The blood supply of the epidermis in psoriasis

Rosina et al. provide a reminder of the characteristic pattern of the capillary bed in psoriasis. It is true that capillary microscopy was originally focused on the nail bed, but, when I reviewed this topic in 1969 and 1970, there was already a substantial use of long working distance lenses and the whole body was frequently examined and, already too, TV cameras were being attached. We knew the capillary shape described by Rosina et al. and were impressed, as they are by its uniformity in psoriasis. Holti in 1964 studied this extensively. We noted this uniformity only in the typical clinical lesion. It was not observed at the edge of an evolving lesion, nor in the early phases of generalized pustular psoriasis. Sometimes chronic lichenification, mycoses fungoides, or vascular naevi could have a low power field identical in appearance but not consistently. Of course, the nailfold continues to be the site of study even with videomicroscopy described diminution of density and dimensions in psoriasis which they ascribed to injury.

Many, having noticed that the unaffected skin had higher rates of blood flow, looked to see if normal skin had more capillaries with “psoriatic” morphology. We emphasized that, of course, we were looking at blood columns and not at the invisible capillary wall. I noted that resolving psoriasis had capillaries empty of blood but on obstructing their emptying they would fill and the characteristic pattern could be observed. The rate of complete resolution was faster in the upper part of the body and especially slow in the legs. This explains in part why studies of blood flow in resolving plaques is less informative than morphology. The development of the pattern could be inhibited by local pressure or adrenaline and the epidermal changes could also be inhibited. All of this and others work is reviewed in Ryan. In this 1980 text there is a hypothesis which may have become more relevant and that is that the psoriasis is demanding more oxygen than is consistently supplied by this pattern of blood supply; even though the basal flux in the plaque exceeds maximum flux in normal skin. Consequently it is a condition that suffers from hypoxic injury. Those interested in the behaviour of the epidermis with respect to growth factors like vascular endothelial growth factor (VEGF) might reconsider this as might the antioxidant evangelists. It is not too different to the discussion by Ryan et al. in this Journal of the paper by Procaccini et al.

One of the issues is the Koebner phenomenon and

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vulnerability. Psoriasis and its characteristic capillary are found to develop at sites of injury nearest an existing plaque. The vasculature of psoriasis is vulnerable in a way that normal blood supply is not. Slight trauma causes bleeding (Auspitz'sign) and close inspection with in vivo microscopy shows that thrombosis is as common as bleeding. This led to studies of fibrinolysis in psoriasis. It was pointed out that the neutrophils that are such a feature of psoriasis are inhibitory of fibrinolysis but demanding of oxygen. We were less concerned about dendritic cells and lymphocytes in those days, but, of course, any activated system needs oxygen. This is a balance between supply and demand and our point is that the epidermis in psoriasis coupled with neutrophils takes all Hern et al. postulate that the capillary bed takes only 2-10% of the skin’s blood supply but that the flow through the deeper dermis in psoriasis is increased some 10-13 fold. Sympathetic and local vasoconstriction is intact in psoriasis plaques. Further vasodilation above the high normal is also possible.

There have been several studies of new thermolytic devices (lasers) to destroy only the most superficial blood vessels, reviewed and studied by Hern et al. They refine and quantify what we attempted 30 years earlier but hardly give us more understanding. It is partially a cure for psoriasis but alters total skin blood flow very little.

Endothelial proliferation is normally infrequent and an increase is confined to the ascending limb of the capillary in psoriasis, which, as shown by Braverman and Yen, are arteriolar and should have sufficient oxygen needed for mitosis. The venous arm may well behave differently, and like all the venous system be well equipped to manage slow flow. The role of VEGF and its form as a permeability factor, its stimulus by leukocytes that are such a feature of psoriasis are inhibitory of fibrinolysis but demanding of oxygen. We were less concerned about dendritic cells and lymphocytes in those days, but, of course, any activated system needs oxygen. This is a balance between supply and demand and our point is that the epidermis in psoriasis coupled with neutrophils takes all Hern et al. postulate that the capillary bed takes only 2-10% of the skin’s blood supply but that the flow through the deeper dermis in psoriasis is increased some 10-13 fold. Sympathetic and local vasoconstriction is intact in psoriasis plaques. Further vasodilation above the high normal is also possible.

References

Systemic antimycotic treatment for children
State of the art

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In this issue of the Giornale Italiano di Dermatologia e Venereologia, Pau et al. publish an elegant study on the efficacy and tolerability of oral terbinafine in pediatrics patients affected by tinea capitis and tinea corporis due to tricophyton mentagrophytes and microsporum canis.1 Their conclusion is that terbinafine is a highly effective drug for the treatment of cutaneous fungal infections in children but regarding tinea capitis caused by m. canis additional studies are needed to better standardize dosage and treatment cycles. Terbinafine is an allylamine that was discovered in 1974.2 Oral terbinafine was first approved for use in the United Kingdom in February 1991, in Canada May 1993 and in the United States in May 1996.3 When terbinafine is administered orally, 70% to 80% of the dose is absorbed, with the bioavailability not being significantly affected by food intake, after a single terbinafine dose maximal plasma concentrations are achieved within 2 h, steady-state levels are reached after 10 to 14 days,4 high concentrations of terbinafine are found in the stratum corneum, sebum and hair. When terbinafine is administered orally, the drug has been detected in the stratum corneum as early as 24 h after commencing therapy.5

Terbinafine is first detected in the deeper layers of the stratum corneum and probably reaches this site by epidermal diffusion. In the hair it has been detected within 1 week of starting therapy. The early detection in hair may be due to delivery of the drug to hair via the sebum, the drug may become incorporated into hair by hair matrix cells. When terbinafine is administered for 14 days, the drug has been detected in hair for at least 50 days. As in Pau et al.’s results it should be noted that terbinafine may be more effective against tinea capitis caused by endothrix organisms such as trichophyton mentagrophytes compared with ectotrix infections like m. canis.

In our experience at the Department of Dermatology and Cutaneous Surgery at the L. Miller School of Medicine, private and indigent Pediatrics Dermatology Clinics oral terbinafine is considered a second line agent. While extensive safety and efficacy studies are acknowledged, the use of oral terbinafine in children is “off label”. Further package inserts state that the safety and efficacy of lamisil have not been established in pediatric patients.

In the United States trichophyton tonsurans is the most prevalent organism. Treatment is usually initiated with griseofulvin at 20-25 mg per kg per day in 1 or 2 doses. It is routinely given with milk or a fatty
meal for duration of 6 weeks. Keratolytics and/or antifungal shampoos are often an adjunct therapy. The success rate of such treatment is usually highly satisfactory. In those instances where griseofulvin is counter indicated, ineffective or associated with significant side effects, then terbinafine becomes an off label option. The usual doses are 62.5 mg per day for children less than 20 kg; 125 mg per day for 20-40 kg and 250 mg per day for those greater than 40 mg.

It was very interesting to read Pau et al. personal experience with 50 patients in the attempt to address the best terbinafine dose and duration regimen to achieve a complete cure and lack of recurrence of the cutaneous and hair pediatrics dermatomycoses. Their effort follows the ones of Lipozencic in the 2002 and of Gupta in the 2003. The issue of dermatomycoses treatment in children was recently reproposed by Roberts and Friedlander from the Children’s Hospital and Health Center and University of California San Diego Medical Center, San Diego, USA with their article in Pediatrics Annals addressing the need of a safe and efficacious short-term treatment to replace or be an alternative to the standard griseofulvin approach. We are pleased to notice that Pau et al. article successfully follows the attempt to establish a safe and efficacious novel treatment for the dermatomycoses in children.

References

Cytokeratin profile as a clue to origin and differentiation in cutaneous squamous cell carcinoma

E. ALESSI 1, D. FANONI 1, E. BERTI 2

Aim. Several types of squamous cell carcinoma (SCC) of the skin are recognized, even if SCCs in situ such as Bowenoid actinic keratosis and Bowen’s disease are histopathologically almost indistinguishable and the existence of entities such as trichilemmal carcinoma is still discussed. The aim of this study was to verify if the determination of the cytokeratin (CK) profile could be useful to better characterize some cutaneous SCCs.

Methods. We studied CK1, 5, 6, 7, 8, 10, 14, 15, 16, 17, 18 and 19 expression in normal epithelial structures of the skin and in Bowenoid actinic keratosis, Bowen’s disease, Bowen’s carcinoma, trichilemmal carcinoma, keratoacanthomatous SCC, acantholytic SCC, conventional cutaneous SCC and malignant proliferating onycholemmal cyst.

Results. The most significant findings were: (a) CK17 positivity in lower follicle; (b) focal positivity for CK19 in the basal layer of the outer root sheath near the bulge; (c) CK7 positivity with CK19 negativity in the sebaceous lobules; (d) CK19 positivity in the secretory portion of both apocrine and eccrine sweat glands; (e) CK10 negativity with CK17 positivity in the nail matrix and nail bed; (f) focal expression for CK19 in several cases of Bowen’s disease in contrast with its constant negativity in Bowenoid actinic keratosis; (g) the almost superimposable pattern of CK expression in Bowen’s disease, Bowen’s carcinoma and trichilemmal carcinoma; (h) the CK10 and CK17 positivity in keratoacanthomatous SCC; (i) the lack of CK10 expression in acantholytic SCC, conventional SCC and malignant proliferating onycholemmal cyst.

Conclusion. The study was in favour of a common origin of Bowen’s disease, Bowen’s carcinoma and trichilemmal carcinoma from pluripotential cells of the acrothrichium and of keratoacanthomatous SCC from the lower follicle. It also showed that a strong and diffuse positivity for CK17 may be useful to differentiate keratoacanthomatous SCC from trichilemmal carcinoma in doubtful cases and that the search for CK10 expression may be useful to distinguish between well differentiated and poorly differentiated SCCs of the skin. This last statement is not true for onycholemmal carcinomas because CK10 is negative in the normal nail matrix and nail bed.

Key Words: Cytokeratin - Skin - Epithelia - Skin neoplasms.

Cytokeratins (CK) are the major structural proteins of epithelial cells, including keratinocytes.1 They form a complex family of at least 30 polypeptides, which are distinguishable from one another on the basis of molecular weight and are numerically classified as such.2 The CK profile of an individual cell is dependent on the epithelial cell type, the differentiation program and the rate at which the tissue cells are turning over.3 CKs are usually conserved during malignant transformation, so that their identification can help to establish the origin of the malignancy.4

CKs may be detected immunohistochemically and the recent generation of a number of highly specific monoclonal antibodies against single CKs, that work
on formalin-fixed, paraffin-embedded tissue, prompted us to study the pattern of CK expression in normal epithelial structures and some squamous cell carcinomas (SCC) of the skin.

Our purpose was to establish if this determination could be useful to better characterize the origin and the degree of differentiation of the tumors studied.

Materials and methods

Material

We first determined CK1, 5, 6, 7, 8, 10, 14, 15, 16, 17, 18 and 19 expression in normal epithelial structures of the skin from 3 biopsies performed on the scalp, 2 on the trunk, 1 on the axilla and 1 on the nail unit. We then examined: (a) 12 cases of SCC in situ, 5 of which localized in sun-exposed areas and classified as Bowenoid actinic keratosis on the basis of the presence of solar elastosis and sparing of skin appendages; and the other 7 classified as Bowen’s disease on the basis of their location in areas unexposed to sun and/or involvement of the pilar infundibulum; (b) 2 cases of invasive carcinoma composed of islands of squamoid and basa-loid cells without horny pearl formation classified as Bowen’s carcinoma; (c) 7 cases of invasive carcinoma showing foci of cytologically atypical clear cells and/or foci of pilar-type keratinization collected during the last 13 years and classified as trichilemmal carcinoma; (d) 3 cases of raised verrucous epithelial tumors showing a central keratin-filled crater and peripheral squamoid proliferation classified as keratoacanthomatous SCC; (e) 2 cases of invasive carcinoma showing foci of cytologically atypical clear cells and/or foci of pilar-type keratinization collected during the last 13 years and classified as trichilemmal carcinoma; (f) 6 cases of invasive growths from the epidermis consisting of irregular masses of atypical epidermal cells, focally keratinising in the form of horny pearls, that proliferate downward into the dermis, classified as malignant proliferating onycholemmal cyst.

Clinical data are reported in Table I. Reagents used are reported in Table II.

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Ab: antibody; CK: cytokeratin; Dako: Dako Cytomation, Productionsovej 42, DK-2000 Glostrup, Denmark; ACSC: Accurate Chemical Scientific Co, Westbury, NY, USA; Novo: Novocastra Laboratories LTD, D.B.A. Italia s.r.l., Segrate (MI); Sigma: Sigma-Aldrich, 3050 Spruce St., St. Louis, Mo, USA.
Method

All reactions were performed by one of us (D. F.). The method was as follows.

Tissue samples were fixed in buffered paraffin, dehydrated, embedded in paraffin wax and sectioned. After deparaffinizing and rehydrating, each tissue section was immersed in citrate buffer 0.01 M, boiled 3 times for 5 min into a pressure cooker and washed with TBS buffer according to Cattoretti et al. Then, each section was incubated with normal rabbit serum for 10 min and then with the specific monoclonal antibody overnight at +4°C, washed with TBS pH 7.6 and incubated in rabbit mouse Dako Z259 1:30 at room temperature for 1 h. After incubation with the secondary antibody (RAM), it followed a new wash with TBS pH 7.6 and incubation with the enzymatic immunocomplexes APAAP (alkaline phosphatase and monoclonal antialkaline phosphatase, Dako D651) 1:50 at room temperature according to Cordell et al. These last 2 passages (RAM and APAAP) were repeated twice, but in the second passage the APAAP dilution was 1:100 and the incubation was made at room temperature for 30 min. A new fuchsin-based solution containing naphtol AS-B, phosphate sodium salt, levamisole and Na nitrite was used as enzyme substrate. Finally, each section was counterstained in Mayer’s Hematoxylin solution and coverslipped.

Results

Cytokeratins expression pattern in normal epithelial structures of the skin

Results are reported in Table III.

In our view, the most significant findings were: (a) CK17 positivity in the deep part of the infundibulum, isthmus and, especially, in the lower portion of the hair follicle outer root sheath (Figure 1A); (b) the focal CK19 positivity in the basal layer of the isthmus and lower portion of the hair follicle outer root sheath near the bulge (Figure 1B); (c) CK7 positivity (Figure 1C) with CK19 negativity in the sebaceous lobules; (d) CK19 positivity in the secretory portion of both eccrine and apocrine sweat glands; (e) CK10 negativity with CK17 positivity (Figure 1D) in the nail matrix and nail bed.

Cytokeratins expression pattern in cutaneous squamous cell carcinomas in situ studied

Results are reported in Table IV.

The most significant findings were: (a) CK6 negativity in 4 of 5 cases of Bowenoid actinic keratosis and the almost constant CK6 expression in Bowen’s disease (Figures 2A, B); (b) the focal expression of CK19 in 5 of 7 cases of Bowen’s disease (Figures 2C, D).
Figure 1.—Suprabasal positivity for CK17 in lower follicle (A); focal positivity for CK19 in the basal layer of lower follicle near the bulge (B); positivity for CK7 in the sebaceous lobules (C); CK17 positivity in the nail bed (D).

**Table IV.**—CK expression pattern in the cutaneous squamous cell carcinomas in situ studied.

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<td></td>
<td>7</td>
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</table>

SCC: squamous cell carcinoma; BAK: bowenoid actinic keratosis; BD: Bowen’s disease; ND: not done; f: focal; *: single cell positivity.
Results are reported in Table V.

In our view, the most significant findings were: (a) the almost superimposable pattern of CK expression in Bowen’s carcinoma and trichilemmal carcinoma, that, interestingly, also showed focal CK19 positivity in the majority of cases (Figures 3A, B); (b) CK10 and 17 positivity in keratoacanthomatous SCC (Figures 3C, D); (c) CK10 negativity in acantholytic SCC, cutaneous conventional SCC and malignant proliferating onycholemmal cyst.

Discussion and conclusions

Cytookeratins expression pattern in normal epithelial structures of the skin

As reported by other authors,9, 10 the epidermis and the upper part of the infundibular epithelium can not be differentiated on the basis of their CK profile, while the deep part of the infundibulum, isthmus and lower portion of the hair follicle outer root sheath show a distinctive CK profile especially because of their positivity for CK17. The CK17 positivity, that is suprabasal, was found to be particularly evident in
lower follicle. Therefore, in our view, a strong CK17 expression in a tumor could indicate its origin from lower follicle.

Likewise, CK7 positivity with CK19 negativity in the sebaceous lobules, CK19 positivity in both apocrine and eccrine sweat glands, and CK10 negativity with CK17 positivity in the nail matrix and nail bed could be used to identify sebaceous (Figures 4A, B), apocrine-eccrine (Figures 4C, D) or onycholemmal origin of a tumour, respectively.

As regards the weak CK19 expression in the basal layer of the isthmus and lower portion of the hair follicle outer root sheath, an explanation could be the reported presence at this level of epithelial stem cells migrating from the bulge.11, 12

**Cytokeratins expression pattern in squamous cell carcinomas in situ studied**

Taking into account that: (a) bowenoid actinic keratosis and Bowen’s disease are possibly different in origin, the first one originating from epidermal keratinocytes and the second one from germinial cell of the pilar outer root sheath and the pluripotential cells of the acrotrichium;13 (b) solar-induced cancerization is worldwide accepted for bowenoid actinic keratosis, while a viral-induced cancerization has been suggested not only for anogenital Bowen’s disease but also for cases of cutaneous Bowen’s disease;14-16 (c) invasive SCC originating from bowenoid actinic keratosis is a keratinising tumour forming horny pearls in its early stage, while SCC originating from Bowen’s disease does not show horny pearl formation and occasionally shows adnexal differentiation,13, 17-19 we expected to find some clear differences in the pattern of CK expression between these 2 entities. On the contrary, only a subtle difference seems to exist, namely the almost constant positivity for CK6 with frequent weak positivity also for CK19 in Bowen’s disease, while the 5 cases of bowenoid actinic keratoses studied were negative for CK19 and only 1 of them showed weak positivity for CK6.

CK6 was found to be positive together with CK16 not only in Bowen’s disease but also in all invasive SCCs studied, both CKs having been considered as markers for hyperproliferative keratinocytes.20 Therefore, CK6 expression in 6 of 7 cases of Bowen’s disease and its lack of expression in almost all bowenoid actinic keratoses examined could be only a sign of a higher tendency to hyperproliferation in the first disorder in comparison with the second one.
The focal but frequent expression of CK19 is more interesting in order to establish the origin of Bowen’s disease considering the positivity for this CK in stem cells migrating from the bulge. A weak positivity for CK19 in cases of Bowen’s disease is not new having already been reported. Taking into account that in Bowen’s disease the positivity for CK19 is associated with positivity for CK10, while a typical marker for the lower follicle such as CK17 is negative, an acceptable hypothesis is that Bowen’s disease originates from pluripotential cells of theacrothrichium as suggested for Bowen’s carcinoma. Is this is true, the tumor could be more properly defined as acrothrichial carcinoma in situ.

Cytokeratins expression pattern in the other cutaneous squamous cell carcinomas studied

Bowen’s carcinoma and trichilemmal carcinoma showed a superimposable pattern of CK expression. This pattern was also superimposable to that of Bowen’s disease with the only exceptions of: (a) the only focal positivity for CK10 in one case of Bowen’s carcinoma and 4 cases of trichilemmal carcinoma with CK10 negativity in 1 case of trichilemmal carcinoma; (b) the focal positivity for CK17 in 2 cases of trichilemmal carcinoma. Based on these findings, we think that not only Bowen’s carcinoma but also trichilemmal carcinoma could be viewed as an evo-

Figure 3.—Trichilemmal carcinoma on the left thigh (A) and its focal positivity for CK19 (B); keratoacanthomatous SCC (C) and its strong positivity for CK17 (D).
lutive phase of Bowen’s disease. Different routes of differentiation toward pilosebaceous and apocrine unit could easily explain their different histopathologic patterns. The partial or total lack of CK10 expression in some cases of Bowen’s carcinoma and trichilemmal carcinoma was associated to poor differentiation. Both Bowen’s carcinoma and trichilemmal carcinoma are basically CK10 positive tumours, but this characteristic may be lost during sdifferentiation as already suggested for invasive malignancies.21

The study was in favour of an origin of keratoacanthomatus SCC from lower follicle because of its strong and diffuse CK17 positivity. This result is in agreement with the market histopathologic similarities between keratoacanthomatus SCC and a tumour worldwide considered to be follicular in origin such as keratoacanthoma. Interestingly, all the keratoacanthomatus SCCs studied showed also strong expression of CK10 which seemed to be a marker of differentiation.22, 23 This last statement is confirmed by the constant CK10 negativity in acanholytic SCC and cutaneous conventional SCC, which are invasive and poorly differentiated tumours.

Lastly, malignant proliferating onycholemmal cyst 24 showed positivity for CK6 and 16 and focally also for CK17 as does the normal nail bed. Interestingly CK10 was negative in spite of the high differentiation of this...
tumour because CK10 is not expressed in nail matrix and nail bed.

In conclusion, we found that the search for CK expression may be useful to better characterize the origin and the degree of differentiation of the SCCs studied. In particular: (a) the almost superimposable CK profile in Bowen’s disease, Bowen’s carcinoma and trichilemmal carcinoma with CK19 expression in the majority of cases seemed to be in favour of a common origin of these entities from pluripotential cells of the acrotrichium; (b) the strong positivity for CK17 in keratoacanthomatomatos SCC could indicate its origin from lower follicle; (c) the diffuse positivity for CK17 in relation to keratoacanthomatos SCC could indicate its origin from pluripotential cells of the acrotrichium; and trichilemmal carcinoma with CK19 expression in particular: (a) the almost superimposable CK profile in Bowen’s disease, Bowen’s carcinoma and trichilemmal carcinoma with CK19 expression in the majority of cases seemed to be in favour of a common origin of these entities from pluripotential cells of the acrotrichium; (b) the strong positivity for CK17 in keratoacanthomatos SCC could indicate its origin from lower follicle; (c) the diffuse positivity for CK17 in relation to keratoacanthomatos SCC could indicate its origin from pluripotential cells of the acrotrichium; and trichilemmal carcinoma with CK19 expression in contrast with its constant absence in keratoacanthoma.

Our results are not comparable with others of the literature because, to the best of our knowledge, no similar study in different cutaneous SCCs with a wide panel of single CKs has yet been performed.

Riassunto

OBIETTIVO. Si riconoscono numerose varianti del carcinoma spinocellulare della cute, ma carcinomi in situ come la cheratosi solare Bowenoid e il morbo di Bowen sono istologicamente quasi indistinguibili e l’effettiva esistenza del carcinoma trichilemmale risulta ancora discussa. Lo scopo di questo lavoro era verificare se la determinazione del profilo citocheratinico in alcuni carcinomi cutanei potesse essere utile per caratterizzarli meglio.

METODI. È stata studiata l’espressione delle citocheratine (CK) 1, 5, 6, 7, 8, 10, 14, 15, 16, 17, 18 e 19 in primo luogo nelle strutture epiteliali normali della cute e successivamente nella cheratosi solare Bowenoid, nel morbo di Bowen, nella monomorbo di Bowen, nel carcinoma di Bowen, nel carcinoma trichilemmale, nel carcinoma spinocellulare cheratoacantomatoso, nel carcinoma spinocellulare acantolitico, nel carcinoma spinocellulare cutaneo convenzionale e nella cisti onicolemmale proliferante maligna.

RISULTATI. I risultati maggiormente significativi sono stati di: (a) espressione della CK17 nella guaina epiteliale esterna in sede soprabasale, particolarmente intensa nella parte profonda del follicolo; (b) focale espressione in sede basale della CK19 in vicinanza del punto di attacco del muscolo eretore del pelo; (c) espressione della CK7 con mancato riscontro della CK19 nei lobuli sebacei; (d) espressione della CK19 nei glomeruli sudoripari sia eccrini sia apocrini; (e) espressione della CK10 con mancato riscontro della CK17 nella matrice e nel letto ungueale; (f) focale espressione della CK19 in numerosi casi di morbo di Bowen in contrasto con il suo costante mancato riscontro nella cheratosi solare Bowenoid; (g) uno profilo citocheratinico quasi sovrapponibile nel morbo di Bowen, nel carcinoma di Bowen e nel carcinoma trichilemmale; (h) espressione sia della CK10 sia della CK17 nel carcinoma spinocellulare cheratoacantomatoso; (i) mancata espressione della CK10 nel carcinoma spinocellulare acantolitico, nel carcinoma spinocellulare cutaneo convenzionale e nella cisti onicolemmale proliferante maligna.

CONCLUSIONI. I risultati dell’indagine sono a favore di una possibile origine comune del morbo di Bowen, del carcinoma di Bowen e del carcinoma trichilemmale da cellulare epiteliale pluripotente dell’acrotrichio e del carcinoma spinocellulare cheratoacantomatoso dalla porzione profonda del follicolo. Lo studio ha anche evidenziato che un’intensa e diffusa espressione della CK17 risulta nei casi dubbi a favore del carcinoma spinocellulare cheratoacantomatoso rispetto al carcinoma trichilemmale e che la ricerca dell’espressione della CK10 appare utile per distinguere i carcinomi spinocellulari ben differenziati da quelli con scarsa differenziazione, salvo nei carcinomi onicolemmali, essendo la CK10 negativa nella matrice e nel letto ungueale normali.

Parole chiave: Citocheratine - Epiteli - Cute - Cute, neoplasie.

References

NM23 protein expression in primary melanoma correlates with disease-free interval and with survival: a ten-year follow-up study

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Aim. nm23 is considered a metastasis suppressor gene for some human cancers. Decreased expression of nm23 mRNA was found in several metastases of melanoma, and such a lower expression in metastases correlated to the patient’s shortened survival. Since the prognostic role of the expression of NM23 protein in primary melanoma is controversial, the aim of this study was to determine the utility of NM23 protein as an immunohistochemical indicator of prognosis in patients affected with primary melanoma.

Methods. The expression of NM23 protein was analysed in 30 primary skin melanomas. NM23 protein was detected on paraffin sections using an immunoperoxidase technique. The follow-up period included evaluation of the disease-free interval at 5 and 10 years and of the survival outcome at 5 and 10 years.

Results. We found that: 1) expression of NM23 protein correlated positively with disease-free interval within 5 years (P<0.01) and 10 years (P<0.01); 2) expression of NM23 protein correlated positively with survival within 5 years (P<0.01) and 10 years (P<0.01).

Conclusion. In this study, NM23 protein correlates inversely with melanoma progression. However, clinical trials including larger numbers of patients should be performed to confirm the favourable prognostic role of NM23 in melanoma.

Key Words: Melanoma- NM23 protein- Survival- Metastases- Prognosis.

The incidence of melanoma is increasing at a rate greater than that of any other malignancy.1 Over the past 30 years, the identification of more accurate predictive factors has led to multiple modifications of the American Joint Committee on Cancer (AJCC) staging system for melanoma.2-5 The most widely used morphological prognostic indicators, such as melanoma thickness and ulceration,5 give consistent information about survival outcome when applied to groups, but they may not have ideal predictive value in the individual case, because a subgroup of thin melanomas metastasize at a relatively early stage in their development.6 A constant research of new prognostic parameters is, therefore, very important to define a correct prognosis.

The nm23 gene, discovered in 1988 by Steeg et al., is a putative metastasis-suppressor gene.7 In fact, low levels of nm23 RNA 8, 9 or NM23 protein 10-14 have been correlated with poor prognosis in breast cancer patients. In addition, low levels of nm23 RNA or NM23 protein expression have also been correlated with metastasis in hepatocellular carcinoma,15 diffuse large B cell lymphoma,16 lung cancer,17 nonsmall cell lung
cancer, gastrointestinal stromal tumors, oral squamous cell carcinoma. However, no similar inverse relationship with metastatic potential has been observed in colonic carcinoma, neuroblastoma, or even in breast cancer. The role of nm23 in the progression of melanoma is still unclear. By comparing 7 murine K-1735 melanoma cell lines that exhibited either low or high metastatic potential in spontaneous or experimental metastasis assays, low-metastatic cells were found to contain ten-fold higher levels of nm23 RNA, and to express higher levels of the 17 Kda NM23 protein. Similarly, the highly metastatic K-1735 TK melanoma cell line showed, when transfected with nm23, a significant reduction in metastatic potential in vivo. On the other hand, no correlation was found between nm23 RNA levels and tumor metastatic potential in murine B16 melanoma cells. Finally, microcell-mediated transfer of chromosome 6 (containing the murine nm23 gene) into C8161 melanoma cells suppressed metastasis but did not inhibit tumorigenicity.

In man, studies about nm23 mRNA levels in metastatic melanomas have shown significantly lower levels in patients developing metastasis within 2 years of diagnosis than in those with less aggressive disease, and longer survival for patients with higher levels of nm23 mRNA. These data allowed to hypothesize that NM23 protein could be involved in contrasting melanoma progression and that low levels of NM23 expression could identify patients with high risk of early metastasis. However, studies evaluating immunohistochemical staining of NM23 protein in melanocytic lesions have not always mirrored the findings of earlier mRNA studies. In fact, although some papers

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demonstrated a correlation between staining and survival outcome, or disease-free interval, other studies found no correlation between NM23 staining and the scarcity of metastasis or the survival outcome.

The aim of this study was to determine the utility of NM23 protein, evaluated by immunohistochemical staining, as a prognostic marker of melanoma. In particular, we asked for the possibility of a relationship between NM23 protein expression in primary melanoma and both disease-free interval and ten-year survival outcome.

### Materials and methods

**Patients**

The study population comprised 30 cases of invasive melanoma reported during the period 1990-1995 in the Cancer Registry of Parma University Hospital. The informations about follow-up and survival outcome were obtained from the Oncologic Center of Parma University Hospital.

Thirty primary cutaneous melanomas were examined, derived from 17 males and 13 females, between 29 and 85-years-old (Table I). Such cases were clas-
sified on the basis of the final version of the AJCC melanoma staging guidelines 5 (Table I). All cases had a follow-up period of 120 months after the excision of the primary tumor, other than 5 cases: 1 lost at the follow-up after 10 months, 1 after 15 months and 3 after 60 months. During the follow-up period, disease relapse (local recurrence, lymph-node and/or visceral metastasis) or the exitus of the patients were evaluated (Table II).

**Immunohistochemical identification of NM23 protein**

Formalin-fixed, paraffin-embedded blocks were obtained from the Pathology Department of Parma University Hospital. Immunohistochemical staining was made using an immunoperoxidase technique, according to a previously described routinary procedure.25 For morphological control, a parallel set of sections were stained with haematoxylin and eosin. For positive controls, dermal adnexal structures as well as nevi were utilized.40 For negative control, seriated sections were incubated, other than with the primary antibody, with normal rabbit serum.

**Semiquantitative analysis of the labeling**

Staining was assessed in a semiquantitative fashion. In fact, 2 parameters were considered, namely, (i) the extent of staining expressed as percentage of tumor cells showing a positive reaction, and (ii) the intensity of labeling graded with reference to the positive internal control (dermal adnexal structures); this last parameter was graded as + (weak intensity), ++ (moderate intensity), and +++ (strong intensity).

**Statistical analysis**

The relationship between NM23 protein expression and the disease-free interval or the survival outcome were obtained by Kaplan-Meier method, using the program “Statistica” of the Statsoft Inc.

**Results**

**NM23 protein expression in melanoma**

Table II shows that 7 cases resulted complete NM23 protein-negative, in 4 cases the percentage of positive cells was between 0% and 10% with a weak staining intensity, in 3 cases the percentage of positive cells was between 10% and 50% with a weak staining intensity, and in 16 cases the percentage of positive cells was greater than 50% with a moderate or strong staining intensity (Figure 1). Positive controls were immunostained, whilst controls for antibody were negative.

![Figure 1](image_url)
Relationship between NM23 protein expression and melanoma progression

NM23 PROTEIN AND DISEASE-FREE INTERVAL

First, we looked for a relationship between the expression of NM23 protein and the appearance of lymph-node and/or visceral metastases. In particular, we considered the time between the excision of primary tumor and the appearance of the first macrometastasis (either lymph-nodal or visceral).

As shown in Figure 2 and Figure 3, patients with a number of NM23-positive cells in the primary tumor
greater than 50% showed a disease-free interval within 5 and 10 years significantly longer (P < 0.01) than patients with a number of positive cells in the tumor lower than 50%.

**NM23 PROTEIN AND SURVIVAL OUTCOME**

Moreover, we looked for an association between the 60-month or 120-month survival and the percentage of NM23 protein-positive cells. As shown in Figure 4 and Figure 5, patients with a percentage of positive cells greater than 50% in the primary tumor had a survival significantly longer (P < 0.01) than patients with a percentage of positive cells lower than 50%.

**Discussion**

This study demonstrates that the expression of NM23 protein in the primary melanoma is inversely correlated with the metastatic potential, and directly correlated with the survival within 60 and 120 months.

First, a direct relationship is herein shown between percentage of NM23-positive melanoma cells and disease-free interval. A series of studies, generally investigating \(nm_23\) RNA rather than NM23 protein, demonstrated that \(nm_23\) in melanoma correlates inversely with metastasis. In fact, former studies showed that murine low-metastatic melanoma cells lines contained ten-fold higher \(nm_23\) RNA levels than high-metastatic cells lines and expressed higher levels of the 17KDa NM23 protein.\(^{31}\) Moreover, transfection of murine \(nm_23\) cDNA in murine high-metastatic melanoma cells lines allowed to reduce their metastatic potential.\(^{32, 34, 44}\) In man, studies about \(nm_23\) RNA levels in metastatic melanoma have shown significantly lower levels in patients developing metastases within 24 months of diagnosis than in those with less aggressive disease.\(^{35}\) Therefore, although one study showed that \(nm_23\) mRNA levels were not different in primary melanoma cells lines or in metastatic melanoma cells lines,\(^{40}\) the majority of studies agreed upon the evidence that \(nm_23\) mRNA levels are inversely correlated with the metastatic potential of melanoma. However, studies evaluating immunohistochemical staining of NM23 protein, other than \(nm_23\) RNA, in melanocytic lesions showed contrasting results about the relationship between percentage of NM23 protein-positive cells and appearance of macrometastases. In fact, whereas some immunohistochemical studies didn’t show an inverse relationship between these 2 parameters,\(^{38-40, 45}\) others, on the contrary, demonstrated that percentage of NM23-positive cells correlates with disease-free interval.\(^{37, 42, 46}\) The latter result was obtained in the present study too: it is obligatory to admit, however, that in our study merely a limited number of metastatic melanoma cases (only 14) was studied.

This study demonstrates that NM23 protein expression in melanoma cells correlated with survival outcome within 60 and 120 months from excision of primary melanoma. However, this relationship is still debated. In fact, some studies didn’t show any association between immunohistochemical NM23-positivity and survival outcome.\(^{41-43}\) On the contrary, other studies demonstrated a direct relationship between these 2 parameters: specifically, cases developing a shorter survival showed a percentage of NM23-positive cells lower than 50%,\(^{6, 37}\) and not only the extension but also the staining intensity of NM23 protein was significantly associated with survival outcome.\(^{6}\) On the basis of the latter and our data, it’s possible to hypothesize a potential prognostic role of NM23 protein in the tumoral progression, although the number of cases studied at this time is still too scarce to achieve definite conclusions.

This is the first study focusing on a ten-year survival period in melanoma patients investigated for NM23 expression. Intriguing, in a cohort of breast carcinoma-affected women, 80% of women with high NM23 expression were alive after 10 years compared to 25% with low expression, whereas at 5 years the survival proportions were 86% and 46% respectively.\(^{10}\) It is worth that, in our cohort of melanoma patients, the only 2 men who died in the period between 60 and 120 months of follow-up had both an absolutely NM23-negative primary tumor (patients n. 14 and 23).

**Conclusions**

This study, in conclusion, showed that the immunohistochemical positivity of NM23 protein in primary melanoma is positively correlated with the disease-free interval and the survival outcome. We must underline, however, that, whereas some studies showed similar results, others showed contrasting results, as above mentioned. Furthermore, even studies about the expression of NM23 protein in tumors other than melanoma lead to contrasting results: some studies, in fact, showed
that high levels of NM 23 protein are positively correlated with a good prognosis,\textsuperscript{10, 11, 15, 47} while other studies show that high levels of NM23 protein are correlated with poor prognosis.\textsuperscript{26, 48} In this regard, we agree that the functions of gene \textit{nm}23 are probably not fully understood. In fact, the mechanism of action of NM23 in metastasis suppression was hypothesized to involve diminished signal transduction downstream of a particular receptor;\textsuperscript{49} the downregulating genes suspected to be involved in NM-23-induced metastasis suppression, however, are complex, and were hypothesized to be associated with cell adhesion, motility and possibly certain tumor/metastasis suppressor members of SWI/SNF-related matrix-associated proteins 2 and 5 and PTEN.\textsuperscript{50} Further uncertainty in the mechanism of action of NM23 was led by the demonstration that p53 is a regulator of \textit{nm}23, but such a regulation is different in different cell types, and this is an important component in the molecular mechanisms of tumor metastasis.\textsuperscript{51}

Hopefully, the availability of larger casuistries will help in better understanding the relationship between immunohistochemical NM23 protein expression in melanoma and disease-free interval and/or survival.

\textbf{Acknowledgements}.—Many thanks are due to N. Campanini for excellent technical assistance.

\textbf{Riassunto}

L’espressione della proteina NM23 nel melanoma primario è correlata con l’intervallo libero da malattia e con la sopravvivenza: dieci anni di follow-up

\textbf{Obiettivo.} \textit{nm}23 è considerato un gene soppressore delle metastasi per alcuni tumori umani. Una ridotta espressione dell’inRNA di \textit{nm}23 è stata riscontrata in diverse metastasi di melanoma, con una correlazione fra espressione di tale proteina e sopravvivenza dei pazienti. Dal momento che il ruolo prognostico dell’espressione della proteina NM23 nei melanomi primari è controverso, lo scopo di questo studio è stato determinare l’utilità della proteina NM23 come indicatore immunohistochimico della prognosi in pazienti affetti da melanoma primario.

\textbf{Metodi.} L’espressione della proteina NM23 è stata analizzata in 30 melanomi primari. Sono state utilizzate sezioni in paraffina e la tecnica dell’immunoperossidasi. Il periodo di follow-up includeva la valutazione dell’intervallo libero da malattia dopo 5 e 10 anni e la sopravvivenza dopo 5 e 10 anni.

\textbf{Risultati.} È stato dimostrato che: (i) l’espressione della proteina NM23 è correlata positivamente con l’intervallo libero da malattia a 5 anni (\textit{P}<0,01) e 10 anni (\textit{P}<0,01); (ii) l’espressione della proteina NM23 è correlata positivamente con la sopravvivenza a 5 anni (\textit{P}<0,01) e 10 anni (\textit{P}<0,01).

\textbf{Conclusioni.} In questo studio, la proteina NM23 correla inversamente con la progressione del melanoma. Dovranno essere eseguiti, tuttavia, studi clinici che includano vaste casistiche di pazienti per confermare il ruolo prognostico favorevole di NM23 nel melanoma.

\textbf{Parole chiave.} Melanoma - Proteina NM23 - Sopravvivenza - Metastasi - Prognosi.

\textbf{References}

**FERRARI**

**NM23 PROTEIN EXPRESSION IN PRIMARY MELANOMA CORRELATES WITH DISEASE-FREE INTERVAL AND WITH SURVIVAL**


Perspective evaluation of a four-year period of application of a follow-up protocol for melanoma patient management

G. PELLACANI 1, P. GIANNELLI 2, C. LONGO 1, S. BASSOLI 1, S. SEIDENARI 1

Aim. Although numerous follow-up protocols have been proposed, there are no widely accepted guidelines for melanoma (MM) follow-up. In 2000, the Emilia-Romagna Melanoma Group agreed upon guidelines for the MM follow-up, considering histological thickness and disease stage. The purpose of this study was the perspective evaluation of this follow-up protocol, in order to verify the efficacy to identify metastases and the effectiveness of the therapeutic approaches.

Methods. During a 4-year period (2001-2004), 176 MM patients were supervised according to our follow-up protocol.

Results. Thirty-nine patients underwent disease progression, showing in transit metastases in 15 cases, lymph node metastases in 11 cases, and distant metastases in 13 cases. At the end of the study 11 patients were dead for the complications of the disease. Disease progression was related with MM thickness, tumour stage and presence of ulceration, with a significant proportion of visceral localization in ulcerated MMs. A low rate of metastases was observed for MMs of the upper limbs, chest and abdomen. Concerning the metastasis identification, the majority of the recurrences were diagnosed by the physician, by means of instrumental examination or during clinical examination. A great part of the first recurrences underwent surgery, with a significant proportion of complete responses for loco-regional ones, whereas distant metastases, although frequently diagnosed before becoming symptomatic, showed a poor response to the treatments.

Conclusion. This study represents a critical intermediate analysis of the application of the Emilia-Romagna protocol after 4 years of methodical application.

Key Words: Melanoma, diagnosis - Follow-up - Metastases.

The rates of morbidity and mortality due to skin tumours in the Caucasian population has remarkably increased in the last few decades.1, 2 Since 1997, dermatological departments and services in Emilia Romagna have taken care of the collection of epidemiological data on melanoma (MM) observing an increment in its incidence.3 Moreover, more than 30% of MMs recorded in Emilia Romagna were thicker than 1.51 mm, and their incidence was stable in spite of information, prevention campaigns, and improvement in MM diagnosis. Since MM prognosis is tightly correlated with thickness, the follow-up of patients at high risk of recurrences is of utmost importance, also in consideration of the low efficacy of treatment of the advanced disease.4, 5 In consideration of the poor prognosis and the low therapeutic effectiveness in advanced stage disease, in the last decade new approaches, such as the research of subclinical lymph nodal metastasis by means of selective lymphadenectomy and the adjuvant immunotherapy with interferon alpha 6, 7 have been proposed, with the aim to improve the prognosis to high risk patients. Moreover, systematic instrumental and clinical follow-up protocols have been proposed in order to rule out operable asymptomatic metastases.
At present, there are no widely accepted guidelines for MM follow-up, even if it is common opinion that patient follow-up is necessary to provide an early diagnosis of a progressive disease.8 Protocols proposed so far took different aspects into account, such as the ability of early identification of metastases and the cost/benefit ratio, linked to the poor treatment efficacy in advanced MM. Many of the proposed follow-up protocols suggested the periodical patient self-examination and medical clinical inspection, rather than instrumental examination, based on the retrospective observation that the majority of recurrences were detected by the patients or were symptomatic, leading to searching of medical advice.9-13 There is also a disagreement on the duration of the follow-up owing to the fact that most recurrences were detected during the first 3 years after the radical surgery, even if there are cases of metastases detected after many years from the operation.14, 15

Recently, the Dermatology German Society proposed guidelines for follow-up, based on a prospective analysis of a cohort of MM patients including whole skin inspection, palpation of superficial lymph nodes, instrumental examination, such as abdominal sonography, chest X-ray and computerized tomography (CT), and blood tests, scheduled at different intervals according to the stage of the disease.16 In this study only in few cases symptoms of metastasis were first discovered by the patient, emphasizing that a careful examination is able to identify numerous still asymptomatic metastases, whereas clinical examination and imaging techniques were useful for the diagnosis of the majority of the recurrences. Abdominal and lymph node sonography enabled the identification of metastasis treatable by surgical approach, whereas, chest X-ray was useless, predominantly detecting untreated lesions.17 In high risk MMs CT resulted useful especially for studying areas inaccessible with physical or sonographical examination, showing a superior sensibility than chest X-ray for the diagnosis of pulmonary metastases.

The problem of MM patient follow-up was considered in the Italian medical community, leading to the proposal of CNR guidelines in 2003.18 Periodical clinical controls, with a variable frequency depending on tumour stage, were considered necessary, whereas lymph node and hepatic sonography and chest X-ray were left to medical decision. Additional tests or instrumental exams can be required in presence of revealed or reported symptoms or warning signals.18

On the other hand, a tight clinical and instrumental follow-up, comprehensive of sonography, chest X-ray, TC and total body scintigraphy have been applied by the Turin Melanoma Centre, since 1975. From the analysis of the patient data base, the authors found that 33.8% of patients undergone recurrences occurring as first site in most cases at locoregional sites and in a smaller, but not irrelevant part, at distant locations.19 Based on the protocol proposed by the Turin Melanoma Centre and on literature data, in 2000, the Emilia-Romagna Melanoma Group, represented by all the University and Hospital Dermatological Departments of the Emilia Romagna (Piacenza, Parma, Reggio Emilia, Modena, Bologna, Ferrara, Ravenna, Forlì-Cesena, Rimini, San Marino Republic), agreed upon guidelines for MM follow-up, considering histological thickness and disease stage. A shared protocol has been fixed and used in hospitals and universities since 2001.20

The purpose of our study consisted in the perspective evaluation of the follow-up protocol on MM, upon which the Emilia Romagna group has agreed. In details, the target was to verify the efficacy of the precocious singling out of metastasis by means of clinical and instrumental examinations. Moreover, the frequency and typology of progression was considered and correlated to the characteristics of the primary MM. The therapeutic approach and its effectiveness was also reported. This study represents a critical intermediate analysis of the application on the Emilia-Romagna protocol after 4 years of methodical application.

Materials and methods

The study represented an experimental clinical evaluation based on the use of record charts. The gained parameters constitute a descriptive and critical analysis, done in a perspective way, on the effectiveness of the Emilia-Romagna protocol.

Population of the study and collected data

The main rule for the inclusion consisted in the possibility of being operated according to radical surgery at the Department of Dermatology of the University of Modena and Reggio Emilia. Patients with a middle or high risk of recurrences were sent to our Department for the staging phase, including, if indicated, sentinel
lymph node biopsy, and total body and brain TC. Necessary conditions to be eligible for the study were histopathological confirmation of MM and the completion of all the regular staging examinations. All enlisted patients underwent follow-up examination, according to the protocol. For each patient a data sheet was filled in conformity with the following parameters: 1) birth date; 2) gender; 3) date of tumour excision; 4) histological examination reporting the Breslow’s thickness, ulceration and regression; 5) MM’s site; 6) report of the number of positive lymph nodes and presence of distant metastases at tumour staging; 7) TNM and American Joint Committee on Cancer (AJCC) stage.\(^\text{21}\)

All the patient referred to our centre for MM excision or staging during the period from the 1\(^{\text{st}}\) January 2001 to the 31\(^{\text{st}}\) of December 2004, were considered. The study was closed on September 2005 in order to have a minimum follow-up period of 9 months. During follow-up period each case of recurrences was recorded. For each patient 1) the site of metastases, 2) the source of disease notification (disease notified by the patient as symptomatic; disease revealed by clinical exams or clinical instrumental techniques), 3) the new TNM, 4) the kind of therapeutic approach (surgery, chemotherapy, radiotherapy or local hyperthermia combined with chemotherapy) and 5) its effects, pointing out the disease state (complete or partial response, stable or progressive disease), were considered. All data were collected and analyzed using Microsoft Excel, which permitted the creation of an electric sheet containing available information for the analysis.

**Follow-up protocol**

The Emilia-Romagna protocol consisted in minimal texts to be done periodically by each patient as shown in Table I. Eventual other tests/controls can be added for specific cases at doctor’s advice. Independently from the belonging stage, guidelines recommended examinations at least until the 10\(^{\text{th}}\) year from the first excision. During the consultation, the patient was educated by the doctor on clinical characteristics of MM and recurrences, and on the methods of recog-

<table>
<thead>
<tr>
<th>Staging</th>
<th>Examinations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clinical examination</td>
<td>Once a year for 10 years</td>
</tr>
<tr>
<td>IA</td>
<td>Clinical examination</td>
<td>Twice per year for 5 years, then once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>Laboratory examinations</td>
<td>Once per year for 5 years</td>
</tr>
<tr>
<td></td>
<td>Sonography:</td>
<td>Twice per year for 5 years</td>
</tr>
<tr>
<td></td>
<td>— locoregional lymph node</td>
<td>Once per year for 5 years</td>
</tr>
<tr>
<td></td>
<td>— abdomen</td>
<td>Once per year for 5 years</td>
</tr>
<tr>
<td></td>
<td>Chest X-ray</td>
<td>Once per year for 5 years</td>
</tr>
<tr>
<td></td>
<td>Total body + brain CT</td>
<td>—</td>
</tr>
<tr>
<td>IB, IIA, IIB, IIC</td>
<td>Clinical examination</td>
<td>Thrice per year for 3 years, then twice per year until the 5(^{\text{th}}) year, finally once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>Laboratory examinations</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>Sonography:</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>— Locoregional lymph node</td>
<td>Twice per year for 5 years, then once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>— Abdomen</td>
<td>*Twice per year for 5 years, then once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>Chest X-ray</td>
<td>*Twice per year for 5 years, then once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>Total body + brain CT</td>
<td>*Once per year in spite of 1 sonography and 1 X-ray</td>
</tr>
<tr>
<td>IIA, IIB, IIC</td>
<td>Clinical exam</td>
<td>3 times per year for 3 years, then twice per year until the 5(^{\text{th}}) year, finally once per year until the 10(^{\text{th}}) year</td>
</tr>
<tr>
<td></td>
<td>Laboratory exam</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>Sonography:</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>— locoregional lymph node</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>— abdomen</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>Chest X-ray</td>
<td>Once per year for 10 years</td>
</tr>
<tr>
<td></td>
<td>Total body + brain CT</td>
<td>Once per year for 5 years</td>
</tr>
<tr>
<td>IV</td>
<td>At doctor’s discretion</td>
<td>—</td>
</tr>
</tbody>
</table>

* Abdomen sonography and chest X-ray can be substituted by total body + brain CT once per year
nition of signs and symptoms. Particular emphasis was turned to the skin self-examination. In our study, in order to compare the effectiveness of the different instrumental methods of research, we choose the option of a total body and brain CT alternated with abdomen sonography and chest X-rays every 6 months for stage IB and II patients.

In stage IV examinations were executed at doctor’s discretion, according to the site and number of metastases.

**Treatment protocol for the metastases**

The therapeutic approach was divided into surgical excision, chemotherapy and radiotherapy. If possible, radical surgery was chosen, while chemotherapy and radiotherapy were reserved to unresectable metastases. As chemotherapy, dacarbazine 250 mg per m²/die for 5 days/week for 4 weeks was chosen as front line therapy. As the second line treatments cisplatin and/or vinblastin and/or fotemustine was employed singularly or in combination, with or without addition of immunotherapy (IL-2 and IFN-α). Local recurrences at the limbs, not operable for spread and number, were treated with local hyperthermia combined with chemotherapy (Melphalan). Radiotherapy was reserved for unresectable brain metastases. Concerning not operable metastases found in patients already treated with first and second line chemotherapy, it was opted for a symptomatic palliative therapy.

**Results**

From 1st January 2001 to 31st December 2004, 176 patients subjected to MM’s excision at the Department of Dermatology of the University Modena and Reggio Emilia have been supervised by the service of Day Hospital, according to the follow-up protocol of the Emilia-Romagna.

The study population included 78 males and 98 females. The average age was 60 years for both sexes, with a standard deviation of 17 years ranging between 22 and 94 years. The patients were supervised for an average period of 34 months, ranging between a minimum of 9 months and a maximum of 57 months. The average histological thickness of the excised lesions was 2.27 mm with a standard deviation of 2.09 mm and a range of 0.15-13 mm. After the completion of the staging phase, inclusive of selective lymph node biop-

<table>
<thead>
<tr>
<th>AJCC</th>
<th>Total (%)</th>
<th>F</th>
<th>M</th>
<th>Progressive disease (%)</th>
<th>Exitus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 (1.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IA</td>
<td>48 (27.3)</td>
<td>23</td>
<td>25</td>
<td>1 (2.1)</td>
<td>0</td>
</tr>
<tr>
<td>IB</td>
<td>37 (21)</td>
<td>12</td>
<td>25</td>
<td>6 (16.2)</td>
<td>1</td>
</tr>
<tr>
<td>IIA</td>
<td>25 (14)</td>
<td>8</td>
<td>14</td>
<td>2 (14.2)</td>
<td>0</td>
</tr>
<tr>
<td>IIB</td>
<td>24 (13.6)</td>
<td>12</td>
<td>12</td>
<td>1 (10.8)</td>
<td>0</td>
</tr>
<tr>
<td>IIC</td>
<td>8 (4.5)</td>
<td>5</td>
<td>3</td>
<td>2 (10.8)</td>
<td>1</td>
</tr>
<tr>
<td>IIIA</td>
<td>19 (10.8)</td>
<td>11</td>
<td>8</td>
<td>3 (10.8)</td>
<td>0</td>
</tr>
<tr>
<td>IIIB</td>
<td>3 (1.7)</td>
<td>2</td>
<td>1</td>
<td>1 (1.7)</td>
<td>0</td>
</tr>
<tr>
<td>IIC</td>
<td>9 (5.1)</td>
<td>5</td>
<td>4</td>
<td>3 (5.1)</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>1 (0.6)</td>
<td>1</td>
<td>0</td>
<td>0 (0.6)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>176 (100)</td>
<td>78</td>
<td>98</td>
<td>39 (22.2)</td>
<td>11</td>
</tr>
</tbody>
</table>

Legend: F = patients from female, M = patients from males, Progressive disease (%) = patients with progression of disease, Exitus (%) = patients who died due to disease.

During the study 39 out of 176 patients underwent disease progression, showing recurrences as satellites and/or in transit metastases in 15 cases, as lymph node metastases in 11 cases, and as distant metastases in 13 cases (7 in the lung, 5 in the liver, 2 in the brain, presenting in one patient 2 contemporary localizations) (Table III). During the follow-up 3 patients out of 39 were dead because of metastases complications, while in 13 patients secondary metastases have been diag-
nosed (1 in transit, 2 lymph nodal, 6 pulmonary, 3 hepatic, 4 encephalic, and 2 spleen recurrences, in 5 cases with multiple localizations). In the group of 13 patients who have had a progression of the disease, currently 8 were dead during the follow-up because of the disease.

Disease progression was related with MM thickness and tumour stage (Table IV). The majority of recurrences (69.2%) were diagnosed in patients with MMs thicker than 2 mm. The risk for developing a recurrence was related to the pT stage, with a progression in more than 40% of patients with a pT3b or greater.

The presence of demonstrated histological ulceration resulted as an unfavourable factor of prognosis since a progression was noticed in 40.5% of lesions with ulceration against 17.3% of lesions without (Table V). Moreover, an increased risk for visceral first localizations was observed in ulcerated lesions compared with non ulcerated ones (18.9% vs 4.3%).

The primary site of MM was linked to the frequency of recurrences and to their localization, as shown in Table VI. Head-neck MMs showed the greatest mean tumour thickness and the highest risk of recurrence (36.8%), with secondary localizations equally distributed in locoregional and distant sites. MMs of the palm and soles had an high rate of recurrences predominately locoregional. MMs on the back and lower limbs showed recurrences nearly in the 20% of cases, but for localizations on the back locoregional and distant metastases were equally distributed, while in the lower limbs MM recurrences were found exclusively in locoregional sites. Localizations on the upper limbs, chest and abdomen were minimally responsible for recurrences, although did not significantly differ in tumour thickness with respect to lower limb and back MMs.

### Table III.—Type and site of the recurrences.

<table>
<thead>
<tr>
<th>Localization</th>
<th>1st recurrence</th>
<th>Further recurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>In transit</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Lung</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Brain</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>18</td>
</tr>
</tbody>
</table>

Cases with contemporary involvement of 2 or more sites 1 5

### Table IV.—Locoregional and visceral recurrences according to the melanoma pT stage.

<table>
<thead>
<tr>
<th>PT</th>
<th>Total (%)</th>
<th>LN + at staging (%)</th>
<th>1st recurrence locoregional (%)</th>
<th>1st recurrence at distance (%)</th>
<th>Progressive disease (%)</th>
<th>Exitus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tis</td>
<td>2 (1.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T1a</td>
<td>49 (27.8)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T1b</td>
<td>3 (1.7)</td>
<td>0</td>
<td>(33.3)</td>
<td>0</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>T2a</td>
<td>45 (25.6)</td>
<td>10</td>
<td>5</td>
<td>(2.2)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>T2b</td>
<td>9 (5.1)</td>
<td>3</td>
<td>0</td>
<td>(33.3)</td>
<td>(22.2)</td>
<td>(22.2)</td>
</tr>
<tr>
<td>T3a</td>
<td>22 (12.5)</td>
<td>4</td>
<td>6</td>
<td>(4.5)</td>
<td>(9.1)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>T3b</td>
<td>16 (9.1)</td>
<td>6</td>
<td>6</td>
<td>(6.3)</td>
<td>(0)</td>
<td>(6.3)</td>
</tr>
<tr>
<td>T4a</td>
<td>17 (9.7)</td>
<td>2</td>
<td>4</td>
<td>(17.6)</td>
<td>(17.6)</td>
<td>(17.6)</td>
</tr>
<tr>
<td>T4b</td>
<td>9 (4.9)</td>
<td>2</td>
<td>(11.1)</td>
<td>(33.3)</td>
<td>(22.2)</td>
<td>(22.2)</td>
</tr>
<tr>
<td>Tx</td>
<td>4 (2.3)</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>176 (100)</td>
<td>31</td>
<td>26</td>
<td>13</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

*% in respect of the total number of MMs; ° %in respect of the MMs belonging to that pT stage
Table V.—Locoregional and visceral recurrences according to the histologic ulceration.

<table>
<thead>
<tr>
<th>PT</th>
<th>Total (%)</th>
<th>LN + at staging (%)</th>
<th>1st recurrence locoregional (%)</th>
<th>1st recurrence at distance (%)</th>
<th>Progressive disease (%)</th>
<th>Exitus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non ulcerated</td>
<td>139</td>
<td>20</td>
<td>18</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(79)</td>
<td>(14.4)</td>
<td>(12.9)</td>
<td>(4.3)</td>
<td>(6.5)</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Ulcerated</td>
<td>37</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(29.7)</td>
<td>(21.6)</td>
<td>(18.9)</td>
<td>(10.8)</td>
<td>(13.5)</td>
</tr>
</tbody>
</table>

*% in respect of the total number of MMs; °% in respect of the MMs belonging to the corresponding group.

Table VI.—Locoregional and visceral recurrence according to the site of melanoma insurgence.

<table>
<thead>
<tr>
<th>Melanoma site</th>
<th>Thickness (mean±DS)</th>
<th>Locoregional recurrence</th>
<th>At distant recurrence</th>
<th>No recurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head-neck</td>
<td>3.75±3.23</td>
<td>3 (10.5%)</td>
<td>5 (26.3%)</td>
<td>11 (63.2%)</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>1.63±1.26</td>
<td>1 (3.8%)</td>
<td>1 (3.8%)</td>
<td>26 (92.4%)</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>1.84±1.49</td>
<td>10 (18.2%)</td>
<td>1 (1.8%)</td>
<td>44 (80%)</td>
</tr>
<tr>
<td>Chest and abdomen</td>
<td>2.26±2.31</td>
<td>1 (7.7%)</td>
<td>1 (7.7%)</td>
<td>11 (84.6%)</td>
</tr>
<tr>
<td>Back</td>
<td>1.97±1.67</td>
<td>5 (12.5%)</td>
<td>4 (10%)</td>
<td>32 (77.5%)</td>
</tr>
<tr>
<td>Palm-plantar</td>
<td>2.88±2.11</td>
<td>6 (26.3%)</td>
<td>1 (5.3%)</td>
<td>13 (68.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100%)</td>
<td>13 (100%)</td>
<td>137 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table VII.—Modalities of identification of the recurrences.

<table>
<thead>
<tr>
<th>Total (%)</th>
<th>1st Recurrence type</th>
<th>Further recurrence type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient’s symptoms</td>
<td>6 (15)</td>
<td>4 in transit</td>
</tr>
<tr>
<td>Clinical examination</td>
<td>12 (30)</td>
<td>10 in transit</td>
</tr>
<tr>
<td>Instrumental examination:</td>
<td>22 (55)</td>
<td>1 in transit</td>
</tr>
<tr>
<td>Sonography</td>
<td>8 (36.4)</td>
<td>6 LN</td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>1 (4.5)</td>
<td>1 Lung</td>
</tr>
<tr>
<td>Total body + brain CT</td>
<td>13 (59.1)</td>
<td>1 in transit</td>
</tr>
</tbody>
</table>

Table VII described the type of recurrence matched with the source of identification. In over the half of cases, the first recurrence was diagnosed by means of instrumental examinations. On the other hand, 12 recurrences (32.4%), mainly in transit metastasis, were individuated during routinely clinical examination of the patient. Just 4 out of 37 patients self-reported symptoms and/or signs correlated with a recurrence. Further metastatisation was diagnosed by instrumental analysis in all cases but 1, found during clinical examination. In transit metastases were mostly identified by the physician by clinical inspection (10 cases out of 15 cases), while lymph nodal and visceral metastases were best diagnosed by instrumental examination.
Sonography seemed effective in diagnosing lymph nodal and hepatic metastases (8 out of 13 recurrences), whereas CT enabled the identification of the majority of lung metastases compared with chest X-ray. Brain metastases and spleen localization were recognized by CT, with the exception of 2 cases of brain metastasis diagnosed on the basis of symptoms referred by the patients.

Table VIII showed the employed treatments and their effectiveness, evaluated 3 month after the therapeutic protocol conclusion by means of total body and brain CT scans. Twenty-six first metastases underwent surgical excision, obtaining a complete resolution in 12 cases (4 lymph node and 8 in transit recurrences), a partial resolution in 6 cases (4 lymph node and 2 in transit recurrences), and a progression in 8 cases (3 lymph node, 3 in transit, 1 lung and 1 liver recurrences). Chemotherapy was given for 11 first localizations, obtaining 2 complete responses in patients with in transit metastases by means of local hypertermia with Melphalan and 3 partial responses in patient with lung recurrences, whereas a progression was observed in 6 cases referred to 3 lung and 3 liver localizations. None of the 2 patients treated with radiotherapy for brain localization had benefit from the therapy. One patient with a liver localization withdrew from our protocol.

### Discussion and conclusions

In front of MM metastases the medical choice is oriented on the radical removal, when possible, because it offers to the patient the best perspectives, both regarding the quality of life and the possibility of surviving. Therefore, instrumental and clinician patient follow-up have been proposed in order to rule out operable asymptomatic metastases.

Numerous follow-up protocols, generally based on retrospective data, have been proposed, which markedly differ if were held in greater consideration the socio-economical or the medical aspects of the survey. In 2003 Garbe et al. perspective evaluated the efficacy of a follow-up protocol showing that a systematic screening of the patients allowed to find metastases before the symptoms appearance in many cases, both for locoregional and visceral ones.

In our study we applied since the 1st of January 2001 a pre-established follow-up protocol to all the MM patients supervised in the Day Hospital service of our Clinic, systematically recording data on primary tumour characteristics, recurrence site and diagnostic and therapeutic procedures.

The population examined in the follow-up represents 69.3% of the totality of 254 invasive MMs in
the Province of Modena recorded by the Modena Cancer Registry during the 4 year study period. According to previous data,23 the frequency of progression was strictly correlated to the histological thickness, to the stage of the disease, to the presence of ulceration at histology, and to the site of insurgence of the primary tumour. We observed a recurrence in 22.2% of patients, with a further progression in 1/3 of those. During the course of the study 11 patients died as a result of MM metastases, in 3 cases in consequence of the first recurrence, while the other 8 of a visceral widespread of the disease, pointing out how MM progressions present a poor prognosis. Although the MM can virtually create metastases in every organ or tissue, the locoregional district was mainly interested (65.1% of the cases) from the first recurrences, in agreement with the literature’s data (Table V).19, 23, 24

Considering also the positive elective and selective lymph node biopsies, the proportion of locoregional recurrences increases to 76.6%, since 20 patients with positive lymph node at staging did not present progression, demonstrating that the disease mainly spread through contiguity and/or lymphatic way.23 Considering first recurrences in visceral sites, they were found in lung, liver and brain.

Interestingly, 9 out of the 13 patients with first visceral localization did not present previous locoregional recurrences, corresponding to a direct haematric spread in a consistent percentage (23%) of the progressions, while in the remaining 4 cases positive sentinel lymph nodes were found. Moreover, the presence of ulceration seemed strongly correlated with a bad prognosis, increasing the risk of progression, with a greater probability to have visceral localizations (Table V). Therefore, an accurate patient check up, comprehensive of CT total-body and brain, seems to be recommended for MMs with ulceration, independently from the histological thickness. On the other hand, for patients with non ulcerated MM clinical examination and lymph node sonography represented the most relevant investigations, especially for tumours thicker than 2 mm which presented a progression in 14 out of 39 cases (35.9%), compared with the 8 progressions in the 96 thinner ones (8.3%).

From our data it emerges that the risk and type of recurrence was also influenced by the site of the primitive tumour (Table VI). The high risk of recurrence observed for MMs localized at the head/neck and palms/soles could be related to the higher thickness. Whereas, a low rate of recurrence was recorded in lesions of the upper limbs, chest and abdomen, although the mean thickness was similar to the other sites. A predominant locoregional metastatization derived from MMs of the palm and soles and of the lower limbs, whereas distant recurrences were more frequently observable in patients with MMs of the head and neck and of the back.

Concerning the metastasis identification, our data confirmed previous observations,16 since the majority of the recurrences were diagnosed by the physician, during clinical examination in the 30% of cases and by means of instrumental examination in 55% of cases. In our population, patients were able to find 4 out of 15 in transit metastases and 2 brain metastases arisen as first recurrence. Probably owing to their early recognition, a great part of the first recurrences underwent surgery, with a significant proportion of complete responses for locoregional recurrences. On the other hand, distant metastases, although frequently diagnosed before to became symptomatic, showed a poor response to the treatments.

In conclusion, the strategy of surveillance examined in this study leaded to a high proportion of early metastasis recognition, although an effective treatment was recorded only for locoregional recurrences. Ultrasounds resulted equally effective, compared with CT, for the identification of lymph node and liver metastases, whereas CT appeared to be superior to chest X-ray for lung metastasis recognition. These data suggest that patients with ulcerated MMs, MMs of the head-neck and back and cases with positive sentinel lymph node at staging (stage III subjects), should be accurately investigated both for locoregional and visceral metastases, whereas a particular attention to locoregional sites should be paid to thick non ulcerated MMs of the limbs, chest, abdomen and palm and soles.

Although our data are based on a short period, the critical analysis of the follow-up protocols is of great importance in order to modify opportunely the rules in accordance to the resulting data, enabling the improvement of the quality of the service sparing non necessary examination and focusing on the most informative and effective investigations. Obviously, conclusive data could derive from a greater number of cases followed for a longer period, suggesting the opportunity of a multicenter trial.
Perspective evaluation of a four-year period of application of a follow-up protocol

Pellacani

Riassunto
Valutazione prospettica del Protocollo Emiliano-Romagnolo di follow-up dei pazienti affetti da melanoma dopo 4 anni di applicazione

Obiettivo. Attualmente non esistono linee guida comune mente accettate di follow-up dei pazienti con melanoma, nonostante numerosi protocolli siano stati proposti negli anni. Nel 2000 il Gruppo Emiliano Romagnolo sul melanoma ha concordato su linee guida di follow-up basate sullo spessore istologico e studio della malattia. Lo scopo dello studio era la valutazione prospettica dei risultati dell’applicazione del protocollo, verificandone l’efficacia nella identificare metastasi e l’effetto del relativo approccio terapeutico.

Metodi. Nel corso dei 4 anni di studio, sono stati seguiti secondo le indicazioni del protocollo 176 pazienti affetti da melanoma.


Personal experience on antibiotic resistance of propionibacteria in Ferrara

V. BETTOLI 1, A. BORGHI 2, R. ROSSI 3, M. FERRONI 2, F. RIGOLIN 3, A. VIRGILI 2

Aim. Antibiotics reducing the number of propionibacteria on the skin represent powerful agents in the treatment of inflammatory acne. The widespread use of both oral and topical antibiotics to treat acne has resulted in the dissemination of propionibacterial-resistant strains. Failure of therapy associated with the selection of antibiotic-resistant propionibacteria is a well recognized concern. The aim of the study is to determine the prevalence of skin colonization by antibiotic-resistant propionibacteria in a large number of acne patients attending our department and to monitor changes over a six-year period (April 2000-October 2005).

Methods. From April 2000 we have tested the susceptibility to the most commonly used antiacne antibiotics of the propionibacterial strains carried by the patients with acne, proposed to be treated with antibiotic. Propionibacterial samples were obtained from the skin surface of the face of 1,579 acne patients using a moistened swab. The swabs were used to inoculate agar plates containing selective concentrations of tetracycline, minocycline, erythromycin and clindamycin, as well as antibiotic-free control plates. After 7 days of anaerobic incubation at 37 °C, a semiquantitative method (a scale from 0 to 5+) was applied to estimate the amount of growth in the presence of each antibiotic.

Results. Propionibacteria were isolated in 1,508 of 1,579 patients sampled. The prevalence of carriage of isolates resistant to at least one antibiotic was 55.9%. Resistance to erythromycin was the most common in all years ranging from 58.8% in 2000 to 38.5% in 2005 (mean prevalence 47.7%); resistance to clindamycin ranged from 44.1% in 2000 to 32.2% in 2005 (mean 39.2%). Thirty-five percent of the isolated strains were resistant to both erythromycin and clindamycin. Rates of resistance to tetracyclines were very low (1.9% to tetracycline and 0.6% to minocycline). Mild reduction in resistance to erythromycin and clindamycin was noticed over the period of samples collection. The highest rates of resistance were found in older patients and slightly higher rates in males than in females.

Conclusion. The available data show a wide distribution of propionibacterial strains resistant to erythromycin and clindamycin was noticed over the period of samples collection. The highest rates of resistance were found in older patients and slightly higher rates in males than in females.

Key Words: Acne - Propionibacteria - Antibiotic resistance.

Antibiotics play a major role in acne therapy, working both as bacteriostatic-bactericidal agents on propionibacteria 1 and as direct anti-inflammatory agents.2, 3 Antibiotics currently used to treat papulo-
Pustular acne are oral tetracyclines, macrolides, sulfonamides and topical macrolides (erythromycin) and clindamycin. The available agents are suitably used in combination with topical retinoids, to target various pathogenic factors involved in acne.4

Antibiotic therapy for acne has to be given for prolonged periods of time, usually several months; the longer the exposure, the greater is the selection and overgrowth of resistant strains of propionibacteria. In P. acnes, antibiotic resistance is acquired by mutations within genes encoding components of the target to which the drug binds. In particular, erythromycin resistance is associated with any one of 3 distinct point mutations within the gene encoding the peptidyl transferase centre of 23S rRNA. Each mutation confers a different cross-resistance pattern to macrolides, lincosamides and type B streptogramins, the so called MLS antibiotics.5,6 In the case of tetracycline, resistance is associated with a mutation in the 16S rRNA of the small ribosomal subunit at E. coli equivalent base 1058.7

Over the past 30 years, the widespread use of antibiotics to treat acne has resulted in significant dissemination of resistant strains of propionibacteria. In clinical practice, the consequence of propionibacterial antibiotic-resistance consists of a reduced response to treatment with both the corresponding and the cross-resistant antibiotics.1,9-11 Skin carriage of resistant propionibacteria may be associated to poor therapeutic outcome to topical as well as to oral antibiotic treatments.1,10 Although Eady has given advice in clinically recognising patients who may carry antibiotic resistant organisms,9 however, in practice, identification of patients carrying resistant strains is very difficult. Undoubtedly, the recognition of specific resistances may only be achieved by the evidences provided by propionibacterial culture and subsequent antibiogram.

This is the reason why from April 2000, in cooperation with the Laboratory of Microbiology of our hospital, we tested the antibiotic sensitivities of propionibacteria strains carried by the acne patients who needed antibiotic treatments. Antibiotic prescriptions have been consequently driven by the laboratory results.

In the present study, we evaluate the prevalence of skin colonization by antibiotic-resistant propionibacteria among acne patients sampled between April 2000 and October 2005. We have analysed the propionibacterial resistance to each of the tested antibiotics also analysing the results by years, age-related populations and sex.

Materials and methods

Propionibacterial samples were obtained from the skin surface of the face of the acne patients using a moistened swab. The whole face was rubbed with a transport swab moistened in wash fluid (0.075 mol L-1 sodium phosphate buffer, pH 7.9) containing 0.1% Triton-X 100. Swabs were used to inoculate plates containing selective concentrations of tetracycline (5 µg/mL-1), minocycline (5 µg/mL-1), erythromycin (0.5 µg/mL-1) and clindamycin (0.5 µg/mL-1) as well as antibiotic-free control plates, always inoculated last. The base medium was TYEG agar containing 2% tryptone, 1% yeast extract agar, 0.5% glucose and 2 µg/mL-1 furazolidone to inhibit the growth of staphylococci. After 7 days anaerobic incubation at 37 °C, a semiquantitative method was used to estimate propionibacterial population densities by recording the level of growth on isolation plates. Bacterial growth was assigned a score of 0-5+ where 5+ denoted confluent growth, 4+ denoted > 101 colonies to semiconfluent growth, 3+ indicated 51-100 colonies, 2+ indicated 11-50 colonies, and 1+ indicated ≤10 colonies.12 The level of propionibacterial resistance in the plates containing antibiotics was evaluated as follows: no resistance = S (sensitive); mild resistance = MR (1-50 colonies); intermediate resistance = I (51-100 colonies); relevant resistance = R (101 to confluent). It should be emphasized that this method is semiquantitative and was used in this study as it can be employed in situations that are unsuitable for the quantitative sampling method of Williamson and Kligman.13

All patients were sampled for propionibacterial resistant to erythromycin and clindamycin sensitivity (1 579 patients). Because of prescription strategies and availabilities of the different antibiotics in the Italian market, tetracycline was tested from April 2000 to December 2000 and again from 2002 up to now (1 084 patients). Minocycline was tested until 2002 when we stopped its use (528 patients).

Results

Between April 2000 and October 2005, a total of 1 579 acne patients (521 males and 1 058 females) of average age 21 (range 12-42) were sampled for propionibacterial resistant strains. Viable propionibacteria were recovered from 1 508 out of 1 579 (95.5%) sampled patients. Resistant strains were found on the
Personal experience on antibiotic resistance of Propionibacteria in Ferrara

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facial skin of 843 patients, which represent 55.9% of the colonized patients. Resistance to erythromycin was the most prevalent (mean prevalence during the considered period 47.7%), while the prevalence of clindamycin-resistant propionibacteria was slightly lower (mean prevalence 39.2%) (Table I). The proportion of patients carrying strains resistant to erythromycin dropped from 58.8% in 2000 to 38.5% in 2005, while the rates of resistance to clindamycin dropped from 44.1% in 2000 to 32.2% during 2005. Combined resistance to both antibiotics was present on 35% of the acne patients carrying resistant propionibacteria. Thirteen percent of patients carried erythromycin-resistant, clindamycin-susceptible strains and 4% of patients were colonized by clindamycin-resistant, erythromycin-susceptible strains. Resistance to erythromycin and clindamycin was much more common than resistance to the tetracyclines (1.9% and 0.6% rates of propionibacteria resistant to tetracycline and minocycline respectively).

A slight difference in prevalence of antibiotic resistance was found in males in comparison with females (59.9% and 54% respectively) (Table II).

<table>
<thead>
<tr>
<th>Table I.—Number and percentage of patients carrying propionibacterial strains susceptible and resistant to each antibiotic: yearly results.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ery</strong></td>
</tr>
<tr>
<td>Number of patients (%)</td>
</tr>
<tr>
<td>Susceptible</td>
</tr>
<tr>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Cly</strong></td>
</tr>
<tr>
<td>Number of patients (%)</td>
</tr>
<tr>
<td>Susceptible</td>
</tr>
<tr>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Min</strong></td>
</tr>
<tr>
<td>Number of patients (%)</td>
</tr>
<tr>
<td>Susceptible</td>
</tr>
<tr>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Tet</strong></td>
</tr>
<tr>
<td>Number of patients (%)</td>
</tr>
<tr>
<td>Susceptible</td>
</tr>
<tr>
<td>Resistant</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Table II.—Propionibacterial resistance related to the sex of the patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number and percentage of colonized patients with any resistant isolates</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Resistant: mild resistance + intermediate resistance + relevant resistance

<table>
<thead>
<tr>
<th>Table III.—Age distribution of skin colonization by antibiotic-resistant propionibacteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range (years)</td>
</tr>
<tr>
<td>10-14</td>
</tr>
<tr>
<td>15-17</td>
</tr>
<tr>
<td>18-20</td>
</tr>
<tr>
<td>21-24</td>
</tr>
<tr>
<td>25-29</td>
</tr>
<tr>
<td>&gt; 30</td>
</tr>
</tbody>
</table>

Resistant: mild resistance + intermediate resistance + relevant resistance
The lowest prevalence of propionibacterial resistance (Table III) was found among 10-14-year-old patients (46%), while 68% of the patients older than 30 years were colonized by resistant strains.

**Discussion and conclusions**

In 1976, a study on 1,000 acne patients exhibited no propionibacterial antibiotic resistance. The first report of resistance to antiacne antibiotics appeared in the USA in 1979, P. acnes resistant strains were found in 1 in 5 patients treated topicaly with either erythromycin or clindamycin. Since then, propionibacteria resistant to one or more antiacne antibiotics have been isolated all over the world.

The continuous monitoring carried out in Leeds for nearly a decade showed a steady increase in prevalence of propionibacterial resistance from 34.5% in 1991 to a peak of 64% in 1997. The emergence of resistance coincided with the introduction in UK of topical antibiotic formulations in treatment of inflamed acne, while the increasing number of patients colonized by resistant strains recorded during the aforesaid period may reflect the increasingly widespread prescription of both oral and topical antibiotics. As erythromycin and clindamycin bind to different regions of the same target within the bacterial cells, the majority of erythromycin-resistant strains are cross-resistant to clindamycin. In Leeds studies, resistance to erythromycin and clindamycin was more common in all years than resistance to tetracyclines. In each year, rates of resistance to clindamycin and erythromycin were similar, but resistance to clindamycin was always lower than resistance to erythromycin.

A multicentre study recently conducted in 6 European countries with the aim of estimating the size of the resistance problem in Europe, found that, overall, 2/3 of patients were colonized with resistant strains. Prevalence rates of skin colonization by antibiotic-resistant propionibacteria were different among the 6 countries studied, clearly mirroring the treatment histories of the sampled patients. The lowest prevalence was found in Hungary (50.8%), which also presents the lowest rate of patients previously treated with any antibiotics (18%), while the highest prevalence was noticed in Spain (93.6%), 84% treated with antibiotic therapy. The European study showed, in agreement with Leeds' findings, that combined resistance to clindamycin and erythromycin was much more common than resistance to tetracyclines. These data seem to strongly correlate with prescribing habits.

The chief aim of the present paper has been to evaluate both prevalence and distribution of propionibacterial resistance in patients affected by inflamed acne attending the acne clinic in Ferrara.

From more than 5 years (April 2000 - October 2005) we sampled all acne patients who needed antibiotic treatment in order to detect any skin carriage of antibiotic-resistant propionibacteria. This practice, in addition, allowed monitoring of propionibacterial resistance among acne patients referred to our department.

Resistant strains were found on the facial skin of 55.9% of the colonized patients. Resistance to erythromycin was the most prevalent, while the prevalence of clindamycin-resistant propionibacteria was slightly lower (Table I). In accordance with the data available in the literature, resistance to erythromycin and clindamycin was much more common than resistance to the tetracyclines. In Italy tetracyclines have usually been prescribed for less than 3 months and this could explain the low rate of resistance evidenced. In addition, there are data suggesting that topical erythromycin and clindamycin may be more selective than the oral tetracyclines.

Topical erythromycin, alone or in combination with topical retinoids, was the most frequently used antibiotic among the studied patients, about 40% of our patients had been previously treated with it. Approximately 14% of the sampled patients had received oral tetracyclines.

Comparing our annual rates of prevalence of resistant propionibacteria to each antibiotic, there seems to be a decrease in both erythromycin and clindamycin-resistances over time (erythromycin-resistance rate was 58.8% in 2000 and 38.5% in 2005; clindamycin-resistance rate was 44.1% in 2000 and 32.2% in 2005). This fall in resistance rates might be at least partially explained by a local change in prescribing practices that was encouraged in an attempt to prevent the rise in resistance rates. Available guidelines could help physicians improve the way they use antibiotics to treat acne. Prolonging antimicrobial treatment for as short a time as possible, withdrawing antibiotics once inflammation is controlled, using in combination with topical retinoids, using benzoyl peroxide for a minimum of 5-7 days between antibiotic courses to eliminate resistant organisms from the skin, avoiding the concomitant use of oral and topical therapy with chem-
Antibiotico resistenza propioniatterica a Ferrara

Obiettivo. Gli antibiotici, riducendo il numero di propionibatteri sulla superficie cutanea, rappresentano un efficace presidio nel trattamento dell’acne infiammatoria. L’impiego su larga scala di antibiotici, sia sistemicamente che topicamente, per la cura dell’acne ha determinato la diffusione di ceppi di propionibatteri resistenti. La conseguenza della selezione di ceppi resistenti consiste essenzialmente nel fallimento della terapia antibiotica. L’obiettivo dello studio è la definizione della prevalenza di ceppi antibiotico-resistenti all’interno di una vasta popolazione di pazienti acneici affetti alla Sezione di Dermatologia di Ferrara, seguendone contestualmente le eventuali variazioni nel corso di 6 anni (april 2000 - ottobre 2005).

Metodi. A partire da aprile 2000 è stata testata la suscettibilità ai più comuni antibiotici utilizzati nella terapia dell’acne dei ceppi di propionibatteri veicolati dai pazienti acneici con indicazione al trattamento antibiotico. I campioni di propionibatteri sono stati ottenuti dalla cute del volto di 1 579 pazienti acneici mediante l’impiego di un tampone inumidito. Quanto prelevato per mezzo dei tamponi è stato inoculato in terreni di coltura contenenti concentrazioni selettive di tetracicline, minocicline, eritromicina e clindamicina, oltre che in una piastra di controllo. Dopo 7 giorni di incubazione in anaerobiosi a 37°C è stata valutata con metodica semiquantitativa (scala da 0 a 5+) l’entità della crescita delle colonie in presenza di ciascun antibiotico.

Risultati. Sono stati isolati propionibatteri in 1 508 dei 1 579 pazienti testati. La prevalenza di ceppi resistenti ad almeno un antibiotico ammonta al 55,9% del totale. La resistenza all’eritromicina è risultata la più comune in tutti gli anni dello studio, variando dal valore di 58,8% nel 2000 a 38,5% nel 2005 (prevalenza media 47,7%); la resistenza alla clindamicina è compresa tra 44,1% dell’anno 2000 e 32,2% del 2005 (media 39,2%). Il 35% dei ceppi isolati è risultato resistente contemporaneamente a eritromicina e clindamicina. Si è rilevata una resistenza molto bassa alle tetracicline (1,9% alla tetracicline e 0,6% alla minocicline). Nel lasso temporale in cui sono state condotte le analisi è stata registrata una sensibile riduzione della resistenza sia all’eritromicina che alla clindamicina. Questo andamento è imputabile probabilmente a una crescente sensibilità dei dermatologi locali al problema dell’antibiotic resistenza e a una maggiore aderenza alle raccomandazioni impartite dalle attuali linee guida in tema di prescrizione e gestione della terapia antibiotica nella acne. Le più alte percentuali di antibiotico-resistenza sono proprie delle fasce di età più elevate, mentre una prevalenza soltanto leggermente superiore è documentata nei soggetti di sesso maschile rispetto a quelli di sesso femminile.

Conclusioni. I dati mostrano la rilevante prevalenza di ceppi di propionibatteri resistenti a eritromicina e clindamicina nella popolazione di pazienti acneici indagati. Allo stato attuale, viceversa, le percentuali di resistenza alle tetracicline risultano basse. Quanto riscontrato supporta il significato dell’esame colturale nella definizione della sensibilità agli antibiotici dei propionibatteri isolati dai pazienti acneici. Questa procedura è considerata utile sia per un’adeguata gestione terapeutica dell’acne sia per lo studio del fenomeno della resistenza batterica.

Parole chiave: Acne - Propionibatteri - Antibiotico-resistenza.

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rially dissimilar antibiotics, using oral isotretinoin for resistant cases, are just some of antibiotic prescribing recommendations.

The prevalence of antibiotic resistance resulted higher in males than in females (59.9% and 54% respectively) (Table II); a better compliance of females patients to previous treatments for acne should be a possible explanation for this finding.

Another aspect of interest arises from the analysis of the age distribution of the prevalence of propionibacterial resistance (Table III). The lowest prevalence was found among 10-14-year-old patients (46%), while 68% of the patients older than 30 years were colonized by resistant strains. This relevant gap is most likely explained by the higher rate of previous treatment with antibiotics among the oldest patients.

In conclusion, the present study shows the wide diffusion of resistant strains of propionibacteria among acne patients of our department. The large number of patients sampled allows a precise definition of the problem in Italy, also because 25% of the patients come from districts far from Ferrara. A correlation between the distribution of the resistances and the available antibiotics is plausible. We agree with the recommended strategies available in literature to minimize and overcome resistances during antibiotics treatments. Some help in suggesting the presence of propionibacterial resistance may come from clinical outcome of acne under antibiotic therapy, but the availability of laboratory data is much better in supporting physicians in antibiotic prescription, thus increasing the probability of more successful and quicker therapeutical results in acne.

Riassunto

Antibiotico resistenza propioniatterica a Ferrara

Obiettivo. Gli antibiotici, riducendo il numero di propionibatteri sulla superficie cutanea, rappresentano un efficace presidio nel trattamento dell’acne infiammatoria. L’impiego su larga scala di antibiotici, sia sistemicamente che topicamente, per la cura dell’acne ha determinato la diffusione di ceppi di propionibatteri resistenti. La conseguenza della selezione di ceppi resistenti consiste essenzialmente nel fallimento della terapia antibiotica. L’obiettivo dello studio è la definizione della prevalenza di propionibatteri antibiotico-resistenti all’interno di una vasta popolazione di pazienti acneici affetti alla Sezione di Dermatologia di Ferrara, seguendone contestualmente le eventuali variazioni nel corso di 6 anni (april 2000 - ottobre 2005).

Metodi. A partire da aprile 2000 è stata testata la suscettibilità ai più comuni antibiotici utilizzati nella terapia dell’acne dei ceppi di propionibatteri veicolati dai pazienti acneici con indicazione al trattamento antibiotico. I campioni di propionibatteri sono stati ottenuti dalla cute del volto di 1 579 pazienti acneici mediante l’impiego di un tampone inumidito. Quanto prelevato per mezzo dei tamponi è stato inoculato in terreni di coltura contenenti concentrazioni selettive di tetracicline, minocicline, eritromicina e clindamicina, oltre che in una piastra di controllo. Dopo 7 giorni di incubazione in anaerobiosi a 37°C è stata valutata con metodica semiquantitativa (scala da 0 a 5+) l’entità della crescita delle colonie in presenza di ciascun antibiotico.

Risultati. Sono stati isolati propionibatteri in 1 508 dei 1 579 pazienti testati. La prevalenza di ceppi resistenti ad almeno un antibiotico ammonta al 55,9% del totale. La resistenza all’eritromicina è risultata la più comune in tutti gli anni dello studio, variando dal valore di 58,8% nel 2000 a 38,5% nel 2005 (prevalenza media 47,7%); la resistenza alla clindamicina è compresa tra 44,1% dell’anno 2000 e 32,2% del 2005 (media 39,2%). Il 35% dei ceppi isolati è risultato resistente contemporaneamente a eritromicina e clindamicina. Si è rilevata una resistenza molto bassa alle tetracicline (1,9% alla tetracicline e 0,6% alla minocicline). Nel lasso temporale in cui sono state condotte le analisi è stata registrata una sensibile riduzione della resistenza sia all’eritromicina che alla clindamicina. Questo andamento è imputabile probabilmente a una crescente sensibilità dei dermatologi locali al problema dell’antibiotic resistenza e a una maggiore aderenza alle raccomandazioni impartite dalle attuali linee guida in tema di prescrizione e gestione della terapia antibiotica nella acne. Le più alte percentuali di antibiotico-resistenza sono proprie delle fasce di età più elevate, mentre una prevalenza soltanto leggermente superiore è documentata nei soggetti di sesso maschile rispetto a quelli di sesso femminile.

Conclusioni. I dati mostrano la rilevante prevalenza di ceppi di propionibatteri resistenti a eritromicina e clindamicina nella popolazione di pazienti acneici indagati. Allo stato attuale, viceversa, le percentuali di resistenza alle tetracicline risultano basse. Quanto riscontrato supporta il significato dell’esame colturale nella definizione della sensibilità agli antibiotici dei propionibatteri isolati dai pazienti acneici. Questa procedura è considerata utile sia per un’adeguata gestione terapeutica dell’acne sia per lo studio del fenomeno della resistenza batterica.

Parole chiave: Acne - Propionibatteri - Antibiotico-resistenza.

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Adverse cutaneous reactions to cardiovascular drugs: the experience of the Department of Dermatology in Cagliari

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Aim. Adverse drug reactions (ADR) represent a heterogeneous group of diseases, often responsible for admission or complication during hospitalization. Risk of ADR increases in elderly and medicated patients, with a high prevalence of cardiovascular diseases. The aim of this prospective study was to investigate the frequency, clinical pattern and course of cutaneous adverse reaction to cardiovascular drugs.

Methods. From October 1999 until November 2004 all adverse cutaneous reactions to drugs were recorded on magnetic support, including hospitalized and outpatients of the Dermatology Department of Cagliari University. Cases related to cardiovascular drugs were further investigated for final causality assessment following the international criteria and algorithm of the World Health Organization (WHO) Collaborating Centre for Drug Monitoring.

Results. Four-hundred and nine consecutive patients affected by cutaneous ADR were studied. Antihypertensive drugs were responsible for 8.5% of the overall cases with ACE inhibitors and hydrochlorothiazide being the most reported. Exanthematous eruptions and urticaria-angioedema were the main clinical forms, followed by photosensitivity, pityriasis rosea-like eruption and lichenoid dermatitis, but several life-threatening cases were also observed, including Stevens-Johnson syndrome, toxic epidermal necrolysis and drug rash with eosinophilia and systemic signs. Due to the extension of the eruption and severity of symptoms hospitalization was required in 80% of cases. The main therapeutic measures involved drug discontinuation, antihistamine administration in all cases, supportive care and general corticosteroids in unresponsive severe eruptions and angioedema, intravenous high dose immunoglobulines in 1 case (toxic epidermal necrolysis).

Conclusion. Adverse cutaneous reactions to cardiovascular drugs are frequent, often severe and should not be underestimated in the risk-benefit evaluation of long-term treatment, especially in elderly and medicated patients.

KEY WORDS: Dermatitis, adverse drug reactions - Cardiovascular agents - Risk factors.

Adverse cutaneous reaction is one of the most common undesired effects during systemic pharmacological therapy and often requires admission or is responsible for complications during hospitalisation.\textsuperscript{1-4} A decrease in the incidence of adverse drug reactions (ADRs) seems unlikely at present as increased average life expectancy and the constant rise of available drugs on the market should predictably lead to a corresponding rise in the number of cases and in the variety of possible reactions to any one specific drug.\textsuperscript{5} Cardiovascular drugs represent one of the most widely used groups. These include different categories for a few of which a discreet incidence of adverse cutaneous reactions has been reported.\textsuperscript{6-9} Cardiac problems and arterial hypertension are very frequent conditions whose incidence and need for specific pharmacological treatment increases with the patient’s
Age. A combination of drugs is frequently required to effectively control the pathology and the simultaneous presence of other systemic illnesses and/or metabolic disorders that can increase individual susceptibility to adverse reactions is just as frequent. Advanced age and high dosages are both risk factors for the onset of adverse reactions to drugs, therefore, cardiac and/or patients with hypertension under treatment could represent a category at risk and hence should be monitored very closely from a pharmacological viewpoint. The frequency of adverse cutaneous reactions associated to the treatment of cardiovascular disorders is reported in literature as being extremely variable, ranging from 3-4% to 19-24% but it does not highlight the specific role played by the involvement of the skin in these adverse events.

A prospective study to collect all the adverse cutaneous reactions associated to cardiovascular drugs in order to analyse their type, gravity and course has been carried out at the Pharmacovigilance Centre at the Dermatological Clinic of the University of Cagliari.

### Materials and methods

From October 1999 until November 2004 all adverse cutaneous reactions to drugs were recorded on magnetic support, including hospitalized and outpatients of the Dermatology Department of the University of Cagliari. Particular attention was paid to avoid duplicating cases. Information charts in compliance with those established by the Italian Health Ministry were used and an original copy was sent to the national network of Pharmacovigilance in accordance with current laws. Cases correlated to the use of cardiovascular drugs were selected from the general information and examined separately. Data on the clinical picture, pathological and pharmacological histories, predisposing factors, therapy and course of illness were obtained. Diagnosis of adverse reaction was reached based on clinical criteria, the time span between drug administration and onset of adverse reaction, past history, chemical-laboratory and instrumental investigation to exclude any alternative diagnosis. Skin biopsy was only carried out in dubious cases. Allergy and pharmacological anamnesis as well as previous episodes of reactions to drugs were obtained by interview directly from patients, family members and from family doctor when more precise details were required. 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 administration of a drug and developing a clinical picture. Cases where the contemporary use of cardiovascular drugs with those with a higher level of responsibility for adverse reaction such as antibiotics and non-steroidal anti-inflammatory drugs were excluded from the study.

### Results

Four-hundred and nine cases of adverse cutaneous reaction from drugs were documented over a 5 year period at the Dermatology Clinic of the University of Cagliari. Of these 35 (8.5%) were associated to the exclusive use of cardiovascular drugs. More specifically (Table I), they involved 26 women and 9 men average age 67. In 28 cases (80%), the intensity of the manifestations required hospitalization of the patients in our clinic, in 3 cases the reactions occurred while patients were already in other structures, whilst in 4 cases adverse reactions were observed in outpatients units. The responsible category of drugs was considered to be primarily the ACE inhibitors, followed by hydrochlorothiazide in formulations in which the diuretic was associated with ACE-inhibitors and/or angiotensin II antagonists; cases ascribable to other diuretics, beta blockers, antiaggregants, statins, vasodilators, amiodipine and amiodarone were observed sporadically (Table II).

The spectrum of clinical symptoms was extremely polymorphic showing the following order of frequen-
ADVERSE CUTANEOUS REACTIONS TO CARDIOVASCULAR DRUGS

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Table II.—Adverse cutaneous reactions to cardiovascular drugs: category, generic names and clinical manifestations.

<table>
<thead>
<tr>
<th>Total cases</th>
<th>Drug category</th>
<th>Generic name (N.)</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>ACE-inhibitors</td>
<td>Cilazapril (1)</td>
<td>Urticaria – angioedema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enalapril (1)</td>
<td><em>Pityriasis rosea</em>-like eruption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fosinopril (2)</td>
<td>Urticaria - angioedema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lisinopril (3)</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perindopril (2)</td>
<td><em>Pityriasis rosea</em>-like eruption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramipril (1)</td>
<td>Steven-Johnson syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Steven-Johnson syndrome</td>
</tr>
<tr>
<td>8</td>
<td>Hydrochlorothiazide + ACE-inhibitors or angiotensin II antagonists</td>
<td>Captopril + Hydrochlorothiazide (3)</td>
<td>Maculo-papular exanthema (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lisinopril + Hydrochlorothiazide (2)</td>
<td>Urticaria - angioedema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramipril + Hydrochlorothiazide (1)</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candesartan + Hydrochlorothiazide (1)</td>
<td>Vasculitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Irbesartan + Hydrochlorothiazide (1)</td>
<td>Photosensibility</td>
</tr>
<tr>
<td>4</td>
<td>Diuretics</td>
<td>Furosemide (2)</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Furosemide + Spironolactone (2)</td>
<td>Urticaria – angioedema</td>
</tr>
<tr>
<td>2</td>
<td>Beta-blockers</td>
<td>Atenolol (1)</td>
<td>Psoriasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carvedilol (1)</td>
<td>Lichenoid dermatitis</td>
</tr>
<tr>
<td>1</td>
<td>Calcium channel blockers</td>
<td>Amlodipine</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td>Antiarrhythmics</td>
<td>Amiodarone</td>
<td>Photosensibility</td>
</tr>
<tr>
<td>4</td>
<td>Aggregation inhibitors</td>
<td>Ticlopidine (2)</td>
<td>Urticaria - angioedema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Warfarin (4)</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetylsalicylic acid (1)</td>
<td>Erythroderma</td>
</tr>
<tr>
<td>3</td>
<td>Vasodilators</td>
<td>Nitroglycerin (1)</td>
<td>Photosensibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pentoxifylline (1)</td>
<td>Photosensibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Natifurofufine (1)</td>
<td>Urticaria - angioedema</td>
</tr>
<tr>
<td>2</td>
<td>Hypolipidemic agents</td>
<td>Atorvastatin (1)</td>
<td>Maculo-papular exanthema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluvastatin (1)</td>
<td>Maculo-papular exanthema</td>
</tr>
</tbody>
</table>

Hyperprespillia, considered to be among the main causes of gravity, was present in 8% of the cases, while hematic eosinophilia was present in 27% of the cases. Further clinical-chemical and routine instrumental tests were within the norm with the exception of the drug rash with eosinophilia and systemic signs (DRESS) case where renal and hepatic function were moderately altered in the absence of previous symptoms. Histological tests were carried out only in severe and/or in dubious cases and the clinical suspicion of toxidermia from drugs was always confirmed.

Previous episodes of pharmacological intolerance were highlighted from case histories in 27% of cases. These were generally ascribable to the same category of drugs responsible for the ongoing reaction and this was considered an involuntary rechallenge with regard to causality judgement.

The level of correlation, according to the WHO criteria, between taking the drug and the onset of clinical manifestations was certain in 14.2% of cases, probable in 60% and possible in 18%. The only recognised risk or predisposing factors were advanced age (77.5%) and elevated use of the drugs (31.4%).

Therapy included suspension of the drug with the introduction of a general antihistaminic symptomatic therapy associated with systemically administered corticosteroids in aggravating and/or angioedema cases. In more severe cases all the necessary support measures, based on laboratory tests in accordance with...
indications suggested in literature, were taken to affront the condition of the skin with constant monitoring and integration of the hydro-electrolytic and protein equilibrium. Despite this, in 2 cases of Stevens-Johnson syndrome and in 1 of TEN a progressive and dramatic worsening of the clinical picture was seen which lead to death in 2 cases and the decision to administer 400 mg/kg die of IgG intravenously for 5 days in the third case. This therapy resulted in a rapid halt of the auto-aggressive process and to a slow but complete recovery of skin and mucous function. Clinical recovery was obtained in 94% of cases in a time lapse ranging from 4 to 41 days.

Discussion

The results of this study, even if they have little statistical importance due to the absence of data relative to the consumption of drugs in the same period of time, highlight a discrete frequency of adverse cutaneous reaction to cardiovascular drugs (8%). They also confirm the initial impression matured during the Dermatology Clinic’s Pharmacovigilance daily activity, which in turn lead to the realisation of this study. The Dermatology Clinic, part of the Local Health Authority n. 8 in Cagliari, has about 476 000 patients. It is, therefore, plausible to take into consideration that 1/100 000 inhabitants will encounter adverse cutaneous reaction to cardiovascular drugs. In this type of study, the contemporary use of numerous other drugs is often a confounding factor and it is difficult to determine the effective responsibility of any specific proximate principle. For this reason, all reactions where cardiovascular drugs were not the principle cause were excluded. The rigidity of this exclusion criteria further underlines the possibility that the true incidence of adverse reaction to cardiovascular drugs is underestimated. The distinct prevalence of ACE-inhibitors, alone or in association with hydrochlorothiazide, compared to other categories of cardiovascular drugs is not surprising, given the high incidence of adverse reactions caused by these drugs, but it does indicate the need for an effective re-evaluation of risk and therapeutic benefit. The minor frequency attributed to other categories of antihypertensive drugs could be due to their being prescribed less, as ACE-inhibitors benefited immediately from widespread diffusion from the moment they became available. The ability to induce adverse cutaneous reactions has been reported for every category of cardiovascular drugs and it is possible that the rare likelihood of the event occurring is conditioned solely by the number of people exposed to the drug. The clinical picture has never been evocative of the drug in question with a notable polymorphism of manifestations. Further considerations are raised by the frequency of adverse reactions with the concomitant use of hydrochlorothiazide, in commercial formulas containing ACE-inhibitors and more recently with angiotensin antagonists. Hydrochlorothiazide itself can cause the observed clinical pictures and the highest number of adverse cutaneous reactions from the combination of hydrochlorothiazide and amiloride were reported in
a now dated review. Specific investigations are needed to re-evaluate safety profiles of this drug and its use in combination with other proximate principles especially considering the difficulty in identifying the effective responsibility of any single drug in cases of adverse reactions. Hospitalization was required in most cases (80%), due to the extent and severity of the reactions irrespective of the nature of the drug, with a variety of clinical pictures indicative of the often diagnostic difficulties that adverse reactions to drugs pose. Atypical reactions simulating pityriasis rosea, photodermatitis, and lichen planus, normally considered rare were well represented in case studies observed. This fact may have been conditioned by the family doctor’s ability to identify the more common cutaneous reactions without the need to consult a specialist to isolate the responsible drug when faced with rashes or urticaria. On the other hand, chronic aggravating clinical pictures with polymorph and atypical manifestations, as with the more severe forms, such as vasculitis, Steven-Johnson syndrome (Figure 2), TEN (Figure 3) and DRESS (Figure 4), require complex classification in terms of specific therapy which is often long term. Diagnosis of adverse reactions to drugs is a complex problem due to scarce knowledge of pathogenic mechanisms and to a lack of availability of predictive diagnostic tools. The intrinsic and extrinsic criteria of the WHO algorithm is useful in clinical practice to arrive at a rapid judgement of the cause, which is often expressed in standardized terms of universal interpretation. However, the importance of re-exposure to the drug, though not ethically feasible nor desirable in clinical practice, is essential to reach judgement with certainty. Consequently, judgement was probable in most represented cases (60%), which, however, offers a high and reliable level of responsibility. Fourteen percent of reactions were considered certain where involuntary re-exposure to the drug occurred, in patients who, in their anamnesis, referred previous adverse cutaneous reactions whilst taking the suspected drug. These events not only occurred after an interval of years, as would be justified by a natural tendency to forget previous events or hope they did not reoccur, but in some patients the adverse reactions occurred only a few months earlier with increasing gravity ranging from exanthematic or urticarial episodes to severe reactions such as Stevens-Johnson syndrome in 2 cases. This underevaluation

Figure 2.—Perindopril Steven-Johnson’s syndrome.
of the iatrogenic potential accounts for the patients lack of information, responsibility which, although delicate, at present lies entirely in the hands of the family doctor or specialist and would merit more attention.

Even though reactions have shown a benign course with a tendency to resolve themselves in most cases, symptomatic therapy and intense monitoring were almost always necessary with hospitalization lasting on average 9 days. Prognosis was particularly conditioned by patient’s advanced age and by persistent systemic pathologies which rendered their general conditions even more unstable. On the other hand, the 2 deaths make us reflect on the need to increase therapeutic options as the general support measures suggested in current guidelines alone do not appear to be sufficient to help overcome the auto-aggressive process, especially in elderly patients. Although controversial, the use of intravenous immunoglobulin in high doses is a therapy based on documented pathogenic mechanisms even if they are not exclusive. Personal positive experience, even if limited to one case, confirms the efficacy of this therapeutic approach in grave forms in which the suspension of the causative drug alone, is insufficient to block the progression of the autoimmune process.

Conclusions

The frequency of adverse cutaneous reactions during therapy with cardiovascular and, in particular, antihypertensive drugs would suggest a population at risk and would merit intense systemic monitoring in terms of pharmacovigilance. Precious information regarding drug safety profiles is currently provided by postmarketing studies. However, this primarily con-
Concerns drugs that have only recently been put on the market and do not provide comparative information on different categories of drugs used for the same indications, especially about known proximate principles used over the years, important information which, on the contrary, could emerge from specific studies on the population. Some not easily recognised clinical pictures, the severity and extension of cutaneous reactions in elderly patients are conditions that require early intervention by a specialised dermatologist to reach a quick and correct diagnosis in order to prescribe the necessary therapeutic measures. Close collaboration between family doctors and specialists is desirable to develop a culture of an appropriate use of drugs and to qualify the activity of pharmacovigilance, if we consider that every iatrogenic pathology represents an intrinsic risk for the medical profession and knowledge in this field can only improve the patients conditions of health and quality of life.

Riassunto

Reazioni avverse cutanee da farmaci cardiovascolari: esperienza della Clinica Dermatologica di Cagliari

Obiettivo. Le reazioni avverse da farmaci rappresentano un eterogeneo gruppo di patologie, spesso causa di ricovero o di prolungamento della degenza in pazienti ospedalizzati. Il rischio di sviluppare una reazione avversa aumenta nei pazienti anziani, con un’alta prevalenza di patologie cardiovascolari, che assumono contemporaneamente numerosi farmaci. Lo scopo di questo studio prospettico era indagare la frequenza, le manifestazioni cliniche e il decorso delle reazioni avverse cutanee associate alla somministrazione di farmaci cardiovascolari.

Metodi. Dall’ottobre 1999 al novembre 2004 sono state registrate su supporto magnetico tutte le reazioni avverse cutanee da farmaci osservate presso la Clinica Dermatologica dell’Università degli Studi di Cagliari, sia nei pazienti ricoverati sia in quelli ambulatoriali. I casi associati all’assunzione di farmaci cardiovascolari sono stati ulteriormente studiati ai fini del giudizio di causalità, formulato in accordo con i criteri internazionali e l’algoritmo suggerito dal Centro di Monitoraggio del Farmaco dell’Organizzazione Mondiale della Sanità.

Risultati. Di 409 reazioni avverse cutanee osservate, l’8,5% erano associate all’assunzione di farmaci cardiovascolari, con gli ACE inibitori e l’idroclortiazide tra i farmaci maggiormente riportati. Le principali forme cliniche sono risultate le reazioni esantematiche e l’orticaria-angioedema, seguite dalla fotosensibilità, dalle eruzioni pitiriasi rosealike e dalla dermatite lichenoide, ma non è stata trascurabile la frequenza anche di reazioni severe quali la sindrome di Stevens-Johnson, la necrofisi epidermica tossica e la sindrome da ipersensibilità o drug rash with eosinophilia and systemic signs (DRESS). L’estensione e la gravità dei sintomi hanno comportato l’ospedalizzazione nell’80% dei casi. Le
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principali misure terapeutiche adottate sono risultate la sospensione/sostituzione del farmaco responsabile e la somministrazione di anti-istaminici in tutti i casi, terapia di supporto e corticosteroidi per via sistematica solo nelle eruzioni severe e nell’angioedema, le immunoglobuline ad alto dosage per via endovenosa in 1 caso (necrolisi epidermica tossica).

Conclusioni. Le reazioni avverse cutanee da farmaci cardiovascolari sono frequenti, spesso severe e non dovrebbero essere sottovalutate nella valutazione del rapporto rischi-benefici di un trattamento a lungo termine, specialmente nel paziente anziano che assume contemporaneamente numerosi farmaci.

PAROLE CHIAVE: Reazioni avverse cutanee da farmaci - Farmaci cardiovascolari - Fattori di rischio.

References

A new categorizing scheme for skin types

L. BAUMANN

The current understanding of skin types is based on the pioneering work of Helena Rubinstein in the early 1900s. However, this system, which identifies 4 different skin types - dry, oily, combination, and sensitive - does not adequately address the numerous and growing array of needs presented by modern patients. While the skin care product market has expanded exponentially into a billion-dollar industry, with commensurate improvements in sophistication in product formulation based on a continually growing knowledge base, the dermatologic establishment has lagged behind, failing to make discernible progress in more accurately characterizing skin types. Such an advance would assist patients in finding and using the skin care products most suitable for their particular skin type. Based on several years of clinical experience, I have developed the “Baumann Skin Typing System” contingent on the results of a 64-item questionnaire self-administered by patients. The system and corresponding questionnaire are founded on current classifications of skin based on 4 parameters: dry or oily; sensitive or resistant; pigmented or nonpigmented; and wrinkled or tight (unwrinkled). These categories are not, of course, mutually exclusive. Therefore, 16 possible Baumann skin types are identifiable from the permutations found among the standard 4 skin-type parameters.

The first codified descriptions of different skin types - dry, oily, combination, and sensitive - were presented by Helena Rubinstein in the early 1900s. In the ensuing century, the skin care product market has grown into a colossal billion-dollar industry. It is shocking that we are still using an outdated skin typing system developed over 80 years ago! A more complete skin typing system is needed to assist patients in selecting the most suitable formulations for their particular skin type. Clearly, all products do not work for all skin types. Over the last 9 years of clinical practice, I have concluded that rather than 4 skin types, there are actually 4 skin-type parameters and potential permutations of these parameters yield 16 different skin types. These variations emerge from identifying skin based on 4 parameters or dichotomies: dry vs oily; sensitive vs resistant; pigmented vs nonpigmented; and wrinkled vs tight (or unwrinkled). Determination of the Baumann skin type is made using a questionnaire that is published in the book The Skin Type Solution. While most skin types will favor one end of each spectrum, these categories really depict a continuum and many patients may be identified as having borderline skin types. Interestingly, traveling or moving to a different environment can affect certain skin types (e.g., a borderline dry type may not experience skin dryness until the winter in a northern climate; a borderline resistant type may not have acne or skin rashes unless it is induced by stress, at which point the skin reacts in a more sensitive manner). This article will briefly discuss some of the underlying science pertinent to each
of the 4 skin parameters. Some ideal product choices for particular skin types will also be included.

Skin hydration: dry vs oily

There are several factors involved in the development of xerotic or dry skin, which is characterized by a dull gray white color, rough texture and an elevated number of ridges. The relative condition or status of the stratum corneum, natural moisturizing factor (NMF), hyaluronic acid (HA), and sebum production all play a role in the level of skin hydration. The stratum corneum, which is composed of ceramides, cholesterol, and fatty acids, is the skin barrier responsible for keeping water in the skin, thereby hydrating the largest organ, and keeping exogenous elements from penetrating to internal organs. The 3 primary constituents of the stratum corneum actually surround the barrier in a watertight lipid bilayer and, when present in the proper proportion, form the barrier that acts comparably to a brick wall in which the keratinocytes would represent the bricks and the lipid bilayer, the mortar. Transepidermal water loss (TEWL), which leads to skin dehydration and a dry appearance, can be engendered by various defects or deficiencies in the stratum corneum. The skin barrier can be genetically deficient or disturbed by any of several external compounds, such as detergents, acetone, chlorine and other chemicals, as well as prolonged water immersion.

Skin with an impaired stratum corneum would be both dry and sensitive because besides being incapable of retaining water, it would be more susceptible to damage from external sources such as plants, chemicals and even water. Further, cholesterol-lowering drugs are well known to cause xerosis. Topical skin care products designed for dry skin concentrate on repairing the 3 key constituents of the stratum corneum: ceramides, cholesterol, and fatty acids. Repair or replenishment is also possible through diet, given that cholesterol and fatty acids can be derived from a healthy diet. Consumption of evening primrose oil, borage oil, or omega-3 fatty acids has the potential to assist in improving dry, sensitive (DS type) skin by replacing the essential skin barrier constituents. Barrier repair moisturizers are especially appropriate for this type.

A DS type that suffers frequently from erythema and pruritus also likely has a defect in the stratum corneum. People with this skin type are more susceptible to developing eczema. In addition to the above recommendations, DS types are advised to avoid harsh foaming detergents that can strip lipids from the skin. Those with very dry skin should also use humidifiers when possible in low-humidity environments, avoid prolonged baths especially in hot water or chlorinated water, and moisturize frequently (2-3 times a day). DS types with just slightly dry skin likely have a minor imperfection in the skin barrier and probably experience irritation in the winter, in low-humidity environments, and/or upon using a harsh foaming soap.

Found in keratinocytes, NMF is a substance derived from the hydrolysis of the protein filaggrin that holds water inside the cells, making them plump. Filaggrin confers structural support in the dermal layers, assisting in providing strength to the skin; in the epidermal layers, the protein is broken down into NMF, which has a potent capacity to bind water, keeping it in the keratinocyte. The pace at which filaggrin is broken down into NMF is affected by environment or climate conditions. For instance, after several days of transition from a high-humidity to low-humidity environment, an individual’s skin will manufacture more NMF, helping the skin hold onto water. Further, UV radiation was shown in the 1970s to disrupt the enzymatic hydrolysis of filaggrin to NMF. Consequently, reducing sun exposure is understood as one way to ameliorate xerosis of filaggrin to NMF. Conversely, reducing sun exposure is understood as one way to ameliorate xerosis due to low NMF levels. Presently, however, there are no known methods for spurring the breakdown of filaggrin thereby increasing NMF levels. It is important to note that NMF deficiency can cause dry skin, but it does not increase skin sensitivity. Indeed, an individual experiencing low NMF levels typically falls into the dry, resistant (DR) category. Skin that is resistant and just slightly dry is likely supported by an intact stratum corneum; the dryness more likely is due to low NMF or sebum secretion. Skin hydration in these circumstances can be improved through the use of moisturizing lotions and creams, avoiding harsh detergents such as those found in foaming cleaners, and avoiding UV exposure.

HA, which can bind 1 000 times its weight in water, is another substance found in the skin that holds onto water. One of the fundamental building blocks of the skin, HA helps confer structure, providing plumpness to the skin. Aged skin is characterized by reduced levels of HA, yielding a less plump, more dehydrated appearance. Such a deficiency in HA, while imparting dryness, does not increase sensitivity. It is important to
note that HA does not penetrate the skin, so topical
products containing HA do not comprise a viable ther-
apy. The production of HA can be enhanced, how-
ever, through oral supplementation with glucosamine;
wrinkles can be temporarily ameliorated via superfi-
cial or deeper injections of HA. Low HA levels are
often associated with the dry, wrinkled (DW) skin
type.

Sebum production is also believed to potentially play a role in the manifestation of xerosis. Sebaceous
gland secretions contain wax esters, triglycerides, and
squalene, which protect the skin from the environ-
ment. Fats derived from sebum prevent TEWL through
the formation of a lipid film over the skin surface, and
thereby might protect the skin from dryness. Low
sebaceous gland activity is not correlated with the occurrence of xerosis, however, and the effects of
sebum on dry skin conditions have not been elucidat-
ed. In fact, decreased sebum production is an uncom-
mon complaint of patients; rather, many more complain
about increased sebum production, which results in
oily skin, and possibly acne. Sebum production levels
can be influenced by diet, stress and hormones, as
well as heredity. In a study of 20 pairs each of identi-
cal and nonidentical like-sex (to eliminate hormonal
considerations) twins, identical twins had virtually
identical sebum excretion rates, despite significantly
different acne severity, whereas the nonidentical twins
differed significantly in terms of sebum excretion rates
and acne severity, suggesting the interplay of both
genetic and environmental influences. Postmenopausal
women often experience xerosis correlated with
reduced sebum production as a function of changes in
hormone levels; hormone replacement therapy can
normalize sebum production. Small pores, and mini-
mal acne history along with dry skin is characteristic
of the DR skin type.

Skin that is slightly oily and straddles the sensi-
tive/resistant threshold is ideal in terms of skin hydra-
tion. Such skin is characterized by an intact skin bar-
rier, sufficient NMF levels, and adequate sebum secre-
tion such that acne development is less likely. People
with oily, resistant (OR) skin rarely suffer from acne.
However, stress or hormonal fluctuations can induce
acne in such skin types. In these circumstances, oral
contraceptives, when appropriate, can be used to pre-
vent hormonal fluctuations, and a benzoyl peroxide-
containing wash can be used pre-emptively before an
expected stressful situation. Individuals with slightly
oily or very oily skin that is also sensitive (OS) likely
suffer from acne or rosacea. In addition, this skin type
that is accompanied by wrinkled (W) skin with exten-
sive sun damage is more prone to develop rosacea.
OS types are fortunate insofar as they are better able
to tolerate products that DS types cannot.

Skin sensitivity: sensitive vs resistant

Sensitive skin can best be described as hyperreactive
skin characterized by a weaker stratum corneum leav-
ing it more vulnerable to exogenous factors (i.e., envi-
ronmental influences such as cold, heat, temperature
variation, and wind, as well as pollution and exces-
sive use of topical agents) and more prone to reacting adversely to such factors. These reactions, in turn, are
characterized by a disrupted stratum corneum and a
tendency to experience exaggerated neurosensory
responses to topically applied products.

Resistant skin is characterized by a potent, solid
stratum corneum that imparts protection to the skin,
providing a shield around the skin cells thereby keep-
ing out allergens and irritating substances. Such skin
is rarely associated with erythema (unless sunburned)
or acne (unless induced by stress or hormonal changes).
The application of cosmetic products seldom elicits complaints from people with resistant skin; such types
can typically use any skin care formulation without
developing any adverse reactions. Unfortunately, these
patients may also fail to derive any benefit from skin
care products because the formulations are not suffi-
ciently potent to penetrate the stratum corneum of
resistant skin.

Whereas it seems almost irrelevant as to which prod-
ucts a person with resistant skin might use (in terms of
potential adverse reactions, not efficacy), great care
must be taken in selecting appropriate products for
sensitive skin. Indeed, sensitive skin is a dynamic
process that is reported to occur in as much as 40% of
the population. Healthy, premenopausal women com-
prise the majority population segment complaining of
sensitive skin and the incidence of sensitive skin
appears to decline with age. It is not a subclinical man-
ifestation of contact allergy, but can be divided into 4
distinct subtypes all of which feature inflammation as
the common denominator. Consequently, all products
formulated to treat sensitive skin are intended to alle-
viate and eliminate the cause(s) of inflammation.
Specifically, though, treatment must address the nature
of a patient’s particular sensitive skin subtype: the acne subtype (predilection for developing acne, blackheads, or whiteheads), the rosacea subtype (tendency to experience recurrent flushing, facial erythema, and sensation of warmth or heat), the stinging subtype (characterized by complaints of stinging or burning skin), or the allergic subtype (characterized by erythema, pruritus, and skin flaking). Such considerations should be taken into account when considering the plethora of cosmetic products touted for activity in sensitive skin.

Exaggerated skin reactivity is most frequently linked with the face, which relates to both the acne and rosacea sensitive skin subtypes. Both conditions have been associated with increased skin sensitivity or reactivity.

Acne type

The etiology of acne is characterized by 3 primary features: elevated sebum production; clogged pores (adhering of dead skin cells inside the hair follicles, which might be further stimulated by increased sebum production); and the presence of the bacteria Propionibacterium acnes. The pathogenetic pathway proceeds as increased amounts of sebum cause dead skin cells in the hair follicles to stick together, thus clogging the follicle and forming a papule or pustule. Then, P. acnes enter the hair follicle, attacking the amassed sebum and dead skin cells, triggering the release of cytokines and other inflammatory factors. This engenders the inflammatory response that results in the characteristic redness and pus. Acne prevention is geared toward attacking the 3 primary etiologic factors, namely reducing sebum production (with retinoids, oral contraceptives or stress reduction), unclogging pores (with retinoids, AHAs, or BHA), and eliminating bacteria (with benzoyl peroxide, sulfur, antibiotics, or azelaic acid). However, not all therapeutic regimens are suitable for all sensitive acne skin types. The OS skin type is the one most likely to develop acne. For these patients, salicylic acid (BHA) and benzoyl peroxide are often the best therapeutic options, unless erythema and stinging is also exhibited, in which case the anti-inflammatory salicylic acid is optimal as a single therapy.

Rosacea type

Characterized by facial redness, flushing, pimples, and the formation of telangiectases in the face, rosacea typically affects adults between 25 and 60 years of age. This condition, which itself is classified into several subtypes, is seen most often in fair-skinned people prone to blushing and flushing, particularly in association with strong emotion. The etiology of rosacea is not clearly understood. Helicobacter pylori, the bacteria implicated in stomach ulcers that also affects nearly half of the world’s population, has long been considered a possible causative agent in rosacea pathogenesis. However, no definitive link has been shown. Rosacea patients with inflammatory papules and facial erythema are advised to get tested for H. pylori, nevertheless, because treatment with oral antibiotics for H. pylori has been shown to improve rosacea outbreaks. Topical skin care for rosacea focuses on preventing exacerbation of the condition. There is no cure. OS types with fair skin, who also have W skin with a strong history of sun damage, are the most likely to develop rosacea. For OS patients with rosacea, products that contain feverfew (such as the Aveeno Ultra calming line), the anti-inflammatory quadrinone (such as the Cutanix products), or the licorice extract licochalcone (such as the Eucerin products) are effective options. For DS rosacea patients that experience erythema but no stinging, products containing sulfacetamide (Rosanil, Rosac, Rosula, Avar), or azelaic acid (such as Azelex and Finacea) may be suitable choices. Despite the moderate success seen in the amelioration of rosacea symptoms from topical prescription medications (e.g., Elidel, Rosanil and Plexion cleansers, Metro Gel, and Rosac cream), intense pulsed light therapy may represent the most significant advance in the arsenal of rosacea treatments.

Stinging type

This subtype of sensitive skin refers to nonallergic stinging that results from what is believed to be amplified neural sensitivity. Referred to as “stingers”, patients with such skin experience subjective cutaneous irritation in reaction to particular ingredients to which others do not respond. Several tests can be used to identify the propensity to experience stinging. In particular, the lactic acid stinging test is a widely accepted standard method for evaluating reported subjective cutaneous irritation. In 5% and 10% lactic acid concentrations, the lactic acid stinging test has been used to show that a subset of patients experience stinging sensations in the treated nasolabi-
al fold area unlike healthy controls.\textsuperscript{17, 18} The stinging sensation is not necessarily associated with erythema. Rosacea patients that experience facial flushing have a greater tendency to feel stinging in response to lactic acid.\textsuperscript{19} Many patients have reported experiencing stinging without redness or irritation, though.\textsuperscript{20} Sensitive “stingers” are advised to avoid the following products or ingredients: benzoic acid, bronopol, cinnamic acid compounds, Dowicel 200, formaldehyde, lactic acid, propylene glycol, quaternary ammonium compounds, sodium lauryl sulfate, sorbic acid, urea, vitamin C, and alpha hydroxyl acids (particularly glycolic acid).

\textbf{Allergic type}

The fourth type of sensitive skin is the allergic subtype. A recent epidemiologic survey in the UK indicated that, over the course of a year, 23\% of women and 13.8\% of men experience an adverse reaction to a personal care product (\textit{e.g.}, deodorants and perfumes, skin care products, hair care products, and nail cosmetics).\textsuperscript{21} Patch testing is typically used to identify allergies to cosmetic ingredients. This technique has also revealed that up to 10\% of dermatologic patients patch tested manifest allergic responses to at least one cosmetic product ingredient.\textsuperscript{21} This figure is probably an under-representation because most patients do not consult a physician upon reacting to new cosmetic products. Patients aged 20-60 years account for 80\% of reported reactions, with the majority occurring in women.\textsuperscript{22} Preservatives and fragrances are the most common allergens in skin care products. The International Fragrance Association (IFRA) has begun work with dermatologists to develop and market safe fragrance-containing products. Given the growing popularity of aromatherapy, this endeavor is likely to benefit many people upon the successful use of fragrance without causing a significant number of allergic reactions.

As the frequency of exposure to ingredients increases along with exposure to an increasing array of ingredients, an individual becomes more likely to develop an allergy to cosmetic ingredients. Allergic reactions to topical allergens are most frequently seen in patients with an impaired \textit{stratum corneum}, manifested by dry skin.\textsuperscript{23} Therefore, in terms of the new skin typing system, most sensitive skin patients with the allergic subtype are DS types.

\textbf{Skin pigmentation: pigmented vs nonpigmented}

This parameter does not consider ethnicity; rather, it measures the proclivity to develop unwanted dark spots on the face or chest. For instance, a white person with freckles and red hair as well as a black person with melasma would be categorized as pigmented (P) types. The P skin category is most often correlated with the W type in fair-skinned individuals because of the causal link between solar exposure and rhytides and solar lentigines. Many dark-skinned individuals have pigmented tight (PT) skin, though, by dint of a lesser propensity to wrinkle. It is important to note, though, that not all dark-skinned patients suffer from pigmentary problems. Many exhibit even skin tones and no hyperpigmentary spots. Such individuals have nonpigmented (N) type skin.

Several dyspigmentations qualify as dark spots that evoke cosmetic concerns among patients, including ephelides, melasma, solar lentigines, seborrheic keratoses, nevi, moles, etc. Some of these conditions are avoidable, such as ephelides, melasma, and solar lentigines, which can be prevented and treated with skin care products and procedures. Twenty-one percent of dermatologic visits are made by patients seeking such treatments and more than 80,000 people in the US alone annually buy over the counter (OTC) skin care products to reduce or eliminate such discolorations (Data on file Galderma).

Individuals with P type skin are best treated with formulations that contain hydroquinone, kojic acid, arbutin, tyrostat and mulberry extract. Vitamin C and retinoid usage is also appropriate for people that have oily resistant pigmented and wrinkled (ORPW) or dry resistant pigmented and wrinkled (DRPW) skin. Products containing soy (such as the Aveeno Positively Radiant line and Neutrogena Visibly Even products) or niacinamide (such as Olay Regenerist and Olay Total Effects or the products by Niadyne) successfully prevent the return of undesired hyperpigmentations. Of course, the best method to prevent the avoidable forms of skin pigmentation is to curtail or avoid sun exposure, given that UV exposure increases melanosome transfer from melanocytes to keratinocytes.\textsuperscript{24} Because most formulations fail to block both UVA and UVB rays, sunscreens are less effective and even broad spectrum products that hinder both UVA and UVB are unable to block all solar rays. That said, sunscreens remain important for skin protection in terms of fighting photoaging and skin cancer and, specifically, at reducing unwanted pigmentary changes as well as wrinkles.
Skin aging: wrinkled vs tight

Although there are some products and procedures that can ameliorate the appearance of hyperpigmentations and behavioral options that can help one avoid the development of such alterations, the wrinkled/tight (W/T) skin parameter is actually the only 1 of the 4 skin-type parameters completely within an individual’s control. Specifically, in reference to the P/N parameter, an individual cannot change the genetic aspect of skin aging but can alter one’s lifestyle to eliminate or reduce behaviors known to spur extrinsic aging, namely excessive use of alcohol, poor nutrition, smoking, and, most importantly, sun exposure. In fact, sun exposure is routinely cited as the source for 80% of facial aging.

Wrinkles, the primary manifestation of facial skin aging (solar lentigos are another significant manifestation), result from damage that occurs in the dermal layers of the skin. Most skin care products lack the capacity to penetrate deeply enough into the dermis to repair the damage at the base of wrinkles, however. Exceptions to this state of affairs include retinoid products approved by the US Food and Drug Administration (FDA) for the indication of wrinkle correction. Several studies demonstrate that these retinoids, Renova and Avage, which are available through prescription, are effective in ameliorating wrinkles. Consistent use of topical antioxidants likely prevents some extrinsic aging as well. Treatment options for wrinkle prevention focus on stopping the erosion of collagen, elastin, and HA since aged skin is known to possess less of all of these fundamental structural constituents. Therefore, most antiaging products are designed to salvage these components, though no known products or procedures can yet effectively restore elastin. Further, antiaging regimens are also intended to reduce inflammation since inflammation can foster the erosion of collagen, elastin, and HA.

Skin aging can be reduced through various behavioral modifications. Specifically, avoiding sun exposure, cigarette smoke, and pollution will benefit all skin types as will eating a diet high in fruits and vegetables, taking antioxidant supplements, and using sunscreen. Regularly using prescription retinoids can also benefit all W types. Differin is likely best suited for sensitive wrinkled (SW) types. For oily wrinkled (OW) types, a retinoid in gel form is recommended; for DWs, a cream retinoid. Dermatologic procedures (particularly the injection of Botox or Reloxin) can prevent wrinkles caused in areas of movement by significantly reducing movement in those areas. Changing lifestyle habits can change a skin type from a W to a T.

The topical antioxidants that can benefit W types include idebenone (found in Prevage), ferulic acid (found in Skinceuticals C E Ferulic), vitamin C (such as the La Roche Posey product Active C), and mushroom extract (found in the Dr. Andrew Weil for Origins Plantidote Mega Mushroom Face Serum). Oral antioxidants such as polypodium leucotomos (found in Heliocare) or pomegranate (found in Murad Pomepol Sunguard Supplement) may also be helpful.

In line with the argument that not all skin care formulations are suitable for all skin types, it should be noted that sunscreen recommendations must also be tailored to skin type. Generally, people with oily skin prefer gel or powder sunscreens whereas individuals with dry skin tend to prefer creams. Sensitive skin types may benefit from using physical sun blocking agents such as zinc oxide and titanium dioxide while resistant types can use chemical sunscreens. Avobenzone, a UV A blocker, can cause stinging in sensitive skin types. Pigmented types that have darker skin may choose a tinted sunscreen product to avoid the violet hues associated with the white thicker opaque sunscreens. All types should use a high sun protection factor sunscreen whether they have a tendency to wrinkle or not.

Conclusions

A new system of skin categorization based on 4 fundamental parameters of describing skin expands our understanding of particular skin proclivities. Acknowledging that the 4 skin parameters - dry or oily, sensitive or resistant, pigmented or nonpigmented, and wrinkled or tight - are not mutually exclusive allows for a more nuanced picture of skin types, with 16 permutations of skin types emerging. The more accurate identification of skin types that is yielded by answers to a 64-item self-administered questionnaire facilitates the selection, by practitioner and patient alike, of the most suitable topical products to treat the patient’s particular skin type and conditions, enhancing overall skin health.

Riassunto

Un nuovo schema di classificazione dei tipi cutanei

L’attuale suddivisione dei tipi cutanei è basata sul lavoro pionieristico di Helena Rubinstein, eseguito nei primi del
A NEW CATEGORIZING SCHEME FOR SKIN TYPES

BAUmann

900. Tuttavia, questo sistema, che identifica 4 diversi tipi di cute - secca, grassa, mista e sensibile - non è più in grado di soddisfare le crescenti e numerose esigenze presentate dalle pazienti attuali. Mentre l'industria cosmetica si è espansia in modo esponenziale, fatturando miliardi di dollari, migliorando continuamente la qualità della formulazione dei prodotti sulla base delle conoscenze di base in continuo aumento, l'aspetto strettamente dermatologico non ha seguito questo progresso e non è stato in grado di caratterizzare più accuratamente i tipi cutanei. Un progresso in questo settore aiuterebbe le pazienti nella scelta e nell'utilizzazione dei prodotti più adatti per la cura del loro specifico tipo di cute.

Sulla base di diversi anni di esperienza clinica, ho sviluppato il “Bauman Skin Typing System”, che tiene conto dei risultati ottenuti da un questionario con 64 domande autosomministrato alle pazienti. Il sistema e il corrispondente questionario si basano sull'attuale classificazione della cute che tiene conto di 4 parametri: cute secca o grassa; sensibile o resistente; pigmentata o non pigmentata; rugosa o liscia (non rugosa). Naturalmente, queste categorie non sono mutualmente esclusive. Di conseguenza, con il sistema Bauman è possibile distinguere 16 tipi cutanei nell'ambito dei 4 tipi standard.

Parole chiave: Tipi cutanei - Cute - Questionari.

References

Primary cutaneous B-cell lymphomas (PCBCLs) represent an heterogeneous group of lymphoid malignancies with various clinicopathologic presentation and prognosis. Primary cutaneous lymphomas are defined as malignant lymphomas confined to the skin at presentation after complete staging procedures. Thus, staging investigations are mandatory for all patients. The classification of cutaneous lymphomas published by the World Health Organization (WHO) and the European Organization for Research and Treatment of Cancer (EORTC) – Cutaneous Lymphoma Project Group recognizes four main types of PCBCL: follicle center lymphoma and marginal zone B-cell lymphoma are in a group characterized by an indolent clinical behavior (five-year survival >90%), whereas diffuse large B-cell lymphoma, leg type, and diffuse large B-cell lymphoma, other (this last including several rare variants) are in a group with intermediate clinical behavior (five-year survival 50-60%). Management of patients depend on precise classification as well as on clinical presentation (e.g., solitary or multiple tumors, age, general conditions, etc.). The main therapeutic strategies for PCBCLs include local radiotherapy, surgical excision, anti-CD20 antibody (rituximab), interferon-alfa, and systemic chemotherapy (usually CHOP). It must be stressed that systemic chemotherapy is needed only rarely in cases of PCBCL with indolent behaviour. Cases associated with infection by Borrelia burgdorferi may be managed with antibiotic treatment. Selected patients may be followed-up at regular intervals according to a so-called “watchful waiting” strategy.

**KEY WORDS:** Skin - B cell cutaneous lymphoma, diagnosis - B cell cutaneous lymphoma, therapy.
Most patients with CBCL are diagnosed primarily by dermatologists, as extracutaneous symptoms and signs are very rare at onset of the disease. Thus, dermatologists should be conversant with the clinico-pathologic features of this group of disorders, in order to be able to establish the diagnosis at an early stage. Additionally, as aggressive treatment modalities are needed only in selected cases, these patients may be managed primarily in dermatology departments with special expertise in cutaneous lymphomas.4-6

Primary cutaneous B-cell lymphomas can arise at skin sites affected by acrodermatitis chronica atrophicans, and may be linked to infection by Borrelia.8 In fact, Borrelia DNA sequences have been demonstrated by polymerase chain reaction (PCR) analysis in skin lesions, particularly in cases of cutaneous marginal zone B-cell lymphoma. However, association with Borrelia infection may be linked to specific species of the microorganism and/or to particular regions of the world, as a PCR study from the United States did not reveal any positivity for Borrelia DNA.9 Although it has been suggested that other infectious agents may play a role in the etiology and/or pathogenesis of PCBCL, compelling evidence for association with microorganisms other than Borrelia is lacking.10, 11 The role of T lymphocytes and accessory cells in the development of PCBCL has been investigated in the past,12, 13 but at present the exact mechanism of recruitment and proliferation of neoplastic cells within the skin is not completely understood. In fact, although T lymphocytes are a constituent of so-called skin-associated lymphoid tissue (SALT), providing an explanation for the high percentage of primary cutaneous T-cell lymphomas, B lymphocytes and the corresponding microenvironment are not present in the skin under normal conditions.

Cutaneous B-cell lymphomas may arise rarely in the setting of immunodepression due to infection with the human immunodeficiency virus I (HIV-I) or to immunosuppressive therapy, or during long-term treatment with methotrexate.4, 14-17 Many of these cases are associated with infection with the Epstein-Barr virus (EBV) or the human herpes virus 8 (HHV-8). Cases due to immunosuppression after transplantation may respond to discontinuation of the immunosuppressive treatment.

**Classification**

In the WHO-EORTC classification, PCBCLs have been divided into four major types.1 Follicle center lymphoma and marginal zone B-cell lymphoma are in a group characterized by an indolent clinical behavior, whereas diffuse large B-cell lymphoma, leg type, and diffuse large B-cell lymphoma, other (this last including several rare variants) are in a group with intermediate clinical behavior. Although in the past some authors suggested that PCBCLs may represent an homogeneous group of disorders,18-21 the 4 diagnostic categories listed in the WHO-EORTC classification have been widely accepted and provide a common basis for diagnosis of these diseases, thus allowing a meaningful comparison of cases observed in different centers as well as in different countries.22, 23 Besides the entities listed in the WHO-EORTC classification, there are rare reports of other types of B-cell lymphoma occurring in the skin.24 It must be emphasized that, in addition to PCBCLs, the skin can be a site of secondary involvement for practically all types of extracutaneous (usually nodal) B-cell lymphomas and leukemias.4, 5, 25 Consequently, complete staging procedures must be performed in all

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**Table I.** — WHO-EORTC classification of primary cutaneous lymphomas.

<table>
<thead>
<tr>
<th>Cutaneous T-cell and NK-cell lymphomas</th>
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<tbody>
<tr>
<td>Mycosis fungoides</td>
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<tr>
<td>Mycosis fungoides variants and subtypes</td>
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<tr>
<td>— Folliculotropic mycosis fungoides</td>
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<tr>
<td>— Pagetoid reticulosis</td>
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<tr>
<td>— Granulomatous slack skin</td>
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<tr>
<td>Sézary syndrome</td>
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<tr>
<td>Adult T-cell leukemia/lymphoma</td>
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<tr>
<td>Primary cutaneous CD30+ lymphoproliferative disorders</td>
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<tr>
<td>— Primary cutaneous anaplastic large cell lymphoma</td>
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<tr>
<td>— Lymphomatoid papulosis</td>
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<tr>
<td>Subcutaneous panniculitis-like T-cell lymphoma</td>
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<tr>
<td>Extramedullary NK/T-cell lymphoma, nasal type</td>
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<tr>
<td>Primary cutaneous peripheral T-cell lymphoma, unspecified</td>
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<tr>
<td>— Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional)</td>
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<tr>
<td>— Cutaneous γδ T-cell lymphoma (provisional)</td>
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<tr>
<td>— Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma (provisional)</td>
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<table>
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<th>Cutaneous B-cell lymphomas</th>
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<tr>
<td>Primary cutaneous marginal zone B-cell lymphoma</td>
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<td>Primary cutaneous follicle center lymphoma</td>
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<tr>
<td>Primary cutaneous diffuse large B-cell lymphoma, leg type</td>
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<tr>
<td>Primary cutaneous diffuse large B-cell lymphoma, other</td>
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<td>— Intravascular large B-cell lymphoma</td>
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<th>Precursor hematologic neoplasm</th>
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<tr>
<td>CD4+/CD56+ hematodermic neoplasm (blastic NK-cell lymphoma)</td>
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patients with a confirmed diagnosis of B-cell lymphoma involving the skin. These include a complete blood examination, bone marrow biopsy, and computed tomographic (CT) examination of the chest, abdomen, and pelvis. Primary cutaneous lymphomas are defined as “malignant lymphomas confined to the skin at presentation after complete staging procedures”.

Distinction of some PCBCLs from reactive cutaneous infiltrates may be very difficult (this is particularly true for marginal zone B-cell lymphoma). Although clinicopathologic correlation and ancillary techniques may provide important informations on phenotype, clonality, and genetic features of the infiltrate, in some cases a precise diagnosis may be impossible. The term “atypical lymphoid proliferation” is used sometimes to refer to lesions in which the diagnosis is not clear-cut. Patients are put on short-term follow-up controls, and eventual new lesions are biopsied.

Primary cutaneous follicle center lymphoma

Primary cutaneous follicle center lymphoma (PCFCL) is defined as the neoplastic proliferation of germinal center cells confined to the skin. It represents a very common subtype of PCBCL, and is observed usually in adults or elderly patients. Preferential locations are the scalp and forehead or the back. The prognosis of patients with cutaneous PCFCL is favorable. Recurrences are observed in up to 50% of the cases, but dissemination to lymph nodes or internal organs is rare. It seems that cases of PCFCL, diffuse type arising on the legs may have a worse prognosis than those arising at other body sites. On the other hand, the difference in prognosis may be due to the difficulties in separating clearly some cases of PCFCL, diffuse type from those of diffuse large B-cell lymphoma, leg-type.

Clinically, patients present with solitary or grouped erythematous to deep red papules, plaques, or tumors which, especially on the trunk, can be surrounded by erythematous patches and papules (Figures 1, 2). In the past, lesions located on the back were referred to as Crosti’s lymphoma or reticulohistiocytoma of the dorsum. The skin lesions are usually asymptomatic, and systemic symptoms are very rare.

Histopathology shows nodular or diffuse infiltrates within the entire dermis, often extending into the subcutaneous fat. The epidermis is usually spared. A clear-cut follicular pattern with formation of neoplastic germinal centers can be observed in a minori-
ty of cases only \(^4, 32, 39\) (Figure 3). However, a higher percentage of cases show at least some follicular architecture. In the cases with a follicular pattern, the neoplastic follicles show morphologic features of malignancy including a reduced or absent mantle zone, the lack of tingible body macrophages, and a monomorphism of the follicles — so-called ‘dark’ and ‘clear’ areas are no longer recognizable. \(^32, 40\) Lesions presenting with a purely diffuse pattern of growth are characterized by predominance of medium-large cleaved cells (large centrocytes) (Figure 4) and may be misdiagnosed as cutaneous diffuse large B-cell lymphoma, but the prognosis of these cases is similar to that of lesions of PCFCL without large cell morphology. \(^1, 35, 41\)

Morphologic variants of PCFCL include those with a predominant spindle cell morphology (sometimes showing a sclerotic or desmoplastic stroma), \(^42-45\) and cases in which a few B-cell blasts are admixed with numerous T-lymphocytes (‘T-cell rich B-cell lymphoma’), a type of PCBCL that in the skin probably represents a rare morphologic variant of PCFCL. \(^4, 46\)

The tumor cells of PCFCL express B-cell-associated antigens (CD20, CD79a). When follicles are present, they are characterized by an irregular network of CD21+ follicular dendritic cells. Bcl-6 is positive in virtually all cases, irrespective of the pattern of growth. In all cases with a follicular growth pattern, and in a minority of those with a diffuse growth pattern, neoplastic cells stain positive also for CD10. The presence of small clusters of Bcl-6+ and/or CD10+ cells outside the follicles is considered strongly suggestive of a diagnosis of follicular lymphoma. \(^32, 40\) In cases with large cell morphology (predominance of large cleaved cells), the main criteria for differential diagnosis from diffuse large B-cell lymphoma, leg-type include predominance of large cleaved cells over large round cells, and lack of Bcl-2 and MUM-1 expression.
(in PCFCL MUM-1 is either negative or expressed by a small minority of neoplastic cells). The clinical aspect of these cases is also helpful, as the great majority of them present with the features of so-called Crosti’s lymphoma (tumors and nodules on the trunk, usually the back, surrounded by erythematous papules and patches). PCFCL, diffuse type should be distinguished also from cases of *Borrelia burgdorferi-* induced lymphocytoma cutis, a reactive condition that may be mistaken for a large B-cell lymphoma, with catastrophic consequences for the patients.

In the overwhelming majority of cases, staining for the protein product of *BCL-2* (Bcl-2) yields negative results, representing a major difference from follicular lymphomas arising within lymph nodes. When present, Bcl-2 expression is confined usually to a small minority (<30%) of the neoplastic cells, and only very rarely shows the strong, uniform positivity characteristic of nodal follicular lymphoma. A useful, though counterintuitive diagnostic immunohistochemical feature is the lower degree of proliferative activity in malignant follicles as detected by the MIB-1 (Ki-67) antibody, contrasting with the strong MIB-1-positivity observed in reactive ones.

The interchromosomal 14;18 translocation, typically found in nodal follicular lymphomas, is present only in a very small percentage of cases of PCFCLs. In fact, presence of the interchromosomal 14;18 translocation or expression of Bcl-2 should raise suspicion that the patient has a systemic lymphoma involving the skin. Standard molecular analyses are of limited value in the diagnosis and differential diagnosis of PCFCL, and somatic mutations may hinder the evaluation of immunoglobulin heavy chain (Iκ) gene rearrangement. Data obtained with DNA microarrays revealed that the genetic signature of PCFCL, diffuse type differs from that of diffuse large B-cell lymphoma, leg type.

**Primary cutaneous marginal zone B-cell lymphoma**

Primary cutaneous marginal zone B-cell lymphoma (PCMZL) has been recognized as a distinct variant of low-grade malignant PCBCL closely related to mucosa-associated lymphoid tissue (MALT)-lymphomas. Cases classified in the past as primary cutaneous immunocytoma or primary cutaneous plasmacytoma represent most likely examples of PCMZL with prominent lymphoplasmacytic or plasmacytic differentiation, respectively, and the terms “cutaneous immunocytoma” and “cutaneous plasmacytoma” have been avoided in the new WHO-EORTC classification. Patients are adults, but children can be rarely affected as well. Preferential locations are the upper extremities or the trunk. The prognosis of PCMZL is excellent. In a study of 32 patients, none developed lymph node or internal involvement after a mean follow-up of more than 4 years.

Clinically, patients present with recurrent red to red-brown papules, plaques, and nodules (Figure 5). Generalized lesions can be observed in a small number of patients. Ulceration rarely, if ever, occurs. Skin lesions are usually asymptomatic, and systemic signs and symptoms, such as fever, night sweats, weight loss, and malaise (B-symptoms), are not present. In some instances, resolution of lesions may be accompanied by secondary anetoderma, due to loss of elastic fibers in the area of the tumor infiltrate.

Histology of PCMZL shows nodular or diffuse infiltrates involving the dermis and, rarely, the subcutaneous fat. The epidermis is spared. A characteristic pattern can be observed at scanning magnification: nodules of lymphocytes, sometimes containing reactive germinal centers, are surrounded by a pale-staining
population of medium-sized cells with indented nuclei, inconspicuous nucleoli, and abundant pale cytoplasm – variously described as marginal zone cells, centrocyte-like cells, or monocytoid B-cells. In addition, plasma cells (at the margins of the nodules), lymphoplasmacytoid cells, and occasional large blasts are observed. It must be clearly underlined that in most cases of PCMZL neoplastic lymphocytes are only a minority of the infiltrate, which is dominated by reactive T- and B-lymphocytes, admixed often with eosinophils and in some cases with a granulomatous reaction. In addition, the neoplastic population in most cases is not monomorphous, but consists rather of cells with different morphology (marginal zone cells, lymphoplasmacytoid cells, plasma cells) (Figure 6A). Cases with predominance of lymphoplasmacytoid lymphocytes were classified as cutaneous immunocytoma in the past (Figure 6B). In these cases, PAS-positive intranuclear inclusions (Dutcher bodies) are sometimes observed and represent a valuable diagnostic clue. In a few cases, the predominant cell type are plasma cells. These cases have been classified as primary cutaneous plasmacytoma in the past, but are considered now to be the most likely variants of PCMZL.

The centrocyte-like cells stain positively for CD20, CD79a, and Bcl-2, and are negative for CD5, CD10, and Bcl-6. In the overwhelming majority of cases, intracytoplasmic monotypic expression of immunoglobulin light chains (either κ or λ) can be observed (Figure 7).

A monoclonal rearrangement of the \( J_H \) gene can be observed in the majority of cases (60-80%). A peculiar t(14;18)(q32;q21) has been detected recently in a subset of PCMZLs, as well as in lesions of MALT lymphomas arising in organs other than the skin. Other genetic aberrations include aneuploidy, trisomy 3 and rarely the t(11;18). However, in more than 50% of the cases no molecular abnormalities have been found.

Primary cutaneous diffuse large B-cell lymphoma, leg type

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCLLT) represents a type of PCBCL that is characterized by predominance of large round cells (centroblasts, immunoblasts) positive for Bcl-2. It has an intermediate prognosis, and it occurs almost exclusively in elderly patients, predominantly women. This cutaneous lymphoma is located on the leg in over 80% of cases, hence the term adopted by the WHO-EORTC classification. The prognosis of PCDLBCLLT is less favorable than that of other types of primary cutaneous B-cell lymphoma, with a five-year survival rate of approximately 50-60%. In the past, prognosis of cutaneous diffuse large B-cell lymphomas had been linked to several factors including Bcl-2 expression, morphology of the cells, number of lesions at presentation, and location on the legs. A recent study, however, demonstrated that accurate classification according to the new WHO-EORTC categories is the single most important prognostic criterion, and that other features are
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CERRONI

Histology shows dense, diffuse infiltrates within the entire dermis and subcutis. Involvement of the epidermis by clusters of large atypical cells, simulating the Pautrier’s microabscesses found in cutaneous T-cell lymphoma, can be observed in some cases (B-cell epidermotropism), representing a potential diagnostic pitfall. The neoplastic infiltrate consists predominantly of immunoblasts and centroblasts (large round cells) (Figure 9). Cases of diffuse large B-cell lymphoma with predominance of large cleaved cells are classified among the PCFCLs. Reactive small lymphocytes are few, and mitoses are frequent. Based on the common finding of immunoglobulin gene hypermutations, it has been proposed that most cases of PCDLBCLLT represent large cell lymphomas originating from postgermin al center lymphocytes.

Neoplastic cells are positive for B-cell markers (CD20, CD79a), but there can be (partial) loss of antigen expression. MUM-1 is strongly expressed in most cases. This marker is useful in the differential diagnosis of PCDLBCLLT from PCFCL, diffuse type (in these last cases MUM-1 is usually either negative or expressed by a small minority of cells). Staining for Bcl-2 is positive in all cases (Figure 10). In fact, cases with prominent round cell morphology and negative staining for Bcl-2 are classified as primary cutaneous diffuse large B-cell lymphoma, other (PCDLBCLO). It may be that these last cases represent either a phenotypic (Bcl-2-negative) variant of PCDLBCLLT, or a morphologic (round cell) variant of PCFCL, diffuse type. Bcl-6 does not provide differential diagnostic

of little or no relevance when cases are stratified into specific categories. Complete staging investigations are mandatory before a diagnosis of PCDLBCLLT can be established.

Clinically, patients present with solitary or clustered erythematous or red-brown nodules, located primarily on the distal aspect of one leg (Figure 8). In some patients, lesions may arise on both lower extremities. Ulceration is common. Small erythematous papules can be seen adjacent to larger nodules. It must be emphasized that tumors with similar morphologic and phenotypic features can arise in areas other than the lower extremities.
clues, as it is positive in the vast majority of cases of PCFCL, PCDLBCLO, and PCDLBCLLT.

The tumors demonstrate monoclonal rearrangement of the JH gene. The interchromosomal 14;18 translocation is not present. Phenotypic analyses as well as genetic data obtained by fluorescence in situ hybridization (FISH) or microarray chip technologies revealed that PCDLBCLLTs show clear molecular differences from PCFCL, diffuse type, confirming the need of classifying these cases separately. Some of the aberrations found in PCDLBCLLT are similar to those observed in diffuse large B-cell lymphomas of the lymph nodes.

Primary cutaneous diffuse large B-cell lymphoma, other

This group consists of rare cases of cutaneous large B-cell lymphoma that do not fit into the category of PCDLBCLLT, and includes cases of diffuse large B-cell lymphoma with a round cell morphology but without Bcl-2 expression (clinicopathologic features intermediate between PCFCL, diffuse type and PCDLBCLLT), intravascular large B-cell lymphoma, and rare examples of large B-cell lymphomas in the setting of immune suppression (e.g., plasmablastic lymphoma). Cases of primary cutaneous B-cell lymphoma with predominance of large round cells but lacking Bcl-2 expression are classified in this group. Other histopathologic and immunohistochemical features of these cases are intermediate between those of PCDLBCLLT and PCFCL, diffuse type, suggesting that these cases represent a morphologic or phenotypic variant of these 2 groups.

Intravascular large B-cell lymphoma is a malignant proliferation of large lymphocytes within blood vessels. Most cases have a B-cell phenotype, but a T-cell variant has been reported. In rare patients, the skin may be the only affected site, although more often there is systemic dissemination from the onset, including often lesions located within the central nervous system. Clinically, patients present with indurated, erythematous or violaceous patches and plaques, preferentially located on the trunk and thighs. The clinical appearance is not typical of cutaneous lymphoma, and it may sometimes suggest a diagnosis of panniculitis or purpura. Interestingly, in some cases intravascular large B-cell lymphoma has been observed confined to lesions of cherry hemangiomas. Histopathologically it is characterized by a proliferation of large atypical lymphocytes filling dilated blood vessels within the dermis and subcutaneous tissues. It has been reported that the prognosis of intravascular large B-cell lymphoma limited to the skin is better than that of the disseminated form, but only a very limited number of cases has been studied.

Plasmablastic lymphoma is a rare lymphoma arising usually in the oral cavity in patients with severe immunosuppression, especially HIV-related. It is often associated with infection by HHV-8. It is characterized by a proliferation of plasmablasts (large eccentric nuclei, abundant cytoplasm, prominent nucleoli). The neoplastic cells are positive for CD38 and CD138, and express monotypic immunoglobulin light chains.

B-Lymphoblastic Lymphoma

B-lymphoblastic lymphomas are malignant proliferations of precursor B lymphocytes. Reports of patients presenting with B-lymphoblastic lymphoma confined to the skin (primary cutaneous B-lymphoblastic lymphoma) have been published. It should be emphasized, however, that all patients should be treated for a systemic disease, even in the absence of documented extracutaneous involvement at the time of presentation. B lymphoblastic lymphoma is highly aggressive, and prognosis of untreated patients is poor. It has been suggested that expression of CD34, an antigen known to be present on the surface of normal hematopoietic stem cells as well as in a subset of immature thymocytes, is associated with a longer disease-free survival in patients with acute lymphoblastic lymphoma/leukemia compared with those who do not express CD34, thus implying a prognostic significance for this antigen.

In contrast to the other cutaneous B-cell lymphomas, primary cutaneous B-lymphoblastic lymphoma shows a clear predilection for children and young adults. Clinically, patients present with solitary, large erythematous tumors, commonly located on the head and neck. Patients with primary skin disease often have asymptomatic lesions of a few weeks duration. Those with secondary skin lesions may have systemic symptoms (e.g., weight loss, fever, fatigue, malaise, night sweats). The serum level of LDH is often elevated, reflecting the aggressive and often systemic nature of the disease.
Histologically, B-lymphoblastic lymphoma shows a monomorphous proliferation of medium-sized cells with scanty cytoplasm and round or convoluted nuclei with fine chromatin. A starry sky pattern is commonly seen at low power, due to the presence of macrophages with inclusion bodies (‘tingible bodies’). Another characteristic feature is the arrangement of neoplastic cells in a ‘mosaic stones’ pattern (Figure 11). Mitoses and necrotic cells are abundant. It must be stressed that histological features alone do not allow differentiation of lymphoblastic lymphomas of B-cell phenotype from those of T-cell lineage. Immunohistology demonstrates positive staining for TdT, CD10 and the cytoplasmic µ-chain of immunoglobulins, as well as, in most cases, for CD20 and CD79a (Figure 12). CD20 is negative in the pre-pre-B cell variant, which is CD34+. Lesions in most patients also express CD99, and some are positive for CD43. Molecular analyses usually show a monoclonal rearrangement of the J_H gene and a polyclonal pattern for the T-cell receptor (TCR) gene, but a lack of rearrangement of the J_H gene or monoclonal rearrangement of both the TCR and J_H genes may be observed.

Other B-cell lymphomas involving the skin

As already mentioned, extracutaneous (usually nodal) B-cell lymphomas may present with secondary spread to the skin. In many instances, morphologic aspects are similar to those of the cutaneous counterparts, thus complete staging investigations belong to the routine management of these patients. Some cases, on the other hand, do not have a primary cutaneous counterpart. The most important among these last is B-cell chronic lymphocytic leukemia (B-CLL). Lesions of B-CLL are characterized by dense, homogenous infiltrates of small lymphocytes. It is possible to confirm the diagnosis by immunohistology, demonstrating an aberrant CD20+/CD5+/CD43+ phenotype of neoplastic cells. Molecular analyses show the presence of a monoclonal population of B lymphocytes in the vast majority of cases. It should be reminded that B-CLL may present at sites of cutaneous inflammation (e.g., at sites of previous herpes simplex 1/2, herpes zoster, or Borrelia infection). Although the overall prognosis of B-CLL is generally not affected by skin involvement, patients with large cell transformation (so-called Richter syndrome) have a very aggressive course with poor prognosis. Richter syndrome may be clonally related to the original haematological neoplasm, or may represent occurrence of a large cell lymphoma clonally unrelated to the B-CLL.

Treatment

The most appropriate treatment modality for patients with PCBCLs is selected upon exact classification of the lymphoma, analysis of results of staging investigations, and evaluation of the overall condition of the patient (Table II). Patients with secondary cutaneous manifestations of extracutaneous B-cell lymphoma should be treated in a hematological, not der-
matologic, setting, and will not be discussed in what follows. The only exception is represented by specific manifestations of B-CLL arising at sites of previous herpes simplex 1/2, herpes zoster, or Borrelia infection, which may be treated with surgical excision (or other non-aggressive modalities).

Before reviewing the major therapeutic approaches, it must be emphasized that in some cases patients with low-grade malignant PCBCL can be managed conservatively with a so called watchful-waiting strategy, similar to that which is often adopted for indolent B-cell lymphomas and leukemias at extracutaneous sites. Follow-up examinations in these patients should be performed at least every 6 months or at the onset of new lesions and/or new symptoms, in order to treat the patient as soon as it is necessary. Many patients managed conservatively with a watchful-waiting strategy experience a prolonged course and long survival, and do not need aggressive treatment.

Most patients with low-grade PCBCLs (PCFCL, PCMZL) and who have a solitary or few lesions can be treated by local radiotherapy, simple surgical excision, or surgical excision followed by radiotherapy of the surgical field. In most cases 20 to 30 Gy of conventional orthovoltage X-rays are sufficient, but relapses are relatively frequent (Figures 13, 14). It has been reported that recurrences are less frequently seen in patients treated by radiotherapy with wide margins (about 10-20 cm beyond clinically apparent lesions). This approach seems to be justified for so called Crosti’s lymphoma, a type of PCFCL arising on the back in which erythematous nodules, papules and patches surrounding the tumor represent specific infiltrates that can extend far beyond the main bulk of the lesion. Radiotherapy with wide margins, however, does not seem to be justified for lesions other than Crosti’s lymphoma, as margins of about 5 cm usually suffice. Surgical excision is a valuable therapeutic modality for patients with solitary, well circumscribed lesions. In these patients, relapse rates do not seem to be higher than in patients treated with more aggressive modalities such as local radiotherapy. In addition, particularly in patients with PCMZL, relapses often occur at cutaneous sites distant or completely unrelated to that of the primary lesion, thus questioning the value of local radiotherapy in these cases (Figure 15).

In recent years, a few reports have appeared on low-grade PCBCLs that were treated with systemic antibiotics, achieving a complete resolution in at least a percentage of the patients. This type of treatment is conceptually analogous to that adopted for Helicobacter pylori-associated MALT lymphomas of the stomach, which in their early stages can be cured by eradication of H. pylori infection. Complete response to antibiotic therapy has been observed recently in some patients with Borrelia-associated PCBCL. Although not yet corroborated by adequate data, antibi-

Table I.—Treatment of different entities of cutaneous B-cell lymphoma.

<table>
<thead>
<tr>
<th>Entity</th>
<th>First line treatment (s)</th>
<th>Second line treatment (s)</th>
<th>Other possibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous marginal zone B-cell lymphoma</td>
<td>Surgical excision</td>
<td>Rituximab, Interferon-α, Radiotherapy</td>
<td>“Watchful waiting” Antibiotics (Borrelia burgdorferi+) Chemotherapy, Surgical excision</td>
</tr>
<tr>
<td>Cellular follicular lymphoma</td>
<td>Radiotherapy</td>
<td>Rituximab, Interferon-α</td>
<td>Chemotherapy, Surgical excision</td>
</tr>
<tr>
<td>Cutaneous diffuse large B-cell lymphoma, leg-type</td>
<td>Radiotherapy, chemotherapy</td>
<td>Rituximab, Combination therapies (§)</td>
<td>Combination therapies (§); Bone marrow transplantation</td>
</tr>
<tr>
<td>Cutaneous diffuse large B-cell lymphoma, other*</td>
<td>Chemotherapy</td>
<td>Radiotherapy</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Intravascular large B-cell lymphoma</td>
<td>Same as for extracutaneous disease</td>
<td>Treatment planned based on extracutaneous disease</td>
<td></td>
</tr>
<tr>
<td>Cutaneous B-lymphoblastic lymphoma</td>
<td>Same as for systemic disease; skin lesions at sites of previous infections (e.g., herpes, Borrelia) may be managed by surgical excision, laser vaporization, or other nonaggressive modalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific skin manifestations of extracutaneous B-cell lymphoma</td>
<td>Treatment planned based on extracutaneous disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific skin manifestations of B-CLL</td>
<td>Same as for systemic disease; skin lesions at sites of previous infections (e.g., herpes, Borrelia) may be managed by surgical excision, laser vaporization, or other nonaggressive modalities</td>
<td></td>
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#) Choice of a given option depends on classification, number of lesions, distribution of lesions, general conditions, first presentation vs relapse/persistence, and results of adjunctive studies such as PCR analysis for Borrelia burgdorferi. §) Usually chemotherapy in association with rituximab. *) Treatment depends on specific diagnosis; options reported here are meant for cases that do not fit into a specific category (e.g., plasmablastic lymphoma, methotrexate-associated lymphoma, etc.).
otic treatment of patients with PCBCLs should be considered before more aggressive therapeutic options are discussed, particularly in countries endemic for *Borrelia* infection. It is important to treat patients at onset of the disease, because in later stages lesions may no longer be sensitive to systemic antibiotics. PCR analysis of *Borrelia* DNA is a rapid test that should be performed in all patients with PCBCL from endemic areas, in order to identify those who would more likely benefit from early antibiotic treatment. A latency time of 3-4 months between antibiotic treatment and resolution of skin lesions is usually observed.

Another treatment modality for low-grade malignant PCBCL is subcutaneous or intralesional administration of interferon, in particular interferon alfa-2a.\textsuperscript{100, 102, 106, 108-114} Interferon alfa has both a direct antitumor effect and an immunomodulatory activity. Although some favorable results have been reported, it seems that treatment with interferon is associated with a complete response only in about 50% of patients. Therapy with interferon should be considered for patients presenting with multiple lesions at different body sites, such that local radiotherapy becomes difficult to administer. Usually a dose of 3 million units is administered subcutaneously 3 times per week for longer periods (at least 6 months). The main side-

Figure 13.—Primary cutaneous follicle center lymphoma. (A) Large tumors on the scalp. (B) Complete resolution after radiotherapy.

Figure 14.—Primary cutaneous follicle center lymphoma. Recurrent lesions on the scalp after radiotherapy.

Figure 15.—Primary cutaneous marginal zone B-cell lymphoma. Small recurrence on the arm distant from the surgical scar of the primary excision.
effects include leucopenia, nausea, asthenia and a depressive syndrome.

Intralesional or systemic injection of anti-CD20 monoclonal antibody (rituximab) has been recently used to manage patients with indolent PCBCL. Systemic treatment is performed by intravenous administration of 375 mg/m² of rituximab controlled by a perfusor. The treatment is given once weekly for 4 consecutive weeks. Intralesional administration should be considered only in cases presenting with a few (up to a maximum of 4), localized lesions, and should be administered as follows: 10 mg of the drug are injected intralesionally in each single lesion, and the treatment is repeated 3 times during 1 week. A new cycle can be repeated after 3 weeks if necessary (several cycles can be performed). Therapy with anti-CD20 monoclonal antibody represents a valid alternative to established treatments, especially in patients who present relapse after radiotherapy, or who present with disseminated skin lesions. Anti-CD20 monoclonal antibody can also be used in combination with other treatment modalities, especially in cutaneous B-cell lymphomas with more aggressive behaviour (PCDLBCLLT). The main limitation of this treatment is represented by the high costs. Although patients experience a depletion of immune-competent CD20+ B lymphocytes, the treatment is usually well tolerated. Circulating B lymphocytes return to the baseline value after 4 to 6 months. Side-effects are usually mild and include mainly fever, malaise and nausea, but severe adverse reactions have been reported rarely after systemic administration.

Patients with disseminated lesions of PCFCL, diffuse type or PCDLBCLLT need more aggressive treatment modalities such as systemic chemotherapy. The chemotherapeutic regimen used most frequently consists of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). In the absence of signs of transformation into high grade lymphoma or of extracutaneous involvement, systemic chemotherapy is not indicated in patients with PCMZL. Chemotherapy may be used also in patients with relapsing PCFCL not responding to conventional therapies. Local radiotherapy or systemic chemotherapy are the treatments of choice for patients with PCDLBCLLT. However, administration of systemic chemotherapy in these patients is often complicated by their age and overall conditions. Therapy with anti-CD20 monoclonal antibody may represent an alternative. Systemic chemotherapy is the treatment of choice for patients with intravascular large B-cell lymphoma, regardless of results of staging investigations. Finally, patients with primary cutaneous B-lymphoblastic lymphoma should be treated in a hematological setting. Aggressive chemotherapy (with or without bone marrow transplantation) should be administered, even in patients with negative staging at presentation.

Conclusions

PCBCLs represent an heterogeneous group of lymphoid malignancies arising in the skin in the absence of systemic manifestations at presentation. Prognosis and treatment depend on exact classification into one of the categories listed in the recent WHO-EORTC classification for cutaneous lymphomas. Dermatologists should be aware of these disorders that in most cases are diagnosed and treated in a dermatologic setting.

Riassunto

I linfomi primitivi cutanei a fenotipo B rappresentano un gruppo eterogeneo di neoplasie linfocitarie con differente presentazione clinico-patologica e con prognosi variabile. La definizione di linfoma primitivo cutaneo implica l’assenza di manifestazioni extracutanee documentata da esami di staging completi effettuati alla presentazione. La classificazione dei linfomi cutanei pubblicata recentemente dalla “World Health Organization” (WHO) assieme alla “European Organization for Research and Treatment of Cancer” (EORTC) – Cutaneous Lymphoma Project Group divide i linfomi cutanei a fenotipo B in quattro gruppi principali: i primi due gruppi sono composti dai linfomi B follicolari e dai linfomi B marginali e sono caratterizzati da un andamento indolente (sopravvivenza ai 5 anni >90%), mentre i linfomi B a grandi cellule-tipo della gamba ed i linfomi B a grandi cellule-altri sono caratterizzati da una prognosi intermedia (sopravvivenza ai 5 anni del 50-60%). Il trattamento dei pazienti dipende dalla classificazione e dalla presentazione clinica (lesioni solitarie o multiple, età, condizioni generali, ecc.). Le principali strategie terapeutiche includono la radioterapia locale, l’escissione chirurgica, l’anticorpo monoclonale anti-CD20 (rituximab), l’interferone-alfa e la chemoterapia sistemica (generalmente CHOP). Occorre tuttavia sottolineare che i pazienti con linfoma primitivo cutaneo B indolente richiedono solo raramente una chemoterapia. I casi associati ad infezione da Borrelia burgdorferi possono essere trattati con terapia antibiotica. Alcuni pazienti si possono giovare per periodi di tempo variabili di una cosiddet-
ta strategia di “attesa vigile” con controlli regolari ma senza terapia specifica.

Parole chiave: Cute - Linfomi cutanei a cellule B, diagnosi - Linfomi cutanei a cellule B, terapia.

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Emerging agents for onychomycosis

B. M. PIRACCINI, M. IORIZZO, G. RECH, A. TOSTI

Onychomycosis is the most common nail disease and describes the invasion of the nail by fungi. In most cases, treatment of onychomycosis requires systemic antifungals, with duration of administration ranging from 2 to several months. Cure rates range from 60% to 80%, and relapses are not rare (up to 20% of patients). New antifungals providing higher cure rates and less relapses are, therefore, auspicious. A review of onychomycoses and their treatment is made and the new antifungal drugs that are currently on study or already available are presented.

KEY WORDS: Voriconazole - Ravuconazole - Posaconazole - Antimicrotics - Nails.

Onychomycosis affects more frequently toenails than fingernails and this may be explained by the growth rate which is 3 times slower for toenails than fingernails.

Predisposing factors for onychomycosis include old age, diabetes, HIV infection, peripheral vascular impairment and peripheral neuropathies, podiatric abnormalities, sports activities and traumatic nail disorders. It has also been suggested that susceptibility to dermatophyte nail infection might be inherited as an autosomal dominant trait.

Different clinical patterns of onychomycosis result from the way by which fungi colonize the nail. Type of nail invasion depends on responsible fungus and host susceptibility. Fungi responsible for onychomycosis are listed in Table I.

Distal subungual onychomycosis

This is the most common type of onychomycosis, most frequently due to T. rubrum.

Fungi reach the nail unit through the hyponychium and invade the nail bed spreading proximally. The skin of the palms and soles is frequently involved, being the primary site of infection.
Clinically the nail shows distal subungual hyperkeratosis and onycholysis. The onycholytic area appears yellow white in colour. Yellow streaks along the lateral margin of the nail and/or presence of yellow onycholytic areas in the central portion of the nail (dermatophytoma) are associated with poor response to systemic antifungals.

The NDM _Aspergillus_ sp., _Fusarium_ sp. and _Scopulariopsis brevicaulis_ may produce a distal subungual onychomycosis (DSO) that usually involves 1 toenail, with diffuse nail invasion associated with periungual inflammation. DSO due to _Acremonium_ sp. usually affects 1 or 2 toenails and presents as one or few longitudinal white streaks extending proximally from the distal margin. The clinical picture is similar to that of a dermatophyte onychomycosis.

In DSO due to _Scytalidium_ sp., the periungual tissues and nail plate show a black pigmentation.

### Proximal subungual onychomycosis

_Fungi_ reach the nail unit through the undersurface of the proximal nail fold and are typically located in the ventral portion of the nail plate. Clinically, proximal subungual onychomycosis (PSO) presents as an area of leukonychia in the proximal portion of the nail plate. The nail plate surface is normal, being spared by _fungi_.

This type of onychomycosis is most frequently caused by molds, but it may also be caused by _T. rubrum_, especially in HIV patients where it is a marker of the disease.

PSO due to _Aspergillus_ sp., _Fusarium_ sp. and _Scopulariopsis brevicaulis_ is typically associated with marked painful inflammation of the periungual tissues. The affected nail shows a deep milky-white or yellow discoloration that starts from the proximal nail and often rapidly spreads to involve the whole length of the nail. Periungual tissues are edematous and red. Some patients complain of periodical inflammatory flares with purulent discharge, especially when _Aspergillus_ is the responsible agent.

### Superficial white onychomycosis

The infection is on the dorsal surface of the nail plate. The classical form of superficial white onychomycosis (WSO) is usually caused by _T. interdigitalis_ that possesses keratinolytic enzymes able to metabolize the hard keratins of the superficial nail plate. Clinically, the nail shows one or more small white opaque patches that can be easily scraped off and may coalesce gradually covering the whole nail plate. _Tinea pedis interdigitalis_ is frequently associated.

In HIV-infected patients, WSO is usually due to _T. rubrum_ and is not only seen in the toenails but can also affect the fingernails. Clinically, the affected nail appears diffusely opaque and white, with the leukonychia often reaching the proximal portion of the nail. A similar clinical picture of deep WSO is seen in children with _T. rubrum_ WSO and in WSO due to NMD.

The most common species of NDM known to be able to invade the superficial nail plate are _Fusarium_ sp., _Aspergillus_ sp. and _Acremonium_ sp. Mold WSO usually affects a single toenail, mainly the big toe. The diffuse and deep nail plate involvement makes it difficult to distinguish a deep WSO from a PSO progressed superficially. As seen in the other types of onychomycosis due to NDM, periungual inflammation may be associated, but usually without pus discharge.

_Scytalydium dimidiatum_ can be responsible for a rare variety of superficial onychomycosis, black superficial onychomycosis, in which the superficial patches on the nail plate are black in colour.

### Endonyx onychomycosis

_Fungi_ invade the nail via the nail plate free margin. Instead of infecting the nail bed, the fungus immediately penetrates the nail plate keratin.

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**TABLE I.—Agents responsible for onychomycosis.**

<table>
<thead>
<tr>
<th>Common</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>Epidermophyton floccosum</td>
</tr>
<tr>
<td>Trichophyton interdigitale</td>
<td>Microsporum audouinii</td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>Microsporum gypseum</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>Trichophyton schoenleini</td>
</tr>
<tr>
<td>Trichophyton soudanense</td>
<td>Trichophyton tonsurans</td>
</tr>
<tr>
<td>Trichophyton violaceum</td>
<td></td>
</tr>
<tr>
<td><strong>Non-dermatophytic molds</strong></td>
<td>Acremonium spp.</td>
</tr>
<tr>
<td>Scopulariopsis brevicaulis</td>
<td>Aspergillus spp.</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>Onichcola canadensis</td>
</tr>
<tr>
<td>Scytalidium spp.</td>
<td></td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
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**PIRACCINI**

**EMERGING AGENTS FOR ONYCHOMYCOSIS**

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**GIORNALE ITALIANO DI DERMATOLOGIA E VENEREOLOGIA**

-Aprile 2006

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This rare type of onychomycosis is caused by *T. soudanense* and *T. violaceum*. The nail plate is diffusely opaque and white in the absence of onycholysis and subungual hyperkeratosis. Plantar infection may be associated.

**Total dystrophic onychomycosis**

This type of onychomycosis may rarely occur as a primary condition. Most commonly, in fact, represents the secondary evolution of untreated DSO, PSO, deep WSO and endonyx onychomycosis (EO). Primary total dystrophic onychomycosis (TDO) is usually due to *Candida*, and typically affects immunocompromized patients, such as patients with chronic mucocutaneous candidiasis (CMCC), iatrogenic immunodepression or HIV infection. In CMCC, onychomycosis is associated with inflammation of the proximal nail fold, nail matrix, nail bed and hyponychium. The nail bed is hyperkeratotic and the nail plate is extremely thick and dystrophic. Complete destruction of the nail plate may be observed. Several nails are generally affected, both fingernails and toenails. Oral candidiasis is often associated.

In HIV positive patients and in iatrogenic immunocompromised individuals clinical presentation of *Candida onychomycosis* is less severe, with only one or few fingernails affected.

*Candida albicans* has been frequently isolated from the subungual area of onycholytic nails and from the proximal nail fold in chronic paronychia. In both these conditions, however, *Candida* colonization is a secondary phenomenon and systemic antifungicides do not cure the nail abnormalities.

**Treatment of onychomycosis**

Treatment depends on the clinical type of the onychomycosis, the number of affected nails and the severity of nail involvement. The goals of antifungal therapy are mycological cure and a normal looking nail. Mycological cure should always be evaluated at the end of treatment, while clinical cure may require some more months to be achieved.

Topical nail lacquers containing 5% amorolfine and 8% cyclopioxolamine are effective as monotherapy in the treatment of classic WSO and of DSO limited to the distal nail of a few digits. They are also utilized in severe onychomycosis in combination with systemic antifungals to reduce duration of treatment and increase cure rate.

DSO, EO, PSO, TDO and deep WSO always require a systemic treatment.

Systematic treatment with terbinafine, itraconazole or fluconazole produces mycological cure in more than 90% of fingernail and in about 60-80% of toenail dermatophyte infections. Onychomycosis due to *Fusarium* sp. and *Scopulariopsis brevicaulis* are very difficult to cure, while *Aspergillus* sp. responds very well to antifungals.

**New antifungals**

New antifungal agents are currently studied for the treatment of systemic infections in immunocompromized patients. Some of them may have a role in onychomycosis.

**Voriconazole**

Voriconazole (UK-109,496) is a triazole antifungal agent, structurally related to fluconazole. It was approved by the Food and Drug Administration (FDA) in May 2002 and is indicated for the primary treatment of acute invasive aspergillosis, as salvage therapy for severe systemic infections by *Scedosporium apiospermum* and *Fusarium* sp., and for refractory *Candida infections*.

As with all azole antifungal agents, voriconazole inhibits the fungal cytochrome P450-mediated 14-alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. The inhibition of P450 14-alpha-demethylase is dose-dependent and, compared to fluconazole, provided with an increased potency. The accumulation of 14 alpha-methyl sterols is added to the loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity of voriconazole.

Voriconazole is metabolized by the human hepatic cytochrome P450 enzymes, CYP2C19, CYP2C9 and CYP3A4, with less than 2% of the dose excreted unchanged in the urine. The major metabolite of voriconazole is the N-oxide. It has minimal antifungal activity and consequently it does not contribute to the overall efficacy of voriconazole.

Voriconazole is active both in oral and intravenous administrations. It is available as a lyophilized powder for solution for intravenous infusion, film-coated tablets.
for oral administration, and as a powder for oral suspension. Dosages are: 200 mg twice daily per os and 3 to 6 mg/kg every 12 h intravenously.

Levels of voriconazole are significantly reduced by the concomitant administration of rifampin, ritonavir, carbamazepine and long-acting barbiturates.

Coadministration of voriconazole increases levels of: sirolimus, terfenadine, cisapride and ergot alkaloids.

Coadministration of voriconazole with this drugs is, therefore, contraindicated.

It is necessary to monitor or adjust the dose of the following drugs when coadministered with voriconazole: cyclosporine, methadone, tacrolimus, warfarin, oral coumarin anticoagulants, statins, benzodiazepines and sulfonylureas.

The interactions of voriconazole with cimetidine, ranitidine and macrolide antibiotics are minor or no significant, so they do not require dosage adjustment.

The most common side effects of voriconazole are visual disturbances that affect 40% of patients and include abnormal vision, color vision change and photophobia, elevations of liver enzymes (20% of patients) and skin rashes (6%). Side effects often lead to discontinuation of voriconazole therapy. Liver function tests should be evaluated at the start of and during the course of the treatment. Acute renal failure has been observed in severely ill patients.

The mechanisms underlying the dermatologic side-effects of voriconazole are unknown.

A photosensitivity reaction may occur with long-term treatment. A case of photoaging caused by voriconazole therapy has been reported in a 15-year-old patient. Voriconazole has never been tried for the treatment of onychomycosis.

**Ravuconazole**

Ravuconazole (ER-30346 and BMS-207147) is a second generation triazole derivative related with voriconazole, currently undergoing Phase II clinical trials.

Ravuconazole has been described in both intravenous and oral formulations. It has broad spectrum in vitro potency and in vivo efficacy against a wide range of fungal pathogens.

In vitro ravuconazole is active against important fungal pathogens, including Candida spp., Aspergillus fumigatus, Cryptococcus neoformans, as well as dermatophytes: Trichophyton mentagrophytes, T. rubrum, Microsporum canis. Activity against dermatophytes is greater than that of itraconazole and fluconazole.

As with all azole antifungal agents, ravuconazole works principally by inhibition of cytochrome P450 14-alpha-demethylase (P45014DM). It’s potency and binding affinity for P45014DM is similar to that of itraconazole.

The metabolism is similar to that of voriconazole. Ravuconazole will be available in both intravenous and oral formulations.

The most common adverse effects of ravuconazole are abdominal problems (15%), headache, dizziness and skin abnormalities (10% of patients).

Gupta et al. reported the use of ravuconazole at different dosages for the treatment of distal subungual onychomycosis. The dose of 200 mg daily for 12 weeks was the most effective. Percentage of cure (59%) was not higher than that obtained with terbinafine and itraconazole and, therefore, ravuconazole 200 mg/day does not appear to be a better option for onychomycosis treatment than the available antifungals. Other clinical trials on ravuconazole in DSO are currently underway.

**Posaconazole**

Posaconazole is a new triazole, formerly known as SCH 56592, that is structurally related to itraconazole. This drug is in Phase I studies to assess safety and antifungal efficacy.

Posaconazole shows potent broad-spectrum activity against opportunistic fungal pathogens like Candida spp., Cryptococcus neoformans, Aspergillus spp., Fusarium spp., dermatophytes and zygomycetes. Posaconazole works principally by inhibition of cytochrome P450 14-alpha-demethylase (P45014DM) and is a significantly more potent inhibitor of sterol C14 demethylation than itraconazole.

SCH 56592 has been formulated in only oral tablet and suspension preparations.

No data are available about the possible use of posaconazole for the treatment of onychomycosis. SCH 56592 has been formulated in only oral tablet and suspension preparations.

**Conclusions**

The new antifungals agents ravuconazole, posaconazole, voriconazole are very promising for the treatment of systemic fungal infections. Their possible use in onychomycosis is still to be assessed.
An optimal systemic antifungal for onychomycosis should possess the following characteristics: easy way of administration, optimal tolerability, rapid and long-lasting effect. At the moment, the new antifungals do not seem to possess these qualities, but further studies are necessary to definitely indicate their role in onychomycosis therapy.

**Riassunto**

**Agenti emergenti nell’onicomicosi**

L’onicomicosi rappresenta la patologia ungueale più comune ed è caratterizzata dall’invasione dell’unghia da parte di funghi. Nella maggior parte dei casi il trattamento dell’onicomicosi richiede una terapia antifungina sistemica, la cui durata può variare da 2 a diversi mesi.

Il tasso di guarigione varia dal 60% all’80% e le recidive non sono rare (sono auspiche). Sono auspicabili nuovi farmaci antifungini in grado di aumentare il tasso di guarigione e di diminuire quello delle recidive. Questo articolo rivede le onicomicosi e il loro trattamento, presentando inoltre i nuovi farmaci antifungini che sono attualmente in fase di studio o già disponibili.

**Parole chiave:** Micosi - Farmaci antifungini - Voriconazole - Ravuconazolo - Posaconazolo.

**References**

Guidelines of the Italian Association for Non Invasive Diagnosis in Dermatology

Linee guida della Associazione Italiana di Diagnostica Non Invasiva in Dermatologia

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General principles

1) Dermoscopy represents a second level examination aimed at improving the noninvasive diagnosis of pigmented skin lesions that resulted equivocal by naked eye observation (I level examination), therefore, dermoscopy represents a part of the overall diagnostic procedure. The dermoscopy report, therefore, supplements the medical report that should be always made after each dermatological consultation.

2) The prescription of a dermoscopy examination is appropriate and must be accepted by the specialist that performs dermoscopy when made by an other physician (general practitioner, dermatologist or other medical specialist); the prescription must indicate what lesion/s should be examined.

On the contrary, the prescription of dermoscopy examination cannot be considered appropriate if suggested by the patient himself that asks the doctor to examine one or more lesions judged clinically banal by the specialist and otherwise not selected for the examination.

Furthermore, the prescription of dermoscopy examination cannot be considered appropriate if it doesn’t indicate accurately the lesion/lesions to be examined. In this case (for example: prescription of a generic dermoscopy examination for multiple atypical nevi…) it might be advisable—with the consent of patient—changing the prescription from dermoscopy (this type of examination provides a specific sanitary ticket, different from that of the general examination) in a dermatological examination for melanoma screening.
This would permit to avoid diagnostic delay in the case where the patient will eventually show a suspicious lesion; in that case, the specialist will use dermoscopy—if needed—according to his personal judgement.

3) Dermoscopy can be performed in the following 2 cases (a) as a consequence of a specific medical prescription or (b) during an examination aimed to melanoma screening according to personal judgement of the examiner. Two different forms for medical report are enclosed.

4) Informed consent: according to the italian law by decree n. 196 of 30 June 2003 “Code in the matter of protection of personal data” that simplifies the previous rule about the Privacy, it is not considered necessary the acquisition of the written consent for the execution of the diagnostic examination; it is regarded sufficient the verbal consent after adequate information about the modality and the aim of the medical test (art. 79).

5) Minimal equipment for dermoscopy
a) 10X Minimal magnification.
b) Hand-held dermoscope with diode illumination (to be preferred to that traditional with incident light).
c) For digital dermoscopy (by means videodermoscope or videocamera) it is not presently possible to establish the qualitative minimal threshold to consider the image sufficiently informative for the diagnosis. In our opinion the image quality should not be worse than that of an analogic image obtained with an hand-held dermoscope (standard procedure in the recent literature on dermoscopy) and then digitalized.

6) The examiner is obliged to give a medical report in the following 2 cases: a) for any dermoscopy examination required by a sending doctor irrespective of the diagnostic outcome; b) in the case of possible malignancy identified by dermoscopy that has been made on the discretion of the dermatologist in the screening.

To the contrary, the dermoscopy report should not be considered mandatory when dermoscopy did not reveal neither malignant nor suspicious lesion. In that case, the report will concern the overall outcome examination (Appendix I).

7) In order to improve the communication with the patient, it is advisable to base the report more on the overall clinical-dermoscopic findings than on dermoscopic examination alone. Indeed, we suggest to pay attention to the main clinical-anamnestic characteris-tics of the lesion analized regarding dermoscopy as a step of a wider clinical opinion. Recent literature as well as clinical experience demonstrate that the combination of dermoscopic and clinical anamnestic data help in minimizing the risk of incorrect management of a pigmented lesion. The existence of the so called “featureless melanomas”, i.e. melanomas lacking dermoscopic features of malignancy (about 8% of all the melanomas), can hamper the role of dermoscopy as gatekeeper of decision for removal. In some cases, excision has to be decided in accordance with lesion’s history and clinical features. Moreover, the “ugly duckling sign” may seldom help in selecting a lesion to be removed even if not associated with clear-cut dermoscopy features of malignancy.

8) Even if an evidence-based demonstration remains to be done, it is opinion of the authors of these guidelines that dermoscopy should be of competence of the dermatologist more than other medical specialists. According to the point discussed before, the optimized use of dermoscopy can be obtained only by means the integration of dermoscopic features with the most relevant clinical-anamnestic informations. The diagnostic performance achieved by a non dermatologist doctor can be affected by the low experience in examining the skin and, therefore, in taking into account additional points seldom needed to select the appropriate lesion’s management in those cases difficult to diagnose.

9) Delivery of the report to the patient. From a medico-legal point of view every instrumental tests must be communicated to the patient by means of a written report signed by physician; this report represents the only document juridically valid.

10) Preservation of the report. The law in force (Ministry of Health Memorandum n. 61 of 19 December 1986) obliges to preserve a copy of the report in a proper archive at least for 20 years; the suitable means are: the chemical copying paper, the photocopy or the print in double copy. If the report is drawn up in digital file—for example by means of a software included in the videodermoscope—it is possible to preserve a copy of the report in this format. These data, both in paper and digital format, are submitted to the regulations provided in the Art. 15 (security of the data) of the italian law n. 675 of 1996 and in the DPR n. 445/2000 about the preservation and transmission of administrative documents; the digital registration must be made at least on 2 distinct data medium preserved
in different premises. In the deliberation of the National Center for Informatics in the Public Administration (Centro Nazionale per l’Informatica nella Pubblica Amministrazione, CNIPA) n. 11 of 19 February 2004 there are further and more recent instructions about optical registration and document conservation. The paper copy registered must be signed by the reporting physician. It is necessary to designate a person responsible of the data-base that knows the conservation state of data and the access procedures.

11) Delivery to patient of the print of the dermoscopic images. In spite of the fact that the presence in the report of the lesion’s image is advisable, the Guarantor for the Privacy pointed out that the Art. 13 of the law 675/96 don’t provide the necessary delivery to the patient of the documents or the medium preserving data, but it obliges, more precisely, the person responsible of the data processing to extract them from the own archives and documents all the informations in paper or digital format concerning the petitioner and to relate them with a manner suitable to easily understand the data. Therefore, it is the written report signed by the physician the only document with medico-legal validity. Viceversa the delivery to the patient of the dermoscopic image has to be regarded as optional. If given to the patient, the image should have a minimal qualitative level in order to be representative of the description made on the report. Anyway, if the image is given to the patient, it can be advisable to specify that the description and the diagnosis written in the report have been made on the basis of the in vivo observation.

Moreover, being dermoscopy a diagnostic procedure that needs to be integrated with the anamnestic-clinical data in order to have an optimized management of the lesion, the dermoscopic image cannot be in itself sufficient to support the conclusions and the management of the lesion written in the report. The file copies of the recorded images should be delivered to the patient if these are required expressly.

12) Storage and preservation of the dermoscopic images: in accordance with that established by the Ministry of Health Memorandum n. 61 of 19 December 1986, it can be assumed—on the analogy of what established for X-ray photographs—that the dermoscopic images must be kept for 20 years at least; this is true also for dermoscopic images acquired by means of analogical technology and recorded on others medium (photographic negative film or photographic paper).

13) The problem of the acquisition and preservation of the image in the case of hand-held dermoscopy: although the authors of these guidelines agree about the need to record and preserve the image anyhow the dermoscopic examination is performed, this procedure has to be considered necessary only if a digital technology (videodermoscopy, video-camera) has been used, while it is only advisable in the case of analogical examination. Indeed, in the latter case the equipment used for hand-held dermoscopy doesn’t permit the registration of the images. In this case the dermoscopic examination can be comparable to ophthalmoscopic or otoscopic examination by means of an optic traditional equipment, that requires a specific report but usually does not require the acquisition of the image as diagnostic support.

The structure of the report

In order to create a uniformity for drafting among Italian dermatologists, a standard clinical-dermoscopic report was made. The clinic-dermoscopic report should include 2 different parts: a general part and an analytical part (Appendices I, II).

General part

Clinical-dermoscopic screening of the cutaneous lesions; this has to be filled in every patient. The aim is to inform the patient and/or the referring doctor about the present of eventual risk factors for melanoma, to make education about prevention of skin cancers, to suggest eventual future medical examination for check-up.

This part can represent the only component of the medical report when dermoscopy has been performed on the discretion of the dermatologist performing the screening and the diagnostic outcome was negative. Concerning guidelines for skin examination aimed at skin cancer screening, we suggest to offer to the patient the chance of a total skin examination including the anal-genital area. In the case of refusal, it should be pointed out in the report. The scalp should be examined in all cases.

Analytical part (Clinical-dermoscopic report of an index lesion submitted to dermoscopy)

This part must be compiled in all cases of dermoscopic examination specifically asked from another physician and in all cases where the examination -
performed on the discretion of the dermatologist during a screening examination - yielded positive results (i.e. suspicious or clearly malignant lesion to be removed).

This procedure is summarized by means of the following algorithm (Figure 1):

A) Screening examination (general examination of all the pigmented lesions with dermoscopy possibly performed at the discretion of the dermatologist):

1. Examination aimed to melanoma screening with manual dermoscopy according to personal judgement of the examiner
   
   No suspicious lesion evidenced
   
   Dermoscopy report not necessary
   
   Suspicious pigmented lesion - according to dermoscopic parameters evidenced - to submitted to excisional biopsy or follow-up
   
   Dermoscopy report necessary

2. Dermoscopic examination (manual or digital), as consequence of a specific medical prescription
   
   Dermoscopy report is mandatory irrespective of the diagnostic outcome

3. Generic prescription of dermoscopy examination that doesn’t indicate accurately the lesion/lesions to be examined
   
   The prescription can be changed in dermatological examination for melanoma screening
   
   The report will concern the overall outcome examination (see point 1)

A1) No evidence of pigmented lesions that needs a diagnostic verification by means excisional biopsy or careful follow-up.

   Type of report:
   — General part: yes
   — Analytical part: no

A2) Evidence of one or more lesions that needs further investigations.

   Type of report:
— General part: yes
— Analytical part: yes

B) Dermoscopic examination of a specific lesion following medical request (screening examination not made).

Type of report:
— General part: no
— Analytical part: yes

**Contents of the report**

**General part**

The report must be compiled on the headed paper of the Physician/ Medical Structure that performed the examination. The report should include—for educational purpose—a brief explanation about the modalities of the skin self-examination and about the rules for the early diagnosis of melanoma.

It must contain:

A) Patient’s personal data:
— Name.
— Surname.
— Date of birth.
— Occupation.
— Date of the examination.

B) Melanoma risk factors identified:
— Familial history of melanoma (First degree relatives).
— Presence of clinically atypical nevi.
— Personal history of melanoma.
— Total number of melanocytic nevi >50.
— Repeated sunburns before the adulthood.
— Intensive and prolonged sunexposure.
— Phototype I-II according to Fitzpatrick

C) Outcome of clinical and dermoscopic examination:
— No suspicious lesions identified:
  — Possible suggestions:
  — Periodical skin self-examination
  — Next examination scheduled after ..........months
— Lesion/s with suspicious diagnostic features: see enclosed form with specific dermoscopy report

D) Space for notes
E) Education about the skin self- examination.

**Suggested formula:**

— We suggest to perform a periodical skin self-examination. Please not forget to examine also anal-genital area and the scalp, this latest possibly with wet hair and with the help of a comb. In the case of modification in size, shape or colour of a pre-existent nevus, or appearance of a new nevus with looks different from the other nevi, it is advisable to seek a timely dermatological examination.

F) Stamp and sign of the physician on each copies.

**Analytical part**

A compilation of a form for each lesion submitted to dermoscopy is needed. A single form is used for every kind of lesion (melanocytic or not). The form will include all dermoscopic parameters both melanocytic and not melanocytic, and a specific space for the diagnosis.

Also the analytical part must be compiled on the headed paper of the Physician/ Medical Structure when performing the examination.

The report must be contain:

A) Patient personal data
— Name
— Surname
— Date of birth
— Date of examination
B) Numbering
The numbering is independent for each patient, and it is represented by progressive number of the examined lesion.

C) Accurate description of the lesion localization - in descriptive manner as well as through the indication of the localization on the figure - (Figure 2).
Moreover, it is advisable to mark directly the lesion indicated in the report (dermographic pen) on the patient skin either for the identification at home – also to make easier the follow-up from relatives or friends – and for lesion’s identification in case of suggestion for immediate excision.

D) Measurements of two major diameters of lesion in mm.

E) Analytical contents:
1) Melanocytic lesions.
   — Symmetry-Asymmetry (shape, colour, structure).
   — Global pattern.
   — Local pattern.
2) No melanocytic lesions.
   — Local pattern.

F) Diagnostic conclusions
A qualifying point of the report are the conclusions that should summarize all the anamnestic, morphologic and dermoscopic data supporting the suggested lesion’s management. With the purpose of safeguard the patient from the risk of leaving a melanoma unexcised, the lesion’s management should be regarded as the greatest point to be addressed. Sometimes, it can be necessary to suggest the removal of a lesion associated with history of change or high degree of suspicious on clinical examination even if this lesion cannot be classified as definitely malignant by dermoscopy.

It is advisable from a medical-legal point of view to include in the report possible factors that may have hampered an accurate lesion’s examination (presence of hematic crust, presence of intense diffuse pigmentation, ecc.).

Suggested formula:
The clinical – dermoscopic examination of the lesion suggest……………

E) Indications
The indications must be indicated as necessary.
— Periodical skin self-examination
— Further clinical-dermoscopic examination
— Diagnostic verification by means excisional biopsy
— Surgical excision for treatment

F) Education about the skin self-examination
Suggested formula (see above general part)

G) Stamp and sign of the physician on each copies.

References
APPENDIX I.
Medical report of a screening examination for melanoma: suggested form

Title and address of the structure

Name………………………………………………………………………………… Birth date …/……/……

Address………………………………………………….. Examination date……/……/……

RISK FACTORS FOUND:

— familiarity for melanoma (I grade relatives) — numerous sunburns in childhood
— personal history for melanoma — skin type I and II according to Fitzpatrick
— clinically atypical nevi
— number of melanocytic nevi > 50

Dermoscopic examination has been performed

❒ No ❑ Yes

The dermoscopic examination has been performed by means of equipment:

❑ analogical ❑ digital

Outcome of examination

— Absence of suspicious pigmented skin lesions in the examined skin
  — We recommende the skin self-examination
  — We recommende next examination in ………..months
— Presence of suspicious pigmented lesions (specific dermoscopy report enclosed)

Notes:…………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………

…………………………………………………………………………………………………………………………

We recommende you to perform a periodical skin self-examination. Do not forget to examine also anal-geni
tal area and the scalp with wet hair and withthe help of a comb. In the case of modification in size, shape or
colour of a preexistent nevus, or the appearance of a new nevus looking different than already present nevi,
it is advisable to seek a timely dermatological examination.

Stamp and signature of Physician
APPENDIX II
Medical report of a dermoscopy examination of a selected lesion: suggested form

Name…………………………………………………………………. birth date …….../…../…….
DERMOSCOPIC REPORT no. ........................................examination date …../…../…….
Lesion site………………………………………………………………….. Dimensions: mm. ____X ____mm.

Referred appearance/growth / changes during the latest months:  ☐ No  ☐ Yes

Symmetry
Asymmetry  shape  colour  structures
Global pattern  reticular  globular  homogeneous
  compound  multicompound  starburst
  parallel  lacunar  aspecific

Local pattern  atypical pigment/pseudopigment network ☐
  irregular streaks ☐  irregular dots/globules ☐
  irregular blotches ☐  blue-whitish veil ☐
  regression structures ☐  ipo/depigmentation ☐
  maple leaf-like areas ☐  spoke wheel structures ☐
  ovoid grey-blu areas ☐  horny pseudocysts ☐

Vascular pattern  pseudofollicolar openings ☐
  arborescent ☐  hair-pin like ☐
  garland-like ☐  comma-like ☐
  dotted ☐  atypical ☐

Diagnostic Method
Pattern analysis ☐  ABCD rule ☐  7-point checklist ☐  7-FFM ☐

Dermoscopic examination
  analogical ☐  digital ☐  Equipment:…………………………

Diagnostic conclusions: the clinical-dermoscopic examination of the lesion suggests……………………………………
………………………………………………………………………………………………………………
………………………………………………………………………………………………………………

Indications: it is needed to submit such neoformation to
— further clinical-dermoscopic examination after …………..months
— diagnostic verification by means excisional biopsy
— surgical excision for treatment
— Periodical skin self-examination

Stamp and signature of the Physician
Familial Mediterranean fever (FMF) is a hereditary disease that especially affects people living around the Mediterranean Sea. The most serious complication is amyloidosis, which can lead to terminal renal failure. We report the case of a 48-year-old man who presented in our clinic with recurrent episodes of erysipelas-like rashes located at the right gluteal area, always associated with fever and sometimes with orchiepididymitis, abdominal pain and arthritis. The clinical diagnosis of FMF was confirmed by the finding of the mutated marenostrin-encoding fever (MEFV) gene, using the PCR technique. The attacks were successfully avoided by continuous administration of colchicine per os 0.5 mg 3 times daily.

**KEY WORDS:** Familial Mediterranean fever - Erysipelas-like rash - Colchicines.

Familial Mediterranean fever (FMF) is a hereditary disease that especially affects people living around the Mediterranean Sea. Two independent French and American teams discovered the gene mutations responsible for the disease in 1997. It encodes for a protein named pyrin/marenostrin involved in the homeostasis of the inflammatory mechanisms. The main mutations have been identified and are, therefore, accessible for molecular screening.²

FMF is characterised by recurrent fever and abdominal pain, often associated with pleuritis, synovitis or exanthema. The most serious complication is amyloidosis, which can lead to terminal renal failure.³ The attacks and complications can be avoided by life-long administration of colchicine.

**Case report**

We report the case of a 48-year-old Greek man who was hospitalised in our clinic for recurrent episodes of fever (38-40°C) and erysipelas-like exanthema of the left lumbar and gluteal area (Figures 1, 2). Clinical examination also revealed congenital hemangioma of the left abdomen site and unilateral, erythematous and tender swelling of the scrotum. The patient mentioned similar attacks several times per year, monthly or bimonthly, during the last 30 years. He was hospitalised several times for recurrent episodes of scrotal swelling and aseptic arthritis associated with high fever. Each paroxysm lasted 48-96 h with peak intensity occurring within the first 12 h. The patient’s family history was negative.

Routine blood tests during the acute attacks were non-specific. Levels of acute phase reactants (C-reactive protein) and erythrocyte sedimentation rate were elevated and returned to reference values in about 4-5 days. Histopathological examination of the skin lesions showed nonspecific features of urticaria. Rectal biopsy excluded the presence of amyloidosis.
The clinical diagnosis of FMF could not be confirmed by PCR specific test. The patient started therapy with colchicine 0.5 mg 3 times per day (1.5 mg daily). One year after the initiation of the colchicine, he is free of febrile attack, exanthema or any other symptom (Figure 3).

**Discussion and conclusions**

The diagnosis of FMF is often difficult. Out of all patients with FMF, 50-60% are younger than 10 years old, 80-95% are younger than 20 years old, and 5-10% are older than 20. The disease is rare in persons older than 40 years old. Our patient is 40 years old, but signs and symptoms have been present since the age of 10.

As much as 50% of patients report erysipelas-like rashes at the lower extremities, particularly below the knees. A well demarcated, erythematous, warm rash, ranging from 15 to 50 cm² may develop and be accompanied by swelling. Several other nonspecific skin lesions may be seen in FMF. Rash and fever may be the only manifestations of the attacks. The diagnostic criteria are short febrile attacks recurring at various intervals and pain in the abdomen, chest and joints or skin rash. Since no specific test for FMF is available, the diagnosis must be based on precise clinical criteria and genetic tests.

Nonsense or missense mutations in the MEFV (Mediterranean fever) gene appear to cause the disease. This gene produces a protein called pyrin (derived from the association with predominant fever) or marenostrin (derived from the phrase “our sea”, due to the Mediterranean origin of most patients). After the successful cloning of the MEFV gene, researchers have developed a rapid test for the most common mutations. The role of E148Q pyrin gene mutation in the development of FMF remains inconclusive. Some authors believe it causes the disease, whereas others favour the concept of a noncausative role. In Greece it seems to be significantly frequent, but in our case the test proved to be negative.

The protein is expressed mostly in neutrophils, but its exact function is not known. The protein may function as an inhibitor of chemotactic factor (C5a) or perhaps of interleukin. Patients with normal pyrin/marenostrin levels may have the ability to deactivate the
target chemotactic factor when it is produced in response to an inflammatory stimulus. However, patients with FMF lack this ability, resulting in uninhibited activity of the chemotactic factor and episodes of inflammation (with associated fever) in the peritoneum, pleura and joints. Presumably, these inflammatory episodes lead to the excess production of amyloid A acute phase protein and reactant serum amyloid A with subsequent deposition at the kidneys; however, only patients with specific MEFV haplotypes develop amyloidosis.

Colchicine is effective in preventing attacks of FMF and the development of amyloidosis. The most important aspects of medical care are to make the correct diagnosis and therapy. In patients who do not respond to colchicine, the use of interferon-α or the TNF-blocking drug, etanercept may be effective.

Our patient had an immediate and excellent response to colchicine.

FMF is frequently accompanied by erysipelas-like exanthem. These should alert the physician to the correct diagnosis of this systemic disease.

**Riassunto**

Febbre familiare mediterranea. Una sfida diagnostica

La febbre familiare mediterranea è una malattia ereditaria che colpisce particolarmente le popolazioni che vivono nell’area Mediterranea. La complicanza più grave è rappresentata dall’amiloidosi, che può portare a un’insufficienza renale terminale. Viene descritto il caso di un paziente di 48 anni che si è rivolto alla nostra clinica a seguito di episodi ricorrenti di un’eruzione cutanea simil-erisipela localizzata a livello del gluteo destro, sempre associata a febbre e talvolta a artrite. La diagnosi clinica di febbre familiare mediterranea è stata confermata dall’identificazione di una mutazione del gene MEFV codificante per la febbre mediterranea. La somministrazione continua di colchicina per os (0,5 mg 3 volte al dì) ha consentito di evitare gli episodi ricorrenti.

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**Parole chiave:** Febbre mediterranea - Erisipela - Colchicine.

**References**

Tinea capitis in an adult woman masquerading as a worsening of dermatomyositis

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Dear Editor,

Tinea capitis (TC) is a common fungal infection, usually affecting children between 3 and 7 years of age. Only 3% to 5% of TC occurs post puberty. Adult cases of TC are very uncommon. We present a case of TC in a woman affected by dermatomyositis (DM).

A 66-year-old Caucasian woman was observed at the Department of Dermatology, University of Pavia. She presented itch, scaling scalp eruption, as well as hair loss of recent onset. The previous year, the patient had been diagnosed affected by dermatomyositis (DM). At that time she had a moderate muscles weakness, particularly of the proximal ones, and a heliotrope rash involving her face, with eyelid edema and eponychial telangiectases. Muscle enzyme levels were high. Electromyography revealed an abnormal pattern with myopathic pattern of the motor unit action, potential myopathic recruitment pattern, increased insertional and spontaneous activity. DM was diagnosed and a treatment with daily 50 mg prednisone and 100 mg azathioprine started. Since then, she could keep DM under control, tapering prednisone to 25 mg every other day, in association with daily 50 mg azathioprine.

At the time of the observation the patient showed areas of scalp alopecia with ill defined margins (Figure 1A), as well as diffuse scales and crusts (Figure 1A, close-up view). An interesting fact in her social history was that she owned 5 cats and 1 dog; some of them presented hairless lesions. On the basis of the clinical picture and of the patient's social and medical history, the first hypothesis of a worsening of scalp lesions of DM was excluded, and a fungal infection was suspected. Wood's light examination of the scalp produced a bright, yellow-green fluorescence of the hair. Microscopic examination showing an ectothrix infection of the hair shaft and cultures positivity for Microsporum canis confirmed the diagnosis of TC. For the photosensitising characteristic of griseofulvin, an 8-weeks treatment with daily itraconazole 100 mg per os and topical econazole 2% lotion was started. After 4 weeks we observed a remarkable reduction of erythema and scaling, whereas for the regrowth of hairs 3 months (Figure 1B) were needed. A moderate hair loss, as a consequence of DM, was, however, persistent.

TC is uncommon in adults and usually accounts for less than 3% of all TC cases. In Italy, during the last century, a progressive change of the dermatophitic flora causing TC has been found, with a gradual decrease of anthropophilic fungi and a rapid increase of zoophilic fungi. In our case, TC resulted as inoculation from animals. As an infection in adults is rare, TC is often not considered in the case of adults with hair loss. Other different diseases are erroneously diagnosed: discoid lupus erythematosus, Brocq pseudo-area, folliculitis decalvans, viral infections, impetigo, lichen planus pilaris, bacterial folliculitis, pustular psoriasis and DM; in particular, in our case, the alopecia was diagnosed as a worsening of scalp lesions of DM. Microscopic direct exams and fungal cultures represent gold standard methods for a correct diagnosis.

The authors have no conflict of interest to disclose. This case has been presented at the 77th National Congress of the Società Italiana di Dermatologia e Venereologia (SIDEV), 2002, May 15-18, Palermo, Italy.

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As an infection in adults is rare, TC is often not considered in the case of adults with hair loss. Other different diseases are erroneously diagnosed: discoid lupus erythematosus, Brocq pseudo-area, folliculitis decalvans, viral infections, impetigo, lichen planus pilaris, bacterial folliculitis, pustular psoriasis and DM; in particular, in our case, the alopecia was diagnosed as a worsening of scalp lesions of DM. Microscopic direct exams and fungal cultures represent gold standard methods for a correct diagnosis.

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DM, together with the therapeutic immunosuppression, may have impaired the immune defence mechanisms, making our adult patient into a susceptible target and contributing to the development of this unusual and severe dermatophytosis.

Currently, many experts consider griseofulvin to be the drug of choice for TC, and it is also the only drug Food and Drug Administration approved for this indication. Griseofulvin is a photosensitising drug, and can make worse the characteristic photosensitivity of DM. The rapid and excellent results of itraconazole in our patient support the view that this drug could be a useful alternative to griseofulvin for TC, in particular when associated to photosensitising diseases.1

References


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The efficacy and safety of topical gel containing clindamycin phosphate and zinc acetate in the treatment of acne vulgaris

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Dear Editor,

Topical antibiotics are very effective for the treatment of mild to moderate acne. Either erythromycin or clindamycin are known to be effective against Propionibacterium acnes.1, 2 The beneficial effect of the zinc component in the treatment of acne has been related to several mechanisms including; anti-inflammatory effects, antibacterial effects (antipropionibacterial) and modulation of epithelial differentiation.2, 4

The Leeds revised grading system is a simple useful, rapid means of assessing and follow-up acne patients, this grading system is based on a 12 colour photographs of facial acne which are ranked in order of severity. The criteria for severity included extent of inflammation, range and size of inflamed lesions and associated erythema.5

This 12 weeks study was performed to evaluate the efficacy and safety of topical gel containing clindamycin phosphate and zinc acetate (Zindaclin® 1% gel, Straken Ltd, UK) in the treatment of mild to moderate acne.

At the end of treatment improvement was found in 69% of patients; 55% of patients showed marked improvement and in 14% partial improvement was reported. Insufficient response to treatment was present in 31% of patients. The treatment was well tolerated.

Side effects were mild and transient and included dry skin in 17% and mild, transient erythema and itch in 5% of
patients. One patient suffered from marked facial irritation and left the study.

Zindaclin® 1% gel containing 1.2% clindamycin phosphate and 0.5% zinc acetate in a new topical product for the treatment of acne vulgaris.

The beneficial effect of Zindaclin® 1% gel in the treatment of acne may be related to the synergistic effect of clindamycin phosphate and zinc acetate and due to the ResiDerm® technology.

Zindaclin® formulation is based on the ResiDerm® technology which is a drug delivery technology in which topically active agents are complexed with zinc ions to improve dermal penetration and reduce transdermal penetration of the drug into the systemic circulation.

After 3 months of treatment improvement was found in 69% (55% showed marked improvement) of patients, using Zindaclin® 1% gel once daily.

The drug was well tolerated and side effects were mild and transient.

References


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