adequate wash-out period from all medications that could possibly interfere with urticaria and allergic conditions, patients entered a three-day run-in prior to the start of treatment (at visit 2). During the run-in, patients scored their pruritus, number of hives, and the impact of their disease on daily activities and sleep in a daily
diary. Patients with pruritus and an overall CIU condition of at least moderate severity at the end of the run-in period were assigned to DL treatment.

Patients with urticaria primarily due to physical or other known aetiological factors, pregnant or nursing women, and patients with concomitant asthma, drug or food allergies, or atopic dermatitis were excluded.

Study medication

DL (Aerius®; Schering-Plough) was administered orally at a dose of 5 mg o.d. (1 tablet) for 28 consecutive days. Patients were instructed to take the drug in the morning, after completion of their diary, with no regard to the timing of meals.

DLQI

The DLQI is a self-administered questionnaire measuring QoL over the previous week in adult patients with skin diseases. It consists of 10 questions scored from 0-3 and grouped in 6 domains, and has been validated in several languages, including Italian (http://www.dermatology.org.uk). Patients completed the questionnaire on treatment days 1 (baseline, before treatment initiation), 7, 14, 21 and 28. The DLQI score was calculated by summing the score for each question (minimum 0, maximum 30) – the higher the score, the more QoL is impaired. The primary outcome measure was the mean change from baseline in overall DLQI score at day 28. The mean changes from baseline at weekly intervals and in individual DLQI domains were also measured as secondary efficacy variables.

Other outcome measures

Patients assessed disease activity during the run-in and treatment periods by scoring in their daily diaries, using four-point scales (0-3), the following items: pruritus and number of hives (twice daily, morning [a.m.] and evening [p.m.], and interference with sleep (a.m.) and daily activities (p.m.). The daily mean score of pruritus and number of hives was then calculated as the mean of the a.m. and p.m. ratings. Overall CIU condition and global response to therapy were evaluated jointly by the investigator and patient after reviewing the diaries on days 14 and 28, using a four-point scale (0=None, 3=Severe) for CIU condition and a five-point scale (1=complete relief, 5=treatment failure) for therapeutic response. The overall condition of CIU was also evaluated at the consent visit and at visit 2 (prior to start of treatment).

Safety assessments

Vital signs were recorded at every visit. Laboratory evaluations were performed at screening and at the end of the study. All adverse events (AEs) were recorded and graded with respect to severity and potential relation to the study drug.

Sample size and statistical analysis

It was calculated that a sample size of 246 patients would allow estimation of the reduction in the percentage of the DLQI score after 28 days of treatment (two-sided, 95% confidence interval for the difference of means) with a significance level of 5% and assuming a standard deviation of the difference of 40.

Quantitative variables were described by the number of available and missing observations, mean, median, standard deviation, range (minimum and maximum), and first and third quartiles, whereas qualitative variables were described by frequency and percentage. Missing values were tabulated with their frequency, but were not included in the calculation of percentages. The overall statistical analysis was conducted on an intention-to-treat (ITT) basis. An analysis of covariance (ANCOVA) model was used, with the baseline DLQI score as the covariable and the DLQI score change (day 28 minus baseline) as the dependent variable. The ANCOVA model was also used to analyse secondary efficacy variables. Paired t-test and signed-rank test were used to assess whether changes produced during the study were different from zero. Categorical variables, such as the overall CIU condition assessment and the therapeutic response, were analysed descriptively and estimated with a 95% confidence interval following an exact binomial method (AS System release 8.02; Cary, NC, USA).

Results

Baseline characteristics

A total of 282 patients were enrolled at 28 Italian investigational sites (see Participating Centres): 27 were screening failures, while 255 were admitted to the treatment phase and represent the ITT population.
Demographic and CIU characteristics at baseline are summarised in Table I. Most patients had a moderate CIU condition, with moderate pruritus and up to 20 hives. The mean overall DLQI score at baseline was 9.4 (±5.4). The mean overall DLQI score was significantly higher (P<0.001) in patients with severe CIU (11.3±6.4) than in patients with moderate CIU (8.4±4.6). Baseline pruritus score (a.m./p.m.) was also significantly different between the 2 groups: 1.9±0.4 in moderate and 2.3±0.4 in severe patients (P<0.001).

**Efficacy analysis**

After 28 days of treatment, DL significantly reduced the mean overall DLQI score from 9.4 (±5.4) at baseline to 3 (±4.1) (66.1%; P<0.001). The reduction was highly significant after only the first 7 days of DL therapy (41.8%; P<0.001). The DLQI scores for moderate and severe patients before and after treatment are shown in Figure 1A. There were no significant differences in the DLQI mean percentage reductions at all time points during DL treatment between patients with moderate and severe CIU (67% and 63%, respectively, at day 28) (Figure 1B). Analysing the DLQI domains separately, the mean percentage reduction was highly significant for all DLQI domains, especially for the 'symptoms and feelings' domain, which was the most affected at baseline (Table II).

The distribution of the patient population across the DLQI domains showed that, at baseline, the impact of CIU on QoL was moderate in 40% of patients and very large in 29.4%. After 28 days of treatment, the impact was none to small in 79.3% of patients (Table III).

The mean pruritus score was significantly reduced within 24 h of the first dose of DL (mean percentage change of a.m. score at day 2 from baseline was 33%; P<0.001), and was further reduced up to day 28 (mean percentage change of a.m./p.m. score from baseline

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**Table I.** — *Demographic and disease characteristics at baseline.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Moderate CIU</th>
<th>Severe CIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td>Male 87 (34.1)</td>
<td>Female 168 (65.9)</td>
</tr>
<tr>
<td>Age, years Mean (median)</td>
<td>43.5 (42)</td>
<td>20.3 (8)</td>
</tr>
<tr>
<td>Duration of CIU, months Mean (median)</td>
<td>18-79</td>
<td>1.5-360</td>
</tr>
<tr>
<td>Duration of CIU, months Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hives, n (%)</td>
<td>1-6 58 (22.7)</td>
<td>7-20 115 (45.1)</td>
</tr>
<tr>
<td>Pruritus, n (%)</td>
<td>1-6 58 (22.7)</td>
<td>7-20 115 (45.1)</td>
</tr>
<tr>
<td>Pruritus, n (%)</td>
<td>Moderate 158 (62)</td>
<td>Severe 97 (38)</td>
</tr>
<tr>
<td>DLQI, Mean±SD</td>
<td>9.4±5.4</td>
<td>8.4±4.6</td>
</tr>
<tr>
<td>DLQI, Mean±SD</td>
<td>9.4±5.4</td>
<td>8.4±4.6</td>
</tr>
</tbody>
</table>

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Figure 1. — *Mean DLQI total score before and after treatment in patients with moderate and severe CIU (A). Mean percentage decrease in DLQI total score versus baseline, in moderate and severe CIU patients (B).*
Desloratadine 5 mg once daily improves quality of life in chronic idiopathic urticaria

Table II.—Mean percentage change in DLQI domain scores, and effect of each domain on the percentage decrease in the total score.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Mean baseline score</th>
<th>Mean final score</th>
<th>Mean % reduction versus baseline*</th>
<th>Relative impact on mean % decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall DLQI</td>
<td>9.4</td>
<td>3</td>
<td>-68.1</td>
<td>100%</td>
</tr>
<tr>
<td>Symptoms and feelings</td>
<td>3.4</td>
<td>1.2</td>
<td>-67.6</td>
<td>35.9%</td>
</tr>
<tr>
<td>Daily activities</td>
<td>1.9</td>
<td>0.6</td>
<td>-68.4</td>
<td>20.3%</td>
</tr>
<tr>
<td>Leisure</td>
<td>1.5</td>
<td>0.4</td>
<td>-73.3</td>
<td>17.2%</td>
</tr>
<tr>
<td>Work and school</td>
<td>1.1</td>
<td>0.3</td>
<td>-72.7</td>
<td>12.5%</td>
</tr>
<tr>
<td>Personal relationships</td>
<td>1.2</td>
<td>0.4</td>
<td>-66.7</td>
<td>12.5%</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.3</td>
<td>0.1</td>
<td>-33.3</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

*P<0.001 versus baseline

Table III.—Patient distribution across DLQI bands before and after desloratadine (DL) treatment.

<table>
<thead>
<tr>
<th>DLQI score</th>
<th>Baseline</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 (no to small effect)</td>
<td>26.1%</td>
<td>79.3%</td>
</tr>
<tr>
<td>6-10 (moderate effect)</td>
<td>40%</td>
<td>13.1%</td>
</tr>
<tr>
<td>11-20 (very large effect)</td>
<td>29.4%</td>
<td>7.6%</td>
</tr>
<tr>
<td>21-30 (extremely large effect)</td>
<td>4.5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

at day 7 was 57.4%, at day 14 62.5%, and at day 21 66.6%; P<0.001 versus baseline at all time points. No significant difference was observed in the mean percentage decrease of the mean pruritus score at all time points between patients with moderate and severe CIU. The mean final score was 0.5±0.6 in moderate and 0.7±0.9 in severe patients.

The reduction in the mean number of hives score followed a similar pattern to that seen in the mean pruritus score over the whole treatment period (–20.4% a.m. score day 2; –49% at day 7; –56% at day 14; –61.2% at day 21; –69% at day 28; P<0.001 versus baseline at all time points).

A marked improvement was observed in both interference with sleep and interference with daily activities (Figure 2); these improvements were evident from the early treatment phase. The overall CIU condition also improved significantly over the treatment period, with 79.7% of patients showing no to mild disease at the end of treatment. The global response to therapy, evaluated jointly by patient and investigator, indicated a complete or marked response in 61.9% of patients after 14 days of DL treatment and in 73.2% of patients by the end of the study period. Furthermore, patients demonstrating a complete/marked response to treatment reported a significantly greater percentage reduction in the overall DLQI score than patients with moderate to null response to treatment (Figure 3).
correlations between the improvement in the DLQI score and the improvement in the scores of pruritus, number of hives, interference with sleep and daily activities, and overall CIU condition were all highly significant (Table IV).

Safety analysis

Treatment-emergent AEs occurred in 80 patients (31.4%). The most frequent AE was headache (n=35; 13.7%), followed by nausea (n=8; 3.1%) and somnolence (n=7; 2.7%). Severe treatment-emergent AEs were reported in 4 patients (1.6%). No serious AEs occurred. Three patients discontinued the study owing to related AEs (2 headache and 1 treatment failure). No clinically significant changes in blood pressure, heart rate or laboratory parameters were observed during the study.

Discussion and conclusions

This is the first study to assess the effects of DL 5 mg o.d., administered for 4 weeks, on QoL, as measured by DLQI, in moderate to severe CIU patients. In the study population, the mean DLQI score at baseline was 9.4, an intermediate value compared with those reported in previous studies. The major impact on the overall score arose from the ‘symptoms and feelings’ domain, which explores the impact of symptoms and of the feelings of embarrassment and self-consciousness. Our study results demonstrate that DL is effective in significantly reducing the overall DLQI score, as well as each single domain score, with the most important contribution to the overall DLQI decrease being the effect on the ‘symptoms and feelings’ domain. The reduction in DLQI score was very rapid, being statistically significant as early as 1 week after treatment initiation, and reaching 66% by the end of the four-week treatment period. The improvement in the QoL of patients was accompanied by a significant and rapid improvement in the signs and symptoms of CIU, namely pruritus and number of hives. In the case of pruritus, although baseline severity differed markedly between patients with moderate and severe disease, the extent of improvement after DL treatment was comparable, with almost complete relief being achieved in both patient subgroups. The reduction in DLQI score paralleled the clinical improvement of CIU in terms of symptoms (pruritus, hives), overall disease conditions and treatment response, as assessed by both patients and investigators, with highly significant correlation coefficients. These results are consistent with those of previous studies, which also demonstrated a highly significant correlation between changes in DLQI scores and clinical outcomes in patients with CIU treated with fexofenadine. Taken together, these data suggest that clinical improvement in CIU associated with antihistamine treatment has a significant impact on patients’ QoL. These findings seem to exclude, at least in CIU, the speculation, based on the lack of correlation between QoL scores and clinical measures, that objective assessment of severity and extension of dermatitis by means of standardised scoring systems may not adequately reflect a patient’s state of disease or the precise outcome of the intervention.

Little has been published so far on the effects of antihistamine treatment on the QoL of patients with chronic urticaria. To our knowledge, only fexofenadine was shown to improve DLQI in a similar CIU patient population, with a reduction in overall DLQI score not exceeding 50% after 4 weeks of treatment with fexofenadine 60 mg twice daily.

In our study, the DLQI was sufficiently sensitive to discriminate between moderate and severe CIU, the mean DLQI score at baseline being 8.4 in moderate CIU patients and 11.3 in severe patients. These values are consistent with the DLQI score bands recently proposed by Hongbo et al., where a score of 6-10 reflected a moderate effect on patients’ life and one of 11-20 a very large effect. Despite the baseline difference, in the present study the DLQI score at the end of treatment was about 3 in subgroups of both moderate and severe patients, indicating a small impact on QoL in all patients. The comparable efficacy of DL, in terms of both improvements in QoL and relief of pruritus, in both patient subgroups suggests that the 5 mg o.d. dosage is also efficacious in patients.
with more severe symptoms, and no that no higher dose is required for any patient subgroup.

It has been suggested that, in the patient’s perception, a change in DLQI score of up to ±4 corresponds to almost the same, or hardly any change, in overall QoL, and a change of ±5 or more is needed for patients to feel at least a little or moderately better or worse. These indications emerged from questions answered by patients with inflammatory skin diseases – mainly psoriasis, atopic dermatitis and acne. In our CIU population, the average DLQI reduction was 5.7 in the subgroup with moderate disease at baseline and 7.8 in the subgroup with severe disease, indicating, according to the Khilji hypothesis, that the reduction in DLQI score was perceived by patients as an improvement in their general wellbeing. The finding that a significantly greater reduction in DLQI scores was reported by patients showing greater treatment response (symptoms absent or scarcely troublesome) provides further supporting evidence for this theory.

The assessment of the interference of CIU with sleep was of particular interest, as this is an important aspect of QoL that is not specifically investigated by DLQI domains. The correlation observed between the improvement in sleep and the decrease in overall DLQI score following DL administration suggests that reduced sleep interference is one of the treatment effects contributing to the global improvement in QoL.

Our study suffers from the limitation of its noncontrolled design. However, the present results are in close agreement, in terms of both the extent and rapidity of the effects observed, with the clinical efficacy data reported in previous double-blind, placebo-controlled studies performed in comparable CIU patients. In our opinion, this finding reduces the bias of the single-arm design and, in addition, supports the consistency of DL efficacy and the reliability of the effects of treatment on QoL. It could also be argued that the transferability of our results to office practice is limited by the selection criteria of our study, which included CIU patients with an acute disease flare in a secondary referral setting. In this respect, it should be noted that the impact of skin disease on the QoL of patients treated in a primary care setting has been shown to be comparable to that of secondary referral patients.

DL 5 mg o.d. administered for 4 weeks was safe and well tolerated in CIU patients. The AE profile confirms the results of previous DL trials, in terms of both the type and incidence. Moreover, the improvement not only in sleep, but also in daily activities, associated with DL treatment seems to confirm the absence of sedation.

In conclusion, our study shows that DL, administered at the dose of 5 mg o.d. for 4 weeks, significantly improves QoL, pruritus and hives in CIU patients, irrespective of disease severity, and with a rapid onset of action. In patients with CIU, DL seems to improve not only clinical symptoms, but also the subjective perception of general wellbeing.

Acknowledgements. The authors are grateful to R. Perego, MD, for her skilful cooperation in the preparation of the manuscript.

Riassunto

Desloratadina 5 mg una volta al giorno migliora la qualità di vita nell’orticaria cronica idiopatica

Obiettivo. Desloratadina (DL) è un farmaco efficace per il trattamento dei sintomi dell’orticaria cronica idiopatica (OCI), ma vi sono pochi dati circa il suo impatto sul benessere generale del paziente. L’obiettivo principale è stato misurare l’effetto di DL sulla qualità di vita dei pazienti con OCI utilizzando l’indice dermatologico della qualità di vita (Dermatology Life Quality Index, DLQI). Gli obiettivi secondari erano: la valutazione degli effetti di DL sul prurito e sul numero di ponfi, sul sonno e sulle attività quotidiane, sul quadro complessivo dell’orticaria nella valutazione della risposta terapeutica. Sono anche state valutate la sicurezza e la tollerabilità di DL.

Metodi. Sono stati trattati con 5 mg di DL una volta al giorno, per 28 giorni, 255 pazienti d’età compresa tra 18 e 75 anni con OCI da moderata a grave. I pazienti hanno registrato quotidianamente il punteggio relativo alla severità dei segni e sintomi e hanno compilato un questionario sull’indice dermatologico della qualità di vita all’inizio del trattamento e dopo 7, 14, 21 e 28 giorni. Al 14° e 28° giorno sono state valutate le condizioni generali della OCI e la risposta terapeutica congiuntamente dagli sperimentatori e dai pazienti.

Risultati. Dopo 28 giorni di trattamento DL ha significativamente ridotto rispetto al basale il punteggio globale medio dell’indice dermatologico della qualità di vita da 9,4 (±5,4) a 3 (±4,1) (-66,1%; P<0,001), senza alcuna differenza significativa tra i pazienti con OCI moderata e grave. Il miglioramento della qualità di vita è risultato significativo (P<0,001) ad ogni valutazione (dopo 7, 14, 21 giorni) e per tutti i parametri contemplati dall’indice dermatologico della qualità di vita. Il punteggio relativo a tutti i segni e sintomi si è ridotto significativamente a partire dal secondo giorno di trattamento, mostrando una correlazione altamente significativa con il punteggio dell’indice dermatologico della qualità di vita.

Conclusioni. Il trattamento con DL 5 mg una volta al giorno per 4 settimane migliora significativamente i sintomi e la qualità di vita dei pazienti con OCI indipendentemente dal-
la gravità della malattia, e il suo effetto compare entro i primi 2 giorni di trattamento.

Parole chiave: Antistaminici - Orticaria cronica - Dermatologia life quality index (DLQI) - Desloratadina - Qualità di vita.

References
Epidemiologic data about polymorphous light eruption in Italy


Aim. Polymorphous light eruption (PLE) is the most common idiopathic photodermatosis. It describes a broad clinical spectrum with chronic recurrences. It is often characterized by non scarring pruritic erythematos papules, vesicles or plaques. UV exposure is the main pathogenetic factor. The aim of this study was to evaluate the prevalence of PLE in Italy, the main clinical features and the clinical course and recurrences in a Mediterranean population.

Methods. The study was carried out on 4,416 subjects in 8 Dermatological Units in Italy, distributed over the whole country. Subjects were required to fill a simple questionnaire (43 questions) exploring the following topics: phototype and phenotype, and modalities of solar exposure. In the subjects with a previous PLE another questionnaire was submitted to investigate the clinical features of PLE, number of recurrences, familiar, pathological and pharmacological anamnesis. The study was carried out in healthy volunteers, not affected by any dermatological disease.

Results. Among the 4,416 apparently healthy subjects who filled out the survey, 212 gave a history consistent with a diagnosis of PLE. The PLE prevalence was 5.89% without significant differences among the Dermatological Units distributed at different latitudes in our Country. The coalescent papules type of PLE was the most common clinical picture (36.4%); the body site most frequently affected was the trunk (61.1%). On the contrary, chronically sun exposed body site (i.e. the face) is affected just in few cases. Also people chronically sun exposed developed PLE less frequently than occasionally sun exposed people. Sometimes, PLE developed after a particularly intense sun exposure (37.7% of PLE).

Conclusion. No correlations with drug assumption or environmental chemical compound have been underlined.

Key words: Polymorphous light eruption - Photodermatosis - Epidemiology.

Polymorphous light eruption (PLE) is extremely common. The prevalence of PLE is reported to range between 5% and 21%. The onset of symptoms is usually in the first 3 decades and, in most series, females outnumber males. 1-4
It is characterized by seasonal occurrence and usually starts to appear in late spring or during holidays in sunny regions. The time interval between the beginning of sun exposure and the outbreak of the skin eruption may range from one hour to a few days, with the most common interval being 1-2 days. Lesions may be situated on any light exposed areas, with a predilection for the forearms, the antero-lateral surfaces of the upper arms and the v-area of the chest. Usually the initial episode occurs after an intense sun exposure.

Several morphological variants of PLE have been described. PLE is usually characterized by a particular clinical pattern: a papular type; a papulovesicular type; a plaque-type or, less frequently, a vesiculobullous or eczematous type.

Itching is almost always present. The disease generally follows a chronic course.

In some patients the severity of PLE decreases as summer progresses. It is uncommon that PLE disappears spontaneously. In most cases it follows a chronic course.

Etiology is unknown. A delayed hypersensitivity reaction to an antigen induced by radiation would seem possible. The most important diagnostic tool is phototesting with the aim of inducing an isomorphic response.

The aim of this study was to assess the PLE prevalence, clinical characteristics and course in a group of people representatives of the Italian population.

Materials and methods

Survey population

The study was carried out in 8 centres distributed in northern as well as in southern Italy: Genoa, Brescia, Milan, Verona, Perugia, Siena, Rome and Naples. A total of 4,416 subjects ranging from 16 to 48 years of age were interviewed. People were healthy subjects selected at random from persons accompanying familiar or friends in the Dermatological Units above indicated.

Questionnaire design

The questionnaire included 43 questions to determine prevalence, clinical characteristics and course of PLE. The questionnaire had 2 parts. The first, a screening questionnaire, detailed age, sex, skin type, phenotype (eye color, hair color, skin color) sun habits. In the second part only individuals experiencing symptoms of PLE who recognized their own eruption in one of the pictures showed (Figure 1-4). The second part assessed the natural history of their disease, time of onset, time of disappearance, affected skin areas, use of sunscreens, recurrences, other contemporaneous disorders or medications.

Analysis of data

Data were collected during winter from January 2004 through May 2004. People selected had been affected with a skin eruption similar to that showed in the photos: 1) PLE with coalescent papules (Figure 1); 2) PLE plaque type (Figure 2); 3) with small papules (Figure 3); 4) a vesiculo-papular type (Figure 4). People were only allowed to ask if a question was unclear. No person refused to respond.

Statistical analysis

Data collected were analyzed by SPSS software version 11.0.

Univariate descriptive analysis and crosstables were performed; \( \chi^2 \) values were performed to test any statistical association.

Results

Our results showed that in the Italian population the most frequent phenotype was characterized by brown hair (72.4%), brown eyes (60.3%), no solar lentigo (61.1%) and no freckles (68.6%); 0.9% of population was skin type I, 29.4% skin type II, 48.0% skin type III and 21.5% skin type IV.

A percentage of 78.2% of people interviewed were not exposed to sun during working hours; 46.9% sun exposed for 2-3 h each day for recreational activities. The main data collected about sun exposure during holidays are shown in Table I.

Cases with PLE

A total of 212 subjects has been identified as affected by PLE (4.8%). The prevalence of PLE in our population determined as mean of percentage among the
The phenotype and phototype of subjects with a history of PLE was not significantly different from unaffected people.

The distribution of different types is shown in Table II.

PLE was almost always characterized by pruritus (78.2%).

The trunk is the skin area most frequently affected (61.1%) while face is interested only in 7.7% of cases.
PLE started after a sunlight exposure more intense then usually in 37.7% of our population, while 42.7% did not confirm this datum.

PLE appeared even if sunscreens had been used from the first day of sun exposure (48.1% of people) and also in skin areas protected by them (47.7% of subjects).

In 46% of population eruption lasted for 2-3 days.

Data about recurrences are shown in Table III.

Other interesting data collected showed that only in 20.2% of cases any drug was assumed in the period of PLE eruption, only in 3.8% of cases cosmetics products had been used and only in 5.9% of cases people had been exposed to surrounding chemical compounds.

**Discussion and conclusions**

On the basis of both the anamnesis and the recognition by subjects interviewed of their eruption being similar to one of that showed in the photos (Figure 1-4) the diagnosis of PLE has been done. We think that only PLE-like lesions expression of a “forme fruste” of Lupus erythematosus cannot be recognized with this criterium. On the other side, some rare clinical variants of PLE, such as the hemorrhagic and the prurigo-like forms, have not been included in our survey, but statistical data obtained would have not been different.

In Italy the prevalence of PLE (mean of prevalence among the 8 centers participating in the study) is 5.89%. This prevalence is a little lower than in other studies 2,8 in which PLE has been estimated as ranging from 10% to 20% of the population. On the other hand, PLE has a wide geographical distribution, but its incidence varies from 15% in UK to 5% in Australia.1 These differences can be due to different UV concentrations as well as to racial differences.

The most frequent clinical type was characterized by coalescent papules (36.4%) and the skin area more frequently affected was the trunk (61.1% of subjects). There was no statistical difference crossing different clinical types and skin areas affected (χ² value, NS).

It is interesting to underline that patients affected by PLE often expose to sun for recreational activities (2-3 h a day in 46.9% of subjects) (χ² value=16.5; P<0.02) while chronic sun exposures due to work are rare (no sun exposures due to work in 78.2% of PLE cases). These data could explain the lack of natural mechanism of photoprotection in subject with PLE.

PLE patients seem to have a high level of knowledge about sun exposure modalities worldwide suggested: actually they sun tan during holidays for a few hours a day (41.4%), in particular during early hours in the morning (35.3%). However, 82.2% of cases have previously burned during the life.

It is interesting to underline that 37.7% of cases experienced a particularly intense sun exposure just before the first PLE eruption.

The fact that PLE occurred even if sunscreens had been utilized (48.1% of cases) (χ² value=16.5; P<0.02) while chronic sun exposures due to work are rare (no sun exposures due to work in 78.2% of PLE cases). These data could explain the lack of natural mechanism of photoprotection in subject with PLE.

PLE, as already known, has a chronic course and it recurs each year (31.4%). But very often (45.2% of cases) it improves spontaneously after some days even if sun exposures continue.

Usually, PLE recurs in particular during summer (52.7% of cases) and only 7.7% of subjects are affected on the face. These last data, in particular the frequent sparing of the facial skin, that is chronically sun exposed, and the gradual improvement of the rash during the summer are possibly related to the development of tolerance mechanisms: suppression of the

### Table III.—PLE recurrences.

<table>
<thead>
<tr>
<th>Frequency of recurrences</th>
<th>Number of days between sun exposure and PLE eruption</th>
<th>Period of recurrences</th>
<th>PLE remission</th>
<th>PLE course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only one time=19.2%</td>
<td>One day=25.5%</td>
<td>Only during summer=52.7%</td>
<td>Remission after some days=45.2%</td>
<td>Improvement after some days=34.7%</td>
</tr>
<tr>
<td>Sometimes=28.9%</td>
<td>2-3 days=35.6%</td>
<td>Only during other seasons=0%</td>
<td>Remission only at the end of the summer=18.0%</td>
<td>No improvement 33.5%</td>
</tr>
<tr>
<td>Every year=31.4%</td>
<td>More than 4 days=9.6%</td>
<td>During summer and other seasons=10.1%</td>
<td>Unknown=36.8%</td>
<td>Unknown=31.8%</td>
</tr>
<tr>
<td>Unknown=20.5%</td>
<td>Unknown=29.3%</td>
<td>Unknown=37.2%</td>
<td>Unknown=20.5%</td>
<td>Unknown=29.3%</td>
</tr>
</tbody>
</table>
immune mechanisms of PLE and increased thickening of the stratum corneum.5 9

In our data, there is not a clear cut correlation among sex, absence of lesions on face and improvement of the eruption during the summer period. Since these 3 criteria have been identified as the main criteria to distinguish a benign summer light eruption from a true PLE, we agree with Leroy et al.10 These authors state that the identification of a benign summer light eruption is not correct since PLE is characterized by a continuous spectrum, ranging from the benign eruptions improving during summer to more severe eruptions recurring each year with a persistent course during summer without tendency to amelioration.

Moreover, the last data of the questionnaire show that there is no correlation between PLE and drugs (only 20.2% of subjects affected took drugs) ($\chi^2$ test NS); PLE and cosmetic substances (3.8% of cases utilized them before sun exposure) (NS) and PLE and chemical compounds (5.9% of association) (NS).

Risultati. Dei 4 416 soggetti intervistati, 212 avevano una storia che orientava per una DPS. Pertanto, la prevalenza della DPS in Italia sembra attestarsi al 5,89%, senza differenze significative tra i diversi Centri Dermatologici distribuiti nel territorio nazionale a diverse latitudini.

La varietà clinica di DPS a “papule coalescenti” è la più comune (36,4%); l’area corporea più colpita sembra essere il tronco (61,1%), mentre il viso viene interessato solo in pochi casi. I soggetti cronicamente esposti al sole tendono a sviluppare la DPS meno frequentemente di quelli che si fotospongono in maniera occasionale. Nel 37,7% la DPS sarebbe comparsa dopo un’esposizione al sole particolarmente intensa.

Conclusioni. Non esiste alcuna correlazione tra DPS e assunzione di farmaci né tra DPS e sostanze chimiche ambientali.

Parole chiave: Dermatite polimorfa solare - Fotodermatiti - Epidemiologia.

References
Sequential treatment of psoriasis with infliximab followed by cyclosporin
Preliminary results of an open-label prospective study

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Aim. The efficacy and tolerability of a sequential treatment consisting of infliximab followed by cyclosporin A (CsA) was evaluated in patients with severe psoriasis, resistant to conventional therapies, including CsA.

Methods. Patients suffered from chronic plaque psoriasis with psoriasis activity and severity index (PASI) ≥ 16, who had failed to respond to standard treatments and to CsA 3-5 mg/kg/day, received intravenous infliximab 5 mg/kg at day 0 and at week (W) 2. From W2 they were treated with CsA 3 mg/kg/day up to W24. Apart the days devoted to infliximab treatment, subsequent visits were scheduled at W6, W12 and W24, assessing PASI and collecting information on tolerability.

Results. Ten patients, with a baseline PASI of 22.25 (range: 16-29.4), received and completed the study treatment. The mean reduction of PASI was 56% at W2 and 82% at W6. At W6 all patients achieved a PASI-50 response and 7 of them a PASI-75 response. The sequential monotherapy with CsA sustained the clinical response in most cases, with a constant PASI-50 response in 9 out of 10 patients (PASI-75 in 6 patients at W12 and 5 patients at W24). The mean improvement of PASI as compared with baseline was 75% at W12 and 63% at W24. Treatment regimen was well tolerated and no serious adverse events were observed.

Conclusion. The results of this preliminary prospective open-labelled experience suggest that treatment with only 2 infliximab infusions caused a striking improvement of psoriasis, which can be sustained by the sequential use of CsA 3 mg/kg/day in patients who had failed to respond to CsA ≥3 mg/kg/day. It can not be ruled out that infliximab can help to restore the clinical response to conventional treatments previously found as ineffective.

KEY WORDS: Plaque psoriasis - Infliximab - Cyclosporin A.
pies and/or had failed to respond to these therapies. All the patients were to have obtained an inadequate response (<25% PASI improvement) to standard doses of CsA used for at least 3 months. Exclusion criteria included concomitant inflammatory skin diseases and other clinical forms of psoriasis other than plaque psoriasis, any contraindications to the use of infliximab and CsA, previous treatment with infliximab, concomitant therapy with medications capable of interfering with psoriasis or with CsA metabolism, as well as nephrotoxic drugs. Any treatment active on psoriasis was to be stopped for an appropriate period of time prior to the baseline evaluation. After a detailed information about treatment modalities and characteristics, a written consent was obtained before the start of infliximab therapy.

Treatment regimen consists of the following sequential phases:

— two intravenous infusions of infliximab 5 mg/kg, at baseline and at week (W) 2, respectively;
— CsA 3 mg/kg/day, in 2 divided doses after meals, continuously for 22 weeks, from W2 up to W24.

During the 24-week treatment period, any topical and systemic therapies active on psoriasis, phototherapy and sun exposure were considered prohibited. Only non-medicated emollients and shampoos were allowed. Visits occurred at the baseline, W2, W6, W12, and W24, when psoriasis severity was evaluated by means of PASI and a complete physical examination, with measurement of vital signs, was performed. After the screening phase, laboratory routine examinations were made a few days before W2 and then at monthly intervals. Patients were asked to constantly monitor their blood pressure values. Discontinuation of CsA treatment was required in case of relapse (PASI of at least 50% of the value registered at the baseline) at any time.

After treatment, patients entered a 24-week observational period, in which rescue treatments were left at the discretion of dermatologists, and underwent 2 other scheduled visits, at W36 and W48, respectively, for clinical assessment. Further optional visits were carried out during the entire 48-week study period, if needed, for any reason (i.e., worsening of psoriasis, adverse events, anomalies of laboratory parameters, willingness of patients to use rescue treatments).

Results

A total of 10 patients, 8 males and 2 females with a mean age of 44 (range: 23-57) and a baseline PASI of 22.25 (range: 16-29.4), were found to meet all the eligibility criteria. As concerns the inadequate response to prior treatment courses with CsA, 3 patients had an unsatisfactory effect with doses of 4-5 mg/kg/day for 3-4 months, whereas the others failed to respond to CsA 3-3.5 mg/kg/day, which was used after tapering off the daily dose in order to control the side effects developed with higher doses (e.g., arterial hypertension, increase of serum creatinine and liver enzymes, gastric disturbances). In these cases, the dose of 3-3.5 mg/kg/day notably improved tolerability with complete resolution of adverse events but it was associated with an insufficient clinical response.

All the 10 patients started treatment between October and December 2003 and completed the therapeutic regimen as scheduled, with the visit at W24 taking place before exposure to sunlight. The therapeutic effect of the 2 infliximab infusions was dramatic (Figure 1), causing a mean reduction of PASI of 56% at W2 and 82% at W6, 4 weeks after the second infusion and the start of CsA. The sequential monotherapy with CsA 3 mg/kg/day up to W24 sustained the clinical response in most cases, with a constant PASI-50 response (≥ 50% improvement in comparison to baseline) and 7 of these had an improvement of at least 75% (PASI-75) (Table I). The sequential monotherapy with CsA 3 mg/kg/day up to W24 sustained the clinical response in most cases, with a constant PASI-50 response in 9 out of 10 patients (PASI-75 in 6 patients at W12 and 5 patients at W24, Table I). The mean improvement of PASI as compared with
Sequential Treatment of Psoriasis with Infliximab Followed by Cyclosporin

VENA

baseline was 75% at W12 and 63% at W24. In a case only, PASI changed less than 50% during CsA monotherapy but the patient refused to discontinue treatment because he was overall satisfied by treatment results, having experienced a notable relief of pruritus and a discrete improvement of PASI (46% at W12 and 40% at W24).

During the follow-up observational period, 4 patients used topical drugs as rescue treatment for lesions localized in critical areas: intermittent use of corticosteroids for face, folds or scalp, vitamin D analogues or keratolitics for scalp, palms or soles. All the patients were exposed to sunlight for a variable period of time, including the patient who previously had not achieved the PASI-50 and who instead obtained a PASI-75 response after sun exposure. Only a patient relapsed at W36 (PASI value=75% of the baseline value) and was excluded from the subsequent evaluation, requiring systemic treatment. Visit at W48 occurred between October and December 2004, when a PASI-50 response still persisted in the 9 evaluable patients, with 3 patients maintaining a PASI-75.

Details of PASI change over the 24-week treatment period and the subsequent 24-week observational phase are summarized in Figure 1 and Table I.

Treatment regimen was well tolerated and no serious adverse events were observed. An infusion reaction was observed only once in a patient receiving the second infusion: it consisted of mild hypotension and flushing, and was promptly controlled by slowing the infusion. Laboratory examinations did not reveal any clinically significant changes in the study population.

Discussion and conclusions

Infliximab is an anti-TNF-alpha chimeric monoclonal antibody which is effective as monotherapy for refractory moderate to severe plaque psoriasis. The recommended regimen in this indication consists of intravenous administration of 5 mg/kg at weeks 0, 2 and 6, followed by maintenance infusions at 8-week intervals. Treatment with infliximab induced a dramatic improvement of skin lesions, with marked effects already observed after the first infusions. Even a single infusion was found to be very effective on psoriatic skin lesions. The long experience gained in rheumatoid arthritis has provided numerous data about the combination of infliximab with methotrexate. Only a few reports exist supporting the possibility of using CsA combined to infliximab in patients in whom methotrexate treatment was contraindicated or adding CsA to infliximab/methotrexate combination in recalcitrant cases of rheumatoid arthritis. It is not known if CsA may reduce or delay the development of neutralizing anti-infliximab antibodies, as shown for methotrexate.

No patient experienced an improvement less than 25% from baseline. Total patients evaluable were 10 at each visit with the exception of W48 when they were 9.

<table>
<thead>
<tr>
<th>Visit</th>
<th>% Mean PASI improvement from baseline (No. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25-49%</td>
</tr>
<tr>
<td>Week 2</td>
<td>4</td>
</tr>
<tr>
<td>Week 6</td>
<td>0</td>
</tr>
<tr>
<td>Week 12</td>
<td>1</td>
</tr>
<tr>
<td>Week 24</td>
<td>1</td>
</tr>
<tr>
<td>Week 36</td>
<td>1</td>
</tr>
<tr>
<td>Week 48</td>
<td>0</td>
</tr>
</tbody>
</table>

Our preliminary results suggest that treatment with only 2 infusions of infliximab 5 mg/kg can cause a striking improvement of psoriasis which may be sustained by the sequential use of CsA 3 mg/kg/day in patients who previously were nonresponders to the same dosages of CsA or higher. Induction therapy with 3 infusions of infliximab was found to cause persistent improvement of psoriasis in some cases, but the time of persistence of the clinical response after 2 infusions has never been evaluated. It can not be ruled out, however, that infliximab can enhance in a synergistic way the activity of CsA or may favour to restore the response to CsA in patients who failed to respond to variable dosages of the drug. The therapeutic regimen reported in this paper was designed and used during a period in which treatment with infliximab had not standardized practical guidelines in psoriasis and was not reimbursed, so that the cost of the drug was directly borne by our hospital units, after regulatory approval on a case-by-case basis. Therefore, the assessment of this regimen was also aimed to obtain pharmacoeconomic data about the possibility of ‘infliximab-sparing’ approaches.

CsA is one of the most used drugs to treat moderate to severe psoriasis, whose efficacy and safety profiles are well-established. The daily dosage is of crucial importance in determining both the clinical response and side effects. During treatment with CsA, side effects are
usually well controlled by dosage adjustment or temporary interruption, according to their severity. Unfortunately, in some patients, the reduction of the daily dosage necessary to improve the tolerability may result in an unsatisfactory therapeutic effect. The dose of CsA used as maintenance treatment in the regimen herein presented was well tolerated by all patients and was never responsible for the dose-related side effects previously experienced by some patients with high dosages.

Although the data of the subsequent 24-week follow-up period were likely to have been influenced by climatic factors (summer months and related habit of sunbathing), they can suggest some considerations which can be useful in clinical practice. In fact, most patients had their psoriasis improved enough to allow them to expose their skin to sunlight without aesthetic or psychologic problems and all but one did not relapse up to W48. The use of rescue therapies in the observational period was limited to topical medications applied to localized skin areas, thus preventing a relevant interference with total PASI values.

In conclusion, the results of this prospective open-labelled experience suggest that treatment with only 2 infliximab infusions caused significant improvement of psoriasis, which can be sustained by the sequential use of CsA 3 mg/kg/day in patients who had obtained an insufficient response to CsA at daily doses \( \geq 3 \) mg/kg. Controlled studies on larger patient samples are certainly needed to confirm these preliminary results, as well as the chance of recovering the response to conventional therapies after infliximab treatment.

**Riassunto**

**Trattamento sequenziale della psoriasi con infliximab seguito da ciclosporina: risultati preliminari di uno studio prospettico in aperto**

**Obiettivo.** Lo scopo di questo studio prospettico in aperto è stato valutare l’efficacia e la tollerabilità di uno schema di terapia sequenziale con infliximab seguito da ciclosporina (CsA), in pazienti con psoriasi severa, con PASI\( \geq 21\), residente a terapie tradizionali, inclusa CsA a dosi \( \geq 3 \) mg/kg/die. **Metodi.** Il trattamento consisteva nell’uso di 2 infusione e.v. di infliximab 5 mg/kg, rispettivamente al giorno 0 e alla settimana (W) 2, seguite dalla somministrazione di CsA 3 mg/kg/die da W2 fino a W24. Le visite, che includevano valutazioni di efficacia e tollerabilità, sono state eseguite al basale, a W2, W6, W12 e W24. **Risultati.** Dieci pazienti, con un valore di PASI basale di 22,25 (range: 16-29,4) hanno completato il trattamento come programmati. La riduzione del PASI rispetto al basale è stata pari al 56% a W2 e all’82% a W6. A W6 tutti i pazienti hanno raggiunto una risposta PASI-50 e 7 di questi il PASI-75. La monoterapia sequenziale con CsA ha consentito il mantenimento della risposta clinica nella maggioranza dei casi, con una risposta costante PASI-50 in 9 casi (PASI-75 in 6 pazienti a W12 e 5 pazienti a W24). Si è osservato un miglioramento del PASI medio del 75% a W12 e del 63% a W24. La tollerabilità al trattamento è apparsa positiva.

**Conclusioni.** Questi risultati preliminari suggeriscono che già con 2 infusioni di infliximab si ottiene un miglioramento sensibile della psoriasi, con possibilità di mantenimento dei risultati in seguito alla monoterapia sequenziale con CsA 3 mg/kg/die in pazienti che avevano precedentemente presentato una risposta insoddisfacente alla stessa CsA a dosi \( \geq 3 \) mg/kg/die. Non si può escludere che infliximab possa favorire il recupero della risposta a terapie convenzionali dimostratisi prima inefficaci.

Parole chiave: Psoriasi a placche - Infliximab - Ciclosporina A - Terapia sequenziale.

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Videocapillaroscopic study in psoriatic patients treated with tacalcitol

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Psoriasis presents well-documented microcirculatory alterations such as the presence of edematous dermal papillae containing tortuous and dilated capillary loops often with extravasated erythrocytes, mononucleate cells and neutrophils. In the intrapapilla, the loop does not have a normal hairpin-like tendency, but it is arranged horizontally in a sinuous manner and appears dilated. The ascending and descending sections of the loop usually twist once or twice thereby taking on a glomerular aspect. There are no accessory anastomoses between these dilated glomerular-like capillaries and the surface vascular plexus. The capillary portion at the papillary crest and the descending portion are decisively wider than the ascending portion; in fact it increases from a diameter of approximately 6-9 µm to a diameter of 8-16 µm, even though the vascular wall thickness remains the same as that of normal skin capillaries.

Video-capillaroscopy (VC) helps to identify such modifications in the microvascular architecture. Dilated, elongated, convoluted capillaries with a glomerular type aspect (i.e. basket-weave form) arranged parallel to the cutaneous surface can be observed in the psoriatic plaque. At the edge of the psoriatic plaque, the capillary loops are elongated with a “hairpin” aspect,

Aim. An analysis was made of the modifications of the superficial capillary bed in a psoriatic plaque and the perilesional healthy skin during treatment with tacalcitol by means of video-capillaroscopy (VC).

Methods. Twenty-four patients suffering from psoriasis vulgaris over no more than 15% of the body were studied for a period of 3 months during treatment with 4 mg/g tacalcitol ointment. Once a psoriatic plaque had been selected, a visual clinical assessment was made and capillaroscopic measurements were taken with VC at the time of recruitment (T0), after 4 weeks (T1), after 8 (T2) and after 12 weeks (T3).

Results. The lesions studied gradually improved and at the end of the study the psoriatic plaques had disappeared in 12 patients. Even the diameter of the “basket” type capillaries had reduced significantly (average diameter: 67.64 at the beginning of the study; 34.08 at the end of the study), but only in 4 patients did the surface capillary plexus reappear with altered capillary loops. In the apparently healthy perilesional skin, 14 patients had abnormally elongated capillary loops. At the end of treatment, only 4 patients still had altered capillary loops.

Conclusion. Tacalcitol proved to be effective in reducing the clinical and capillaroscopic alterations. Nevertheless, the microcirculatory improvement does not perfectly follow the clinical improvement. We also noted that the tacalcitol action on the microcirculation is not only limited to the area of application, but also extends to the surrounding areas.

KEY WORDS: Psoriasis, drug therapy - Microcirculation - Tacalcitol.
arranged parallel to the cutaneous surface aligned with a centripetal tendency. The subpapillary venous plexus cannot be observed because of acanthosis. The calibre of the vessels appears increased, 12-13 µm compared to the 5-6 µm of the capillaries in the healthy skin. The latter has a “mesh-like” capillaroscopic aspect, that is the aspect of the surface capillaryplexus, as the papillary capillary loops perpendicular to the cutaneous surface are not visible.

The first studies relating to modifications of the microcirculation following treatment for psoriasis date back to 1981 with Horacek’s electron microscope researches following UVB therapy. In 1985 Klemp and Staberg studied the modifications in the blood flow during antipsoriatic treatment. In the same year, Braverman and Syble described the modifications of the capillary plexus following treatment with methotrexate and topical steroids. In 1989 Trevisan et al. observed the modifications of the psoriatic microcirculation at the periungual fold following PUVA therapy. In 1994 Strumia et al. submitted a preliminary study with VC in which they observed the reduction in length and tortuosity of the capillary loops after 6 weeks of tacalcitol treatment. Still in 1994 Berardesca et al. used laser Doppler velocimetry and trans-epidermal water loss to compare two drugs such as calcipotriol and clobetasol.

In this study, taking the idea from the work by Strumia et al., the modifications of the superficial capillary bed in a psoriatic plaque and the perilesional healthy skin during treatment with tacalcitol were analysed by means of VC.

**Materials and methods**

Twenty-four patients suffering from psoriasis vulgaris involving no more than 15% of the body area participated in this study: 14 male and 10 female patients aged between 21 and 64 years (average age: 43 years), who had not been taking any form of treatment for psoriasis for at least 4 weeks. Excluded from this study were pregnant women or those who were breast-feeding and patients suffering from heart disease or severe hepatosis, kidney disorders, hypercalcemia and tumours.

The duration of the study was scheduled over a 12-week period. Each patient was recommended to apply tacalcitol ointment 4 mg/g once a day with precision only on the psoriatic plaques. The use of any cosmetic or topical drug, systemic therapy for psoriasis or phototherapy was prohibited. Any treatment already in course for existing pathologies (hypertension, diabetes, disthyroidism) was continued.

At the first visit (T0) the plaque that was going to be the subject of this study was identified, precise anatomic points were selected, an image of the anatomic region with the lesion was acquired, likewise a capillaroscopic image of the centre of the plaque and a capillaroscopic image of the healthy perilesional skin at a distance of 2 cm from the lesion edge. From each capillaroscopic image of the centre of the plaque the diameter of 3 different “baskets” was measured and the average was then calculated. In the healthy skin, 3 measurements of the calibre of the reticular vessels were taken and the average was calculated. Reference was made to the plaque studied with the VC for the clinical assessment, summing up the values obtained after having assigned a score of between 0 and 4 to the erythema, infiltration and scaling.

The clinical assessment and capillaroscopic measurements were repeated by using the noted reference points on the same site after 4 weeks (T1), after 8 weeks (T2) and after 12 weeks (T3).

**Table I.—Diameters of the “baskets” expressed in µm and calibre of the capillaries forming the network of the superficial capillary plexus and length of the altered loops expressed in µm during topical treatment with Tacalcitol.**

<table>
<thead>
<tr>
<th>Tacalcitol</th>
<th>Basket diameter</th>
<th>Capillary network</th>
<th>No. of patients</th>
<th>Alterated ansae</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>67.6±11.06</td>
<td>7.16±1.02</td>
<td>14</td>
<td>24.28±8.82</td>
</tr>
<tr>
<td>T1</td>
<td>48.5±10.42</td>
<td>6.41±0.51</td>
<td>8</td>
<td>19.6±3.21</td>
</tr>
<tr>
<td>T2</td>
<td>37.5±9.43</td>
<td>6.24±0.35</td>
<td>6</td>
<td>17.74±4.43</td>
</tr>
<tr>
<td>T3</td>
<td>34.0±8.71</td>
<td>6±0</td>
<td>4</td>
<td>16±5.65</td>
</tr>
</tbody>
</table>

A DERMASCOPE® digital capillaroscope was used for imaging, consisting of a central body with a 100 Watt cold halogen lamp fitted with a manual luminous intensity control device; a probe consisting of a 2 m long flexible cable enclosing an optical fibre bundle, a connection cable between the video signal processing unit in the central body and the optical terminal and a video signal processing unit. In turn, the terminal consisted of a colour microcamera and a support to hold the various 300x “contact” type lenses used.
Before carrying out the capillaroscopic test, a drop of citron oil was applied to point where the probe was going to be applied to eliminate any reflections of light and allow the topmost layers of the epidermis to be seen.

Statistical analysis

The statistic significance of the microcirculatory reduction and clinical improvement was calculated by means of Student’s t test, assuming a reduction of greater or equal to 30% in the basket diameter as an effective reduction.

Results

At the beginning of the study, the average clinical score of the 24 patients was 7.25±1.86. The lesions studied improved progressively with a score of 3.91±0.91 at T1, 2.16±0.71 at T2 and 0.5±0.52 at T3. In 12 patients the psoriatic plaques had disappeared by
the time the study had been completed, whereas the remaining 12 had slight erythema or scaling.

The reduction in the capillaroscopic alterations at the psoriatic plaque and skin at 2 cm from the lesion are summarised in Table I.

In all 24 patients at the time of recruitment T0, a psoriatic capillaroscopic pattern was observed at the centre of the plaque with “basket” like convolute capillaries (average diameter 67.64) (Figure 1). At the end of the treatment, significant modifications were observed in the reduction of the “basket” diameter (average diameter 34.08) (test $t=6.83$ P>0.01) (Figure 2).

In 4 patients out of the 12 who had clinically healed, there was a return to the visualisation of the surface capillary plexus, even though altered capillary loops remained. The other 8 patients in whom the plaque was no longer objectively observed, typical psoriatic capillaroscopic alterations were still evident.

At the beginning of the study, in the healthy perilisional skin of 20 patients examined, a mesh-like aspect was observed, whereas in 4 there were abnormally elongated capillary loops (Figure 3). Abnormally elongated loops were also visible in 10 patients whose surface capillary plexus was observable. At the end of treatment, only 4 subjects still had altered capillary loops, however these were reduced compared to their size at the beginning of the study (Table I and figure 4).

**Discussion**

Elongated and convolute capillaries with a basket-like appearance at the centre of the plaque and non-observable surface capillary plexus are typical capillaroscopic aspects of psoriasis. Such alterations seem to be the expression, besides a compensatory response, of the microcirculation to the increased cellular turnover, as well as a consequence of the expression of numerous growth factors and chemokines secreted by the psoriatic keratinocytes. In fact, an increased secretion of Tissue Growth Factor alpha (TGF-α) and Keratinocyte Growth Factor (KGF) has been demonstrated, leading to an increased keratinocyte proliferation and therefore major metabolic demand, as well as Vascularendothelial Growth Factor (VEGF) that directly stimulates neoangiogenesis.

Tacalcitol or 1,24-dihydroxycholecalciferol, a vitamin D analogue with activity for vitamin D receptors (VDR) of 1.25(OH)$_2$D$_3$, has a marked capacity to induce differentiation and reduce cellular proliferation. Its activity on psoriasis is also due to an anti-inflammatory and immunomodulating activity that leads to a reduction in the number of polymorphonucleates, CD$^+$ and T cells at the psoriatic plaque. Moreover, the drug has the ability to reduce the number and activity of Langerhans cells, to reduce the release of interleukin-8, to increase that of interleukin-10 and to regulate the cellular distribution of integrin. The capacity to induce keratinocyte differentiation, thereby reducing cellular proliferation also reduces the metabolic demands of the epidermis, eliminating one of the neovascularisation stimuli. Even the anti-inflammatory and immunomodulating action has an effect on microcirculation by reducing the chemotactic stimulus as regards the T-cells with a minor induction of epidermal changes. Further action on the microcirculation is undoubtedly determined by the capacity to reduce IL-8 release, known as a neoangiogenetic factor.

In this study, tacalcitol proved to be effective in reducing the clinical and capillaroscopic alterations. Nevertheless, microcirculatory improvement did not perfectly follow clinical improvement (Figure 5).

At the end of the 12 weeks of treatment, the plaque in 12 patients had clinically healed, but only 4 returned to a normal capillaroscopic picture.

The reason for this discrepancy could be found in a slower “restitutio ad integrum”, i.e. full recovery of the capillary alterations compared to regularisation of keratinocyte proliferation or marked sensitivity of the endothelial cells to keratinocyte alterations and presence of growth factors. In an apparently clinically healthy skin, minimum alterations in keratinocyte proliferation or minimum concentrations of growth factors...
that are able to maintain the neoangiogenetic stimulus could still be present.

Although application of the topical drug was carefully limited to the psoriatic plaque, a reduction in the calibre of the vessels forming the superficial capillaryplexus and a reduction in the length of the altered capillaries in patients who had them was noted on the perilesional healthy skin. This could be the result of the drug going through the circulatory flow: absorption of tacalcitol during topical therapy is known\(^\text{11}\) and therefore it is not surprising to see its action away from the area of application. To be checked is whether diffusion of the drug at the epidermal level in the surrounding areas of the site of application could have influenced our observation or whether improvement of the psoriatic plaque could have determined a reduction in the production of growth factors and mediators with a consequent modification in the microcirculatory alterations in the perilesional skin.

Observation of the discrepancy between clinical and capillaroscopic improvement and the presence of microcirculatory alterations in healthy skin and their reduction following treatment lead to think over the definition of “healed” as far as the psoriatic plaque is concerned. Can a patient be considered in clinical remission and therefore suspend treatment when the psoriatic plaque could have determined a reduction in the caliber of the vessels forming the superficial capillaryplexus and a reduction in the length of the altered capillaries in patients who had them was noted on the perilesional healthy skin. This could be the result of the drug going through the circulatory flow: absorption of tacalcitol during topical therapy is known\(^\text{11}\) and therefore it is not surprising to see its action away from the area of application. To be checked is whether diffusion of the drug at the epidermal level in the surrounding areas of the site of application could have influenced our observation or whether improvement of the psoriatic plaque could have determined a reduction in the production of growth factors and mediators with a consequent modification in the microcirculatory alterations in the perilesional skin.

Observation of the discrepancy between clinical and capillaroscopic improvement and the presence of microcirculatory alterations in healthy skin and their reduction following treatment lead to think over the definition of “healed” as far as the psoriatic plaque is concerned. Can a patient be considered in clinical remission and therefore suspend treatment when the clinically visible plaques have disappeared or is necessary to wait for the other components, such as microcirculation, to return to normal? In 1982 Irwin et al.\(^\text{13}\) demonstrated that there is no epidermal hyperplasia without vascular proliferation. The VC is certainly an excellent, fast and comfortable tool to assess microcirculation and therefore it is suggested as the ideal instrument to decide on the strategy to suspend ongoing treatment of psoriasis.

Riassunto

Studio videocapillaroscopico in pazienti psoriasici trattati con tacalcitol

Obiettivo. Abbiamo analizzato con videocapillaroscopia a sonda ottica le modificazioni del letto capillare superficiale nella chiazza psoriasica e nella cute sana perilesionale durante il trattamento con tacalcitol.

Metodi. Abbiamo studiato per 3 mesi 24 pazienti affetti da psoriasi volgare con interessamento non superiore al 15% della superficie corporea durante il trattamento con tacalcitol 4 mg/g unguento. Scelta una chiazza di psoriasi è stata eseguita una valutazione clinica visiva e misurazioni capillaroscopiche con VCSO, al momento dell’arruolamento (T0), dopo 4 settimane (T1), dopo 8 settimane (T2) e dopo 12 settimane (T3).

Risultati. Le lesioni studiate sono progressivamente migliorate e al termine dello studio in 12 pazienti le chiazze psoriasiche erano scomparse. Anche il diametro dei capillari convoluti tipo “basket” si sono significativamente ridotti (diametro medio: iniziale 67,64; finale studio 34,08), ma solo in 4 pazienti si è osservato il ritorno alla visualizzazione del plesso capillare superficiale con anse capillari alternate. Nella cute perilesionale clinicamente apparentemente sana 14 soggetti presentavano anse capillari abnormemente allungate. Al termine del trattamento, soltanto 4 soggetti presentavano ancora anse capillari alternate.

Conclusioni. Il tacalcitolo si è dimostrato efficace nel ridurre le alterazioni cliniche e capillaroscopiche. Tuttavia, il miglioramento microcirculatorio non accompagna perfettamente quello clinico. Abbiamo anche notato che l’azione del tacalcitolo sul microcircolo non si limita solo alla superficie di applicazione, ma si estende anche alle aree contigue.

Parole chiave: Psoriasi, terapia farmacologica - Microcircolazione - Tacalcitol.

References

Head lice: ex vivo videodermatoscopy evaluation of the pediculocidal activity of two different topical products

F. LACARRUBBA 1, B. NARDONE 1, M. MILANI 2, G. BOTTA 2, G. MICALI 1

Aim. The aim of this study was to evaluate by videodermatoscopy the efficacy and rapidity of pediculocidal activity in 2 different products indicated in the treatment of head lice.

Methods. Ten tests on 10 adult samples of Pediculus humanus capitis were performed. Head lice were taken from 3 subjects with active head lice infestation and placed in 10 Petri’s capsules. After an initial videodermatoscopy observation (of approximately 180 s duration) to evaluate the viability of the parasites, on 5 out of 10 a synergized pyrethrin thermophobic foam was applied; on the other 5 a coconut and anise oil based-spray was applied.

Results. In all performed tests with thermophobic foam product the absence of movements of the parasites within 10 s from the contact with the product was observed, while the absence of peristalsis was noted within 60 s. On the contrary, with the essential oil based-product the parasites were alive also after a continued observation of 120 min after the application of the product.

Conclusion. Our experience suggests that the videodermatoscopy is a valid researching tool to evaluate the efficacy and the time of action of topical products with pediculocidal activity.

KEY WORDS: Pediculosis, diagnosis - Videodermatoscopy - Pediculosis, drug therapy.

called natural products with mechanical action (e.g. essential oil) or with systemic drugs, such as antibiotics (trimetoprim) or ivermectin.1

However, for many of these products, data about their real therapeutic efficacy or rapidity of action are not available at the moment; moreover, evaluation methods up to now have been based on very simplistic criteria (i.e. clinical examination before and after treatment).

Videodermatoscopy (VD) is a new instrumental methodology allowing a rapid, exhaustive and non-invasive in vivo observation of the skin: this tool provides high quality images with magnifications ranging from X 4 to X 1000. Both static and dynamic images obtained through a colour microvideo camera can be displayed on a monitor and recorded on PC.

A study with VD about the pediculocidal efficacy and rapidity of action of 2 different products indicated in the treatment of head lice was performed at the Dermatologic Clinic, University of Catania (Italy). A formulation of synergised pyrethrin in thermophobic foam (Milice®; Mipharm) was compared to a coconut and anise oil based-spray, with a mechanical action obtained by suffocation (Paranix®; Chefaro).
Materials and methods

Ten tests were performed on the same number of adults’ specimens of *pediculus humanus capitis* taken in 3 subjects with head lice infestation using a fine-tooth comb. Each louse was placed in a Petri’s capsule with gauze on the bottom in order to improve the VD visualization.

VD examination was performed by a videodermatoscopy Hirox Hi Scope KH 2200 equipped with zoom lens at magnification ranging from X 10 to X 600.

An initial observation by VD of 180 s duration was performed to evaluate movements and peristaltic intestinal activity (that is visible in transparency) as indicator of lice viability. After this time, a minimal quantity of pyrethrin thermophobic foam was applied on 5 parasites and on the other 5 parasites the oil based-spray was applied. The activity of parasites were then observed and recorded for 120 continuous minutes.

Results

In the case of pyrethrin thermophobic foam product, in all performed tests, the absence of movements of lice were observed within 10 s from the contact with the product; the absence of peristalsis were noted within 60 s. With the essential oil based-product the lice were alive also after a continuous observation of 120 min after the application of the product (in the packaging the time for optimal product activity is indicated as around 15 min).

Discussion and conclusions

Pediculosis is due to *pediculus humanus capitis*, a blood-sucking insect and specific parasite of humans. Pediculosis affects people aged 4-14 years. This parasitosis generally does not involve relevant complications, even if it is possible to observe impetigo with an associated local retro-auricular adenopathy due to scratching in response to severe itching.

The evidence based medicine shows that natural pyrethrin, permethrin and malathion are effective in the treatment of head lice. Synergised natural pyrethrin and permethrin are the first choice for the topical treatment of head lice thanks to their safety profile. However, also for these molecules with proved pediculocidal activity is always more frequently described the appearance of resistance resulting in the inefficacy of head lice treatment. This phenomenon is more frequent in some geographic area where it is a real problem. Furthermore, cross-resistance to permethrin and malathion was described. The cause of therapeutic failure in head lice treatment is frequently not due to resistance but to the incorrect use of topical products.

Some products containing a pediculocidal active compound are vehicolated in a formulation not suitable for this purpose. Clinical evidences show that shampoo formulations are less effective than cream, lotion or foam formulations: this is due to the low concentration of the active compound and to the very short time of contact.

VD is a noninvasive diagnostic tool very useful in dermatology. VD is used in a great number of cutaneous diseases thanks to its remarkable versatility and reliability, including cutaneous parasitosis. Recently, VD was used in a study to monitor *in vivo* the efficacy of a topical antiscabetic treatment and the optimal timing of this drug application. VD can be used as a diagnostic tool in head and pubic lice infestation: it permits an easy identification of parasite and eggs when these are not easy to identify to the naked eye. VD permits also an *in vivo* evaluation of the movements and physiology of lice and eggs. Isolation of an adult parasite permits in ex vivo evaluation of the movements and physiology of lice and eggs. Through the isolation of *pediculus humanus capitis* (that cannot be reared in laboratory conditions) and through VD evaluation is also possible to assess the efficacy and rapidity in pediculocidal activity of topical pediculocides.

In this study pediculocidal action of a synergized pyrethrin thermophobic foam was highlighted in a very short period of time: absence of parasite movements were observed within 10 s from the contact with the product; the absence of peristalsis was noted within 60 s. Data showing in details the time of action of substances or drugs indicated in head lice treatment, including pyrethroids substances are not available at the moment. This study did not assess the evaluation of a possible ovicidal activity which will be performed in a future trial.

Our experience suggests that VD represents a valid research tool for the evaluation of efficacy and time of action of topical pediculocides. A further and future
use of VD could be represented by the study of possible lice resistance to commonly used substances with pediculocidal activity to contribute to the identification of alternative and appropriate therapeutic options.

Riassunto

Pediculosi del capo: valutazione ex vivo tramite videodermatoscopia dell'azione e della rapidità pediculocida di due differenti preparati topici

Obiettivo. Scopo del presente studio è stato valutare tramite videodermatoscopia l'efficacia pediculocida e la rapidità di azione di 2 differenti prodotti indicati per il trattamento della pediculosi del capo.

Metodi. Sono stati eseguiti 10 esperimenti su altrettanti esemplari adulti di Pediculus humanus capitis, prelevati da 3 soggetti con pediculosi del capo e mesi in capsule di Petri. Dopo un'osservazione iniziale in videodermatoscopia di circa 180 s al fine di valutare la vitalità dei parassiti, su 5 di essi veniva applicato un prodotto a base di piretrine sinergizzate in formulazione in mousse termosensibile, su altri 5 veniva applicato un prodotto a base di olio di cocco e anice in formulazione spray.

Risultati. Nel caso del prodotto in mousse è stata osservata in tutti i test eseguiti l'assenza di movimenti del parassita entro 10 s dal contatto con il prodotto e della peristalsi intestinale entro 60 s. Nel caso del prodotto a base di olio essenziale, i parassiti rimanevano vitali anche dopo un'osservazione continua di 120 min dopo l'applicazione.

Conclusioni. La nostra esperienza indica come la videodermatoscopia rappresenti un valido strumento di ricerca per la valutazione dell'efficacia e dei tempi di azione di prodotti topici ad azione pediculocida.

Parole chiave: Pediculosi, diagnosi - Videodermatoscopia - Pediculosi, terapia farmacologica.

References

Aim. The aim of the study is to evaluate the efficacy and tolerability of a new kind of combined peeling for melasma.

Methods. This new combined peel consists in using salicylic acid 25% in alcoholic solution and trichloroacetic acid 10% in gel, subsequently in the same session. We used this technique to treat 15 female patients, aged from 18 to 45 years, with phototype from II to IV according to Fitzpatrick scale; they were affected by epidermic (8 patients), dermal (4 patients) or mixed-depth (3 patients) melasma. For the evaluation of the type and the severity of melasma we used MASI score and Wood’s lamp.

Results. We obtained an excellent esthetic results, in 3–4 settings at 3–4 weeks interval: in fact, we observed complete resolution in patients with epidermic melasma, significant regression of the hyperpigmentation in mixed-depth melasma (more than 50% decrease in MASI score) and a mild regression in dermal melasma (more than 25% decrease in MASI score). No side effects were observed. In patients with total resolution no relapses were observed after 6 months from the end of the treatment.

Conclusion. In this study we have evaluated the efficacy and tolerability of the combined peel, proving that it is useful, reliable and safe in the treatment of the melasma, in all skin types.

Key words: Melasma - Peel - Salicylic acid - Trichloroacetic acid.

Melasma is a common skin hyperpigmentation disorder, occurring most frequently in adult females, and with a difficult resolution with dermocosmetic therapy.

Until now different peels have been used to treat melasma; these include salicylic acid, trichloroacetic acid (TCA), glycolic acid, Jessner’s solution, pyruvic acid and phenol.¹

Results however are not always satisfactory, making occasionally the melasma worsening, especially because of postinflammatory hyperpigmentation, persistent erythema and hypertrophic scars.

The aim of our study was to evaluate the efficacy and tolerability of a combined peel in the treatment of melasma.

Materials and methods

Our combined peel consists in using subsequently in the same session 2 different chemical agents, salicylic acid 25% in alcoholic solution and TCA 10% gel.

We enrolled 15 female patients (aged from 18 to 45 years) that were visited at our outpatient consultation for cosmetic dermatology; their phototype was from II to VI according to Fitzpatrick scale and they had centrofacial and malar melasma (Table I).

For the evaluation of the severity of melasma we utilized MASI (Melasma Area and Severity Index)²
score and Wood’s lamp. To calculate MASI the face was divided into 4 areas: forehead (30%), right malar (30%), left malar (30%), chin area (10%).

The examination with Wood’s lamp, used to determine depth of pigmentation, showed an epidermic melasma in 8 patients (clinically melasma is light brown and its appearance is enhanced by Wood’s light), a dermal melasma in 4 patients (clinically melasma is dark-brown to grey and its appearance is not enhanced by Wood’s light) and a mixed-depth melasma in 3 patients (clinically melasma is dark-brown and its appearance is not enhanced by Wood’s light).

The melasma had developed during pregnancy in 3 patients and during treatment with oral contraceptives in 4 patients. All patients did not utilize photoprotection.

We treated all the 15 patients with combined peel in 3-4 settings at 3-4 weeks interval. We decided to treat our patients from October to May, to avoid the risk of a post-peel hyperpigmentation due to the sun exposure in summer.

The used technique includes different phases:

1. pre-peel phase: 3 weeks before the peel the patient was treated with bleaching agents (Kligman’s trio: hydroquinone 4%, hydrocortisone 0.5% and retinoic acid 0.1% in 100 mg of base cream), applying it on the affected area every evening;

2. cleansing: immediately before applying the chemical peel the skin was cleaned with physiologic solution;

3. salicylic acid application: the alcoholic solution of salicylic acid 25% was applied using a brush in order to obtain a uniform and homogeneous dispersion;

4. salicylic acid removal: salycilic acid 25% in alcoholic solution has a limited action since, after the evaporation of the alcohol, in few seconds, the salicylic acid precipitate remaining on the skin and needs to be removed with physiologic solution;

5. TCA 10% gel application: the gel was applied

<table>
<thead>
<tr>
<th>Age</th>
<th>Phototype</th>
<th>Type of melasma by Wood’s light</th>
<th>Distribution of melasma</th>
<th>MASI (before)</th>
<th>MASI (after)</th>
<th>Duration of melasma</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 y.</td>
<td>III</td>
<td>E</td>
<td>M</td>
<td>60%</td>
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<td>4 months</td>
<td>Caucasian</td>
</tr>
<tr>
<td>39 y.</td>
<td>III</td>
<td>E</td>
<td>M</td>
<td>60%</td>
<td>0%</td>
<td>3 years</td>
<td>Caucasian</td>
</tr>
<tr>
<td>37 y.</td>
<td>II</td>
<td>E</td>
<td>C</td>
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<td>0%</td>
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<td>Caucasian</td>
</tr>
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<td>21 y.</td>
<td>II</td>
<td>E</td>
<td>M</td>
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</tr>
<tr>
<td>32 y.</td>
<td>IV</td>
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<td>C</td>
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<td>0%</td>
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</tr>
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<tr>
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<td>M</td>
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<tr>
<td>45 y.</td>
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<td>D</td>
<td>M</td>
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<td>40%</td>
<td>5 years</td>
<td>Caucasian</td>
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<td>D</td>
<td>C</td>
<td>90%</td>
<td>0%</td>
<td>1 year</td>
<td>Caucasian</td>
</tr>
<tr>
<td>34 y.</td>
<td>II</td>
<td>D</td>
<td>C</td>
<td>90%</td>
<td>75%</td>
<td>1 year</td>
<td>Caucasian</td>
</tr>
<tr>
<td>37 y.</td>
<td>IV</td>
<td>D</td>
<td>M</td>
<td>60%</td>
<td>40%</td>
<td>8 months</td>
<td>Caucasian</td>
</tr>
<tr>
<td>40 y.</td>
<td>III</td>
<td>M</td>
<td>M</td>
<td>75%</td>
<td>25%</td>
<td>2 years</td>
<td>Caucasian</td>
</tr>
<tr>
<td>42 y.</td>
<td>V</td>
<td>M</td>
<td>C</td>
<td>10%</td>
<td>10%</td>
<td>1 year</td>
<td>Black</td>
</tr>
<tr>
<td>45 y.</td>
<td>IV</td>
<td>M</td>
<td>M</td>
<td>50%</td>
<td>10%</td>
<td>4 years</td>
<td>Caucasian</td>
</tr>
</tbody>
</table>

RM = right malar; LM = left malar; F = forehead; E = epidermic; D = dermal; M = mixed-depth; C = centrofacial; M = malar.
with a cotton tipped applicator proceeding by single esthetic-anatomic subunits, to control and modulate the frosting evolution. We looked for a white-pink frosting, in order to reach the epidermis and the superficial layers of papillary dermis;

6. inactivation: since in the epidermic melasma we need a white frosting, while in dermic or mixed-depth melasma we need a pink frosting, the frosting was modulated using physiologic solution, to inactivate the TCA 10% gel;

7. post-peel phase: the post-treatment regimen consisted of cutaneous hydration with moisturizers, and daily use of high-spectrum sunscreen [an ultraviolet (UV) A/B 50 sunscreen]. After the 10th day, the patient started again to use bleaching agent (Kligman’s trio) applying it every evening in the affected area;

8. long term protection phase: patients were instructed to avoid the sun exposure and use a high-spectrum sunscreen.

Results

Results were evaluated 3 months after the beginning of treatment.

The response in each patient was graded as:

— no response, no change in MASI score at the end of the treatment;
— mild response, less than 25%;
— moderate response, from 25% to 50%;
— good response, from 50% to 75%;
— very good response, more than 75% decrease in MASI score.

All the 8 patients (100%) with epidermic malar melasma (Figure 1) obtained a very good response, with a complete regression of the skin hyperpigmentation (Figure 2).

Only one (25%) of the 4 patients with dermal melasma (Figure 3) had a complete clearing, the remaining 3 patients (75%) only obtained a mild response (less than 25%) (Figure 4).

All the 3 patients with mixed melasma (100%) had a good response (50% to 75%) with a significant reduction of the hyperpigmentation.

We should underline that at the 3rd day after the combined peel, the skin of the patients had a dry and brown colour, lightly wrinkles, minimal limitation of facial expressions, movements and mild itch. These are not side effects, but consequences of the coagulative necrosis of cells in epidermis, that are necessary to depigmentate the skin affected by melasma.

All the patients with total resolution (53.3%) and with mild or good response were examined every
month for 6 months and no relapses or worsening were observed.

Possible side effects of combined peeling are hyperpigmentation, persistent erythema and reactivation of Herpes Simplex virus infection. No side effects were observed either during or after the treatment.

Discussion

Already in the past Brody,3 Monheit 4 and Coleman5 proposed combined peels: in fact, combining a lower concentration of TCA with another less potent superficial peeling agent, we gain the required level of skin damage with a lower risk of side effects.

The aim of our combination is to favour penetration of TCA at low concentration, using salicylic acid as an exfoliant agent. This association has a softer action than a single strong chemical agent and permits good therapeutic results with minimal risk of side effects.

In particular we utilized salicylic acid as a keratinolytic agent to remove epidermal keratinocytes, including pigmented keratinocytes, and to enhance the topical penetration of TCA gel 10% which causes a coagulative necrosis7,8 limited to the epidermis and the superficial layers of papillary dermis.

This induces renewal of the epidermis by accelerating epidermal turnover, by stimulating and increasing the growth of normal and undamaged cells underneath the lesion, thus leading to the resolution of the cutaneous hyperpigmentation.

Although the epidermis and the papillary dermis can be regenerated by simply injuring the cornified layer by superficial peeling, their effect is very slow and multiple treatments are required to improve pigmentation disorders.9

Utilizing this combined “cold” peel we obtained an excellent esthetic result in only 3-4 settings separated each other by 3-4 weeks interval.

Moreover, this technique is ideal for melasma permitting to treat all Fitzpatrick skin types, because the dynamics of the frosting (intensity, tonality, homogeneity, speed) allows to modulate the deepness of action, making it possible to use this technique also in patients with a high phototype.

The main advantage of combined peel is the lack of inflammatory reaction that could cause a post-inflammatory hyperpigmentation.

For this reason patients with epidermic melasma may have a complete resolution of the hyperpigmentation and patients with dermal or mixed melasma may improve also, because the combined peel makes the skin more receptive to topical bleaching regimens.

Conclusions

In conclusion, we underline that all the different phases of our regimen are important for the efficacy of treatment. In the pre-peel phase the bleaching agents disperse pigment granules in keratinocytes and interfere with pigment transfer. The combined peel cause a complete or partial clearing of melasma and without inflammation. In the post-peel phase the photoprotection permits to avoid relapses.

Riassunto

Peeling di combinazione con una soluzione di acido salicilico al 25% e di gel di acido tricloroacetic al 10% per il trattamento del melasma

Obiettivo. L’obiettivo dello studio è di valutare l’efficacia e la tollerabilità di un nuovo tipo di peeling di combinazione per il trattamento del melasma.

Metodi. Questo nuovo peeling di combinazione consiste nell’utilizzo di una soluzione alcolica con il 25% di acido salicilico e di un gel al 10% di acido tricloroacetic, applicate in successione nella stessa seduta. Abbiamo usato questa tecnica per trattare 15 soggetti di sesso femminile, di età compresa tra 18 e 45 anni, con fototipo Fitzpatrick compreso tra II e IV, che erano affette da melasma epidermico (8 pazienti), dermico (4 pazienti) o misto-profondo (3 pazienti). Per valutare il tipo e la gravità del melasma abbiamo utilizzato il punteggio MASI e la lampada di Wood.

Risultati. Abbiamo ottenuto un eccellente risultato estetico con appena 3-4 sedute ad intervalli di 3-4 settimane. Infatti, si è avuta risoluzione completa del melasma epidermico, una regressione significativa dell’iperpigmentazione nel caso di melasma misto-profondo (dimostrazione del punteggio MASI superiore al 50%), e una lieve regressione del melasma dermico (dimostrazione del punteggio MASI superiore al 25%). Non sono stati osservati effetti collaterali. A distanza di 6 mesi dal trattamento, nelle pazienti con risoluzione totale del melasma non sono state osservate recidive.

Conclusioni. In questo studio abbiamo valutato l’efficacia e la tollerabilità del peeling di combinazione, provando che esso è utile, affidabile e sicuro per il trattamento del melasma in tutti i fototipi cutanei.

Parole chiave: Melasma - Peeling - Acido salicilico - Acido tricloroacetic.
COMBINED PEEL WITH 25% SALICYLIC ACID SOLUTION AND 10% TRICHLOROACETIC ACID GEL FOR MELASMA

DE PADOVA

References

Advancing vaccination strategies for prevention and therapy of dermatological diseases

P. WALDEN

Vaccinology increasingly incorporates the rapidly accumulating new knowledge of the molecular and cellular pathology of diseases, and of the components and mechanisms of cellular and humoral immune responses. With the changes in paradigms, new vaccination strategies are developed and explored for infectious diseases, cancer, autoimmune disorders and allergies. While these strategies are still at early stages of development, vaccination is expected to become an important tool not only for prevention but also for treatment and management of diseases. Future vaccines are bound to be molecularly defined and accurately tailored to induce most effective immune responses. Diseases of the skin are, increasingly, targets for vaccine development. Moreover, because of its easy accessibility and its constitution as a major immune organ, future developments will aim for vaccine designs suitable for delivery via the skin.

KEY WORDS: Allergy - Antigens - Autoimmune diseases - Bacteria - Neoplasms - Epitope - Infections - Parasite - Lymphocytes, T cells - Toll-like receptor - Vaccines.

Ever since the successes of the small pox vaccination campaigns were acknowledged by the medical and scientific communities in the XIXth century, vaccines were viewed as magic bullet for basically every disease.1 Numerous vaccination attempts were initiated for both prevention and therapy of infectious diseases, cancer, allergies and autoimmune disorders. The outcomes of these trials were mostly disappointing. Nonetheless, following internationally coordinated vaccination campaigns one major scourge of mankind, small pox, is considered extinct now. A number of other infectious diseases could be checked and current campaigns aim at eradicating all vaccine-preventable infectious diseases. Despite these successes, only 25 infectious diseases are targeted by licensed vaccines (www.who.int). These are mostly viral infectious and some bacterial toxins. There is no approved vaccine for prevention of infection by bacteria, protozoa and helminths. And, despite intense efforts over the past one and a half century, cancers and autoimmune diseases are still not controllable by vaccination. On the other hand, great progress has been made in regard to allergic disorders with effective hyposensitization protocols now available for a number of type I allergies.

Vaccinology has largely been an empirical discipline developed by trial and error, in most cases, testing the pathogenic agent itself as vaccine, sometimes with severe side effects. Only recently, knowledge of antigenic structures recognized by antibodies and T cells and of the mechanisms of immune regulation have been incorporated in vaccine development.2 With the systematic dissection of the immune responses against infectious diseases, cancer, autoimmune disorders and allergies, and the identification of the rel-
The skin is the largest and most exposed organ of the body, route of entry for various pathogens and affected by many diseases as primary or secondary site of manifestation. A number of these diseases are considered possible targets for preventive or therapeutic vaccination. The skin is also a large immune organ particularly suited to sense and battle pathogenic intruders and in-born pathologic developments. As such, the skin has moved into the focus of vaccinology as possible target site for vaccines not only for skin but also internal diseases, and new strategies for dermal vaccination are being developed.

This review will sum up some of the most recent developments in vaccinology with focus on the skin as site of disease manifestation and vaccination.

**Vaccine targets**

**Infectious diseases**

Infectious diseases are still the major burden of mankind with enormous losses of lives and, for every individual, of life quality many times and often extensively or continuously during lifetime. Infectious diseases inflict huge economic costs for all countries and severely block or slow the economic and social developments in regions endemic for protozoal diseases, HIV, diseases caused by helminths and others. The vast majority of infectious diseases are not controlled by vaccination. Rather, hygiene measures such as providing clean drinking water, clearing sewage and waste, and avoiding unprotected contact to infected individuals are the most important safeguards against infection. For some 25 of the most wide-spread infectious diseases effective vaccines exist. Nonetheless, still many, especially children, die from these diseases. With intense international efforts, the World Health Organization (WHO) and Global Alliance for Vaccination and Immunization (GAVI; www.gavi alliance.org) run and coordinate world-wide vaccination campaigns aimed at eradicating these vaccine-preventable infectious diseases. But there are many diseases for which there are no vaccines. Among these are viral diseases such as AIDS, avian influenza and SARS, protozoal diseases such as malaria, leishmaniasis and sleeping sickness, diseases caused by helminths such as river blindness and schistosomiasis and a large number of bacterial diseases. Many infections once believed checked reappear with new epidemics. Among these are insect-borne diseases such as malaria, leishmaniasis and sleeping sickness that vigorously recurred with the ban on the insecticide DTT. New diseases such as SARS or fatal variants of avian influenza have arisen and rapidly spread through international traffic. Among the most prevalent infectious skin diseases that are primary targets for vaccine development are oral and genital herpes, and human papilloma virus infections. Both clusters of diseases are highly immunogenic viral infections that affect high proportions of the populations in developed and developing countries alike. Various efforts are undertaken to develop vaccines against herpes and warts but there are still enormous problems in the development of vaccines for these causative agents, not least because of the lack of understanding of the basic immunology of infections by the large DNA viruses and of the molecular cell biology of their long latencies. Most individuals respond effectively with strong humoral and cellular immune responses to herpes family viruses such as EBstein Barr virus (EBV) and Citomegalovirus (CMV), and the infections are usually well controlled, however, without sterile immunity. Oncogenic subtypes of human papilloma viruses are closely associated with cervical and penile cancers. It is generally believed that vaccines against these viruses can prevent these cancers. Substantial progress has been made in recent years in the development of vaccines for the human papilloma viruses HPV 16 and HPV 18 that are thought to be causative agents for cervical cancers. The initial observations from a clinical trial with virus-like particle vaccines of the papilloma virus capsid protein L1 are
induction of neutralizing anti-viral antibodies and clear-cut reduction of the occurrences of premalignant conditions. These observations, thus, strongly support the expectations that HPV vaccines can confer protection from HPV-related intraepithelial cervical neoplasia.\textsuperscript{14, 15} If successful, HPV vaccines will be the first case of preventive cancer vaccines.\textsuperscript{11} It is estimated that 20\% to 25\% of all cancers have a direct or indirect microbial etiology. Targeting the infections in these cases by vaccination is believed to interfere with the malignant development and prevent the cancers. Following-up on the promising observations, new vaccine designs for HPV are developed and tested that, in addition to neutralizing antibodies, also induce T cells against epitopes of the oncogenic E6 and E7 gene products of HPV 16 and 18.\textsuperscript{16-18} While the early observations with preventive HPV vaccines seem to justify the high hopes that prevention of cervical cancer might be possible, therapeutic vaccines for these malignancies have failed to show any effect.\textsuperscript{18} This failure mirrors the observations from animal model experiments with transplantation tumors that vaccines that can prevent the establishment of the tumors usually have no effects on established tumors.

Bacterial and fungal infections are highly prevalent and have a great impact on the affected individuals. These infections are caused by highly pathogenic microbes as well as common bacteria that can turn invasive and pathogenic in cases of immune deficiencies or in response to certain cytokines produced by immune cells. Such induction of bacterial pathogenicity by immune responses has recently been shown for \textit{Pseudomonas aeruginosa} a common bacterium that is responsible for a large fraction of nosocomial but also for increasingly occurring community acquired infections with severe courses.\textsuperscript{19, 21} The extent of and the possible involvement of the skin in such immune-induced or immune-enhanced pathogenicity of common micro-organisms is currently under intense research. Bacterial and fungal infections can persist for a long time, often life-long and cause substantial financial burden for the community. Preventive measures such as vaccination are of obvious importance and, taken into consideration the above-mentioned interplay of immune responses and pathogenicity of bacteria, need to be tailored carefully to avoid exacerbation of the pathological effects by specific types of immune responses. Moreover, some of these microbial diseases such as staphylococcal and many fungal infections may develop complications by triggering remission or exacerbation of atopic dermatitis.\textsuperscript{22} Besides the fact that no preventive vaccine is available yet for bacterial and fungal infections,\textsuperscript{2} primary localization and propagation of many infections at the outer skin with restricted accessibility for the immune effector systems is a fundamental problem. It is not clear yet whether immune interventions or immune prophylaxis can prevent or cure these kinds of infectious diseases. Some other bacterial infections develop inside the skin and, thereby, are within the reach of the immune system. \textit{Mycobacterium leprae} the causative agent of leprosy induces strong immune responses. However, these immune responses are not protective as the disease inevitably progresses with its terribly disfiguring effects. Animal studies suggest that the type of helper T cell response might shift the disease either in direction of disfiguring lepromatous or less devastating tuberculoid manifestations.

Of even higher complexity is the immunology of protozoal skin infections caused by the different leishmania species. In the cases of several of the parasites, the infection is confined to the skin. Always, however, also in cases of visceral or mucosal manifestation, the skin is the primary port of entry of the parasites. The immediate local immune responses at this site may decide whether or not the immune system can fend off the infection and mount protective immunity. The quality of the initial cutaneous immune responses, thus, may be a matter of life or death for the patient. The skin is also the site of secondary infection by \textit{Leishmania donovani} after cure from the initial visceral manifestation of the disease, a condition known as Post-Kala Azar Dermal Leishmaniasis (PKDL). In Sudan, PKDL is observed in a large fraction of the individuals some weeks after they recovered from visceral leishmaniasis. In India the frequency of PKDL is low and it occurs usually years after the initial visceral disease. In both situations it is a skin disease associated with more or less extensive depigmentation. It is not clear why the same parasite that is responsible for the life-threatening visceral leishmaniasis causes a much milder skin disease at its second appearance but development of a partial organ-specific immunity during the initial infection might well be part of the explanation. It is also not clear whether PKDL is caused by reinfection with new parasites or by parasites persisting in the skin. Active immunization in the form of the age-old practice of leishmanization has been suc-
cessfully used in the Near East all the way through to Central Asia to prevent cutaneous leishmaniasis known as oriental sore. Very recent studies in regions in Northern India that are highly endemic for visceral leishmaniasis or Kala Azar have shown that the frequencies of seropositivity for the leishmania antigen K39 is much higher than the prevalence of manifest disease. These observations indicate that there is a state of immunity to leishmaniasis established in the course of natural infection and strongly support the efforts to develop leishmania vaccines. But the heterogeneous outcomes of the infections by leishmania parasites also emphasize that the development of vaccines needs to incorporate the large body of information on the immune responses to leishmania infection that has been accumulated over the past years. Infection by leishmania parasites induces vigorous humoral and cellular immune responses involving all cell types of the immune system. The pathology is complicated by host-disease relationships that are dynamically evolving in the course of infection and during the life-cycle of the parasites. Key determinants of these relationships are elaborate immune evasion mechanisms enacted by the parasites, the involvement of vector systems highly specialized for particular leishmania and host species, the existence of extra-human and obscured human reservoirs, and explosive propagation of the parasites. Notwithstanding these complexities, great progress has been made in understanding the immune mechanisms associated with protection or with the failure of the immune defenses. Earlier generalizations from studies with mouse models and Leishmania major which is associated with cutaneous leishmaniasis suggested that Th 1-type helper T cell responses are correlated with resistance to disease and Th 2-type responses with susceptibility. However, more recent studies indicate that curative immune responses involve all arms of the immune system including innate immune cells, specific antibody responses and CD4 T cells of both helper T cell types as well as CD8 effector T cells. From these observations it appears necessary that preventive as well as therapeutic vaccines against protozoal diseases induce massive broad-range immune responses with parasite antigen-specific antibodies, T cells of all helper and effector types, strong innate immune reactions, and various immune-regulating and effector cytokines. On this basis, the agenda of vaccine development is twofold. First, early blockade of infection with strong antibody responses against the promastigote form of the parasites as they are injected by the bite of the insect vector. Second, induction of effector T cell responses against antigens presented by the infected macrophages that activate these macrophages to kill their intruders via mechanisms involving active oxygen species. The first of these strategies is the only possibility to prevent entry of the parasites into the host cell. It is, however, not clear whether neutralizing antibodies can indeed block the parasites. The latter goal for anti-parasite vaccines is derived from mouse model experiments that show that cure from Leishmania donovani infection is correlated with a Th1- and Th2-type CD4+, and CD8+ T cell responses and that macrophages induced to oxidative bursts can kill the parasites. It is hereby particularly important to identify T cell epitopes of parasite antigens that are presented by infected cells. Moreover, recent observations suggest that the course of the infection may also be influenced by the saliva and immune responses against salivary components of the insect vector. Pre-existing immunity against these components seem to support protective immune response. If this is confirmed, insect antigens may become relevant constituents of leishmania vaccines. Considering the large body of information on anti-leishmania immunity and the requirements that leishmania vaccines have to meet, many details of the vaccine design still need to be worked out before meaningful trials can be planned.

In summary, the number of infectious diseases that can be prevented by vaccination is still very limited. It has become clear that effective preventive or curative immune responses against infectious agents, especially viruses, and intracellular bacteria and protozoa, depend on combinations of antibody and effector T cell responses, and that vaccines have to control the direction of the helper T cell responses in addition to inducing the effector cells and cytokines required for protection.

Autoimmune diseases and allergies

In contrast to other diseases, the goal in cases of autoimmune diseases and allergies is to specifically suppress or re-direct on-going pathogenic immune responses. The therapeutic vaccines should address B cells, and disease-associated helper and the regulatory T cells rather than effector T cells in these immune reactions. The identification of autoantigens and
The use of such epitopes, rather than unfractionated immune tolerization, for the treatment of allergies, desensitization, meaning immune modulation and strategies against allergies. These strategies of immune intervention for disease prevention and cure,\textsuperscript{36-41} The hope is that it may become possible to utilize T cell epitopes as specific immune modulators. Upon suitable modifications, they may be used as antagonists of the CD4+ T cells that drive the pathogenic immune responses. Alternatively, the T cell receptor families of the pathogenic T cells can be attacked by vaccination to raise cytolytic T cell responses against the autoimmune T cells.\textsuperscript{42-45} These two strategies have been tested in mouse and rat models and the corresponding attempts to prevent or reverse autoimmune disease yielded some interesting successes. The translation of these approaches into therapies for autoimmune diseases in man, however, is still far off. In contrast to the situation with autoimmune diseases, there is rapid progress in the development of therapeutic vaccination strategies against allergies. These strategies of immune desensitization, meaning immune modulation and immune tolerization, for the treatment of allergies,\textsuperscript{46} are increasingly being based on identified T cell epitopes.\textsuperscript{47,48} The use of such epitopes, rather than unfractionated allergen preparations, will substantially improve the quality of therapeutic vaccines and help to control the effects of the immune interventions. Many of these attempts are based on the hypothesis that a disbalance of Th1/Th2-type immune responses is responsible for allergies\textsuperscript{49,50} and, consequently, aim at redirecting the polarization of the helper T cell responses in order to correct the disbalanced immune states.\textsuperscript{51} Other concepts aim at inducing regulatory T cells (Treg) responses to down-regulate aberrant T cell activities, both in cases of autoimmune diseases and allergies.\textsuperscript{52-54}

Cancers

The hypothesis that the immune system is responsible for protection against cancer and plays an important part in rejection of tumors has already been proposed in the XIX\textsuperscript{th} century, however, it was not until the late sixties and seventies of the twentieth century that the instruments of anti-tumor immune responses began to become unraveled. Macfarlane Burnet was the first to propose that thymus-dependent, viz, as we know today, T cell-dependent immune reactions are the basis for immune surveillance and tumor rejection.\textsuperscript{55,56} But it took another twenty years, with the discovery of the first tumor-associated T cell epitopes and antigens, before it became possible to dissect the specific immune mechanisms against cancer.\textsuperscript{57,58} Today it is clear that CD8+ T cells are the most important effector cells in tumor immunity and with the identification of some 300 different tumor-associated epitopes for these cells derived from about 100 different antigens we have now a relatively advanced understanding of the specificities of immune responses against cancer.\textsuperscript{59,60} This knowledge has been translated into the design of vaccines for induction of tumor-specific T cell responses. With that, tumor immunology has transformed vaccinology and has complemented the earlier antibody-focused approach to vaccine development with new concepts for T cell vaccines.\textsuperscript{3,59,61,62} Also, vaccination in cases of cancer is mostly a therapeutic intervention despite very encouraging attempts towards prophylactic anti-cancer vaccines for cervical cancer\textsuperscript{15} and the hope that many of the cancers with microbial etiology may be prevented by prophylactic vaccines. As therapeutic vaccination the specific immune histories of the patients are expected to impact the outcome of the treatment and have to be taken into consideration in vaccine design.

Comparing the specificities of antibody and T cell responses against tumor cells, the HLA-restricted mode of antigen recognition allows T cells to scan a much wider range of antigenicity than accessible to antibodies. The HLA peptidome of cells, that is the totality of HLA-bound peptides, reflects not only the proteome of the cells but also the specific rates of turnover of every protein. It is often phrased that the HLA present the degradomes of the cells rather than their proteomes.\textsuperscript{63-67} The turn-over of the proteins is very sensitive to the physiological states of the cells, the HLA peptidomes reflect alterations within as well as in the environment of the cells. A higher turn-over of some proteins associated with a higher proliferation rate will, thereby, result in an altered HLA peptidome compared to resting cells. All this allows T cells to sense various pathological changes in the cells, a capacity that can be exploited for vaccine development.\textsuperscript{64} On the other hand, the HLA polymorphism significantly complicates the task of developing T cell vaccines in that the specificities of the responses are far more individualized as it is the case for serological responses. In effect T cell vaccines need to be designed differently.
than vaccines for antibody-based immunity. The physiology of antigen-presenting cells and the molecular mechanism of antigen processing have to be incorporated in the vaccine design as well as the specific requirements for the collaboration of the precursors of tumor-specific cytolytic effector cells with T helper cells.

The first tumor-associated antigens and HLA class I-restricted T cell epitopes for human cancers were determined by expression cloning of cDNA from tumor cells of a melanoma patient together with HLA genes into COS cells, testing these doubly transected cells with T cells of the same patient and sequencing the cDNA of transfectants that specifically induce T cell responses. The first identified antigen was MAGE, a member of a new class of proteins whose expression is confined to tumor cells and testis. In the following years different families of these so called cancer-testis antigens were identified but, despite intense efforts, no functions have yet been determined for the MAGE proteins. Testis is an immune-privileged organ, meaning, it is not accessible to CD8+ T cells. The antigens, therefore, are relatively tumor-specific and considered preferred antigens for cancer vaccines.

Now, different technologies are employed to identify cancer-associated antigens and T cell epitopes. The most successful approach involves bioinformatics to predict potential T cell epitopes in the sequences of identified or suspected tumor-associated antigens that are validated in T cell assays. Powerful but technically very demanding alternatives are biochemical approaches that involve extraction and sequencing by mass spectrometry of peptides bound to the HLA of tumor cells. These analyses of HLA peptidomes provide new insights into the physiology of the tumor cells and allow to identify antigens that are relevant for the pathology of the tumors. For use in cancer vaccines, some tumor-associated T cell epitopes were modified to improve HLA binding and, thereby, the efficiency of T cell activation. Such altered epitopes are more efficient than their natural counterparts in inducing responses by specific T cells, however, in some cases it turned out that T cells induced by such altered epitopes did not or only inefficiently recognize and destroy tumor cells that present the natural epitope. Such problems can be circumvented by complete de novo design of peptides that efficiently activate T cells originally raised against the tumor cells. Such so called mimotopes, mimetics of natural epitopes, can be designed to recruit and activate tumor-specific T cells most efficiently. The potentials of such mimotopes have been demonstrated in animal models for melanoma as well as in clinical applications as therapeutic vaccines for cutaneous lymphoma.

The tumor-associated antigens known to date are, with very few exceptions, normal self-antigens. Antigens such as the melanoma-associated antigens tyrosinase, tyrosinase-related proteins (TRP), MART-1/Melan-A and S100 constitute the most numerous group followed by the cancer-testis antigens. For some tumors, for instance hepatocellular carcinoma or colon carcinoma, embryonic antigens like α-fetoprotein (AFP) or carcinoembryonic antigen (CEA), respectively, have been found aberrantly expressed. Tumors with viral involvement express viral antigens that, as foreign antigens, are ideal targets for cancer vaccines. The most relevant examples here are, as mentioned above, human papillomavirus antigens in cervical carcinoma. For melanoma, the latter two categories of tumor-associated antigens play no role. Tumor-specific neoantigens that once were believed to be the main focus of anti-tumor immune response have rarely been found. A mutation in cdk4 of melanoma cells of a patient is one of the few examples. It, thus, seems that mutations giving rise to such neoantigens play no relevant role in tumor immunology. The exceptions might be idiootype sequences of the T cell receptor of T cell lymphomas and of the immunoglobulins of B cell lymphoma that are explored as possible targets for cancer vaccines in cases of lymphoma, also cutaneous T and B cell lymphomas. In any case, tumor-specific neoantigens are individualized and not suited for widely applicable vaccines. By and large, the tumor-associated antigens and T cell epitopes known to date classify tumor-specific immune responses as autoimmune responses. Notwithstanding the fact that nearly all tumor-associated antigens are not tumor specific but also expressed and presented by non-malignant cells, these antigens can be and are being used for cancer vaccines.

With the identification of tumor-associated T cell epitopes, new techniques for detection, characterization and enumeration of T cell responses against these antigens have been developed and are being used for immune monitoring in the context of clinical vaccination trials. These new tools include HLA oligomers loaded with the T cell epitope of interest that are used to detect all T cells with given specificity, and intra-
cellular cytokine staining with subsequent flow cytometry as well as ELISpot that detect T cells specifically responding to their cognate antigens. With the help of these tools natural anti-cancer immune responses as well as the immunological effects of vaccination and other therapeutic interventions are analyzed. As most tumor-associated antigens have been identified for melanoma, melanoma research is the forerunner in tumor immunology and cancer vaccinology and the vast majority of the clinical vaccination trials have been and are being conducted with melanoma patients. In most cases these were advanced stage patients with metastasized tumor and a history of repeated treatment failures. Such patients are generally not expected to respond well to therapeutic vaccination. Nonetheless, the trials have yielded significant evidence for the potential effectiveness of vaccination therapy for the treatment of cancer. Cancer vaccines regularly induce tumor-specific T cell responses and, in some cases, also clinical responses in cancer patients. However, despite frequent induction of tumor-specific T cells in the vaccinees, these immunological responses only in a few cases correlate with clinical responses. These failures of the tumor-specific T cells to exert an effect on the tumors have been correlated with immune evasion by the tumor cells with either loss of antigenicity or immune suppression. To reduce the risk of antigen loss, vaccines with complex antigenicity have been designed and tested in clinical trials. Hybrid cell vaccination that uses fusions of a patient’s tumor cells with allogenic dendritic cells as vaccines, is the most extensively studied vaccine design that represent a complex tumor antigenicity. Multiantigen immune monitoring of these hybrid cell vaccination trials demonstrate induction of high frequencies of tumor-specific T cells with a broad range of specificities for tumor-associated antigens. Nonetheless, the frequencies of objective clinical responses was not significantly improved. Instead, immune escape variants of the tumor cells were detected in tumor lesions progressing under therapy that have lost expression of multiple antigen, of HLA or of the transporter associated with antigen processing (TAP). It thus seems that multiantigen tumor vaccines efficiently induce broad immune specificities that select tumor cells with a correspondingly broad pattern of immune evasion. Although only few objective clinical responses were recorded, the median survival time of the patients was more than twice that expected from clinical experiences and a high fraction of the patients experienced stable disease. Therapeutic vaccination, thus, seems to be suited for maintenance therapy where cure is not possible. Since the adverse effects of vaccination therapy are minimal and restricted to slight inflammatory responses at sites of vaccine injection, vaccination could become an alternative to conventional treatment modalities for maintenance in advanced stage cancer patients. Despite some reports of vaccination-induced complete responses in advanced stage cancer patients, it is questionable whether therapeutic vaccination will become a dependable curative therapy for late stage cancers. The basic problem that limits the efficiency of cancer vaccination is the heterogeneity of the tumor cells that increases with tumor mass and time of tumor development. With their heterogeneity the immune evasion capacity of the tumor cells increases with time. Therapy failures due to the selection of therapy-resistant tumor cell variants is also reported for chemotherapy and radiotherapy. To circumvent these problems combinations of different but compatible and mutually complementing therapeutic principles should be considered. In contrast to advanced disease stages, early stages of cancer might be less prone to immune evasion and more sensitive to therapeutic vaccination. At early cancer stages curative therapeutic vaccination is conceivable. However, no sufficiently informative clinical trials with early stage cancer patients have been conducted yet to test these options. In addition to melanoma, initial studies have demonstrated that cutaneous lymphoma is susceptible to therapeutic vaccination. Due to a lack of identified tumor-associated antigens and T cell epitopes, other skin cancers have not yet been addressed with therapeutic cancer vaccines. Nonetheless, there is indirect and very suggestive evidence that they could be targeted by vaccination therapy as well. Among the observations supporting this notion are tumor regression in cases of basal cell carcinoma upon applications of immune modulators such as imiquimod and CpG that are known to act via Toll-like receptors (TLR) to activate antigen-presenting cells. This option is currently tested for actinic keratosis as well and has yielded very promising initial results. The exact immunological, cellular and molecular mechanisms of these clinical responses upon treatment with TLR agonists still need to be worked out.
Although natural anti-cancer immune responses are mainly mediated by T cells, it may well be possible to induce antibodies against tumor cells with therapeutic effects. Tumor cells often have an altered molecular composition of their surfaces, in particular, altered glycosylation patterns. The identification of such altered glycosylations may be exploited to develop strategies for active antibody-directed immunization against tumor-specific cell surface molecules.\(^1\)\(^2\) Antibody-based therapies may be interesting alternatives and complementation of T cell-based strategies. Moreover, for cancer with microbial etiologies such as papilloma viruses in cervical or some head-and-neck cancers, hepatitis B and C viruses in hepatocellular carcinoma, human T lymphotrophic virus in T cell lymphoma, EBV in lymphoma or nasopharyngeal carcinomas or *Helicobacter Pylori* in MALT lymphoma, prevention of infection by neutralizing antibodies may contribute to immune protection.\(^2\)\(^3\) In these cases, for other infectious diseases, vaccines should induce neutralizing antibody as well as cytokine T cell responses.

**Vaccine design and delivery**

All commercial vaccines are for infectious diseases and aim at the induction of neutralizing antibody against the infectious agent itself, this is the case for anti-viral vaccines, toxoids produced by bacteria. They were all developed empirically. They consist of either the inactivated, sometimes attenuated, infectious agent or a subunit thereof, alum is added as an adjuvant to enhance the efficiency of the immune induction. With the elucidation of the specificities of the immune reactions over the past two decades and the identification and dissection of immune dominant epitopes for B cells and T cells, the development of vaccines of molecularly defined components has become possible. As a result, only few such molecularly defined vaccines have been tested in clinical trials, none has been approved yet for routine application.

Besides the antigenic determinants recognized by the T and B cells, the requirements of the regulatory cell interactions in the induction of immune responses need to be considered in the development of new vaccines. Challenging the antibody-focused approaches to vaccine development, recently reported studies indicate that protective immunity against many infectious diseases such as viral, mycobacterial or protozoal infections depends on efficient antibody as well as CD8+ effector T cell responses. This is particularly true for the immune reactions involved in recovery and cure from disease.\(^2\) Immunity to cancer is mostly T cell-mediated; immunity, antibodies play no relevant part. Only in the case of cancers with microbial etiology, both antibody and cell responses are expected to be important for immunity. The induction of both types of immune responses, cellular responses mediated by B cells through antibodies and effector-cellular immune responses by CD8+ T cells, T cell help is required.\(^2\)\(^6\)\(^8\) T cells do not respond to antigen directly but to processed antigen presented by the HLA molecules on antigen-presenting cells. It has become clear now that antigen-presenting cells involved in the induction of immune responses need to fulfill specific requirements and, besides presenting the antigenic epitopes for the T cells in the correct HLA contexts, HLA class I molecules for CD8+ and HLA class II molecules for CD4+ T helper cells, they have to provide additional signals via specific sets of costimulatory receptors and counter receptors as well as cytokines such as IL-12. Although all cells that express HLA class II molecules can serve as antigen-presenting cells, dendritic cells are best equipped for the task and need to be addressed by vaccines.\(^1\)\(^1\)\(^9\)\(^1\)\(^0\) The dendritic cells are critical in induction and orchestration of cellular immune responses. In all, molecularly defined vaccines should contain three essential constituents. First, epitopes or antigens for the effector cells, cytotoxic CD8+ T cells or B cells, second, epitopes or antigens for helper T cells, third, adjuvants for active modulation of the antigen-presenting cells. In addition, vaccines composition should include adjuvants with reservoir functions to control the bio-availability of the vaccine.

Different vaccine formulations have been proposed to incorporate these constituents. Besides whole viruses or pathogenic cells, complete lysates, subunit preparations from the pathogens or tumor cells, recombinant proteins, peptides, virus-like particles, DNA and RNA-based vaccines were developed and are being tested, mostly in animal models, few already in clinical trials.\(^1\)\(^2\)\(^1\)\(^2\) Epitopes for HLA class I-restricted effector CD8+ T cells are peptides of eight to ten amino acids which are products of limited proteolysis from proteins expressed by the cell. These peptides are bound by HLA class I molecules and presented at the cell surfaces.\(^5\)\(^9\) Epitope for HLA class II-restricted T
helper cells have eleven and more amino acids and are derived from internally expressed as well as extracellular antigens. While the epitopes for T cells are peptides, antibodies can bind molecules of virtually any chemical class. Often they recognize conformational rather than simple linear determinants. Based on the elucidation of the structure of the antigenic determinants for many antibodies, various strategies for the synthesis of conformationally defined vaccine components are being developed. However, these strategies have not been translated into synthetic vaccines yet. More often, recombinant proteins are prepared and tested as vaccine antigens. Most successfully, virus-like particles (VLP) are explored for this purpose. VLP can also be altered chemically or by gene-technological means to include additional epitopes besides the determinants of the viral core proteins.

To enable T-B cell collaborations, the epitopes for both cell types need to be in one molecule. This notion, known as the epitope linkage concept, is one of the basic concepts of immunology. It is based on experiments that have shown that for induction of secondary antibody responses which involve antibody class switch, hapten as model antigenic determinant for the B cells need to be covalently linked to a carrier protein that contains epitopes for T helper cells. For T cell collaboration, that is the interaction of T helper cells and precursors of CD8+ cytolytic T cells there is also an epitope linkage requirement. However, in contrast to T-B cell interaction the epitopes for the two T cell types need not be covalently linked. They have to be presented by the same antigen-presenting cell. Helper T cell antigens for efficient induction of CD8+ T cells can be part of the vaccine antigen but in many situations it may improve the potency of the vaccines if other antigens are added. Recall antigens like tuberculin, potent foreign antigens like KLH or allogeneic HLA class II molecules have been used as helper antigens for the induction of CD8+ T cells. Whatever the design of the vaccine, it is advantageous to include a large number of epitopes for the targeted immune cells in order to cope with the heterogeneous immunogenetics of human populations and to broaden the scope of the immune responses. Complex antigens, mixtures of antigens or peptides and polyvalent epitopes, called polytopes, have been developed for this purpose. In cancer vaccinology, comparisons of single epitope vaccines with complex vaccine antigens carrying various epitopes have shown the superiority of complex antigens in inducing immune responses to the tumors. In all cases where T cells are to be addressed by the vaccines, complex antigenicity is essential to sufficiently cover the HLA immunogenetics of the human target populations.

Infectious agents used as vaccines have often sufficient immune-stimulatory capacity to induce protective immune responses. Nonetheless, the effectiveness of the vaccines can be enhanced by addition of adjuvants. When defined components, especially synthetic products are used as vaccine antigens, however, activators of the dendritic cells can be essential for the vaccination effects. The recent discovery of Toll-like receptors (TLR) in mammals and the finding that their ligands are compounds of long known immune stimulators used as adjuvants has made it possible to develop molecularly defined modulators of antigen-presenting cell functions. TLR are ancient and highly conserved key components of the innate immune system that share structural elements with the Toll protein of *drosophila*. They are differentially expressed by different cells of the immune system including B cell, monocytes, macrophages and dendritic cells, but not by T cells. The signaling pathways addressed by the TLR concurs in parts with the interleukin-1 signaling pathway and involves the transcription factor NF-ÎB. The effects of signaling through TLR is expression of costimulatory molecules and proinflammatory cytokines. The resulting innate immune responses attract various cells of the immune system including T cells. Particularly important is the activation of dendritic cells with their functions in the induction and coordination of T cell-mediated immune responses.

So far 11 mammalian TLR have been identified, and their cellular distributions and ligand specificities unraveled. The natural ligands are microbial products of diverse chemical classes. As examples, TLR2 binds, among many other molecules, lipopeptide of various pathogens and peptidoglycans of Gram-positive bacteria. TLR3 binds double-stranded viral DNA, TRL4 lipopolysaccharide, TLR7 and single stranded viral RNA and TLR9 unmethylated CpG motifs of bacterial DNA. TRL8 had initially been found to bind synthetic imidaziquinolines such as imiquimod that is in clinical use for treatment of warts and tested in clinical trials for treatment of basal cell carcinoma and actinic keratosis.
as TLR 4, 7, 8 and 9 are found in endosomes and bind viral or microbial components that become accessible only upon disintegration of the viruses, bacteria or protozoons. Depending on the cellular distribution of TLR expression, different cytokines and different other cell types are induced. In humans TLR 7, 8 and TLR9 are expressed by plasmacytoid dendritic cells that, when activated by the corresponding TLR ligands, mature to potent antigen-presenting cells with increased expression of HLA class II molecules and costimulatory molecules, and secretion of T cell-stimulatory cytokines including IL-12. Similarly, the TLR9 agonist CpG (CpG-ODN) has strong adjuvant activity in vaccines for infectious diseases and tumors. It is well established now that the efficacy of DNA vaccines, at least in parts, depends on the presence of CpG motifs in their sequences. A hepatitis B vaccine that includes CpG oligonucleotides has been tested successfully in orangutans that developed efficient protective antibody responses. Vaccine without the CpG oligonucleotides was not effective. Other examples for the efficacy of CpG in supporting induction of antigen-specific immune responses are mouse model experiments with the E7 protein of the oncogenic papilloma virus HPV-16 that resulted in induction of E7-specific cytotoxic T cells and protection from HPV 16-related cancers. Currently, the first clinical trials with hepatitis B virus envelop proteins plus CpG-ODN are being conducted. Besides induction of CD8 T cells via enhancing the co-stimulatory capacity of dendritic cells, down-regulation of regulatory T cells (Tregs) is discussed as possible principle of TLR activity. TLR agonists can stimulate dendritic cells via TLRs such that regulatory T cell function becomes blocked and, thereby, immune responses against pathogens unblock.

Some pioneering work on the potential clinical application of imiquimod and other TLR agonists has focused on tumor models such as a murine colon carcinoma model. Recent results of studies with women treated with topical imiquimod for cervical intraepithelial neoplasia indicate that imiquimod induces T cell responses with broadened specificity of the responding CD8+ T cells. Different TLR differ in their capacity to induce specific CD8 responses. Studies with ligands for TLR 2 and 4 have produced conflicting results, in some experiments efficient induction of CD8+ T cells was achieved, in others not. Ligands for TLR3, 5 and 7 have moderate adjuvant effects whereas TLR9 most dramatically increased CD8 responses. Despite such promising data, it still needs to be established what the optimal dose and timing is for the application of TLR agonists as adjuvants in therapeutic or prophylactic vaccination. Further studies are needed especially in the light of recent reports of extensive TLR engagement leading to immunological anergy and the respective adverse effects.

The route of delivery is crucial for the success of vaccination. Most vaccines available to date have to be injected, in some few cases oral application is possible but not used anymore. Such invasive vaccinations are problematic. Not only that they cause discomfort for the vaccinees and may be traumatic experiences especially for children. In many developing countries the needles and syringes are used several times for different individuals. WHO reports that in some regions more than 80% of the vaccination equipment is reused (www.who.int). This practice born from poverty and restricted resources bears a substantial risk of spreading infections. Especially in regions with high prevalence of HIV and hepatitis virus infections the risk of vaccination-related dissemination of disease is very high. WHO, therefore, calls for intense research to replace the current invasive vaccination practices by non-invasive applications. Besides some attempts in direction of oral vaccines, transdermal routes are being explored and transdermal vaccination is high up on the agenda of vaccine development. These developments build on the new techniques for delivering substances through the skin, some pioneered by cosmetics industry. The skin, thereby, is not only an easily accessible site and transdermal application, not only a convenient route for vaccination, it is also a major immune organ that provides all the cellular requirements for efficient utilization of the vaccines. In contrast to muscle, currently the main target organ for vaccine application, the antigen-presenting cells required for induction of all aspects of protective
immune responses are present in abundance and the lymphocytes to be activated are readily recruited to the skin. It is foreseeable that future vaccinations involve application of patches or crèmes rather than the use of needles. Moreover, almost all vaccines available to date require prime-boost schemes that cause enormous logistic problems. To overcome these problems, intense research is on-going to develop simplified schemes that combine immediate and delayed antigen release so that priming and boosting vaccines can be delivered simultaneously.

In conclusion, while today vaccines in clinical use as well as the recent developments tested in clinical trials are complex natural products, future vaccines are expected to be defined synthetic products containing the above-discussed constituents. The skin, thereby, is not only the site of manifestation of diseases that in future may be preventable by vaccination. It is also the immunologically well-equipped outer wall of immune defense against infections and, therefore, prospectively the site for transdermal vaccine delivery.

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References


UV A1 phototherapy

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Non ionizing radiations with wavelengths ranging from 340 to 400 nm (UV A1 waveband) have peculiar photophysical, photobiological and phototherapeutical effects. UV A1 penetrates deeply into the skin and modulates the biological and immunological activity of both dermal and epidermal circulating as well as resident cells. Unlike UVB (260-320 nm) and UV A2 (320-340 nm), UV A1 photobiological effects are mediated exclusively by oxidative photochemical processes leading to the formation of singlet oxygen and other reactive oxygen species (ROS). The main targets of ROS are cell membranes and cytoplasmic organelles, particularly mitochondria, that are rich of structural molecules with several double bonds, e.g. lipids and aminoacids. UV A1 phototerapy proved to be an effective and well tolerated therapeutic option in the treatment of atopic dermatitis, localized scleroderma, mastocytosis, systemic lupus erythematosus, mycosis fungoides, chronic graft versus host disease and a growing number of other skin diseases, characterized by dermo-epidermal infiltrates of normal as well as neoplastic lymphocytes, mastocytes and/or eosinophils as well as altered metabolism of collagen. There is no general agreement on the optimal treatment protocol. UV A1 is delivered at fixed doses without progressive dose adjustments. However, protocols differ in the UV A1 dosage, the number of weekly exposures, and total number of exposures. UV A1 therapy is always well tolerated and episodes of erythemogenic reaction are rare. Long term adverse effects are unknown but the hazard of skin carcinogenicity seems negligible because UV A1 does not damage directly DNA.

KEY WORDS: UV A1 - Phototherapy - ROS - Cytokines - Dermatitis, atopic.

In 1981, a novel filtered metal halide lamp with high output strictly confined to the UV A1 (340-400 nm) waveband was invented.1

In the following years, substantial progress was made in clarifying the photobiological, photophysical and phototherapeutical effects induced by this particular waveband.

Mechanisms of action

In comparison to UVB (280-325 nm) and UV A2 (320-340 nm), UV A1 penetrates deeper into the dermis and affects the biological and immunological activity of both epidermal and dermal cells, whether circulating or resident.

The mechanisms of action are also different. Unlike UVB and UV A2, UV A1 is not able to isomerize urocanic acid and does not cause direct anaerobic effects.
It mainly works through photochemical aerobic reactions leading to the generation of singlet oxygen and other reactive oxygen species (ROS), \textit{i.e.} superoxide anions, hydroxyl radicals and hydrogen peroxide. These activated molecules damage amino acids through oxidation, particularly tryptophan, tyrosine, histidine, methionine and cysteine leading to the denaturation of structural proteins, enzymes and receptors.

The auto-catalytic process of peroxidation of unsaturated membrane phospholipids and cholesterol leads to the formation of hydroxide derivatives of these lipids and a broad group of aldeidic compounds, the most important of which being malonyldialdehydes.\textsuperscript{2,3} DNA bases damaged through oxidation produce many photoproducts,\textsuperscript{4} such as 8-hydroxi guanine, 5-hydroxycytosine and tynime glycol. However, their mutagenic activity is held low because the base excision repair (BER) enzymatic system is able to repair the DNA damages quickly and efficiently. This enzymatic system consists of a glycosidase, the replication protein A (RPA), and an endonuclease (adenyl AP) together with the proliferating cell nuclear antigen (PCNA). In addition, UVA\textsubscript{1} induces pyrimidine dimers, though in negligible amounts without biologic importance.

UVA\textsubscript{1} exposures also induce cross-links between DNA strands and proteins as well as breaking single-strand DNA through a Fenton reaction.\textsuperscript{4} Since these DNA alterations do not inhibit cell growth, they are considered transient products of the repairing process.\textsuperscript{5} UVA\textsubscript{1} exposures may result in immunomodulatory effects such as the modification of transcription and release of several cytokines, the enhancement of the expression of cell-surface receptors and adhesion molecules and the selective induction of apoptosis of immunocompetent cells.\textsuperscript{6}

Increased m-RNA and protein expression of IL-1\textalpha, IL-6, IL-8, IL-10, TNF-\alpha \textsuperscript{7,9} and proopiomelanocorticotropin (POMC)-derived peptides, including \alpha-MSH,\textsuperscript{10} may be observed in UVA\textsubscript{1} irradiated keratinocytes. \alpha-MSH has many anti-inflammatory effects, \emph{e.g.} inhibition of IL-1 or TNF-\alpha-mediated proinflammatory effects, and immunosuppressive effects, \emph{e.g.} inhibition of cell-mediated immune responses.\textsuperscript{11}

UVA\textsubscript{1} exposures enhance the expression of nitric oxide synthase-2 (NOS-2) in endothelial cells of normal human skin and in keratinocytes of psoriatic skin. The extent of the enhancement is comparable with the expression achieved by stimulation with pro-inflammatory cytokines IL-1\beta, TNF-\alpha and IFN\gamma.\textsuperscript{12}

UVA\textsubscript{1} enhances the synthesis of prostaglandins (mainly PGE\textsubscript{2} and leukotrienes, activates phospholipase, and promotes the growth of Langerhans cells and dendritic cells. PGE\textsubscript{2} is a potent immunosuppressant that affects the expression of co-stimulatory molecules on the surface of antigen presenting cells and thereby prevents the activation of selected T cell subsets, especially Th1-like cells.\textsuperscript{13} It has also been demonstrated that the improvement of skin status in patients with atopic dermatitis treated with medium-dose UVA\textsubscript{1} is associated with the modulation of the expression of cathepsin G in the dermal inflammatory infiltrate.\textsuperscript{14} This is a serine protease that may attack laminines, proteoglycans, collagen I and insoluble fibronectine, inducing several biological effects: provocation of pro-inflammatory events, degradation of the basement membrane, destruction of the tissue inhibitor of matrix metalloproteinases (MMP) and enhancement of the endothelial permeability.

UVA\textsubscript{1}-induced upregulation of ICAM-1 expression on the surface of keratinocytes is mediated by an oxidation mechanism\textsuperscript{8,9} which may be modulated through cellular glutathione levels. UVA\textsubscript{1}-induced transcription of AP-1, c-Fos, c-Jun and NF-kB not only affects several inflammatory and immune activities but also the activation of metalloproteinase.\textsuperscript{15}

ROS and singlet oxygen generation cause so-called ”early” or pre-programmed cell death through an apoptotic mechanism that does not require de novo protein synthesis, takes less than 20 min and acts on the proteins which constitute the mitochondria, such as cytochrome-C.\textsuperscript{16} This process leads to the immediate opening of the cycloporine A-sensitive (”S” site) mitochondrial megapore.

Such early cell death of neoplastic lymphocytes has been observed in B and T-cell lymphoma skin infiltrate. The apoptotic process also leads to the release of IFN-\alpha. The following immunomodulation and the increased number of T-lymphocytes can partially explain the phototherapeutic efficacy of UVA\textsubscript{1} in the treatment of immune diseases.\textsuperscript{17}

Like PUVA and UVB, UVA\textsubscript{1} can also kill keratinocytes by a ”delayed” or programmed cell death. This apoptotic mechanism takes at least 4 h, and works by damaging the DNA, and the subsequent up-regulation of p53 (with the activation of Bax and 8-cas-
pase, and down-regulation of bcl-2), transcription of AP1 and expression of Fas-L.18-20

However, in vivo trials showed that 3xMED of UV A1 irradiation causes far fewer apoptotic sunburn cells than 3xMED of NB-UVB or solar irradiation.21

We can see a correlation between the decrease of bcl-2, the increased expression of p53 and the considerable reduction of either normal or pathological T-lymphocytes. These events are probably critical in the successful treatment of atopic dermatitis, mycosis fungoides and other T-cell mediated dermatosis.22, 23

Unlike UVB, UV A1 cannot significantly reduce the number of epidermal Langerhans cells even if it can reduce their size.24

Furthermore, UV A1 increases the number of CD34+ dermal dendritic cells and causes their accumulation in the draining lymph nodes by a mechanism that requires only IL1-β.25 This contrasts with UVB activity where the presence of TNF-α is also required. Therefore, it is likely that, unlike PUVA and UVB, UV A1 targets dermal dendritic cells and not Langerhans cells.26

Unlike UVB, UV A1 radiation induces the synthesis and the expression of metalloproteinases (e.g. MMP-1, MMP-2 and MMP-3) in human dermal fibroblasts through an autocrine mechanism involving the UV A1 inducible cytokines IL-1 and IL-6. Thus UV A1 phototherapy-induced softening and disappearance of sclerotic lesions such as morphea and keloids may result from induction of MMP.11 Finally UV A1 does not reduce only the release of histamine from basophils and mastocytes, but also the number of dermal mast cells present.30

UV sources and action spectrum

Nowadays, UV A1 irradiation sources are equipped with both fluorescent and filtered metal halide lamps. The fluorescent lamps have a low irradiance (5-10 W/cm² at skin level) and deliver low UV A1 doses (usually around 10-30 J/cm²) within 30-60 min. High-output metal halide sources have a much greater irradiance (80 W/cm² at skin level) and can irradiate medium (40-70 J/cm²) or high doses (up to 130 J/cm²) in treatment sessions of 30-45 min.31

Unfortunately, metal halide UV A1 irradiation units are very expensive to buy and operate. In addition, they require elaborate cooling systems and careful maintenance. Therefore, their use is limited to a few highly specialized centers of phototherapy in Europe and the USA.32

Unlike UVB and PUVA phototherapies, UV A1 is delivered at fixed doses without progressive dose adjustments.33

Clinical indications

UV A1 phototherapy has proved to be an effective and well-tolerated therapeutic option in the treatment of a growing number of inflammatory diseases, characterized by dermo-epidermal infiltrates of normal or neoplastic T-cells, mastocytes and/or eosinophils and diseases where the metabolism of collagen is altered.34-38 The efficacy of UV A1 has been demonstrated by randomized and controlled studies in the treatment of atopic dermatitis and LES (unfortunately, the LES studies come from a single source and a low number of patients were enrolled).

As regards dosages, different regimens have been proposed: low (10-20 J/cm²), medium (50-60 J/cm²), and high (100-130 J/cm²) doses,39 though at present there is no general agreement about which would be best.

In controlled clinical trials for the treatment of acute and severe atopic dermatitis, medium doses of UV A1 proved to be superior to low doses while being just as effective as high doses.40, 41 Combined UV A-B phototherapy was found more effective than low-dose UV A142, 43 but inferior both to medium44 and high dose UV A1. High doses of UV A1 were also found more effective than the topical application of fluocortolone.45

Medium doses of UV A1 are more effective than NB-UVB in the treatment of acute lesions of atopic dermatitis but less effective in the treatment of chronic manifestations.46

UV A1 is just as effective as topical PUVA in the treatment of chronic atopic dermatitis but it is preferable in that it is easier to perform.46-54

The reduction of skin inflammation and the improvement of the disease condition are closely linked to the significant decrease of the eosinophilic count in the blood, serum levels of the eosinophilic cationic protein (ECP)48 and soluble IL-2 (sIL-2R) and IL-4 (sIL-4R) receptors 44, 48-51 and a reduction of dermal mast cells and intraepidermal IgE-bearing Langerhans cells.52, 53

Low doses of UV A1 have been used in the treat-
ment of skin lesions associated with LES because they not only modify the pathogenetic mechanisms inducing immediate apoptosis, but promote the repair of UVB and UVA2-induced DNA damage, interfere with the translocation of extractable nuclear antigens (e.g. RoSSA), and reduce the IL12 levels (through the release of IL10) as well as the eosinophil serum levels.37, 55

An improvement of several extra-cutaneous clinical parameters has also been observed in these patients, e.g. morning stiffness, headache, arthralgia, asthenia and some laboratory parameters considered as disease activity markers (leukopenia and serum iger of anti-nuclear antibodies).37

However, patients affected with LES should be carefully monitored in order to opportunely diagnose phototoxicity.

In the treatment of other diseases, the efficacy of UVA1 is supported only by single case reports or controlled trials enrolling small numbers of patients. Therefore, until we have further information, UVA1 phototherapy must be administered exclusively to patients that are resistant to or do not tolerate standard therapies.

UVA1 irradiation proved to be effective in the treatment of patients affected by cutaneous T-cell lymphoma (CTCL) not only in 1A and 1B stages of the disease, but also in the tumoral and erythrodermic stages. UVA1 appears particularly useful for patients showing gastrointestinal or systemic toxicity due to oral psoralen administration.56-60

Medium dose UVA1 was found effective also for large-plaque parapsoriasis,59 pityriasis lichenoides et varioliformis acuta 61 and pityriasis lichenoides chronica.62, 63

In these skin diseases, the therapeutic activity of UVA1 seems to be related to direct effects on the cutaneous inflammatory infiltrate since unexposed lesions do not respond.63

UVA1 phototherapy quickly reduces itching and clears skin lesions in patients affected by sub-erythrodermal pityriasis rubra pilaris 64 and Netherton Syndrome.65

Although UVA1 phototherapy showed some efficacy in the treatment of psoriasis, it can be considered a viable alternative to either UVB or PUVA only in the case of HIV+ patients since it does not induce promoter genes of HIV replication.66

In anecdotal case reports, medium or high dose UVA1 phototherapy proved to be effective in the treatment of inflammatory diseases characterized by dermal infiltrate such as idiopathic mucinosis follicularis,67 cutaneous sarcoidosis,68, 69 hypereosinophilic syndrome,70 generalized granuloma annulare 71, 72 and extragenital lesions of lichen sclerosus et atrophicus 73, 74 (this last seems to be responsive also to low-dose UVA1 75).

Both low-dose and medium-dose UVA1 phototherapy are effective in the management of patients affected by maculo-papular mastocytosis 76 although pigmentary changes do not disappear. Clinical improvement is accompanied by a marked reduction of both lesional skin mast cells and histamine levels present in the urine.

Prolonged treatment cycles with high-dose UVA1 are effective for localized scleroderma 77 in adults and pansclerotic morphea in children,78 while low-dose UVA1 is less effective,25, 79, 80 but results are very good if associated with b.i.d. application of calcipotriol cream.81

Medium 82 or low-dose 83 UVA1 exposures cause progressive softening of the skin 82 and healing of peripheral piecemeal necrosis 83-85 in patients affected by systemic sclerosis (SSc). These patients showed an improvement of functional parameters related to disease activity, including passive joint mobility, skin temperature, and cutaneous elasticity. This clinical improvement is also accompanied by improvements in ultrasonic parameters and histological features. The therapeutic effects of UVA1 may be demonstrated by immunohistochemical investigations that show the induction of collagenases and a reduction of the lymphocyte infiltrate. 83, 86-88

A recent case-report indicates that UVA1 phototherapy may improve keloids and hypertrophic scars 84 and some clinical symptoms related to POEMS syndrome such as sclerodactyly, flexion contractures, and skin thickness.85

Medium-dose UVA1 phototherapy was found effective also in the treatment of sclerodermic as well as lichenoid lesions caused by graft-versus-host disease (GVHD).90, 91 Furthermore, UVA1-induced clinical improvement has permitted the use of immunosuppressive therapies by reducing the risk of drug induced toxicity and opportunist infections.92, 93

Protocol of treatment

Before engaging in a new treatment cycle patients must sign a written consent form that informs them
of the possible risks of the therapy as well as their alternative therapeutic options.

UVA1 phototherapy must be absolutely excluded when one or any of the following criteria are present: congenital DNA-repair defects; porphyrias; congenital defects of skin pigmentation; dysplastic nevus syndrome; former cutaneous melanoma; severe heart disease; therapy with photosensitizing drugs.

Other though minor criteria for the exclusion of this therapy are: under age patients; a personal or family history of non-melanoma skin cancer; family history of melanoma; severely sun damaged skin; former radiotherapy; therapies with arsenic and BCNU; poor compliance.

Treatment protocols are very simple: UVA1 is delivered at fixed and sub-erythemigenic doses without progressive dose adjustments. A phototest for the determination of the erythematous threshold as well as a photoprovocative test are recommended only if a photodermatosis is suspected.

Even if there are no comparative trials currently available for most of the possible applications of UVA1 phototherapy, a medium-dose seems to be just as effective as high-dose UVA1 phototherapy. Therefore medium-dose regimens are preferentially delivered because of lower cumulative dosages, costs and side effects.

Low-dose regimens are exclusively recommended in the treatment of LES, because medium and high-dose UVA1 could trigger the disease.

Five exposures a week are indicated in the case of atopic dermatitis while 3 exposures a week on alternate days are suggested for other dermatoses.

Treatment should be continued until complete clearing occurs, or, in the case of partial improvement, until there is no further amelioration in spite of one additional week of treatment. Alternatively, other authors suggest a predetermined number of exposures: 15 in the case of atopic dermatitis and 30 for dermal sclerosis.

There is no evidence to show whether maintenance cycles of treatment are useful in preventing recurrences. Therefore, they should be avoided not only because of cost, but more importantly because of the greater risk of cumulative skin toxicity.

Side effects

Skin dryness and mild itch are often reported by patients undergoing UVA1 phototherapy, but are usually responsive to emollient creams. Other acute side effects are uncommon and limited to the exacerbation of latent photosensitive diseases or relapse of a herpes simplex infection.

The major potential long-term adverse effects are photaging and skin cancer.

UVA1 induces photaging through photobiological mechanisms that are not completely known; although mitochondrial DNA mutations may play a relevant role.

UVA1 induces SCC-like tumours in chronically exposed mice. UVA1 induced cancerogenesis involves biological mechanisms that seem to be different from UVB even if the resulting skin tumours are the same. In particular, a wide spectrum of p53 mutations are observed after UVB but not after UVA1 exposures.

However, the magnitude of this risk in UVA1 treated patients is still unknown and long term trials enrolling a wide range of patients would be needed in order to clearly understand the risks involved. Nonetheless it is generally agreed that a standardization of dosage regimens and an accurate dosimetry would reduce these potential long term hazards.

Conclusions

After a review of published trials and single case reports the following guidelines can be suggested:

— UVA1 mainly induces type II, oxygen-dependent photochemical reactions;
— unlike UVB and PUVA, UVA1 penetrates into the dermis and modulates the biological and immunological activities of both epidermal cells and dermal cells;
— UVA1-induced biological and immunological effects are different from those induced by UVA2, UVB and PUVA;
— UVA1 phototherapy is expensive because of the high costs of UVA1 irradiation units and the high power consumption (up to 24 KW); this machinery requires constant and careful maintenance; a single treatment session may last up to 50 min even with the most powerful irradiation units;
— before starting a new treatment cycle, patients must sign a written consent form that informs them of the possible risks of the therapy and their eventual therapeutic options;
— treatment protocols are very simple: a preventive phototest is not required; UVA1 is delivered at
fixed, sub-erythemigenic doses without progressive dose adjustments.

— comparative studies have shown that medium-dose UVA1 regimens (50-60 J/cm²) are as effective as high-dose regimens (100-130 J/cm²) and more effective than low-dose regimens (10-39 J/cm²) in the treatment of atopic dermatitis. As far as other therapeutic indication are concerned, medium-dose UVA1 seems as effective as high-dose UVA1 even if no comparative studies are available. Since there is no evidence that confirm the higher efficacy of high-dose UVA1, medium-dose regimens are preferred because of their lower cumulative dosages, costs and side effects. Neither medium-dose nor high-dose regimens are recommended in the treatment of LES;

— the real risk of long term side effects, e.g. photaging and skin cancer, is not at present known;

— randomized and controlled studies supporting UVA1 efficacy have been reported only for atopic dermatitis and LES (however good results in the treatment of LES come from a single source and the number of studied patients was low);

— UVA1 phototherapy is preferable over NB-UVB in the treatment of acute exacerbations of atopic dermatitis but not for chronic lesions;

— LE affected patients must be treated with low-dose regimens and must be carefully monitored in order to opportunely diagnose photocytotoxicity or systemic clinical manifestations;

— in other suggested indications, the use of UVA1 is supported only by single case reports or uncontrolled or small trials. Therefore UVA1 phototherapy should be administered exclusively to patients who do not tolerate, or are not responsive to standard therapies;

— therapeutical results reported in the treatment of diseases characterized by lymphocyte or mastcells dermal infiltrate or by deficiencies in fibroblastic activity are particularly relevant and promising;

— for the treatment of psoriasis, UVA1 must be reserved for HIV+ patients only;

— criteria for the absolute exclusion of UVA1 phototherapy are: congenital DNA-repair defects; porphyrias; congenital defects of skin pigmentation; dysplastic nevus syndrome; former cutaneous melanoma; severe heart diseases; photosensitizing drugs;

— contraindications that require an evaluation between risk and benefit are: under age patients; personal history of epitheliomas; family history of melanoma; numerous UV exposures in the past; former radiotherapy or therapies with arsenic and BCNU; poor compliance.

Riassunto

Fototerapia UVA1

Le radiazioni ionizzanti con lunghezza d’onda compresa tra 340 e 400 nm (UVA1) hanno effetti fotofisici, fotobiologici e fototerapeutici peculiari. Gli UVA1 penetrano profondamente nella cute e modulano l’attività biologica e immunitaria sia delle cellule dermiche ed epidermiche circolanti che di quelle residenti. Diversamente dagli UVB (260-320 nm) e dagli UVA2 (320-340 nm), gli effetti fotobiologici degli UVA1 sono mediati esclusivamente da processi fotochimici ossidativi che portano alla formazione di ossigeno singololetto e di altre specie di ossigeno reattivo (ROS, reactive oxygen species). Il bersaglio principale delle ROS è rappresentato dalle membrane cellulari e dagli organuli citoplasmatici, in particolar modo dai mitocondri, che sono ricchi di molecole strutturali con diversi doppi legami, quali i lipidi e gli aminoaclidi. La fototerapia UVA1 si è dimostrata essere un’opzione terapeutica efficace e ben tollerata per il trattamento della dermatite atopica, della sclerodermia localizzata, della mastocitosi, del lupus eritematoso sistemicco, della micosi fungoide, della malattia cronica da riletto del trapianto verso l’ospite. Essa inoltre si è dimostrata efficace per un numero crescente di altre patologie cutanee, caratterizzate da infiltrati dermo-epidermici di linfociti normali o neoplastici, mastociti e/o esosinofili, così come per le alterazioni metaboliche del collagene. Al momento non c’è un accordo generale su quale sia il protocollo ottimale di trattamento. Gli UVA1 vengono somministrati a dosi fisse, senza aggiustamento progressivo del dosaggio. Tuttavia, i protocolli differiscono per quanto riguarda il dosaggio, il numero di esposizioni settimanali e il numero totale di esposizioni. La terapia UVA1 è sempre ben tollerata e sono rari gli episodi di reazione eritematosa. Gli effetti collaterali a lungo termine non sono noti, ma il rischio di carcinogenicità cutanea sembra trascurabile, dal momento che gli UVA1 non danneggiano direttamente il DNA.

Parole chiave: UVA1 - Fototerapia - ROS - Citochine - Dermatite atopica.

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Human immunodeficiency virus and dermatology - a focus on special diseases and a review of the literature

H. BELTRAMINELLI, P.H. ITIN

Cutaneous disorders can be seen in any stage of the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), including a wide spectrum of diseases which, owing to the rapid development of anti-HIV drugs, have changed relevance during the history of HIV infection. With the advent of highly active antiretroviral therapy, many skin disorders have improved or disappeared completely, whereas others may even worsen as the patient's immune status begins to recover, a phenomenon known as the immune reconstitution syndrome. Disorders such as Kaposi's sarcoma and oral hairy leukoplakia are typical but not specific for HIV infection. When recognized, HIV infection needs to be determined serologically. Seborrheic dermatitis or atypical herpes simplex are commonly associated with HIV infection but they can often be present without the infection. Dermatological manifestations such as generalized skin rash are a presenting feature in 40% of cases of acute HIV infection. In 10% of HIV patients, stomatologic findings are the first clinical sign of the disease and can help to establish a diagnosis of HIV infection. Most cutaneous disorders in the setting of AIDS share common features: an unusual presentation with atypical localization, widespread eruption, sudden exacerbations, resistance to treatment and often a chronic course; some reflect the patient's underlying immune status; manifestations of disseminated and extensive disease typically co-present with laboratory findings of lower CD4+ cell count and/or high viral load. Some HIV-associated systemic fungal infections (without skin correlations) and some adverse drug reactions (with evident skin manifestations) may be life-threatening. Moreover, many drugs can produce several adverse effects and possible interactions. Hence, in the setting of HIV, a high index of suspicion is a vital for establishing early diagnosis and instituting prompt, effective treatment.

KEY WORDS: Human immunodeficiency virus - Acquired immunodeficiency syndrome - Antiretroviral therapy - Marker lesion - Skin.

History and epidemiology

The history of human immunodeficiency virus (HIV) infection began in 1981 when an outbreak of an unusual combination of Pneumocystis carinii pneumonia and mucosal candidiasis was observed in healthy young men in Los Angeles, California.1 The HIV was later identified, and a growing number of patients showed typical disease patterns associated with impaired cellular immune function. Taking a systematic approach to skin diseases in the setting of HIV, this article focuses on diseases associated with highly active antiretroviral therapy (HAART), which has been widely used in western Europe since 1996-1997.

The HIV infection pandemic was initially limited to communities such as men who have sex with men (MSM)-in fact, the acquired immunodeficiency syndrome (AIDS) was first called gay-related immunodeficiency (GRID), injection drug users, commercial sex workers and the poor. Current trends indicate,
however, an increasing shift in the incidence of HIV to heterosexuals. Noteworthy is that systematic screening of blood donations since 1985 virtually eliminated the risk of HIV transmission through blood transfusion.

The epidemiology of HIV and HIV-related diseases and the social problems they cause have changed over the course of the history of the disease. In the past, when treatment was unavailable, HIV patients were socially discriminated and isolated from the community. As new treatment modalities became available, however, discrimination diminished and epidemiologic data shifted parallel with the introduction of modern drugs. But in recent years, a new clinical situation, the so called immune restoration syndrome, has been observed in patients with good response to HAART, where diseases like herpes zoster or Mycobacterium avium intracellular infections flare up because of the patient’s better immunological response to the microorganism. HAART has led to a marked decrease in HIV-related morbidity and mortality: the annual AIDS mortality has dropped by 75% since 1995; opportunistic infections in general, and serious cases in particular, are less frequent; mucocutaneous diseases have declined dramatically. Despite this good news, new problems such as the fat redistribution syndrome have emerged, along with a rise in the incidence of skin cancers (e.g. squamous cell carcinoma, basalioma and Bowen’s disease).

With the implementation of interventions to reduce the risk of vertical transmission of HIV in pregnant women, the rate of vertical transmission in Europe fell from 15.5% by 1994 to 2.6% after 1998. Worldwide about 40 million people (17.5 million women, 2.3 million children) are currently living with HIV infection; more than 25 million have died since 1981; about 4.9 million new infections occurred in 2005 alone. Globally, HIV is now the fourth leading cause of death; 3.1 million people died from HIV in 2005. There is a large gap in the data between the poor and the rich countries. While all report an increasing number of people living with AIDS, in the former this is because of a lack of prevention campaigns and a result of not using a condom during sex, either of which may be associated with particular cultural, socioeconomic, linguistic and administrative barriers. In the latter, HAART reduced HIV-related mortality to levels comparable with the rates in non-HIV-infected reference populations. So people feel healthier and are more sexually active. In addition, the number of non-HIV-infected persons at risk for the infection may be increasing among a young generation now sexually active who did not experience the initial AIDS epidemic and are either unaware or simply ignore the risk of HIV infection. Swiss data, like those from other western countries, show an increase in AIDS and HIV infections during the late 1990s, followed by a decrease that has stabilized since 2002. More recently, however, the rate of new infections is increasing again. The largest group of HIV-infected persons are those who have heterosexual contact (about 60% in total); this increase probably reflects the general upward trend in infections attributed to heterosexual sexual contact in the developed countries; regrettably the incidence of HIV infection among MSM is still rising.

**General considerations**

During the last 20 years, the spectrum of HIV-related skin manifestations has changed, subsequently influencing dermatological differential diagnosis. HIV-positive patients have more skin problems (92% of HIV cases) than a similar non-HIV-infected collective. Stern showed that on average HIV-positive patients have 3.7 different dermatological diagnoses. Dermatologic clinical findings can be markers and sometimes detectors of hidden HIV infection. The common features of the majority of skin diseases in HIV-positive patients are the unusual clinical presentation, with atypical and wider localization, sometimes mimicking other dermatologic diseases, uncharacteristic course with treatment resistance and relapses. Because of this atypical presentation, skin biopsy for histological analysis is recommended to verify clinical diagnosis; when there is clinical suspicion of infection, a microbiological study should be ordered as well, particularly in nodular, pustular or ulcerated clinical presentations. Knowledge of the spectrum of these skin disorders is a challenge for daily clinical practice.

A frequent clinical observation is that the incidence and severity of skin disorders occurs more often as the body’s immune function deteriorates; however, statistical data confirming this observation are limited, and the correlation between the two is still controversial. One study showed a statistically significant correlation between a low CD4 cell count in HIV patients with various skin disorders and a higher CD4 cell
count in asymptomatic HIV patients. A study from Thailand showed that during the early stage of HIV infection (CD4 cell count >500/µL), patients may typically have xerosis and seborrheic dermatitis, and in advanced stages (CD4 cell count <200/µL) patients have more opportunistic infections such as multidermatomal herpes zoster and penicillinosis (typical in tropical countries). Interestingly, no cases of Kaposi’s sarcoma or skin tumors were identified in this study, perhaps because of the low prevalence of Kaposi’s sarcoma among Asians, and the prevalence of drug eruptions was unexpectedly low.

A study from Spain on a cohort of 1161 HIV-positive patients (74% were injection drug users) showed some correlations between advanced stages of HIV disease with lower CD4 cell count and certain dermatoses associated with an increased risk of developing AIDS and increased mortality. Oral candidiasis and seborrheic dermatitis were the most common disorders, followed by xerosis, drug eruptions, dermatophytosis, papular eruption of AIDS; others included genital herpes, herpes simplex (Figure 1), oral hairy leukoplakia (OHL) (Figure 2), molluscum contagiosum, verruca vulgaris, onychomycosis, folliculitis in...
candidiasis, staphylococcal folliculitis, abscesses, Kaposi’s sarcoma (Figure 3), idiopathic pruritus, diffuse alopecia, psoriasis, skin hyperpigmentation, mucosal hyperpigmentation. Although herpes zoster (Figure 4) did not appear to be associated with impaired immunity, it could, however, be considered a marker of poor prognosis as it was associated with higher mortality. Most of these results were in line with those of other studies from the 1990s.

Jordan et al.13 showed that in adults, and particularly in children, HIV-specific CD8 cells lack perforin, an important CD8 cell component which, when lost, may be associated with progressive HIV disease, suggesting that a lack of effector cell properties in HIV-specific CD8 cells contributes to a lack of immune control in HIV.

Increased serum IgE levels and eosinophilia have been almost exclusively described in HIV patients with low CD4 cell count. Paganelli et al.14 showed that CD8 cells functionally mimic Th-2 type CD4 cells and may account for hyper-IgE and eosinophilia in the absence of CD4 cells.

Data from a prospective Swiss cohort study from 199615 on 357 HIV patients showed tinea (43.7% of patients) as the most frequent dermatological problem, followed by xeroderma (37.8%), seborrhoic dermatitis (32.5%), candida stomatitis (Figure 5), verruca vulgaris, folliculitis, herpes simplex, condyloma acuminatum and molluscum contagiosum. These data are similar to those from other cohort studies of German-speaking Europe.16 It is well known that xerosis, OHL, molluscum contagiosum, oral candidiasis and Kaposi’s sarcoma are cutaneous markers of HIV disease with an important immunodeficiency.17

Before and after highly active antiretroviral therapy

During the 1990s, a series of new antiviral drugs and combinations of various active compounds profoundly changed the approach to treating HIV patients. After treatment failure or resistance, the therapeutic regimen was usually replaced with new medications and/or new combinations. After zidovudine monotherapy of the early 1990s, combination therapy with other reverse transcriptase inhibitors became available; after 1995 HIV-1 protease inhibitors with potent anti-HIV activity became widely available and were added to these combinations. There are 4 different classes of antiretroviral drugs: nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and fusion inhibitors (FI). Modern HAART is a combination of at least 3 drugs, typically including either a PI, or a non-nucleoside analogue reverse transcriptase inhibitor, and 2 nucleoside analogue reverse transcriptase inhibitors. The goal of this treatment is to achieve maximum viral suppression for as long as possible, while minimizing side effects and preventing the development of drug resistance. With the introduction of HAART, the prevalence of several HIV-associated opportunistic infections and malignancies changed remarkably, including regression of oral candidiasis, Kaposi’s sarcoma and OHL,18 except for mycobacterial diseases,19 oral condylomata and herpes simplex virus infection, this last of which seems to persist. The morbidity and mortality of AIDS patients declined dramatically. Sterne et al.4 showed that HAART, compared with no treatment, was more beneficial with longer duration of treatment, but was less effective in patients whose presumed mode of transmission was injection drug use. The reason for the clinical improvement was probably the result of partial immune reconstitution with improved cellular immune system, as demonstrated by the more persistent, higher CD4 cell count as compared with previous antiviral monotherapy. The decreasing incidence of opportunistic infections may also have been due to advances in prophylactic treatments. The prevalence of most noninfectious skin diseases did not change after the introduction of HAART.20

Prins et al.21 showed that in the developed countries there is little evidence for sex differences in the rate of disease progression before and during the HAART era. Notably, with the availability of effective treatment, HIV-infected women of reproductive age have increasingly decided to have children.

Seoane Reula et al.22 observed a significant decrease in the prevalence of mucocutaneous manifestations during HAART treatment in HIV-infected children (follow-up every 3 months over 22 years); some children had no treatment at the beginning of the study, others received antiviral monotherapy followed by combined therapy, and then by HAART. During HAART an increase in the CD4 cell count was observed, while during monotherapy or combination therapy there was no decrease in cell count and even a small increase in mucocutaneous manifestations.

Maurer et al.23 studied the effect of HAART on HIV-infected women in the U.S.; studies on women...
with HIV infection are important because their proportion of the population living with HIV infection is rising (17.5 million women worldwide of a total of 40.4 million people); the women receiving HAART had less eczema, folliculitis, tinea pedis and xerosis than those who did not receive HAART, independently of CD4 cell count.

The incidence of Kaposi's sarcoma dropped precipitously after the introduction of HAART. HAART may prevent the risk of developing non-Hodgkin's lymphoma but not that of Hodgkin's lymphoma or other nonacquired immunodeficiency syndrome-defining cancers. HIV patients are at high risk for human papilloma virus (HPV) related cancers (i.e., cervical and anal cancers) and they are not clearly affected by CD4 cell count or HAART; nonmelanomatous skin cancer, which is also associated with cutaneous HPV types, was found to be three-fold higher in a Swiss HIV cohort study, but the number of HIV patients with this tumor is still about 10-fold less than among organ transplant recipients. Other data on the impact of HAART on the natural history and outcome of HIV-associated malignancies are limited.

In our previous study we found that the prevalence of seborrhoeic dermatitis is influenced neither by initial CD4 cell count nor by antiretroviral treatment. Epidemiological data from a Swiss cohort study of the most frequent skin diseases before and after HAART are shown in Table I.

Interestingly, herpes zoster appeared more frequently after HAART; this rise was attributed to the immune reconstitution phase. Another interesting observation is that eosinophilic folliculitis appeared not only in patients with low CD4 cell count but also in those who had successfully completed HAART. This dermatosis is another new addition to the immune reconstitution syndrome. Other diseases that show a flare-up after starting HAART are infections from herpes viruses, cytomegalovirus, mycobacteria, cryptococci, inflamed cutaneous warts, molluscum contagiosum demodex folliculitis and inflammatory diseases such as sarcoidosis, eosinophilic folliculitis, atop dermatitis and tattoo intolerance. The immune reconstitution syndrome is a transitory worsening of disease that appears generally 3 to 6 months after HAART has been started.

Although the overall survival of HIV patients since the introduction of HAART has improved, mortality remains higher among HIV-positive patients than in the general Swiss population. Caution is warranted when interpreting these data because the long-term effect of HAART is still unclear. In most studies that have reported successful treatment, follow-up was limited to 1 year or less, and most trials focused on increased CD4 cell count and viral response to treatment. But these outcome measures are not the only ones that correlate with AIDS improvement or death, since other elements such as improved medical care, early detection of HIV patients and the increasing use of prophylactic drugs may also play a critical role. For ethical reasons, placebo-controlled studies versus HAART are not possible.

### Antiretroviral drugs and cutaneous side effects

HIV patients have a higher incidence of drug reactions than those unaffected by HIV. The reasons for this difference are many: first, HIV patients take many different drugs daily (antiretrovirals, antibiotics, antimycotics, and other medications) over years, so they are concurrently exposed to several substances with various potential interactions; second, it is known that viral infection in general, and HIV in particular, predisposes to skin reactions; third, drug reactions are more common in the setting of immunosuppression. In addition, HIV-infected patients have more CD8 cells in their otherwise normal skin compared with controls. There are short- and long-term side effects, the risk varies from drug to drug, from drug-class to drug-class, from interactions among different drugs and from patient to patient. Certain antiretroviral agents are associated with specific cutaneous manifestations. The most common NRTI-induced dermatological side effects are hypersensitivity (with multisystem involvement) to abacavir and hyperpigmentation (reversible and relatively dose-dependent) from zidovudine. Cutaneous problems account for a significant portion of NNRTI-related adverse events, including mild rash or severe exantheme (Stevens-Johnson syndrome, [SJS])

<table>
<thead>
<tr>
<th>Disease</th>
<th>Before HAART (%)</th>
<th>After HAART (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida stomatitis</td>
<td>41.7</td>
<td>12</td>
</tr>
<tr>
<td>Oral hairy leukoplakia</td>
<td>34.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Seborrheic dermatitis</td>
<td>32.5</td>
<td>13.0</td>
</tr>
<tr>
<td>Folliculitis</td>
<td>21.8</td>
<td>10.9</td>
</tr>
<tr>
<td>Reactive syphilis serology</td>
<td>18.5</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Table I.—Epidemiology of the most frequent skin diseases before and after highly active antiretroviral therapy (HAART). Results of the Swiss cohort study.
from nevirapine, pruritic rash from delavirdine; major side effects caused by PI are retinoid-like effects (recurrent paronychia, pyogenic granulomas, curly hair, alopecia), lipodystrophy from indinavir, and mild skin rash from amprenavir. The most common cutaneous side effect of the newest class of antiretrovirals (FI) is injection site reactions (98% of patients!). These and other less frequent HAART side effects have been described in detail by Kong and Myers.30

A major adverse effect of HAART is the lipodystrophy syndrome, which includes loss of peripheral fat (lipoatrophy) in the face, limbs and buttocks, and/or central obesity; other characteristic features are dorsocervical obesity (buffalo hump), lipomas and breast hypertrophy; some HAART agents such as stavudine (d4T), and PI in general, are more likely to induce lipodystrophy. Typically, the symptoms appear within 6 to 12 months after the start of treatment. The pathogenesis of lipodystrophy is poorly understood, but recent studies have shown an association with mitochondrial-DNA depletion caused by NRTI.31 Partial reversibility of lipoatrophy was obtained after switching from the agent zidovudine or stavudine (d4T) to abacavir, but other features of the lipodystrophy syndrome showed no improvement from the switch.32

Sexually transmitted infections

Preventing and treating sexually transmitted infections (STIs) reduces the risk of HIV transmission, especially among persons with numerous sexual partners, such as sex workers and their clients. Infection with syphilis, gonorrhea, Chlamydia, trichomoniasis and genital herpes, which cause breaks in mucosal tissues and inflammation, increase the chance of HIV transmission during unprotected sex. The broken mucocutaneous barrier in the lesions of these infections plays an important role. In 2001 Auvert et al.33 demonstrated a strong association between HIV and herpes simplex type 2. McFarland et al.34 and Del Mar et al.35 suggested that the 2 viruses favor each other, with each boosting the odds that a person will contract and transmit the other.

Wearing a latex condom is the most efficient way to reduce the sexual transmission of HIV and other STIs. Most people in general, and young persons in particular, tend to know very little about STIs, hence part of HIV awareness campaigns should be specifically addressed to young people and promoted as an example of youth-friendly services. We believe that the ignorance about STIs in general and about HIV infection and AIDS in particular is the main reason for the rise in the number of infected patients in the developed countries. People know that the risk of dying from HIV infection is much less now than it was during the first years of the history of HIV. Some believe AIDS can be definitively cured or managed and survived, while forgetting that the risk of transmission, and hence of contracting HIV infection is lifelong. In addition, the risk of infection by heterosexual contact is underestimated, owing to the misconception that HIV infection is only a risk for particular communities such as sex workers, injection drug users and MSM. Some HIV-infected persons stop using a condom, believing that a low viral load renders them noninfectious. A different kind of prevention campaign showing a generally healthy looking HIV-positive patient maybe just that kind of new message to raise awareness.

Focus on particular noninfectious HIV-related cutaneous disorders

Early human immunodeficiency virus manifestations and the skin

Acute or primary HIV infection (i.e. acute retroviral syndrome, HIV seroconversion syndrome, primary HIV infection) is a transient (a)symptomatic illness with a broad spectrum of clinical manifestations. In the majority of cases a high index of suspicion is needed to diagnose it. Most patients (40-90%) present with mononucleosis-like symptoms in the first week of acute HIV infection, but symptoms are often unspecific, and some patients have an asymptomatic seroconversion. Although more than 50% of patients with acute HIV infection are symptomatic, over 90% go undiagnosed at the first medical visit.36 Misdiagnosed cases miss the chance to institute early treatment; furthermore, early diagnosis is important because administration of antiretroviral treatment during acute HIV infection has been demonstrated to improve the subsequent clinical course of the disease and to boost the CD4 cell count.37 HAART initiated during primary virus infection may reduce the number of long-lived, latently infected lymphocytes that act as a reservoir for HIV.38 Patients with acute HIV infection seem to have a more heterogeneous spectrum of the virus
than those with chronic infection, thus potentially improving their response to HAART. Early recognition of HIV infection is important for preventing the spread of the disease, since primary infected patients have high titers of HIV virus in the blood and genital secretions, and are also particularly contagious. Many new HIV infections occur during this early phase from contact with persons with high viral load. The signs and symptoms of acute HIV infection usually present within days to weeks after initial exposure and last about 2 weeks. The commonest nondermatological signs are fever, fatigue, headache, lymphadenopathy, pharyngitis, myalgia, arthralgia, weight loss, gastrointestinal troubles and night sweats. Interestingly, 87% of HIV-infected persons remember symptoms which could be associated with an acute retroviral syndrome. The pathogenesis of periungual erythema is unknown. Ruiz-Avila et al. suggested that angiogenic factors produced by HIV, which are implicated in the pathogenesis of Kaposi's sarcoma and bacillary angiomatosis, may be responsible for the telangiectasias seen in HIV patients; these factors included the Tat protein, interleukin-6 and oncostatin M.

Erythema of the proximal nailfold

In a previous study, we analyzed skin diseases associated with HIV infection in addition to those discussed above; 2.4% of the patients showed asymptomatic marked periungual redness, 2 of which had dilated capillaries. Periungual erythema (Figure 6) is an unspecific finding which may be found in systemic lupus erythematosus, dermatomyositis and scleroderma; it can be a very early manifestation of diabetes mellitus, other rare associations such as the sequelae of cytotoxic therapy, atopy or of working on coffee plantations. Periungual erythema has been reported repeatedly in several conditions with severe immunodeficiency such as graft-versus-host disease, Langerhans' cells histiocytosis and Wiskott-Aldrich syndrome. In 1989 we reported the first patient with HIV infection, periungual lichenoid papules and marked erythema. The pathogenesis of periungual erythema is unknown. Ruiz-Avila et al. suggested that angiogenic factors produced by HIV, which are implicated in the pathogenesis of Kaposi's sarcoma and bacillary angiomatosis, may be responsible for the telangiectasias seen in HIV patients; these factors included the Tat protein, interleukin-6 and oncostatin M.

Pruritic papular eruption

The pathogenesis of pruritic papular eruption (PPE), despite 2 decades of reports, is still unclear. Clinical manifestations are a severe pruritus associated with firm, discrete, erythematous urticarial papules localized on the extremities, the face and sometimes the trunk; the excoriated papules heal with scarring hyperpigmentation (Figure 7). Histopathologic findings show a mild to moderate dermal perivascular and perineural lymphomononuclear infiltrate with eosinophils and mast cells (which could justify the intense pruritus), immunohistochemical characterization of T-cell subpopulations show a clear predominance of CD8 cells compared with healthy skin; the predominance of CD8 cells in PPE lesions does not simply represent a reflux of peripheral blood, as the number of CD8 lymphocytes in the lesions is higher than in healthy skin. Pathophysiologically, PPE has been linked to insect bite reactions as a hyperallergic response against mosquito saliva or against another antigen. The incidence of the disease increases as the CD4 cell count decreases, so PPE can be regarded as a cuta-
neous marker of advanced HIV infection, nevertheless, there are cases of PPE as an initial manifestation of HIV. In Brazil for example, PPE has been found in 11.7% of patients with HIV infection and is considered there as a marker of HIV infection. Most patients have been described in Haiti, Brazil, Thailand and Africa, but rarely reported in Europe and North America. The differential diagnosis of this disease includes staphylococcal folliculitis, eosinophilic folliculitis, demodex folliculitis, drug eruptions, arthropod bite reactions, photodermatitis, crusted scabies and secondary syphilis. Because of the evident scarred excoriations similar to prurigo, HIV patients with PPE suffer additional stigmatization. The persistent pruritus is usually refractory to topical steroids and oral antihistamines.

**Eosinophilic folliculitis**

Eosinophilic folliculitis (EF) develops in the setting of abnormal host immunity, mostly in individuals with end-stage HIV infection with low CD4 cell count, and it is a marker for individuals with a high risk of developing opportunistic infections. Unlike some authors who believe that EF and PPE are the same entities, we think they constitute 2 different diseases because of clinical and pathological differences and by a different course during HIV infection.

EF is a disease of the immune reconstitution syndrome and shows flare-ups after HAART is begun. Paradoxically, it has even been thought to improve with HAART. The clinical manifestation of EF is a pruritic papulopustular eruption involving the trunk, limbs and face (Figure 8). The bulk of documented cases are MSM. Often there is a peripheral blood eosinophilia. The pathophysiological mechanism underlying EF is unknown. The suggested cause is a hypersensitivity reaction against Demodex folliculorum and Pityrosporum or against some constituent of sebum acting as an autoantigen. This last hypothesis is supported by histological evidence for a lytic degeneration of the sebaceous glands. Other histological findings are follicular inflammation with lymphocytes, eosinophils and neutrophils. Immunohistology shows a T-cell lymphocytic infiltrate with marked CD8 cell predominance.

EF should be differentiated from other types of folliculitis, Ofuji’s disease and PPE.

Treatment of EF is frustrating; some patients improve with topical steroids, antihistamines, antimycotics, retinoids and UV-therapy. Currently there is no gold standard. Recent studies have reported a positive effect of topical tacrolimus. In our experience, administration of systemic retinoids is the most efficient therapy.

**Oral lesions and human immunodeficiency virus**

Oral lesions are readily visible; oral inspection is a simple and a highly important step in assessing dermatological status. When HIV status is unknown and HIV testing is difficult, as in developing countries, certain oral lesions provide a strong indication of the possible presence of HIV infection; they can also be considered early clinical features. Oral/lymphoid candidiasis are indicators of a depleted immune system and signs of progression to AIDS, for this reason they are included in the CDC classification criteria. Oral candidiasis, Kaposi’s sarcoma and lymphoma were among the earliest described lesions of AIDS seen in homosexual and bisexual men.

Most HIV-related oral lesions correlate chiefly with HIV viral load, independently of CD4 cell count; nevertheless, studies have shown an evident association of oral candidiasis and OHL with low CD4 cell count. Many have confirmed that oral lesions are remarkably reduced under the effect of HAART, probably as a result of an expression of a reconstituted immune system; a reduction in opportunistic infections was also demonstrated.

Many oral diseases are similar in clinical appearance, making the right diagnosis difficult. With an atypical presentation, as in HIV patients, the differential diagnosis includes pseudo-OHL, chronic hyperplastic candidiasis, herpes simplex, lichen planus, graft-versus-host reaction, idiopathic leukoplakia, white sponge nevus, leukokeratosis nicotinica, geographic tongue, marked edema and trauma. The diagnostic steps are microscopic study for mycosis, viral scraping for microbiological determination or punch biopsy. We recommend serological HIV test for lesions suggestive of HIV infection.

**Future**

There is new evidence that the prevention programs initiated in many countries are finally helping to bring
new discoveries, in the context of clinical practice and research. This has led to the rapid development of antiretroviral drugs, and advancements in the treatment of HIV infection have been accompanied by an increased awareness of the consequences of HIV infection. The incidence of new infections among different groups (e.g., MSM and bisexual men) that have shown a recent rise in the incidence of new infections.

The past 2 years, worldwide access to the expensive antiretroviral treatment has improved markedly. Despite the progress witnessed in some regions, however, the situation has changed slowly in the poorest countries of Latin America and the Caribbean, vast parts of Asia and virtually all of sub-Saharan Africa.

Indications are that some treatment gaps will narrow further, but not as quickly as to contain the epidemic. Universal change of current conditions will require the coordination of many different approaches to treatment, prevention and care, all of which needs to be done urgently. But this is part of a larger long-term challenge that will require overcoming such serious problems as stigma, discrimination, gender inequalities and other human rights violations, together with the social injustices caused by AIDS. These are extraordinary challenges that demand extraordinary responses and, very importantly, long term vision.

A special direction in future prevention campaigns in western Europe will be to target HIV information to different groups at risk for HIV infection, such as immigrants from countries with a high prevalence of HIV/AIDS (notably sub-Saharan Africa), young people, persons with multiple sex partners, and other groups (e.g., MSM and bisexual men) that have shown a recent rise in the incidence of new infections.

### References


Acantholytic changes may be induced in vivo and in vitro by a growing list of drugs. Nifedipine (a widely used antihypertensive drug acting as a calcium channel blocker) seems to have an acantholytic effect in vitro. We report a case of a 74-year-old man, affected with intermediate basal cell carcinoma of his right clavicular area, in whom the histologic examination showed suprabasal acantholytic clefts in the perilesional epidermis. The patient’s history revealed only a mild arterial hypertension that had been treated with 10 mg nifedipine for 10 years. Long-term administration of nifedipine, the absence of fixed intercellular antibodies by direct immunofluorescence (DIF) and circulating anti-Desmoglein-1 and anti-Desmoglein-3 (anti-Dsg 1-3) autoantibodies by ELISA, the lacking of any blisters or erosive lesions, and the histologic findings suggest a diagnosis of in vivo nifedipine-induced acantholysis.

KEY WORDS: Acantholysis - Nifedipine - Pemphigus - Carcinoma, basal cell.

Acantholytic changes may be induced in vivo and in vitro by a growing list of drugs. Nifedipine is a widely used antihypertensive drug with an acantholytic effect in vitro.1

The complex and still partially understood action mechanism seems to be related to an inhibitory interference of the drug with enzymes involved in the keratinogenesis and calcium-dependent adhesion molecules like desmogleins.1-3 Furthermore, as described by Kim et al., nifedipine may cause the outcome of pemphigus mainly in genetically predisposed subjects.2

Case report

A 74-year-old retired man had been suffering for 3 years from an asymptomatic irregularly roundish neof ormation located on his right clavicular region. Over the years the lesion had ulcerated and covered with crusts, and enlarged to reach a diameter of about 2 cm. He was referred to our department.

The patient’s clinical history did not reveal any significant finding except a mild arterial hypertension that had been treated with 10 mg nifedipine per day for 10 years.

Clinical examination showed a nodular ulcerated lesion suggesting a diagnosis of basal cell carcinoma.

An excisional biopsy was performed and histologic examination displayed masses of basaloid cells, areas of squamous metaplasia with concentric keratinization (Figures 1, 2), leading to a diagnosis of intermediary basal cell carcinoma. Unexpectedly, the histologic picture also showed areas of suprabasal acantholysis with acantholytic cells in the perilesional epidermis (Figure 1).
Neither acantholytic genodermatoses (Hailey-Hailey disease, Darier disease) were revealed by the clinical history nor clinical signs of pemphigus or other acquired acantholytic diseases (Grover’s disease) were present.

Direct immunofluorescence (DIF), using fluorescein-labelled antibodies to human IgG, IgA, IgM, and C3, of the perilesional biopsy specimen did not display any intercellular deposits. In addition, no anti-Desmoglein-1 and anti-Desmoglein-3 (anti-Dsg 1-3) autoantibodies were detected by immunoenzymatic assay (ELISA). HLA typing did not reveal any pemphigus correlated antigens.

In addition we ruled out by histologic evaluation the other benign or precancerous cutaneous lesions characterized by localized acantholysis such as acantholytic actinic keratosis, acantholytic seborrheic keratosis, acantholytic acanthoma and warty dyskeratoma. Acantholytic actinic keratosis is mainly characterized by the presence of hyperkeratosis with parakeratosis, loss of underlying granular layer, epithelial dysplasia and solar elastosis. These morphological features are not identifiable in the present case.

In acantholytic seborrheic keratosis, the acantholysis is generally focal, but the lesion elsewhere shows the characteristic morphologic picture including horny cysts, variable proliferation of basaloid and squamous cells, acanthosis, papillomatosis and hyperkeratosis.

Acantholytic acanthoma should be easily differentiated from our case because this lesion displays acanthosis, papillomatosis, hyperkeratosis and dyskeratosis.

Likewise, we ruled out the warty dyskeratoma because it is composed of widely dilated cystic lesion, often associated to hair follicle, containing keratinous debris mixed with grains of Darier.

A further biopsy was carried out, after the patient’s informed consent, on apparently healthy skin of his back, but histologic examination did not show acantholysis or other morphologic changes.

The patient’s history (nifedipine long-term administration), the absence of fixed intercellular antibodies by DIF and circulating anti-Dsg 1-3 autoantibodies by ELISA, the lacking of any blisters or erosive lesions, and the histologic findings suggested a diagnosis of in vivo nifedipine-induced acantholysis in a non genetically pemphigus prone-patient.

The patient was advised to change nifedipine with another antihypertensive drug and to undergo periodical check-ups.

**Discussion**

Acantholysis is a morphofunctional change occurring in stratified epithelia, characterized by the loss of intercellular cohesion with subsequent disjunction of epithelial cells.

An exhaustive explanation on the mechanisms involved in acantholysis is lacking at present. Nevertheless, several data indicate that acantholysis can be induced both in vivo and in vitro by 2 main mechanisms: a) immune acantholysis induced by specific pemphigus antibodies which, binding to antigens target on keratinocyte cell membrane, cause the activation of proteinases leading to cellular dyshesion; b) biochemical acantholysis induced by some drugs and
chemical substances able to link with keratinocyte cell membrane and to interfere with cell-cell adhesion molecules thus producing a disjunction of keratinocytes.

The acantholytic potential of nifedipine has been documented in vivo \(^1,3\) as well as the possibility that it may cause the outbreak of pemphigus foliaceus.\(^2\)

The process of keratinogenesis needs the presence of certain enzymes, such as keratinocyte transglutaminase and gamma-glutamyl transpeptidase, whose activity requires free calcium ions.\(^9,11\) These enzymes seem to play a pivotal role in cell-cell cohesion and aggregation of keratinocytes to form a stratified epithelium. Nifedipine, acting as a calcium channel blocker inhibiting transmembrane calcium ion influx,\(^1,3\) may interfere with calcium availability thus provoking biochemical acantholysis.

In the present case, factors supporting the hypothesis of biochemical acantholysis are the presence of a HLA genotype not related to pemphigus and the absence of fixed intercellular antibodies by DIF and circulating anti-Dsg 1-3 autoantibodies by ELISA.

**Conclusions**

The lacking of a genetic predisposition might explain the presence of acantholysis only, with no clinical signs of pemphigus.

The association of acantholysis with intermediary basal cell carcinoma is intriguing. In fact, a similar case was reported in the literature,\(^12\) concerning the finding of an intermediate basal cell carcinoma adjacent to suprabasal acantholytic clefts, where acantholysis was linked with the use of another antihypertensive drug (enalapril).

We hypothesize that, in the case presented, besides nifedipine, additional factors might have influenced skin susceptibility to acantholysis, such as a locally immunodepression (locus minoris resistentiae) and antigen subtle changes on keratinocyte cell membrane induced by the presence of the near basal cell carcinoma. In fact, our case displayed acantholytic changes only in the skin area adjacent to the basal cell carcinoma, whereas no morphological changes were detectable in other cutaneous sites.

Further clinical observations and investigations are needed to completely elucidate the pathogenic mechanisms of induced acantholysis.

**References**

Mohs surgery is a surgical and anatomo-pathological technique that permits complete eradication of malignant skin tumors, namely basal cell carcinomas, with an extensive local growth and without metastatic diffusion. This is obtained through an examination of the whole perimeter thanks to horizontal sections of the specimen after careful mapping. The practical procedure is described and the possible variants of Tübingen method and the delayed technique are discussed. Indications and limits of this technique are discussed with some clinical cases. Finally, considerations are made about the increasing need of this technique due to spreading inappropriate nonsurgical treatments for basal cells carcinomas. Much more clinical centers in Italy are needed in the future that can use Mohs surgery.

**KEY WORDS:** Mohs surgery - Basal cell carcinoma - Tübingen method - Delayed technique.

Mohs surgery (MS) or microscopic controlled surgery is a particular technique with peculiar executive and histopathologic modalities. This method, in fact, is the only one able to eradicate malignant skin tumors, without metastatic diffusion (namely basal cell carcinomas) with an extensive local growth, with a percentage of eradication higher than with conventional surgical techniques (from 97% to 99%) and a saving of healthy tissue otherwise not obtainable.

**Discussion**

At the beginning, MS, conceived by F. Mohs in 1930, provided tumoral tissue chemical fixation *in vivo* followed by excision for histologic examination, after mapping. Histologic examination was executed with horizontal cutting plans. Difficulties in the management of zinc chloride’s caustic effects in some weak anatomic sides, the pain after its application and, in particular, the evolution of cryostatic techniques, permitted the passage to the fresh tissue techniques, now used.

MS, often used in the United States, especially in private clinics, probably has not been sufficiently understood in Europe. This poor interest is due to technical, economical and logistic reasons as much as to its high costs. Moreover, the lack in specialised medical and technical staffs, the inadequate organisation between surgical and anatomo-pathological phases (that must be simultaneous and tightly binded) and the ignorance of best oncological treatments in dermatology lead to
undervalue the potential aggressiveness of basal cell carcinoma.³

All those behaviours lead to wrong treatments and increasing relapsing lesions that will need MS for complete eradication.⁴

Today in USA private practice, the technique is often used mainly for small lesions and the dermatologist is the only protagonist including the histologic examination. In Italy and in other European countries, because of sanitary laws, histological examination must be done only by anatomo-pathology specialists. So, a tight collaboration between these specialists and the dermatologists is needed.

In Italy, the only public centre practicing MS regularly is the Dermatologic Clinic of Novara. In our hospital this treatment has been used for over 15 years with more and more frequency, increasing to a hundred of cases every year, with a total number of more than 900 surgical treatments (regularly registered) with the collaboration between us and the Anatomo-Pathology Unit.

This outcome, obtained with the improvement of procedure, technical and human resources, led to an increasing number of patients coming from all Italian regions due to the diffusion of information about our activity.

The great number of patients causes a long waiting time, so we hope that more clinics in the National Health System will be able to perform MS in the future.

The best solution could be the establishment of more centres, at least one in the center and one in the South of Italy, in order to avoid too much expenses travels for patients needing MS.

**Execution principles and method**

MS technique is based on the observation that the tridimensional development of neoplasia is often irregular and unpredictable, if it is based only on its clinical macroscopic aspect.⁵, ⁶

In these conditions, surgical respect limits can be excessive in some points but insufficient in others. Neoplastic topography can not be solved by normal diagnostic instruments used in vivo (for example cutaneous echography): microscopic dimensions of the irregular limits of some neoplasias need, necessarily, a histologic examination.

Complete eradication can be obtained by the observation of all the boundaries of surgical sample. This is not guaranteed using normal histological sections because of randomised vertical and perpendicular cuts normally used (Figure 1).

Complete perimeter examination, obtainable by sectioning vertically all the sample, does not permit localization of neoplasia persistence in vivo.

The innovation of MS was to perform horizontal sections of the sample maintaining orientation during histopahologic phases permitting the correlation with the correspondent point mapped on the surgical wound.⁷, ⁸

Thus, each histologic point corresponds to a surgical wound point in vivo (Figures 2, 3). Practical application of this technique is characterized by different phases.⁹-¹¹

A) Mapping of the lesion in vivo: the observational examination permits to identify the clear or suspected clinical limits of the neoplasia. Then, the surgical limits should be defined by using a dermographic pen, drawing them a few millimetres from clinical edges (normally a 1-2 mm distance is used) (Figure 4). This aim of this minimal distance is to save the majority of normal tissue as possible (especially in some esthetic regions such as face) and it can be done thanks to histologic support. The behaviour in conventional surgery is different because of the wider security limits requested.

After perimeter delimitation, the main axes of the area can be drawn, with dermographic pen, usually vertical and horizontal, obtaining 4 quadrants.
If the area of each quadrant is greater than 1 cm, it would be better to perform other subdivisions following a quadrangular network.

B) Mapping on documentation support: the picture obtained should be drawn schematically on a paper that will be put into clinical diary as a document used for the following procedures and for carrying the sample to the histological examination (Figure 5).

Documentation can be taken also with a camera or polaroid for the same purpose.

It is then possible to proceed to numbering the projected pieces and draw the colour of the margins.

C) Local anesthesia by infiltration or regional (if there are great lesions).

D) “Bowl shaped” cut along the perimeter using a No. 5 blade lancet. Before the complete excision of the lesion from its seat, make incisions along the lines previously drawn by dermographic pen, being careful to create the marks also on the surrounding skin, for an easy orientation in the subsequent phases (Figure 6).

E) Dressing of surgical wound is done after a careful hemostasis, waiting for histological examination.

F) The specimen, carefully oriented, is placed on the mapped supporting paper and then cut as previously planned. Each piece is numbered (tidily arranged on the drawn plan) and each edge is pictured by a different colour in order to obtain a perfect orientation (Figure 7).

Special colours, freezing and histology-colouring resistant, are used to paint specimens.

G) Specimens are then sent to the Pathology Unit on their paper support. Here, they are inverted (bottom up) and put in special containers and frozen. Histological cryostatic sections, are stained with hematoxylin and eosin. The first sections will be representative of deeper situation (also of the edges).

The first sections will mainly give a picture of the deep situation and the precise detection of the possible persistence of tumor on the bottom of the operating rubble.

The following sections allow to observe the whole perimeter of the lesion showing where the tumor reaches the colored margins (Figure 2).

The specimens are completely cut horizontally from the bottom to the epidermic surface.12
H) After examination of all pieces sections, the pathologist marks on the attached paper-map the points in which the tumor reaches bottom or sides, and sends back the drawing to the surgeon.

I) The drawing allows to establish in which exact points it is necessary to widen the excision. Any new removal is 4-5 mm wide from the margin (Figure 8). A new map is drawn showing the correspondent increases, the numbering of pieces (continuing from the previous ones) and the plan of coloration of the margins of new pieces removed.

L) New pieces are carefully laid down on the prepared map sheet with the relevant numeration and then the margins are painted (Figure 9). In order to maintain orientation and due to irregular shapes of the new specimens, only the margin of the external cut is marked using different colours.

M) The pathologist proceeds to new frozen sections from the depth towards the surface and to microscopical examination.

N) The procedure is repeated till the external margins are negative for neoplasm.  

O) The most suitable dermosurgical reconstruction is chosen (direct suture, flaps or graft).

**Different techniques**

**Method of Tübingen**

When the specimen is of remarkable dimensions in extension and thickness, the conventional technique is difficult to perform due to the very high number of sections needed. In order to overcome such a disadvantage, a variation in the technique has been proposed by the University of Tübingen, allowing the same precise indication on margins and depth with a limited number of sections. This technique, also named “the cake method”, has the entire piece horizontally cut only in the lower layer obtaining, therefore, indications of the invasion in depth with the examination of the first sections. The margins are cut tangentially in a vertical way in order to have a clear view of the possible peripheral persistence of neoplastic expansions (Figure 10).
Figure 6.—Surgical gap after removal of the lesion. The radial cutaneous incisions are made in correspondence to the division of the removed fragments.

Figure 7.—Fragments of the specimen are positioned on a paper sheet with the map having cure to maintain the correct guideline and coloring the margins in differentiated way.

Figure 8.—Plan of increase in 2 fields on histologic indication.

Figure 9.—Positioning of pieces of increase on map with the coloration of the margins.
The “deferred” method

Another possible variation of the technique is the so-called “deferred Mohs”. This option is an expedient due to the technical impossibility to obtain from the pathologist reports in short times or in the same day. In this case, the technique is the same as the one of the traditional MS. Numbered and coloured pieces are normally fixed and examined in some days (but with the typical horizontal sections of Mohs).

The main disadvantage of this method is represented by the necessity to maintain open the surgical gap for more days waiting for the pathologist’s response. The formation of granulation tissue makes the reading of possible new cuts more difficult and uncertain. Moreover, remarkable expansions of operating times (logistics and surgical) give to this method a second choice position.

Indications for Mohs micrographic surgery

As we have seen, MS has great costs in terms of human and economic expenses: an accurate selection of treatable cases is, therefore, needed in public health structures.

The choice criteria will first of all take care of the probability for a determined tumor to overcome the clinical limits, of particular anatomical locations where maximum saving of tissue is needed, and of the possible recurrence of some types of tumors, for instance:

- a. sclerodermiform basal cell carcinomas often have badly defined limits and irregular and unpredictable radial extension;
- b. nodular basal cell carcinomas can infiltrate underlying structures and muscular layers;
- c. also other neoplasms with unpredictable infiltrating modality of expansion (i.e. dermatofibrosarcoma protuberans and extramammary Paget) can take benefit from this technique. Particularly dermatofibrosarcoma, usually needing very large precautionary demolitions is better cured with this method also thanks to histochemical help to detect any neoplastic fragment left;
- d. delicate and esthetic sites of the face need the maximum saving of healthy tissue (i.e. periorificial regions, nose, eye, etc.), but also other critical regions can take benefit from this technique (for instance external genitalia);
- e. recurrent tumors (in great part due to previous incomplete excisions or wrong treatments without histopathologic examination) often show an anomalous spreading modality along the previous scars or along anatomical plans in depth and are scarcely valuable only on clinical examination. Only MS allows an exact ablation.

Limits of Mohs surgery

The limits of MS are represented by the impossibility to remove with reasonable certainty all the tumor when it reaches layers of other tissues or organs that cannot be removed, oriented and examined under frozen sections (like in case of invasion of bones, deep organs, nervous structures, nasal and oral cavities, eye etc.).

Another limit is represented by tumors with tendency to metastasize (i.e. squamous cell carcinoma, melanoma, merkelomas, sarcomas etc.)

Conclusions

In conclusion, we hope that a greater awareness of necessity and importance of radical excisions in dermatological oncology will spread in dermatologist’s practice. We confirm the necessity of an important place for MS, also with its limits, in the National Health System and in dermosurgical practice.

This occurrence will be able to reduce remarkably
the morbidity and mortality still observed for basal cell carcinomas. Meanwhile, any effort should be made not only in oncologic prevention campaigns but also in the sensibilization of the Sanitary Administrations to demonstrate the social benefits of this technique of treatment in spite of its great economic engagements.

**Riassunto**

**Chirurgia di Mohs oggi in Italia**

La chirurgia di Mohs è una tecnica chirurgica in grado di eradicare neoplasie maligne cutanee non metastatizzanti in fase di crescita estensiva locale. I principi su cui essa si basa sono: l’esame di tutto il perimetro dell’exeresi mediante sezioni orizzontali del pezzo asportato e la mappatura precisa del pezzo stesso. Vengono descritte in dettaglio le procedure adottate nell’esecuzione pratica con il supporto di alcuni casi esemplificativi e le sue possibili varianti tecniche (metodo di Tübingen e metodica “differita”). Vengono discussi le indicazioni e i limiti all’applicazione della chirurgia di Mohs con considerazioni sui campi di impiego anche al di fuori delle indicazioni classiche. Si considerano, infine, i possibili sviluppi dell’applicazione della metodica con una sempre più ampia richiesta di utilizzo a causa della diffusione di trattamenti non chirurgici per gli epiteliomi e, quindi, la necessità di aumentare in Italia il numero di centri che praticano la chirurgia di Mohs.

**Parole chiave:** Chirurgia di Mohs - Carcinoma basocellulare - Metodica di Tübingen - tecnica di Mohs differita.

**References**

Regression induced by highly active antiretroviral therapy of a psoriasiform dermatitis revealing HIV-infection

A. VIRGILI, E. ALTIERI, M. M. LAURIOLA, M. CORAZZA
Section of Dermatology, Department of Clinical and Experimental Medicine, University of Ferrara, Ferrara, Italy.

Dear Sir,

A 54 year-old man presented a non-itchy dermatitis characterized by erythematous scaly papules and plaques on the limbs (Figure 1). The dermatitis, starting since 3 months, was unresponsive to topical steroids.

During the previous year, the patient was admitted to the Hematology Center for acute thrombocytopenia (11 000 PLT mm⁻³) and was treated with HAART for HIV infection (CD4+ 28 mm⁻³ and CD4/CD8 0.08). After 1 month of therapy, CD4+ increased to 66 mm⁻³, CD4/CD8 to 0.08, viremia (HIV-RNA/mL) decreased from >100 000 to 1 880 copies/mL and PLT count increased to 171 000 mm⁻³.

Table I.—Modification of hematological tests during follow-up.

<table>
<thead>
<tr>
<th>Test</th>
<th>Before HAART</th>
<th>After 1 month of therapy</th>
<th>After 3 months</th>
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<tbody>
<tr>
<td>CD4+ (mm⁻³)</td>
<td>28</td>
<td>85</td>
<td>66</td>
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<tr>
<td>CD4/CD8</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viremia (mL)</td>
<td>&gt;100 000</td>
<td>1 880</td>
<td>&lt;50</td>
</tr>
<tr>
<td>PLT (mm⁻³)</td>
<td>11 000</td>
<td>77 000</td>
<td>171 000</td>
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The paper has already been presented at the 1° Congresso Regionale Dermatologi Emiliano-Romagnoli. Bologna, March 20, 2004.

Table II.—Histopathology of HIV-associated psoriasis.

<table>
<thead>
<tr>
<th>Classic form</th>
<th>Psoriasiform dermatitis</th>
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<tbody>
<tr>
<td>Parakeratosis</td>
<td>Focal parakeratosis</td>
</tr>
<tr>
<td>Regular acanthosis</td>
<td>Irregular acanthosis</td>
</tr>
<tr>
<td>Neutrophils within the horny layer (Munro microabscesses)</td>
<td>Fewer Munro microabscesses</td>
</tr>
<tr>
<td>Spongiform pustules of Kogoj</td>
<td></td>
</tr>
<tr>
<td>Absence of the granular layer (agranulosis)</td>
<td></td>
</tr>
<tr>
<td>Elongation of dermal papillae</td>
<td></td>
</tr>
<tr>
<td>Perivascular infiltrate (lymphocytes, histiocytes, neutrophils) in the upper dermis</td>
<td>Perivascular and diffuse lymphocytic infiltrate with occasional macrophages and multinucleated giant cells</td>
</tr>
</tbody>
</table>

Figure 1.—Psoriasiform dermatitis of the thigh in HIV-positive patient. Figure 2.—Skin biopsy showing focal parakeratosis and acanthosis, perivascular infiltrate of lymphocytes, neutrophils and plasma cells in the upper dermis. HE-20x.
Two histopathological patterns of HIV-associated psoriasis are recognized: the classic form and a so called psoriasiform dermatitis (Table II). Some authors underline that lymphocytolysis, dermal cellular infiltrate, mainly CD8+ lymphocytes and macrophages, relative decrease of T lymphocytes and increase of plasma cells within the inflammatory infiltrate have been considered peculiar histological aspects of HIV-related psoriasis.

HIV infection induces CD4+ T-cell depletion, nevertheless in cutaneous inflammatory infiltrates of psoriasis reactive B cells, namely plasma cells, are increased. In fact, in patients with acquired immunodeficiency there is a polyclonal B cell expansion and an expression of plasma cells in peripheral tissues with unknown functional significance.

Therefore, the presence of numerous plasma cells within the biopsy of a psoriatic lesion may indicate an underlying HIV infection.

In our case, the histological features and the presence of plasma cells in the dermal infiltrate were suggestive of psoriasiform dermatitis.

In the therapy of HIV-related papulosquamous disorders, topical drugs (steroids, tars and dithranol) are unsatisfactory and systemic treatments are debated (UVB, PUVA) or even dangerous (immunosuppressive drugs). In addition to inhibition of the HIV reverse transcriptase, AZT also interferes with DNA synthesis repressing keratinocyte proliferation. This antipsoriatic effect is dose-dependent, long-lasting, though long-term relapses may occur.

In our case, thrombocytopenia was correlated with HIV infection and underwent an evident improvement after the beginning of HAART. Thrombocytopenia can be seen in 60% of the HIV-positive patients. Its pathogenesis is debated; in addition to an autoimmune hypothesis, a direct viral action can be supposed on the megakaryocytopenosis.

Thrombocytopenia, often unrecognized, should be considered a clue to suspect HIV infection, especially when associated with other signs.

In conclusion, recognition of an associated skin disease may lead to early HIV diagnosis, thereby reducing the risk of transmission, initiating appropriate antiviral therapy and prolonging the disease-free interval prior to the development of AIDS.

References
Chronic hypertrophic perianal herpes in HIV-infected individuals

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Dear Editor,

Genital herpes is particularly frequent in HIV-1 infected individuals, showing rather an ulcerative than vesicular or erosive aspect.1 Less frequently, it can assume a combined aspect, both ulcerative and vegetant.2 During these last years, we have seen 4 HIV-infected individuals presenting under this last appearance (Figure 1). They were all men who had sex with men (MSM) and were naïve for high active antiretroviral therapy (HAART), in spite of a long time from HIV-1 disease diagnosis. In all the subjects the lesions were localized in the perianal region. Immunological data showed a lymphocyte CD4+ count that ranged from the lowest values of 54/mm³ of a subject, to the highest ones of 384/mm³ of another, while the HIV-RNA viral load varied from 27 900 copies/mL to 94 700 copies/mm³ (Table I). To exclude condylomata lata, external genital warts, mycobacteriosis, chancroid, herpetics lesions or neoplasms, we performed a panel of examinations and a biopsy.3 In the first group, only the Tzanck test resulted positive in all the patients.4 The histological examination revealed in all cases an ulcerated lesion with a chronic telangiectatic tissue of granulation and a large number of plasma cells and eosinophils; at the borders of the ulcer, multinucleated keratinocytes were observed with greyish nuclei and a margined chromatin, within a hyperplastic epidermis (Figure 2). In 2 subjects, the lesions showed a marked pseudoepitheliomatous hyperplasia of the epidermis. Finally, a dense infiltrate mainly plasmacellular was often seen throughout the dermis. The immunohistochemical examination revealed polyclonality for the k and λ

Figure 1.—Patient P.R. Ulcerative and vegetant lesions in the perianal area.

Figure 2.—Patient P.R. A partially eroded epidermis, with a dense plasmacellular infiltrate in the dermis (EE 100×).
light chains of the plasmacytoid cells. Lastly, all 4 patients showed a poor response to oral acyclovir, as reported in the literature (5% of HIV-infected persons), so they underwent a treatment with acyclovir i.v. (5 mg/kg/every 8 h daily) combined with HAART, with a partial response.\(^5\)

In conclusion, the presence of nodular or nodulo-ulcerated chronic lesions in the perianal or genital region in HIV-positive patients should lead the clinicians to exclude the herpetic aetiology and differently, they should invite the patients with an unknown HIV serostatus presenting similar lesions to perform a HIV test.

References


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<table>
<thead>
<tr>
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<th>Sex</th>
<th>Age</th>
<th>Risk</th>
<th>No. of lesions</th>
<th>Duration of symptoms (months)</th>
<th>CD4/mm(^3)</th>
<th>HIV-1 RNA c/mL</th>
<th>HAART</th>
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<tr>
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<td>M</td>
<td>60</td>
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