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Mycology: an update

M. PAPINI [1], E. M. DIFONZO [2], N. ASTE [3]

[1]Dermatology and Venereology
University of Perugia, Dermatology Clinic
Terni University Hospital, Terni, Italy
[2]Clinic of Dermatology
University of Florence, Florence, Italy
[3]Clinic of Dermatology
University of Cagliari, Cagliari, Italy

Fungal infections of the skin and nails are a common global problem. The majority of the studies focusing on these infections show that up to 20-25% of the world's population has skin mycoses, which makes them one of the most frequent forms of infection.

Their epidemiology is constantly evolving due mostly to the relevant migratory movements of recent years, but also because of profond changes in socio-economic conditions and lifestyles. The increase of vulnerable subjects, such as people of very advanced age and patients undergoing immunosuppressive therapy, inevitably leads to an increase in opportunistic fungal infections characterized by a high morbidity and mortality.

In addition to these socio-demographic aspects causing the emerging of new pathogens, new clinical manifestations are reported worldwide as well as novel views on the delicate mechanisms of the host-parasite interaction open up innovative scenarios in the understanding of the immune response and tolerance to fungi.

Newly developed diagnostic techniques such as molecular biology, new treatment modalities such as photodynamic therapy, as well as the increase in non-dermatophytic mould infections, will undoubtedly change the epidemiology and the management of many skin mycoses.

Nevertheless, skin mycoses are too often considered a disease of low impact, especially the superficial forms. It is wrongly believed that they are easily diagnosable and treatable diseases, and the interest of many Italian dermatologists in these infections has much weakened in recent years.

These considerations have helped the Editor of the Giornale Italiano di Dermatologia e Venereologia to renew interest in skin mycoses dedicating this special issue to dermatological mycology.

This monograph will begin with an excellent paper written by Luigina Romani on immune resistance and tolerance to fungi, delineating novel insights into the complex dynamics of the host-fungus interaction. The second paper comes from a team of recognized experts in the field of veterinary mycology. Annabella Moretti *et al.* will focus on dermatophyte infections in pets and other domestic animals in close contact with humans, offering important information that can be of great help in dealing with zoonotic fungal transmission. In his paper, Aldo Morrone will highlight the clinical problems arising from migration. This phenomenon notably influences our clinical practice in the field of mycology, leading to the observation of a growing number of unusual clinical aspects and aetiological agents considered rare in our country.

Corresponding author: M. Papini, Dermatology and Venereology, University of Perugia, Dermatology Clinic, Terni University Hospital, viale Tristano di Joannuccio 1, 05100 Terni, Italy.
E-mail manuela.papini@unipg.it

Special attention will be paid to specific clinical pictures of skin mycoses such as tinea atypica presented in the comprehensive review by Laura Aztori *et al*., but also *Malassezia* infections by Elisa Difonzo *et al*., skin and nail fungal infections in diabetic patients by Manuela Papini *et al*., and subcutaneous mycoses by Clara Romano. New therapeutic modalities will be masterly discussed by Bianca Piraccini and Claudia Gianni in their paper, which will offer updates on the management of onychomycosis, and by Gabriella Calabrò in her accurate description of the potential of photodynamic therapy in the treatment of nail fungal infections.

We sincerely trust and hope that this issue will be of help in updating Italian dermatologists, and hopefully others from abroad, about the constantly evolving and fascinating topic of dermatological mycology.

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Immune resistance and tolerance to fungi

L. ROMANI

Fungal diseases represent an important paradigm in immunology, since they can result from either the lack of recognition or over-activation of the inflammatory response. Current understanding of the pathophysiology underlying fungal infections and diseases highlights the multiple cell populations and cell-signaling pathways involved in these complex conditions beyond the dysregulated chaos in which fungal infection and disease are perceived. A systems biology approach that integrates investigations of immunity at the systems-level is required to generate novel insights into this complexity and to decipher the dynamics of the host-fungus interaction. Recent advances in the immune response to fungi have highlighted the cellular and molecular mechanisms of immune adaptations that maintain homeostasis with the fungal biota and its possible rupture in fungal infections and diseases. Functionally distinct modules of immunity, *i.e.*, resistance and tolerance, evolved for the achievement of the best-fitted host-fungus interaction in mammals, are now essential components of the host-fungus interaction in the vertebrate host.

KEY WORDS: Fungi - Microbiology - Fungi, immunology.

Department of Experimental Medicine and Biochemical Sciences
Pathology Section, University of Perugia
Polo Unico Sant'Andrea delle Fratte, Perugia, Italy

Of the 1.5 million fungal species, only a few hundred are pathogenic to mammals. Fungal diseases in mammals often reflect impaired immune function, and fungi did not emerge as major pathogens for humans until the late 20th century. For example, candidiasis was uncommon until the 1950s, when thrush was associated with the introduction of antibiotics that disrupted bacterial flora. Similarly, diseases such as cryptococcosis, aspergillosis, and histoplasmosis were rare until recently, when their prevalence increased with the human immunodeficiency virus epidemic and the development of immunosuppressive therapies.[1, 2] Even more, it has been forecast that global warming will bring new fungal diseases for mammals.[3] Invasive infections, referred to as invasive fungal diseases (IFD),[4] continue to be a serious problem in patients with hematologic disorders and solid organ [5] and hematopoietic stem cell transplantation.[6] IFD have been reported in non high-risk, *sensu strictu*, patients, such as patients with the H1N1 [7] or *Mycobacterium tuberculosis* [8] infection, hyper IgE syndrome,[9-11] and anti-TNF alpha therapy.[12]

Corresponding author: Department of Experimental Medicine and Biochemical Sciences – Pathology Section, University of Perugia, Polo Unico Sant'Andrea delle Fratte, 06132 Perugia, Italy.

The dynamics of the host-fungus interaction

Fungi can interact with hosts in multiple ways, establishing symbiotic, commensal or pathogenic relationships with plants, animals or humans. Indeed, the mycobiome, that is the fungal biota in an environment, is an important component of the human microbiome. By interfacing with other biomes, as well as with the host, the mycobiome probably contributes to the progression of fungus-associated diseases and plays an important role in health and disease.[13] Despite the intimate contact of fungi with the human host, fungal diseases in immunocompe-

tent hosts are fairly uncommon, indicating that fungi have evolved particular adaptation mechanisms to the host allowing them to persist relatively unnoticed by the immune system.[14] This "peaceful" coexistence may digress into overt disease under conditions of immune deregulation. The ability to colonize almost every niche within the human body involves specific reprogramming events to adapt to environmental conditions, fight for nutrient acquisition and utilization and deal with stresses generated by host defense mechanisms.[14, 15] Genomic and transcriptomic approaches have revealed the interconnection between metabolism, morphogenesis, and the response to stress in adaptation to the host environment,[16-18] thus linking the pathogenesis of fungal diseases to host adaptation.[19, 20] Such adaptations can improve parasite fitness but can also create potential therapeutic targets.[21] Indeed, disease onset is often critically dependent on the ability of fungi to adapt through reversibly switch morphotypes, a trait that has forced the host immune system to continuously evolve its repertoire of cross-regulatory and overlapping antifungal host responses at different body sites.[22] Thus, in the context of a dynamic host–fungus interaction, the strategies used by the host to limit fungal infectivity are necessarily assorted in order to cope with the multitude of fungal survival strategies; in retaliation, fungi have developed their own elaborate tactics to evade or modulate host defenses and to survive.

Most fungi, such as *Aspergillus fumigatus*, *Cryptococcus neoformans*, and the thermally dimorphic fungi (*Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis, Penicillium marneffei,* and *Sporothrix schenckii*), are ubiquitous in the environment and humans are exposed by inhaling spores or small yeast cells. Probably, they have gained their pathogenic potential in environmental niches through antagonism with competing microorganisms, including predators. The crosskingdom interactions in nature are considered to be important for the evolution and maintenance of microbial virulence toward human.[23] In the case of commensals, such as *Malassezia* spp. and *Candida albicans*, co-evolution with their mammalian hosts for millions of years implicates the existence of complex mechanisms of immune adaptations and, likewise, of sophisticated mechanisms to antagonize immunity. Thus, fungi as other microbes (be symbionts, commensals or true pathogens) could be regarded as elicitors, perpetuators, modulators and terminators of inflammatory responses, thus likely contributing to the increased incidence of chronic inflammatory and autoimmune diseases in high-income countries. Applying systems biology approaches to this complex process has resulted in a better appreciation of the intricate cross-talk provided by temporal changes in mediators, metabolites, and cell phenotypes underlining the coordinated processes.[24]

Fungi and inflammation: evolving concepts

As in autoimmunity and chronic inflammation, an imbalance between pro- and anti-inflammatory signals may prevent successful host/fungal interaction, thus leading to infection and disease.[25] Indeed, despite the occurrence of severe fungal infections in immunocompromised patients, clinical evidence indicates that fungal diseases also occur in the setting of a heightened inflammatory response, in which immunity occurs at the expense of host damage and pathogen eradication.[26] Although inflammation is an essential component of the protective response to fungi, these have evolved ways to exploit and subvert it, thereby affecting their ability to persist in the host and pathogenicity.[27] A hyper-inflammatory response does, in fact, enhance virulence of some fungi. This is well illustrated by the commensal lifestyle of *Malassezia* spp. in the normal skin, possibly due to the down-regulation of inflammation *via* TGF-β1 and IL-10.[28] In contrast, in atopic dermatitis and psoriasis, the skin barrier enhances release of allergens and molecules involved in hyper-proliferation, cell migration, and disease exacerbation. Additional fungal diseases are also critical examples of such bidirectional influences between infection and immune-related pathology. For example, in chronic mucocutaneous candidiasis (CMC), *C. albicans* yeasts persist in recurring lesions of the skin, nails, and mucous membranes.[29] Although CMC has occasionally been associated with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (a condition of dysfunctional T cell activity), evidence has highlighted the contribution of deregulated inflammation and immune responses to disease pathogenesis.[30, 31] Chronic disseminated candidiasis (CDC) is typically observed during neutrophil recovery in patients with acute leukemia and requires protracted antifungal therapy. However, the

efficacy of adjuvant corticosteroid therapy in these patients supports the pathophysiological hypothesis that CDC belongs to the spectrum of fungus-related immune reconstitution inflammatory syndrome.[32] These observations highlight a truly bipolar nature of the inflammatory process in infection, at least by specific fungi. Early inflammation prevents or limits infection, but an uncontrolled response may eventually oppose disease eradication. Thus, fungal diseases represent an important paradigm in immunology since they can result from either the lack of recognition or over-activation of the inflammatory response.

In this regard, fungal sensing of IL-17A has recently been described as a possible mechanism by which host inflammation may favor fungal infectivity and pathogenicity.[33] Indeed, sensing of IL-17A induced artificial nutrient starvation conditions in *C. albicans* and *A. fumigatus* resulting in increased adhesion and filamentous growth that clinically translates in a dramatic increment of biofilm formation and fungal virulence.[33] This suggests that commensals or ubiquitous fungi have evolved a contingency–based system during co-evolution to guarantee their persistence in an inflammatory host environment.

The main implication of these findings is that, at least in specific clinical settings, it is a heightened inflammatory response that likely compromises a patient's ability to eradicate infection, and not an "intrinsic" susceptibility to infection that determines a state of chronic or intractable diseases.[25] The conceptual principle highlighting a truly bipolar nature of the inflammatory process in infection is best exemplified by the occurrence of severe fungal infections in patients with chronic granulomatous disease,[34] cystic fibrosis [35] or with immune reconstitution inflammatory syndrome (IRIS),[36] an entity characterized by local and systemic inflammatory reactions that can result in quiescent or latent infections manifesting as opportunistic mycoses. IRIS responses are also found in otherwise immunocompetent individuals and likely associates with disease severity in paracoccidioidomycosis,[37] blastomycosis [38] or *Malassezia* folliculitis.[39] Additionally, a high incidence of fungal infections and sensitization to *Aspergillus* spp. has been described in the hyper–IgE syndrome in which increased levels of proinflammatory gene transcripts have been found.[40, 41] These observations suggest that an inflammatory loop compromising a patient's ability to eradicate infection, and not an "intrinsic" susceptibility to infection, seems to be at work, at least in specific clinical settings, the manipulation of which may offer strategies to control or prevent exacerbations of these diseases.

Resistance and tolerance mechanisms of antifungal protection

We have entered an exciting transition period from studying the molecular and cellular bases of fungal virulence to deciphering the mechanisms of immune activation and tolerance to them within the microbiota. A two–component antifungal response has emerged that includes resistance, *i.e.*, the ability to limit fungal burden, and tolerance, *i.e.*, the ability to limit the host damage caused by either the immune response or other mechanisms. Evolutionary conserved from plants to vertebrates,[42] this new concept may help to define the best fitness in response to fungi and its integration into new medical practices. The immune system protects from infections primarily by detecting and eliminating invading pathogens through a variety of host resistance effector mechanisms.[22, 43] Resistance is meant to reduce pathogen burden during infection through innate and adaptive immune mechanisms, whereas tolerance mitigates the substantial cost to host fitness of resistance. Even in the absence of overt tissue damage, resistance mechanisms commonly occur at a cost to normal tissue function, thus causing immunopathology. This means that the optimal immune response is determined by the balance between efficient pathogen clearance and an acceptable level of immunopathology.

Inflammation is an essential process required for immune resistance, particularly at mucosal tissues, during the transition from the rapid innate to the slower adaptive response. However, the downside of this powerful mechanism of protection against fungi is the collateral damage to the host. These side-effects may be more devastating than infection itself. Thus, the ability to tolerate a pathogen's presence is a distinct host defense strategy that may have evolved to favor protective mechanisms without pathogen killing.[22, 43] A plethora of tolerance mechanisms, despite less known relative to resistance mechanisms, protect the host from immune-or pathogen-induced damage.[44, 45] Therefore, the term tolerance is semantically used here to refer to the multitude of anti-inflammatory mechanisms, including immunological

tolerance, that is, unresponsiveness to self-antigens. At this stage however, whether "unwanted" immune responses against "self", environmental antigens and commensal microorganisms occur is not clearly defined, although there is evidence that fungal sensitization contributes to auto-reactivity against self-antigens due to shared epitopes with homologous fungal allergens.[46]

Mechanisms of resistance

Immunity to fungi is a dynamic interplay between every arm of the immune system. Innate immune mechanisms are used by the host to respond to a range of fungal pathogens in an acute and conserved fashion. The constitutive mechanisms of innate defense are present at sites of continuous interaction with fungi and include the barrier function of body surfaces and the mucosal epithelial surfaces of the respiratory, gastrointestinal and genito-urinary tracts.[22] Microbial antagonism, defensins, collectins and the complement system realize the strict fungus specificity of the constitutive mechanisms and provide opsonic recognition. Multiple cell populations and cell-signaling pathways are involved in the antigen-independent recognition of the fungus by the innate immune system. Pattern recognition receptors (PRRs) for fungi include Toll-lile receptors, C-type lectin receptors, nucleotide oligomerization domain-like receptors and NALP3 inflammasome.[22] Both murine and human studies have confirmed the association of susceptibility to fungal infections and diseases with genetic deficiency of selected PRRs.[47]

Receptors on phagocytes not only mediate downstream intracellular events related to clearance, but also participate in complex and disparate functions related to immunomodulation and activation of immunity, depending on cell type. Monocytes, macrophages, neutrophils, as well as normally non–phagocitic cells, such as endothelial and epithelial cells, mostly contribute to the innate immune response through phagocytosis and pathogen killing. In contrast, pathogen engulfment by dendritic cells (DCs) activates their maturation and release of specific signature cytokines to orchestrate the differentiation of naïve T lymphocytes into appropriate T helper (Th) subtypes, thereby shaping the adaptive response. To achieve optimal activation of antigen–specific adaptive immunity, it is first necessary to activate the pathogen-detection mechanisms of the innate immune response. However, by hyper-inducing proinflammatory cytokines, facilitating tissue damage or impairing protective immunity, PRR activation itself is a double-edged sword and this may explain why PMNs, although essential in initiation and execution of the acute inflammatory response and subsequent resolution, may act as double-edged swords, as the excessive release of oxidants and proteases may be responsible for injury to organs and fungal sepsis.[25]

Well-balanced Th1 and Th17 cell responses are crucial in antifungal immunity and facilitate phagocytic clearance of fungal recognition. Combined deficiency of the Th1 and Th17 pathway predisposes to fungal diseases,[31, 48] thus emphasizing the important role played by both pathways in resistance against fungi.[22, 49, 50] A dominant Th1 response correlates with the expression of protective immunity to fungi [22, 51-53] and vaccines.[51, 54] Through the production of the signature cytokine IFN-γ and help for opsonizing antibodies, the activation of Th1 cells is instrumental in the optimal activation of phagocytes at sites of infection. Therefore, the failure to deliver activating signals to effector phagocytes may predispose patients to overwhelming infections, limit the therapeutic efficacy of antifungals and antibodies and favor fungal persistency.[22] Recently, the phenotype of the peripheral CD4+T-cell response to *Cryptococcus* was associated with disease severity and outcome in HIV-associated cryptococcal meningitis and IFN-γ/TNF-α-predominant responses were associated with survival.[55]

Th17 are present in the human T cell memory repertoire to fungi [56-59] inborn errors of human IL-17 immunity underlie CMC.[60] However, CMC patients with and without AD–HIES have defective Th17 [61] but also Th1 [62, 63] responses. This could be explained with the notion that Th17 cells, although found early during the initiation of an immune response, are involved in a broad range of both Th1-and Th2-dominated immune responses.[64, 65] In terms of effector functions, although the ability of IL-17A to mobilize neutrophils and induce defensins may contribute to a prompt and efficient control of the infection at the different body sites, the IL-17RA/IL-17A pathway has been shown to be either essential [64, 66, 67] or not [65, 68] in candidiasis. This suggests that the activity of IL-17A/IL-17RA axis may depend on stage and site of infection, likely contingent upon environmen-

tal stimuli activating cells producing cytokines of the IL-17 family, including IL-22. It is intriguing that Th17 responses are down regulated by *C. albicans.*[69] Regardless of the contribution of this phenomenon to infection or commensalism, this finding suggests that Th17 responses are finely tuned by fungi, as the failure to down-regulate Th17 may eventually result in chronic inflammation and failure to resolve the infection.[70, 71] The mechanisms that linked inflammation to chronic infection have been credited to the offending potential of IL-17A that, although promoting neutrophil recruitment, impeded the timely restriction of neutrophil inflammatory potential [72] while directly promoting fungal virulence.[33] Thus, the Th17 pathway could be involved in the immunopathogenesis of chronic fungal diseases where persistent fungal antigens may maintain immunological dysreactivity. This may happen in autoimmune polyendocrine syndrome type 1 patients (APS-1) and Aire-deficient mice [73] were an excessive Th17 reactivity was observed. This finding apparently conflicts with the presence of autoantibodies against IL-22, IL-17A and IL-17F observed in these patients.[74, 75] Although correlated to susceptibility to CMC, these antibodies were also present in patients without CMC. In addition, despite the presence of antibodies to type I IFN, APS-I patients do not appear prone to recurrent viral infections. It has instead been shown that autoantibodies to pro–inflammatory cytokines may act as beneficial autoimmunity in their ability to dampen proinflammatory mediators and restrict self–destructive immunity.[76]

Recent evidence indicate that IL-22, a member of the IL-10 cytokine family, may play a crucial role in the innate immune resistance and local protection in mucocutaneous fungal diseases.[65, 77] IL-22, involved in the modulation of tissue responses during inflammation, is produced by Th22, Th1, Th17, NK-22, NKT and innate lymphoid cells (ILC)3 and acts on cells of non-hematopoietic origin, particularly epithelial cells. In the skin, IL-22 induces keratinocyte proliferation and epidermal hyperplasia, inhibits terminal differentiation of keratinocytes, and promotes the production of antimicrobial proteins.[78] In the gut, IL-22 regulates intestinal homeostasis and mucosal wound healing *via* the activation of epithelial signal transducer and activator of transcription 3 (STAT3). Through the exploitation of primitive antifungal defense mechanisms, IL-22 was crucially involved in the control of Candida growth at mucosal sites in conditions of Th1 and Th17 deficiency.[65] Produced by ILC3 cells expressing the aryl hydrocarbon receptor (AhR), IL-22 directly targeted gut epithelial cells (ECs) to induce STAT3 phosphorylation and the release of S100A8 and S100A9 peptides known to have anticandidal activity and anti-inflammatory effects.[65] Thus, due to dominant-negative mutations of STAT3, patients with autosomal dominant hyper-IgE syndrome (AD-HIES) patients have a defective Th17 [61] that is likely amplified on ECs where STAT3 mutation compromises the IL-22 effects. IL-22 also mediates antifungal resistance and epithelial protection in experimental and human vulvovaginal candidiasis (VVC) as well as in recurrent VVC (RVVC). In RVVC, functional genetic variants in IL22 genes were found to be associated with heightened resistance to RVVC, and they correlated with increased local expression of IL-22. Not only are naturally occurring IL-22$^+$ cells highly enriched at mucosal sites, where continuous exposure to fungi occurs, but also memory *C. albicans* – specific IL-22$^+$CD4$^+$ cells are present in humans [79] and defective in patients with CMC.[80] Thus, IL-22$^+$ cells, employing ancient effector mechanisms of immunity, may represent a primitive mechanism of resistance against fungi under a condition of limited inflammation. The fact that IL-22 production in the gut is driven by commensals (see below) also provides novel mechanistic insights on how antibiotic-related dysbiosis may predispose to candidiasis.[77]

Mechanisms of tolerance

Treg cells

The exposure to fungi requires the generation of a controlled immune response in the host that recognizes and controls them, limits collateral damage to self-tissues and restores a homeostatic environment. A number of clinical observations suggest an inverse relationship between IFN-γ and IL-10 production in patients with fungal infections.[22] High levels of IL-10, negatively affecting IFN-γ production, are detected in chronic candidal diseases,[62] in the severe form of endemic mycoses and in neutropenic patients with aspergillosis.[81] Thus, high levels of IL-10 have been linked to susceptibility to fungal infections.[82] However, given its prominent effect on resolution of inflammation, IL-10 production may be a consequence,

rather than the cause, of the infection.[81] This predicts that, in the case of chronic fungal infections dominated by non-resolving, persisting inflammation, IL-10 produced by Treg cells acts as homeostatic host-driven response to keep inflammation under control. Treg cells with anti-inflammatory activity have been described in fungal infections of both mice [81, 83] and humans.[84, 85] In experimental fungal infections, inflammatory immunity and immune tolerance in the respiratory or the gastrointestinal mucosa were all controlled by the coordinate activation of different Treg cell subsets, exerting a fine control over effector components of innate and adaptive immunity.[81] Seen in this context, the Treg/IL-10 axis is a dangerous necessity the failure of which may lead to detrimental inflammation. However, as the Treg responses may handicap the efficacy of protective immunity, the consequence of Treg activity is less damage to the host but also fungal persistence and immunosuppression, eventually.[83, 85] Thus, by controlling the quality and magnitude and effector innate and adaptive responses, the spectrum of Treg cell activities may go from "protective tolerance", defined as a host's response that ensures survival of the host in a trade-off between sterilizing immunity and its negative regulation limiting pathogen elimination to overt Immunosuppression.[81] Taking a step further, this suggests that the interaction between fungi and the host immune status may determine their position from commensals to pathogens, and this position can change continuously.

Tryptophan metabolism

The enzyme indoleamine 2,3-dioxygenase 1 (IDO1) and its downstream catabolites sustain the delicate balance between Th1/Th17 pathways and Treg cells, by providing the host with adequate protective immune mechanisms without necessarily eliminating the pathogen or causing undesirable tissue damage.[86] As a result of their ability to induce differentiation of Treg cells and inhibit Th17 cells, IDO1 is critical to cell lineage commitment in experimental fungal infections and contributes to the overall outcome of inflammation, allergy and Th17-driven inflammation in these infections. Under these circumstances, the Th17 pathway, by inhibiting tryptophan catabolism, may instead favor pathology and provides evidence accommodating the apparently paradoxical association of chronic inflammation with fungal disease.[34]

IDO1 is a "metabolic" enzyme conserved through the past 600 million years of evolution. Initially recognized in infection because of antimicrobial activity ("tryptophan starvation" of intracellular parasites), IDO1 is now widely recognized as suppressor of acute inflammatory responses and regulator of mammalian immune homeostasis.[87] Not surprising, IDO1 may represent an evasion mechanism for microbes that establish commensalism or chronic infection.[87] In their capacity to induce Tregs and inhibit Th17, IDO1-expressing DCs and ECs and kynurenines revealed an unexpected potential in the control of inflammation, allergy and Th17-driven inflammation in these infections.[72, 88]

Microbiota regulation of resistance and tolerance to fungi

Commensal-driven mucosal responses are up-regulated in IDO1 deficiency [89] and IL-22 responses are up-regulated in conditions of defective adaptive immunity [65] and IDO deficiency.[77] AhR is a ligand-activated transcription factor that mediates IL-22 production.[90] A variety of indole derivatives act as endogenous ligands for AhR [91] and are generated through conversion from dietary tryptophan by commensal intestinal microbes.[92] Recent evidence has shown that AhR is involved in the (patho)physiology of skin including the regulation of skin pigmentation, photocarcinogenesis, and skin inflammation.[93] Of interest, the ability of *Malassezia*-derived indoles to activate AhR correlated with local immunoregulation [94] and pathogenicity in seborrheic dermatitis.[95] Similarly, metabolomics has revealed that bioactive indoles with Ahr agonist activity are also present in mice with candidiasis.[77] Thus, the trpyptophan metabolism pathway is exploited by commensals and the mammalian host to increase fitness in response to fungi *via* induction of resistance and tolerance at the skin and mucosal surface (Figure 1). The new findings support a model in which the IL–22 axis controls the initial fungal growth (*i.e.*, resistance) and ECs homeostasis likely exploiting primitive antifungal effector defense mechanisms. In contrast, the exploitation of the IFN-γ/IDO 1axis for functional specialization of antifungal regulatory mechanisms (*i.e.*, protective tolerance) may have allowed the fungal microbiota to co-evolutes with the mammalian immune system, survives in

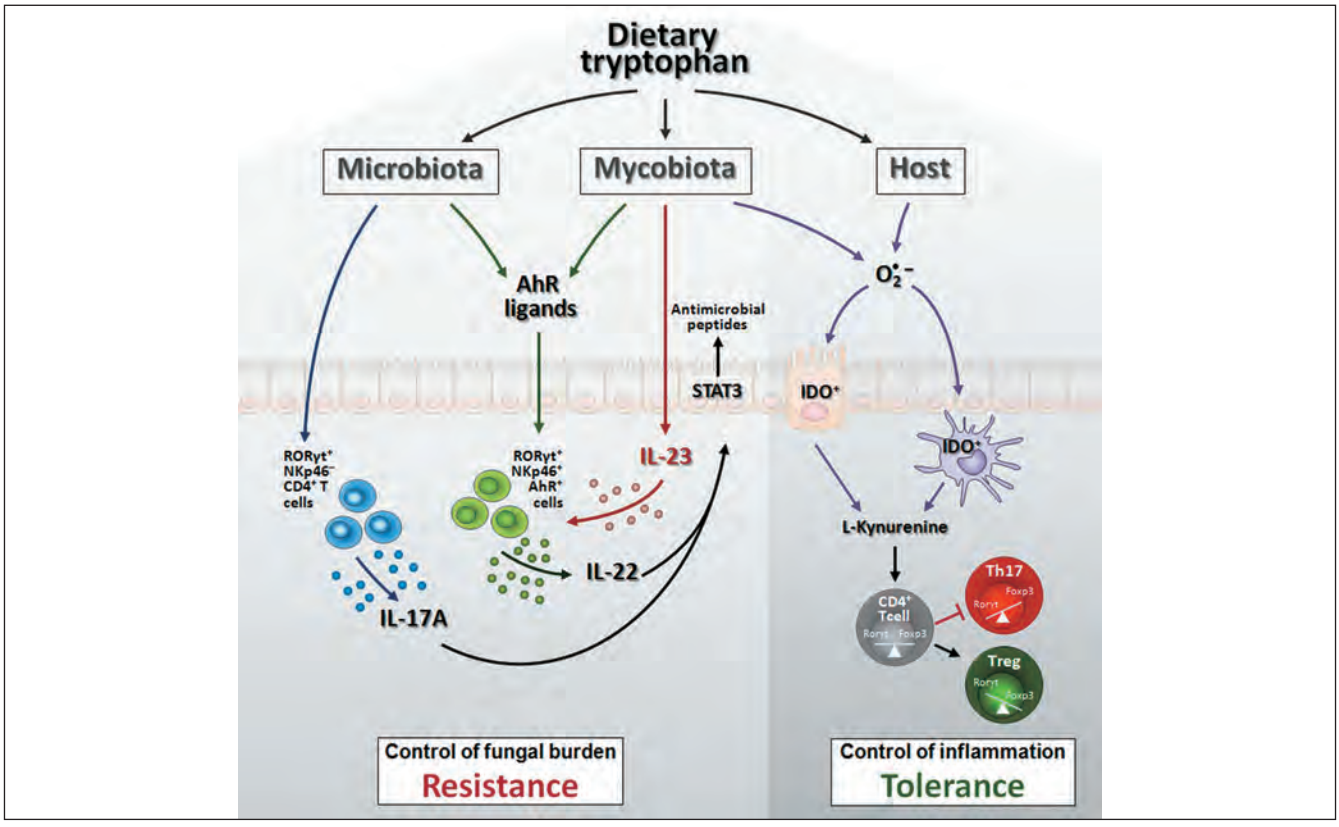

Figure 1.—Resistance and tolerance to fungi and its regulation by tryptophan. The tryptophan metabolism pathway is exploited by the mammalian host and commensals (including fungi) to increase fitness in response to fungi through resistance and tolerance. At mucosal surfaces and skin, the fungal biota promotes the production IL-22, *via* IL-23 and aryl hydrocarbon receptor (AhR) ligands, by CD3$^-$NKp46$^+$retinoic acid-related orphan receptor-γt (RORγt)$^+$AhR$^+$ innate lymphoid cells. By contrast, NKp46$^-$ cells produce IL-17A. IL-22 targets epithelial cells, leading to activation of signal transducer and activator of transcription 3 (STAT3) and, together with IL-17A, to production of antimicrobial peptides. Various indole derivatives, which are generated through conversion from dietary tryptophan by commensal intestinal microorganisms, act as endogenous ligands for AhR, and thereby contribute to IL-22 production. Fungus-induced activation of tryptophan catabolism by indoleamine 2,3-dioxygenase (IDO) expressed by dendritic cells and epithelial cells leads to the production of immunologically active compounds that induce the transcription of FOXP3 and suppress the transcription of RORγt. These findings support a model in which the AhR/IL-22/IL-17A axis control initial fungal growth (i.e., resistance) and epithelial cell homeostasis. By contrast, the exploitation of the IFN-γ/IDO axis for functional specialization of antifungal regulatory mechanisms (*i.e.*, tolerance) may have allowed the fungal microbiota to evolve with the mammalian immune system, survive in conditions of inflammation and prevent dysregulated immunity. The balance between resistance and tolerance to fungi may accommodate the spectrum of host/fungus relationships, ranging from protection and immunopathology to fungal persistence and immunosuppression.

conditions of high-threat inflammation and prevents dysregulated immunity.[86] The two pathways, although non-redundant, are reciprocally regulated and compensate each other in the relative absence of either one,[65] consistent with the theme that adaptive immunity depends on innate immunity but innate immunity requires adaptive regulation. Clinically speaking, this finding not only helps to explain the association of fungal infections with dysbiosis but also points to the essential help the microbiota may provide in fungal colonization and pathogenicity in immunodeficient patients.

The contribution of fungi to immune tolerance

It is not surprising that many of the strategies mammalian hosts have developed to coexist peacefully with their microbiota can be hijacked or manipulated by commensals but potentially harmful fungi to ensure their own survival. Manipulation of the regulatory network of the host by the fungal microbiota is one such mechanism to ensure fungal survival.[81, 96, 97] Zymosan and *C. albicans* activated the tolerogenic program in gut macrophages [98] and DCs [97, 99] resulting in the activation of

Treg–dependent immune tolerance. ECs may also play critical roles by expressing PRRs for fungi and providing the machinery required for the induction of T-cell tolerance.[100] In the normal skin, the fungus *Malassezia* down-regulates inflammation *via* TGF-β1 and IL-10, and establishes itself as a commensal. In contrast, in atopic dermatitis and psoriasis, the skin barrier acts to enhance release of allergens and molecules involved in hyper-proliferation, cell migration, and disease exacerbation. In the case of *C. albicans*, besides commensalism, the induction of tolerance also resulted in amelioration of gut inflammation.[97] Thus, similar to symbionts, the fungal microbiota may contribute to the balance of inflammation and tolerance to the benefit of both parties, at mucosal surfaces and at distant sites.[101] Thus, host regulatory responses may contribute to the transition from symbionts to pathobionts. In this scenario, it has important translational implications the clear distinction of conditions in which yeasts are cause (that is, required for disease) as suggested,[102] trigger (that is, not required, but may favour disease progression) or sign (that is, pathogenicity is promoted by the host failure) of persistent, non–resolving inflammation and associated clinical diseases.

Conclusions: translating basic knowledge into clinical perspectives

Vaccine development

The level of our understanding of fungal–host interactions has progressed to the point where vaccines against both primary fungal pathogens and the prevalent opportunistic fungi are becoming a reality.[103, 104] The nature of the antifungal vaccine, the route of antigen delivery and the mode of antigen routing and presentation are important for determining the success of a fungal vaccine. Indeed, recent evidence has highlighted striking differences in antigen presentation pathways in DCs leading to the activation of distinct protective immune responses to fungal vaccines.[105, 106] The data highlight how understanding memory at a basic level, including information obtained from suitable animal models, may be exploited to personalize vaccination strategies against fungal diseases.

Genetic screening for susceptibility and resistance to fungi

There is undeniable evidence that genetic variants within recognition molecules involved in innate immunity may account, in part, for the inherited differences in human susceptibility to fungal infections.[107, 108] Although the dissection of complex genetic traits modulating susceptibility to fungal infections is complex, the contribution of host genetics may hold the key to elucidate genetic markers for IFD occurring in high-risk patients. Understanding which patients are at highest risk of developing a life-threatening infection is at present a major deficiency, and genetic markers will probably assist in risk assessment.

Novel immune-based antifungal therapies

The last decennia have brought important progress in the development of more effective and safe antifungal agents.[109] However, medical treatments that increase host resistance, such as antibiotics, place selective pressures on pathogens. As tolerance mechanisms are not expected to have the same selective pressure on pathogens, new drugs that target tolerance will provide therapies to which pathogens will not develop resistance. Thus, challenging existing paradigms with new perspectives from the crosstalk between fungi and the immune system will eventually lead toward the discovery of "commensal signatures" and the development of multi–pronged therapeutic approaches for mucosal and systemic fungal diseases. Breakthroughs in understanding how mucosal homeostasis is established, maintained or disrupted in the presence of fungal exposure and/or colonization should be sources of new therapeutics and drugs targeting specific inflammatory or metabolic endpoints. In essence, limiting inflammation, *via* PRR agonism/antagonism, to stimulate a protective immune response to fungi should pave the way for rational design of novel immunomodulatory therapies. Tryptophan metabolites are also likely candidate as potent regulators capable of simultaneously activating antifungal resistance and taming overzealous inflammatory host responses.[72] By the use of multidisciplinary approaches based on whole-genome immunogenetics, cutting-edge "omics" techniques, advanced bioinformatics and systems biology applied to immune profiling, it will be possible to challenge

existing paradigms in the fields of fungal immunology and immunopathology, thereby leading to the discovery of immune-based therapeutic approaches for mucosal and systemic fungal diseases.

Riassunto

Immunità e tolleranza ai funghi

Le malattie da funghi, ivi comprese le infezioni, rappresentano un paradigma importante in immunologia, essendo causate sia da un'aumentata sia da una ridotta attività immunologica. È evidente che le risposte immuni ai funghi sono regolate a molti livelli, regolatori ed effettori, talora percepiti come altamente caotici. Solo un approccio di studio basato sulla biologia dei sistemi può fornire indicazioni e spiegazioni utili al superamento di tale percezione nonché alla comprensione dei meccanismi di interazione dinamica ospite/fungo. In tal senso, recenti acquisizioni in ambito sperimentale e clinico hanno messo in luce sia la complessità di detta interazione sia la sua semplificazione in "moduli" di risposta antifungina. Resistenza, capacità cioè di controllo immunologico dei funghi commensali e non, e tolleranza, capacità cioè di regolare l'entità di dette risposte di resistenza, fanno parte del lessico attuale nell' interazione ottimale ospite/fungo.

Parole chiave: Funghi - Microbiologia - Funghi, immunologia.

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Dermatophytosis in animals: epidemiological, clinical and zoonotic aspects

A. MORETTI [1], F. AGNETTI [2], F. MANCIANTI [3], S. NARDONI [3], C. RIGHI [2], I. MORETTA [1], G. MORGANTI [1], M. PAPINI [4]

[1]Department of Biopathological Science and Animal and Alimentary Production Hygiene University of Perugia, Perugia, Italy
[2]Department of General Diagnostic and Animal Welfare Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy
[3]Section of Parasitology and Parasitic Diseases Departemnt of Veterinary Science University of Pisa, Pisa, Italy
[4]Dermatological Unit of Terni Department of Medical-Surgical Specialties University of Perugia, Terni, Italy

Aim. **Dermatophytosis are the most frequent fungal infections of pets and livestock and play an important role in animal and human health due to their zoonotic potential. Another important aspect of these infections is linked to the economic consequences in farm animal and fur production systems. An overview of dermatophytosis in animals is described in this paper. Epidemiological, clinical and zoonotic aspects are addressed, considering individual species, both pets and farmed animals.**

Methods. **In particular, most recent investigations in the field of animal mycology, carried out in Central Italy, are reported, with particular reference to rabbit, ruminants, horse, dog, cat and some wild species.**

Results. **The information in this article show how dermatophytes infect a wide range of animals which may be in contact with human beings either directly or indirectly. Consequently they are frequently a source of infection for human beings who, vice versa, may sometimes become contagious for animals.**

Conclusion. **Fungal pathogens derive their power to spread from contamination of the animal's habitat – whether the animal is a conventional pet or not, a farm animal or living in the wild. Thus if treatment of the animal or human patient is to achieve optimal efficacy, it needs to be associated with adequate environmental measures.**

KEY WORDS: Tinea - Animals - Epidemiological studies.

Dermatophytosis accounts for many of the fungal infections that dermatologists and veterinarians are called upon to treat, involving as it does humans, pets, livestock and wild animals. Constant changes in human-animal relationships are determined by evolving human needs and customs which create complex zoo-anthropological values. Animal over-crowding is nowadays a major problem in rural and urban areas. On farms livestock overcrowding is aimed at meeting the growing demand for food supplies, while the satisfaction of cultural, emotional and psychological needs is responsible of pet overcrowding in towns. Both instances create opportunities for animal-human transmission of pathogen micro-organisms, with health and epidemiological consequences that are sometimes hard to manage. Major difficulties arise with "animalization" of the environment, which means a widespread dispersion of hair, feathers, dropping and other organic matters that creates an optimal medium for exuberant proliferation of pathological micro-organisms potentially targeting humans and animals in the territory. Zoonotic consequences are facilitated by poor compliance with hygiene and health regulations as well

Corresponding author: F. Agnetti, Department of General Diagnostic and Animal Welfare, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, via G. Salvemini 1, 06126 Perugia, Italy. E-mail: f.agnetti@izsum.it

as by abandoned stray animals. At the same time suburban areas may become transition zones for contact with wild animals like wild boar, foxes, river rats, mice and other rodents, and thus become channels that allow extra-urban infections to penetrate into cities. Knowing about, and being aware of, the spaces that surround human beings means we can provide more in-depth control, treatment and prevention of health and hygiene emergencies in men and animals.

The attitudes of people who cope with these problems have improved greatly over the years, thanks to profound socio-cultural modifications. Farmers have become more professional and more attentive to animal well-being. Animal lovers in the general population have become more aware as the social and emotional value of pets was recognized. Individual knowledge of health and hygiene has increased, creating demands not only for better levels in the individual but also for prevention of any potential health hazard in the environment. These new attitudes have focussed attention on all the major assessment criteria for epizoonotic fungal control, which implicates a remarkable commitment of human and financial resources involving several branches of human and animal health. It has already yielded interesting data on the epidemiology, pathogenesis, clinical features, diagnosis, therapy and prevention of fungal infections.

An in-depth analysis of the animal's habitat is crucial for making a predictive risk assessment of transmission to the receptive host. Epidemiological charts with the distribution, prevalence and incidence of various species of fungi in diverse animal species are essential. They provide an organic picture of any given situation in a determined area and enable a coordinated targeted strategy to be developed. They also provide information about risk factors and triggers of the fungal infection and monitor the epidemiological impact over time. Furthermore, predictive risk chart can foster collaboration and exchange of information among the diverse structures engaged in safeguarding human and animal health.

Given this background this review focused on dermatophytosis in animals and, by association, in humans. Understanding the clinical pictures in animals as well as the complex epidemiological and management issues will provide the dermatologist with in-depth information for treating and managing the infection in humans. We hope it will also stimulate and motivate closer and more frequent collaboration between human dermatologists and vets.

Materials and methods

Dermatophytosis in animal species

In animals and humans *Epidermophyton* (exclusively human), *Microsporum* and *Trichophyton* (prevalently animal), are the three types of fungi which underlie dermatophysosis, with each including several species. Animals risk being infected by many species of dermatophytes which are mostly animal-specific but may be ground-specific and occasionally even human-specific.[1-6] Although these parasites are not animal species-specific, some have developed a predominant predilection for the particular animal species which was probably their original host. Evolution modified dependence on the primitive host, giving rise to a range of biological behaviour patterns, *Microsporum (M.) nanum* for example is swine-specific, *Trichophyton (T.) gallinae* is bird-specific and *T. equinum* is horse-specific. On the contrary, *M. canis*, who was probably originally a feline-specific parasite, can infect currently all pets and humans. Sources of human infection include pets and animals on farms, in laboratories and in the wild, some of which are clearly workplace-related and give rise to occupational diseases.

Rabbit

Very common in rabbits, dermatophytosis is nearly always caused by *T. mentagrophytes* var. *mentagrophytes*. Adult rabbits are practically always healthy carriers and they constitute the fungal reservoirs. Kits, which are quickly infected through contact with their doe's abdomen, often develop overt fungal disease because of their immature immune systems. At the age of about one month, at the start of weaning, the clinical signs of fungal disease manifest as a crusting dermatitis on the head (face, eyelids and ears), nails, legs and back. Initially lesions are not always circular; they are non-itchy, alopecic, and erythematous with dry, yellowish-white scales (Figure 1). Subsequently they may spread to other body parts, becoming ichthyosis-like in some cases. When severe hyperkeratosis and widespread crusted lesions are present, particularly on the snout, ear edges and legs, the dermatitis needs to be distinguished from sarcoptic mange. With physiological moulting at 60-70 days kits improve in health, sometimes even markedly but remain carriers and infected fur is always a source of contagion.

Figure 1.—One-month old rabbit with dermatophyte lesions.

Dermatophytes have a prime opportunity to colonize and spread in rabbit farms which produce meat for human consumption.[7] Several Italian and European epidemiological investigations emphasized the high prevalence and incidence of fungal infections on farms and discussed adverse consequences. It is noteworthy that Italy is the largest rabbit meat producer in the European Union, accounting for 44% of the market. Italian rabbit farms vary in socioeconomic conditions and individual ownership. They are very heterogeneous in size, organization, technical and technological equipment. Intensive rearing are at high risk of fungal infections because of overcrowding, confinement in small cages, high temperatures and humidity levels. With no ventilation and suitable lighting systems the environment becomes a reservoir of resistant fungal spores and keratin material from infected animals. Furthermore, the high sensitivity to stressors perturbs rabbits and lower natural defence systems. Stressors include drafts, bright lights, noise, change of cages, overcrowding, high animal density per cage, and a poor imbalanced diet. Under these conditions, dermatophytosis, a typical condition-driven disease, colonizes and spreads exponentially. In fact Italian and European epidemiological studies reported 10-90% of animals displayed clinical symptoms and a mean of 56% were diseased, even after adjustments had been made to allow for the different types of farms and levels of hygiene. Fungal infections are shockingly persistent as the agent is usually refractory to any control measures that are put in place. Clinical manifestations tend to peak consistently in a seasonal spring and autumn pattern and whenever animals are particularly stressed. Analysis of species that are involved in disease outbreaks and isolated healthy carriers showed rabbits are highly vulnerable to *T. mentagrophytes* and up to 70% are carriers, followed by *M. canis* and *M. gypseum.*[8, 12] Epidemiological investigations in Umbria, Tuscany and Emilia-Romagna delineated similar epidemiological patterns, finding dermatophytosis in 60% of farms and 93% positivity for *T. mentagrophytes.*[13] If intensive farming predispose to dermatophytosis infection, the different options in biological farming seem to provide promising results.[14]

Widespread dermatophytosis in young rabbits impacts profoundly on animal health and on farm profits. Infected animals are more likely to succumb to secondary bacterial infections, especially by *Staphyloccus spp*. and have lower indices in prolificacy and growth as well as less commercial value. Indeed the severely infected usually have to be discarded, thus reducing productivity and causing financial loss. Furthermore, since the infection is highly contagious, farm workers are almost always infected, leading to loss of working days and more money losses for the business. Despite these adverse consequences, veterinary surgeons often find farmers are unwilling to carry out constant monitoring, implement disease management strategies and repeat environmental treatments. Farmers often do not comprehend the need for these health interventions, since dermatophytosis in never fatal and only affects the skin for a limited period of time. Finally, since very few antifungal agents are available for systemic use because of the residue risks in products destined for human consumption, focusing on treating the environment is crucial for disease control and farming profits.

Besides rabbit farms, pet rabbits can also carry and transmit fungi to humans. Since rabbits are becoming more popular as pets and are often found in pet-shops, fairs, shows and so on, opportunities are more frequent for contact with people who could become infected. Chincillas (*Chinchilla brevicaudata*), another rodent species that are as popular as rabbits as pets and in fur production, are extremely vulnerable to dermatophyte infection.[15] Recent findings reported that *T. mentagrophytes* is the most commonly encountered fungus in pet rabbits and chinchillas. It is isolated in the fur of asymptomatic animals, with

a prevalence of up to 10%.[16, 17] *M. gypseum* and *M. canis* were also found in chincillas with dermatophytosis.[18] One Umbrian investigation into a chincilla farm found *T. mentagrophytes* skin lesions in approximately one-third of animals and isolated the fungus in 18% of the other asymptomatic animals.[19]

Cattle

T. verrucosum (var. *album, discoides, ochraceum*) is the main etiological agent in bovine dermatophytosis. It causes an endo-ectothrix type of hair infection with non-fluorescent mega-spores. The fungus colonizes not only beef and dairy cattle but also buffaloes, sheep and goats. *T. mentagrophytes* var. *mentagrophytes* can also cause ringworm in cattle and its diffusion within traditional farms is usually related to the presence of small rodents and/or lagomorphs.

Modern intensive battery farms are the main reservoir of *T. verrucosum* as conditions favour its proliferation. The animals most often affected are those imported from abroad and arriving in Italy stressed by the journey and the change in diet. Extremely exploitative conditions on farms further perturb these cattle, adversely affecting their immune defence. Within about a month of arrival they manifest extensive dermatophyte infection, especially if they were healthy carriers, or are put into a fungal- infested byre, where *T. verrucosum* can persist and infect for up to four years.[20] Most outbreaks of fungal disease occur in fall and winter. In fact, inside housing increases the risk of contagion because of byre over-crowding and contact with infected objects like mangers and walls. Cattle under one year old are most vulnerable and suffer from more severe disease. The fungus localizes preferentially on the head, upper chest, rump, dewlaps, intermaxillary space and around the eyes (Figure 2). Lesions are numerous but rarely cover the entire body or merge in confluence. Clinically, alopecia ringworm lesions in cattle are scaly and crusted, reaching a thickness of 8-10 mm. They are about 10-50 mm in diameter with distinct margins. Over time the lesions exfoliate, exposing dry, airless, floury looking skin. Small intradermic abscesses may form around infected follicles due to frequent secondary bacterial infections, dysplasia and probably accessory gland occlusion. Lesions will resolve within 4-5 weeks, particularly when isolated, or otherwise persist for 8-12 weeks. When cattle go out to pasture at the end of winter the disease disappears.

Figure 2.—Three-month old calf with dermatophyte lesions on its head.

Italian epidemiological data refer principally to the Po valley area, where cattle farming is widespread. Dermatophytosis was found in 19-30% of herds (with peaks up to 50-100%) and was demonstrated that it retarded growth in calves and reduced milk production. Skin lesions caused by dermatophytes were also observed in 15% of farm workers.[21] In Umbria fungi infestation was found in 4.5% dairy cattle and 31.2% beef cattle. Moreover, 38% of the farm workers tested positive for *T. verrucosum.*[22-24] In Tuscany, prevalence of infection was 85% in dairy and 91% in beef cattle and at least one case of clinical infection in workers was observed on each farm.[20] Lack of prophylaxis with *T. verrucosum* vaccination accounts for the high infection rates in Italy as bovine dermastophytosis was markedly reduced in Germany and other countries with use of vaccination protocols.

The high prevalence of dermatophytosis in cattle causes, as in rabbits, major profit losses. Weight is reduced by an estimated 10-13 kg per butchered animal in beef cattle and milk yield is lower in dairy cattle. Furthermore, poor hide quality for the leather industry make hides unsuitable for tanning so they are discarded or fetch much lower sums. In fact, skin inflammation residuals are present even when skin lesions have disappeared. The British Leather Confederation estimated losses due to poor quality hides at £ 35 million annually, with dermatophytosis accounting for 5%. The Swedish Association of Hide Producers consequently offered monetary incentives

to farmers to activate suitable immunization programmes.

Ringworm in cattle is very contagious for humans. Farmers, veterinary surgeons, artificial insemination experts and each individual that is in contact with a diseased animal are at high risk of contagion. Employees with *T. verrucosum* – related occupational dermatophytosis are entitled to two-week sick-leave which creates difficulties for farm management as temporary workers have to be sourced, and increases National Health Service costs as employees have to be treated as do family members who may also become infected.[25, 26] The incidence of human *T. verrucosum* infection varies from country to country.[27] Socio-economic conditions, cultural backgrounds and lifestyles concur to facilitate animal-human transmission and race the incidence to 16% in Ethiopia and 33% in Iran. In Europe, incidences range from 0.7-3.1% in Spain, to 1.8% in Greece and 1.3% in Poland. Italian surveys in 1993 and 1995 registered an incidence of 0.9-2.1% in Lombardy and 0.1% in Latium.[28] In humans the disease mainly manifests in two forms: sycosis (*tinea barbae*) and *tinea corporis*, which are usually localised on exposed body parts.

Sheep are usually more resistant to dermatophyte infection. Although *T. verrucosum* is the main infective agent a recent study in Tuscany detected for the first time skin lesions caused by *T. mentagrophytes* var. *mentagrophytes.*[30] Interestingly, since anti-fungal drugs are not approved for sheep, the authors proposed topical treatment based on herbal products, *i.e.*, a mixture of *Thymus serpillum* 2%, *Origanum vulgare* 5% and *Rosmarinus officinalis* 5% in almond oil, which successfully stopped fungal growth.

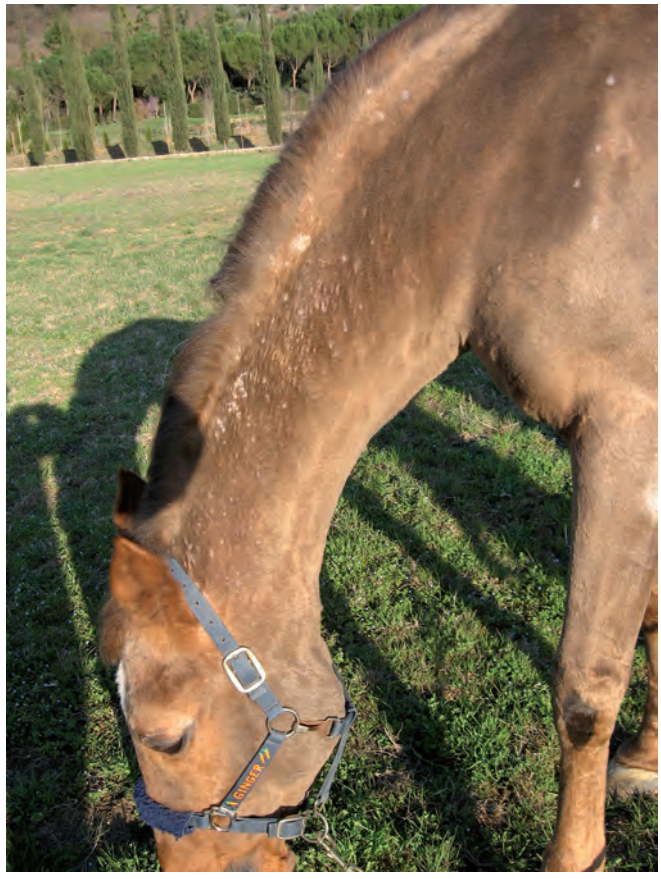

Figure 3.—Elderly Italian Sella mare with widespread *T. equinum* lesions on its neck. Its age (24 years) and immune system deficit due to other concomitant systemic diseases (Cushing Syndrome and chronic piroplasmosis) could have increased susceptibility to fungal infection.

Horses

The growing popularity of horse-riding sports and recreation in the past few years has focused attention on fungal skin lesions because of the health consequences of increased horse-human contact. In horses dermatophytosis predominates during the winter and can quickly develop into an epidemic. Contagion is easy in confined environments and/or in livery stables or riding schools as fungal spores can be transmitted from a diseased animal or healthy carrier to others. Young horses are susceptible to ringworm which is found on the upper chest, flanks, shoulders, body areas that are exposed to trauma and contact with saddles, bridles and harness. *T. equinum*, *M. canis* and *T. mentagrophytes* are the most common fungi infecting horses, but *M. gypseum* infections are also sometimes observed.[25] *M. praecox* was also found as isolated saprophytes from healthy animals [31] and more recently, *T. bullosum*, a new species, was occasionally isolated in horses from Africa and Asia.[32] In 2011 skin lesions due to *M. praecox* were observed in a jockey [33] and in 2012 *T. bullosum* dermatophytosis was reported for the first time in a man and was probably transmitted through contact with an infected donkey in rural France.[32]

Ringworm caused by *T. equinum* and *M. canis* are typically dry forms of the disease. Onset, particularly in young animals is characterised by small tufts of spiky hair which soon fall out, leaving an alopecic ar-

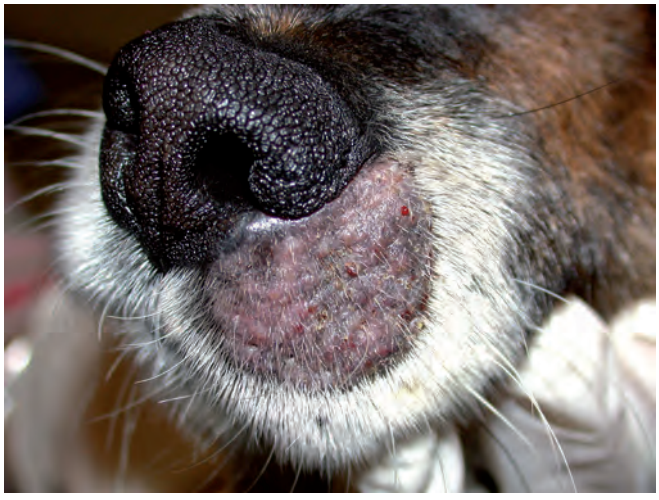

Figure 4.—Dermatophyte Kerion in a dog. Courtesy of Dr. Federico Leone, Clinica Veterinaria Adriatica, Senigallia, Ancona, Italy.

eas covered by fine, flaky, greyish scales with an underlying dry integument (Figure 3). Lesions are generally few in number, limited in size, rarely reaching 25 mm in diameter and non-itchy unless the animal develops a type 1 hypersensitivity reaction associated with infiltrating, exudative lesions. Miliary dermatitis and kerion, which are generally due to *T. mentagrophytes*, may first involve localized areas like the head and withers before spreading to the chest. After hair is shed, the skin appears very inflamed, infiltrated and covered with small follicular pustules which, when squeezed, emit itchy-grey-reddish material. Finally, superficial eschar forms, below which the scarring process begins. Dermatophytosis is not very common in horses in Umbria. An epidemiological investigation into 10 privately owned stables with 200 horses of different breeds that were used for sport and recreation found 9% of healthy animals tested positive for fungi (*T. equinum* 6%, *T. mentagrophytes* 3%).[22]

Dogs

As dogs are more and more often chosen as pets they play a major role in the transmission of fungal infections. Some breeds like the Jack Russell Terrier, Yorkshire Terrier and Pekingese are particularly vulnerable to dermatophytosis and pups often present with disease symptoms. Traumatic skin lesions as well as scratching because of flea- and other ectoparasite-related itching facilitate fungal infiltration.

Figure 5.—Onychomycosis in a dog: nail and nail bed involvement. Courtesy of Dr. Federico Leone.

Feline and canine dermatophytosis causes so many different clinical pictures that they could reasonably be included in the differential diagnosis of all skin diseases in cats and dogs. Canine dermatophytosis is characterized by typical round alopecic lesions and brittle hairs. The scaly crusted lesions may be single or multi- focal and are rarely symmetrical. Local or widespread folliculitis may be observed, with or without foruncolosis. Other clinical signs include dry seborrhoea, focal or multi-focal crusted dermatitis with a well-defined erythematous margins, kerion, onychomycosis and/or paronychia. Signs and symptoms vary greatly with the host-fungus interaction. In dogs *M. canis*-related dermatophytosis usually presents with more marked inflammation than in cats. Scaly or papulo-pustular and crusted facial lesions are sometimes associated with loss of pigmentation in the truffle- and rostral areas and are very similar to those observed in cases of pemphigus foliaceus. *M. gypseum* or *T. mentagrophytes* often cause kerion which presents as a deep, infiltrated inflammatory swelling, with a damp, ulcerated pus-exuding surface (Figure 4) and is often associated with secondary bacterial infection. These infections frequently develop on the face and limbs of hunting

and truffle dogs that spend a lot of time outdoors in contact with the ground.

Onychomycosis is very rare in dogs and usually caused by *M. gypseum* or *T. mentagrophytes*. The nail becomes brittle, loses its shape with periungual inflammation usually developing (Figure 5).

Cases of human ringworm due to transmission of animal dermatophytes have increased in Umbria over the past years. Strong interdisciplinary co-operation among veterinary and medical dermatology researchers has illustrated the links between animal and human health sectors. Research focussed on dogs in rural and urban habitats who were brought by their owners to veterinary clinics for non-dermatological diseases or routine vaccination [35] as well as dogs without skin lesions that were in kennels or pounds.[36] Results showed ringworm in 10% of dogs brought to veterinary clinics, while 10% of apparently healthy animals tested positive in cultures on hair removed by brushing. The most common fungal agents were *M. canis, M. nanum, T. verrucosum* and *T. mentagrophytes,* with no significant differences in distribution whether habitats were urban or rural or whether other animals lived there or not.

Cats

Clinical pictures in cats are very polymorphous. Persians are particularly vulnerable to dermatophyte infection and breeders need to tackle the problem rationally and efficiently to limit financial loses and damage to their reputations. Factors facilitating dermatophytosis include incorrect diets, age, concomitant systemic diseases, stress from various sources, pregnancy or immune-suppressive therapies. Alopecic and inflamed lesions are uncommon and healthy carriers are often found. In fact felines tolerate *M. canis*, the most frequently isolated dermatophyte which has its natural reservoir in the cat. This dermatophyte is found in over 90% of fungal infection in cats, even if *M. gypseum*, *M. persicolor* and *T. mentagrophytes* can also be isolated.[25] Typical lesions observed in kittens are non-inflammatory alopecic areas, with central desquamation, which are surrounded by brittle or easy to extract fur. Lesions localize preferentially on areas that are most in contact with the healthy carrier mother cat while feeding, *i.e*., face, ears and legs. Other forms are characterized by small, crusted scaly, sometimes itchy, lesions. Other aspects are miliary-like dermatitis and

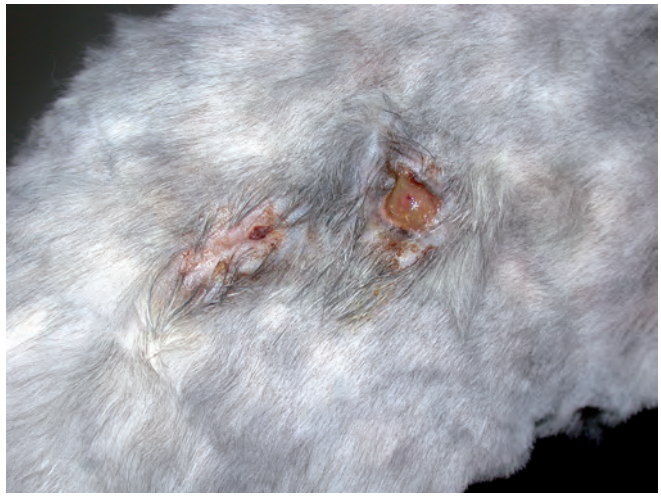

Figure 6.—Pseudomycetoma in a Persian cat. Courtesy of Dr. Federico Leone.

ring-shaped lesions with inflammation or papules on the periphery and fur regrowth in the center. In cats with viral immunodeficiency (feline AIDS), weak animals and those who have undergone inappropriate cortisone therapy, widespread alopecia may be associated with desquamation, inflammation, crusted lesions and severe itch. Dermatophytic pseudo-mycetoma is a rare granulomatous, exudative infection of deep tissues. Single or multiple cutaneous nodules, firm and painless at palpation, are usually found on the back and neck. Nodules are in blue or purplish colour, without alopecia or erythema and may result in fistula formation. This form of dermatophytosis is mainly found in Persian cats because of their genetic predisposition to it (Figure 6).

Feline transmitted *M. canis* is the most common etiological agent of *Tinea capitis* and *Tinea corporis* in Italy, Greece and Spain. Peak incidence of the infections occurs between April and September, which coincides with the period when most kittens are homed. Children and women are most affected. The diseases many be transmitted through fomites, as shown by an investigation into an open-air public swimming pool in Umbria after an outbreak of *M. canis*-related *Tinea corporis* in children using the pool.[37]

Interesting studies were conducted in Tuscany in 2002 and 2013 to investigate pet cats and dogs.[40, 41] The 2002 study collected 10,678 dermatological samples (7650 feline; 3028 canine) over the years 1986-2000, while the 2013 study, which was de-

signed to investigate *M. gypseum* distribution, examined 15,684 samples (10,187 feline; 5497 canine). All the animals presented clinical signs suggestive of ringworm. Positivity for dermatophytes was observed in 23% of the animals, specifically in 18.7% canine and in 24.7% feline specimens. *M. canis* was isolated in respectively 83% and 97% of dogs and cats, *M. gypseum* in respectively 13% and 2.6%, and *T. mentagrophytes* in 5.5% and 0.2%. Toy breeds had a significantly higher (P<0.001) prevalence of *M. canis* infections while *M. gypseum* was mostly found in sporting (hunting) breeds, as was *T. mentagrophytes* (6.7%). *M. canis* was isolated from long-haired cats in a ratio of 2:1 *vs.* short-haired cats. The feline recovery rate from *M. canis* was significantly higher in autumn and winter than in summer and spring, while *T. mentagrophytes* did not display seasonal distribution.

Wild animals

Besides native species of wild animals other species are bred for human consumption or hunting on Italian soil. This recent policy was designed to enhance the value of marginal, abandoned or underused areas and has indeed improved the economy in many areas of the country. At the same time, however, gradual loss of woodlands to thoughtless, opportunistic development, exaggerated anthropization and less farming have created areas inhabited by wild animals, pets and humans where less solid barriers facilitate transmission of pathogens, including fungi.[42-44] Although the health status of animal species which are officially part of a traditional veterinary service is constantly monitored, woodland animals are not given the same attention. Consequently to quantify dermatophyte infection in wild animals we have, over the past few years, examined several species including sinanthropic animals living in the Umbrian border areas with Tuscany and the Marches.

In Tuscany, the first study was conducted on adult free-ranging red foxes (*Vulpes vulpes* L.) without cutaneous lesions, in the provinces of Pisa and Livorno, which were killed in a population control program.[45] About 15% resulted positive for several fungal species including zoonotic pathogens, such as *T. mentagrophytes* and its perfect state *Arthroderma benhamiae*, *M. gypseum* (1.03%), and *M. canis*. Since these animals could harbor dermatophytes without manifesting clinical signs, they might be a source of infection. *T. mentagrophytes* is found in many animal species, especially rodents and lagomorphs, which foxes often prey upon. *M. gypseum*, a common soil saprophyte, was an expected finding on wild fox hair. Isolation of *M. canis* was, however, of particular interest as it had previously been isolated from rodents living in a heavily anthropogenic rural area by Mantovani *et al*.,[43] but not from red foxes. In Tuscany researchers' attention also focused on naturalized coypus (*Myocastor coypus* or river rat) and indigenous brown rats (*Rattus norvegicus*), which were caught in the Fucecchio Marshes, a protected wetland area, during a legal population control program.[46] *M. gypseum* and *T. mentagrophytes* were identified in 29.6% coypus and 46.7% rats. These animals may play a greater role in spreading keratinophilic fungi and people exposed to contact with their coats should take all necessary precautions to protect themselves from mycotic agents.

Hares (*Lepus europaeus*, Pallas 1778), in the Province of Pisa, Tuscany, were also investigated as dermatophyte carriers in a five-year study.[47] Samples from numerous apparently healthy European hares were tested for dermatophytes and related keratinophilic fungi. Overall, 7.5% hares were positive for *M. gypseum*, *T. mentagrophytes*, *T. erinacei* and *M. canis*. Although brown hares seem to play a limited epidemiological role as dermatophyte carriers, many people may be exposed to the infection during hunting and trapping of hares.[48]

Other wild animals, including some sinanthropic species, coming from the border area between Umbria and the Marches were also investigated as part of the Umbrian Regional Monitoring Plan.[49, 50] They included numerous deer (*Capreolus capreolus*), foxes, river rats, wolves (*Canis lupus*), and squirrels belonging both to the exotic species *Sciurus carolinensis* and to the native species *Sciurus vulgaris*. Several dermatophytes were isolated including *T. mentagrophytes* in 15.8% hares, 25% foxes, 14.6% river rats and 14.3% squirrels, *M. gypseum* in 4.7% hares, *M. canis* in 7.5% foxes, *M. coke* in 14.3% squirrels. Deer were all dermatophyte-negative.

These findings confirm dermatophytes are present in woodlands and may spread into urban areas through the wild animal-pet-man bridge. Attention will focus on foxes and river rats because of their prevalent sinanthropy in future health monitoring

programmes. Given the distribution of *S. carolinensis* in Umbrian parks and gardens and the grey squirrel's adaptability and friendliness towards humans, there seems to be a large contact interface between them, pets and humans which could facilitate rapid spread of *M. cookei*.

Results and discussion

Dermatophytosis is undoubtedly the most frequently encountered fungal pathology in veterinary medicine. In farming it is associated with notable costs. Indeed the potential losses are such as to justify outlay on constant monitoring and suitable treatments for the environment and animals.

In the country and at home human beings are considered to be at "biological risk". A close daily relationship with pet dogs or cats, ever more frequent presence of predisposing factors in the host, multiple users of sanitation equipment and community lifestyles all facilitate contagion, particularly in children and weak subjects of all ages with impaired immune function. Animals are at risk on farms because fungus proliferation and host colonization are facilitated by physical and environmental factors such as overcrowding, poor nutrition, inadequate stall clearing, introduction of new animals, and high temperature and humidity levels. When a farm is infected, the farm workers are always more or less severely infected and carry the risk of contagion home to their family members.

Conclusions

This review showed how dermatophytes infect a wide range of animals which may be in contact with human beings either directly or indirectly. They may be transmitted across animal species in diverse environments. Consequently they are frequently a source of infection for human beings who, vice versa, may sometimes become contagious for animals. Fungal pathogens derive their power to spread from contamination of the animal's habitat – whether the animal is a conventional pet or not, a farm animal or living in the wild. Thus if treatment of the animal or human patient is to achieve optimal efficacy, it needs to be associated with adequate environmental measures.

Riassunto

La dermatofitosi negli animali: aspetti epidemiologici, clinici e zoonotici

Obiettivo. La dermatofitosi è l'infezione fungina più frequente negli animali da compagnia e in quelli da reddito, svolgendo un ruolo importante sia per la salute animale che per quella umana, causa il suo potenziale zoonotico. Un altro aspetto importante legato a tale patologia è dato dalle conseguenze economiche nei sistemi di produzione di pellicce e nelle industrie conciarie. In questo articolo viene redatta una panoramica delle dermatofitosi nelle principali specie animali. Sono trattati aspetti epidemiologici, clinici e zoonotici, con particolare riferimento sia ad animali domestici che selvatici.

Metodi. In particolare, sono riportate le più recenti ricerche in campo micologico animale, svolte nel Centro Italia, con particolare riferimento al coniglio, ai ruminanti, al cavallo, al cane, al gatto e ad alcune specie selvatiche.

Risultati. Le informazioni riportate in questo articolo mostrano come i dermatofiti siano in grado di infettare una vasta gamma di animali, i quali a loro volta possono entrare in contatto direttamente o indirettamente con gli esseri umani. Di conseguenza, essi sono spesso fonte di infezione per l'uomo, ma l'uomo stesso, viceversa, può a volte diventare fonte di contagio per gli animali.

Conclusioni. La capacità dei patogeni fungini di diffondersi e conservarsi trovano nell'habitat degli animali notevoli possibilità, sia che si tratti di animali domestici convenzionali o non, che di animali d'allevamento o selvatici. Di conseguenza, per perseguire un'ottimale efficacia del trattamento del paziente umano o animale, è necessaria un altrettanto adeguato intervento di carattere ambientale.

PAROLE CHIAVE: Tinea - Animali - Studi epidemiologici.

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Migration and mycoses

A. MORRONE

San Camillo Forlanini Hospital, Rome, Italy

In recent years, the incidence of fungal infections of the skin, one of the most frequent forms of infection, has been steadily increasing in Europe. One of the main factors contributing to this increase is the gradual raise of migratory flows towards Europe. In the last decades Italy has witnessed an ever-increasing growth of the migrant population, and has become, to this day, one of the European countries with the highest number of immigrants. This phenomenon has had significant implications in clinical practice of dermatologic mycology as it is increasingly common to see unusual clinical isolate causal agents absent in our latitudes until a short time ago. This review provides an update on the epidemiology, classification, pathogenesis, clinical manifestations and treatment of the most important dermato-mycosis observed in the immigrant population, through the most typical cases, investigated by microscopic and cultural findings. These diseases continue to expand and are often difficult to detect. The special relationship between host-environment interaction-parasite plays a crucial role as, even more than in other categories, it is widely widespread among the immigrants.

Key words: Emigration and immigration - Health - Mycoses.

The rising incidence of new infectious diseases and the re-emergence of diseases that had been eradicated or considered rare in Italy pose a major challenge for public health. With the steady flow of human migration from south to north and international trade from east to west, the epidemiologic patterns of infectious diseases are gradually changing.[1, 2] One example is the recent outbreak of *chikungunya*. Although an epidemic described in Indonesia in 1779 was perhaps attributable to the same viral agent, the first known outbreak was reported in Tanzania in 1952. Since then, various *ckikungunya* epidemics have repeatedly occurred in Asia and Africa. In Europe, the first indigenous cases were reported in Emilia Romagna, Italy, in August 2007.[3]

Another striking example is airport malaria: a cluster of 4 cases of airport malaria were reported in France during the summer of 1999. Case analysis found that airport malaria can also be transmitted to people without the risk of occupational exposure, such as residents living near airports.[4] These instances signal two broad epidemiologic trends of dermatologically important mycoses imported from tropical regions and the rising incidence of superficial skin mycoses closely correlated with immigration to and within Europe.

Italy is the main port of entry for many migrants and numbers among the countries with the highest rates of new arrivals. This influx has markedly changed clinical skin mycology, as dermatologists now face an ever-growing number of unusual clinical presentations and etiological agents once considered rare. Today, it is increasingly common to observe both indigenous and non-indigenous fungal pathogens once considered rare such as *Trichophyton soudanense* and *T. violaceum*. In contrast, with the blurring of geographical barriers, it is not uncommon to see typically Mediterranean dermatomycoses in African countries, such as *Microsporum canis*, which was once considered rare in Africa.

Corresponding author: A. Morrone, San Camillo Forlanini Hospital, Rome, Italy. E-mail: a.morrone@scf.gov.it

Finally, the increasing frequency of immunosuppression-associated conditions, though highly variable in the general population, places immigrant patients at greater risk of exposure to fungal diseases. In brief, recognition of cutaneous manifestations is essential for early diagnosis. Skin involvement can be either primary or secondary to dissemination caused by a fungemia. Deep systemic/opportunistic mycoses are more difficult to diagnose because of their marked clinical polymorphism and rarity.

Migration and health

Historically, human migration has brought with it complex sociopolitical problems that have repercussions on the delivery of medical health services. Immigration from developing countries has only recently become a reality for Europe, leaving governments unprepared for the socioeconomic and public health consequences of migration and mobility. The World Health Organization (WHO) defines a "human mobile population" as any person who, for various reasons, moves from one country to another, or more precisely, permanently or temporarily crosses the borders of their state. Therefore, not only do immigrants in the strict sense fall into this group, but also migrant workers, asylum seekers, refugees, cross-border workers, travelers and tourists. According to the latest WHO figures, there were 1.35 billion migrants in 2012, approximately 214 million of which were migrants in search of work, a growing reservoir, as compared to the 70 million recorded for the 1980s.

About 214 million people, or 3.1% of the world population, are currently living temporarily or permanently outside their country of origin. This figure includes migrant workers, permanent immigrants, refugees and asylum seekers but not the growing irregular migration flows.[5] Here a distinction needs to be made between registered or non-registered migrants and irregular migrants. Migrants, specifically registered migrants, are those whose entry, residence, and type of employment in the host or transit country are known and authorized by the host country's authorities.

Irregular migrants and undocumented (sometimes inappropriately referred to as "illegal" or "illegals") have entered the host country without authorization and/or live there beyond the period allowed; for example, visitors, tourists, students or workers with temporary contracts. In addition, a further distinction should be made between "voluntary" and "forced" migrants. Voluntaries have migrated spontaneously, although the decision may have been made under economic or other pressures, like labor migrants, foreign students or in cases of family reunion. Forced migration refers to "movements of refugees and people who move within a country (because of conflicts), as well as people moving as a result of natural or environmental disasters, chemical or nuclear, famine, or development projects".[6]

As in other European Union (EU) countries, immigration to Italy is now a structural phenomenon, is steadily increasing, and has become a widespread social phenomenon. Immigrants to Italy come from many different areas, mainly from less developed countries. Recent years have seen a rapid rise in immigration from Central and Eastern Europe (60% of stay requests), followed by North Africa. Since the mid 1980s, owing mainly to its geographical position and lack of immigration legislation, Italy has been the gateway to Europe for thousands of immigrants. Furthermore, the annual number of arrivals has increased, making Italy, with its 6 million migrants, one of Europe's most important immigration countries, along with Germany, Spain, France, and the United Kingdom. As the inflow of immigrants continues to increase, an evaluation of its sociocultural, health and economic effects and the implementation of appropriate programs and policies have become ever more critical.

Within the public health sector, knowing the epidemiological profile and studying the utilization of and access to medical services by immigrants are key to determine and monitor their health needs and remove health care barriers. Because migrants constitute a specific risk group, they should be the target of specific health policies.[7]

Today, the number of migrants residing in Italy almost equals the number of Italian emigrants in the world. According to official statistics, there were 5,011,000 foreigners in Italy at the end of 2011, or 6.2% of the total population, or 1 immigrant every 12 Italian citizens. During the 1980s, central Italy received the highest number of foreigners; however, recent trends show that more and more migrants reside in the northern regions. Currently, the North

is home to 59.9% of all migrants, followed by the Center with 27%, and the South (including Sicily and Sardinia) with 13.5%. Rome and Milan are the preferred cities of residence, with a foreign-born population of 12% and 11%, respectively, and it is expected that Milan's share will grow more quickly than Rome's. The ten major cities by number of migrants in relation to the percentage of their population are: Prato 12.6%, Brescia 10.2%, Rome 9.5%, Pordenone 9.4%, Reggio Emilia 9.3%, Treviso 8.9%, Florence 8.7%, Modena 8.6%, Macerata, and Trieste 8.1%. Among foreign-born nationals, most migrants come from Romania (997,000), Morocco (506,369), Albania (491,495), China (277,570), and the Ukraine (223,782).[6, 7]

Overall, the majority of migrants come from Europe: 5 out of 10 foreign-born migrants are European, 2 are African, 2 are Asian, and 1 is American. Migrants from Eastern Europe number about 1 million: Ukrainians and Albanians are the two main groups from outside the EU, 5.2% and 11%, respectively, making up 3% of the total migrants from this region. The Romanian (11.9% of total migrants) and Polish populations (3.2% of total migrants) are the two largest immigrant groups from EU countries.

In Europe, Moroccans (10.3%) account for the majority of migrants from Africa, Chinese (4.9% of total migrants) and Philippinos (34%) from the Asia-Pacific region, and Peruvians (2.2%), Ecuadorians (2.1%) and Brazilians (1.4%) from South America. Because migrants living in Italy come from many different countries, it is not surprising that different religions coexist. Christians make up 49.1% of all migrants, followed by Muslims with 33.2%, and Eastern religions with 4.4% of the population.[8-11]

Fungal infections in the migrant population

The fungal infections mainly found in the migrant population can be classified in six groups:

1. cutaneous mycoses and superficial dermatophytes with low potential for dissemination
2. superficial candidiasis
3. cutaneous mycoses with potential opportunistic dissemination
4. fungal sinusitis with skin involvement
5. skin involvement secondary to disseminated fungal infection
6. subcutaneous and deep mucosal infections

Infections by dermatophytes and their classification

Dermatophytosis (or tinea) is caused by three genera of dermatophytes: *Trichophyton*, *Microsporum*, and *Epidermophyton*. Depending on their natural habitat, they are classified as anthropophilic, zoophilic, or geophilic organisms. Generally, anthropophilic fungi cause superficial dermatomycoses with relatively low inflammatory activity because of the immunological balance between the fungus and its human host.[12, 13] Household dust may act as a reservoir of anthropophilic dermatophytes, preserving dermatophyte spores for years. Zoophilic dermatophytes are found in animals but are also sporadically transmitted to humans by cats, dogs (*Microsporum canis*), guinea pigs, and rabbits (*Trichophyton mentagrophytes* var. *granulosum)*.

Zoophilic dermatophytes causing so-called cuddly-toy mycoses (because of the mode of infection in children and adolescents) have a high affinity for the child's head hair. They are associated with highly inflammatory and potentially highly contagious skin infections.

Geophilic fungi grow in the soil and only sporadically infect humans.[13] When they do, they produce high to low inflammation. Strains of *Microsporum gypseum*, the most common geophilic pathogen, cultured from humans are more virulent than those from the soil, accounting for occasional epidemic spread under appropriate conditions.[14]

There are approximately 100,000 species of fungi distributed worldwide. The majority of fungal infections seen in temperate and tropical countries are superficial skin infections. The most common pathogens encountered in dermatological practice are dermatophytes, yeasts, and molds.

There are approximately 40 different species of dermatophytes. They are characterized by their ability to digest keratin and are classified in three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. The majority of superficial fungal skin infections are caused by five or six species of dermatophytes, of which *Trichophyton rubrum* is the most common [15, 16] (Table I).

The clinical manifestations of fungal infections caused by superficial dermatophytes do not differ within the Italian population. *Microsporum*, *Epidermophyton*, and *Trichophyton* are the main fungal species and can cause superficial skin infections

TABLE I.—*The main dermatophytes responsible for cutaneous mycoses.*

Anthropophilic	Zoophilic	Geophilic
T. rubrum	M. canis	M. gypseum
T. interdigitale	T. mentagrophytes	
T. floccosum		
T. tonsurans		
T. violaceum		
T. soundanense		
T. schoenleinii		

(stratum corneum) of the nail and hair terminals.[17] In most cases, even in persons living in extremely debilitated and poor hygienic conditions, like most migrants, they are unable to invade deep tissues or lead to clinical dissemination.

In recent years, however, an increasing number of superficial cutaneous mycoses caused by unusual fungi endemic to tropical regions have been reported. Increasingly common are dermatomycoses caused by *T. violaceum* and *T. soudanense*.[18, 19] *T. soudanense* and *T. violaceum* have frequently been isolated in Italy - by Manca-Pastorino in the Sardinian countryside in the 1930s - and were always associated with extreme poverty and degraded hygienic conditions.[20] *T. soudanense*, an anthropophilic species found mainly in Africa and less frequently in Australia, Brazil, and Israel, can cause tinea capitis, corporis, pedis and unguium (Figure 1).

T. violaceum, an anthropophilic species widespread in Africa and the Mediterranean, is responsible for tinea corporis and especially tinea capitis in adulthood (Figure 2). The main dermatomycoses observed in migrants are tinea pedis in onychomycosis, followed by tinea cruris, tinea corporis, and tinea capitis, all of which can easily worsen when untreated. They are characterized by scaly bald patches on the scalp, rarely seen in Italian subjects (Figures 3-6). In foreign-born patients, tinea corporis may manifest with unusual clinical features or locations, making clinical diagnosis more difficult. Patchy or large plaques (>20 cm), annular or polycyclic lesions can be observed, sometimes with a fine scaly surface, which can mimic many other skin diseases of non-fungal origin (Figures 7, 8). In such cases, direct diagnosis by microscopic examination and bacterial culture is essential. In immunocompromised adult migrant patients, the dermatophyte may invade the hair follicle, leading to folliculitis, tinea capitis and Majocchi granuloma.[21] Clinically, it is characterized by patchy alopecia or rounded plaques, covered with fine whitish scales.

Most mycoses are caused by fungi that reside in nature, but there appears to be a considerable inter- and intracontinental variability in the incidence of fungal infections throughout the world. Furthermore, different geographical locations favor different infection patterns. *Trichophyton rubrum, T. interdigitale* (*mentagrophytes* var. *interdigitale*), *M. canis, M. audouinii, T. tonsurans*, *and T. verrucosum* account for most dermatophytoses globally, but the attack rates and incidence of specific mycoses can

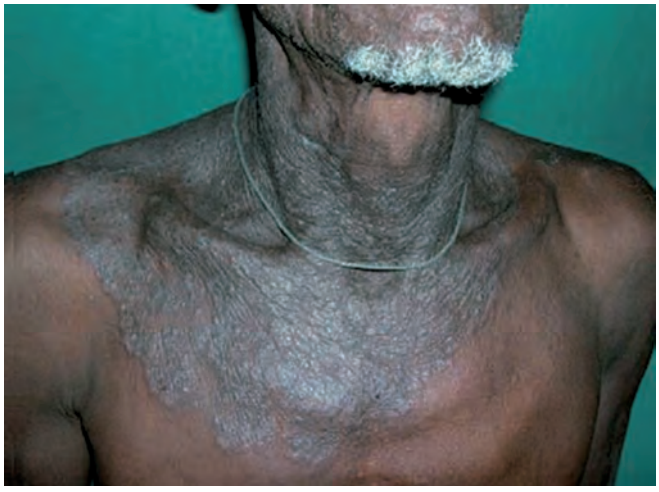

Figure 1.—Tinea corpis large with poor scaling lesions components.

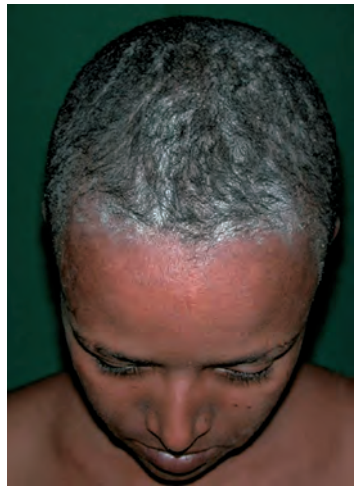

Figure 2.—Tinea capitis.

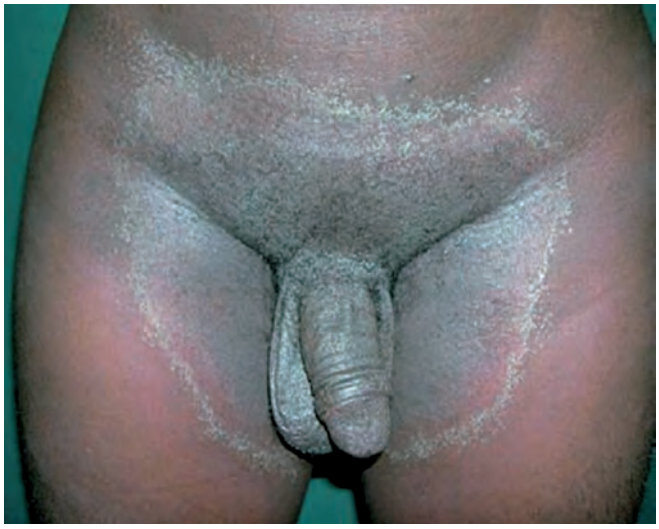

Figure 3.-Tinea cruris in HIV-positive patient.

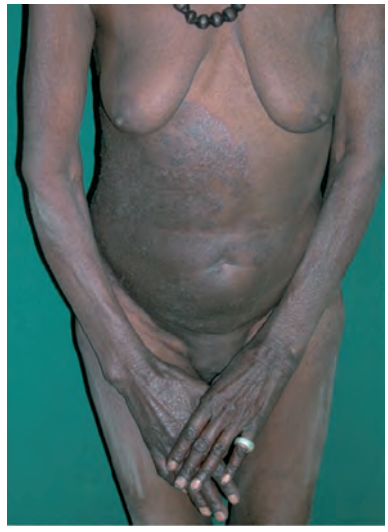

Figure 4.—Tinea corpis.

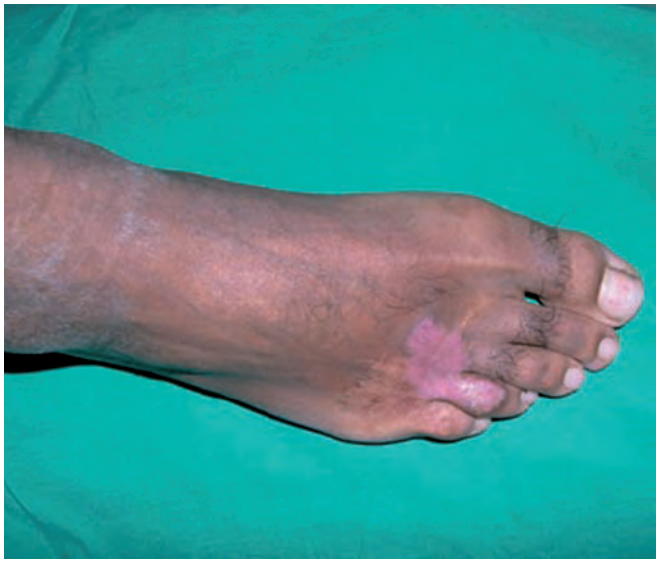

Figure 5.—Chromomycosis. Spill erythematous to purplish clear limitations to the dorsum of the foot.

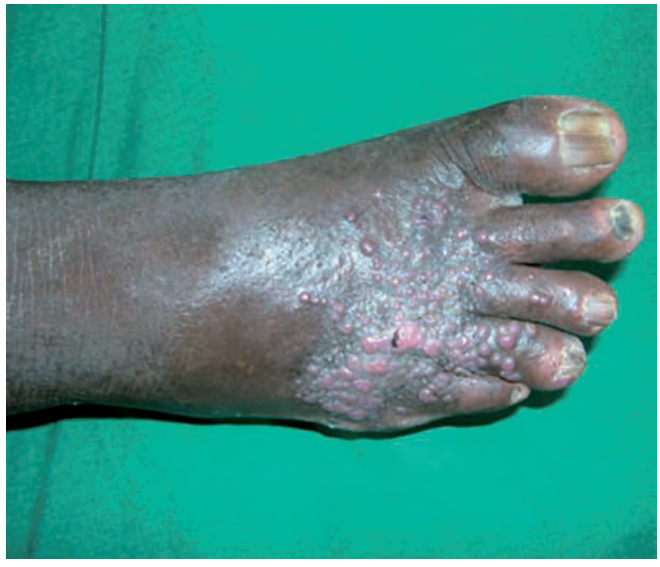

Figure 6.—Chromomycosis. Papular and nodular lesions with a predisposition for confluence on the top of the foot.

vary widely. The highest incidence of infection with *T. rubrum* is reported for Europe, whereas infection with *T. mentagrophytes* is more commonly reported in Asia.

Increasingly frequent, tinea pedis caused by infection with *T. rubrum* and *T. interdigitale* (*mentagrophytes* var. *interdigitale*) seems to be more prevalent in highly developed countries due to attendance at sports and fitness facilities, increasing prevalence of obesity and diabetes mellitus, and an ageing population. *Microsporum canis* is a major agent of tinea capitis in the developed world and could be related to mass tourism to endemic regions (such as the Mediterranean) and close contact between humans and companion animals. Several dermatophyte species are geographically limited. *T. violaceum* is endemic to certain parts of Eastern Europe, Africa, Asia, and South America but not North America. *T. soudan-*

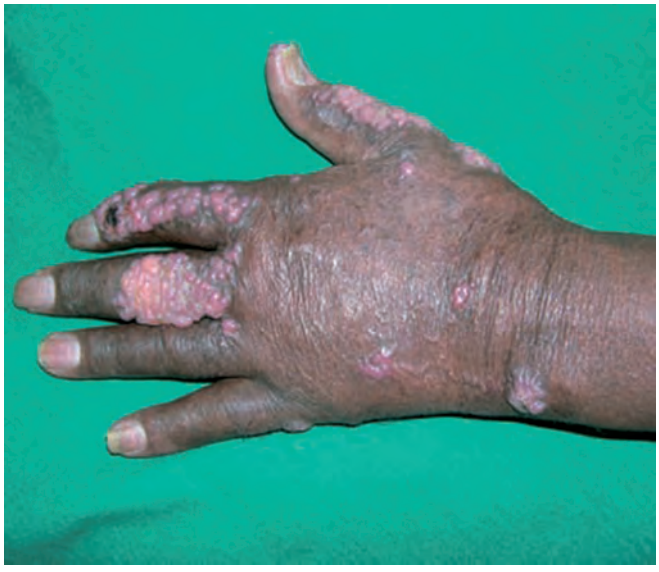

Figure 7.—Chromomycosis. Confluent erythematous papules on the back of the hand.

Figure 8.— Chromomycosis. Extensive lesion of verrucous appearance combined with major lymphatic stasis.

ense is a common endemic cause of tinea capitis in northwestern tropical Africa and West Asia. The sporadic appearance of both species in Western Europe and the United States provides evidence for the ongoing evolution of dermatophytosis epidemiology in relation to international travel, immigration, and shifts in population demographics.

In Africa, fungal infections are among the most commonly diagnosed skin diseases. The pathogen spectrum and the clinical manifestations differ from those seen in Europe. The anthropophilic dermatophyte *T. audouinii* is the most prevalent pathogen, together with *T. violaceum* and *T. soudanense*. *Trichophyton gourvilii* is an endemic, partly geographically restricted, anthropophilic dermatophyte, and its predominance in tinea capitis is characterized by patches of grey hair. The black dot type, caused by the endotrichus fungi *T. tonsurans* and *T. violaceum* (also endemic in Africa), is widespread. Favus, caused by *T. schoenleinii*, has been occasionally observed. This pathogen was a widespread trigger of family epidemics in Europe in the 18th century. Today, it has almost disappeared from developed European countries but is still found in densely populated areas of Africa. *T. soudanense* seems to be a common cause of tinea capitis in northwestern tropical Africa. *T. rubrum* and *T. mentagrophytes* are less common and are associated with tinea corporis and tinea cruris (or tinea axillaris in women) in particular. In 2005, Hay *et al*. estimated the overall incidence of tinea in sub-Saharan Africa to be 78 million.[22]

Superficial candidiasis

Skin infections with *Candida* in foreign patients living in substandard sanitary conditions can produce clinical pictures different from those we would normally recognize and may be associated, albeit rarely, with an increased risk of dissemination. Superficial candidiasis includes intertriginous candida, vaginitis, balanitis, perleche, and paronychia. Oral candidiasis may precede the spread of infection to the esophagus, leading to candidemia in neutropenic patients. In the management of migrant patients with oral candidiasis, a co-existing immunocompromised state should be ruled out; if present, its cause will need to be determined and prompt systemic antifungal prophylaxis initiated.[23, 24]

Opportunistic cutaneous mycosis

Disruptions of skin surface integrity may serve as a door for many microorganisms including fungi. Given the poor housing and sanitary conditions in

which thousands of immigrants are forced to live in our country, the occurrence of skin infections coupled with the risk of transmission to cohabitants is a growing public health concern. Furthermore, irregular employment, often precluding access to public health services due to the precarious legal status of many migrants, is one of the many factors contributing to the impairment of skin barrier function. A recent 1998 law (Legge Turco-Napolitano) will theoretically grant these patients full rights to medical services. Often, work-related microtrauma to the skin can lead to infection by opportunistic fungal species, especially in neutropenic persons. Superficial cutaneous mycoses caused by opportunistic pathogens can more easily invade the deep tissues and result in fungemia, which is facilitated by the ability of some opportunistic fungi to invade the vascular endothelium.[25]

Researchers have shown that fungal paronychia of the toes is the most common form of opportunistic infection by fungi of the skin.[26] The main species involved in this type of paronychia are *Fusarium* and *Aspergillus*. Clinically, fungal paronychia is manifested by erythema and edema of the periungual skin, and can quickly involve the whole toe. Sometimes, scab formation can be observed owing to the ability of *Aspergillus* to invade the vascular endothelium, resulting in local skin necrosis (eschar).

The differential diagnosis of fungal paronychia is broad. Although paronychia is more often caused by bacteria, scab formation indicates a possible fungal origin, concomitant onychomycosis, neutropenia, and lack of response to antibiotics. Eschar of mycotic origin can also be found in other skin locations such as the extremities following injury, with the risk of fungal superinfection.

Clinical presentations of fungal cellulitis are more uncommon and can be caused by *Aspergillus*, *Rhizopus, Candida, Fusarium* spp. or *Criptococcus.*[23, 27] The clinical picture is comparable to bacterial cellulitis. For this reason, the presence of petechiae or purpura should always raise the suspicion of a mycotic origin.

Rarely, opportunistic fungi may produce unusual skin lesions such as subcutaneous nodules, abscesses, or folliculitis. Nonspecific skin lesions will require diagnostic confirmation by culture of tissue fragments obtained with skin biopsy. However, because they are common skin saprophytes, the detection of *Aspergillus* and *Fusarium* in a culture medium does not establish a definitive diagnosis. Cutaneous infection by opportunistic fungi may provide a "nest" for the development of fungemia and the involvement of other organs.[28] The literature reports that in 33% of cases of disseminated infection by *Fusarium* the skin is the entry route.[29, 30]

Hematogenous dissemination usually occurs in migrant patients with severe neutropenia and it is associated with high mortality. In Italy, unusual opportunistic infections may be caused by *Histoplasma* or *Coccidioides* spp. Also in these cases, the neutropenic patient is at greater risk of hematogenous dissemination from an unknown entry site. Special care should be taken in treating immunocompetent patients who develop an infection and may risk future reactivation of fungal infection in cases of immunosuppression.

Fungal sinusitis with cutaneous involvement

Migrant patients with invasive fungal sinusitis may present with involvement of the overlying skin, leading to fungal cellulitis of the cheek or orbital region. Fungal cellulitis may be the first sign of an underlying fungal sinusitis. Fungal sinusitis can occur not only in neutropenic patients but also consequent to therapy in those with uncontrolled diabetes mellitus. The most common etiological agents are *Aspergillus, Rhizopus*, and *Mucor* species.[31, 32]

Symptoms are initially nonspecific and include fever, cough, headache, and nasal congestion. Focal events such as sinus pain, periorbital edema, and nasal secretions are more rare in the initial phase.[32] Skin involvement occurs through dissemination of the fungus to the overlying skin and is manifested by erythematous, edematous plaques on the cheeks, nose or periorbital regions, entirely indistinguishable from bacterial cellulitis. Skin biopsy allows for correct diagnosis and identification of the nature of the infection. Fungal sinusitis can act as a reservoir for future dissemination and fungal infection following multiple organ involvement.

Cutaneous manifestations secondary to disseminated fungal infection

The main fungi responsible for secondary cutaneous dissemination are *Aspergillus, Candida, Fusarium, Mucor, Rhizopus,* and *Criptococcus* spp.,

of which *Candida* and *Aspergillus* are the two most common causal pathogens. Whatever the causal pathogen, dissemination inevitably occurs in patients with severe neutrophilia.[33] Disseminated aspergillosis is a frequently fatal disease in the critically ill (mortality 75%).[33-35] The primary localization is usually the lungs, while *Aspergillus* sinusitis or primary skin infections are less frequent. The primary cutaneous manifestations are characterized by a patch or plaque that can develop inside an eschar; the skin lesions from secondary skin involvement are characterized by numerous patches or plaques (diameter, 2-3 cm) associated with fever.[25, 29, 34] Establishing a diagnosis is difficult because blood cultures for *Aspergillus* often test negative; therefore, timely skin biopsy for histology and culture is essential.

Dissemination of *Candida* species occurs more frequently in the immunocompromised but it may sometimes be found even in the immunocompetent.[36] The esophagus as the entry site usually shows chronic solutions of continuity. In the course of fungemia, febrile myalgia is present and secondary skin involvement is present in 13% of cases. Papules or pink dermal nodules (diameter, 5-10 mm) that do not develop centrally or eschar or necrosis may be observed, sometimes containing purpuric elements. The lesions are numerous and located on the trunk and proximal extremities, while the head and neck are usually spared. Skin biopsy for histological examination and culture may provide an important diagnostic aid.[37]

Dissemination of *Fusarium* spp. is less frequent but nevertheless plays an important role for the dermatologist because of skin involvement in 75% of the scattered forms.[32] Primary infection by *Fusarium* can manifest as sinus infection, pneumonia or primary cutaneous infection (paronychia eschar or digital).

During the course of fungemia, the patient is febrile and myalgias may be present; skin lesions are characterized initially by papules or plaques that develop erythematous, centrally purpuric lesions followed by eschar formation. Sometimes these lesions can become hemorrhagic in thrombocytopenic patients. Multiple lesions are usually located on the extremities but may also be found on the trunk. The mortality associated with its dissemination is 80%. Prompt diagnosis is important; skin histology and culture are necessary for early diagnosis. The literature reports that skin lesions caused by infection with *Fusarium* appear 5 days before positivization of blood cultures.[32, 33]

Rarely, *Mucor* and *Rhizopus* spp. are responsible for fungemia and even more rarely of skin lesions secondary to dissemination. Clinically, it manifests with erythematous lesions (diameter, 2-4 cm) with a purple center that can sometimes evolve into scabs.[38, 39] The disseminated form of *Criptococcus* is quite common.

Fungal pathogens are distributed worldwide. The most common fungi are found in the soil, rotting wood and decaying vegetation. Infection usually spreads through inoculation of the fungus into the skin after an injury that damages skin integrity. Even handling timber or sitting on wooden benches in Finnish saunas was traced back to infection with *F. pedrosoi*.[44]

The sites most frequently infected are the lower extremities, less often the shoulders, chest, back or face. A high prevalence of infection in males has been reported, probably owing to more numerous opportunities for men to be in contact with the soil and a higher susceptibility to trauma during work. Transmission of the fungus from person to person or from animals to humans has not been demonstrated.

Clinical manifestations

The typical form of chromomycosis (dermatitis verrucosa) manifests itself at the site of inoculation following traumatic injury; however, the injury may have been so remote that the person no longer remembers it. The primary lesion manifests as sometimes itchy, small scaly pink papules. With time, months or years, new lesions appear as nodular, violet verrucous carcinomas in the same or adjacent areas. The lesions have a characteristic tendency to grow and regroup. The older lesions take on a cauliflower and warty appearance on the surface, with small ulcerations or "black dots" of mucopurulent material. Sometimes, new satellite lesions can develop as a result of treatment or by diffusion through the lymphatics, leading to the growth of large confluent verrucous plaques. There is also a clinical variant characterized by confluent papules with flat, raised active margins and a tendency to keloid formation in the central part. In advanced cases, fibrosis can be extensive, causing lymphatic stasis and marked edema. Typically, fistula formation or invasion into the underlying muscles and bones is unnoticed, and hematogenous

spread with involvement of lymph nodes, liver and lungs is rarely possible.

Diagnosis

All types of chromomycoses produce characteristic sclerotic bodies in tissues and exudates. These are typically more abundant in samples taken from warty lesions than in the material from flat lesions. In the material obtained by scraping the lesions, examined in a solution of 10-20% KOH on a slide preparation for light microscopy, "copper coins" appearing as pigmented sclerotic bodies can be observed. Exudates in the fungus may appear as long branched hyphal filaments. In contamination with bacteria or other fungi, direct examination of the samples should be confirmed by culture in Sabouraud glucose agar containing chloramphenicol and cycloheximide and maintained at 25-30° C for 4-6 weeks.

Treatment

In the early stages of the disease, when the lesions are small and few, wide deep surgical excision or liquid nitrogen cryotherapy is effective. However, because most of the cases seen in clinical practice are already at an advanced stage, medical treatment is needed. Medical therapy, however, is often disappointing: the response to antimycotics depends on the nature of the fungus. The best results have been obtained with combination therapy with itraconazole-flucytosine and terbinafine.[45]

Intracutaneous and deep phaeohyphomycoses

Phaeohyphomycoses are caused by phaeoid or melanized fungi characterized histopathologically by dermatiaceous septate hyphae that grow in the tissues. Though distributed worldwide, infections are more prevalent in warm climates. The most frequent causal fungi are *Exophiala jeanselmei, Wangiella dermatitidis, Alternaria* spp., *Cladosporium* spp., *Curvularia* spp., *Phialophora* spp., *Aureobasidium, Dactylaria*, and *Drechslera.*[46]

The initial lesion is a subcutaneous cyst at the site of traumatic implantation of the fungus; the lesion is solitary, well encapsulated and asymptomatic. Disseminated phaeohyphomycosis begins in the lungs and then spreads to the brain, skin and other organs.

Some forms of phaeohyphomycosis are not primary; among these is cutaneous alternariosis which primarily affects the immunocompromised and migrant patients. The lesions are nodular or may have the appearance of granulomatous cellulitis or purulent papules.

Mycetoma

Definition

Mycetoma or Madura foot is a local, chronic, slowly progressive, destructive and often painless infection of the skin, subcutaneous tissue, fascia, bones and muscles. After inoculation of the fungus, often through contact with the ground or with decaying plants, the infection usually affects the foot, hand or injured site, giving rise to local edema with granulomas and multiple fistulas containing granules of various color.

This chronic granulomatous disease manifests with nodular lesions, later with sinuses and fistula that discharge purulent material containing granules. In advanced stages, muscle and bone involvement leads to deformity and functional limitations. Several different bacterial (actinomycetes) or fungal (eumycetes) etiologic agents are recognized. Numerous fungal species are responsible for mycetoma, the most commonly isolated are *Madurella mycetomatis, M. grisae, Acremonium kiliense, A. cell anemia,* and *Pseudoallescheria boydii.*[47]

Epidemiology

Mycetoma is commonly found in the mycetoma belt, between latitudes 30° north and 15° south, and most frequently observed in India, Mexico, Niger, Saudi Arabia, Senegal, Somalia, Sudan, Venezuela, Yemen, and Zaire. More and more often, mycetoma is also observed in temperate regions.[48, 49]

Clinical manifestations

Mycetoma is observed more frequently in men between the ages of 20 and 40 years. The male: female ratio is 5:1. It occurs more frequently in farmers and rural workers and generally more often in people exposed to penetrating wounds from thorns and splin-

ters. The most common site of infection is the foot, particularly the dorsum. Systemic disorders are rare; fever indicates possible bacterial superinfection. Other areas of the body in contact with the ground at work, rest or lying position include the hands, torso, arms, head, thighs, and buttocks. Initial clinical manifestations are painless small papules or nodules on the sole or dorsum of the foot which gradually increase in size (Figure 9). The skin lesions swell and break, forming fistulas. As the infection spreads, lesions develop near one another, with healing of old fistulas and new fistulas opening elsewhere (Figures 10, 11). With time, months or years, as the infection spreads into the deep tissues, including bone, generalized painless edema develops (Figures 12, 13). The course is progressive, with soft tissues prone to recurrent cycles of edema, suppuration and scarring. Finally, a swollen formless mass develops, consisting of destroyed tissue with numerous fistulas through which the granules are discharged. The

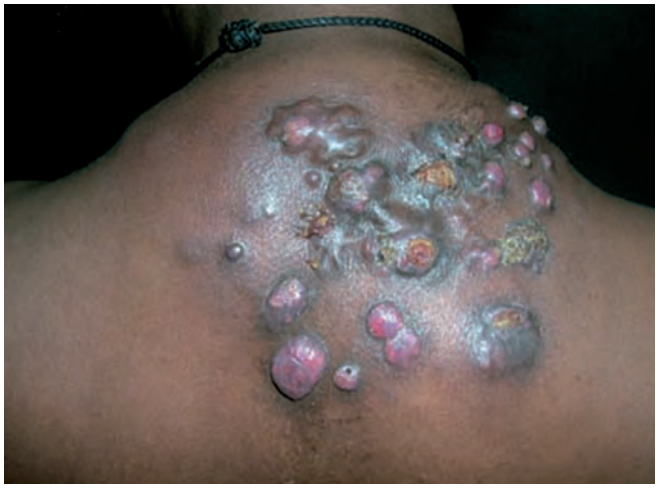

Figure 9.—Mycetoma. Nodular lesions with a tendency to grouping the back.

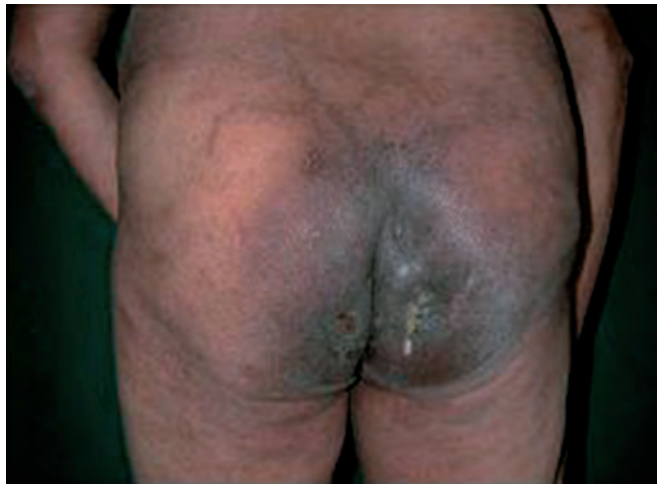

Figure 10.—Mycetoma. Edematous plaque with fistula to the buttock.

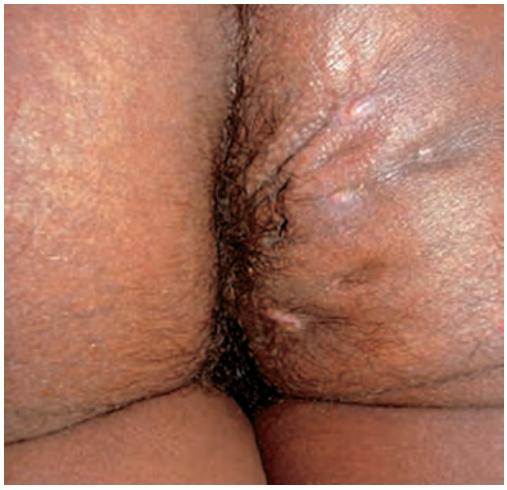

Figure 11.—Mycetoma. Edematous plaque with fistula to the buttock.

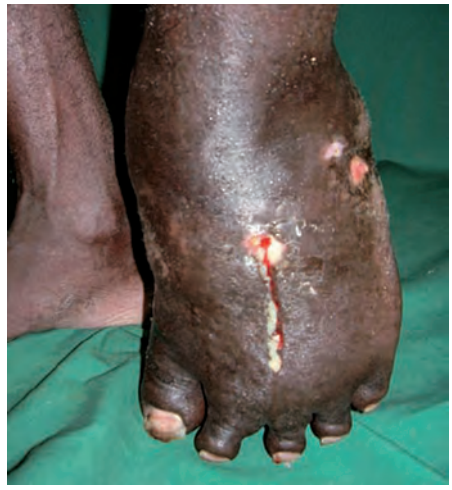

Figure 12.—Mycetoma. Full involvement the foot with formation of fistulas.

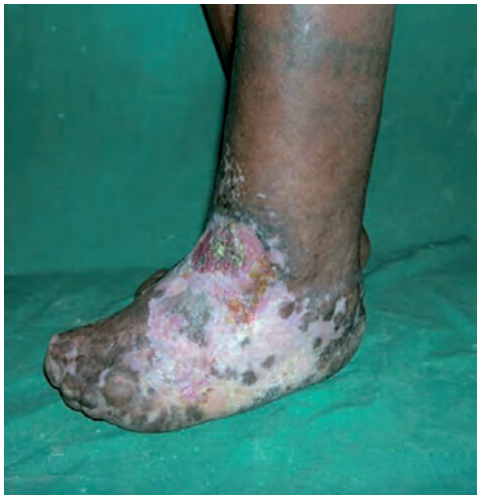

Figure 13.—Mycetoma. Involvement of tissues underneath with clear events of cicatrization and fistulas.

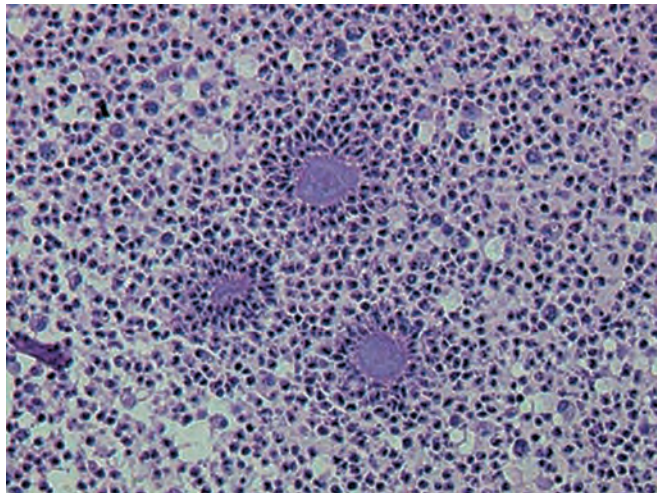

Figure 14.—Sporotrichosis. Some ulcerated erythematous nodules willing linearly.

spread of infection by continuity or blood contamination is remarkable.[50]

Diagnosis

A hallmark triad of signs (firm edema and multiple sinus tracts that drain pus containing granules) characterizes advanced mycetoma. The granules found in the sinus tracts (diameter, 0.2-3.0 mm) can be black, white, yellow, pink or red depending on the organism that produces them. The granules are difficult to identify in histopathological sections. A more precise diagnosis of the species causing the disease can be obtained by culture of the granules and isolation of the microorganism. Wedge biopsy provides an excellent sample for histological examination and culture (Figure 14).

Treatment

The course of antifungal treatment should last for several months, often together with surgical excision, which should be limited to avoid causing disability.

Sporotrichosis

Definition

Sporotrichosis is caused by *Sporothrix schenckii*. It has a worldwide distribution but is particularly prevalent in rural areas of tropical and subtropical regions. Infection occurs through the skin and results from trauma or contact with animals or plants carrying the spores of the fungus.

Epidemiology

Sporotrichosis occurs worldwide. The majority of cases are reported for the tropical and subtropical regions of the Americas. *S. schenckii* is found in the soil, plants or their derivatives (straw, wood). Epidemic forms have been ascribed to exposure to timber, thorny plants, hay, straw, moss, and armadillos.

Clinical manifestations

The most common forms of sporotrichosis arise from inoculation of the fungus into the skin after trauma. In the majority of cases, the initial lesion occurs at the distal extremities, but any area of the body can be affected. The peculiar preference for the colder parts of the body is due to the well-known intolerance of some strains of *S. schenckii* to temperatures above 37° C. The initial lesions are papular and nodular, often erythematous, ranging in size from a few millimeters to 2-4 cm. The lesions may have a smooth or warty aspect, and often ulcerate with elevated erythematous margins (Figure 15). The initial lesions can spread along the lymphatics, giving rise to a clinical picture at a distance like that seen in the

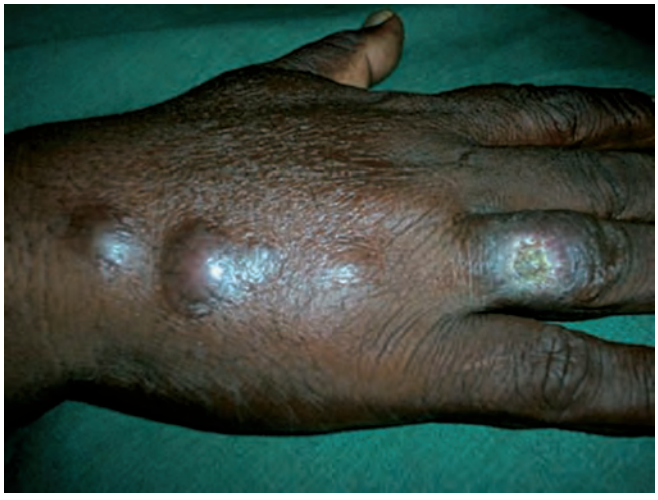

Figure 15.—Sporotrichosis. Some ulcerated erythematous nodules willing linearly.

primary inoculation. Usually, secondary lesions do not affect the lymph nodes, although lymphadenopathy may be present. The lesions are painless even after ulceration. The initial lesion may remain solitary and take on a warty appearance or resemble chronic pyoderma, sometimes mimicking pyogenic granuloma. Clinical variants are associated with increased scar volume.[51] The disseminated form is rare and is manifested by subcutaneous abscesses, visceral and bone lesions.

Diagnosis

Diagnosis is easily made by culture of material taken from the infected site.

Therapy

The drug of first choice is itraconazole at a dose of 100-400 mg/day.

Blastomycosis

Definition

Blastomycosis is a chronic granulomatous suppurative infection caused by *Blastomyces dermatitidis*. It is particularly endemic in certain regions of the American continent.

Epidemiology

Thorough knowledge of the incidence and epidemiology of blastomycosis is hampered by the lack of a sensitive and specific skin test, as well as the difficulty of establishing the ecological niche of *B. dermatitidis*. It is still endemic in the southwestern and central south states of the United States. More and more, cases have been reported for Africa, Central and South America, India, and the Middle East.

Clinical manifestations

Blastomycosis is typically contracted through the lungs and is often subclinical. Hematogenous dissemination may lead to the involvement of organs other than the lungs, such as the skin, bones, and genitourinary tract. The cutaneous form is the most common manifestation of extrapulmonary disease, being reported in 40-80% of cases. Even in cases where extrapulmonary blastomycosis is observed, usually in association with the active pulmonary form, skin involvement may also occur alone. Some patients with cutaneous blastomycosis can be noted to have pulmonary symptoms. Two types of skin lesions are distinguished: warty lesions localized to exposed areas and ulcerative lesions. The early manifestations of infection are small papulopustular lesions that slowly spread to form areas of crusted lesions ranging from gray to purple in color. The older lesions may show a central depression with scar formation, while the peripheral ones have a tendency to form microabscesses with scabs harboring mucopurulent material. The second type of lesions are ulcerative lesions in which the initial scab spreads to form a superficial ulcer with a bed of easily bleeding granulation tissue. Both types of lesions can be present simultaneously.[52]

Diagnosis

Diagnosis is based on culture and microscopic examination that shows budding yeasts with spherical, thick double contoured cell walls (diameter, 3-25 uM).

Therapy

The treatment of choice is itraconazol at a dose of 200-400 mg/day.

Coccidiodomicosis

Definition

Coccidioidomycosis is a chronic disease caused by *Cocciadioides immitis*. The disease typically occurs in the desert areas of North and South America.

Epidemiology

Coccidioidomycosis is endemic in certain parts of North, Central and South America. In the United States alone an estimated 100,000 people will be affected each year. Increasingly, cases outside endemic areas have been reported: typically migrants from endemic areas, travelers who have visited endemic areas, or cases of reactivation of previously acquired infections in individuals who resided in endemic areas or infections acquired through exposure to substances coming from these areas. The fungus grows in the soil where it is found in mycelial form. When mature, alternate cells and hyphae form a barrel. These hyphae, called arthroconidia, fragment easily, allowing the airborne spores to disperse into the environment and the soil (saprophytic cycle). They may also be inhaled by animal hosts, where spores then swell and reproduce by forming endospores.

Clinical manifestations

The main entrance is mostly through the lungs and rarely the skin. The course of primary infection is characterized by nonspecific cutaneous manifestations including erythema nodosum and erythema multiforme on the trunk and upper limbs. In disseminated coccidioidomycosis the skin is a common target organ. Cutaneous manifestations are variable: papules, pustules, plaques, nodules, ulcers, abscesses or proliferative lesions. The differential diagnosis includes tuberculosis and other deep mycoses. Coccidioidomycosis should be suspected whenever there is an abnormal increase in scar volume, in which cases the differential diagnosis will include sarcoidosis and sporotrichosis.

Diagnosis

Histological examination shows large spherules (diameter, 20-80 mM) containing numerous endospores (diameter, 1-4 mM): the spherules are present in the granulomatous lesions, the pus containing asexual endospores, and the sputum.[52]

Therapy

Depending on symptom severity, amphotericin B (0.4-0.6 mg/kg/day) is administered. Oral itraconazole is active at a dose of 200-400 mg/day.

Paracoccidioidomycosis

Definition

Paracoccidioidomycosis is caused by *Paracoccidioides brasiliensis* and is endemic in most South American countries. It is one of the major systemic mycoses in Latin America. It usually manifests as a chronic, progressive infection in adult males. Although the primary infection is preferentially localized to the lungs, dissemination is common, mainly localized to the skin, the mucous membranes, the reticuloendothelial system, and the adrenal glands.

Epidemiology

Paracoccidioidomycosis is a chronic progressive infection. It mainly affects the elderly and is geographically limited to certain areas of Latin America. It is considered a rare imported infection in Europe. Paracoccidioidomycosis is endemic to Latin America from Mexico to Argentina, primarily in areas with tropical and subtropical forests where the temperatures are mild and the relatively high degree of humidity is constant throughout year. Cases of paracoccidioidomycosis have also been reported in North America, Europe, and Asia. Its distribution by age and gender is peculiar: rare in children and adolescents, frequent in young adults. Males are affected much more frequently, with an average male to female ratio of 15:1. This contrasts with the rate of infectivity as determined on the basis of skin reactivity to paracoccidioidina, which is similar in both genders. In prepubertal children, however, there are significant differences between the two genders. It has been suggested that the lack of difference between the genders observed in the adult population could be related to the inhibitory activity of estrogen on conidia or on the transition to the mycelial form. The infection has a long latency period, as demonstrated by imported cases, in some of which the disease

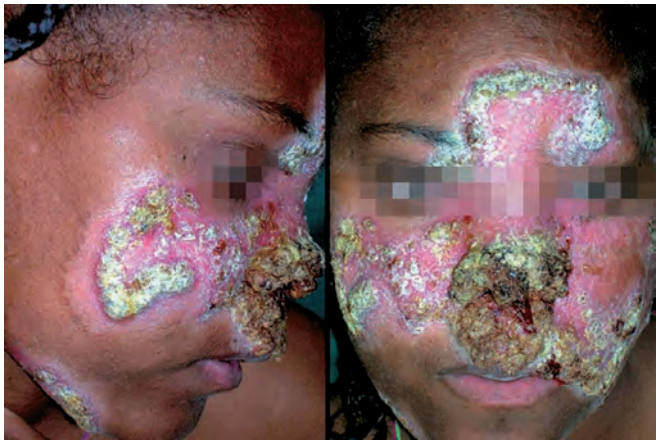

Figure 16.—Paracoccidioidomycosis. Confluent erythematous scaling lesions to the face.

was discovered after 30 years. Paracoccidioidomycosis should alert European mycologists, clinicians and pathologists that migrant patients or people who have travelled or lived outside Europe may harbor paracoccidioidomycosis or other imported mycoses.

Clinical manifestations

The initial lesion is usually confined and it can remain clinically silent in the lungs. Early clinical manifestations are often the result of fungal dissemination in the body, particularly to the oral mucosa and throat, skin and lymph nodes. The mucous membranes show infiltrated plaques with yellowish-white specks of exudate and sometimes with ulceration (Figure 16). The facial and truncal skin is more frequently affected than other areas. The dermatological syndrome is polymorphic, with isolated or grouped papules, nodules and abscesses, and swelling and suppuration of regional lymph nodes. The central nervous system, liver, and spleen may also be affected as the disease becomes chronic and progresses toward death within a few months or years.

Diagnosis

Biopsy for histology and culture is often diagnostic in cases of skin involvement. Gomori methenamine silver stain (GMS) is useful for the visualization of fungi. Fungal cells appear spherical, very large (diameter, 10-60 μm) and have multiple budding.

Therapy

Paracoccidiodomicosis is the only systemic fungal infection treatable with sulfa drugs. It can also be treated with amphotericin B and various imidazole derivatives, in particular ketoconazole.[53]

Penicilliosis

Definition

Penicilliosis is caused by *Penicillium marneffei.* Once considered rare, its occurrence has increased due to AIDS. It is now the third most common opportunistic infection (after extrapulmonary tuberculosis and cryptococcosis) in HIV-positive individuals in endemic areas of Southeast Asia.

Epidemiology

The majority of infections reported in the literature concern immunocompromised patients or residents who have stayed in Thailand, southern China or other areas of Southeast Asia. Penicilliosis is apparently acquired through the inhalation of spores, but the natural reservoir is not yet known.

Clinical manifestations

From a primary lung infection, the disease spreads to other organs, leading to splenomegaly and osteolytic lesions. The skin lesions are polymorphic, appearing as pustules, chronic ulcers, multiple papules, acneiform-like or molluscum contagiosum.

Diagnosis

Diagnosis is based on the characteristic aspect of *Penicillium marneffei* on microscopic examination of a biopsy taken from skin lesions or exudate from the skin lesions. The elliptical cells (diameter, 2-3 x 6-8 uM) are found inside phagocytes. Equally characteristic is the diffuse red halo surrounding the colony in the agar medium.

Therapy

The drug of first choice is itraconazole at a dose of 200 mg/day.[55]

TABLE II.—*Subcutaneous and deep mycoses.*

Mycosis	Etiological agent	Characteristics
Cromomycosis	Different species of fungi dematiacei dimorphic saprophytic soil including: Cladosporium carrionii Fonsecaea compacta, F. pedrosoi, Phialophora verrucosa	Infection of the skin and subcutaneous resulting from trauma. Verrucoidi injury. Can spread through the lymphatic involvement with pulmonary and cerebral.
Feoimycosis	Different species of fungi dematiacei dimorphic saprophytic soil including: Bipolaris spicifera, Exophiala jeanselmei, Phialphora parasitic, Wangiella dermatitidis	Form subcutaneous nodules with ulceration and possible verrucoid injury. Deep form: pneumonia, brain abscesses, endocarditis, dissemination
Mycetoma	Mushrooms saprophytic soil and vegetation including: Madurella micetomatis and M. grisea	Infection resulting from trauma with subcutaneous nodular lesions with a propensity to colliquative and release of granules
Blastomycosis	Dimorphic fungus present in the vicinity of the rivers	Pulmonary infection with Blastomyces dermatitidis possible involvement as a result of dissemination to the bone, skin, viscera
Dimorphic fungus coccidioidomycosis	Coccidioides immitis	Respiratory infection in benign course and self-healing. Fatal systemic disease in immunocompromised
Paracoccidioidomycosis	Dimorphic fungus	Pulmonary infection with Paracoccidioides brasiliensis dissemination of the oral and nasal mucous membranes. Possible involvement for dissemination to the lymph nodes, skin, viscera
Penicilliosis	Penicillium marneffei	Pulmonary infection with possible involvement for dissemination to the reticuloendothelial system, skin and bones
Sporotrichosis	Sporothrix schenkii	infection resulting from trauma with skin lesions and subcutaneous nodule - ulcerative. Possible spread
Mucormycosis	Several fungi of the order Mucorales including: Rhizus oryzae, R. microsporus	There are many clinical situations including the shape of rhino- orbital- cerebral, cutaneous, pulmonary. possible spread
Rinosporidiosis	Rhinosporidium seeberi	affects the nose, palate, nasopharynx
Lobomycosis	Fungus lievitiforme: Loboa loboi	Nodules with tendency to ulcerative

TABLE III.—*Etiology and characteristic of eumicetomi.*

Causative agent	Appearance	Granule diameter (mm)
Madurella mycetomatis	Blacks, hard, oval or lobulated	0.5-1
Mardurella grisea	Blacks, from soft to hard, oval and lobulated	<1
Acremonium falciform	White-yellow, soft, variable shape	<1.5
Acremonium kiliense	White-yellow, soft, variable shape	<1.5
Pseudoallescheria boydii	White-yellow, soft, oval	1-2
Exophialia jeanselmei	Blacks, soft, irregular shape	<1
Pyrenochaeta romeroi	Blacks, hard, oval, lobular	0.5-1.5
Leptosphaeria senegalensis	Blacks, soft, irregular shape	1
Neotestudina rosatii	White, soft, variable shape	0.5-1.5
Fusarium moniliforme	White, fluffy	<1
Fusarium solani	White, oval	<1.5
Aspergillus nodulans	White, soft, oval and lobulated	<0.6
Corynespora cassicola	Blacks, round, oval and elongated	<1

Mucormycosis

Definition

Mucormycoses are a group of diseases caused by fungi of the order Mucorales. Many different species have been implicated as etiological agents producing similar clinical syndromes. The most common fungi are *Rhizus* spp., and *R. oryzae* and *R. rhizopodiformis microsporus* var. in particular, followed by fungi belonging to the genera *Absidia, Mucor, Rhizomucor, Saksenaea, Mortierella, Cunninghamella, Syncephlastrum* and others.

Epidemiology

Mucoraceae are ubiquitous fungi found in decaying materials. Because of their rapid growth and spore-producing ability, inhalation of conidia is extremely common. Although these fungi grow in many ecosystem niches, and in spite of the wide distribution of these fungi, human disease is limited in most cases to the immunocompromised or persons with diabetes mellitus or previous trauma.

Clinical manifestations

Various clinical manifestations have been described, the most common being rhino-orbital-cerebral infection in diabetic ketoacidosis and neutropenia in association with hematologic malignancy. Bronchopulmonary and intestinal involvement are also possible.[56] In diabetics, primary cutaneous mucormycosis can arise in skin ulcers covered with contaminated dressings. *R. oryzae* has been isolated from patients with renal failure reeceiving dialysis and those with hemochromatosis. The administration of desferrioxamine was found to be a risk factor for the onset and spread of the disease because it facilitates the growth of *Rhizopus* (mainly *R. microsporus*) and antagonizes the action of amphotericin B. Cutaneous mucormycosis primarily affects the epidermis and the dermis, with necrosis secondary to vascular invasion. In addition, patients with pulmonary or other forms of mucormycosis may develop skin lesions at sites distant from the primary site of infection. Secondary skin involvement is the expression of fungemia, which is almost never detected on blood testing. The affected area appears as an erythematous sore with varying degrees of central necrosis.

Diagnosis

Bacterial culture is the best method to identify the fungus. Histolopathology demonstrates pleomorphic thin-walled hyphae (diameter, 5-25 mM) with irregular branching that may occur at right angles and invade blood vessels, inducing thrombosis and disseminated fungal emboli in other organs.[56]

Therapy

Amphotericin B is the only drug with some activity against mucormycosis.

Rhinosporidiosis

Rhinosporidiosis is an infection caused by *Rhinosporidium seeberi*. The disease is typical of tropical and subtropical regions, mainly South India, Sri Lanka, South America, and Africa. It is thought to be transmitted by exposure to the pathogen during bathing in stagnant water pools. The lesions are localized mostly to the nose, palate or nasopharynx. More rarely, the conjunctiva, lips, ears, face, genitals, and rectum may be affected. Clinically, it manifests with soft polypoid formations pink to purple in color that may be pedunculated, papillomatous or warty, and bleed easily, accompanied by epistaxis and obstructive dyspnea. Histological examination shows large sporangia (diameter, 0.3-3 mm) containing endospores (diameter, 6-7 microns). This fungus is uncultivable. Differential diagnosis includes viral warts and condyloma acuminate.[60, 61]

Lobomycosis

Lobomycosis, also called keloid blastomycosis, a rare infection described in Central and South America, is caused by *Loboa loboi*. It is a chronic, localized fungal disease of the skin and subcutaneous tissues. It begins with a papule that may evolve slowly until a nodule varying in color forms (Figure 17), followed by other elements which develop at distant sites probably due to diffusion through the lymphatics. The head and limbs are most often affected. Diagnosis is histological, as this fungus is uncultivable. Numerous round, thick-walled fungal cells are found in intradermal histiocytic granulomas, where they form chains of various elements (diameter, 1-12 mM) joined by tight bridges. Antifungals are ineffective. Only surgery is curative.[62]

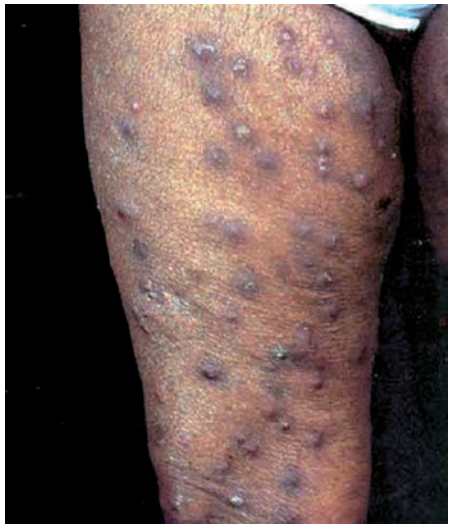

Figure 17.—Lobomicosi. Multiple nodular lesions the lower limb.

Histoplasmosis

Histoplasmosis is acquiring attention in nonendemic areas due to increased travel and immigration, being the most common systemic mycosis acquired by European travelers. Epidemiological studies show that the incidence of histoplasma infection in such patients may be higher than previously believed and a wide clinical spectrum of disease may be observed.

Histoplasmosis is caused by *Histoplasma capsulatum* var. *capsulatum*. It is distributed worldwide, but some tropical regions and the southern United States are the typical endemic areas. The disease is contracted through the inhalation of spores present in soil contaminated by the feces of infected birds or bats. It affects individuals of all ages and is common among people with AIDS.

Disseminated histoplasmosis manifests with chronic mucocutaneous involvement showing granulomatous, ulcerative or verrucous lesions. The disease can resemble angular stomatitis or gingivitis, with well-defined ulceration of the palate or tongue. Vegetating lesions of the skin or mucous membranes may arise very late, sometimes up to 20 years after a clinically silent primary infection.

Culture is thought to be useful, although it does not allow to differentiate this form from histoplasmosis caused by *H. duboisii*. Histological examination shows the presence of unicellular small fungi (diameter, 1-3 mm), arranged in extracellular clusters within uninucleate macrophages. These elements need to be distinguished from leishmaniasis, *Toxoplasma*, and *Pneumocystis carinii*.[59]

The treatment of choice is itraconazole at a dose of 100-200 mg/day until the lesions clear and then at a dose of 100 mg/day until completion of 6 months of treatment.

Clinicians in nonendemic areas may encounter patients with a diagnosis of histoplasmosis, and although histoplasma infection can have a varied and nonspecific clinical presentation, imported histoplasmosis may have two distinct profiles. Previously healthy travelers may be exposed in endemic areas and develop acute forms of the disease with a favorable outcome. Immigrants from endemic areas who may be immunocompromised due to HIV infection may experience reactivation of latent disease and develop disseminated forms with high mortality rates. This infection should be considered in the differential diagnosis of diseases affecting immigrants.[63]

African histoplasmosis

African histoplasmosis, characterized by large yeast, is due to Histoplasma capsulatum var. duboisii. The disease derives its name because endemic in hot humid west-central Africa. The skin lesions frequently arise on the chest and face, where they appear as hemispherical lenticular or umbilical papules or nodules, abscesses, ulcers, or scars. The lesions resemble those seen in osteoarticular tuberculosis: granulomatous lesions over the joints (wrists, knees, jaw) and over boney areas (skull, sternum and vertebrae). Bacterial culture allows the isolation of H. capsulatum but is unable to distinguish the two varieties (H. duboisii versus H. capsulatum). The two fungi can be differentiated in tissues by histological examination. H. duboisii is larger (diameter, 8-15 mum) and often presents unique lateral budding and double cell configurations inside plurinucleate giant cells.[53, 54]

Amphotericin B is effective at a total dose of 2-4 g administered over a course of 4 months; ketoconazole (200 mg 2-3 times a day) and itraconazole (200 mg/day) have also proved effective.

Conclusions

Fungal disease is the leading cause of disease in migrant patients who often present with unusual

clinical manifestations and findings, rendering accurate and timely diagnosis difficult. In addition, as the occurrence of fungal infections once considered rare in Italy and the identification of unusual etiologic pathogens become increasingly common, physicians will need to become familiar with a variety of skin diseases conventionally classified as tropical in origin.

Riassunto

Migrazione e micosi

Negli ultimi anni l'incidenza delle micosi cutanee superficiali è in costante aumento in Europa. Uno dei principali fattori che ha contribuito a questo incremento è rappresentato dal progressivo aumento dei flussi migratori. In particolare l'Italia, negli ultimi decenni, ha assistito a una sempre maggiore crescita della popolazione migrante sul proprio territorio, diventando, ad oggi, uno dei paesi europei con maggior numero di immigrati. Questo fenomeno ha avuto notevoli implicazioni nella pratica clinica della micologia dermatologica in quanto è sempre più frequente osservare quadri clinici inusuali e isolare agenti eziologici, non presenti, fino a poco tempo fa, alle nostre latitudini. Scopo del presente lavoro è quello di aggiornare sull'epidemiologia, la classificazione, la patogenesi, le manifestazioni cliniche e la terapia delle principali dermato-micosi osservate nella popolazione immigrata, attraverso i casi più tipici, indagati mediante accertamenti microscopici e colturali. Trattasi di patologie in continua espansione, spesso di difficile individuazione, soprattutto in considerazione del peculiare rapporto di interazione ospite-ambiente-parassita che, ancor più che in altre categorie, risulta significativo nella popolazione degli immigrati.

PAROLE CHIAVE: Emigrazione e immigrazione - Salute - Micosi.

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Tinea atypica

L. ATZORI, M. PAU, N. ASTE

Dermatology Departments
Cagliari University, Cagliari, Italy

Although usually simple, the diagnosis of dermatophyte infection is sometimes neglected. Variations in clinical presentation (*tinea atypica*), mimicking other skin diseases depend on many factors, partially due to the dermatophyte's characteristics, and a combination of patient's pathological but often physiological conditions, such as excessive washing or sun exposure. The physician's misdiagnosis and eventual prescription of steroids or other incongruous treatments further induce pathomorphosis (*tinea incognito*), longstanding disease and delayed recovery. This review describes the morphology of some atypical dermatophyte infections, in an attempt to compare and correlate changes to the normal features of the disease by site of involvement. The risk factors and predisposing conditions are also analysed to provide a reasoned interpretation of morphology and therefore evoke the diagnostic suspect in atypical cases. Periodical training is the clue to improve dermatologist expertise in what is the first-sight ability to make a diagnosis, perform the correct assessments and consequent therapy in daily practice.

KEY WORDS: Tinea - Epidemiology - Risk factors.

Dermatophytes represent the prevailing type of fungi causing infection in humans worldwide,[1-12] and the diagnosis is usually simple, rapidly confirmed by the execution of direct microscopy, or by dermoscopy and confocal microsocopy, as recently suggested.[13-17] Beside, longstanding disease and neglected cases periodically arrive to the dermatologist's office, most of these patients having made multiple visits and therapies without conclusive results. The surprisingly and embarrassing matter is that very often the final picture and the history of the patient is clearly evocative of a dermatophyte infection for a trained dermatologist, posing the question of why some colleagues have missed the diagnosis. Available literature includes many cases describing dermatophyte infections that mimic other skin diseases,[18-27] and the term *tinea incognito*, which should be *tinea incognita* to respect Latin etymology, has been coined to underline the diagnosing error with the prescription of steroids, or other incongruous treatment such as antibiotics, antivirals, calcineurin inhibitors.[28-42] A general common attitude to prescribe combination treatments and/or corticosteroid topics before confirming the diagnosis (*ex adjuvantibus*) is one of the possible causes of long standing disease,[43] but some published cases confirm that the clinical diagnosis might be difficult from the very beginning and sometimes the misleading presentation is due to general conditions requiring steroids administration or inducing immune-suppression, which are not to be considered a mistake.[18-23] Performing more mycological examinations should avoid underestimation of the disease, but it is probably true that light microscopy is no more available in many general offices, and less dermatologists are trained to recognize dermatophytes. Samples shipping to specialised laboratory is expensive and final report requires time. Improvement of dermatologist ability to recognise atypical cases requires periodical training, giving clues in the patient's history and

Corresponding author: L. Atzori, Dermatologic Clinic, Via Ospedale 54, 09124 Cagliari, Italy. E-mail: laura.atzori@libero.it

physical examination that point towards the presence of dermatophytes and consequent execution of mycological examinations.

Mechanism of dermatophytes pathomorphosis

In the immune competent patient, dermatophytes are the main fungal infection, being primary pathogen, and having developed the ability to metabolize and subside upon keratin.[44] Clinical presentation depends on a mild inflammatory host response to the fungus presence and its metabolic products,[44-46] as dermatophytes colonize a not living tissue, the stratum corneum, and usually do not have an invasive potential. The result is the typical ringwarm pattern, characterized by an erythematous scaling reaction, centripetally progressing as the dermathophyte move from the inoculum to the surroundings spaces, looking for new sources of keratin. Variations in clinical presentation depend on different conditions, combining the fungal invasiveness characteristics and the host response.[45-48] A distinction can be made between those cases in which the disease presentation is misleading from the very beginning (primary atypia), due to intrinsic variations of the pathologic process and the modifications induced by treatments (secondary atypia), usually consequence of a diagnostic error or immune-suppressive therapy (Table I). In daily practice a combination of both situations is frequent, and an attempt to classify the pathology changes with the clinical morphology includes:

Variations due to dermatophytes' characteristics

The dermathophytes involved in atypical presentations are the same as in common tinea, but there are minimal differences in invasiveness.[7, 45] Species usually infecting animals (zoophilic), such as *M.canis* and *T.mentagrophytes* are able to evoke a more inflammatory reaction, with pustules and sometimes vesicles, less tendency to central clearing or relapses with target features. By converse, strains which have completely adapted to humans (anthropophilic), such as *T.rubrum* are able to induce persistent infections even in adults through immunologic tolerance, by activating specific suppressor T cells.[46-49] Although fine mechanisms are still unknown, among the fungal constituents, *T.rubrum* mannose-rich glycoproteins are able to suppress cell-mediated immune reactions,[48-50] better than any other fungal mannans. The functional expression and secretion of proteins, permease and membrane transporters have been documented in dermatophytes,[46-48] up-regulated in the presence of keratin, and under various stimuli including antifungal drugs, correlated with the development of multidrug-resistance, which is an actual worry also in immune-competent patients.[50-52]

The ability to parasite hairs is a possible further cause of pathomorphosis and longstanding course, because the infection moving from the horny layer into the hair follicles reaches the dermis, and the fungal metabolic products, as well as antigens (principally trichophytin) diffusion stimulates a more complex immunological response,[46-48] with purplish papule and pustules appearance, eventually progressing to chronic granulomatous formations, called Majocchi's granulomas.[53-57]

Variations due to the host predisposing conditions

As in any skin disease, the host factors favouring the infection can be divided in general and local conditions.

Local factors influencing the course of dermatophytosis are many, but variations of the skin barrier are the first to be considered. Several studies have provided evidence that the pH environment is important to regulate fungal gene expression and secretory activity.[58, 59] Sebum saturated fatty acids fungistatic

TABLE I.—*Atypical dermatophyte infections: classification of possible mechanisms inducing pathomorphosis and consequent atypical presentation.*

Primary clinical atypia	– Variation in dermatophyte invasion ability – Host genetic susceptibility – Skin Barrier defects (immaturity, previous skin illness, immune defects, etc.) – Anatomical and physiology variation of the site of infection – External factors inducing modification of the fungal growth rate and/or of the host defences (sun exposure, excessive washing, tight-fitting clothes in synthetic materials, warm wet climate)
Secondary clinical atypia	– Inadequate local therapy (cortisone based, acyclovir, tacrolimus, pimecrolimus, etc.) – General immune suppression: HIV patients, transplant recipients, drugs.

activity is a natural defence, which is widely affected by the age and hormonal profile of the patient.[20] The anatomical characteristics of the involved area are also important, because presence of skin folds, sebaceous glands content as well as variable thickness of the horny layer might bar the typical centripetal progression, favor crusting, and inflammation instead of central clearing, vellus hair follicle involvement. These conditions are frequently evoked in *tinea faciei* and adults' *tinea capitis* atypical presentations.[21-24, 60-71] All conditions altering the skin barrier, from immature skin barrier in the newborn [20] to true disease or physiologic conditions, might favour dermatophytes implantation and proliferation, exacerbate inflammation or by converse masquerade the infection under an heavy hyperkeratosis, eventually leading to bullous formation.[72, 73] Therefore, working conditions and life-style habits are quite as important as pathological history: tight-fitting clothes in synthetic occlusive materials, excessive washing, wet warm climate are able to alter the clinical expression of the dermatophyte infection. As regards skin diseases, in a personal experience recently published on atypical dermatophyte infections,[19] about 20% of the patients reported previous cutaneous illnesses including atopic dermatitis, contact dermatitis, lichen planus and psoriasis. Among the controversial risk factors, sun exposure might play a role, especially in outdoors workers.[21-24, 74-76] High doses of ultraviolet radiation (UVR) have well-known inhibitory effects upon dermatophytes, but repetitive low doses might have different effects, joined with an host cell-mediated response inhibition. An *in vitro* experiment suggested the possibility that *T.rubrum* and some geophilic species might evade UV harmful effects.[77] A careful evaluation of professional and recreational activities, as well as the patient's immunological condition is necessary to avoid and/or correct predisposing factors.[5-8, 24, 27-31, 74] From our experience,[19] disease duration is another crucial point, as persistence of the disease along time favour pathomorphosis.

Passing to general predisposing conditions, several studies have investigated a genetic susceptibility, with an autosomal dominant pattern of inheritance, especially for diffuse, longstanding and treatment resistant infections, mainly due to T. rubrum.[79-82] Immunosuppressed individuals, particularly those undergoing transplants [83-87] and chemotherapy, diabetic patients and human immunodeficiency virus-positive patients,[88-92] might experience very diffuse and misdiagnosing dermatophyte infection. Obesity was reported among predisposing factors in tinea pedis and manum.[6] Hormonal alterations, especially menopause in females, and immune-senescence might be relevant in adult tinea capitis.[69-71] Modifications of the clinical picture following the use of topical and systemic steroids, is the most widely reported factor in literature,[28-38] because directly responsible of changes in the virulence of the dermatophyte strains, inhibiting the immunological defenses, increasing neo-angiogenesis, inducing dyschromic alteration, hypertrichosis and skin atrophy. Recently,[26, 40-42] other incongruous topical treatment such as acyclovir, tacrolimus, and pimecrolimus, have been reported as responsible of *tinea incognita*.

As regards investigation for the responsible strains and source of infection, in most of single reports anthropophilic dermatophytes are reported, mainly *T.rubrum*,[25, 34-38] and previous onychomycosis with auto-contamination suggested.[38] In our experience,[19] zoophile dermatophytes were more frequently identified (*M.canis*, *Tr.mentagraphytes* var.*mentagrophytes, T.verrucosum*), the carriers varying from pets to large animals, such as sheep and cattle. Therefore, information about the type of work might be a clue in very atypical cases, especially in adults (farmers, shepherds, housekeeping taking care of domestic animals). Emergency of very inflammatory manifestation from *T. tonsurans* is also reported.[71] The soil (*M. Gypseum*) has become a rare source of infection, but clinical presentation might be very confusing.[19, 39]

Clinical presentation

Clinical atypia is defined as significant variations in clinical signs and pathological features from textbook descriptions of the disease. Atypical dermatophytosis or tinea atypica is characterized by variation in the severity of inflammation and scaling (Table II), as well as extension of the involvement. Usually lesions show more severe inflammatory components, with central scaling and/or follicular papules, vesicle-pustules instead of clearing, sometimes with borders oedematous induration. Hyper-pigmentation and crusting secondary to itching is also frequent (Figures 1, 2). Although frequency evaluation should requires very large study population, our decennial experience suggested that 2.5% of all diagnosed

TABLE II.—*Tinea atypica: clues suggesting a dermatophyte infections.*

Tinea atypica clinical features
– Asymmetric eczematous dermatitis, especially at onset.
– Indolence and very slow disease progression, if compared with its main mimics, such as eczema, impetigo, discoid lupus or polymorphous light eruption.
– Active borders, with serpiginous if not semi-annular erythematous scaling margins, sharply demarcated even if a central clearing is absent and desquamation seems diffuse.
– Presence of small follicular papules and vesicle-pustules, with brittle or fragmented hairs inside the patches.
– Irregular shaped pseudo-alopecic patches, with granulomatous papules and pustules, intermingled with normal hairs when the scalp is involved.

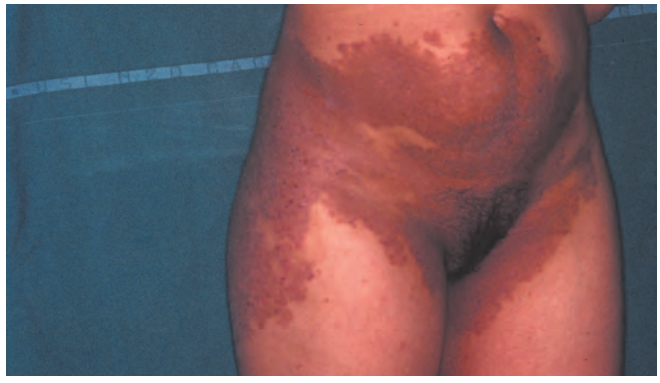

Figure 1.—Very widespread eczematous hyper pigmented dermatophyte infections simulating a contact dermatitis in a young white woman.

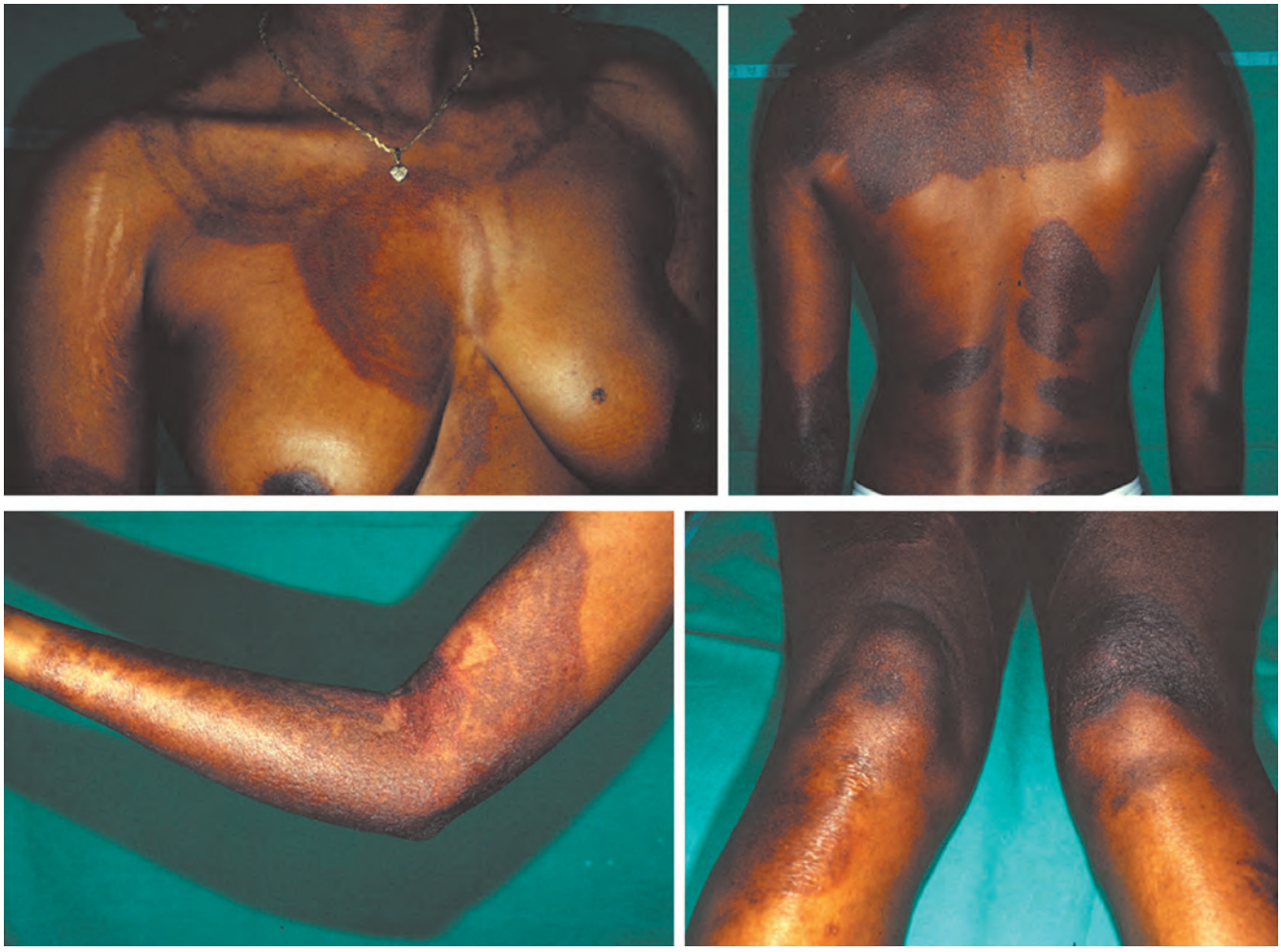

Figure 2.—Widespread tinea showing no central clearing, with diffuse scaling and hyper pigmentation in a black woman.

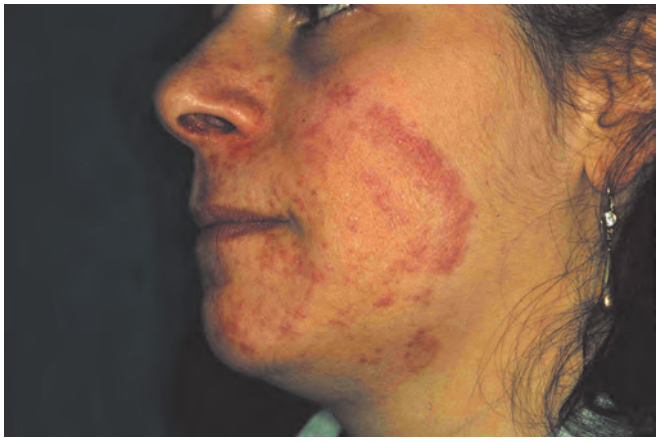

Figure 3.—Erythematous papular-pustular dermatophyte infection simulating a rosacea.

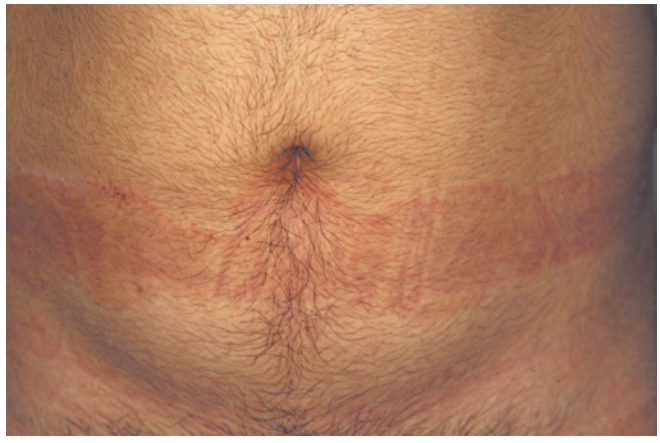

Figure 4.—Eczematous itching dermatophyte infection along the waistline, mimicking a contact dermatitis.

dermatophyte infections are misdiagnosed at onset, corresponding to 1 patient over 100.000 inhabitants/year, with an illness time before diagnosis which encompass the year in many patients, ranging from 6 months to 5 years.[19, 1, 21-23,] Any age can be affected, although atypical presentation is less frequent in the first decade,[19-21] probably because children under the close eye of their parents are visited in relatively short time frames, before the infection underwent consistent pathomorphosis.

Certain variations are highly conditioned by the site of involvement, therefore a classifications considering the typical form and the principal mimickers is suggested on Table III. Tinea faciei is a rare dermatophyte infection limited to the glabrous skin, thus excluding the areas where moustache and beard grow.[22] Although its position as separate entity from tinea corporis is controversial, Tinea faciei very frequently simulates other skin diseases,[21-24, 63-68] both for anatomical conditions, and external factors, such as frequent washing, use of moisturing creams, sun exposure. It is observed more frequently in children as a result of contact with household pets or close quarters activities,[21] and in adults for work exposure or life-style habits.[60-66] Lesions on the face primarily mimic impetigo, per-oral dermatitis and atopic dermatitis in children, rosacea (Figure 3) discoid lupus erythematosus (LED), polymorphous light eruption, seborrhoeic dermatitis, and herpes simplex in adults.[63]

Very widespread dermatophyte infections usually involve the trunk, simulating psoriasis, atopic

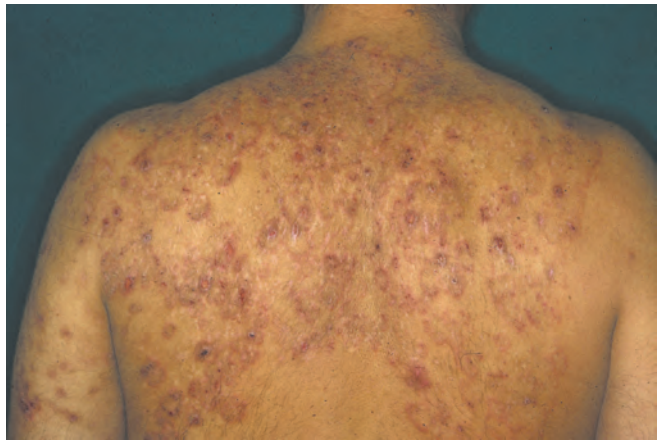

Figure 5.—Neck, shoulders and upper trunk annular erythematous scaling patches, partially covered with crusts and itching erosions, simulating a subacute lupus erythematosus.

or allergic eczema (Figures 1, 2, 4), systemic lupus erythematosus (Figure 5), folliculitis, pityriasis rosea, herpes zoster, but also kaposi's sarcoma.[18, 26-30, 34, 42, 93-95] *Tinea manuum* is frequently misdiagnosed for contact dermatitis.[6, 74] Cases simulating a burn and another mimicking pyoderma gangrenosum were observed on the leg.[19] Adult tinea capitis, although very rare is a misleading conditions, because the pseudo-alopecic patches have very irregulars borders, intermingled with normal hairs, presence of pustules, and crusts simulating seborrhoeic dermatitis, psoriasis, bacterial folliculitis, dissecting cellulitis, lupus erythematosus, Brocq pseudopelade and trichotillomania.[67-71]

TABLE III.—*Tinea atypica: site of involvement and main mimics reported in current literature.*

Atypical Clinical form	Skin diseases simulated by the dermatophyte infection	References
Tinea faciei	– Bacterial Impetigo – Rosacea – Seborrhoeic dermatitis – Perioral dermatitis – Atopic dermatitis – Herpes simplex – Lupus Erythematosus – Polymorphous light eruption	– Alteras *et al.*[25] – Atzori L *et al.*[19] – Aste N *et al.*[63] – Belhadjali H *et al.*[24] – Difonzo EM *et al.*[65] – Jorquera E *et al.*[60] – Lin RL *et al.*[22] – Meymandi S *et al.*[23] – Pravda DJ *et al.*[64] – Romano C *et al.*[61] – Shapiro L *et al.*[62]
Tinea corporis	– Allergy – Contact dermatitis – Folliculitis – Herpes zoster – Kaposi's sarcoma – Lupus Erythematosus – Pityriasis Rosea – Pyoderma gangrenosum – Polymorphous light eruption – Psoriasis	– Aste N *et al.*[26] – Atzori L *et al.*[19] – Brod C *et al.*[94] – Kwon KS *et al.*[93] – Serarslan G [30] – Turk BG *et al.*[29] – Veraldi S *et al.*[73] – Wacher J *et al.*[34] – Ziemer M *et al.*[18]
Tinea pedis T. manuum	– Burn – Bacterial intertrigo – Contact dermatitis – Psoriasis	– Aste N *et al.*[72] – El Fekih N *et al.*[4] – Kiraz N *et al.*[6] – Radev S *et al.*[76]
Tinea capitis	– Alopecia areata – Brocq pseudopelade – Dissecting cellulitis – Erosive pustular dermatosis – Folliculitis – Lupus erythematosus – Psoriasis – Seborrhoeic dermatitis – Trichotillomania	– Aste N *et al.*[69] – Buckley AD *et al.*[66] – Chia C *et al.*[68] – Hryncewicz-Gwòzdz A *et al.*[71] – Morell L *et al.*[70] – Stein LL *et al.*[67]

A possible complication and differential diagnosis to be considered, dealing with chronic inflammatory dermatophyte infections, it is the occurrence of diffuse sterile erythematous papular or targeted macular eruptions, called dermatophytide or id-reactions.[96, 97] The occult origin of the hypersensitivity reaction should be careful sought to unsure correct samples collection, demonstration of the dermatophyte infection, consequent efficacious control of the disease and recovery.

Diagnosis

Atypical dermatophytosis are usually easily confirmed with direct mycological examination, because hyphae and arthrospores are abundantly expressed. An exception might be vellus hairs involvement, in which scales might become deserted and the fungal elements should be underscored scraping papular-pustular elements. Culture is recommended to further evaluate source of contagion and predisposing factors, as well as response to treatment. Recently, fast fungal identification by assay mass spectrometry (matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) has been developed by several independent researchers and preliminary results suggest high sensitivity on skin isolates with limited costs, and promise to be largely adopted as routine laboratory determination in an immediate future.[97-100]

Biopsies for histological tests with haematoxylin-eosin and periodic acid-Schiff (PAS), might be necessary in very extensive or unclear cases to exclude other aetiology, as well as simple super-infections of major dermatitis. Initial negative microscopic and culture tests were experienced also in our centre,[19] and should be repeated and/or completed with histology, when clinical suspicion is high. Mycologically proven complete recovery of the skin manifestations after antifungal therapy is to be considered as final confirmation of the correct diagnosis. Careful evaluation of the nails [19, 38] and timely treatment of onychomycosis is mandatory to avoid relapses.

Therapy

Response to antifungal drugs is generally good, especially with the combination of topical and systemic therapy. Hairs involvement and concomitant onychomycosis require prolonged periods of treatment.

Very localized forms, might be treated with simple antifungal medications, imidazoles and allylamines based 1 or twice daily depending on the drug-delivery characteristics, usually for 2-4 weeks. Extensive and longstanding infections, especially if the vellus hairs are involved, requires systemic therapies for 4 to 5 weeks: griseofulvin remains the reference drug (dosage of 25-30 mg/Kg/daily), but terbinafine (250 mg/daily; in children weighing less than 20 kg 62.5 mg daily, 125 mg daily in those weighing between 20 and 40 kg, and adult dosage when weighing more than 40 kg) or imidazoles (itraconazole 200 mg/daily; fluconazole 5-10 mg/Kg/daily) are usually as effective and more accepted from the patients, especially adults.[19]

Report of multi-drug resistance is a current alarm, which should be taken into consideration especially in *T.rubrum* infections and immune suppressed patients.[51, 52]

Conclusions

Dermathophytes are very ancient fungal species, having adapted to many different conditions, from soil dependency to animal and humans. The fungal ability to hide itself, simulate other dermatologic conditions and maintain illness along times is another natural strategy preserving survival and environmental diffusion. Although the term *tinea incognito* is widely diffused, it is limiting, because it underlines the physician's fault and ascribes to the unwise incorrect prescription of steroids the source of the misleading presentation, while *tinea atypica* better comprehends the numerous variables conditioning an unusual dermatophyte infection appearance, both for primary and secondary pathomorphosis. Dermatologists should be regularly trained to recognised minimal signs differing from typical features in any skin illness, especially when dealing with erythematous scaling dermatitis, present for long periods of time and when previous therapies have proved to be ineffective. Misdiagnosis in tinea atypica can be overcome through a higher use of mycological tests, although sometimes histology remain the final clue.

Riassunto

Tinea atipica

Per quanto in genere semplice, la diagnosi di dermatofitosi talvolta sfugge a causa di variazioni nella presentazione clinica (*tinea atipica*), che simulano altre patologie cutanee e che dipendono da svariati fattori, in parte condizionati dalle caratteristiche del dermatofita e da una combinazione di condizioni patologiche, ma spesso anche semplicemente fisiologiche dell'ospite, come l'eccessiva detersione o l'esposizione solare. L'errore diagnostico e l'eventuale prescrizione di steroidi o altri trattamenti incongrui può ulteriormente indurre la patomorfosi (*tinea incognito*), prolungare la malattia e ritardare la guarigione. Il presente studio descrive la morfologia di alcune dermatofizie atipiche, nel tentativo di comparare e correlare i cambiamenti rispetto alle abituali forme cliniche dell'infezione per sede di coinvolgimento. Sono inoltre analizzati i fattori di rischio e le condizioni predisponenti per suggerire una interpretazione della anomala morfologia e di conseguenza evocare il sospetto diagnostico nei casi atipici. L'aggiornamento periodico è la chiave per migliorare la capacità del dermatologo nel fare la diagnosi a prima vista, nell'eseguire i corretti accertamenti e consigliare la conseguente terapia nella pratica quotidiana.

PAROLE CHIAVE: Tinea - Epidemiologia - Fattori di rischio.

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Skin and nail mycoses in patients with diabetic foot

M. PAPINI [1], M. CICOLETTI [1], V. FABRIZI [1], P. LANDUCCI [2]

[1]Terni Dermatologic Clinic, University of Perugia S. Maria Hospital, Terni, Italy
[2]Internal Medicine Belcolle Hospital, Viterbo, Italy

Diabetes mellitus affects all socioeconomic and age groups and its incidence is rapidly increasing worldwide. The diabetic foot complication represents one of the most complex and serious complications in these patients. Fungal infections can also contribute to the severity of the diabetic foot. The aim of the present study was to evaluate the prevalence of foot skin and toenail mycosis in a group of 75 patients with diabetic foot complication and in a matched control group. Diabetic patients showed onychomycosis in 53.3% and foot skin mycosis in 46.7% of the cases, with a prevalence of both fungal infections significantly higher than that observed in the control group. At least one type of these fungal infections was present in 69.3% of diabetic subjects with a highly significant difference compared to control group (P<0.001). *Trichophyton rubrum* and *Trichophyton interdigitale* were the most common species responsible of both nail and skin infections. *Candida spp*, *Fusarium spp, Aspergillus spp* and other moulds. were found in about 1/3 onychomycosis. Previous toe amputation was significantly associated with both skin and nail mycosis. The present study confirms that both tinea pedis and onychomycosis have a high prevalence in subjects suffering from diabetic foot complication, and that the problem of fungal infections of the foot in diabetic subjects is still highly underestimated. Consequently, there is an important clinical rationale for careful mycological examination of diabetic foot and an adequate treatment tailored for each individual patient according to the fungal species involved.

Key words: Onychomycosis - Tinea pedis - Diabetic foot – Epidemiology.

Diabetes mellitus is a widely diffused disease and its incidence is rapidly increasing worldwide. It is estimated that diabetes afflicts actually an average 7% of the world population and its incidence will rise to 8.3% by the year 2030.[1] This estimation makes diabetes one of the most relevant health problems of the current century.

Diabetic foot complication is a common problem of diabetes mellitus and represents a major cause of morbidity, disability and mortality with relevant economic consequences for the patients, their families, and society. The lifetime risk of a person with diabetes developing foot complications could be as high as 25% and it is estimated that every 30 seconds a lower limb is lost somewhere in the world as a consequence of diabetes.[2] Peripheral neuropathy involving motor, sensory and autonomic fibres, and poor circulation are the main predisposing factors of this condition. Bacterial infection frequently accompanies or follows foot ulceration and can represent a limb- and life-threatening event. Fungal infections could also contribute to the severity of diabetic foot.[3] Several studies [4-10] have investigated the prevalence of fungal foot infections among diabetic patients, showing a prevalence of nail fungal infection ranging from about 20% up to one-third of the cases. The overall risk ratio of diabetic patients having onychomycosis has been estimated as high as 2.77 compared with age- and sex-matched control subjects. Predisposing factors include increasing age, male gender, duration of diabetes, impaired peripheral circulation and

Corresponding author: M. Papini, MD, Clinica Dermatologica, Azienda Ospedaliera S. Maria, Via T. di Joannuccio, 05100 Terni, Italy. E-mail: manuela.papini@unipg.it

neuropathy. Although the presence of onychomycosis is considered a risk factor for severe diabetic foot complications, i.e. gangrene, foot ulcer and osteomyelitis[3,7], the problem of fungal infections in these patients is still widely understimated. Aim of the present study was to evaluate the prevalence of fungal infection of the skin and toenails in a diabetic population with foot complication.

Materials and methods

Participants were recruited over a two-year period among the patients attending the Diabetes Outpatient Service of Terni Hospital for periodic follow-up. The control group included non-diabetic, non-immunocompromised, age- and gender-matched persons recruited among inpatients from either Surgery Department or Internal Medicine Department. Ethics approval was granted by the local Scientific Ethical Committee and all participants signed an informed consent.

Assessment of the diabetic patients and control subjects

All subjects included in the study were asked for the following information: age, gender, racial origin, complete clinical history and list of medications taken. Specific questions about any alterations of skin and nails of the feet and previous diagnosis of skin and nail mycoses were also included. For the diabetic patients, further data from the patients' record on leg neuropathy, peripheral circulation and possible previous foot ulcers and toe amputations; fast blood glucose, glycated haemoglobin values over the past year, serum creatinine, proteinuria, triglycerides and cholesterol, and the treatment received over the last 12 months were recorded.

Both groups underwent a careful clinical evaluation of their toenails and foot skin. The following clinical findings were registered for the foot skin and nails: interdigital fissuration, desquamation, erythema, maceration; plantar hyperkeratosis, erythema, vesicles and/or pustules; tickened nail plate, discoloration, onycholysis, nail ingrowth. If those cases when foot skin and/or toenails appeared clinically abnormal, additional information was obtained about the onset and the evolving of such alterations and treatment received, if any.

Mycological sampling and evaluation

Interdigital and plantar skin scraping and nail clipping samples were obtained from all subjects showing clinical manifestation compatible with skin and/or nail fungal infection.

Mycological examination included direct light microscopy after 20% KOH clearing for the presence of fungal filaments and spores and culture test on Sabouraud peptone-glucose agar with and without cycloheximide, according to the standard techniques. Part of the nail clipping samples underwent histological examination according to technique described by Gianni *et al.*[11]

Skin and nail mycosis were considered confirmed in all patients with direct microscopy and/or histological examination showing fungal filaments, and/or a culture test growing a dermatophyte. In the case of a yeast or non-dermatophytic mould, congruous and recognizable non-dermatophytic fungal spores, filaments or pseudomycelium had to be observed under direct microscopy and/or histological examination. Repeated cultures (at least two) of new specimens growing the same fungal species were requested to validate the isolation of yeasts or non-dermatophytic moulds.

Statistical analysis

Data were expressed as total numbers and percentages. Difference in skin and nail mycosis prevalence between diabetic and non-diabetic subjects were assessed using either the χ^2 test or Fishers' exact test and variance analysis. Differences were considered significant when the P-value was lower than 0.05.

Results

A total of 75 diabetic patients and 75 matched control subjects were enrolled. Both groups comprised 52 Caucasian males with an age range from 41 to 84 (mean age 60.5 years for the diabetic patients and 61.1 for the control group) and 23 Caucasian females aged between 43 and 86 years (mean age of diabetics 68.9 years, mean age of controls 68.3 years). Type I insulin-dependent diabetes was present in 4 patients (5.33%).

Table I shows the prevalence of skin and nail mycoses observed in diabetic subjects and in the con-

TABLE I.—*Numbers and percentages of foot skin mycosis, onychomycosis and onychodystrophy in 75 diabetics with foot complications and 75 control subjects.*

	Diabetic foot subjects N. (%)		Non-diabetic subjects N. (%)		P value (total)
	Total	Men/women	Total	Men/women	
Skin mycosis	35 (46.7)	22/13	11 (14.7)	7/4	<0.01
– interdigital	27	16/11	7	4/3	
– diffuse plantar	7	5/2	4	3/1	
– vesicular-pustular	1	1/0			
Onychomycosis	40 (53.3)	32/7	17 (22.7)	12/5	<0.01
– both feet involved	31	28/3	9	6/3	
– >3 nails involved	18	15/3	2	2/0	
Both skin and nail mycosis	23 (30.7)		9 (12.0)		<0.01
At least one mycosis	52 (69.3)		19 (25.3)		<0.001
Onychodystrophy	30 (40.0)	22/8	28 (30.7)	18/10	n.s.

trol group. Fungal infection of the skin was found in 35 diabetic individuals (46.7%), 22 males and 13 females, and in 11 control subjects (14.7%), the difference being statistically significant (P<0.01, OR 5.09, 95% CI 2.32-11.15). The most common clinical aspect was interdigital desquamation and/or maceration in both groups. The prevalence of onychomycosis in the diabetic and control subjects was 53.3% and 22.7%, respectively, the difference being statistically significant (P<0.01, OR 3.89, 95% CI 1.92-7.89). Toenail mycosis involved both feet in 31 of 40 diabetic patients and in 18 of them more than 3 nails were affected. The presence of both nail and skin mycosis was observed in 30.7% of the diabetic patients; 69.3% of them presented at least one type of fungal infection. Both these values are significant compared with those obtained in the control group.

Distal-lateral subungueal onychomycosis was the most common clinical presentation in both diabetics and control subjects (Figure 1), but diabetic patients showed generally more severe forms characterized by the involvement of a greater portion of the nail plate. Total onychomycosis was present in 31% of diabetics and in 12% of control subjects.

Dermatophytes were isolated from 71.4% of the skin infections and in 75% of onychomycoses observed in diabetic patients, *Trichophyton (T.) rubrum* being the most common species in both type of mycosis (Table II). *Candida* species were responsible for interdigital mycosis in 22.8% of the diabetic patients, while only one of the control subjects was positive for yeast infection. Non-dermatophytic moulds were involved in 15% of the onychomycoses in diabetics and in 11.7% of nail infection in control group.

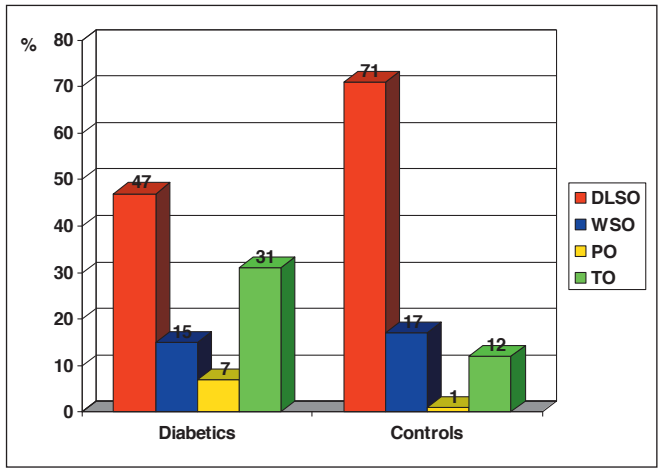

Figure 1.—Frequency of different clinical types of toenail onychomycosis observed in diabetic patients with foot complications and in control subjects.
DLSO: distal-subungual onychomycosis; WSO: white superficial onychomycosis; PO: proximal onychomycosis; TO: total onychomycosis

Table III reports the prevalence of fungal infections according to possible risk factors. Peripheral neuropathy was found to be associated with a higher risk of having both onychomycosis and tinea pedis at the same time, but not with the presence of only one type of these infections. Previous toe amputation was associated with a significant prevalence of both nail and skin mycoses; the association was highly significant when skin and nail involvement coexisted.

Based on clinical history, 16 (21.3%) of the diabetic patients reported a previous diagnosis of toenail fungal infection, mycologically confirmed in only 2 cases. Asked for details of treatment recieved,

TABLE II.—*Fungal species causing foot skin and toenail mycosis in 75 diabetic patients with foot complications and 75 control subjects.*

Fungal organism	Diabetic foot subjects		Non-diabetic subjects	
	Foot skin	Toenails	Foot skin	Toenails
Dermatophytes	25	30	9	13
– *Trichophyton rubrum*	11	16	5	5
– *Trichophyton interdigitale*	12	14	4	7
– *Epidermophyton floccosum*	2	-	-	1
Candida species	8	3	2	1
– *Candida albicans*	8	2	2	1
– *Candida krusei*	-	1	-	-
Non-dermatophytic moulds	1	6	-	2
– *Aspergillus niger*	-	2	-	1
– *Fusarium oxysporum*	1	2	-	-
– *Scopulariopsis brevicaulis*	-	2	-	1
Unidentified (KOH positive, no growth)	1	1	-	1
Total	35	40	11	17

TABLE III.—*Prevalence of skin fungal infection and onychomycosis according to possible risk factors: lower extremity arterial disease (LEAD), neuropathy, foot skin ulcer, toe amputation and poor metabolic diabetes control (mean glycated hemoglobin >8 mg% over the last year)*

	LEAD		Neuropathy		Foot skin ulcer		Previous toe amputation		Poor metabolic control	
	+	-	+	-	+	-	+	-	+	-
No. of patients	40	35	50	25	58	17	9	66	45	30
Onychomycosis	22	18	26	14	36	4	8	32	29	11
P-value	n.s.		n.s.		<0.05		<0.05		<0.05	
Foot skin mycosis	20	15	24	11	29	6	8	27	24	11
P-value	n.s.		n.s.		n.s.		<0.01		n.s.	
Both skin and nail mycosis	16	7	20	3	20	3	8	15	17	6
P-value	n.s.		<0.05		n.s.		<0.001		n.s.	

one subject said that he had received systemic therapy followed by complete recovery, 7 patients were treated topically with only little improvement and 8 subjects did not receive any treatment. The question about the duration of nail changes could not be answered by more than half of diabetic patients with onychomycosis because they were unable to determine the time of appearance of nail alterations, while about one third claimed to have noticed them by more than one year before.

Discussion and conclusions

Diabetes mellitus may present serious complications involving almost all organs and systems, such as neuropathy, peripheral vascular insufficiency, renal and cardiovascular disease, retinopathy, and several skin manifestations. The diabetic foot is one of the most complex and severe of these complications. A combination of several factors contributes to the development of the ulcer, including peripheral neuropathy resulting in altered sensation, trauma, vascular disease causing poor tissue oxygenation and impaired wound healing, and decreased resistance to infection. Skin and toenail mycosis can also contribute to severity of diabetic foot [12].

The present study evaluating fungal infections of the foot skin and nails in 75 subjects with diabetic foot complication indicates a very high prevalence of both these type of infection. Tinea pedis was observed in 46.7% and onychomycosis in 53.3% of these patients versus a prevalence of 14.7% and 22.7% respectively in matched control subjects. Both these figures are statistically significant (P<0.01). Furthermore, diabetic patients suffered from at least one type of foot fungal infetion in 69.3%, while the corresponding figure in control subjects was 25.3%

and this difference is highly significant (P<0.001).

Several studies have evaluated the prevalence of foot onychomicosis in diabetic subjects. As highlighted in the review of Mayser *et al.*,[12] early surveys did not clarify whether the prevalence of onychomycosis was increased in diabetic subjects; however, recent large epidemiological studies clearly indicates that the prevalence of toenail mycosis is significantly higher among diabetic patients than in the non-diabetic population. *Tinea pedis* and onychomycosis in subjects with diabetes mellitus were also associated with an increased risk of secondary bacterial infections, gangrene and/or foot ulcer, and amputation[13-16]. In our study, previous toe amputation was associated with a significant prevalence of both nail and skin mycoses when singly considered, while the association was highly significant when skin and nail involvement coexisted.

A previous diagnosis of tenail fungal infection was reported in more then 1:5 patients, but only half of them underwent antifungal treatment and only one of the latter obtained a complete recovery of the infection. These anamnestic data together with the presence of a large number of individuals with active undiagnosed mycoses indicate that the problem of foot fungal infections of the foot in the diabetics is still highly underestimated. Onychomycosis and tinea pedis in diabetic subjects are not a simple aestethic problem, but a limb-threatening condition if underestimated and left untreated. The presence of the mycotic lesions may result in adjacent nail and/or skin injury and provide a reservoir of microrganisms, thereby further increasing the risk of bacterial infection and other serious sequelae. Diabetic foot complication is the major cause for non-traumatic lower extremity amputations worldwide, and the amputation is usually preceded by a chronic infected foot ulcer. Lower limb amputation has a huge impact in terms of costs and poor quality of life for both the patient and society, but it is a problem that can and must be prevented through appropriate measures.[17] Prevention of diabetic foot ulcers includes, among other recommendations, the systematic nail and foot skin care.[18] A dermatological videat and mycological tests should always be performed in presence of nail alterations and/or clinical signs suggestive of *tinea pedis*, *i.e.* interdigital maceration and/or desquamation, diffuse plantar desquamation or vesicular-pustular lesions. The prompt treatment of skin and nail mycoses should be considered a relevant measure in the prevention of diabetic foot ulcers and in decreasing the amputation risk.

Riassunto

Onicomicosi e tinea pedis in pazienti con piede diabetico

Il diabete mellito è una malattia che affligge tutte le età e tutte le classi socio-economiche e che mostra un'incidenza in rapido incremento in tutto il mondo. Il cosidetto piede diabetico è una delle complicanze più gravi e complesse che si possono osservare nei pazienti diabetici. Le infezioni fungine possono contribuire ad aggravare i problemi connessi con il piede diabetico. Scopo di questo studio è stato quello di valutare la frequenza delle infezioni fungine della cute del piede e delle unghie in un gruppo di 75 soggetti con piede diabetico rispetto ad un adeguato gruppo di controllo. La presenza di onicomicosi è stata accertata nel 53,3% dei pazienti diabetici, mentre micosi della cute del piede era presente nel 46.7% dei casi, con una prevalenza di ciascuna infezione statisticamente significativa. Almeno un tipo di infezione fungina del piede era presente nel 69,3% dei diabetici, mostrando una differenza altamente significativa (P<0,001) rispetto al gruppo di controllo. I miceti più spesso isolati da unghie e cute erano *Trichophyton rubrum* e *Trichophyton interdigitale*, mentre in circa 1/3 dei casi i miceti responsabili di onicomicosi sono risultati essere C*andida spp, Fusarium spp, Aspergillus spp* e altre muffe. Onicomicosi e tinea pedis sono risultate statisticamente più frequenti nei pazienti che avevano subito precedenti amputazioni di uno o più dita dei piedi. I risultati di questo studio confermano l'elevata prevalenza di micosi della cute e delle unghie nei pazienti con piede diabetico, ma indicano anche che queste infezioni sono ancora ampiamente sottovalutate nei soggetti diabetici. È auspicabile, quindi, un più attento controllo clinico e micologico del paziente con piede diabetico, così da individuare e trattare precocemente le eventuali infezioni fungine e prevenire le potenziali complicanze ad esse correlate.

Parole chiave: Onicomicosi - Tinea pedis - Piede diabetico - Epidemiologia.

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Malassezia skin diseases in humans

E. M. DIFONZO [1], E. FAGGI [2], A. BASSI [1], E. CAMPISI [2]
M. ARUNACHALAM [1], G. PINI [2], F. SCARFÌ [1], M. GALEONE [1]

[1]*Division of Dermatology*
Department of Surgery and Translational Medicine
University of Florence, Florence, Italy
[2]*Division of Microbiology*
Department of Clinical and Experimental Medicine
University of Florence, Florence, Italy

Although *Malassezia* yeasts are a part of the normal microflora, under certain conditions they can cause superficial skin infection, such as pityriasis versicolor (PV) and *Malassezia* folliculitis. Moreover the yeasts of the genus *Malassezia* have been associated with seborrheic dermatitis and dandruff, atopic dermatitis, psoriasis, and, less commonly, with confluent and reticulated papillomatosis, onychomycosis, and transient acantholytic dermatosis. The study of the clinical role of *Malassezia* species has been surrounded by controversy due to the relative difficulty in isolation, cultivation, and identification. This review focuses on the clinical, mycologic, and immunologic aspects of the various skin diseases associated with *Malassezia*. Moreover, since there exists little information about the epidemiology and ecology of *Malassezia* species in the Italian population and the clinical significance of these species is not fully distinguished, we will report data about a study we carried out. The aim of our study was the isolation and the identification of *Malassezia* species in PV-affected skin and non-affected skin in patients with PV and in clinically healthy individuals without any *Malassezia* associated skin disease.

Key words: Malassezia - Tinea versicolor - Dermatitis, seborrheic - Dermatitis, atopic - Psoriasis.

The genus *Malassezia* belongs to the phylum Basidiomycota, class Hymenomycetes, order Tremellales, and family Filobasidiaceae.[1] All *Malassezia* species have distinct morphologic characteristics that allow them to be differentiated from other yeasts. In 1996, Guého *et al.* revised the *Malassezia* genus using morphology, ultrastructure, physiology, and molecular biology, and classified the genus into 7 species: *M globosa*; *M restricta*; *M obtusa*; *M slooffiae*; *M sympodialis*; *M furfur*; and *M pachydermatis.*[2] With the development of physiologic and molecular techniques, the genus *Malassezia* was expanded and currently includes 14 species, namely *M. pachydermatitis*, *M. furfur*, *M. sympoidalis*, *M. sloffiae*, *M. globosa*, *M. obtusa, M. restricta, M. dermatis, M. japonica, M. nana, M. yamatoensis, M. equina, M. caprae, and M. cuniculi.*[3] All species are lipid dependent (obligatory lipophilic), except for one non-obligatory lipophilic form, *M. pachydermatitis*, which is primarily zoophilic although it has occasionally been isolated from human skin and has also been implicated in nosocomial systemic infections.[4, 5]

Yeasts of the genus *Malassezia* are part of the normal human skin flora, and the density of colonization is related to the subject's age and activity of the sebaceous glands in different areas of the body.[6-10] *M. restricta* seems to be predominant on the scalp skin, whereas *M. sympodialis* predominates on the trunk and *M. globosa* seem to be evenly distributed on both sites. Other species, such as *M. sloffiae* and *M. furfur*, seem to be much less common in healthy human skin.[10-12] However, due to predisposing factors these yeasts become pathogenic and are associ-

Corresponding author: E. M. Difonzo, Dermatologic Clinic, viale Michelangelo 41, 50125 Florence, Italy.
E-mail: elisa.difonzo@asf.toscana.it

ated with pityriasis versicolor (PV) and *Malassezia* folliculitis.

A large part of pathogenic potential of *Malassezia* is determined by activation of different enzymatic systems. Efficiency of nutrients utilization present on the skin surface and in the sebaceous gland determines simultaneously the density of the *Malassezia* population as well as the quality and quantity of metabolic byproducts. Metabolic byproducts range from irritant or toxicity-free fatty acids to highly bioactive indole derivatives binding to specific cellular receptors regulating the expression of downstream metabolic pathways. The recently reported genome and secretory proteome of *M. globosa* and in part of *M. restricta* provide a molecular basis to understand the adaptions of *Malassezia* yeasts to their environment and to identify pathogenic factors.[13]

The yeasts of the genus *Malassezia* have been associated with a number of other skin diseases, such as seborrheic dermatitis and dandruff, atopic dermatitis, psoriasis, and, less commonly, with confluent and reticulated papillomatosis, onychomycosis, and transient acantholytic dermatosis.[14, 15] The study of the clinical role of *Malassezia* species has been surrounded by controversy due to the relative difficulty in isolation, cultivation, and identification. Moreover, colonizing the seborrheic areas of the skin, a commensal status of *Malassezia* yeasts cannot be clearly distinguished from the pathogenic stage, with the exception of PV where a transition to the pathogen hyphal form can be found. There is also the clinical question about a relationship between particular *Malassezia* species and the aforementioned disorders, as different authors have debated whether *Malassezia* yeasts are of primary pathogenic significance or a secondary phenomenon.

This review will discuss the skin diseases associated with *Malassezia* yeasts and the evidence for individual specie.

Moreover, since there exists little information about the epidemiology and ecology of *Malassezia* species in the Italian population and the clinical significance of these species is not fully distinguished, we will report data about a study we carried out. The aim of our study was the isolation and the identification of *Malassezia* species in PV-affected skin and non-affected skin in patients with PV and, as control, in clinically healthy individuals without any *Malassezia* associated skin disease.

PV

PV is caused by overgrowth and superficial invasion of human skin by *Malassezia*. While most studies suggest that *M. globosa* is a causative agent of this mycosis, several studies showed that *M. sympodialis* and M. *furfu*r were the predominant species.[16-24] According to Lyakhovitsky A *et al.*, the higher pathogenicity of *M. globosa* may be a result from its high lipophilic activity.[20] The different distribution of *Malassezia* species among several studies may be attributed to the sampling technique and the used culture media. The growth rate and medium requirements substantially vary between *Malassezia* species, which would bias analysis. Colonies of *M. globosa* are the smallest and grow more slowly than other *Malassezia* species. They also seem to be more fragile, not growing at higher temperatures, and in contrast to *M. sympodialis*, they do not survive in Dixon broth for long periods. The difference of findings in distribution of *Malassezia* species is also related to ethnic, climatic, and geographic factors.

The exact pathogenesis of PV has not yet been elucidated. *Malassezia* yeasts is regularly present in its yeast phase on the skin of healthy adults and produces the clinical lesions of PV when developing its hyphal phase. The conditions that induce such transformation remain unclear, although the available data at present indicate that it could be caused by changes in local conditions (*e.g.* heat, humidity, sweat, seborrhea, sebum composition) on an individual/genetic predisposition. The possibility of the existence of particular, more virulent strains of *Malassezia* should also be considered. *Malassezia* yeasts are able to produce *in vitro* reactive oxygen species (ROS) which are supposed to play a role in the pathogenesis of the pigmented variant of PV.[25] The hypochromic variant may be explained by toxic effects of lipid peroxides on melanocytes.

M. furfur produces a great variety of pigmented indole derivatives when cultured on a minimal agar medium with tryptophan as sole nitrogen source. Tryptophan aminotransferase (Tam1) was identified as the major enzyme responsible in this biochemical pathway. The role of this enzyme is also underlined by the therapeutic effects of Tam 1 inhibitors such as cycloserine, which may therefore represent a new therapeutic approach to PV. The chemistry and pharmacology of indole derivatives were found to be especially interesting since they might explain differ-

ent symptoms of PV.[13, 26-30] First these pigments (red substances such as pityriarubins and the yellow substance pityriacitrin) explain the broad spectrum of hyperpigmentation in PV and also the fluorescence which is characteristic for the disease but until now not really understood. The multicolor fluorescence of PV lesions described in the literature (yellow-orange–green-yellow) depends if the indole pigment is dissolved in sweat or epidermal lipids. Moreover pityriacitrin, which absorbs UV radiation, may be accountable for reduced UV sensitivity of the depigmented areas due to its mesomeric structure,[27] while lack of inflammation may be caused by the suppressive impact of the pityriarubins on the oxidative burst of human granulocytes.[28, 29] Interestingly and in contrast to other depigmenting diseases such as vitiligo, hypochromic lesions in PV are less sensitive to UV light. Depigmentations is also caused by others indole derivatives, such as indole A, an inhibitor of tyrosinase, and malassezin, that induces apoptosis in human melanocytes.[26] However some authors have invoked in the pathogenesis of depigmentation the role of fungal metabolities with toxic influence on melanocytes, such as dicarboxylic acids, expecially azelaic acid, a competitively tyrosinase inhibitor. [31] Beyond the depigmenting action, malassezin shows the feature of an aryl hydrocarbon receptor (AhR) agonist and induces cytochrome P450 in cultures of rat hepatocytes. *Malassezia* yeasts synthesize other potent ligands of AhR such as indirubin and indolo[3,2-b]carbazole, which inhibit the phenotypic and functional Toll-like receptor-induced maturation of monocyte-derived dendritic cells thus potentially affecting immune surveillance in *Malassezia* associated dermatoses. Moreover, the humoral and cellular immune responses to *Malassezia* yeasts are strongly diminished in patients with PV. The ligands of AhR might act in vivo as immunomodulators in the course of PV, expressing a highly adaptive strategy of *M. furfur* to encounter with the host's defense mechanisms and contributing to the comparably low incidence of inflammatory signs in affected skin lesion.

Etiology of PV: our study

There exists only a little information about the epidemiology and ecology of *Malassezia* species in the Italian healthy subjects and patients with PV. Therefore, the aim of our study was to contribute to the knowledge of the etiology of PV, by studying clinically healthy individuals without any *Malassezia* associated skin disease and patients with PV, in which the clinically non-affected skin of a seborrheic area of the trunk of the same patients was investigated as control.

Materials

Patients with pityriasis versicolor

The samples were collected from 40 Caucasian subjects from Florence and the surrounding area with PV (21 females and 19 males, age range 16-60 years), who visited our outpatient clinic. Patients with a medical history of autoimmune diseases, diabetes, and immunosuppressive treatment, were excluded. Additional exclusion criteria included any treatment against PV for at least one month before the study. In all cases the clinical diagnosis was confirmed by direct microscopy with KOH 20% of the scales that revealed rounded yeast cells and short, squat hyphae (so-called spaghetti and meatballs) (Figure 1).

Table I shows the main clinical-epidemiologic data of these patients. As we can see, the mycosis was recurrent in 27 patients, and one had 6 relapses in a year. One of the main predisposing factors war hyperhidrosis, and the mycosis was familiar in 6 cases. Clinically, in 18 patients the lesions were typical pink, salmon colored or brownish, whereas in the others the lesions were slightly hypopigmented (Figure 2).

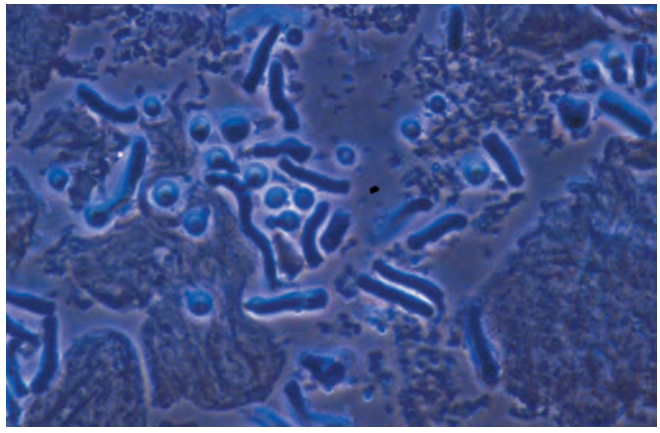

Figure 1.—Direct microscopy (blue cotton stain) of scales of PV: round yeast cells and short hyphae.

TABLE I.—*Principal epidemiological data of 40 patients with PV.*

	N. cases
1st episode	13
Relapse	27
Predisposing factors	
Hyperhydrosis	26
Seborrhea	6
Oily skin	2
PV in family members	6
Skin area involved	
Neck	8
Back	13
Pectoral region	14
Abdomen	2
Diffuse	3
Clinical variety	
Hyperchromic	18
Hypochromic	17
Hypo-hyperchromic	5

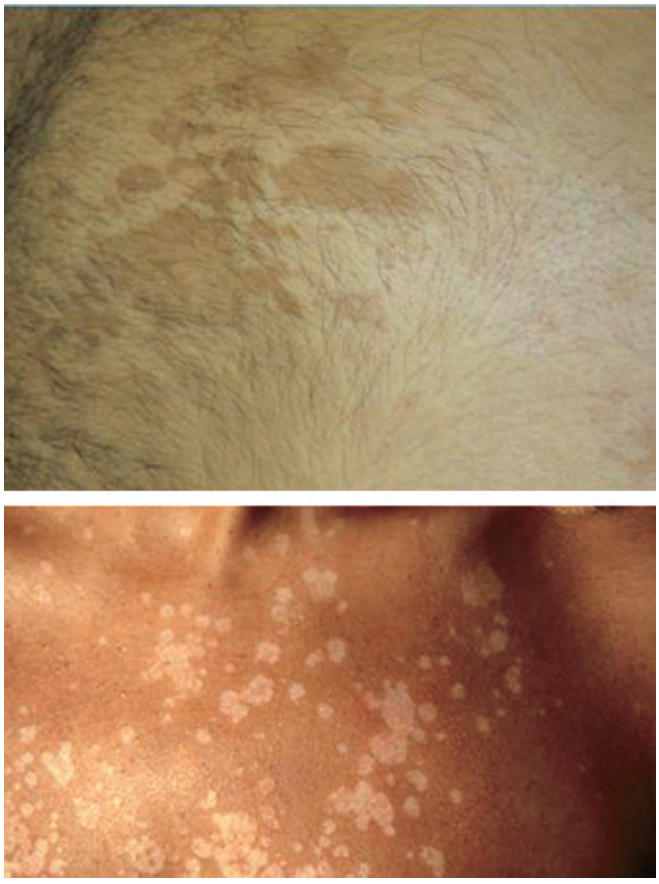

Figure 2.—Hyperchromic and hypochromic lesions of PV.

CLINICALLY HEALTHY INDIVIDUALS

We selected 40 caucasian subjects (12 males and 24 females; age range 16-60 years) from Florence and the surrounding area with negative history for PV and other skin disease *Malassezia* associated.

Methods

COLLECTION OF SCALE SAMPLES

In patients with PV scales were scraped with a sterile blade from lesions and from non-affected skin of the trunk. In control subjects the scales were scraped from the interscapular region. In both groups we scraped a similarly sized areas (approximately 1.5x1.5 cm).

CULTURE TESTS AND IDENTIFICATION OF *MALASSEZIA* SPECIES

The skin samples were immediately incubated on modified Dixon agar for 15 days at 32 °C. [32] Species were identified according to their morphological features and the following physiological tests.

Catalase reaction.—The presence of catalase was determined by using a drop of hydrogen peroxide (30% solution) and the production of gas bubbles was considered as a positive reaction. Lack of catalase activity is a characteristic feature of *Malassezia restricta*.[32]

Tween assimilation test.—According to the method reported by Guillot *et al.*[32, 33] ability to utilize different Tween compounds as a unique lipid supplement by *Malassezia* species was evaluated. A yeast suspension (at least 107 cfu/mL) was made in 2 mL sterilized distilled water and poured into plate containing Sabouraud dextrose agar at 45 °C. The inoculums were then spread evenly. After solidification of each plate, four wells were made and filled with 30 µl of a Tween compound, in Tween 20, 40 and 80, respectively. These plates were incubated for a week at 32°C and the growth was assessed around the individual wells after 2, 4 and 7 days (Figure 3).

Splitting of esculin.—The β-glucosidase activity of different Malassezia species was assayed.[33] A loop of fresh yeast was inoculated deeply in the esculin agar tube and incubated for 5 days at 32 °C.

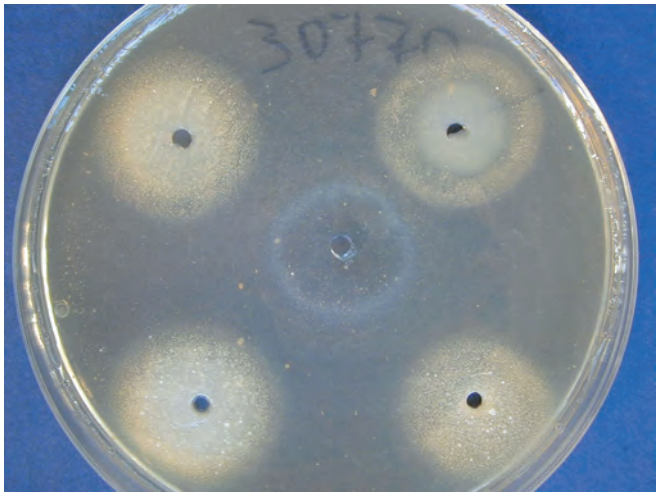

Figure 3.—*M. sympodialis*: tween assimilation test.

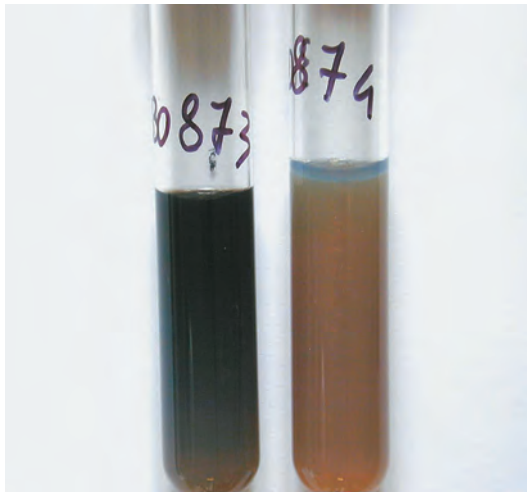

Figure 4.—Splitting of esculin: the test is positive for *M.sympodialis* (left tube; darkening of the medium) and negative for *M. globosa* (right tube).

The splitting of esculin is revealed by darkening of the medium. This test was used to distinguish *M. furfur*, *M. slooffiae* and *M. sympodialis* from other species (Figure 4).

Triptophane test.—A particular metabolic feature of *Malassezia* yeasts is the synthesis of pigments and fluorochromes when tryptophan is the main nitrogen source. Suspensions of yeast were smeared on agar medium.[34] After 14 days (*M. furfur*) and 28 days (*M. pachydermatis*) of incubation, the content of Petri dishes was pureed and extracted with ethyl acetate for 36 h. The extract was filtered over glass wool, evaporated to dryness and dissolved in methanol. The crude extract was separated under UV light (254 nm) by chromatography on Sephadex LH-20 with methanol as eluent. Thin layer chromatography was performed with each fraction on silica gel 60 plates and compared with standards.

Results

Patients with pityriasis versicolor

By culture, *Malassezia* was isolated from PV lesions in 33/40 patients (82.5%). Specifically, as shown in Table II *M. globosa* was isolated in 78.8% of cases: in 13 patients alone and in the other cases in association with *M.sympodialis*. *M.sympodialis* was isolated in other 7 patients. In over half of patients the cultures presented more than 10 colonies (Table III).

The cultures from non-affected skin revealed growth of *Malassezia* in 15/40 patients (37.5%): *M. globosa* was isolated in 66.7% of cases (in 5 patients alone and in 4 in association with *M. sympodialis)*. *M. sympodialis* was isolated in other 5 patients: in 4 cases alone and in the last patient in association with *M. restricta* (Table II). In over half of patients the cultures presented fewer than 10 colonies (Table III).

Table II.—Malassezia *species isolated in patients with PV.*

	Lesional skin N. patients (%)	Non-affected skin N. patients (%)
M. globosa (alone)	13 (39.4)	5 (33.3)
M. globosa +*M.sympodialis*	13 (39.4)	4 (26.7)
M. globosa (total)	26 (78.8)	9 (60)
M. sympodialis (alone)	7 (21.2)	5 (33.3)
M. sympodialis + *M.globosa*	13(39.4)	4 (26.7)
M. sympodialis + *M.restricta*	0	1
M. sympodialis (Total)	20 (60.6)	10 (66.7)

Table III.—*Number of colonies isolated in patients with PV.*

	Lesional skin N. patients (%)	Non-affected skin N. patients (%)
M. globosa		
<10 colonies	8/26 (30.8)	5/9 (55.5)
>10 colonies	18/26 (69.2)	4/9 (44.4)
M. sympodialis		
<10 colonies	9/20 (45)	8/10 (80)
>10 colonies	11/20 (55)	2/10 (20)

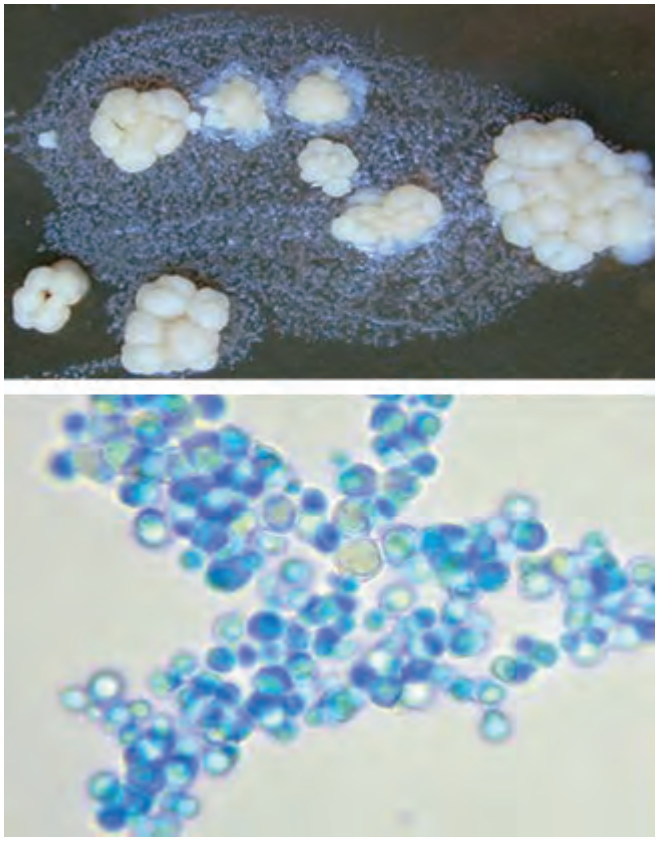

Figure 5.—*M. globosa* culture:"fried-egg" colony morphology and round cells with narrow budding base (Parker's blue ink stain).

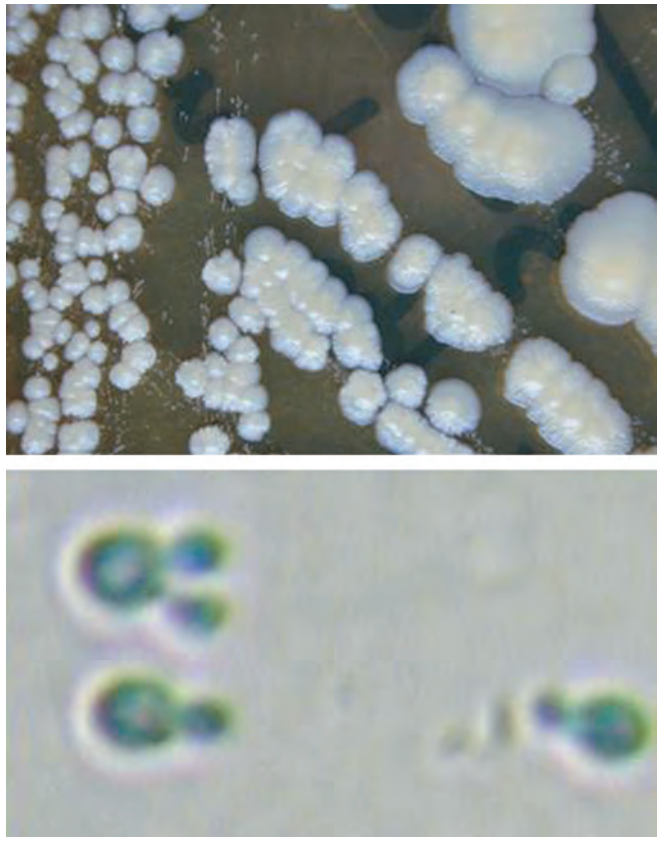

Figure 6.—*M.sympodialis* culture: oval cells with sympodial budding (Parker's blue ink stain).

M. globosa can be identified in most cases by its typical "fried-egg" colony morphology and by the presence of round cells with narrow budding base (Figure 5). The identification was confirmed by the physiological tests (positivity of catalase reaction and negativity of the other tests).

The identification of *M.sympodialis* was suggested by the presence of oval cells with sympodial budding and confirmed by the positivity of catalase reaction, tween assimilation test, splitting of esculin and by the negativity of triptophane test (Figure 6).

Clinically healthy individuals (controls)

The culture test was positive in 21 of the 35 subjects (60%). *M. globosa* was isolated in 61.9% of cases (in 7 patients alone and in 6 in association with *M. sympodialis)*. *M. sympodialis* was isolated in other 8 patients (Table IV). Approximately in 80% of

TABLE IV.—Malassezia *species isolated in control subjects.*

	N. patients (%)
M. globosa (alone)	7 (33.3)
M. globosa +*M.sympodialis*	6 (28.6)
M. globosa (total)	13 (61.9)
M. sympodialis (alone)	8 (38.1)
M. sympodialis + *M.globosa*	6 (28.6)
M. sympodialis (Total)	14 (66.7)

TABLE V.—*Number of colonies isolated in control subjects.*

	No. patients (%)
M. globosa	
<10 colonies	11/13 (84.6)
>10 colonies	2/13 (15.4)
M. sympodialis	
<10 colonies	11/14 (78.6)
>10 colonies	3/14 (21.4)

cases the culture presented less than 10 colonies of either species (Table V).

Conclusions of our study

The culture tests showed growth of *Malassezia* colonies in 82.5% of the patients. The negative responses in the remaining cases can be explained by the presence in the scales of cells with low vitality which adapt poorly to the artificial culture medium. Therefore, we believe that microscope examination is the most sensitive (as well as the simplest and most economical) method for confirming the clinical diagnosis of PV in daily clinical practice.

In this study we isolated *M. globosa* in PV lesions in 78.8% of cases (in 39.4% alone and in 39.4% associated with *M. sympodialis*). We isolated *M.sympodialis* in 60.6% of the cases and specifically, alone in 21.2% and associated with *M. globosa* in 39.4%. Our findings confirm the data reported by other authors in regions with temperate climates such as Spain, Greece and Iran.[17-20]

M. globosa and *M. sympodialis* were also isolated from non-affected skin in 15 of the 40 PV patients, albeit with a lower number of colonies per culture plate.

In the healthy control group the culture test was positive in 60% of subjects, and allowed us to identify *M. globosa* alone or associated with *M. sympodialis* in 61.9% of the cases and *M. sympodialis* alone or associated with *M. globosa* in 66.7%. Our study shows that *M. globosa* and *M. sympodialis* are the two predominant species. Aside from the isolation of *M. restricta* in just one patient, we did not find any other species such as *M. sloffiae*, wich Crespo-Erchiga *et al.* found in Spain, although in a limited number of patients.[18]

Concluding, on the basis of our experience, *M. globosa* and *M. sympodialis* (often in association) are the only two species found on the skin of patients with PV and in healthy subjects. The factors that lead to the transformation into the pathogen hyphal phase and hence clinical lesions remain unclear, although our data also indicate that it could be caused by changes in local conditions (*e.g.* heat, humidity, hyperhidrosis, seborrhea, sebum composition) on an individual/genetic predisposition.

Malassezia folliculitis

Malassezia folliculitis (MF) is an acne-like eruption characteristically located on the "sebaceous" areas of the trunk (shoulders, back, chest). Clinical lesions are small erythematous follicular papules and/or pustules accompanied by intense pruritus. The lesions heal with an easily removable crust. Histological findings demonstrate invasion of the central and deep follicle with large number of yeasts and inflammatory infiltrate consisting of lymphocytes and histiocytes, along with focal rupture of the follicular wall within the destroyed pilosebaceous units.[15, 35] Most frequently MF affects young oil skinned people with acne vulgaris or seborrheic dermatitis. Its development is favored by hot and humid climates, use of antibiotics, above all tetracyclines, and immunosuppression resulting from leukaemia, lymphomas, AIDS, organ transplantation.[36, 37] More recently it has been observed in patients receiving anti-tumor necrosis factor (TNF)-α medication (infliximab) for inflammatory bowel disease, erlotinib, for renal carcinoma, and cetuximab for parotid gland adenocarcinoma.[38-40] Due to the many similarities with other forms of folliculitis and the fact that *Malassezia* yeasts are not routinely cultured, this condition might be underdiagnosed in the daily practice. *M. pachydermatis*, *M. globosa* and *M. furfur* are the predominant causative agents.[37]

Seborrheic dermatitis

Seborrheic dermatitis (SD) is a chronic, widespread skin condition, which is considered a multifactorial disease influenced, in part, by *Malassezia* spp. opportunistic activities, as well as various endogenous and exogenous factors. Pityriasis simplex capitis (so-called "dandruff") is a mild form of scalp SD. The isolation of *Malassezia* species from dandruff and SD lesions varies around the world and the study of the relationship among factors such as gender, age, immunosuppressive condition of the patient and SD development and can lead to a better understanding of this disease. Some authors have stated that *M. globosa* predominate, whilst others have found *M. restricta* or *M. sympodialis* to be the most common species in lesion of SD.[41-48] The pathogenesis and exact mechanisms via which these yeasts cause inflammation are still not fully elucidated. They are rather complex and subject of controversy in literature.[49-51] Most probably *Malassezia* spp. cause seborrheic dermatitis by involving and combining both non-immune and immune mechanisms (nonspecific and specific). Which of these mechanisms will dominate in any single case depends on the number and virulence of the yeasts as well as on the microorganism reactivity. A direct causal link between *Malassezia* yeast and SD has been proposed based on the distribution of *Malassezia* species on the skin of lipid-rich anatomic locations, such as the face, scalp, and trunk, on the presence of an increased number of *Malassezia* cells in affected SD skin compared with SD-free individuals, and on the therapeutic response seen to antifungal agents. Improvement in SD is accompanied by reduction in the yeast on the scalp, whereas recolonization leads to disease recurrence. SD is not associated with microscopic changes of the fungus morphology, and it remains unclear whether SD patients have or not higher *Malassezia* counts than normal controls, although a correlation between yeast density and severity of SD has been reported. It was proposed that lipases may be related to the development of SD and could be considered as virulence factors. Some authors suggest that these enzymes provide the ability to metabolize lipids and to integrate the fatty acids into the fungal cell wall, and thus are very important for growth.[55] The current hypothesis for SD/dandruff pathogenesis associates individual susceptibility with the penetration of irritating *Malassezia* metabolites, such as oleic acid, through a defective epidermal barrier.[56] *M. globosa* produces oleic acid through lipase activity, and the desquamation seen on dandruff skin can be caused by oleic acid, in dandruff-susceptible individuals. Malassezin and indole-3-carbaldehyde, both produced on the skin of patients with *Malassezia* associated SD, have been implicated in immunologic events such as the differentiation of Th17 cells and the production of inflammation and mediation of contact sensitivity in transgenic mice. It was found that compared with healthy controls bioactive indoles that are AhR agonists are selectively produced by *M. furfur* isolates from SD patients.[57] In SD, the lipophilic malassezin and the indolo[3,2-b]carbazole could cross the defective epidermis, reach the granular and spinous layers, and activate the AhR. Subsequent downregulation of the high-affinity epidermal growth factor receptor could trigger SD.

Atopic dermatitis

Despite intense research over the past few decades, the pathogenesis of atopic dermatitis (AD) is still not fully understood. A number of exogenous and endogenous trigger factors have been identified. Gene-environment interactions in genetically predisposed subjects also appear to play a central role. Furthermore, there is evidence for a dysregulation of the cellular and humoral immune response and for defects of skin barrier function in patients with AD. Microorganisms, in particular *Staphylococcus aureus* and *Malassezia* yeasts have been described as contributing to the disease pathogenesis.[58-67] *M. sympodialis* has been reported as the most frequent species in patients with AD, both *M. restricta* and *M. globosa* are thought to play significant roles. Approximately 50% of adults with AD show immediate-type skin reactions and/or have specific serum IgE antibodies against *M. sympodialis*. Sensitization to this yeast occurs almost exclusively in patients with AD. A significant correlation has been reported between the level of these IgE antibodies and the clinical severity in head and neck AD. Recently, autoreactivity to human proteins has been postulated as a pathogenic mechanism for AD. Specifically Balaji *et al.*[68] documented cross-reactivity at the T-cell level between *M. sympodialis* thioredoxin (Mala s 13) and its human homologue (h Trx). These data

indicate that autoreactive T cells are characterized by a cytokine secretion profile encompassing not only the Th1 and Th2 phenotypes but also the Th17 and Th22 phenotypes, which might have relevance in the pathogenesis of AD.

Psoriasis

Reports of efficacy of antifungal drugs in the treatment of scalp psoriasis have suggested a pathogenic role of *Malassezia* species.[69, 70] Currently only a secondary role, at most of an exacerbating factor, can be supported for *Malassezia*.[71-73] *M. restricta* is predominant in patients with psoriasis, in contrast to those with PV.[72] It was suggested that this predominance is caused by the different lipid classes in psoriasis, specifically by a significant increase in ceramides in diseased skin and in cholesterol and squalene. The possible mechanisms by which these organisms may trigger psoriatic lesions have, however, yet to be elucidated. Studies have demonstrated that *Malassezia* can induce the overproduction of molecules involved in cell migration and hyperproliferation, thereby favoring the exacerbation of psoriasis.

Riassunto

Dermatosi umane associate alla malassezia

I lieviti del genere *Malassezia* fanno parte della comune flora saprofita e in particolari condizioni possono causare infezioni superficiali quali la pitiriasi versicolore e una particolare forma di follicolite. Un loro ruolo, inoltre, viene discusso in alcune affezioni quali la dermatite seborroica, la pitiriasi simplex del cuoio capelluto, la dermatite atopica e la psoriasi. Più raramente questi lieviti sono stati associati alla papillomatosi confluente e reticolare, ad onicomicosi e alla dermatosi acantolitica transiente. Lo studio del ruolo della *Malassezia* è comunque oggetto di controversie, anche in conseguenza delle relative difficoltà di crescita su terreni di coltura e di identificazione di specie. In questo lavoro puntualizzeremo gli aspetti clinici, epidemiologici e immunologici delle dermatosi associate alla *Malassezia*. Inoltre, dal momento che i dati sulla epidemiologia e sul significato clinico di questi miceti nella popolazione italiana sono estremamente carenti, riporteremo i risultati di uno studio da noi condotto nella pitiriasi versicolore. Lo scopo di questo studio era l'isolamento e l'identificazione delle specie di *Malassezia* nei pazienti con pitiriasi versicolore su prelievi eseguiti a livello di lesione e a livello di una zona seborroica non lesionale. Per controllo abbiamo scelto prelievi eseguiti a livello del tronco da soggetti clinicamente sani, senza anamnesi positiva per affezioni correlate alla *Malassezia*.

PAROLE CHIAVE: Malassezia - Tinea versicolor - Dermatite seborroica - Dermatite atopica - Psoriasi.

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Subcutaneous mycoses

Part 1: subcutaneous mycoses due to non-dermatophytes

C. ROMANO

Subcutaenous mycoses are increasingly reported in the literature for various reasons. Firstly, life expectancy has increased and even patients with cancer and/or immunodepression live longer, making them susceptible to these infections. Secondly, diagnostic techniques for mycoses have improved. Dermatologists have now begun to suspect subcutaneous mycoses when faced with certain clinical pictures and are aware of the need for histopathological examination and culture of lesion biopsy material on appropriate culture media. This review considers the clinical, histopathological and mycological aspects of the most common subcutaneous mycoses and outlines how to treat them. A better understanding of these mycoses enables early diagnosis and treatment of infections that are sometimes life-threatening.

KEY WORDS: Mycoses - Diagnosis - Mycoses, therapy.

Subcutaneous mycoses are fungal infections that primarily affect the dermis and subcutaneous tissue and are constantly increasing. Some, such as chromoblastomycosis, sporotrichosis and eumycetoma, are mainly described in immunocompetent patients, particularly in geographical areas like the tropics and subtropics. They are often associated with traumatic inoculation of the mycete and are therefore more frequent on exposed areas of skin, such as the limbs and face. They prefer subjects who work in the open air and come into contact with spores in the soil or air. The infection is usually confined to the point of onset, the skin, and rarely disseminates. It is often asymptomatic and chronic, complicating diagnosis.[1]

Corresponding author: C. Romano, Micology Unit, Dermatology Section, Department of Clinical Medicine and Immunological Sciences, University of Siena, viale Bracci 1, Siena, Italy.
E-mail: romanoclara@unisi.it

Micology Unit, Dermatology Section
Department of Clinical Medicine
and Immunological Sciences
University of Siena, Siena, Italy

Other subcutaneous mycoses, such as zygomycosis, manifest at all latitudes, especially in immunodepressed patients. They are potentially lethal due to angioinvasion and often develop rapidly. They have poor prognostic value because skin infection may be derived from infection of internal organs that spread to the skin.[2]

Subcutaneous mycoses are generally characterised by histological findings of granulomatous inflammation in the dermis and subcutaneous layer, where fungal structures, sometimes typical of the causative mycete, may be observed. Two types of subcutaneous mycoses, "mostly localised" and "mostly systemic", can be distinguished. The former may include chromoblastomycosis, sporotrichosis, eumycetoma, phaeohyphomycosis and rhinosporidiosis; the latter cryptococcosis, paracoccidioidomycosis, coccidioidosis, blastomycosis and American and African histoplasmosis.

Frequent subcutaneous mycoses include sporotrichosis, eumycetoma, chromoblastomycosis and phaeohyphomycosis. The latter term, introduced in 1974 by Ajello, indicates fungal infections caused by fungi with dark hyphae, which in the tissues present as melanised filaments.[3] Phaeoid (pigmented) fungi may cause three histologically different types of subcutaneous infections: eumycetoma, chromoblasto-

mycosis and phaeohyphomycosis, which are distinguished on the basis of specific structures found in the tissues. Indeed, histological examination reveals grains in eumycetoma, muriform cells in chromoblastomycosis, and yeasts, pseudohyphae and dark septate hyphae in phaeohyphomycosis.[4] It is not clear why certain mycetes give rise to mycetoma and others to phaeohyphomycosis, nor why the same mycete, such as *Curvularia* or *Exophiala,* may cause histologically and clinically different pathologies, namely eumycetoma or subcutaneous phaeohyphomycosis.[5]

The diagnosis of subcutaneous mycosis must be based on histopathological examination of biopsy specimens taken from the lesions and mycological examination by direct microscopy and culture of pathological material, which often consists of biopsy specimens from lesions, on appropriate culture media (Sabouraud dextrose agar with CAF or gentamycin and without cycloheximide). Molecular biology techniques, which are rapid and reliable but expensive, are not available in all dermatology laboratories due to their high cost and the small number of cases seen at a given clinic.

The aim of this brief review was to summarise the salient features of the most frequent subcutaneous mycoses, so that dermatologists, faced with polymorphic or aspecific clinical findings, can confirm clinical suspicions with appropriate laboratory tests and provide early diagnosis and treatment of these diseases.

Chromoblastomycosis is a subcutaneous mycosis caused by phaeoid fungi. The most frequent, present in soil as saprophytes of plants and decaying wood, with different geographical distributions, are: *Fonsecae pedrosoi* and *Fonsecae compacta, Phialophora verrucosa, Cladophialophora carrionii, Rhinocladiella aquaspersa*, followed by *Exophiala jeanselmei* and *Aureobasidium pullulans*. This mycosis is described worldwide but is more frequent in the tropics and subtropics. Madagascar reports the highest number of cases, followed by Mexico, Brazil, Venezuela, India, Australia and Japan. In China and Latin America, infections due to *Fonsecae* are prevalent. The cases diagnosed in Europe are often imported. The mycetes may penetrate an abrasion on splinters of wood. The lower extremities are the most affected skin areas. Patients are often male agricultural workers between 30 and 60 years of age, who work outdoors without the protection of footwear or clothing.[1] The disease manifests as asymptomatic papular or nodular lesions that evolve slowly, over 5-10 years or more, into centrifugally expanding plaques with a verrucous surface. Lesions may often be crusted or scaly. Nodular, vegetating, lymphangitic and mixed forms have been described. Ulceration and purulent evolution of verrucous lesions are not uncommon, and may indicate pyogenic superinfection. Scarring and keloid formation is also common. Muscles and bones in the affected region are not usually involved. Diagnosis is based on mycological and histological examination of biopsy material. Direct microscope examination of scales or crusts after addition of potassium hydroxide (KOH) shows muriform cells, also known as sclerotic bodies, fumagoid cells or Medlar bodies, having the appearance of double-walled structures, 4-10 μm in diameter, resembling copper pennies. Pathological material sown on Sabouraud agar with or without antibiotic produces velvety or woolly colonies in 20-30 days. Initially dark green or olive-grey, they subsequently become brown or black. The microscopic appearance of the colonies varies according to the mycete isolated. Histopathological examination of biopsy fragments often shows hyperkeratosis and pseudoepitheliomatous hyperplasia in the epidermis. The dermis shows suppurative or tuberculoid granulomatous inflammation with neutrophils, eosinophils, lymphocytes, plasma cells, macrophages and giant multinucleated cells. The dermis contains typical sclerotic bodies, 5-10 μm in diameter, isolated or massed, in the form of brownish morulae, within and outside the giant multinucleated cells or macrophages. Differential diagnosis should consider squamous cell carcinoma, verrucous tuberculosis, Leishmaniasis, and of course other subcutaneous mycoses.[4]

Therapy includes surgery, such as curettage, electrodesiccation, cryosurgery and systemic antimycotics (itraconazole, terbinafine, 5-fluocytosine, i.v. amphotericin B). Response to therapy varies in relation to the extent and duration of lesions. Small new lesions are successfully treated by deep excision or cryosurgery associated with itraconazole (300-400 mg/day for 6-8 months) or terbinafine (250 mg/day for 3 months before surgery and for 6-9 months after). Extended chronic forms often relapse even after surgery associated with systemic antimycotics. In such cases the more effective antimycotics seem to be terbinafine at doses of 250-500 mg/day for 12 months or 5-fluocytosine at doses of 100-150 mg/day until the cultures become negative. Intravenous amphotericin B has also been given at initial doses of 0.1-0.25 mg/kg/day, which may be varied in the eventuality of

nephrotoxicity. Combinations of systemic treatments have also been used, for example 5-fluocytosine (70-100 mg/kg/day orally) and amphotericin B (50 mg i.v. on alternate days) for long periods. The efficacy of posaconazole is under evaluation.[4]

Sporotrichosis is a chronic infection caused by species of the *Sporothrix schenckii* complex. The following have been identified by molecular techniques and have different geographical distributions: *S. schenckii* sensu strictu, *S. lurei, S. globosa*, *S. mexicana*, *S. brasiliensis* and *S. albicans.*[1] *Sporothrix schenckii* is a dimorphic fungus that grows in soil and plants. Various animals, such as cats, dogs, horses and rats, may be vectors of the disease, which manifests mainly in countries with temperate and tropical climates. Farmers, gardeners, veterinarians and forest workers are the occupations most affected. The mycete usually penetrates the skin by traumatic inoculation on splinters of wood, insect stings, animal bites or tattoos. Two types of skin infection may manifest after days or months: a more frequent lymphagitic form and a fixed form. Lymphangitic sporotrichosis manifests with an erythematous nodule that becomes necrotic and ulcerated, producing a pus-like secretion. Further erythematous nodules that develop into ulcers then appear along the path of a lymphatic vessel of the limbs, face or other area exposed to trauma. Fixed sporotrichosis presents as a nodular or verrucous plaque, often ulcerated, without lymphatic dissemination. The nodular lesions are multiple in disseminated cutaneous forms and are usually observed in immunodepressed patients. Extracutaneous forms are possible, and may affect bones and joints, eyes or lungs; they may be disseminated or systemic, the latter spread in the bloodstream and often affect subjects with immunodeficiency. The diagnosis of cutaneous sporotrichosis is based on mycological and histological examination. Direct microscope examination is not always conclusive, as it is rarely positive. Culture of purulent or biopsy material on Sabouraud medium produces creamy grey colonies after 1-3 weeks. The colonies have a cerebriform surface composed of thin microscopic mycelial filaments with pear-shaped spores. The sympodial conidia resemble daisies.[6] Histological examination of biopsy material shows sporotrichoid granuloma consisting of a central microabscess with neutrophils and necrosis, and a peripheral area with prevalence of lymphocytes and macrophages surrounded by plasma cells and fibroblasts. Typical fungal structures in the form of oval or cigar-shaped yeasts or asteroid bodies with an eosinophilic halo, 20 μm in diameter, can sometimes be observed in the inflamed area. The latter are not pathognomonic of sporotrichosis and are thought to be a resistance mechanism of the yeast. Molecular biology techniques enable rapid diagnosis.[7] Differential diagnosis should consider tuberculosis, Leishmaniasis, atypical mycobacterial infections, nocardiosis and other deep mycoses, such as mycetoma, blastomycosis and histoplasmosis. Elective treatment is oral itraconazole at 100-200 mg/day until healing of the lymphocutaneous form and then for an additional 2-4 weeks (*i.e.*, for a total of 3-6 months). Pulsed therapy at 400 mg/day for one week/month for 3-4 months has been proposed.[1] In patients unresponsive to itraconazole, terbinafine has been used at a dose of 250-500 mg/day for 3-4 months. Potassium iodide is still used in some undeveloped countries at doses of 3-6 g/day for 3-4 months because of its low cost.[6] *In vitro* studies suggest that susceptibility to the various antimycotics may be species-dependent.[8]

Eumycetoma is a subcutaneous infection that mainly affects males who work out of doors, often in tropical and subtropical areas of India, Africa and South America. The fungus is present in soil and may penetrate the skin through wounds or abrasions with splinters or acacia thorns. The pathogens may be pigmented or hyaline moulds. The most frequent among the former are *Madurella mycetomatis*, *Madurella grisea*, *Leptosphaeria senegalensis*, *Exophiala jeanselmei* and *Curvularia lunata* that form dark grains in the tissues. Hyaline mycetes include *Acremonium kiliense*, *Acremonium falciforme*, *Acremonium recifei*, *Fusarium solani*, *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus nidulans* and *Pseudoallescheria boydii* (*Scedosporium apiospermum*), which form pale grains in the tissues.[9, 10] Mycetoma manifests with slow-growing papular-nodular lesions or plaques that develop into abscesses and form fistulas exuding serous-hematic material or pus and granules. The most frequent sites of infection are the feet, legs and hands, in that order. In long-standing chronic forms, the infection spreads via the lymphatic system, involving muscles and bones and giving rise to deformities. The grains have diagnostic value. They can be collected in fistulas and observed by light microscopy after adding 20% KOH. Their colour, form and dimensions differ according to the pathogen. The mycete can be identified in culture by sowing pathological material on Sabouraud medium with CAF

and actidione. In the absence of grains, it is useful to perform deep skin biopsy and sow biopsy fragments on culture medium for histological examination. This reveals granulomatous inflammation with a central area rich in neutrophils and grains. The fungal hyphae composing the grains are revealed by PAS or methenamine silver stain. Molecular diagnostic techniques are useful for diagnosis, especially when cultures are negative due to bacterial contamination.[11] The dot-in-circle sign in soft tissue of the foot, observed by MRI, is considered pathognomonic of eumycetoma of the foot.[12] Computed tomography is also used for diagnosis.[13] Differential diagnosis is mainly with respect to actinomycetomas caused by filamentous bacteria, the manifestations of which are usually more inflammatory, with faster evolution and more destructive of bone than those caused by mycetes.[14] Actinomycetes grow in culture on Loewenstein medium. Tumours such as rabdomyosarcoma and synovial sarcoma should also be excluded.[15] Treatment is radical surgical resection of the lesion associated with systemic antimycotic therapy. Drugs successfully used include ketoconazole (400-800 mg/day), itraconazole (400 mg/day) that has fewer side effects, posaconazole (600-800 mg/day) or voriconazole (200 mg twice a day), all administered for many months.[15] In particular, voriconazole healed a case of mycetoma of the foot with areas of osteolysis caused by *Scedosporium apiospermum,* unresponsive to a previous treatment with itraconazole (200 mg/day for 2 years).[16]

Phaeohyphomycosis is caused by phaeohyphomycetes, the most frequent of which are *Curvularia, Exophiala jeanselmei, Exophiala spinifera* and *Alternaria***.**

Curvularia is a ubiquitous dematiaceous fungus found in water and on plants. In the rare reported cases of subcutaneous infections, the patient, usually immunocompetent, recalled being wounded or suffering skin abrasion by a plant before appearance of a nodular lesion, which sometimes ulcerated and formed a crust.[17] On other occasions the lesion manifested as a papule and evolved into a purulent plaque.[18] Histological examination of a biopsy specimen stained with PAS or Grocott shows large septate hyphae and darkly pigmented spores in a context of granulomatous inflammation with epithelioid cells, foreign body giant cells and mononuclear cells in the dermis. Culture of biopsy material on Sabouraud dextrose agar with chloramphenicol produces grey or black cottony colonies composed of typical curved brown poroconidia arising from geniculate condiophora. Therapies include terbinafine (125 mg/day for a month) and in cases of relapse, surgical excision followed by itraconazole (100 mg/day for 4 months) [17] or oral fluconazole (100 mg/day) for 3 months.

Exophiala jeanselmei is a phaeohyphomycete that causes subcutaneous infections in immunodepressed (generally transplant) patients [19] and immuncompetent subjects.[5] Lesions are nodular, cystic and confluent into cyst-like abscesses enclosed in a fibrous capsule. They remain confined to the subcutaneous tissue, which differentiates them from mycetomas caused by the same mycete, which tend to invade muscle and bone. Direct microscope examination on pus shows dark septate hyphae. Culture on Sabouraud dextrose agar produces black or olive brown colonies that microscopy shows to consist of tubular conidiogenous cells with elongated tips and scars (annellides). Ellipsoid conidia arise from the annellides and are disposed in masses at the apices of same or at the sides of conidiophores. Biopsy shows granulomatous subcutaneous infiltrate composed of histiocytes, multinucleated giant cells and lymphocytes. The giant cells contain pigmented hyphae. Treatments include itraconazole (200 mg/day for 5-8 months) and surgical excision.[20]

Exophiala spinifera is a dematiaceous fungus that causes subcutaneous infections with typical erythematous papules or verrucous lesions, abscesses or subcutaenous cysts. In immunodeficient patients, lesions may be single or few, whereas in immunocompetent children they are multiple. The response to various therapies varies widely: surgical excision is successful in some cases,[21] whereas in others, drugs such as itraconazole, fluconazole and terbinafine, or cryotherapy with liquid nitrogen may have no effect.[22]

Alternaria is a phaeoid fungus. All species live in soil as saprophytes and parasites of different plants and may infect exposed skin areas, such as the limbs and face. The spores are transported by air and penetrate the skin through minor traumas. *Alternaria alternata, A. tenuissima, A. chartarum* and *A. infectoria* are some of the species responsible for subcutaneous infections. Lesions may be papular-nodular,[23] nodular [24] (Figure 1), erythematous infiltrating patches,[25] verrucoid lesions [26] or lesions resembling erysipelas.[27] Immunodepressed patients are susceptible, especially heart,[28] bone marrow [29] and renal transplant recipients.[30] Diagnosis is based on histological examination of tissue specimens showing epithelioid granulomata

with a central abscess in the dermis. The granulomata are composed of epithelioid cells, lymphocytes and giant cells. Gomori-Grocott and PAS stain after diastase show hyphae and roundish bodies. Fontana Masson stain may be used to detect pigmentation in the fungal wall. Culture of biopsy material on Sabouraud dextrose agar without cycloheximide produces greyish cottony colonies that subsequently darken (Figure 2). The microscopic appearance of colonies enables the various species to be distinguished on the basis of conidia size and morphology (Figure 3). Rapid identification can be obtained by molecular biology techniques.[31]

All phaeohyphomycetes form melanin-like pigment in culture and in host tissues. This feature facilitates diagnosis by aiding histological identification. It also aids identification in cultures of biopsy fragments on Sabouraud medium with antibiotics.

Rhinosporidiosis is a chronic granulomatous mycosis endemic to India and Sri Lanka, and reported sporadically in America, Europe, Asia and Africa. It is caused by *Rhinosporidium seeberi* that lives in stagnant water. The mycete causes polypoid lesions of the mucosae, especially the nose, rhinopharynx and conjunctiva. Transmission is thought to involve dust, infected clothing, fingers or bathing in stagnant water, when spores penetrate the mucous membranes where they form sporangia. Skin infections are rare and manifest with nodules, often in patients with disseminated forms of the disease.[32] Differential diagnosis should consider other granulomatous diseases of the nose, including infectious forms of mycotic origin, such as histoplasmosis, infection by tubercular mycobacterium and atypical mycobacteria, syphilis, Leishmaniasis, rhinoscleroma, leprosy, sarcoidosis and systemic vasculitis such as Wegener granulomatosis and Churg-Strauss syndrome.[33]

Diagnosis is based on the finding of typical sporangia by cytodiagnostic examination of smears obtained from lesions and observed after addition of KOH or Papanicolau or Giemsa staining, or by histological examination if the sporangia are found in biopsy specimens. Histological examination of biopsy fragments shows inflammatory infiltrate rich in lymphocytes, plasma cells and foreign body giant cells. The sporangia have the appearance of globular cysts with thick walls, variable in size, depending on the stage of maturation, from 10 to 200 μm. They contain endospores. The mycete does not grow in culture. Elective therapy is surgery, whereas response to treatment with dapsone or amphotericin B has often been disappointing.[34]

In first place among "mostly systemic" subcutaneous mycoses we have Cryptococcosis, a deep mycosis caused by *Cryptococcus neoformans*, a ubiquitous capsulate yeast present at all latitudes in soil, trees, dust and pigeon excreta. Two varieties and five serotypes are known: *Cryptococcus neoformans var. neoformans,* with serotypes A, D and AD, and *Cryptococcus neoformans var. gattii* with serotypes B and C. Infections caused by *Cryptococcus neoformans var. neoformans* are more frequent in subjects with immunodepression due to HIV, immunosuppressant treatment or neoplasias;[35] those caused by *Cryptococcus neoformans var. gattii* are more frequent in immunocompetent subjects. The most frequent infections affect the lungs and central nervous system, less often the skin, eyes, abdominal organs, bones and joints. Disseminated forms have also been described. Skin cryptococcoses are more often secondary, linked to spread in the bloodstream from an infection elsewhere in the body, and have negative prognostic significance. In the less frequent primary skin infections, there are no signs of impairment of other organs or systems and the mycete penetrates through the skin as a result of modest traumas.[36] Affecting the face, neck or limbs, the lesions may be single or multiple, papular, nodular (sometimes ulcerating), plaques, granulomas, acneiform, herpetiform, cellulitis-like or resembling molluscum contagiosum. Fingers and face are the most frequent sites in immunocompetent cases, trunk and lower extremities in immunodepressed patients. Diagnosis is based on mycological and histological examination. Mycological examination includes direct microscope observation after adding a few drops of Indian ink to lesion exudate. This highlights globose elements surrounded by a thick capsule, typical of *Cryptococcus*. The ink enhances the halo of the capsule. Milky, ivory-white yeast-like colonies develop on Sabouraud medium, blood agar or chocolate agar, subsequently turning dark and mucoid; they can be identified by microbiological techniques (fermentation of sugars, production of ureases). In recently contracted infections, histology of biopsy fragments shows little inflammatory reaction and a great number of globose elements, 5-20 μm in diameter, surrounded by a capsule hyaline to hematoxylin-eosin, evident with PAS, and that stains red with Meyer's mucicarmine. In long-standing forms, inflammation is aspecific, showing giant cells,

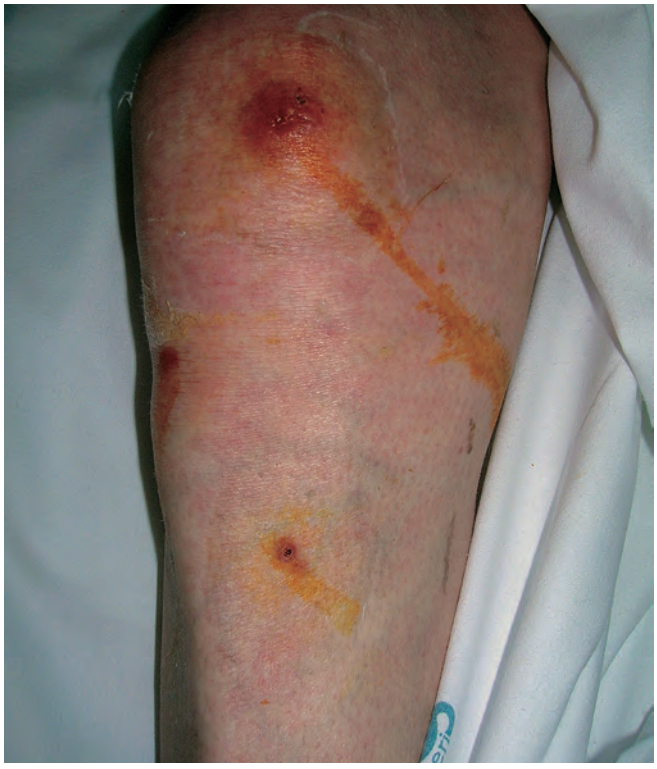

Figure 1.—Nodular lesion in immunodepressed patient.

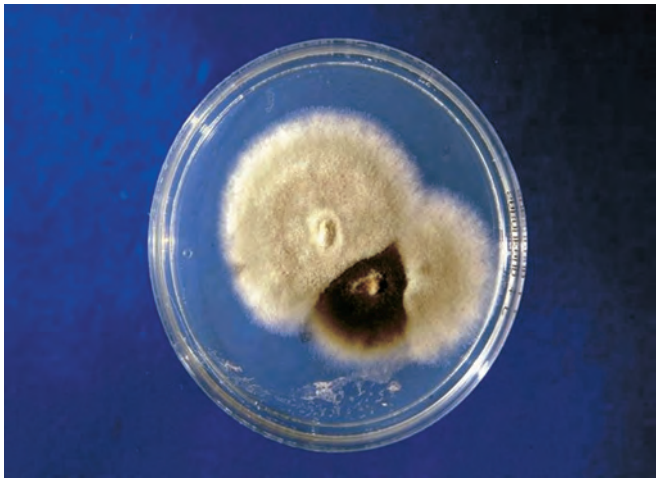

Figure 2.—Macroscopic appearance of *Alternaria* on Sabouraud-dextrose-agar.

lymphocytes and sometimes neutrophils but without suppurative character. In cases of skin infections, it is always important to exclude involvement of other organs and dissemination; this is done by radiological and serological examination. Clinical differential diagnosis should consider tubercular mycobacterium infections and other deep mycoses, especially phaeohyphomycoses. Treatments used include amphotericin B (9.3-1 mg/kg/day), itraconazole (400 mg/day for 6 weeks) [37] or fluconazole (450 mg/day for months).[36] Voriconazole and posaconazole have been proposed as alternatives.[38-40]

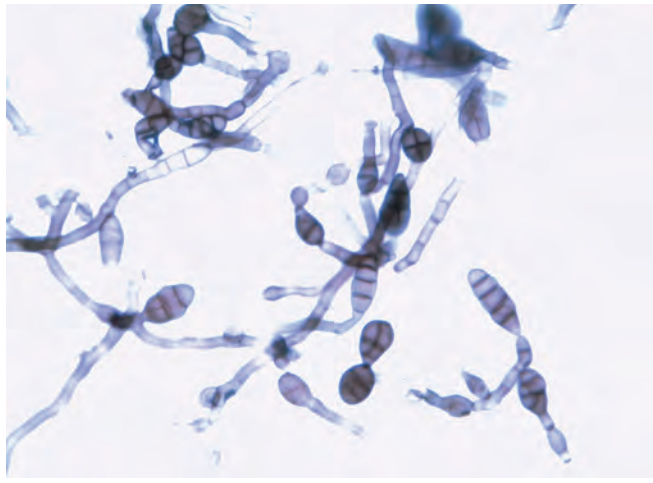

Figure 3.—Microscopic appearance of typical conidia of *Alternaria*.

Paracoccidioidomycosis is caused by *Paracoccidioides brasiliensis* and is endemic to Central and South America from Mexico to Uruguay, with higher incidence in Brazil, Venezuela and Colombia. Cases observed in other geographical areas are generally imported from endemic areas. The mycete is a dimorphic fungus present in soil and armadillos are a major host, infected by inhalation of spores.[41] Affected subjects are more often males, 30-60 years of age, of low socioeconomic level, who work in rural areas. The most common form is lung infection due to inhalation of spores and subsequent possible diffusion to other organs through the bloodstream. In 14% of cases with systemic forms, the infection involves the central nervous system.

Skin manifestations are rarely at the site of the primary postraumatic infection [42] but are more often secondary to infection in other organs disseminated in the bloodstream. The face, lips, nose and oral cavity may be affected. Lesions are papular, papular-pustular, papular-necrotic, ulcerative or sarcoid-like.[43] Forms manifesting with ulcerative or ulcerative and vegetating genital lesions are rare and generally affect

the glans and frenulum.[44] Diagnosis is based on direct microscopic mycological examination of material, obtained by scraping biopsy fragments, and culture. After treatment with KOH, the former method shows round cells with multiple budding. Culture of biopsy fragments for more than 20 days produces cream-coloured cerebriform colonies resembling popcorn. Histological examination shows pseudoepitheliomatous hyperplasia and granulomatous inflammation with typical spheroidal spheres, 1-30 μm in diameter, having a double boundary bearing Mickey-Mouse-like budding spores, 2-5 μm in size. Immunological diagnostics and molecular biology tests (PCR) may be used to assess the course of the disease and response to therapy, especially in extracutaneous forms. Clinical differential diagnosis should consider Leishmaniasis, tuberculoid leprosy, granulomatous syphilis and sarcoidosis. Therapy relies mainly on trimethoprim sulfamethoxazole and imidazolics, such as fluconazole and itraconazole, administered for many months.[44]

Coccidioidomycosis is an infection caused by *Coccidioides* species, dimorphic fungi found in soil and hosted by humans, dogs and horses. Climatic factors also play a non indifferent role (dust storms, droughts). The disease is endemic to dry regions of the SW United States of America and north Mexico. Its incidence is increasing in California and Arizona. The infection is generally acquired by inhalation of airborne conidia from soil. Subjects exposed to dust and soil, farmers and archaeologists are at risk of contracting the disease, which in travellers appears on return from stays in endemic areas.[45] In immunodepressed subjects (HIV-positive or with AIDS, transplant patients treated with immunosuppressants such as cyclosporin and systemic corticosteroids or TNF-alpha antagonist), *Coccidioides immitis* causes lung or disseminated infections involving the central nervous system, especially the meninges, bones, joints and skin. *Coccidioides posadasii* is considered a variety of the Californian strain *C. immitis*.[46]

Skin infections may be of three types: aspecific, primary cutaneous and secondary cutaneous. Aspecific reactive manifestations without histological evidence of fungal structures in biopsy material, include erythema nodosum, erythema multiforme, toxic erythema and rarely Sweet syndrome and interstitial granulomatous dermatitis.

Erythema nodosum expresses a valid cell-mediated response and coincides with the development of delayed hypersensitivity, shown by positivity to the coccidioides skin test. It manifests as painful subcutaneous nodules of the lower limbs. Biopsy reveals septal granulomatous panniculitis that should be distinguished from those due to other causes, especially sarcoidosis and infections, such as streptococcal forms. Erythema multiforme appears early in the course of the disease, often within 48 h of symptoms, with typical target-like lesions. Toxic erythema or acute generalised exanthema, also has rapid onset within two days of appearance of symptoms, precedes the development of antibodies detectable in serum. Lesions are papular, macular, urticarial or morbilliform. Histology of skin biopsies shows spongiotic dermatitis or interface dermatitis with mild perivascular infiltrate composed of lymphocytes, neutrophils, eosinophils but no necrotic keratinocytes. Sweet syndrome combines fever, peripheral blood leukocytosis and eruption of tender red papules and plaques, histologically composed of neutrophils and leukocytoclastic debris. These disappear on recovery from the lung infection and therefore do not require systemic steroid treatment, unlike Sweet syndrome lesions associated with other diseases. This picture must be distinguished from Sweet syndrome associated with hematological malignancies or connective tissue diseases. The rare forms of interstitial granulomatous dermatitis observed during lung infection, which disappear on recovery from respiratory symptoms, present with papules, nodules and plaques characterised histologically by dermal interstitial inflammation with neutrophils, leukocytoclastic debris and eosinophils, and are only an expression of reactivity. Similar clinical pictures may be observed in the course of various disorders, such as systemic diseases (lymphoma, systemic vasculitis).

Cutaneous infection is generally secondary, spreading through the lymphatic system or bloodstream from a lung focus having a months-long history. It manifests as papules, nodules (sometimes ulcerative), granulomatous or exudative plaques,[47] ulcers and abscesses, the latter mainly on the face, especially the nasolabial fold. Facial lesions may indicate involvement of the central nervous system.

Primary skin infection is rare, being observed in only 1-2% of cases of coccidioidomycosis and is usually caused by traumatic inoculation of the mycete. It is diagnosed above all in agricultural workers, typically from splinter injuries, or in laboratory workers due to the high infectiousness of the mycete, present in labs in the form of mycelia[45]. After 1-2 weeks of incubation, painless papular or nodular lesions appear,

sometimes verrucous, ulcerative or plaque-like with central ulcer. The extremities are the elective site of ulcerative nodules, which if multiple may have sporotrichoid disposition along the path of lymphatic vessels, and may sometimes clear up spontaneously. The possibility of disseminated infection should always be excluded by laboratory tests, such as complement fixation and other examinations, in the case of lesions suspected of being primary cutaneous infection. Skin biopsy is essential for diagnosis. Histological examination with hematoxylin-eosin, PAS and silver stain reveals granulomatous inflammation in the dermis with areas of abscesses, necrosis and reactive inflammation resembling vasculitis, containing double-walled spherules that enclose endospores typical of *Coccidioides immitis*. Cottony white colonies grow in culture in 2-6 days, microscope observation of which shows barrel-shaped arthroconidia. The colonies can be identified with DNA probes or detection of specific coccidioides exoantigens. The mycete may be identified by PCR on paraffin-embedded tissue.[45]

Treatment of the secondary cutaneous form relies on amphotericin B in the case of forms with multiple lesions and involvement of other organs. The drug is administered until clinical remission and reduction of serological titres (complement fixation). Otherwise oral treatment with imidazoles, such as itraconazole or fluconazole, can be used. In cases with mild skin and no bone or joint involvement, oral imidazoles are given. In primary forms, itraconazole treatment for various months has been successful.[48]

American histoplasmosis is a systemic infection caused by *Histoplasma capsulatum*, a dimorphic fungus widespread at all latitudes, especially in temperate and tropical climates. It is isolated from soil contaminated with bird and bat excrement and is easily inhaled to cause acute and chronic lung infections, sometimes resembling tuberculosis, with possible subsequent spread of the infection to the CNS, digestive system, urinary tract, bones, joints and eyes. Disseminated forms of infection are possible in subjects with impaired cell-mediated immunity. The skin disease can be secondary or primary. Secondary cutaneous histoplasmosis spreads by contiguity or occurs in the course of disseminated forms; it manifests in 4-6% of patients with histoplasmosis. Cutaneous histoplasmosis is rarely primary due to trauma with direct inoculation of the mycete into the skin in patients with predisposing factors, such as diabetes or immunosuppression.[49, 50] Clinical manifestations include pustules, nodules, ulcers or cellulitis-like plaques, the latter may be multiple in immunodepressed subjects. Sometimes lesions are nodular-ulcerative with satellite lymphadenopathy. Histological examination is necessary for diagnosis and shows granulomatous-suppurative inflammation with histiocytes, neutrophils, lymhocytes and typical yeast-like cells, 3-4 μm in size inside or sometimes outside histiocytes. These structures stain intensely with hematoxylin-eosin, PAS and Gomori reagents. Direct microscopic mycological examination and culture may enable identification of the mycete, which when cultured on Sabouraud medium produces whitish cottony colonies composed of microconidia measuring 2-4 μm and macroconidia measuring 10-15 μm with typical thick budding walls. Surgical excision and systemic antimycotics, including amphotericin B and itraconazole, administered for many months, are the elective therapy.[51]

African histoplasmosis due to *Histoplasma duboisii* is a rare deep mycosis endemic to Africa between the tropics of Cancer and Capricorn and in Madagascar.[52] It involves the skin, subcutaneous tissue and bone. Disseminated infection may involve lymph nodes, spleen, liver, lungs and gastrointestinal tract. Skin lesions are small papules, similar to those of molluscum contagiosum, or ulcerating nodules. Primary and secondary infections with osteomyelitic foci that spread by contiguity or fistulisation of lymph node abscesses are possible. Diagnosis and treatment are the same as for the American form. Histological examination shows granulomatous inflammation containing yeast cells (8-15 μm) larger than those typical of *Histoplasma capsulatum* and distinguished from those of *Cryptococcus* by the absence of the mucicarminophilous halo.

Cutaneous zygomycoses or mucormycoses are caused by organisms of the class Zygomycetes, order Mucorales. They are ubiquitous filamentous fungi having mycelia composed of thick-walled coenocytic hyphae (*i.e.*, without septa). They grow in warm humid climates, in soil, on decaying fruits and decaying organic matter. In nosocomial infections, zygomycetes may be isolated from adhesive tape, ventilation systems and wooden tongue depressors. Zygomycoses manifest at any latitude, preferring males and immunodepressed patients with cancer (especially blood malignancies), organ transplant recipients and patients chronically treated with immunosuppressants. They are potentially lethal due to the angio-

invasive potential of the mycetes, which may cause rhinocerebral, gastrointestinal, lung and disseminated infections, and less often endocarditis, peritonitis, osteomyelitis, renal infections and skin infections.[2] The rhinocerebral, lung and gastrointestinal forms are particularly severe for the same reason, and may lead to thrombosis, ischemia and tissue necrosis.

Skin infections by zygomycetes often evolve rapidly and have a poor prognosis because they usually indicate spread to the skin from foci in internal organs. They may be primary or secondary. In both, the etiological agent is prevalently *Rhizopus orizae*, followed in primary skin forms by *Apophysomyces elegans* and *Saksenaea vasiformis* and in secondary forms by *Mucor*. In primary cutaneous zygomycosis, the infection occurs when the skin is damaged and does not fulfil its barrier function: the mycete penetrates after trauma such as injections, mosquito bites or burns. Patients are usually immunosuppressed, often with blood malignancies or in diabetic ketoacidosis.[53] The mycete may be found on contaminated adhesive tape or catheters, or may penetrate after venipuncture. It may occur in immunocompetent subjects from soil entering the skin by polytrauma, such as motor vehicle accidents. The lesions manifest on the limbs, face and trunk as purplish infiltrated erythematous patches, which become necrotic, ulcerative and purulent. Necrotic scarring surrounded by erythema and induration is the typical clinical picture of cutaneous mucormycosis. Necrotising fasciitis may occur, involving muscles, tendons and bones, and leading to osteolysis. Cutaneous zygomycosis may develop into disseminated zygomycosis through the bloodstream. Differential diagnosis should consider skin infections due to *Aspergillus*, pyoderma gangrenosum and bacterial fasciitis. If diagnosed early, primary zygomycosis has a good prognosis and is treated by extensive surgical debridement, completely removing the necrotic tissue, associated with systemic antimycotics such as amphotericin B and/or posaconazole, for long periods.[54]

Secondary zygomycosis is more frequent than the primary form and has a poor prognosis. The skin is involved by dissemination of infections from internal organs, especially rhinocerebral. Patients are often uncontrolled diabetics with metabolic acidosis, immunological impairment or on desferoxamine and steroid therapy. The secondary form manifests on the face, palate and eyelids with edema, erythema, areas of necrosis, fistulas secreting serum and pus, and ulcers. Osteolysis of the ethmoid and sphenoid bones is possible, as are thrombosis and cranial nerve deficits. Necrotic ulcers due to *Aspergillus* should be distinguished from centro-facial lymphoma and anaerobic bacterial infections. Therapy consists of radical resection of necrotic tissues combined with amphotericin B deoxycholate or liposomal amphotericin B, usually at a dose of 3 mg/kg/day. The latter has fewer side-effects. Posaconazole can be combined with amphotericin.

In both primary and secondary forms, laboratory diagnosis is based on light microscope observation of exudate or pathological material from lesions, which after addition of KOH 20% may reveal non septate (*i.e.*, coenocytic) hyphae, 5 µm wide and 20-50 µm long. When cultured at 25-28 °C on Sabouraud dextrose agar for 3-6 days, zygomycetes produce cottony or woolly greyish white colonies, identified by their microscopic characters. Molecular biology methods are also useful for diagnosis.[55, 56] Histological examination of biopsy fragments shows inflammatory infiltrate with polynucleated neutrophils, plasma cells, areas of necrosis and large non septate hyphae that may invade vein and artery walls and must be differentiated from those typical of *Fusarium* and *Aspergillus* on the basis of size and morphology of fungal structures.

Lobomycosis is a chronic infection of subcutaneous tissue caused by the mycete *Loboa loboi* or *Lacazia loboi*, diagnosed in South America, especially Brazil.[57] The mycete is present as a saprophyte in soil, on plants and in water, and may cause infections in dolphins. It penetrates human skin through bites or stings of arthropods and snakes, or other traumas. Males engaged in agriculture or forestry, typically 40-70 years of age, may manifest painless nodules, confluent into plaques on the legs, arms or ears. Lesions have a vegetating, verrucous appearance and may develop into ulcers after trauma, and sometimes lead to atrophic scarring. Slow spread through the lymphatic system or bloodstream over decades may produce manifestations in sites distant from the original source, and may sometimes be disfiguring. Diagnosis is based on cytological examination of a skin smear obtained by scraping the skin lesion with a scalpel blade, which after adding KOH on a microscope slide, reveals oval or round yeast-like fungal cells, 6-12 µm in diameter, isolated or clustered in chains of 10-20 elements, often connected by tubular projections. The mycete does not grow in culture medium but histological examination of biopsy fragments detects it with stains specific for fungi, such as Grocott, in a context of

granulomatous inflammation, inside multinucleated cells and histiocytes. The fungal bodies are round, often empty, and may contain vacuoles, birefringent to polarized light. Budding yeast-like cells are found but not hyphae. Differential diagnosis should consider Leishmaniasis, lepromatous leprosy, different neoplasias including dermatofibrosarcoma protuberans and other subcutaneous mycoses such as paracoccidioidomycosis. Treatment of localised lesions is surgical, whereas disseminated lesions have been treated for long periods and with variable results, with clofazimine (100-200 mg/day) and itraconazole, or both,[58] and/or surgical excision.[59]

Conclusions

The number of mycetes responsible for subcutaneous mycoses will increase with improvements in the molecular techniques for identifying them and describing their variations. The current monolithic concept of the species is largely based on morphological characteristics.[60] To avoid boring readers, this small review does not include subcutaneous mycoses caused by yeasts. Further reviews may be added in the near future, since the classification, diagnostics and therapy of subcutaneous mycoses is developing rapidly, with new articles and new species of mycetes published every day.

Riassunto

Micosi sottocutanee. Parte 1: micosi sottocutanee da non-dermatofiti

Le micosi sottocutanee sono descritte in letteratura con sempre maggior frequenza per una seri di motivi. In primo luogo la vita media è aumentata ed anche i pazienti affetti da neoplasie e/o immunodepressi hanno migliori aspettative di vita, il che li candida a sviluppare questi tipi di micosi, in secondo luogo si sono affinate le tecniche diagnostiche di queste patologie ed anche il dermatologo ha cominciato a sospettare una micosi sottocutanea in presenza di particolari quadri clinici ed ha appreso la necessità di far ricorso all'esame istopatologico ed alla semina di frammenti bioptici prelevati dalle lesioni e seminati su adeguati terreni di coltura. La presente review pone l'accento sugli aspetti clinici, istopatologici e micologici delle micosi sottocutanee più comuni ed accenna alle terapie consigliate. Una migliore conoscenza di queste micosi può consentire un precoce trattamento di patologie che possono, a volte, mettere in pericolo la vita del paziente.

PAROLE CHIAVE: Micosi - Diagnosi - Micosi, trattamento.

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Update on the management of onychomycosis

B. M. PIRACCINI [1], C. GIANNI [2]

Onychomycosis is a fungal infection of the nail which is highly prevalent in the general population, particularly among older individuals. Patients seek care because the disease is infectious or simply for an esthetic discomfort. The difficulty in treating onychomycosis results from the deep-seated nature of the infection within the nail unit and the inability of drugs to effectively reach all sites. Present treatment options include both oral and topical drugs, with oral therapies giving better outcomes. New derivatives with a favorable risk-benefit ratio and new formulations of older azoles seem to be promising. The research for new drugs or formulations has the objective of discovering new active antifungals or new technologies to facilitate incorporation or persistence of existing antifungal drugs inside the nail plate. In fact, the same antimycotics that heal skin fungal infections are rendered less efficacious in nail disease. This update has the aim to synthesize and focus the therapies currently in use and new therapeutic approaches on onychomycosis. It also summarizes the newer areas of research in the treatment of onychomycosis as photodynamic and laser therapy.

KEY WORDS: Nails - Arthrodermataceae - Onychomycosis.

[1]Division of Dermatology, Department of Experimental Diagnostic and Specialty Medicine University of Bologna, Bologna, Italy
[2]Unit of Dermatology Italian Diagnostic Center (CDI), Milan, Italy

Onychomycosis is the most frequent cause of abnormality of the nail unit and it is responsible for about 50% of all consultations for nail disorders. In special populations, for example in the elderly with peripheral vascular disease or diabetes, the incidence of onychomycosis may have an incidence >40%.[1]

In most cases, this infection is caused by anthropophylic dermatophytes of the *Trichophyton species* and more rarely of *Epidermophyton* and *Microsporum.* Particularly, *Trichophyton rubrum* is the most common cause, followed by *Trichophyton mentagrophytes* var. *interdigitale*.

The non-dermatophyte molds can be involved in onychomycosis as primary pathogens ranging from 1.5% to 22% in the world.[2, 3] *Scopulariopsis brevicaulis, Fusarium spp,* and *Aspergillus spp* are the most common non-dermatophyte molds isolated in onychomycosis of the toenails.[4] Other molds that have been isolated include *Acremonium spp, Alternaria spp, Scytalidium spp* and other less frequent.[5] For the treatment is important emphasize that the onychomycosis of the toenail caused by non-dermatophyte molds alone or in combination with dermatophytes is more difficult to eradicate with standard antifungal therapeutic regimen.[6]

Yeasts represent a last common cause of nail fungal infection in the world, and *Candida albicans* and *Candida parapsilosis* are the two most common species. They are seen in immonodepressed patients and in sever diabetes.

Clinical features of onychomycosis

Dermatophyte fungi may produce several different clinical types of onychomycosis, depending on the modality of nail invasion by the fungus.

Corresponding author: B. M. Piraccini, Division of Dermatology, Department of Experimental, Diagnostic and Specialty Medicine, via Massarenti 1, 40138 Bologna, Italy. E-mail: biancamaria.piraccini@unibo.it

Distal and lateral subungual onychomycosis (DLSO)

DLSO is the most common of the clinical entities and involves invasion from the lateral or distal sites of the nail plate.

The affected nails usually show subungual hyperkeratosis, onycholysis and white or yellow discoloration. Infrequently, brown, black or orange discoloration may also be seen. In certain cases longitudinal streaking of the nail may be seen, called dermatophytoma. These infections are difficult to treat and may require excision of the area and systemic treatment rather than topical therapy. Since the skin of the palms and soles is the primary site of infection, DLSO is usually associated with tinea manum or tinea pedis. (Figure 1). This form of infection is seen with a variety of causative agents including dermatophytes.

Superficial onychomycosis (SO)

SO can present with superficial patches or transverse striae, and may arise from the superficial nail plate or emerge from the proximal nail fold. These bear clinical implications on treatment. In the classic form, previously known as white superficial onychomycosis, dermatophytes colonize the most superficial layers of the nail plate without penetrating it. The affected nail presents multiple friable white opaque spots in a patchy distribution that can be easily scraped away (Figure 2). This form is amenable to topical treatment. In contrast, in the striate form when the infection emerges from the proximal nail fold and with deep penetration of the nail plate, oral therapy is indicated.

Endonyx onychomycosis

Endonyx onychomycosis is characterized by massive nail plate parasitization in the absence of nail bed inflammatory changes. Clinically, the affected nail may show lamellar splitting and a milky white discoloration. The nail plate is firmly attached to the nail bed and there is no nail bed hyperkeratosis or onycholysis. This type of infection is commonly seen with *T. soudanense* but can also be seeing with *T. violaceum.*

Proximal subungual onychomycosis (PSO)

PSO is characterized by a primitive invasion of the nail matrix keratogenous zone through the proximal nail fold horny layer. Fungal elements are typically located in the ventral nail plate with minimal inflammatory reaction. The affected nail shows proximal leukonychia that progresses distally with nail growth. PSO can be divided into either patchy, striate, or secondary to paronychia. Amongst the dermatophytes, these infections are usually caused by *T. rubrum* and are very rare. PSO is, on the other hand, a typical presentation of non-dermatophyte mold onychomycosis, where it is typically associated with periungual inflammation (Figure 3).

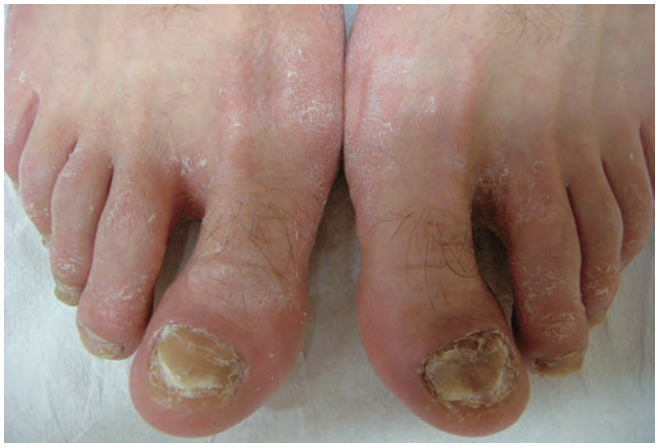

Figure 1.—Distal subungual onychomycosis: diffuse scaling of the periungual skin is due to the associated tinea pedis.

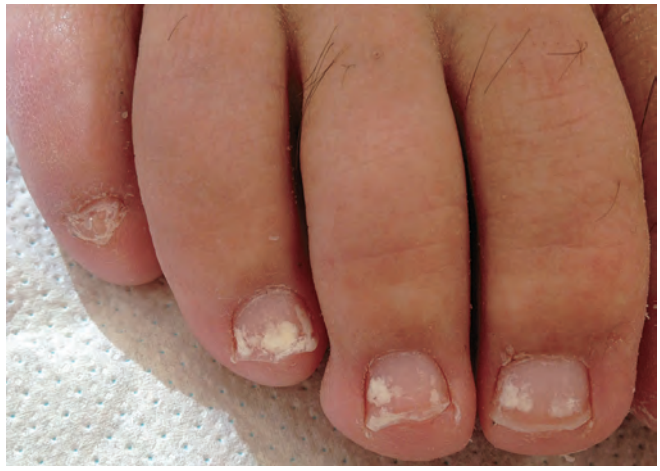

Figure 2.—White superficial onychomycosis.

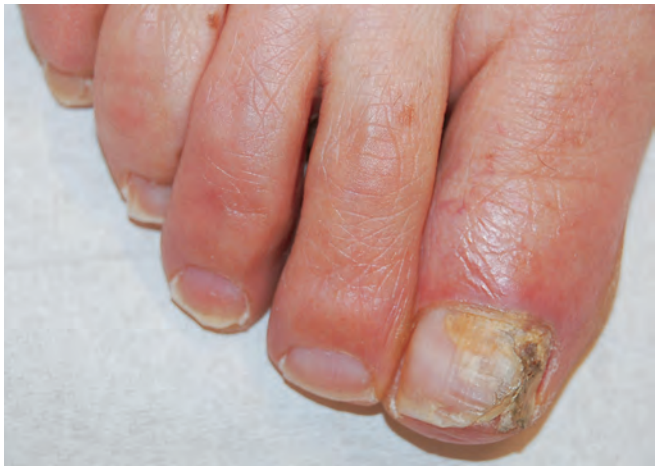

Figure 3.—Proximal subungual onychomycosis due to non-dermatophyte molds: the periungual inflammation is typically associated.

Mixed pattern onychomycosis

Often times different patterns of infection may be seen in the same patient. DLSO may occur with superimposed SO or PSO and SO may occur with superimposed DLSO or PSO. The most common of these are PSO with SO or DLSO with SO. Mixed pattern onychomycosis often requires oral treatment.

Totally dystrophic onychomycosis (TDO)

TDO is the end stage of onychomycosis and can result from DLSO as well as PSO. The nail plate, in these cases, crumbles and the underlying nail bed is thickened

Treatment

Onychomycosis represent about 30% of all superficial mycoses and are the most difficult to treat. Only topical therapy is often not enough and, therefore, systemic treatment must be considered.

The treatment choice depends on the clinical type of the onychomycosis, the number of affected nails and the severity of nail involvement.

The onychomycosis severity index (OSI) was developed in 2011 and provides a fast and effective way to evaluate the extent of onychomycosis and may provide an objective measurement on which to base treatment. Furthermore, the OSI scoring system demonstrates excellent inter-observer reliability. The categories assessed when calculating the OSI are area of involvement, proximity of disease to matrix, and the presence of dermatophytoma or subungual hyperkeratosis greater than 2 mm. First, the area of involvement is determined as the percentage of the nail that is invaded by fungi. Involvement is categorized as 1% to 10%, 11% to 25%, 26% to 50%, 51% to 75%, or 76% or greater with scores ranging from 0 to 5 respectively. Next the proximity of disease to matrix is assessed by visualizing the leading edge of disease proximally. The nail is divided tranversely into quarters. Scores of 1 through 4 are assigned, with 1 for only distal quarter involvement and a 4 for proximal quarter involvement. If the proximal edge extends into the nail fold or if the lunula is involved a score of 5 is given. The third step is to assess the presence of dermatophytoma, defined as the presence of a patch or a longitudinal streak, or subungual hyperkeratosis greater than 2 mm. If either of these are present a score of 10 is given. The OSI is calculated by multiplying the score for the area of involvement by the score for proximity of disease to the matrix and adding 10 if necessary from the third step. Scores range from 0 to 35 with higher numbers correlating with increased severity. Scores of 1 to 5 indicate mild onychomycosis, scores of 6 through 15 indicate moderate onychomycosis, while scores of 16 to 35 indicate severe onychomycosis. A score of zero indicates a cured state.[7, 8]

Topical treatment

Penetration of a topical antifungal through the nail plate requires a vehicle that is specifically formulated for transungual delivery. The two most commonly used agents are amorolfine 5% nail lacquer and ciclopirox 8% nail lacquer. However, agents such as miconazole, tioconazole, ketoconazole, tolnaftate, naftifine and tea tree oil have been tested with varying success rates in the past. Amorolfine nail lacquer is applied once a week, whereas ciclopirox nail lacquer is applied daily. Vehicles include non-water soluble resins or water soluble vehicles, containing hydroxipropylchitosane. This latter vehicle is characterized by its ability to ensure high drug delivery into the nail plate and easy application by the patient.[9] Nail lacquers are effective as monotherapy in the treatment of superficial onychomycosis and of distal subungual onychomycosis, limited to less than 50% of the distal nail.[10, 11] Treatment duration should be 6-12 months. Nail lacquers are also uti-

lized in combination with systemic antifungals [12] or nail avulsion in severe onychomycosis to reduce duration of treatment and increase cure rate.[13]

Recent studies on lipid diffusion enhancers and water soluble biopolymers have shown promises.[9, 14] Terbinafine nail solution and a terbinafine spray using lipid based vesicles currently under development, labeled TDT 067, may be viable treatment alternatives in the future.[14, 15] Other formulations with terbinafine that are undergoing phase II trials include MOB-015 and TMI-358. Luliconazole has completed phase I and IIa testing for treatment of moderate to severe distal subungual onychomycosis with positive results.[17] For the treatment of distal moderate onychomycosis other studies of new topical antifungal are ongoing.[18-21]

Non-pharmacological treatments

Treatments with photodynamic therapy (PDT) using photosensitizers may also prove to be effective treatment options in the future.[22] Laser therapy is also currently being researched and may be effective in the treatment of onychomycosis. FDA approved lasers for onychomycosis include carbon dioxide laser, ND:YAG laser, and the diode 870 nm, 930 nm laser. The carbon dioxide laser is the oldest laser and is infrequently used today. With the Nd:YAG laser small clinical trials have demonstrated mycological cure rates as high as 87.5%.[23-25] Similarly, the diode laser has shown some efficacy in small trials, with mycological cure rates as high as 38% reported at 9 month follow up. Landsman Large randomized control trials need to be conducted to validate the efficacy of these lasers and PDT in the treatment of onychomycosis.[26]

Systemic treatments

Distal subungual onychomycosis that involves greater than 50% of the nail, proximal subungual onychomycosis, and deeply infiltrating white superficial onychomycosis require systemic therapy.[27] Systemic treatment with terbinafine or itraconazole produces mycological cure in more than 90% of fingernail infections and in about 80% of toenail infections. These success rates can be increased by associating a topical treatment with a nail lacquer to the systemic treatment. However, compared to itraconazole, terbinafine has a higher mycological and clinical cure rate and a lower rate of recurrence.[28-30] Terbinafine can be administered as a continuous therapy at 250 mg per day for 12 weeks or an intermittent regimen of 2 pulses of 250 mg/ day for four weeks on and 4 weeks off.[31] Itraconazole is administered as pulse therapy at the dosage of 200 mg twice a day for 1 week a month. The treatment duration is 2 months for fingernails and 3 months for toenails. Sequential treatment with itraconazole and terbinafine has been utilized to increase cure rates: the suggested regimen is 2 pulses of itraconazole 400 mg/day for 1 week a month followed by 1 or 2 pulses of terbinafine 500 mg/day for 1 week a month.

Fluconazole is also used in dermatophyte onychomycosis but is less effective. Various regimens with different results have been reported by several authors in the treatment of the dermatophytic onychomycosis. Treatment doses ranged from 100 mg to 450 mg weekly and 150 mg daily, and durations ranged from 12 weeks to 12 months. Actually, in patients unable to tolerate other oral antifungal agents, the recommended dosage is 150 mg weekly for more than six months, especially for toenails.[32]

Posaconazole and albaconazole, a novel triazole, are newer drugs that could be alternative therapy options.[33, 34]

In cases of lateral nail plate involvement or dermatophytoma, surgical or chemical avulsion of the nail plate combined with topical or systemic treatment is indicated.[12]

Total dystrophic onychomycosis is an extremely recalcitrant entity. Surgical or chemical nail avulsion followed by topical therapy may be a viable treatment option.[35, 36]

Mycological cure can be evaluated at the end of treatment. Evaluation of clinical response, on the other hand, requires several months due to the slow growth rate of the nail. Recurrences and reinfection are not uncommon and vary with type of treatment (rates of 35.7% reported with itraconazole).[30] These may be prevented by topical antifungals on soles and toe webs or with new approaches in sanitizing onychomycosis patient footwear based on antimicrobial properties of ozone gas.[37]

Conclusions

The therapeutic approach for onychomycosis depends mostly on the fungal organism identified

and on how extensive is the area of infection. The treatment choice depends on the clinical type of the onychomycosis, the number of affected nails and the severity of nail involvement. Topical antifungal therapies, sometimes associated with chemical avulsion of the nail, are effective, but if nail involvement is severe, systemic treatment must be considered.

In addition to the known treatments, in recent years new therapeutic approaches in the therapy of onychomycosis have benne developed, as PDT and the use of the lasers, which may represent alternative non invasive techniques. Moreover, pharmacological research is investigating new drugs and new formulations more effective in treating of onychomycosis, but new and more detailed studies are needed to confirm encouraging results.

Riassunto

Aggiornamenti sul trattamento delle onicomicosi

Le onicomicosi sono infezioni fungine delle unghie frequenti nella popolazione generale, soprattutto negli anziani. I pazienti con onicomicosi cercano una cura non solo per il danno estetico, ma anche per evitare un ulteriore contagio ad altre unghie e altre aree cutanee e per il danno funzionale a volte associato. La difficoltà del trattamento è dovuta alla struttura anatomica dell'unghia, difficilmente penetrabile dagli antifungini topici. I trattamenti attuali sono sia topici che sistemici, con migliori risultati dei farmaci per via generale. Alcune novità paiono promettenti, come le nuove formulazioni degli azolici o altri farmaci con un alto rapporto beneficio/rischio. La ricerca sui nuovi farmaci per le onicomicosi è focalizzata su nuovi principi attivi o su formulazioni in grado di veicolare e fare persistere il farmaco all'interno della lamina ungueale. Questo articolo sintetizza lo stato dell'arte del trattamento delle onicomicosi e riassume i nuovi approcci terapeutici, incluse nuove are di ricerca quali quelle con la terapia fotodinamica e i laser.

Parole chiave**:** Unghia - Arthrodermataceae - Onicomicosi.

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Photodynamic chemotherapy in the treatment of superficial mycoses: an evidence-based evaluation

G. CALABRÒ, A. PATALANO, V. LO CONTE, C. CHIANESE

Department of Clinical Medicine and Surgery – Dermatology Federico II University of Naples, Naples, Italy

Photodynamic therapy (PDT) is a constantly evolving treatment modality consisted of a chemical reaction activated by light energy that is used to selectively destroy tissue; it may be considered a particular form of photochemotherapy that uses a photosensitizer, light and oxygen. The combination of the possibility of ablation of lesion with an excellence aesthetic result has allowed the photodynamic therapy an increasing role in the treatment of skin disease, that ranges from skin cancer to cosmetic treatment. Particular attention is paid in the last years to a developing area of research, the antifungal photodynamic therapy. The growing resistance against antifungal drugs has renewed the search for alternative therapies and PDT seems to be a potential candidate. This article provides an extensive review of antifungal photodynamic therapy, its mechanisms and applications in the treatment of superficial mycoses.

KEY WORDS: Antifungal agents - Mycoses - Candida.

Photodynamic therapy (PDT) is considered a photochemical reaction used to selectively destroy tissue; it is a particular form of photochemotherapy that uses a photosensitizer, light and oxygen.

It is a two-stage therapeutic technique in which the use of a topical or systemic sensitizing drug is followed by visible light radiation. The photosensitizers, administered exogenously or formed endogenously, are activated by the light and transfer energy to molecular oxygen, thereby generating reactive oxygen species to induce cell death.[1, 2] For the treatment of gastrointestinal cancer, brain cancer or bronchopulmonary cancer, photosensitizers are administered orally or intravenously. For endometrial cancer or bladder carcinoma they are generally administered by instillation, while for the treatment of skin cancer the drugs are effective when applied topically.[3]

In dermatology PDT was initially investigated to treat non-melanoma skin cancer;[4] it has proved to be a valid therapeutic option in cases of actinic keratosis lesions, basal cell carcinoma and Bowen's disease, with the advantage of being able to treat multiple tumors simultaneously.

More recently, PDT has been indicated for a wide range of dermatological conditions such as photodamaged skin, acne, hidradenitis, scleroderma, psoriasis, warts, leishmaniosis and superficial mycoses (Table I).[5]

Basic principles of PDT

Most modern PDT applications involve three key components: a photosensitizer, a light source and tissue oxygen. The combination of these three components leads to the chemical destruction of any tissues which have either selectively taken up the photosensitizer.

The photosensitizer is accumulated in the target cells and absorbs light of a certain wavelength. The

Corresponding author: Dott.ssa G. Calabrò, Department of Clinical Medicine and Surgery - Dermatology, Federico II University of Naples, Naples, Italy. E-mail: gcalabro@unina.it

TABLE I.—*Research on uses of PDT in the treatment of dermatological disease.*

Disease	Outcome	Author (Ref.)
Actinic keratosis	Results similar to those reported with conventional treatment. Fewer side effects and a faster recovery time.	Braathen *et al.*.[5] Goldman *et al.*.[6] Piacquadio *et al.*.[7] Alexiades-Armenakas *et al.*[8] Smith *et al.*[9] Touma *et al.*[10] Fink-Puches *et al.*[11] Freeman *et al.*[12] Dragieva *et al.*[13]
Basal cell carcinoma	The best result has been described with Methyl aminolevulinate (MAL). Poor results in terms of long-term recurrence rates.	Braathen *et al.*[5] Szeimies *et al.*.[14]
Squamous cell carcinoma	Systemic and topical PDT is a useful diagnostic and adjunctive therapeutic modality.	Braathen *et al.*[5]
Bowen's disease	Efficacy as that achieved with cryotherapy and 5-fluorouracil.	Braathen *et al.*[5]
Acne	Oral use of 5-aminolaevulinic acid (ALA) associated with visible polychromatic light is effective for the treatment of acne. PDT with ALA- Pulsed. Dye Laser may be considered as an alternative to the use of Isotretinoin.	Itoh *et al.*[15] Futsaether *et al.*[16] Taub *et al.*[17] Tzung *et al.*[18] Hongcharu *et al.*[19] Pollock *et al.*[20] Wiegell *et al.*[21] Fabbrocini *et al.*[22] Alexiades-Armenakas *et al.*[23]
Photo-damaged skin	Intense Pulsed Ligh is able to improve different components of photo-damage except actinic keratosis.	Nestor *et al.*.[24] Touma *et al.*.[25] Gold *et al.*.[26] Ruiz-Rodriguez *et al.*.[27] Zane *et al.*.[28] Issa *et al.*.[29]
Bacterial Infection Cutaneous lymphoma Genital condyloma Hailey-Hailey disease Hidradenitis suppurativa Kaposi's sarcoma Leishmaniosis Necrobiosis lipoidica Perioral dermatitis Psoriasis Scleroderma Sebaceous hyperplasia Viral warts	PDT may represent an alternative therapy.	Procaccini *et al.*.[30] Welch *et al.*.[31] Richey *et al.*.[32] Perrett *et al.*.[33] Herzinger *et al.*.[34] Fabbrocini *et al.*.[35]

energy is transferred to oxygen and highly reactive oxygen species - mainly singlet oxygen - are generated. Treating with appropriate light doses, the reactive oxygen species directly lead to cell and tissue damage by inducing necrosis and apoptosis and indirectly stimulate inflammatory cell mediators. Following lower light doses treating inflammatory dermatoses, immunomodulatory effects are induced.

A clinical treatment of PDT can be divided into four phases: administration of the drug, accumulation in the cells, activation of photosensitizer with generation of cytotoxic reactive oxygen species.

In clinical practice, the photosensitizer is administered to patient in such a way as to favour the accumulation in the diseased tissues. Subsequently these are irradiated with visible light of appropriate wavelength that electronically excite the photosensitizer, which passes from the ground state S0 to the singlet excited state S1. The lifetime of the excited state S1 is of the order of nanoseconds, too short to allow effective interaction with molecules surrounding. To return to the singlet ground state S0, the agent photoreactive must disperse the energy and this process can follow different mechanisms, such

as the emission of fluorescence or the dissipation electronic energy through nonradioactive processes of conversion other forms of energy, such as thermal energy, through processes or collisional quenching. This process, forbidden by the rules of selection, is favoured by the effects of spin-orbit coupling. The further descent to the ground state S0, requires a second forbidden transition from the excited triplet T1. The life time of the species is then T1 much longer (micro/milli-seconds), and is sufficient for allow the energy transfer phenomena and charging surrounding molecules, with the generation of cytotoxic species.

These may be formed by means of two types of reactions:

1) Type I reactions: an electron transfer occurs from the photosensitizer in the excited triplet state T1 to biological substrates (unsaturated lipids, steroids, aromatic aminoacids), with resulting in the formation of radical species, which react with molecular oxygen to produce reactive species oxygen species (ROS). These intermediates are highly reactive and strong oxidants, such as the superoxide anion O_2^-, Hydrogen peroxide H_2O_2 and the hydroxyl radical OH;

2) Type II reactions: the energy is transferred from the photosensitizer in triplet state T1 directly to molecular oxygen to form singlet oxygen, which, as indicated by direct and indirect evidence, has a key role in molecular processes underlying the anticancer treatment via PDT.

The two types of reactions do not occur in a mutually exclusive way, but may contribute to the therapeutic effect together. Final mechanism ends in the formation of ROS that results in tissue destruction.[36]

The ROS generated by the photosensitizer can destroy the tissue through three different mechanisms interdependent, which are the damage direct cellular, the damage to the vascular system and the activation of the response immune. To achieve long-term results of treatment is required the action of all mechanisms, although it is not yet clear what is the contribution made by each individual process.

However, the effectiveness of the photodynamic therapy depends on the localization of the photosensitizer and its degree of accumulation in the treated tissue, the time and dose of irradiation of the tissue in addition to the yield of the formation of ROS (Figure 1).

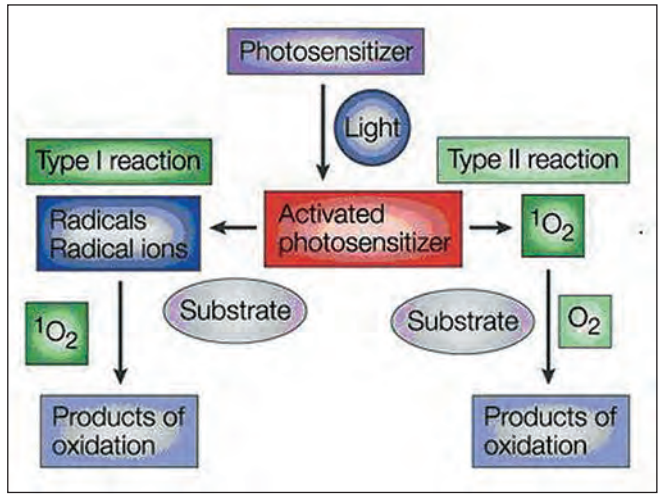

Figure 1.—Schematic representation of the photochemical process of PDT.

Antifungal photodynamic therapy

At the beginning of 20th century, bacterial epidemics were a global and important cause of mortality. In contrast, fungal infections were almost not taken in account. Since the late 1960s when antibiotic therapies were developed, fungal infections increased, and they currently represent a global health threat. The increasing incidence of fungal infection is influenced by the growing number of immunodeficient cases related to AIDS, cancer, old age, diabetes, and organ transplants and other invasive surgical procedures.

Despite improvement of antifungal therapies over the last 30 years, when compared with the number of antibacterial drugs, the number of antifungal agents available for therapy is very restricted and treatment failure is common in clinical practice. The most common reasons of the failure are represented by the long duration, serious side effects, drug-drug interactions, and antifungal resistance.[37, 38]

The great interest in alternative therapies for the treatment of fungal infections derives from the fact that the phenomenon of antifungal resistance is still of major concern in clinical practice.

The growing resistance against antifungal drugs has promoted the search for alternative treatment modalities and PDT appears a potential candidate.[37]

Antifungal photodynamic therapy is a developing

area of research [39] and a majority of the literature in this area is concerned with in vitro experiments. Antifungal PDT is, in fact, an area of increasing interest, as research is advancing 1) to identify the photochemical and photophysical mechanisms involved in photoinactivation; 2) to understand how photoinactivation is affected by key microbial phenotypic elements multidrug resistance and efflux, virulence and pathogenesis determinants, and formation of biofilms; 3) to develop clinically compatible photosensitizers.

PDT against Candida species

The photosensitisation of Candida yeasts inducing cellular damage through the utilisation of several sensitizing compounds has received special attention in several works.[40-43]

In the last years, the effect of PDT has been already demonstrated in the inhibition of germ tube formation,[41, 44] biofilm formation [44]and reduction in adhesion to epithelial buccal cells.[45]

Monfrecola *et al.*[46] evaluated the in vitro- efficacy of 5-aminolaevulinic acid (ALA) -PDT. The authors measured the development of *C. albicans* colonies after application of ALA plus visible light (VIS) irradiation. For the experiment 30 microl of suspension have been incubated in the dark for 3 h, with increasing concentrations of ALA (125, 250, 300, 350, 400, 450, 500, 550, 600, 750, 1000 mg mL^{-1}) and then irradiated with a fixed dose (40 J cm^{-2}) of VIS. Immediately after the irradiative session, the *C. albicans* suspensions were disseminated on dishes containing a Sabouraud agar + CAF medium and cultured in the dark at 27 °C; after 48 h colony development has been measured. The authors concluded that ALA plus VIS light is able to kill *C. albicans* colonies.

Calzavara *et al.*[47] investigated the efficacy of ALA-PDT with an open pilot study enrolling three patients with interdigital mycosis of the feet caused by *C. albicans*. All colonies showed a strong red fluorescence after incubation with 20% ALA water solution and irradiation with Wood's lamp. The treatment protocol provided for the application of a 20% ALA preparation in Eucerin cream ® under an occlusive dressing, followed, 4 h later, by the irradiation of 75 J cm^2 of broadband red light. They observed clinical and mycological examination recovery in one patients after four treatments.

Demidova and Hamblin [48] evaluated the antifungal photodynamic activity on *C. albicans* of Rose Bengal (RB), a new characterized by light absorption at wavelengths of 450-600 nm and usually used for the diagnosis of eye disease. The authors observed log10 reduction of 4 and 6 for cellular densities of 10^7 and 10^6 cells mL respectively.

Junqueira *et al.*[49] evaluated the efficacy of malachite green (MG) on *Candida spp.*; MG is a new cationic dye of the triarylmethane family that showed strong absorption in the red region of the visible spectrum. They demonstrated the PDF with malachite green was effective in *Candida strains* reduction (<1 log10) when irradiated with low doses (26 J cm^2) of gallium-aluminum-arsenide laser.

The photoinactivation efficacy of MG was also investigated by Souza *et al.*[50] The authors evaluated specific effects of photodynamic therapy (energy density 15.8 J/cm^2, 26.3 J/cm^2 and 39.5 J/cm^2) using methylene blue, toluidine blue and malachite green as photosensitizers and low-power laser irradiation on the viability of *C. albicans*. Suspensions of *C. albicans* containing 10^6cells/ml were standardized in a spectrophotometer. For each dye, 120 assays, divided into four groups according to the following experimental conditions, were carried out: laser irradiation in the presence of the photosensitizer; laser irradiation only; treatment with the photosensitizer only; no exposure to laser light or photosensitizer. Next, serial dilutions were prepared and seeded onto Sabouraud dextrose agar for the determination of the number of colony-forming units per milliliter (CFU/mL). The results were subjected to analysis of variance and the Tukey test ($P<0.05$). They demonstrate that PDT using toluidine blue, methylene blue and malachite green were effective photosensitizers in antifungal photodynamic therapy against *C. albicans*, as was low-power laser irradiation alone.

Mitra *et al.*[51] investigated the effectiveness of the photosensitizer meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP-1363) in the treatment of *C. albicans* infection in vitro and its selectivity in an animal model. The efficacy of TMP-1363 in PDT of *C. albicans* in vitro was compared to that of methylene blue using a colony forming unit assay. In vivo infection in the mouse was established by inoculation of *C. albicans* yeast in the intradermal

space of the ear pinna. Two days post-infection, 0.3 mg/mL^{-1} TMP-1363 was administered topically. Thirty minutes after TMP-1363 application, the ears were irradiated at 514 nm using a fluence of 90 Jcm^{-2} delivered at an irradiance of 50 mWcm^{-2}. The ears were excised 2- hours post-irradiation, homogenized, and the organism burden was determined by a CFU assay. In vivo wide field and confocal fluorescence imaging assessed the localization of the photosensitizer in relationship to *C. albicans*. They concluded that photosensitization with TMP-1363 resulted in a greater than three-log increase in killing of *C. albicans in vitro* compared to methylene blue. *In vivo* fluorescence imaging demonstrated a high degree of selective labeling of *C. albicans* by TMP-1363. PDT of infection using TMP-1363 resulted in a significant reduction in CFU/ear relative to untreated controls.

Dovigo *et al.*[52] described the association of of curcumin with light emitting diode (LED) for the inactivation of *C. albicans*. Suspensions of Candida were treated with nine curcumin concentrations and exposed to LED at different fluences. Curcumin-mediated PDT was also assessed against biofilms. They selected the protocol that showed the best outcomes for *Candida* inactivation to evaluate the effect of the preirradiation time (PIT) on PDT effectiveness, the uptake of curcumin by *C. albicans* cells and the possible involvement of singlet oxygen in the photodynamic action. They observed that when compared with the control group, a statistically significant reduction in *C. albicans* viability was observed after PDT ($P<0.05$), for both planktonic and biofilm cultures. Photodynamic effect was greatly increased with the presence of curcumin in the surrounding media and the PIT of 20 min improved PDT effectiveness against biofilms.

The antifungal activity of two new dye (RB and erythrosine) against *C. albicans* was investigated by Costa *et al.*[53] They observed a significant reduction of planktonic cultures (3.45 log10 and 1.97 log10) and of biofilms (<1 log10) after blue LED irradiation (95 J cm^2) using erythrosine and RB respectively. The authors demonstrated that Erythrosine was more effective than RB against *C. albicans* in planktonic cultures. They have pointed out that chemical structures of these dyes may contribute to a greater affinity with the external structure of the yeast cells than other phenothiazine dyes.

Although *C. albicans* is the most prevalent species involved in human infections, other species are also important. It is worth mentioning that *C. krusei* is intrinsically resistant to fluconazole.[54, 55] Considering this fact, the action of PDT against different Candida species is very relevant.

Dovigo *et al.*[56] tested the efficacy of PDT with hotogen, a porphyrin photosensitiser, against four species of *Candida*. They showed that *C. krusei* was not inactivated by any of the associations between light and photosensitiser tested, while *C. albicans*, *C. tropicalis* and *C. dubliniensis* were completely eliminated. The authors underlined how the results achieved in vitro may not reflect the in vivo situation: even in PDT treatment, biofilms are less susceptible to antifungal treatment than planktonic cultures.

In another study, Dovigo *et al.*[57] evaluated the efficacy of PDT against *C. albicans* and *C. glabrata* resistant to fluconazole, and against cells in suspension or in biofilms. They concluded that the fungicidal effect of PDT was strain-dependent; they have pointed out that although PDT was effective against *Candida* spp., fluconazole-resistant strains showed a reduced sensitivity to PDT.

Costa *et al.*[58] obtained interesting results against *C. dubliniensis* using erythrosine and green LED light, evaluating the effect of erythrosine- and LED-mediated PDT on planktonic cultures and biofilms of *C. albicans* and *C. dubliniensis*. Erythrosine concentrations of 0.39-200 μM and LEDs in a 96-well microtiter plate were used to treat planktonic cultures of standardized suspensions (10^6 cells/mL) of *C. albicans and C. dubliniensis*. Biofilms formed by *C. albicans* and *C. dubliniensis* in the bottom of a 96-well microtiter plate were treated with 400 μM erythrosine and LEDs; after PDT, the biofilms were analysed by scanning electron microscopy. They concluded that *C. albicans* and *C. dubliniensis* were susceptible to erythrosine- and LED-mediated PDT, but the biofilms of both Candida species were more resistant than their planktonic counterparts.

PDT against dermatophyte species and nondermatophytic molds

Dermatophyte infections are a common skin disorder and their epidemiological features is different according to the geographical area and have

changed over time as a consequence of several factors, such as migratory streams, lifestyle, socioeconomic conditions and incidence of peculiar comorbidities. Two important restrictions of current therapeutic infections are recurrence of the infections and prolonged treatments.

Photodynamic inactivation of dermatophytes has been studied by Romagnoli et al [59]. Eight strains of dermatophytes (*T. mentagrophytes, T. rubrum, T. tonsurans, M. cookei, M. canis, M. gypseum, E. floccosum and N. cajetani*) were exposed to UVA irradiation after sensitization with two thiophenes (2,20:50 200-terthienyl and 5-(4-OH-1-butinyl) 2,20-bithienyl). A strong and dose-dependent inhibition of the growth of all tested strains was found although a complete inactivation was never obtained.

Smijs *et al.*[60] evaluated the susceptibility of dermatophyte *T. rubrum* of PDT with the use of the light-activated porphyrins deuteroporphyrin monomethylester (DP mme) and 5,10,15-tris(4-methylpyridinium)-20-phenyl-[21H,23H]-porphine trichloride (Sylsens B). Sylsens B and DP-mme are known to be able to kill certain bacteria, Chinese hamster ovary cell and the common fruit fly Droswophila melanogaster.

They also compared the photodynamic activity of Sylsens B and DP mme with that of some other photosensitizers that are well known in the field of PDT: the porphyrins deuteroporphyrin and hematoporphyrin, the drug Photofrin and several phthalocyanines. Cultures of *T. rubrum* growned on malt extract agar (MEA) and made in Dulbecco's modified Eagle medium (DMEM) were incubated with the photosensitizer in test tubes for 30 min at a temperature of 28° C. After incubation, the suspension cultures (2 mL) were illuminated in presence of the sensitizer using a lamp from "MASSIVE", 1X max. 500W-230 V-R7s, IP44.

They demonstrated that PDT of *T. rubrum* in suspension culture with Sylsens B and DPmme resulted in a complete kill of the fungus and that Sylsens B was the most effective sensitizer and showed no dark toxicity. The phthalocyanines and Photofrin displayed a fungistatic effect for about 1 week, whereas all the porphyrins caused photodynamic killing of the dermatophyte. The authors concluded that Sylsens B in an appropriate formulation, it could be a promising candidate for the treatment of various forms of tinea.

Calzavara *et al.*[47] investigated the efficacy of ALA-PDT with an open pilot study enrolling six patients with interdigital mycosis of the feet caused by *T. mentagrophytes* (four patients) and *T. rubrum* (two patients). All colonies showed a strong red fluorescence after incubation with 20% ALA water solution and irradiation with Wood's lamp. The treatment protocol provided for the application of a 20% ALA preparation in Eucerin cream ® under an occlusive dressing, followed, 4 h later, by the irradiation of 75 J cm^2 of broadband red light. They observed clinical and microbiological recovery in three out of six patients after the treatments.

Smijs *et al.*[61] evaluated the photodynamic effectiveness of the porphyrins in a situation that mimics the clinical situation, developing an ex vivo model using human stratum corneum (SC) that offers the possibility of applying PDT at different time points during the germination and subsequent development of *T. rubrum* microconidia. The model was used for two different incubation media, Dulbecco's modified Eagle medium (DMEM) and distilled water. *T. rubrum* cultures were grown on Sabouraud dextrose agar at room temperature for the preparation of a microconidia suspension, that was made using the method of Zurita and Hay. Human SC was inoculated with a microconidia suspension and incubated; at 8, 24, 48 and 72h after spore inoculation, PDT was applied using Sylsens B or DPmme and a lamp from "MASSIVE", 1X max. 500W-230 V-R7s, IP 44. They demonstrated that the PDT susceptibility of *T. rubrum* depended on the time of PDT application after spore inoculation. They underlined how a decrease in susceptibility was observed with increasing time of PDT application for both photosensitizers in DMEM; changing the incubation medium to distilled water resulted in an increased fungicidal effect for Sylsens B and in a decreased effect for DP mme. They concluded that *T. rubrum* is susceptible to PDT in a situation that mimics the clinical situation and that the fungicidal effect of PDT on fungal spores is of particular importance.

Smijs *et al.*[62] have examined the penetration of Sylsens B in healthy and with *T. rubrum* infected skin and investigated the susceptibility of *T. rubrum* to PDT using formulation I and UVA^{-1} radiation (340-550 nm). Skin penetration studies were performed with formulations I and II (Sylsens B in PBS, pH 7.4) applied on dermatomed skin, human stratum corneum (SC), disrupted SC by *T. rubrum* growth and SC pre-treated with a detergent. They

demonstrated that no penetration was observed in healthy skin and that disruption of SC by preceding fungal growth caused Sylsens B penetration at pH 7.4, but not at pH 5.2; however, chemically damaged SC allowed Sylsens B to penetrate also at pH 5.2. UVA-1 PDT was applied ex vivo during two fungal growth stages of two *T. rubrum* strains (CBS 304.60 and a clinical isolate). Both strains could be killed by UVA-1 alone (40 J/cm^2). Combined with formulation I (1 and 10 microm Sylsens B for, respectively, CBS 304.60 and the clinical isolate), only 18 J/cm^2 UVA-1 was required for fungal kill. They concluded that PDT with 10 microm Sylsens B and 18 J/cm^2 UVA^{-1} could be considered as effective and safe.

Piraccini *et al.*[63] proposed a successful strategy treating toenail onychomycosis caused by *T. rubrum* with topical ALA-PDT acid after surgical removal of the nail plate. They treated a toenail onychomycosis of a patient unresponsive to conventional topical treatment, and with conditions that contraindicated administration of systemic antifungal (HCV, warfarin and antihypertensive drugs). Three ALA-PDT sessions were applied under an occlusive dressing for three hours, during a period of 45 days. The nail was irradiated with LED, with emited broadband red light at a wavelength of 630 nm at 37 J/cm ^{2} for 7 minutes and 24 seconds. At the 12 and 24 months follow-up visits, potassium hydroxide examinations and cultures were still negative and the toenails were considered recovered with residual mild traumatic onycholysis.

Watanabe *et al.*[64] described two cases of onychomycosis successfully treated with PDT with topical application of an ointment containing ALA 20%. They first applied a 20% urea ointment directly to the diseased nail surface and covered with a piece of plastic film wrap for 10 hours. Then, a 20% solution of ALA methyl ester in aqueous cream was applied to the treated nails for 5 hours. Subsequently, the treatment site, including proximal and lateral nail folds, was irradiated both horizontally and vertically with pulsed laser light at a wavelength of 630 nm at 100 J/cm^2 using an excimer-dye laser. ALA- PDT was performed once a week. No recurrence was observed clinically at a 3-month follow-up visit.

Sotiriou *et al.* evaluated the efficacy of ALA-PDT in patients with tinea cruris,[65] tinea pedis [66] caused by *Trichophyton* spp. Eight of 10 tinea cruris patients and six of 10 tinea pedis patients obtained a complete remission after 1-3 ALA- PDT treatment, but four tinea cruris patients and three tinea pedis patients had a persistent healing at the 8-week follow-up after the last treatment. The authors underlined how it was unclear why the apparently good therapeutic effect was followed by quick recurrence in almost all patients. They suggested that ''in vivo'' environmental conditions (temperature, humidity and pH of the interdigital skin), could induce a poor cell uptake of ALA and a deficient biosynthesis of PpIX; also the non-uniform delivery of light and/or ALA cream due to the irregular tridimensional shape of this peculiar anatomical area must be taken into account.

Sotiriuou *et al.*[67] also investigated the efficacy of PDT in the treatment of onychomycosis caused by *T. rubrum.* Thirty patients with distal and lateral subungual toenail onychomycosis were first treated for 10 days with 20% urea ointment under occlusion in order to remove nail plate and subungueal hyperkeratosis. Subsequently 20% ALA was applied under an occlusive dressing topically on the entire nail bed; 3 hours later nail was irradiated with red light (570-670 nm) at a light dose of 40 J/cm^2 and a fluence rate of 40mW/cm^2. The treatment was repeated 3 times at 2 weekly intervals. Cure rate of 43,3% and 36,6% was seen 12 and 18 months after treatment, respectively, while clinical and mycological recurrence was seen in other patients.

Aspiroz *et al.*[68] evaluated the effectiveness of PDT in a case of onychomycosis caused by moulds. They treated an onychomycosis caused by *Acremonium sclerotigenum* with 3 sessions of PDT with methyl-aminolevulinate MAL separated by 15 days. They demonstrated that the patient achieved mycological and clinical cure and remains asymptomatic after 12 months of follow-up.

Conclusions

Photodynamic therapy is a minimally invasive approach widely used in dermatological practice for the treatment of several diseases, including infective diseases.

Conventional antifungal strategies have become increasingly ineffective due to the emergence of multidrug resistance among pathogenic microorganisms. The need to overcome these deficiencies

has triggered the exploration of alternative treatments and PDT seems to be a potential candidate.

The susceptibility of *Candida* and *dermatophyte spp*. to antifungal photodynamic therapy under in vitro conditions has been extensively demonstrated. However, for the treatment to be successful in the clinical situation, development of enhanced strategies for delivery of photosensitizers and light to the site of action is required. The clinical efficacy of PDT is strain-dependent and the biological medium can diminish the efficacy of the therapy *in vivo*.

Considering the efficacy of the technique demonstrated *in vitro* studies and the importance of developing new antifungal strategies, this is an area of great interest for future research studies.

Riassunto

Chemioterapia fotodinamica nel trattamento delle micosi superficiali: una valutazione basata sull'evidenza

La terapia fotodinamica (PDT) è una terapia in costante evoluzione che consiste in una reazione chimica attivata dall'energia luminosa utilizzata per distruggere selettivamente i tessuti danneggiati; è considerata una particolare forma di fotochemioterapia che prevede l'utilizzo di tre elementi essenziali: un fotosensibilizzatore, la luce e l'ossigeno. La combinazione della possibilità di ablazione delle lesioni con un eccellente risultato estetico ha consentito alla terapia fotodinamica di assumere un'importanza sempre crescente nel trattamento delle patologie cutanee, estendendo il proprio campo di applicazione dai tumori cutanei ai trattamenti cosmetologici. Una delle più recenti applicazioni della PDT in ambito dermatologico è il trattamento delle micosi superficiali. Il costante incremento della resistenza ai trattamenti antifungini ha spinto, infatti, la ricerca a nuovi trattamenti alternativi e la PDT sembra essere un potenziale candidato. Molteplici sono stati gli studi *in vitro* che hanno dimostrato la sensibilità dei dermatofiti e di *Candida spp.* alla PDT; quelli effettuati *in vivo* sino ad oggi sono poco rappresentativi, essendo limitati in numero e condotti su un numero piuttosto esiguo di pazienti. Considerando l'efficacia della tecnica ottenuta negli studi *in vitro* e l'importanza di sviluppare nuove strategie terapeutiche nel trattamento delle micosi superficiali, la PDT è un ambito di grande interesse per futuri studi clinici di ricerca.

Parole chiave: Agenti antifungini - Micosi - Candida.

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Psoriasis or "Psoriases"?

A. BALATO, L. DI COSTANZO, C. PATRUNO, F. AYALA, M. MEGNA, N. BALATO

Department of Dermatology
University of Naples Federico II, Naples, Italy

Psoriasis is now considered a systemic immunomediated disease with different subclinical types; is it one disease or could it be more appropriate to redefine psoriasis as not a single dermatosis but as multiple dermatoses named "psoriases"? This question is basic to introduce a new interpretation of known concepts regarding psoriasis.

The hypothesis of "psoriases" could be supported by differences in the age of onset. "Early" psoriasis (onset before 30 years) seems to be more common in females, more severe with an unstable course and associated with a psoriasis family history,[1] whereas "late" psoriasis (onset after 40-60 years) have a milder course of progression.[2] Moreover, it has been observed that "early" and "late" psoriasis differ in their genetic background.[2]

In addition, different cytokine patterns underlie distinct subclinical types, reinforcing the idea of multiple dermatoses; *e.g.* Forkhead box P3+ regulatory T cells levels are lower in guttate psoriasis compared with the plaque form,[3] whereas interleukin-33 (IL-33) is induced,[4] loss of IL-36 receptor antagonist protein results in susceptibility to generalized pustular psoriasis [5] and IL-17 mRNA levels were distinctively higher in pustular psoriasis.[6]

How can psoriasis be considered a unique entity when the prevalence greatly differ, depending on the countries? In Europe, prevalence varies from 0.6% to 6.5%, whereas in USA, is 3.15%. In Africa it varies upon geographic location, being lowest in West Africa;[7] dietary habits and absence of specific alleles have been correlate to low incidence of the disease in Africans.[8] Psoriasis is less frequent in China and Japan than in Europe, and is absent in natives of the Andean region of South America.[7] This prevalence variability is usually explained by environment influence on genetic factors. In a recent report we have found that environmental factors may condition the clinical course of psoriasis.[9] A provocative concept may be raised: not one disease influenced by the environment, but distinct diseases! Obviously, genetics represent the basis of psoriasis pathogenesis and genetic variance may precisely sustain "psoriases" hypothesis. The major genetic determinant of psoriasis is psoriasis susceptibility gene 1 (PSORS1). A strong association with the human leukocyte antigen-C (HLA-C) allele, especially HLA-Cw*0602, in the PSORS1 locus has been found. The most significant association exists between HLA-Cw*0602 polymorphism and psoriasis risk in Caucasian and Asian populations. Homozygosity for HLA-Cw*0602 predisposes to earlier onset, but it otherwise does not impact clinical course.[10]

Based on a genetic disposition, psoriasis can cause clinical relevant changes in the skin and joints. In addition, other distinguished medical conditions may simultaneously exist, but without a recognized causal link.[11] The most frequent comorbidities are represented by cardiovascular diseases with asso-

Corresponding author: Dr. L. Di Costanzo, Department of Clinical Medicine and Surgery, Federico II University of Naples, Via S. Pansini 5, 80131 Naples, Italy. E-mail: luisadicostanzo@virgilio.it

ciated risk factors (obesity, arterial hypertension, dyslipidaemia and type-2 diabetes mellitus). Why some psoriasis patients are affected by comorbidities whereas some other not? May be explained by the existence of "psoriases"? Furthermore, "psoriases" could explain different responses to anti-Tumor Necrosis Factor-alpha (TNF-α) therapy; in fact polymorphisms in TNF-α genes have been associated with predictable response to anti-TNF-α therapy in patients with psoriasis.[12] Differences regarding the efficacy of anti-TNF-α, as well as other biological treatments, may be linked to intrinsic peculiarities of the dermatosis, reinforcing the "psoriases" hypothesis. Indeed, it is known that much attention is given nowadays to the development of new biological therapies for psoriasis. The literature is enriching day by day of studies and clinical trials regarding different types of biologics such as monoclonal antibodies against IL-17,[13] IL-17 receptor,[14] and p40 subunit of IL-12 and IL-23.[15] The high number of biological medical products, their diverse mechanism of action and, therefore, diverse molecular targets, as well as the various and changeable percentage of efficacy of these treatments highlight the fact that psoriasis should not be considered as a single entity. There is a plenty variety of psoriasis forms, each one with peculiar pathogenic, clinical and therapeutic features so we should better redefine the concept of psoriasis as multiple dermatoses named "psoriases". All this should be considered in approaching to psoriasis patients, putting the basis to reaching the final goal of the clinician: choosing the right therapy in the right patients at the right time.

This letter was conceived to launch the innovative idea and the original term "psoriases". This proposal is based, in our opinion, on differences regarding: age of onset, cytokine patterns underling distinct subclinical types, geographic distribution, environmental and genetic factors, associated comorbidities as well as response to anti-TNF-α therapy. We have unquestionably discussed just some of the aspects possibly involved, but the intention was to ignite a debate. Although the concept of "psoriases" open the mind to a new interpretation of the literature, we reckon that it needs to be deeper investigated, in order to render it less philosophic and more scientific.

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Topical steroids and corticophobia

A. BELLONI FORTINA [1], L. NERI [2, 3]

[1]Pediatric Dermatology Unit, Department of Medicine University of Padua, Padua, Italy
[2]Department of Clinical and Community Sciences Milan Univerity, Milan, Italy
[3]"Clinica del Lavoro Luigi Devoto" Ca' Granda – Ospedale Maggiore IRCCS Foundation Policlinico, Milan, Italy

Steroids are the topical treatment of choice for the management of acute and chronic skin inflammation in patient with a variety of dermatological conditions. Since Sulzberger and Witten's seminal paper (1952) advocating topical steroids to treat dermatological diseases in the early '50s,[1] their use has radically changed the approach to common inflammatory conditions, particularly psoriasis and eczema. Topical steroids (TS) provide a powerful and fast anti-inflammatory effect, small vessels vasoconstriction, and anti-mitotic action. Additionally they are easy to use and are only marginally absorbed into the systemic blood flow. Due to these promising properties topical steroidal formulations rapidly became very popular. Even though complications of topical steroid use are relatively rare,[2-4] their widespread use corresponded with an increasing public concern toward possible adverse effects, especially in case of prolonged treatment courses.[5-8]

Part of the concerns are possibly due to the fact that TS mechanism of action is not fully understood yet. Both a genomic and a non-genomic pathway has been shown in animal and in vitro experiments. The genomic pathway implies that steroids interact with nuclear receptors and ultimately promote the transcription of genes with anti-inflammatory functions (*e.g.* Tyrosine Amino-Transferase, IL-10, β-adrenergic receptor, etc) whereas they inhibit the activity of pro-inflammatory genes (*e.g.* cytokines, growth factors, prostanoids, etc).[9] The non-genomic mechanism of action is responsible for fast-acting effects and involves the modulation of monocytes, T-cells and piastrine reactivity with no synthesis of new proteins.[9]

Hydrocortisone was the first molecule used as a topical steroid; in the following years several new derivatives had been developed with different equivalent-doses.[10] Since both efficacy and the occurrence of side effects are dose-dependent outcomes, the choice of a specific TS should always balance expected benefits and risks, relative to patients' age, the severity, dimension and localization of skin lesions, and the projected duration of treatment.

The emergence of corticophobia

Given the poor correlation between the likelihood of side-effects and patients' concerns, the negative attitude toward corticosteroids has been described as an irrational fear, a condition called *Corticophobia*. Since patients' misconceptions toward TS therapy is a leading cause of non-adherence,[5, 11] it has been suggested that prednisone-phobia should be considered as a clinical entity, needing specific treatment and

Corresponding author: L. Neri, MD, PhD, Department of Clinical Science and Community Health, Milan Statal University, Via San Barnaba 8, 20122 Milan, Italy. E-mail: luca.neri@unimi.it

TABLE I.—*Classification of "prednisone-phobia".*

Type	©Source:
Interpersonal	Friends, relatives, acquaintances
Bibliographical	magazines and newspapers
Parental	Parents and siblings
Iatrogenic	Physician

Adapted from Patterson *et al.*[5]

management strategies aimed at improving patients' compliance. In order to address the most common sources of irrational fears and unfounded beliefs toward chronic steroid use, Patterson [5] has proposed the classification reported in Table I.

The first study discussing the issue of "steroid-phobia" dates back to 1979, when L. Tuft noted a raising public concerns toward the use of steroids in the management of Asthma vis-à-vis their limited side effects.[12] Since then an excessive reluctance to accept treatment with this class of drugs has been repeatedly reported.[5, 13] Paradoxically, there is evidence that patients' reluctance to undergo a treatment course with steroids is stronger in those with more severe symptoms.[13] A recent survey has shown that 58% of French dermatologists and primary care physicians consider *Corticophobia* a relevant clinical problem.[14] Most dermatologists believe that patients' concerns toward TS prescriptions are even stronger in pediatric contexts. The strength and persistence of public attitudes toward TS gradually modified physicians prescription patterns. Whereas patients' irrational safety concerns may reduce prescription adherence and therapy effectiveness,[14] physicians became more sensitive to patients' preferences and adapted treatment regimens to minimize side effects. Additionally new and effective drugs have been developed in the attempt to satisfy market pressure toward less intrusive treatment courses. This long-lasting effort resulted in safer molecules with greater specificity of action, reduced tachyphylaxis, fast skin clearance, reduced transcutaneous absorption, and low sensitization potential.

Patients' concerns and beliefs toward steroid therapy

The first estimate of the prevalence and antecedents of corticophobia in patients with Atopic Dermatitis (AD) has been published in 2000 from a survey of 200 subjects referred to the Queen's Medical Centre di Nottingham.[8] The authors observed that skin atrophy (34.5%), uncertainties around long-term side effects (24%), systemic exposure through dermal absorption, developmental and growth effects (9.5%) coupled confusion around the equivalent dose of different TS formulations were patients' main concerns.

Even if corticosteroid-related skin atrophy was a common patients' concern, only few studies compared its occurrence either across different steroid classes, treatment duration, anatomical sites, or age groups. In most cases, exposure to high steroids doses was not associated to permanent lesions; however such studies enrolled healthy volunteers only and generalizations to patients groups with likely increased skin permeability could not be established.[2, 3] In a small study on 13 patients with atopic dermatitis aimed at addressing this important issue, no patient developed clinically significant skin atrophy during the treatment course with a powerful steroid applied to the lesions once daily for 20 days.[15]

Nevertheless the concerns around steroids safety profile persisted until nowadays. In a large survey of 1558 patients with a clinical history of atopic dermatitis, only 16% stated no objections in completing a full treatment course with topical steroids. The rest of patients would have refused to adhere to TS prescriptions even though almost all of them (97%) had taken such drugs before. The most common patients' concerns were unsatisfactory effectiveness on the long term (tachyphylaxis), side effects (*e.g.* hyper- or hypo-pigmentation, rosacea-like dermatitis, skin atrophy, infections, telangiectasia, ocular complications, etc), and safety issues raised by primary care physicians.[16] In a later survey,[17] the authors found that about 2 every 5 parents of children with non-eczematous skin disease and mild eczema and 3 every 5 parents of those with moderate/severe disease reported at least some concern about TS safety. Additionally about 50% of parents asked information about steroid-sparing medications (*e.g.* TIMs) in cases of moderate/severe eczema and many of them considered TS therapy as the last resort remedy for persistent eczema only due to its possible side-effects. Parents most frequently reported the fear of skin atrophy and drug-related growth disorders.

In a recent, very small survey, parents of children with AD reported a strong preference for "safer natural therapy", fear of TS side-effects, and a strong

feeling of guilt related to the chronic nature of the disease which cannot be eradicated. Parents reported to rely on trusted persons, positive hospitalization experiences and on the relationship with a trusted dermatologist as key sources of information. Parents also stated that free access to readily available information and to qualified pediatric dermatology services would enhance trust in TS therapy.[18]

Zuberbier and colleagues (2008) further investigated the emotional reactions to TS treatment with a self-administered web-survey among physicians (N.=453), pharmacists (N.=200), healthy volunteers (N.=1632) and patients with allergic rhinitis (N.=406), and AD (N.=401). Evidence of corticophobia was observed in 65% of patients with allergic rhinitis and 43% those with AD pts. A vast majority of patients believed that TS therapy would inevitably cause side effects.[19]

A recent cross-section survey further evaluated emotional and medical correlates of cortico-phobia in 208 parents of children and adult patients with AD. Irrational fear of TS was associated with the need for reassurance, the belief that TS would have a systemic effect, prior adverse events, inconsistent information TS administration procedures, and the desire to self-treat for as short a time period as possible. Most importantly, patients with evidence of cortico-phobia reported poor treatment adherence.[7]

Health education and communication issues

A frequent finding in previous surveys of corticophobia was that patients and physicians partially shared similar concerns toward steroidal therapy even though its benefits in terms of survival and quality of life had been largely proven.[5, 16, 20] Physicians' concerns also might influence treatment decision and patients attitude toward TS therapy.[19] Additionally, it has been shown that only a minority of patients gathered relevant information about TS from health care professionals (primary care physicians or pharmacist) whereas the time spent by dermatologists and nurses in discussing with patients about the treatment was associated to better adherence and health outcomes.[8] In a recent study carried out by the Dermatology Working Group among physicians in the UK, the authors observed a higher rate of treatment failures when doctors advised their patients to "sparingly" or "thinly" apply TS. The authors stated that physicians should inform and periodically supervise patients about correct administration procedures. They also recommend that TS products include clear "fingertip unit" instructions, preferably with images and charts showing the number of units required for specific body surfaces (Table II).[21] Consistent with this evidence, it has been shown that health education modules might strongly improve adherence and clinical outcomes in children with atopic dermatitis.[22]

Even though patients might greatly benefit from health education programs, only a minority of them have sufficient interactions with their doctors to thoroughly discuss their concerns and beliefs toward TS therapy.[17] In a different study parents of children with chronic skin diseases reported to rely on trusted persons, positive hospitalization experiences and on the relationship with a trusted dermatologist as key sources of information. Parents also stated that free access to readily available information and to qualified pediatric dermatology services would enhance trust in TS therapy.[23]

Hence, *Corticophobia* represents a symptom of inefficient physician-patient communication, where both patients' mistrust toward medicine and unsatisfactory clinical effectiveness due to incomplete treatment adherence generate a vicious circle which is often difficult to eradicate. Since all chronic therapy

TABELLA II.—*Dose schedule based on Finger tip units (FTU = Finger Tip Unit) by age class.*

Age	Number of FTU needed to treat surface				
	Hand or genital area	Face and neck	Upper limb	Lower limb	For the chest
3-6 months	0.5	1	1	1.5	1.5
1-2 years	0.5	1.5	1.5	2	3
3-5 years	0.5	1.5	2	3	3.5
6-10 years	1	2	2.5	4.5	5
Adult	1	2	3	6	14

FTU: amount of ointment, cream or other semi-solid dosage form expressed from a tube with a 5 mm diameter nozzle, applied from the distal skin-crease to the tip of the index finger of an adult which corresponds to 0.5 g of cream

may possibly yields side-effects, health care professionals should describe potential risks and benefits of each treatment option and share clinical decision making with patients.

Conclusions

Corticophobia is a widespread phenomenon with profound consequences on treatment adherence which in turn affect overall TS effectiveness. Patients and parents with unfounded fear of TS may under-use or waste prescribed steroids treatments in the attempt to avoid rare or mild side effects. Incomplete, erroneous or misleading information may represent a strong barrier to the uptake of evidence-based treatment regimens in clinical practice. Hence, it is important to understand the prevalence and antecedents of corticophobia in order to develop appropriate health education interventions. Since TS is a key treatment option both in pediatric and adult patients with several dermatologic diseases, they should be reassured that the safety of modern TS therapy is well-established when appropriate regimens are adopted.

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Psoriasis vulgaris does not adversely influence the quality of sleep

G. STINCO [1], G. TREVISAN [2], F. PICCIRILLO [1], N. DI MEO [2], K. NAN [2], L. DEROMA [3], S. BERGAMO [1], P. PATRONE [1]

***Aim*. Sleep could be severely affected in psoriasis because of skin symptoms and psychological repercussions of the disease. The aim of this study was to investigate the influence of psoriasis on sleep.**

***Methods*. A total of 202 patients with psoriasis and 202 healthy volunteers have completed a self-rated questionnaire, the Pittsburgh Sleep Quality Index, which assesses sleep quality and disturbances over a 1-month time interval. The severity of the dermatoses has been evaluated utilizing the PASI score.**

***Results*. In psoriatic patients the Pittsburgh Sleep Quality Index resulted between 0 and 17 (5.56±3.93), in the controls between 0 and 18 (5.13±4.16). No statistically significant correlation was observed between the score of Pittsburgh Sleep Quality Index and PASI. The anti-psoriatic therapy, while causing a marked improvement of lesions and itching, does not affect the quality of sleep.**

***Conclusion*. Although literature indicated that psoriasis negatively affects the quality of sleep, in this study this correlation was not observed.**

KEY WORDS: Psoriasis - Sleep - Pruritus.

[1]Institute of Dermatology
Department of Experimental and Clinical Medicine
University of Udine, Udine, Italy
[2]Institute of Dermatology and Venereology
University of Trieste, Trieste, Italy
[3]Institute of Hygiene and Epidemiology
University of Udine, Udine, Italy

Psoriasis, a chronic and relapsing dermatitis, is responsible of deep alterations in the quality of life, resulting in physical and mental disabilities.[1, 2] Sleep, although has not been widely studied, is one of the activities that are more severely affected due to the cutaneous sensory symptoms of the disease.[3] Many factors may interfere with sleep such as coexisting medical problems and stressors (*e.g.*, social, personal, occupational, etc.) and between them recently Literature has focused on pruritus, which is associated with psoriasis with percentages ranging between 63% and 90%, as one of the most important cutaneous symptoms causing psycho-physical discomfort.[4] Beside that, other psychological factors deriving from the presence of the skin disease, such as guilt, shame, stress, may affect sleep quality.[5] Sleep and stress influence each other in psoriasis. Sleep deprivation increases the production of stress hormones and therefore it can impair sleep quality.[6, 7] Furthermore the cytokines involved in the pathogenesis of psoriasis such as IL-6 and TNF alpha, play an important role also in the pathogenesis of excessive daytime sleepiness, sleep disorders and sleep deprivation.[8, 9] However, there are no studies that test in depth the association between cutaneous symptoms and sleep disturbances.[10]

The aim of the present study was to investigate the influence of psoriasis on sleep, searching for any correlation between sleep quality and the severity of illness, itch and the relationship with treatment.

Materials and methods

A case-control study was performed. Two hundred and two out-clinic and hospitalized patients ad-

Corresponding author: G. Stinco, Institute of Dermatology, University of Udine, Gemona Hospital, Piazza Rodolone 1, 33013 Gemona del Friuli, Udine, Italy. E-mail: giuseppe.stinco@uniud.it

dressing to the Dermatology Clinics of the University Hospital of Udine and Trieste with a diagnosis of psoriasis vulgaris (group A) were enrolled. The only inclusion criterion was the presence of plaque psoriasis; arthropatic psoriasis, pustular psoriasis and other complicated forms of psoriasis have been excluded. Other exclusion criteria were the presence of co-existing medical problems (*e.g.*, obstructive sleep apnea, restless leg syndrome, congestive heart failure, depression) and stessor factors that might affect the quality of sleep. An equal number of age- and sex-matched healthy controls (group B) was recruited between out-clinic patients observed for nevi check-up. Once an informed signed consent to join the study was obtained, all subjects were given a two-parts questionnaire. In the first part, given to both groups, data (gender and age) were recorded and the Italian validated version of "Pittsburgh Sleep Quality Index" (PSQI) questionnaire evaluating the quality of sleep during the month preceding the interview, was given[11,12]. Such questionnaire is easy to handle and can be completed within 5 minutes. PSQI is a self-rating questionnaire investigating at what time the patient usually goes to sleep and wakes up, how long he takes to fall asleep and the actual hours spent in sleep. There is a number of questions about the frequency (assessed with four variables) of several problems that the patients might have experienced in sleep during the last month. Finally, PSQI ends up with a subjective assessment of sleep quality, using a four-point scale. The PSQI results in a global score ranging between 0 and 21, consisting of seven subscores between 0 and 3. The closer to 21 the final score is, the poorer quality the sleep has.

In the second section, a survey given only to psoriatic patients group, the duration of psoriasis, the presence or absence of itching and the effect of the therapy for psoriasis (topical medications, systemic drugs, or phototherapy) on the pruritus and sleep were investigated.

The severity of psoriasis has been always evaluated by the same dermatologist using the "Psoriasis Area and Severity Index" (PASI) score.

This study has been approved by the Internal Board of University of Udine.

Statistical analysis

The categorical variables were described using absolute frequency and percentage frequency, whereas the continuous variable using mean±standard deviation (DS). The chi-square test was used in order to evaluate the association between categorical variables, but in the absence of a sufficient number of data and in the event that might not be valid, Fisher's exact test was used. The difference between the means was estimated using Wilcoxon test because all of the variables were not normally distributed according to Shapiro-Wilk test. For the comparison between means the Student's t test was used for variable that were normally distributed according to Shapiro-Wilk test. A $P<0.05$ was considered statistically significant. All statistical analyses were conducted with the Sas System Software, version 6.2.

Results

In total, 404 questionnaires were completed, resulting in a response rate of 100%.

Group A

The psoriatic patient group consisted of 202 patients, 118 males (58.4%) and 84 females (41.6%), aged between 21 and 87 years (mean age 50.75±16). The average duration of psoriasis was 16 years±10.25, with a minimum of 2 months and a maximum of 46 years of illness. The mean PASI score was 8.96±7.99, 124 patients (61.4%) having a mild psoriasis (PASI score ≤10, mean 7.2±3.1), 62 (30.7%) with a moderate psoriasis (PASI score between 10 and 20, mean 14.3±5.7) and only 16 patients (7,9%) with a severe psoriasis (PASI score ≥20, mean 28.7±6.2). PSQI score was between 0 and 17 (mean score of 5.56±3.93). One hundred thirteen patients (55.9%) had a PSQI score ≤5, 76 (37.6%) had a PSQI score between 5 and 10 and the remaining 13 patients (6.4%) had a score >10. Comparing the PSQI score with the PASI score, not statistically significant relation ($P>0.05$) was recorded, and then no relationship between the severity of psoriasis and impaired quality of sleep was observed (Figure 1). Itch was present in 116 patients (57.4%), absent in the remaining 86 (42.6%). All of the 116 patients with itching were treated with antipsoriatic therapy: 30 with immunosuppressive agents, 25 with phototherapy, the remaining with topical medications. Among these

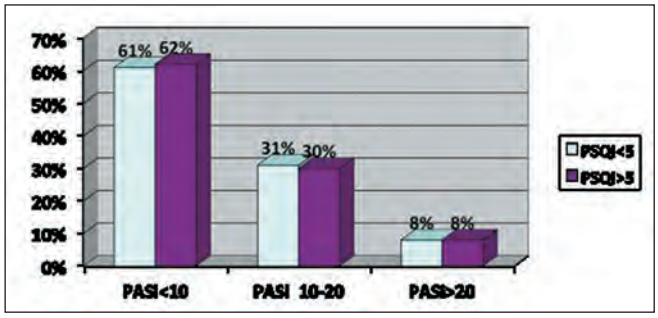

Figure 1.—PASI and PSQI:no relationship between the severity of psoriasis and impaired quality of sleep was observed (P>0.05).

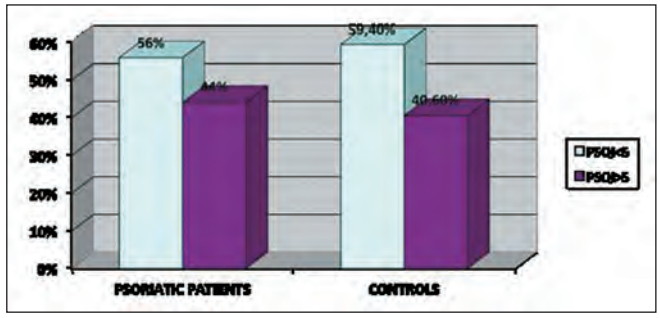

Figure 2.—The figure shows the impairment of the quality of sleep (PSQI≤ or >5) between controls and psoriatic patients. No significant differences were observed between the two groups (P>0.05)

116 patients with pruritus, 82 (70.6%) reported an improvement of pruritus with the current antipsoriatic treatment (P≤0.05) while 34 (29.4%) did not. Among the 82 patients who reported itching improvement due to the antipsoriatic therapy, only 21 patients (25.6%) noticed a concomitant improvement of the quality of sleep. Our study shows that whereas the antipsoriatic therapy and the subsequent clinical improvement is well related to the attenuation of the itching, it does not correlate with the improvement in sleep quality. Most of the patients in which we have observed an improvement of the quality of sleep had a PASI score >10 (18 patients out of 21).

Group B

The control group consisted of 202 subjects, 100 males (49%) e 102 females (51%) aged between 17 and 83 (mean 52.23±12). The PSQI score of the whole control group ranged between 0 and 18 with a mean of 5.13±4.16. One hundred twenty (59.4%) patients had a score of PSQI≤5, 75 (37.1%) a score between 5 and 10, and 7 patients (3.5%) had a score > 10.

Group comparison

The mean age, sex, the absence of other factors that influence the quality of sleep, such as coexisting medical conditions and the PSQI score between psoriatic patients and healthy volunteers were comparable (P>0.05) so that psoriasis does not seem to affect the sleep quality of the patients (Figure 2).

Discussion and conclusions

In Literature the impact of psoriasis on quality of sleep has not been extensively investigated, and sleep disturbances have not been fully detailed. Some studies indicated that psoriasis is significantly associated with sleep disorders and insomnia,[12-14] but the factors impairing sleep in those patients have not been well defined. Several conditions have been considered as potential disturbing factors in psoriatic patients, such as itch, stress,[2, 15, 16] depression and psychologic distress that are very common in patients with psoriasis, obstructive sleep apnea observed in 36% of patients with psoriasis and pain of arthropathic psoriasis.[14, 17] Among them, itching has been identifed as the main responsible for sleep abnormalities in patients with psoriasis.[4, 18, 19] This observation is supported by the existence of intimate "chemical bonds" between pruritus, sleep and psoriasis since some pro-inflammatory cytokines such as IL-6 and TNF-alpha, are involved not only in the pathogenesis of psoriasis but also in sleep deprivation and excessive daytime sleepiness in sleep disorders.[8, 9]

The associations between the above mentioned symptoms and sleep impairment in psoriasis are complex. It is not clear whether sleep disturbance in psoriasis is caused by a single or a combination of these factors. In our study we excluded all patients with medical problems or stressor factors leaving only psoriasis and the eventual associated itch as possible influencing factors of sleep.

According with data in Literature, in our study itch affected 57.4% of the psoriatic patients. There is not a statistically significant difference in the PSQI and consequently in the sleep quality between the

psoriatic patients and controls so that psoriasis does not seem to affect sleep quality. It is possible that the association between sleep disturbance and pruritus might be correlated with scratch intensity and rarely it was so severe in psoriatic patients as in other skin disease. Scratching has been demonstrated to be more related to the physiology of the sleep stage than to the skin disease itself.[20] The antipsoriatic therapy, while causing a marked improvement of lesions and itching ($P \leq 0.05$), does not improve sleep quality ($P \geq 0.05$), confirming that the clinical improvement of psoriasis does not correlate with the improvement of sleep quality. Furthermore our data indicate that the few patients referring a sleep quality improvement with the antipsoriatic therapy, have mostly a mild psoriasis. It could be speculated that patients with a mild psoriasis had an easier and faster remission of the dermatosis, with less psychological distress and consequently improvement of the quality of sleep. Probably not only the skin symptoms, but also the acceptance of their condition, are important factors affecting the psychological wellbeing of a person.[21, 22] In a study Zachariae *et al.* stressed that the most important factor in creating sleep disturbances is the negative psychological impact conferred by the disease and its cutaneous symptoms.[18]

We stress the need for additional investigations, where better tools of assessment of sleep may be used, in order to really understand whether there are actual implications of psoriasis on sleep. So far there is no standardization in the tools used in the various studies. Some investigators used the Brief Pain Inventory, a pain assessment tool easy to use, which quantifies the intensity of pain and disability in patients. This tool investigates whether the patient's sleep is altered by the skin symptoms of the disease, referring to the previous 24 hours.[17] In other studies the 4-Itch item questionnaire is used, which assesses the frequency, severity, location of itching and sleep disturbance caused by itching.[22] Recently Ljoosa *et al.*[23] used the Generale Sleep Disturbance Scales and the Illness Perception Questionnaire to explore whether sleep disturbance and psychological distress might be mediators of the association between skin pain or skin discomfort and health related quality of life. In our study we used the PSQI, a quite convenient instrument to measure the sleep quality, investigating several possible causes of sleep disturbances, not only referring to skin symptoms. We chose the PSQI due to these characteristics, even if the survey is limited to a short period of time (one month) and above all it does not allow to distinguish the various causes that may provoke sleep disturbances. In addition, the PSQI does not detect the quality of the sleep before the illness development and the presence of problems not concerning with the dermatosis itself. Probably the best tool in order to objectively define a sleep disturbance would be the study through the polisonnografy, also proposed in other studies but not yet implemented.

Riassunto

La psoriasi non influenza negativamente la qualità del sonno

Obiettivo. Il sonno può essere severamente disturbato nei pazienti psoriasici a causa dei sintomi cutanei e delle ripercussioni psicologiche della patologia. L'obiettivo di questo studio è indagare l'eventuale influenza della psoriasi sul sonno.

Metodi. In totale 202 pazienti affetti da psoriasi e 202 volontari sani hanno completato il Pittsburgh Sleep Quality Index, un questionario di autovalutazione che indaga la qualità del sonno e gli eventuali disturbi in un intervallo di tempo di circa un mese. La severità della psoriasi è stata valutata utilizzando il PASI.

Risultati. Nei pazienti psoriasici lo score del Pittsburgh Sleep Quality Index risultava compreso tra 0 e 17 (5,56±3,93), nei controlli risultava tra 0 e 18 (5,13±4,16). Non sono state osservate correlazioni statisticamente significative tra gli score del PASI e del Pittsburgh Sleep Quality Index. La terapia anti-psoriasica produce un marcato miglioramento delle lesioni e del prurito, ma non influenza la qualità del sonno.

Conclusioni. Sebbene la letteratura indichi che la psoriasi influisce negativamente sulla qualità del sonno, in questo studio tale correlazione negativa non è stata osservata.

PAROLE CHIAVE: Psoriasi - Sonno - Prurito.

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Efficacy of food supplement to improve metabolic syndrome parameters in patients affected by moderate to severe psoriasis during anti-TNFα treatment

N. SKROZA, I. PROIETTI, N. BERNARDINI, G. LA VIOLA, F. NICOLUCCI, R. PAMPENA, E. TOLINO
S. ZUBER, M. T. MANCINI, V. SOCCODATO, V. BALDUZZI, C. POTENZA

***Aim*. Psoriasis is a systemic inflammatory immune-mediated skin disease. Recently a relationship with metabolic syndrome in terms of psoriasis severity and response to therapy was observed.**

***Methods*. We performed an open-label randomized controlled study to evaluate the role of a nutraceutical containing Q10 coenzyme, Krill-oil, lipoic acid, resveratrol, Vitis vinifera seed oil, vitamin E and selenium in addition to etanercept therapy for patients affected by psoriasis and metabolic syndrome. Forty patients were enrolled and divided into two arms, one receiving only etanercept, one other receiving also the neutraceutical. After a period of 3 months (T1) a second evaluation of the considered parameters was performed.**

***Results*. At T1 statistically significant differences were detected in HDL cholesterol and triglycerides values both comparing the two arms and in the nutraceutical arm.**

***Conclusion*. Our results show that the dietary addiction of the nutraceutical to the etanercept therapy in patients affected by both psoriasis and metabolic syndrome could help to restore the normal lipid profile.**

KEY WORDS: Psoriasis - Metabolic syndrome X - Oxidative stress.

Department of Dermatology "Daniele Innocenzi"
Sapienza University of Rome, Polo Pontino, Rome, Italy

Psoriasis is a systemic inflammatory immune-mediated disease, primarily involving the skin, characterized by chronic-relapsing course and a great impact on quality of life.[1, 2] It is frequently associated with significant comorbidities such as arthritis (24.1%), hypertension (21.2%), dyslipidemia (7.4%) and obesity (11.9%).[3] Recently a tight relationship with metabolic syndrome in terms of psoriasis severity and response to therapy was observed. The link between psoriasis and the different aspects of metabolic syndrome seems to be the state of chronic inflammation mediated by increased levels of TNF alpha, protein-1 (macrophage chemotactic factor), PAI-1 (plasminogen activator inhibitor), IL-6, leptin and adiponectin.[4] Obese patients have a lower response to biologic therapy, even in presence of the same pro-kg dosage, since fat tissue is a constant supply of pro-inflammatory cytokines. Consequently, it's important to consider and control metabolic syndrome when managing psoriasis. Treatments with biological agents (anti-TNF alpha and anti-IL12/23) reduce the risk of developing co-morbidities associated with psoriasis such as metabolic syndrome.[5] The use of nutraceuticals in psoriatic patients could improve metabolic imbalance and oxidative stress together with a correct lifestyle, physical activity, food and alcohol intake.[6]

For this reason we performed an open-label randomized controlled study to evaluate the role of a nutraceutical containing Q10 coenzyme, Krill-oil, lipoic acid, resveratrol, Vitis vinifera seed oil, vitamin E and selenium in addition to etanercept therapy for patients affected by psoriasis and metabolic syndrome (Table I).

Corresponding author: C. Potenza, Department of Dermatology "Daniele Innocenzi", Sapienza University of Rome, Polo Pontino, Via Firenze, 04019 Terracina, Latina, Italy.
E-mail: concetta.potenza@uniroma1.it

TABLE I.—*Nutraceutical composition.*

Composition	mg
Q10 coenzyme	50
Krill oil	300
Lipoic acid	150
Resveratrol	20
Vitis vinifera seed oil	30
Vitamin e	36
Selenium	27.5

Materials and methods

Study population

Forty patients affected by psoriasis and metabolic syndrome attending the Dermatology Department of Sapienza University of Rome - Polo Pontino - Italy, in treatment with etanercept 50 mg/week for a period of 3 to 12 months were enrolled in the study.

Patients with diagnosis of psoriatic arthritis and/or diabetes, age ≥65 year old, Body Mass Index (BMI) ≥30 or ≤20, months of therapy with etanercept >12 or <3, under therapy with anti-diabetes drugs, insulin, lipid lowering agents, statin or anti-hypertensive drugs were excluded from the study.

Metabolic syndrome was considered, according to NCEP ATP III criteria, in presence of 3 or more of the following criteria: fasting glucose >110 mg/dL, blood pressure >130/>85 mmHg, HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, triglycerides >150 mg/dl and central obesity (waistline >120 cm in men and >88 cm in women).[7]

Study population was exactly divided into two arms homogenous for gender, age, weight, height, BMI, Psoriasis Area Severity Index (PASI), Dermatology Life Quality Index (DLQI) and PSODISK scores, months of therapy with etanercept and for the parameters of metabolic syndrome (blood sugar levels, waistline, HDL cholesterol, triglycerides levels, and blood pressure) (Table II).

One arm continued receiving etanercept 50mg weekly; the other arm received etanercept 50mg weekly and 1cp daily of a nutraceutical containing Q10 coenzyme, Krill-oil, lipoic acid, resveratrol, Vitis vinifera seed oil, vitamin E and selenium.

After a period of 3 months (T1) a second evaluation of the parameters of metabolic syndrome, weight, BMI, PASI, DLQI and PSODISK scores was performed.

Informed consent was obtained from each patient, following full written and oral explanation.

Statistical analysis

For the total number of patients and for the two arms, mean values and standard deviation (SD) were calculated considering age, weight, height, BMI, PASI, DLQI and PSODISK scores, months of therapy with etanercept, blood sugar levels, waist-

TABLE II.—*Baseline – T0 (mean values).*

	Controls	Nutraceutical	P value
Males	9	12	>0.05
Females	11	8	
Age	47.85±12.4	49.25±10.2	>0.05
M of T	6.6±2.5	7.0±2.6	>0.05
Height	171.9±9.8	172.35±11.75	>0.05
Weight	80.4±10.6	83.15±9.5	>0.05
BMI	27.15±2.3	28.0±1.8	>0.05
PASI	6.0±2.5	4.5±2.85	>0.05
DLQI	10.9±2.4	11.0±2.15	>0.05
PSODISK	48.4±8.1	46.85±7.0	>0.05
BSL	114.6±5.6	112.8±8.9	>0.05
BP max	135±13.5	137±13.7	>0.05
BP min	84.15±11.9	79.5±11.3	>0.05
HDL-C	43.0±4.0	45.4±7.9	>0.05
TG	130.8±34.1	141.8±32.5	>0.05
WL	94.15±12.0	96.05±11.7	>0.05

M of T: months of therapy with etanercept; BMI: Body Mass Index; PASI: Psoriasis Area Severity Index; DLQI: Dermatology Life Quality Index; BSL: blood sugar levels; BP: blood pressure; HDL -C: high density lipoprotein – cholesterol; TG: triglycerides; WL: waistline.

line, HDL cholesterol, triglycerides levels, and blood pressure. Student's t-test and chi-square test were used for quantitative and qualitative variables, respectively. Statistical analysis was performed with SPSS for Windows, release 13.0 (SPSS, Chicago, IL, USA) and SPSS for Windows, release 19.0. Statistical significance was fixed at P<0.05.

Results

We enrolled 40 consecutive patients (21 male patients and 19 female patients, median age 48.55±11,2).

The arm receiving both the nutraceutical and etanercept consisted of 20 subjects (12 males and 8 females) with a mean age of 49.25±10.2. The control arm (only etanercept) consisted of 20 patients (9 males and 11 females) with a mean age of 47.85±12.4 (Table II).

At T1 statistically significant differences were detected, comparing the two arms, regarding HDL cholesterol (45.1±3.8 in controls and 51.8±8.3 in nutraceutical arm – P<0.05) and triglycerides (147.3±28.2 in controls and 120.4±32.8 in nutraceutical arm – P<0.05) (Table III).

Even if not in a statistically significant way, all considered parameters improved at T1 in the control arm, except for triglycerides levels (130.8±34.1 at T0 and 147.3±28.2 at T1) (Table IV).

After 3 months, an improvement of all the considered parameters was observed in the nutraceutical arm, with statistically significant differences for HDL cholesterol (45.4±7.9 at T0 and 51.8±8.3 at T1 – P<0.05) and triglycerides (141.8±32.5 at T0 and 120.4±32.8 at T1 – P<0.05) (Table V).

At T1, 2 patients among controls and 6 in the nutraceutical arm didn't show anymore the diagnostic criteria for metabolic syndrome (Table VI).

Finally we compared the percentage of improvement of the considered parameters between T0 and T1 in the control arm vs. the nutraceutical one and a statistically significant improvement was found for HDL cholesterol (5.1±7.9% in controls and

TABLE III.—*Follow up – T1 (mean values).*

	Controls	Nutraceutical	P value
Weight	79.3±10.8	82.3±9.5	>0.05
BMI	26.8±2.35	27.7±1.9	>0.05
PASI	5.0±2.5	3.9±2.7	>0.05
DLQI	10.25±2.0	10.2±2.2	>0.05
PSODISK	45.0±9.3	41.9±7.7	>0.05
BSL	111.5±7.7	107.0±10.8	>0.05
BP max	129±11.1	132.25±13.5	>0.05
BP min	79.0±8.5	78.0±10.2	>0.05
HDL-C	45.1±3.8	51.8±8.3	<0.05
TG	147.3±28.2	120.4±32.8	<0.05
WL	92.65±11.6	94.35±11.6	>0.05

TABLE IV.—*Control arm – T0* vs. *T1 (mean values).*

	T0	T1	P value
Weight	80.4±10.6	79.3±10.8	>0.05
BMI	27.15±2.3	26.8±2.35	>0.05
PASI	6.0±2.5	5.0±2.5	>0.05
DLQI	10.9±2.4	10.25±2.0	>0.05
PSODISK	48.4±8.1	45.0±9.3	>0.05
BSL	114.6±5.6	111.5±7.7	>0.05
BP max	135±13.5	129±11.1	>0.05
BP min	84.15±11.9	79.0±8.5	>0.05
HDL-C	43.0±4.0	45.1±3.8	>0.05
TG	130.8±34.1	147.3±28.2	>0.05
WL	94.15±12.0	92.65±11.6	>0.05

TABLE V.—*Nutraceutical arm – T0* vs. *T1 (mean values).*

	T0	T1	P value
Weight	83.15±9.5	82.3±9.5	>0.05
BMI	28.0±1.8	27.7±1.9	>0.05
PASI	4.5±2.85	3.9±2.7	>0.05
DLQI	11.0±2.15	10.2±2.2	>0.05
PSODISK	46.85±7.0	41.9±7.7	<0.05
BSL	112.8±8.9	107.0±10.8	>0.05
BP max	137±13.7	132.25±13.5	>0.05
BP min	79.5±11.3	78.0±10.2	>0.05
HDL-C	45.4±7.9	51.8±8.3	<0.05
TG	141.8±32.5	120.4±32.8	<0.05
WL	96.05±11.7	94.35±11.6	>0.05

TABLE VI.—*Metabolic syndrome (MS) resolution or persistence at T1.*

		Controls	Nutraceutical	P value
MS at T1	Yes	18	14	>0,05
	No	2	6	

TABLE VII.—*Percentage of improvement – control* vs. *nutraceutical arm (mean values).*

	Controls	Nutraceutical	P value
Weight	-1.4±1.7	-1±1.3	>0.05
BMI	-1.4±1.7	-1±1.3	>0.05
PASI	-19.0±23.4	-13.0±16.4	>0.05
DLQI	-5.35±8.3	-7.3±7.5	>0.05
PSODISK	-7.4±7.4	-10.9±7.0	>0.05
BSL	-2.7±4.8	-4.75±10.6	>0.05
BP max	-4.0±7.1	-3.0±9.0	>0.05
BP min	-5.2±10.05	-1.2±10.1	>0.05
HDL-C	+5.1±7.9	+14.8±12.15	<0.05
TG	-10.4±18.8	-18.5±14.1	<0.05
WL	-1.6±1.35	-1.8±1.5	>0.05

14,8±12,15 % in nutraceutical – P<0.05) and triglycerides (-10.4±18.8% in controls and -18.5±14.1 in nutraceutical – P<0.05) (Table VII).

Discussion

Our results show that the dietary addiction of a nutraceutical containing Krill-oil, a pool of antioxidants, vitamin E and selenium to etanercept therapy in patients affected by both psoriasis and metabolic syndrome is effective in increasing HDL cholesterol and reducing triglycerides levels. Improving lipid profile is important to lower the cardiovascular risk and reduce the pro-inflammatory status that is observed in psoriasis.[8] The efficacy of the tested nutraceutical is related to its strong antioxidant activity.

Coenzyme Q10 efficiently prevents proteins and lipids from oxidation, with a synergic activity with vitamin E. Krill oil is rich in Omega-3, Omega-6, Omega-9, phospholipids, dodecaesanoic acid, ecosapentanoic acid and astaxantin. In Krill oil Omega-3 are bound to phospholipids, ensuring a greater bioavailability, and a faster anti-inflammatory activity. Lipoid acid limits ROS formation during Krebs cycle.[9] Resveratrol protects LDLs against the lipid peroxidation, reduces triglycerides levels, induces vasodilation through the synthesis of NO and inhibits cyclooxygenase activity thus reducing inflammation. Selenium is part of the glutathione peroxidase, the enzyme that reduces free hydrogen peroxide to wa-

ter; several studies found a link between low levels of selenium and severity of psoriasis.[10]

Reducing metabolic syndrome parameters and consequently the pro-inflammatory status could help improving clinical outcome in patients affected by psoriasis. In present study PASI and DLQI improved in both groups, even if not significantly, probably due to the short time of observation and to population features (patients started treatment with etanercept at least 3 months earlier). Interestingly, a significant PSODISK improvement in the nutraceutical group was observed; this may be related to a better perception of wellness by patients observing the lowering of metabolic syndrome parameters.

Conclusions

Psoriasis is a complex disease with a great impact on patients' quality of life. Therefore it needs a multisystem approach involving the treatment of cutaneous lesions, metabolic syndrome and other comorbidities, when present. Patients should be also informed about the importance of a correct lifestyle, including physical activity and diet.

We observed that the introduction of a nutraceutical in addition to the systemic therapy in psoriatic patients affected by metabolic syndrome could help to restore the normal lipid profile, therefore completing psoriasis management. This is a preliminary study that needs to be strengthened on a larger cohort for a longer period.

Riassunto

Efficacia di un integratore alimentare nel miglioramento dei parametri della sindrome metabolica in pazienti affetti da psoriasi da moderata a severa durante il trattamento anti-TNFα

Obiettivo. La psoriasi è una patologia infiammatoria sistemica a prevalente impegno cutaneo, frequentemente associata a condizioni quali la sindrome metabolica. Recentemente è stato dimostrato come le citochine infiammatorie rilasciate dal tessuto adiposo influenzino in maniera significativa la risposta alla terapia e la severità delle manifestazioni cliniche.

Metodi. Abbiamo condotto uno studio in aperto controllato randomizzato per valutare l'efficacia di un nutraceutico a base di coenzima Q10, olio di krill, acido lipoico, resveratrolo, olio di semi di vite rossa, vitamina E e selenio in 40 pazienti affetti da psoriasi e sindrome metabolica in trattamento con etanercept. I 40 pazienti arruolati sono stati divisi in due bracci, il primo continuando la terapia in corso con etanercept 50 mg/settimana, il secondo integrando tale terapia con il nutraceutico. Risultati. Dopo un periodo di 3 mesi si è osservato un miglioramento significativo del colesterolo HDL e dei trigliceridi, sia confrontando i due bracci che nel braccio trattato con il nutraceutico.

Conclusioni. I risultati mostrano come l'introduzione di un nutraceutico nella terapia con etanercept in pazienti affetti da psoriasi e sindrome metabolica potrebbe aiutare a ripsristinare il profilo lipidico, contribuendo nel lungo termine al miglioramento della psoriasi e alla diminuzione del rischio cardiovascolare.

PAROLE CHIAVE: Psoriasi - Sindrome metabolica - Stress ossidativo.

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Melanoma in patients younger than 20 years

M. SANLORENZO [1*], S. RIBERO [1, 2*], S. OSELLA-ABATE [1], E. M. BALAGNA [2]
V. CALIENDO [2], G. MACRIPÒ [2], M. G. BERNENGO [1], P. QUAGLINO [1]

***Aim*. Melanoma is rare in children and uncommon in adolescents. Clinical and prognostic factors can differ from adult population. There is often a delay in diagnosis and the therapeutic management is not unequivocally established. The aim of this study was to review our monocentric case series to establish the characteristics of the population and the possible different behaviour of the malignancy compared to adults.**

***Methods*. From 1975 to 2011 we selected 36 out of 43 patients with a diagnosis of melanoma before the age of 20. We reported a female predominance, the most common site of primary lesions for both sexes were the lower extremities and according to adulthood population the most common histotype was Superficial Spreading Melanoma.**

***Results*. None of our patients presented distant metastasis at diagnosis, but 29.4% showed a progression, and the 17.6% died during the follow-up. A significant difference based on gender was found at the multivariate analysis on Disease free survival as well as Breslow thickness, but only Breslow thickness was the only parameter that maintained a role on survival at multivariate analysis when corrected for gender and age. We performed the sentinel lymph node biopsy in 3 patients and they all resulted negative.**

***Conclusion*. Despite our small case series we observed some important differences of melanoma in children compared to adults. It remains difficult to establish the prognostic factors in younger melanoma patients. Similar to adults, the detection of melanoma in an early phase of development, with a low Breslow thickness, is the most important prognostic factor.**

KEY WORDS: Melanoma - Young adult - Survival.

[1]Department of Medical Sciences, Dermatologic Clinic University of Turin, Turin, Italy
[2]Section of Dermatologic Surgery Department of Oncology and Ematology AOU Città della Salute e della Scienza, Turin, Italy

The incidence rate of melanoma (CM) has increased in the past decades, mainly in the industrial countries with white populations.[1, 2] Approximately 2 percent of melanomas occur in patients under the age of 20 years.[3]

Melanoma in children and in adolescents differs from adulthood in many features. Especially clinical and histological factors that are important in adults could have a totally different rule in childhood. The most common site in patients less than 20 years of age are the extremities, followed by trunk and head and neck.[4]

In children and adolescents there is often a delay in diagnosis.[5] It can be due to the atypical presentation as amelanotic lesions, the low index of suspicion and the indisposition in performing a surgical procedure in younger patients. So at the diagnosis, the 20% of patients presented regional lymph node disease or distant metastasis.[6]

Prognosis of melanoma in this population is difficult to establish and few data are reported in literature.[7-9] The objective of this study was to reviewed our case series melanoma in children and adolescents (<20 years), to assess the characteristics of the population and the possible different behaviour of the malignancy compared to adults.

Materials and methods

We selected 43 patients with diagnosis of melanoma before 20 years old from our monocentric data-

*Both authors contributed equally.

Corresponding author: M. Sanlorenzo, Section of Dermatology, Department of Medical Sciences, University of Turin, via Cherasco 23, 10126 Turin, Italy. E-mail: martina.sanlorenzo@hotmail.it

base of 5879 diagnosed between 1975 and 2011. The study was approved by the committee on research ethics at the institution in which the research was conducted and any informed consent from human subjects was obtained as required.

This age-cut off was previously fixed in larger epidemiological studies.[9, 10] Clinical features analyzed were age at diagnosis, gender, Breslow thickness and localization. SLNB has been introduced since January 1999 in our Institution. SLNB inclusion criteria were previously reported.[11] Clinical and imaging follow-up was performed according to the guidelines in use at the time of diagnosis and previously reported.[12-15] The impact of these features on overall survival (OS) and disease free survival (DFS) was analyzed. For all patients DFS was calculated from the surgical excision to the date of first disease relapse, whilst OS was calculated from the surgical excision to the date of death or last check-up. Survival estimates were derived by the Kaplan-Meier method and the statistical comparison was done by the log-rank test.

Statistical analysis

Univariate and multivariate analyses were carried out with the stepwise procedure in Cox model to evaluate the influence of different variables on DFS and OS. Statistical analyses were performed with Stata 11 software.

Results

From our monocentric case series we collected 43 patients younger than 20 years old with a diagnosis of cutaneuos melanoma. Data from 36 patients out of the 43 were completed. Out of these 21 were females and 15 males. The median follow-up was 11.5 years (0.4-39.1 years) and the mean age was 17.4 (±2.9) year old. Only one patient was under 10 year old.

Mean Breslow thickness was 1.72±1.96, no statistically significant difference based on gender was found. The histotype was nodular (MN) for 5 patients, the others presented a SSM (Superficial Spreading Melanoma) two patients presented an *in situ* melanoma.

Only 4 patients presented ulceration of the primary lesion. The ulceration was significantly more represented in melanoma with nodular pattern of invasion (P<0.0001, 3/5 *vs.* 1/31)

Melanoma appeared on legs in the 50% of cases, and no significant difference in site was found between male and female (Table I).

None of our patients presented distant metastasis at diagnosis, whilst one of them presented a lymph node involvement at the first consult.

The 29.4% (10/34) of patients showed a progression (the 2 patients who presented a in situ melanoma were excluded), and the 17.6% (6/34) of the global cohort died during the follow-up. Out of the 10 who presented a relapse during follow-up, 6 were in

TABLE I.—*Clinico-pathological features across gender.*

		Male	Female	Total
Age (median)		16.7 (±3.6)	17.8 (±2.5)	17.4 (±2.9)
Breslow	≤1	5	12	17
	1<Br≤2	6	4	10
	2<Br≤4	2	4	6
	Br>4	2	1	3
Histotype	SSM	9	16	25
	NM	3	2	5
	Others	3	3	6
Ulceration		2	2	4
Site	Head and neck	1	2	3
	Trunk	5	4	9
	Upper extremities	2	3	5
	Lower extremities	7	12	19
1st progression	None	9	17	26
	Regional	3	3	6
	Distant	3	1	4

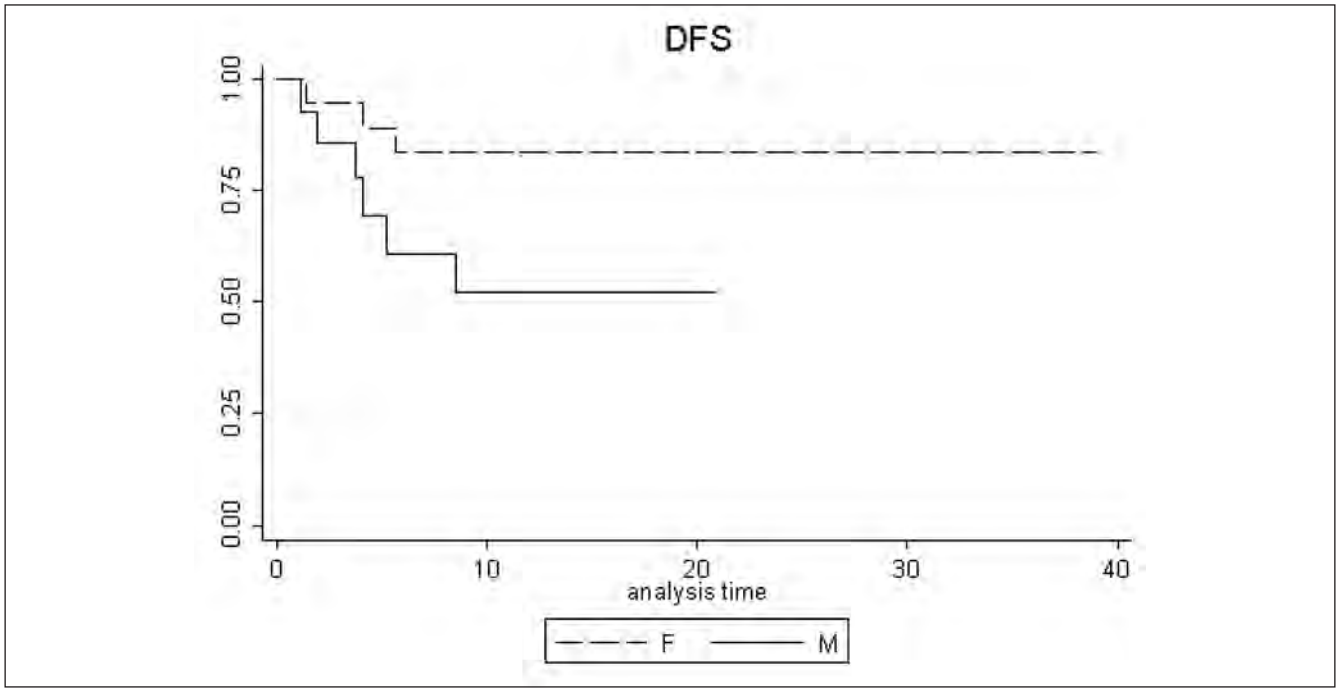

Figure 1.—DFS according to gender.

regional lymph nodes, 2 in the lung, and 2 in the lung and in distant skin. The mean time to relapse was 4 years (1.1-8.5 years) The 6 patients who died during follow-up presented a mean time of OS of 5 years. Ulceration, histotype and location were not significantly correlated with the event relapse or death.

At the univariate analysis on DFS, a significant difference based on gender was found (Figure 1). In fact at 5 years of follow-up the 89.2% of girls were free from recurrence, while only the 71.5% of boys. This difference was not maintained on OS. The OS was 87% at 5 years and 80.9% at 10 years. Breslow thickness was the only parameter that maintained a role in multivariate analysis when corrected for gender and age on survival, with an HR of 1.32 on OS, and an HR of 1.57 on DFS (Table II). Stratified for Breslow thickness, for ≤1 mm the survival was 100% at 5 years, for Breslow thickness between 1 and 2 mm 81.8%, for Breslow 2-4 mm 80% and for Breslow more than 4 mm 66. 6%.

In 14 patients the diagnosis was made after 1998, 3 of them reported a Breslow thickness >1 mm. This gave the indication to submit them to sentinel lymph node biopsy (SNLB). The pathological results of the

TABLE II.—*Multivariate analyses on OS and DFS.*

	OS				
	HR	ES	Z	P	CI
Breslow	1.32	0.19	1.93	0.05	1.00 1.75
Gender	2.61	2.35	1.07	0.28	0.45 15.21
Age	1.36	0.40	1.07	0.28	0.77 2.41
	DFS				
	HR	ES	Z	P	CI
Breslow	1.57	0.30	2.42	0.01	1.09 2.28
Gender	3.49	2.58	1.69	0.09	0.82 14.89
Age	1.01	0.15	0.13	0.90	0.76 1.35

sentinel lymph node referred no presence of metastatic involvement.

Discussion

Cutaneous melanoma is uncommon in teenagers and rare in children, but as in the adult population, in recent decades an increase of incidence has been reported.[9] It can be due to an anticipation of diagnosis thanks to screening campaigns, sensitivity of clinical instruments, as dermoscopy, and parents more aware of the danger of melanoma than in the past. However the always more frequent occurrence could also be considered real thinking about the higher risk in last decades of solar burn already in younger ages due to an intermittent exposition that is now considered one of the principal acquired risk factor of developing a cutaneous melanoma.[16]

Most of the studies regarding melanoma in younger patients are based on Cancer Registries population [9, 10] and therefore they could include a higher number of patients and perform epidemiological studies.

The incidence of melanoma increases with age.[9] Among patients aged 15 to 19 years, melanoma accounts for 7% of all cancers, but just 1% in persons younger than 15 years.[17] It occurs more frequently in adolescents than in younger children [18] and also in our cohort we reported only one patient under 10 years old. In the last decades, in European countries, the incidence rate of melanoma in adults was higher in females than in males, even if recently this could reach a equalization in diagnosis.[19] Different previous papers [9, 20, 21] reported the female predominance in childhood and adolescent and our data confirmed this trend.

The clinical presentation of melanoma in the young is often challenging, several authors found that the clinical characteristics of melanomas that developed during adolescence more closely resemble those seen in adults than they do of those developing in young children,[21] on the other hand clinical-pathological features change the prognostic value if the diagnosis is done in different period of the life time.[8, 22]

The first difference that we observed was on the anatomical location of the lesions. The extremities were the most common primary site with 50% of cases on the legs and without differences across gender. In adults the anatomical pattern of distribution is different, with a predominance of trunk lesions in males, whilst legs are the most frequent site of appearance of a cutaneous melanoma in female.[23] Instead according to adulthood population the most common histotype was superficial spreading melanoma. Some prognostic factors known for adults were not significantly correlated with the event relapse or death in our population.

We reported very few cases of ulceration, and this important prognostic factor for adulthood,[24] did not confirm its relevance. Probably the limited number, due to the rarity of this pathology in children, does not permit an accurate analysis. Nodular melanoma Breslow thickness was higher compared to other types, and out of 5 patients 2 had a regional progression and one died. We reported a mean Breslow thickness of 1.72 mm whilst in a large cohort [9] thickness was lower. Moreover the same author stratified population into different subgroups based on age, reporting a higher Breslow thickness in younger patients compared to older ones. In our monocentric study the small number of patients under 20 year old did not permit to analyze this stratification.

Different previous papers have reported a delay in diagnosis especially in pediatric population, due to the atypical presentation and the low grade of suspicion of parents about the appearance or a modification of a cutaneous lesion.[25, 26] The ABCDE clinical rule often used in adults may be difficult to apply to pediatric skin lesions, because common pediatric lesions, such as pyogenic granulomas, Spitz nevi, and benign nevi that grow as the child grows, can have suspicious features.[6] This delay sometimes could bring to a late diagnosis when a lymphadenopathy or a distant metastasis appear, with a worsening in prognosis. In our experience only one patient presented a not localized disease at the diagnosis, at the first visit a clinically palpable lymph node lymphadenopathy was detected.

The overall survival of our patients are not so different from data previously reported. In fact Strouse [4] reported 93% in 5 years OS in patients <20 years; and the National Cancer Base of United States revealed in patients aged 1 to 19 an overall 5-year survival rates of 98.7% for in situ disease, 93.6% for localized invasive disease, 68.0% for regionally metastatic disease, and 11.8% for distant disease.[6]

Gender influence on prognosis is debated; some authors [4, 27, 28] had reported a better OS in female similarity to adult population,[19, 28] but others [24] did

not find any differences. In our series we observed a better DFS in females, but this advantage does not play a significant statistical role on OS.

Different studies had suggested that younger patients seem to have more frequently a positive SLNB [8] than older patients. We started to perform this technique after 1998 in our institution, according to the AJCC guidelines. So, only 3 of our patients had an indication to perform SLNB according to the criteria of inclusion of this procedures. SLN status in melanoma is a powerful predictor of clinical outcome. They were 7, 14 and 16 years old, and they all resulted negative. Nowadays there are not any recommendation evidences based on the SLN biopsy in young patients, but it is important to identify patients with a potentially metastatic deposit in lymph node at diagnosis.

Conclusions

The conventional treatment of melanoma in children is the same of adult age. The first therapeutic line is surgery, SNLB should be performed according to AJCC classification and there are also studies about the efficacy and safety of adjuvant therapies.[29] However the biological behaviour of melanoma in childhood and adolescence seems to be different from the adults. Clinical presentation usually is different, more frequently lesions are amelanotic and there is a preferential site of distribution on lower extremities in both sexes differently from adulthood. It is likewise difficult to establish the prognostic factors in younger melanoma patients. As well as to adult, the detection of melanoma in an early phase of development, with a low Breslow thickness, remains the most important prognostic factor. So, despite its rarity in childhood, dermatologists and pediatrics should consider this diagnosis also in front of lesions without the typical presentation. Several others studies are required to establish the best diagnostic and therapeutic management in this rare pathology in this population.

Riassunto

Melanoma in pazienti di età inferiore a 20 anni

Obiettivo. Il melanoma cutaneo è una patologia rara nei giovani, i quali differiscono per caratteristiche cliniche rispetto a pazienti adulti affetti da questo tumore. Nei giovani si osserva spesso un ritardo diagnostico e la gestione terapeutica non è cosi univoca. Lo scopo del presente studio è di descrivere la nostra casistica e valutare il diverso comportamento biologico della patologia in confronto con la popolazione adulta.

Metodi. Dal 1975 al 2011 sono stati presi in considerazione 36 di 43 pazienti con una diagnosi di melanoma cutaneo in età inferiore a 20 anni. Riportiamo una prevalenza maggiore nel sesso femminile, il sito principale di insorgenza della lesione primitiva erano gli arti inferiori in entrambi i sessi e il più frequente istotipo risultava essere il melanoma a diffusione superficiale.

Risultati. Nessuno dei pazienti selezionati presentava metastasi a distanza alla diagnosi, ma il 29,4% mostrava una progressione di patologia e il 17,6% andava incontro a decesso per patologia. All'analisi multivariata di sopravvivenza il sesso e lo spessore di Breslow mantenevano un ruolo indipendente sul tempo libero da malattia, mentre solo lo spessore di Breslow si manteneva indipendente sulla sopravvivenza globale quando corretto per sesso ed età. Sono stati sottoposti a biopsia del linfonodo sentinella tre dei pazienti in oggetto risultando tutti negativi per metastasi.

Conclusioni. Nonostante l'esiguo numero della casistica descritta si osservano alcune differenze cliniche nel melanoma dei giovani quando comparati con quelli degli adulti pur risultando difficile valutarne il diverso significato prognostico. Come per gli adulti un limitato spessore di Breslow, indice di una diagnosi in un basso stadio di patologia, resta il principale fattore prognostico.

PAROLE CHIAVE: Melanoma - Giovani - Sopravvivenza.

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Lichen sclerosus and the risk of malignant progression: a case series of 159 patients

G. PAOLINO, C. PANETTA, C. COTA, L. MUSCARDIN, P. DONATI, A. DI CARLO

***Aim and methods*. We analyzed 159 stored specimens of *Lichen Sclerosus* (LS) collected in the period 1999-2011 from 159 patients, in order to evaluate the histological patterns, clinical outcomes and possible associations with malignancies. The histopathologic analysis revealed 145 cases (males and females) with LS alone, 7 in whom penile LS was associated with spinocellular carcinoma (SCC), and 7 in whom LS was associated with a pseudocarcinomatous-hyperplasia (PCH). Extragenital LS was found in 20% (17/85) of the males and 78% (58/74) of the females. In the cases of SCC, immunohistochemical analyses was performed.**

***Results and conclusion*. The results showed very low positivity to p16^{INK4A} and Ki-67; biomolecular PCR was positive in only two cases, and in both cases the non-oncogenic genotype HPV 100 was detected. No important additional risk factors for malignancies were found (*e.g.*, hormones, infections, other autoimmune diseases).**

KEY WORDS: Lichen Sclerosus et Atrophicus - Penile neoplasms - Hyperplasia.

Lichen Sclerosus (LS) is a chronic inflammatory disease that generally affects the genital tract, although any cutaneous site can be involved. The origin of LS remains unclear, yet it is believed that the clinical outcome is influenced by genetic, physiological, and environmental factors. Hallopeau and Darier first described LS at the end of the 19th century, as a variant of lichen planus. In 1928, Stuhmer reported a similar condition, "balanite xerotica obliterans", defining it as a chronic, sclerosing, atrophic process that involved the gland and the foreskin, leading to meatal stenosis and phimosis.[1]

Corresponding author: G. Paolino, Dermatopathological Laboratory, Institute San Gallicano Institute of Rome, via Elio Chianesi 53, 00158 Rome, Italy. E-mail: paolgio@libero.it

Dermatopathological Unit
San Gallicano Institute – IRCCS
Rome, Italy

LS is currently considered to be an autoimmune disorder with a predilection for genital skin, although the exact target antigens have yet to be defined.[2-4] The antigens HLA-DQ7, -DRT7, -DQ8 and -DQ9, as well as BP180 and BP230, seem to play an important role. Other potential etiologic or modifying factors include sex hormones (mostly androgens), infectious agents (*Borrelia burgdorferi*) and local trauma.[5] The etiopathogenetic role of human papillomavirus (HPV) is still a subject of debate.

The most frequent sequelae of LS at the genital level are, in males, phimosis with micturition,[6, 7] scleroatrophy of the genital mucocutaneous tissues, and urethral and genital discomfort, whereas the most common sequelae in females are adhesion and resorption of the labia minora and reduced introitus, causing dyspareunia. The most severe association is squamous cell carcinoma (SCC). In general, the lifelong risk of SCC ranges from 3% to 7% in females and from 4% to 8% in males.[6] However, the percentages reported in the literature vary greatly. This could depend on the fact that the disease has been studied by clinicians with different specialties (*e.g.*, urologists, dermatologists and gynecologists) and using different methods, some of which have been based on clinical criteria and have been either retrospective [8, 9] or prospective,[10] whereas others have been based on histologic series of genital LS [11] or genital SCC.[12]

TABLE I.—*Sites of the lesions in males.*

Area	N.	%	Site	N.	%
Genital	68	80	Prepuce	36	53
			Gland	16	23
			Penile shaft	5	7
			Genital area (undefined)	11	17
Extragenital	17	20	Trunk	12	70
			Upperlimbs	2	12
			Lower limbs	3	18
Total	85	100			

TABLE II.—S*ites of the lesions in females.*

Area	N.	%	Site	N.	%
Genito-anal	18	25	Vulvar	16	89
			Anal	2	11
Extragenital	56	75	Mammary	17	30
			Upper limbs	4	7
			Rrunk	29	52
			Lower limbs	6	11
Total	74	100			

Recently, many studies have reported that HPV infection is an important co-factor of LS.[13, 14] In one study, oncogenic HPV 16 was found in 33% of cases of penile LS.[15] In other studies, HPV has been isolated in 30-54% of cases of penile SCC.[6, 15] More recently, two distinct pathways in the development of SSC of the vulva have been defined: an HPV-independent SCC that arises from LS and an HPV-dependent SCC which has a better prognosis.[16] However, other authors have not confirmed this distinction.[17]

In light of these considerations, we performed a histological reexamination of LS specimens observed in recent years at the Dermatopathology Unit of the San Gallicano Institute in Rome, Italy, with the objective of evaluating the prevalence of malignant lesions in LS, also performing immunohistochemical and biomolecular analyses. Where possible, we also reviewed clinical records to evaluate clinical data and risk factors.

Materials and methods

We examined 159 paraffin-embedded specimens of LS which were stored at the Dermatopathology Unit; the specimens had been collected from 159 individuals (*i.e.*, all cases with clinically suspected LS) and sent to the Unit in the period 1999-2011. The study was approved by the committee on research ethics at the institution in which the research was conducted and any informed consent from human subjects was obtained as required.

We also reviewed the individuals' clinical records to examine their medical history and determine whether or not they had risk factors for SCC (*e.g.*, hormonal therapy, smoking) For the specimens from individuals with SCC, we performed immunohistochemical staining with p16 and Ki67 proteins[14, 15], using a commercial kit (CINtec® PLUS kit) that is normally used for the simultaneous qualitative detection of p16 and Ki-67 proteins in cervical cytology preparations. In the same specimens, a highly sensitive two-step polymerase chain reaction (PCR) was performed, using general GP5+/GP6+ PCR primers and MY11/MY09 consensus primers. The PCR was performed, in the SCC specimens, to identify the possible presence of HPV.

Results

Study population and sites of the lesions

Of the 159 individuals, 85 were males, with a median age of 54 years, and 74 were females, with a median of 64 years. The topographic sites of the lesions, by gender, are reported in Tables I, II. After a LS diagnosis the patients were followed (at the dermatologic clinic) with controls, for a median of 5.5 years; in the first two years, the controls were twice a year, subsequently deferred to one once a year, based on the degree of the disease.

Risk factors

According to the clinical records, none of the women had taken oral contraceptive pills. None of the individuals had reported other autoimmune diseases, such as vitiligo, pernicious anemia, alopecia areata, or diabetes mellitus.[18]

Clinical features

Among males, LS most frequently presented as ivory or sclerotic white plaques (N.=59), ulcerative and erosive or hypertrophic lesions (N.=10), papillomatous plaques (N.=4) and eritematous-eczematous lesions involving the gland and sparing the semimucous (N.=2). Six of the males had had a history of chronic phimosis, and other 2 males had a nonspecific balanitis (Figure 1), as found in other studies.[19, 20]

Among females, at vulvar level, there were white and flat-topped papules (N.=10); in other 5 individuals, atrophic patches were reported, in some cases with a persistent purpuric aspect. In 2 females, there was obliteration of anatomic structures associated with atrophic labia minora, clitoral hood and urethral meatus.

Extragenital sites

For both males and females, the lesions in the extragenital sites appeared as macular lesions or atrophic papules with a "cigarette paper" feature, located prevalently on the trunk, mammary area (women; Figure 2), and the upper and lower limbs. Two women have showed the concomitant presence between extragenital LS and Morphea.

Histological review and associations with malignancies

Histologic re-examination revealed a classic LS pattern in all 159 specimens. In 7 specimens, penile LS was associated with SCC, whereas in other 7 specimens penile LS was associated with a pseudocarcinomatous-hyperplasia (PCH), as found in previous studies.[21, 22] The sites affected by SCC were respectively foreskin (N.=3), gland (N.=3), and penile shaft (N.=1). In one patient, a total penectomy had been performed; in another patient, a partial penectomy had been performed; in both cases, the evaluation of sentinel lymph nodes did not show neoplastic cell populations (Figures 3, 4). Regarding PCH, the site most affected was the preputius (Table III).

The re-examination of histopathologic features revealed that LS showed a thinned epidermis associated with hyperkeratosis and atrophy of the stratum Malpighi with hydropic degeneration of basal cells, together with a wide band of homogenized collagen beneath the dermo-epidermal junction. A lymphocytic infiltrate beneath the homogenized area were also observed (Figure 3).[23] In the 7 cases with an associated malignancy, we found intraepithelial neoplasia (IN)/carcinoma *in situ*, or SCC, the usual type. IN was characterized by an intraepidermical dysplastic change and disorderly maturation of the epidermis with multinucleated and dyskeratotic cells, whereas SCC lesions showed histologically invasive nests of atypical squamous epithelial cells arising from the epithelium; dyskeratotic cells and prominent central keratinization with horn pearls were observed, depending on the degree of differentiation of the tumor.

In the 7 cases of PCH associated with LS, a reactive epithelial hyperplasia was observed, which was characterized by a prominent irregular hyperplasia of the epidermis and adnexal epithelium with tongue-like projections in the dermis, which may vaguely or closely simulate SCC. This pattern is often present in a number of conditions of chronic inflammation/infection, as well as in association with many cutaneous neoplasms.

HPV study and Immunohistochemistry

The HPV test was performed only in the cases of penile SCC. The immune-staining for $p16^{INK4A}$ and Ki-67 showed a very low positivity for p16 (0-33%), whereas only two lesions showed mild (34-67%) positivity for Ki-67 (Figure 5). Positivity for p16 ranged from 26% to 46%.[24, 25] In 2 cases we found positivity for HPV, and in both cases the genotype was HPV 100, which is a non-oncogenic genotype.

Discussion

The exact pathogenesis of LS remains unclear. Its incidence has been reported to range from 1/300 (0.3%) to 1/1000 (0.1%).[18] In a study conducted among 84 female patients, an association with the class II DQ7 antigen was reported.[10] LS recently began to be considered as an autoimmune disorder. Some researchers consider it as a sort of Koebner phenomenon, given that it arises in sites exposed to constant friction, which can trigger LS (it also recurs around vulvectomy scars and circumcision scars). Another pathogenetic factor could be the persistence of urine or smegma in these areas, particularly in older individuals, as also occurs with phimosis and lack of circumcision.[3, 4, 22] Regarding gender, some Authors have described a higher incidence among females,[2] whereas others report a higher incidence among older

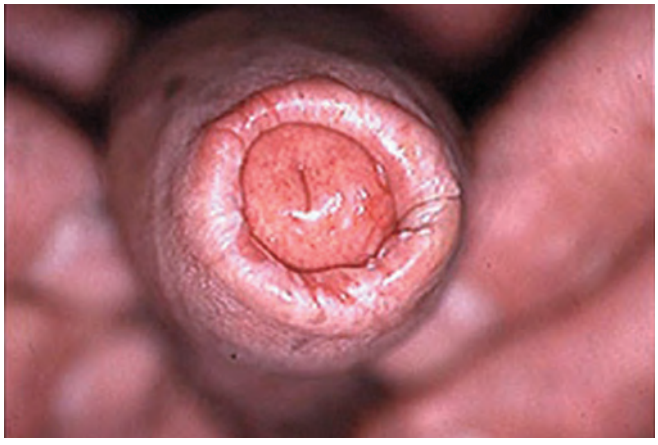

Figure 1.—Penile Lichen Sclerosus

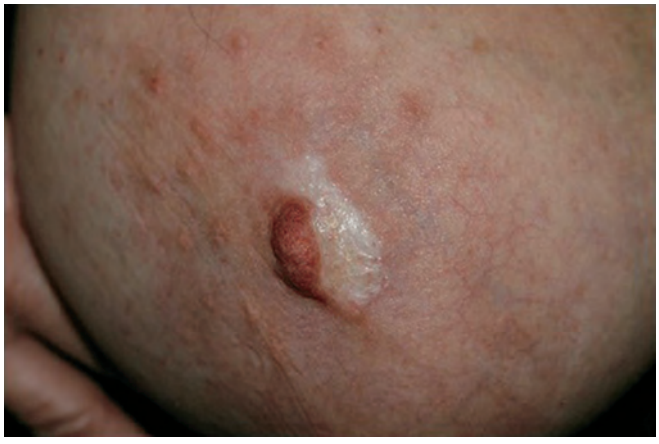

Figure 2.—Extragenital Lichen Sclerosus

white males.[9] In our study, there was a slightly higher prevalence of males (85, compared to 74 females).

With regard to the association with malignancies, in our study 8.2% of the cases of LS in males were associated with SCC, which is consistent with the percentages reported in other studies;[6] however, if we also consider PCH as a premalignancy, the percentage increases to 16.4%, a value epidemiologically more relevant. This figure suggests that a strict follow-up of patients affected by LS should be performed during the course of the disease. Immunochemistry performed on the 7 specimens with SCC showed very low $p16^{INK4A}$ positivity and a mild Ki-67 positivity, whereas HPV testing did not demonstrate high-risk genotypes. These results could

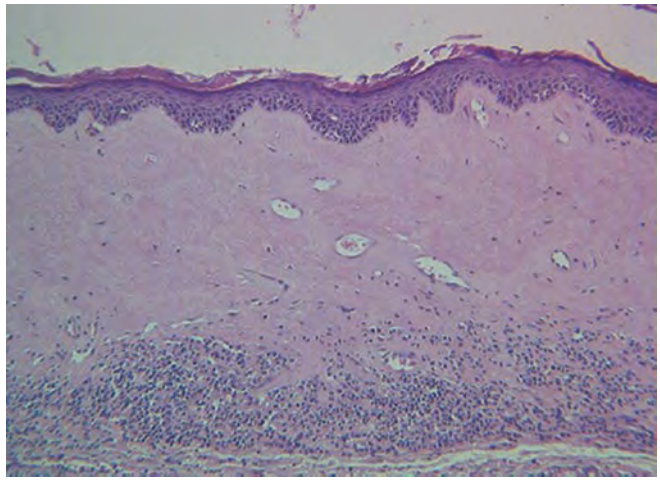

Figure 3.—Lichen Sclerosus Pattern 2 (H-E, 10X: hyperkeratosis of epidermis with atrophic Malpighian layer, edema and homogenization of the collagen in the superficial dermis, band-like lymphocytic infiltrate in the mid-dermis.

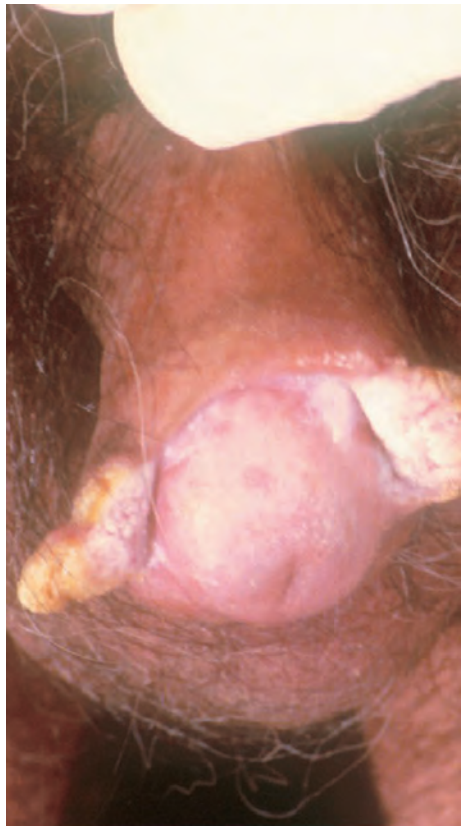

Figure 4.—Penile Lichen Sclerosus with neoplastic transformation.

TABLE III.—*Neoplastic lesions in males by site.*

	LS+SCC (7 males)	LS+PCH (7 males)
Median age (years)	63	68
Site		
Foreskin	3	Preputius 7
Gland	3	
Penile shaft	1	

support the hypothesis of a bimodal malignant progression in penile SCC.[16] No malignancies we found associated with LS in females. This difference observed in our cases between males and females could possibly be to the fact that women prefer to see a gynecologist for their genital complaints; to confirm this hypothesis, we reviewed all of the specimens of vulvar cancer stored at our General Pathology Unit, and we found that 68 of the 127 cases had an underlying LS (52%). Data seem to indicate that probably the different statistics and malignancies incidence in LS reported in the literature could depend by the fact that a unique process is often performed by different specialists (dermatologists, gynecologists, oncologists, surgeons, pathologists etc.)

With regard to extragenital LS, the percentage of cases with extragenital localization has been reported to be 2.5% among males (20% in some reports) [5, 6] and 8-20% among females.[6] In our study, extragenital LS was found in 20% of the males and 84% of the females. No association between extragenital LS and genital LS was found, whereas in other studies 15-20% of individuals had both genital and extragenital LS[3]. The association between SCC and extragenital LS is extremely rare; in fact, only two cases are reported in the literature, and these cases were possibly related to exposure to ultraviolet rays (particularly UVA-1).[26, 27]In two females, we observed an association between extragenital LS and morphea. This is supported by previous observations of different Authors, that have demonstrated through sequential biopsies a transition from LS and morphea. These reports suggest that these diseases could belong to the same spectrum of diseases, in which also acrodermatitis chronic atrophicans is included.[28, 29]

Conclusions

LS is a common chronic inflammatory dermatosis which can occur at any age, in both sexes and all races, although it occurs more often among postmenopausal women, adult men, pre-pubescent girls and boys, and individuals with an increased risk of autoimmune disease.[5] The disease preferentially affects the mucous and semi-mucous membranes of the genitals, although it can frequently affect extragenital areas, as also found in our study.

According to both the literature and our observations, LS seems to play an important role as relative factor for SCC onset, as well as HPV, trauma, poor hygiene and long-lasting application of steroids [30] However, we would need to perform more extensive immunohistochemical and biomolecular analyses among a greater number of individuals with LS and associated malignancies. Moreover, new data are needed to assess more specifically the carcinogenic activity of HPV both in genital LS and in genital SCC, and in particular its natural progression from HPV infection to intraepithelial neoplasia and invasive SCC, as occurs in cervical carcinoma.

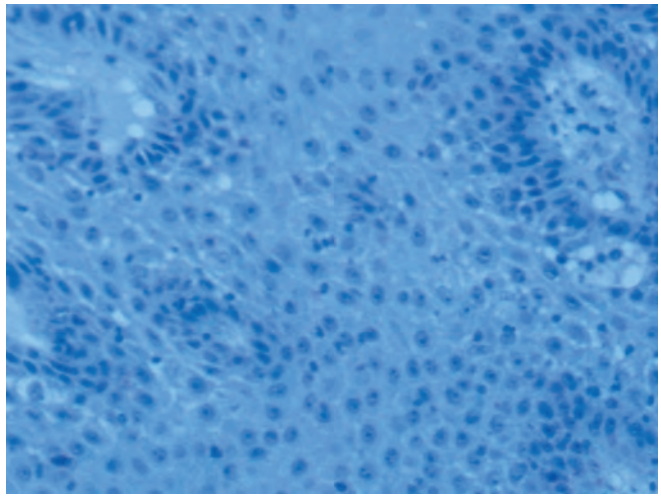

Figure 5.—Very low positivity for immunohistochemical staining with $p16^{INK4A}$ and Ki67 proteins in a case of IN with underlying LS (40 X).

Riassunto

Lichen scleroso e rischio di malignità: serie clinica di 159 pazienti

Obiettivo. Abbiamo voluto svolgere uno studio retrospettivo sul lichen sclero-atrofico (LS) revisionando i preparati istologici ed analizzando le cartelle cliniche di ogni paziente, con diagnosi accertata istologicamente di LS, pervenuto presso il nostro Servizio di Dermatopatologia.

Metodi. Sono stati revisionati 159 preparati istologici di pazienti affetti da LS, pervenuti nel nostro istituto dal 1999 al 2011. Sui campioni di SCC l'analisi è stata perfezionata utilizzando la metodica PCR in associazione ad appropriate indagini immunoistochimiche tramite l'utilizzo di p16^{INK4A}-Ki-67.

Risultati. Sono stati osservati rispettivamente 145 preparati istologici che mostravano un classico pattern istopatologico di LS; associato in altri 7 preparati a carcinoma a cellule squamose (SCC) del pene ed in altri 7 ad un quadro di iperplasia pseudoepiteliomatosa (PCH). I casi di LS extra-genitali erano, rispettivamente, il 20% (17/81) tra i pazienti di sesso maschile e il 78% (58/74) tra i pazienti di sesso femminile. La metodica biomolecolare PCR applicata sui 7 casi di SCC ha evidenziato una positività in solo due preparati ed i genotipi isolati erano entrambi HPV100. L'ulteriore analisi immunoistochimica con p16^{INK4A}-Ki-67 ha evidenziato una scarsa positività per p16^{INK4A}-Ki-67.

Conclusioni. La percentuale dei casi di SCC esaminati nella nostra coorte, è simile ai dati riportati in letteratura con un valore del 8,2% che tuttavia sale al 19% considerando anche le PCH. Abbiamo anche osservato un'elevata percentuale di LS extragenitali. Non abbiamo trovato un'associazione diretta tra carcinoma squamocellulare ed HPV. In base alla letteratura ed in base alle nostre osservazione, il LS resta un importante fattore associativo per l'insorgenza del SCC.

Parole chiave: Lichen scleroso e atrofico - Pene, tumori - Iperplasia.

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The topical vehicle as a key factor in the management of the psoriatic patients' therapy

S. VERTUANI [1], A. D. CVETKOVSKA [1], S. ZAULI [2], A. VIRGILI [2], S. MANFREDINI [1], V. BETTOLI [2]

This review deals with the importance of the topical vehicle as a key factor in the management of the psoriatic patients' therapy.

KEY WORDS: Psoriasis - Therapeutics - Skin diseases.

[1]Department of Life Science and Biotechnology
Medicinal and Health Products Section
University of Ferrara, Ferrara, Italy
[2]Department of Medical Sciences
University of Ferrara, Ferrara, Italy

A vehicle, is defined as the inert medium of a topical formulation that carries the drug substance into the skin. The vehicle itself can have beneficial properties, such as cooling or emolliating, which is evidenced by substantial vehicle responses in clinical investigations. The vehicle can containone or more inert, nontherapeutic excipients. The combination of excipients should be non-allergenic, nonirritating, and cosmetically acceptable.[1]

One of the most significant challenges in topical drug delivery is designing and developing an appropriate vehicle.[2]

During vehicle development the following points are considered:

1. efficient deposit of the drug substance on the skin with optimal distribution;
2. efficient drug substance release;
3. efficient drug substance delivery to the target site;
4. sustain a therapeutic drug substance level in the target tissue for a sufficient duration to provide a pharmacologic effect;
5. be appropriately formulated for the anatomic site to be treated;
6. be cosmetically acceptable to the patient.[2]

Corresponding author: V. Bettoli, Department of Medical Sciences, via Fossato di Mortara 64, University of Ferrara, 44121 Ferrara, Italy.
E-mail: vincenzo.bettoli@gmail.com

Classification of vehicles depends on various physiochemical principles. For example, a vehicle is considered a lotion if it is pourable with viscosity less than 30,000 cp (at 5 rpm and 25 degrees centigrade) and a greater than 50% loss upon drying (Figure 1).

Opposite, the vehicle is considered an ointment if after drying loses less than 20% of its total content and contains more than 50% of hydrocarbon or polyethylene glycol.[3] There is uncertainty in distinguishing one vehicle from another. For example, by definition oil-in-water and water-in-oil emulsions are considered creams. However, hydrophilic substances such as lanolin or cholesterol that are emulsions of oil in water or water in oil are considered ointments.

Vehicles for topical use may be sub classified into monophasic, biphasic, and triphasic forms, based on physical state (Tables I, II).[1]

Classification of topical drug vehicle based on physical state

Preparation for cutaneous use can be divided into three categories:[3]

— powders for cutaneous use;

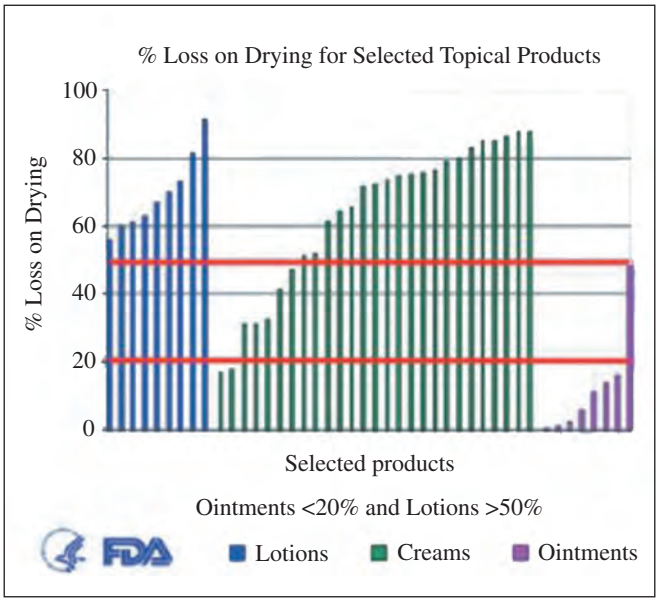

Figure 1.—Classification by percent loss upon drying.

— liquids for cutaneous use;
— semisolid for cutaneous use.

Semi-solid

Semi-solid preparations for cutaneous application are intended for local or transdermal delivery of active substances, or for their emollient or protective action. They are of homogeneous appearance. Semi-solid preparations for cutaneous application consist of a simple or compound basis in which, usually, one or more active substances are dissolved or dispersed. According to its composition, the basis may influence the activity of the preparation. The basis may consist of natural or synthetic substances and may be single phase or multiphase. According to the nature of the basis, the preparation may have hydrophilic or hydrophobic properties; it may contain suitable excipients such as antimicrobial preservatives, antioxidants, stabilisers, emulsifiers, thickeners and penetration enhancers.

Several categories of semi-solid preparations for cutaneous application may be distinguished: ointments, creams, gels, pastes, poultices, medicated plasters, cutaneous patches.

According to their structure, ointments, creams and gels generally show viscoelastic behaviour and are non-Newtonian in character, *e.g.*, plastic, pseudoplastic or thixotropic type flow at high shear rates. Pastes frequently exhibit dilatancy.[3]

Of the semi-solids for cutaneous use ointments, creams and gels are the most common used.[4]

Ointment

An ointment consists of a single-phase base in which solids or liquids may be dispersed and fall into three classes: hydrophobic, water-emulsifying and hydrophilic bases.

Hydrophobic ointments

Hydrophobic ointments can absorb only small amounts of water. Typical bases used for their formulation are hard, liquid and light liquid paraffins, vegetable oils, animal fats, synthetic glycerides, waxes and liquid polyalkylsiloxanes.

Water-emulsifying ointments

Water-emulsifying ointments can absorb larger amounts of water and thereby produce water-in-oil or oil-in-water emulsions after homogenisation, depending on the nature of the emulsifiers: water-in-oil emulsifying agents such as wool alcohols, sorbitan esters, monoglycerides and fatty alcohols, or oil-in-water emulsifying agents such as sulfated fatty alcohols, polysorbates, macrogolcetostearyl ether or esters of fatty acids with macrogols may be used for this purpose. Their bases are those of the hydrophobic ointments.

Table I.—*Vehicle classification by physical state.*

Solid	Liquid	Semisolid
Powder	Lotion	Ointment
Aerosol	Liniment	Cream
Plaster	Solution	Paste
	Emulsion	Gel
	Suspension	Jelly
	Aerosol/foam	Suppository

Table II.—*Vehicle classification by phase.*

Monophasic	Biphasic	Triphasic
Powder	Cream	Foam
Tincture	Ointment	
Solution	Paste	
Lotion		
Gel		
Oil		

Hydrophilic ointments

Hydrophilic ointments are preparations having bases that are miscible with water. The bases usually consist of mixtures of liquid and solid macrogols (polyethylene glycols). They may contain appropriate amounts of water.[3]

Ointment bases: recognized for use as vehicles fall into four general classes: the hydrocarbon bases, the absorption bases, the water-removable bases, and the water-soluble bases. Each therapeutic ointment possesses as its base a representative of one of these four genera.

The choice of an ointment base depends upon many factors, such as the action desired, the nature of the medicament to be incorporated and its bioavailability and stability, and the requisite shelf-life of the finished drug product. In some cases, it is necessary to use a base that is less than ideal from a patient perspective in order to achieve the stability required. Drug substances that hydrolyze rapidly, for example, are more stable in hydrocarbon bases than in bases containing water, even though they may be more effective in the latter.[5]

Typical ointments are based on petrolatum. An ointment does not contain sufficient water to separate into a second phase at room temperature. Water-soluble ointments may be formulated with polyethylene glycol. Ointments are ideal emollients with good skin penetration and adherence to surfaces.

Gel

Gels consist of liquids gelled by means of suitable gelling agents.

Lipophilic gels

Lipophilic gels (oleogels) are preparations whose bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels

Hydrophilic gels (hydrogels) are preparations whose bases usually consist of water, glycerol or propylene glycol gelled with suitable gelling agents such as poloxamers, starch, cellulose derivatives, carbomers and magnesium-aluminium silicates (3).

Types

Single phase gels: gels in which the macromolecules are uniformly distributed throughout a liquid with no apparent boundaries between the dispersed macromolecules and the liquid; double phase gels: gel mass consists of floccules of small distinct particles, often referred to as a magmas. Milk of magnesia (or magnesia magmas).

Particular attention has been drawn in the last decade on oleogels (Lipophilic, Lipogels), semisolid systems that have a hydrophobic liquid as the continuous medium. Their applications were studied in many fields.[6] Recently, lipogels — semisolid ointment-like preparations — have been investigated as vehicles for topical drug delivery.[7, 8] Lipogels are based on fatty components and are obtained by gelling an oleaginous phase with a lipophilic substance. The type and concentration of the gelling agent can affect the structure on which the rheological characteristics of the preparation depend and consequently on the requirements of physical stability and consistency.[9] The method used to produce lipogels was based on the findings of Fukasawa and Aiache for the gelling of vegetable oils. They have intrinsic cosmetics properties because of their lipidic composition that serves as a barrier between the skin and the outside ambient, therefore they improve stratum corneum function because of their rich content in fatty acids (*i.e.*, oleic fatty acid) which are compatible with the epidermis.[10] Nonetheless they are used as vehicles to incorporate active compounds to a therapeutic use. Usually, a higher capacity of releasing the active molecule to the stratum corneum, correspond to a higher efficacy.

Vehicles and psoriasis

Absorption of the drug substances and compliance for topical application are greatly influencing outcames of the topical therapy.

During the last two decades the understanding of the skin barrier function has expanded, elucidating both manner in which drugs penetrate and how the cutaneous formulations efficiently deliver drug substances.[11] This advancement in skin biology has stimulated the development of novel vehicles for skin disease management with both new formulations of old drug substances and new chemical entities.

The nature of the drug substance conducts the development of the vehicle. Ideal vehicles are drug substance-specific.[2] Vehicles need to be tailored according to the features of the drug substances in order to provide efficient drug delivery. During development of the formulation the optimal vehicle needs to assure stability and bioavailability of the drug substances. Based on physicochemical properties of the drug substance, a rational strategy can be developed to create the vehicle. Key factors include: 1) the degree of solubility or insolubility in various excipients; 2) compatibility or incompatibility with potential excipients; and 3) sensitivities to the molecule degradation and instability.

Beyond the physicochemical considerations and requirements for the formulation and its physical container to provide stability and uniformity, formulators also must consider the physical changes that occur as a formulation is applied to the skin. Upon application of a drug formulation on the skin, volatile components such as water, alcohol, and propellants evaporate, thereby concentrating the active drug substance and non-volatile excipients on the skin. During the application process these residual components become mixed with the hydrolipidic film on the skin surface, creating what some call the "secondary formulation".[2] It is from this secondary formulation that the drug substance is typically delivered into the skin.

The mechanical stress during application process is very important because it can alter the initial vehicle matrix and change the thermodynamic properties of the drug.[12] The pharmacokinetic of the drug substance on the skin can be quite variable and it is largely due to the physicochemical properties of the vehicle components.[13] Penetration variability is particularly present for corticosteroids,[14] where the same molecular entity can carry a different potency depending on the vehicle in which it is being delivered.[15]

The release of the drug substance from its vehicle into the underlying skin layers and through to the general systemic circulation, is described by its pharmacokinetics. The physiochemical properties of the formulation influence the pharmacokinetics of the release and absorption of the drug substance.

The time-dependent changes in drug concentration can be affected by multiple factors including:[16]

1. change in the structure and containment of the topical formulation;
2. time-dependent changes in skin barrier integrity;
3. the disease state and its severity;
4. regional variability in the skin barrier;
5. reaction of the underlying viable tissues to the topical preparations;
6. drug substance influences.

Therefore, other aspects in the vehicle should be considered in order to fully explain the delivery of the drug substances.

In order to promote absorption the vehicle often include penetration enhancing ingredients that temporally distrupt the skin barrier, fluidize the lipid channels between corneocytes, alter the partitioning of the drug substance into skin structure, or otherwise enhance delivery into the skin. So called penetration enhancers are drug substance- and vehicle-dependent and therefore are not universally effective. Propylene glycol is a multifunctional excipient in topical formulations, having humectant, solvent, and antimicrobial effects. The mechanism of permeation enhancement by propylene glycol is multiple and varies with concentration and formulation type. Propylene glycol is commonly used as an excipient in topical drug formulations and is found in a majority of formulations of corticosteroids. It is shown to diminish barrier function and often functions as a penetration enhancer. Other penetration enhancers, including detergents and emulsifiers, disrupt the barrier to encourage migration of active drug through the stratum corneum. Oleic acid can be a penetration enhancer in certain formulations and its mechanism is thought to be through fluidizing the intercellular lipids of the stratum corneum. Most penetration enhancing excipients have the potential to be irritating to the skin, depending upon concentration and the other inactive ingredients in a particular vehicle.[16-18]

Our recent results (data not published) point to the importance of the vehicle also in terms of modulation of penetration. Using especially designed nanoemulsifiers it is possible to even concentrate the drug substance in a specific layer of the skin (*i.e.*, dermis).

FDA recognizes eight topical dosage forms: Solution, Suspension, Lotion, Paste, Gel, Ointment, Cream, or "Other," which includes foams, aerosols, powders, patches, etc.[19] In clinical practice the importance of these classification varies, although in many situations the vehicle makes a difference between a good therapeutic effect and a therapy failure. The psoriatic skin has unique reactivity and the ap-

plication of an improper vehicle leads to poor therapeutic outcome leading to signs and symptoms of skin irritation, lack of patient compliance and possible contact dermatitis.

Among the many aspects that must be addressed during the typical four to seven years development period of the vehicle are stability and defined levels of impurities and degradation. The results of AUCs of two different formulations can be comparable but if the formulation does not remain on the affected skin for the required time, the clinical outcome will differ. Unfortunately, regulatory agencies (*i.e.*, FDA) does not require test for clinical utility of a vehicle from generic dosage form,[2] although the properties of the vehicle can profoundly influence and modulate the therapeutic outcome of the topical formulation and change its local as well as systemic safety.

Psoriasis is a disease that is very frequently diagnosed and treated by dermatologists, and is illustrative of prominence of topical therapies in dermatology. The topical therapy continue to be a mainstay of patient management and for clinicians is it a challenge to select the proper topical treatment to meet the therapeutic needs of the patients and optimized therapeutic adherence. Besides other factors, this challenge is due to the importance of the selection of an appropriate vehicle.[20]

The treatment vehicle is an important consideration in topical therapies because their cosmetic acceptability and ease of use affect treatment preference and adherence, with patients generally preferring less messy treatments that are easy to apply.[21, 23]

Feldman *et al*.[24] stated that 'There is one vehicle that is the best one to prescribe for a patient with psoriasis: the vehicle that a patient will best use". Bearing in mind the importance of the treatment vehicle, a series of one-to-one interviews were recently conducted with 150 patients in Germany, Spain and the UK to gain insight into their immediate reaction to a lipophilc gel, a cream and an ointment – all as placebo formulations – when tested on the skin.[25]

These interviews revealed that patients with psoriasis prefer lipophilic gel formulation to ointments for ease of use and cosmetic acceptability. Indeed, when asked to grade the lipophilic gel and ointment for greasiness and stickiness, the gel was considered less greasy and less sticky. The psoriasis patients also indicated challenges related to topical treatment, with many respondents mentioning problems with slow absorption of products into the skin and the need to apply their treatment more than once a day.

According to a recent survey of 483 patients with mild-to-moderate psoriasis across five European countries, the majority (81%) expressed dissatisfaction with their topical medication. The most common complaints included slow absorption (44% of patients), application frequency greater than once-daily (41%) and staining of clothes (34%) and bedding (27%). Clearly, issues of convenience are a high priority for many patients and these seem likely to contribute to the 38% non-adherence cited in this study.[26] This view was expressed by many of the 500 physicians also surveyed: 56% thought that adherence was the greatest challenge they faced. Moreover, 82% of physicians thought that adherence could be significantly improved by making available a non-alcohol topical gel.[26]

To address this point, a new vehicle for calcipotriol/betamethasone dipropionate formulation has been recently developed for the plaque psoriasis as a lipophilc gel that ensures a better cosmetic acceptability despite the ointment formulation. It had to be non-aqueous, consisting of compatible components which ensured stability of both drug substances and at the same time delivered both into the skin. The lipophilic gel contains PSE (polyoxypropylene-15 stearyl ether) as lipophilic solvent for both drug substances [27, 28] and also paraffin liquid that improve the bioavailability of the active compounds.[28] In addition to the confirmed additive effect of the two active ingredients, it is possible that the emollient properties of this lipophilic gel formulation may add to the patient benefits as compare with an alcohol- or water-based formulation.[28]

The two-compound formulation (TCF) calcipotriol plus betamethasone dipropionate (ointment and gel) had a comparable antipsoriatic effect, which was superior to the other products tested. Furthermore, these data indicate that the gel formulation could provide a better treatment option to the well established two-compound ointment for psoriasis patients due to the adherence improvement.[29]

The two-compound gel is a new topical treatment option for psoriasis, and data from phase 2 and phase 3 studies show that it is more effective than the competitor, with fewer AEs, and a rapid onset of action.[30]

The treatment adherence is a major issue in psoriasis and many factors affect adherence to topical treatments, including ease of use and convenience of

application, as well as patient-physician interaction. Prescribing therapy in line with patient preference for treatment vehicle may be a key factor, and improving patient education may help improve adherence, outcomes and QoL of patient with psoriasis.[31]

Conclusions

The importance of the vehicles in dermatology must be highlighted. From the evidence discussed, it is clear that different vehicles, in spite same active principle, determine different patient acceptance, therapy adherence and eventually clinical outcomes. In a topical formulation, the vehicle, on top of the active principles, needs to be taken into consideration when comparing drugs.

Lipophilic gels — as well as ointments — have staying power as they stay on the skin for longer period in the respects of other formulations. This makes them ideal for areas of the body that are in need of extended treatment or must endure a good deal of friction.

Recent European surveys approaching physicians and psoriasis patients, showed that the majority of physicians believed patient adherence to be the greatest challenge in the treatment of psoriasis. The gel formulation calcipotriol plus bethametasone dipropionate, beyond a comparable antipsoriatic effect with the ointment formulation, is able to meet patients' preferences and significantly improve patient adherence and consequently clinical outcomes.

Riassunto

Il veicolo topico come fattore chiave nel trattamento della psoriasi

Il compito di un veicolo topico è quello di portare il principio attivo all'interno della cute. I veicoli vengono classificati in base al loro stato fisico in formulazioni monobasiche, bifasiche e trifasiche. Le preparazioni cutanee per uso topico possono essere distinte in polveri, preparazioni liquide e semisolide. Unguenti, creme, gel e paste appartengono a quest'ultima categoria. L'assorbimento del principio attivo e la compliance del paziente sono fortemente influenzate dal tipo di veicolo utilizzato. La scelta del veicolo dovrebbe dipendere dalle caratteristiche del principio attivo, cioè ogni farmaco dovrebbe avere il suo specifico veicolo per modulare al meglio il rilascio del principio attivo stesso. Infatti, il rilascio e l'assorbimento del farmaco dipendono dalle proprietà psico-chimiche della formulazione. La scelta del veicolo dovrebbe tenere conto anche della cosmeticità del prodotto finale, la quale può influenzare l'aderenza dei pazienti al trattamento e di conseguenza l'efficacia del trattamento stesso. Nei pazienti psoriatici in veicolo maggiormente accettato da un punto di vista cosmetologico è risultato essere il gel lipofilico. Per tale motivo l'associazione topica calcipotriolo/betametasone diproprionato comunemente utilizzata nel trattamento della psoriasi come unguento è stata recentemente sviluppata in formulazione di gel liofilo.

PAROLE CHIAVE: Psoriasi - Trattamento - Cute, malattie.

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Jojoba in dermatology: a succinct review

N. PAZYAR, R. YAGHOOBI, M. R. GHASSEMI, A. KAZEROUNI, E. RAFEIE, N. JAMSHYDIAN

Department of Dermatology, Jundishapur University of Medical Sciences, Ahvaz, Iran

Phytomedicine has been successfully used in dermatology horizon for thousands of years. Jojoba (*Simmondsia chinensis*) is a long-lived, drought resistant, perennial plant with interesting economic value as it is processed for liquid wax production. The jojoba plant produces esters of long-chain alcohols and fatty acids (waxes) as a seed lipid energy reserve. The liquid wax is an important substrate for a variety of industrial applications and is used in skin treatment preparations. The oil from the jojoba plant is the main biological source of wax esters and has a multitude of potential applications. The review of literatures suggest that jojoba has anti-inflammatory effect and it can be used on a variety of skin conditions including skin infections, skin aging, as well as wound healing. Moreover, jojoba has been shown to play a role in cosmetics formulas such as sunscreens and moisturizers and also enhances the absorption of topical drugs. The intention of the review is to summarize the data regarding the uses of jojoba in dermatology for readers and researchers.

KEY WORDS: Dermatology - Jojoba wax - Phytotherapy - Herbal medicine.

Herbal remedies have been used effectively in skin disorders for thousands of years in Europe and Asia.[1] Jojoba (*Simmondsia chinensis*) is a long-lived, drought resistant plant with perennial woody shrub. It is native to the semiarid regions of Southern Arizona, Southern California, and Northwestern Mexico (Figure 1).[2, 3] The jojoba plant produces esters of long-chain alcohols and fatty acids (waxes) as a seed lipid energy reserve. This is in contrast to the triglycerides found in seeds of other plants.[4] These alcohols are significant components in cuticle waxes of plants.[2] Waxes are oxygen esters of fatty alcohols and fatty acids which are the main components of biological systems and perform numerous functions. They are located on the surfaces of plants and animals and play a protective role against various stresses such as desiccation, wetting, and pathogen attack.[4] The weight of jojoba seeds contains over 50% of liquid wax esters. Jojoba liquid wax is a stable, highly lipophilic, non-toxic "oily" material differing from common vegetable oils and animal fats in that it is

Figure 1.—Male jojoba flowers.

Corresponding author: N. Pazyar, Department of Dermatology, Imam Hospital, Azadegan Street, Ahvaz, Iran. E-mail: dr.pazyar@gmail.com

composed mainly (97%) of linear long-chain esters. Jojoba wax has numerous applications in lubricant and personal care formulations.[5] It is also a natural gum base used as a food additive in Japan.[6]

The purpose of this article is to collect the facts from different studies. It provides a narrative review and summarizes the available evidence regarding the dermatologic uses of jojoba.

Materials and methods

A review was performed in three databases of Pubmed, ISI Web of Knowledge and Google Scholar to identify *in vivo* and *in vitro* studies as well as clinical trials on this subject. The limits used were 1) English language; 2) publication date from 1990 to October 2011. The main search terms were "jojoba", "dermatology", and equivalent expressions.

Results

Bioactive components in jojoba

Wax esters are neutral lipids which composed mainly of aliphatic alcohols and acids, with both moieties usually long-chain (C(16) and C(18)) or very-long-chain (C(20) and longer) carbon structure.[7] Analysis of the jojoba wax shows that the main constituents in jojoba wax are various kinds of wax esters, namely eicosenyl octadecenoate (C20:1-C18:1) (1), eicosenyl eicosenoate (C20:1-C20:1) (II), docosenyl eicosenoate (C22:1-C20:1) (III), eicosenyl docosenoate (C20:1-C22:1) (IV) and tetracosenyl eiosenoate (C24:1-C20:1) (V).[6]

Jojoba meal

The jojoba meal is rich in protein. The main jojoba proteins are albumins (79%) and globulins (21%) which show a labile thrombin inhibitory activity. The increased digestibility by boiling may be due to inactivation of the protease inhibitors and proteins denaturation.[8] Simmondsin is a glycoside present in jojoba meal. Food supplementation of 0.5% simmondsin leads to decreasing in food intake of approximately 40% in free-feeding rats. A gustative effect is improbable since simmondsin is tasteless and induce anorexia in rats.[9]

Jojoba oil

Jojoba oil is a pure product with less than 3% triglyceride content and therefore highly resistant to oxidation. Jojoba oil is the main biological source of wax esters and possesses multiple potential applications. It is expensive and for this reason its use has been limited to medical and cosmetics applications as a basis for ointments, creams, and other care products.[10] Many studies have shown the feasibility of incorporating jojoba as an oil phase in formulas containing active compounds to increase the efficiency of topical drugs. It can be used in manufacturing varnishes, inks, waxes, detergents, resins and plastics. Additionally, jojoba is used as a potential low-calorie edible oil and coating material for fruits and pills.[4]

Dermatologic applications of jojoba

Anti-inflammatory

Jojoba liquid wax (JLW) has been shown to have anti-inflammatory properties on rats. It could decrease prostaglandin E2 (PGE2) level in the inflammatory exudates and cause significant reduction of granulation tissue formation in experimental models. Notably, JLW reduces neutrophil infiltration, as indicated by decreased myeloperoxidase (MPO) activity and also decreased nitric oxide (NO) levels and tumor necrosis factor-alpha (TNF-alpha) (Table I).[11]

Antibacterial activity

An experimental study demonstrated that exposure of bacteria for 24 h to the jojoba oil/tea tree oil

Table I.—*Dermatologic Applications of Jojoba*

Anti-inflammatory [11]
Antibacterial [12]
Antiherpetic [13]
Skin aging [14]
Natural wound healing [18]
Synthetic sebum [19]
Cosmetic [20]
Hair straightening [22]
Moisturizer [23]
Emulgel [24, 25]
Enhancing drug delivery [26, 27]

Based on the available data

mixture containing an antimicrobial leads to a loss of their culturability. The antibacterial activity of the jojoba oil/tea tree oil mixture supersedes that of carbol oil. It has been suggested this mixture inhibits bacterial colonization, when used for intermittent self-catherization.[12]

Antiherpetic activity

Yarmolinsky *et al.* demonstrated that *Simmondsia chinensis* leaf extract shows antiviral activity against herpes simplex virus (HSV) infection. It may be indicative of the presence of various components in the extract that have antiviral activity.[13]

Skin aging

The expression of collagen 1A1 and 1A2 reduces in photoaged skin. It has been shown that the basic preparation containing jojoba oil inhibits the UV induced up-regulation of matrix metalloproteinase (MMP)-1 and cause UV-induced down-regulation of collagen1 A1 and collagen1 A2.[14] Therefore, it is hypothesized that jojoba oil may be a useful addition to anti-aging products.

Sunscreen

It has been illustrated that sunscreens currently being used can cause adverse skin reactions; owing to their penetration into skin.[15] New non-permeating sunscreens (NPSUN) are suitable for use in cosmetic and pharmaceutical preparations. Jojoba oil chemical backbone immobilizes UV-absorbing moieties in the human integument. The physicochemical characteristics of NPSUNs allow these derivatives to remain confined to the upper stratum corneum where the sunscreen molecule acts, with no further clearance to deeper epidermal strata or systemic circulation. As an example, no permeation across the skin of methoxycinnamate-NPSUN was observed during 24-hour *in vitro* experiments, after topical application of either unformulated substances or of methoxycinnamate-NPSUNs formulated in jojoba oil. This study also presents jojoba oil as a preventive agent in skin cancer.[16] The jojoba ferulate is a good UV absorber by esterification of the tetrahydroxy wax with trans-4-hydroxy-3-methoxycinnamic acid. Differential scanning calorimetry has indicated that ferulate, its precursor, the tetrahydroxyjojoba wax, and the acetylated jojoba ferulate derivatives have very stable properties from ambient to 100 degrees C temperature.[17]

Natural wound healing

An *in vitro* study on keratinocytes and human dermal fibroblasts has demonstrated that JLW stimulates collagen I synthesis in fibroblasts, while no effect was detected on the secretion of MMP-2 and MMP-9. Therefore it might be used in the treatment of wounds in clinical settings.[18]

Synthetic sebum

The human skin surface and hair are generally coated with a thin film of liquid phase sebaceous lipids that contribute to the cosmetic properties of the skin. Wertz *et al*. examined a standardized and inexpensive synthetic sebum consisting of 17% fatty acid, 44.7% triglyceride, 25% wax monoester (jojoba oil) and 12.4% squalene in human. They demonstrated this synthetic sebum could be useful on the cosmetic of the skin surface or hair, on penetration of chemicals into the skin and in the development of standardized tests of laundry detergent performance.[19]

Cosmetics

Liquid wax esters are used commercially in the cosmetics industry.[20] The seeds of *Simmondsia californica* contain about 50 of light yellow oil. These are used in the cosmetic and pharmaceutical products.[7] It has been shown that jojoba oil potentiates as a dermal permeation enhancer in cosmetic preparations. Jojoba oil is often used in cosmetics as a carrier oil for specialty fragrances.[1, 21]

Hair straightening

Hair straightening is a chemical process by which excessively curly hair is straightened in an irreversible way. Generally, products are formulated as emulsions with high pH value (9.0-12.0), which, after applied on hair, cause considerable damage, making it dry and fragile. It is documented that jojoba oils exhibit conditioning effects. A study on Afro-ethnic subjects revealed that the addition of conditioning agents to the straightening emulsion with ammoni-

um thioglycolate benefit the hair fiber. It mitigates protein loss, protects the hair thread, and improves the resistance to breakage.[22]

Moisturizing

A small pilot clinical study has shown that hydrolyzed jojoba esters K-20W (K-20W) are able to increase skin hydration and improve sensory skin "feel" when included in a variety of skin, hair, and nail care cosmetic/personal care formulations. Glycerol and hydrolyzed jojoba esters are added to obtain a substantial long-acting 24 h skin hydration (moisturizing) effect for topical products. It supports the "proof of concept" that an enhanced, additive role exists between these two ingredients resulting in a long-term (24 h) skin moisturizing effect. The moisturizing potential (glycerol and hydrolyzed jojoba) may prove valuable in the future development of cosmetic and over-the-counter/prescriptive topical products, including new medicaments containing botanicals.[23] The new crop oil from jojoba is a skin-softener similar to sperm whale oil. Tetrahydroxyjojoba wax (THJF) is a stable, colorless, low-melting solid which is considered an excellent emollient.[24]

Emulgel

Emulgel topical formulation is a vehicle of potential for topical delivery of antifungal drugs. According to the *in vitro* studies, drug release from the commercial preparation is lower than some of the prepared jojoba oil-based emulgel formulae. The selected formula shows superior antimycotic activity compared to the commercially available formulation.[24, 25]

Enhancing drug delivery

Wang *et al.* evaluated the effects of aminophylline from cream formulations on human skin *in vivo*. They illustrated that microemulsions containing 10% jojoba oil and 30% corn germ oil were a key driver for the percutaneous absorption of aminophylline.[26] Enhanced systemic absorption *in vivo* and percutaneous penetration *in vitro* after transdermal administration of diclofenac sodium formulated in U-type microemulsion was demonstrated in another study.[27]

Side effects

It has been reported that application of moisturizing cream contain jojoba oil could cause contact dermatitis in human.[28]

Discussion and conclusions

The use of alternative medicine including herbal therapy is increasing dramatically. A few randomized controlled trials have shown promising results in the use of herbal therapies for dermatologic disorders.[1] The anti-inflammatory activity of jojoba oil is mediated through the decrease of prostaglandin E2, myeloperoxidase, nitric oxide and TNF alpha.[11] Importantly jojoba oil can immobilize UV absorption and inhibits UV-induced down-regulation of collagen type 1. Therefore it may be a useful addition to sunscreens and anti-aging preparations.[14, 15] A wound healing effect of JLW may be exerted by synthesis of collagen type I in fibroblasts. Jojoba products can be added to hair straightening emulsions and are considered moisturizer agents in cosmetology.[22, 23] It has been shown that jojoba oil could play a role in absorption of topical drugs and could be used as an emulgel for topical delivery of antifungal drugs.[24, 25]

In sum, we should know what common herbal alternatives therapy exists and which adverse effects can potentially appear so as to counsel patients more effectively. Much more research on this herbal agent is required.

Riassunto

La jojoba in dermatologia: una breve review

La fitoterapia è utilizzata efficacemente in ambito dermatologico da migliaia di anni. La jojoba (*simmondsia chinensis*) è una pianta perenne, longeva e resistente alla siccità che possiede un interessante valore economico quando viene lavorata per la produzione della cera liquida. La pianta di jojoba produce esteri di alcoli e acidi grassi a catena lunga (cere) come riserva energetica lipidica sotto forma di semi. La cera liquida è un sostrato importante per numerose applicazioni industriali ed è utilizzata nei preparati per il trattamento cutaneo. L'olio derivante dalla pianta di jojoba è la principale fonte biologica di esteri della cera e possiede una molteplicità di potenziali applicazioni. La presente review della letteratura suggerisce come la jojoba possieda un effetto antinfiammatorio e possa essere utilizzata nel trattamento di numerose condizioni cutanee, tra

cui le infezioni cutanee, l'invecchiamento cutaneo e la guarigione delle ferite. Inoltre, è stato dimostrato come la jojoba rivesta un ruolo nelle formulazioni cosmetiche come i filtri solari e le creme idratanti e sia in grado di potenziare anche l'assorbimento di farmaci per via topica. Obiettivo della presente review è stato quello di riassumere i dati concernenti gli utilizzi della jojoba in ambito dermatologico per i lettori e i ricercatori.

PAROLE CHIAVE: Dermatologia - Jojoba - Fitoterapia.

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Efficacy of a photolyase-based device in the treatment of cancerization field in patients with actinic keratosis and non-melanoma skin cancer

M. PUVIANI [1], A. BARCELLA [2], M. MILANI [3]

[1]Struttura Semplice di Dermatologia e Dermatologia Chirurgica Ospedale di Sassuolo Sassuolo, Modena, Italy
[2]Servizio di Dermatologia, Nembro, Bergamo, Italy
[3]Direzione Medica ISDIN Italia Milan, Italy

Eryfotona AK-NMSC (ISDIN Spain) is a film-forming medical device in cream or fluid formulation containing the DNA-repair enzyme photolyase and high-protection UV filters in liposomes (repairsomes) indicated in the treatment of cancerization field in patients with actinic keratosis (AK) or non-melanoma skin cancer (NMSC). Photolyase is an enzyme that recognizes and directly repairs UV-induced DNA damage. The most common UV-induced DNA damage is the formation of cyclobutane pyrimidine dimers (CPD). Clinical studies evaluating the histological and cellular effects of Eryfotona AK-NMSC have shown a potential benefit in the treatment of the cancerization field in AK patients. In particular the use of Eryfotona AK-NMSC improves the confocal microscopic appearance of skin at the cancerization field level. In addition, Eryfotona AK-NMSC improves the p53 gene expression at keratinocyte level. In this study we reported a series of 6 cases of patients with AK or NMSC lesions treated with Eryfotona AK-NMSC fluid, both as coadjuvant and as single treatment, applied twice daily in the affected area with photograph documentation. Clinical photographs of the skin lesions at baseline and after Eryfotona AK-NMSC treatment were taken in all cases using a high-definition digital camera. Six patients with multiple AK lesions of the scalp or face with or without NMSC were treated for a mean of 1-3 months with Eryfotona AK-NMSC fluid formulation. Image documentations before and after treatment of this clinical series show a great improvement in AK lesions count and of cancerization field. This clinical series supports the clinical efficacy of the use of photolyase and high-protection UV filters in the treatment of cancerization field and AK lesions in patients with actinic damage.

Key words: Keratosis, actinic - Case reports - Sunscreening agents.

Actinic keratosis (AK) is a common epidermal lesion which can progress to invasive squamous cell carcinoma.[1] Chronic exposure to ultraviolet radiation, particularly in fair-skinned patients, is the most relevant risk factor for the development of AK.[2] Sunscreens have shown to be an effective AK prevention treatment.[3] UV chronic exposure causes mutation in nuclear and mitochondrial DNA, mainly at the tumor suppressor gene p53 level.[4] The most common UV-induced DNA damage is the formation of Cyclobutane pyrimidine dimers (CPD).[5] CPDs are actually the major DNA photoproducts produced by exposure to UV radiation.[6] If left unrepaired, these lesions can lead to replication errors, mutation, and cell death.[7] Photolyase is a light-activated flavoenzyme that binds to pyrimidine dimers in DNA and repairs them in a reaction triggered by electron transfer from the photo-excited flavin cofactor to the dimer.[8] Photoreactivation is carried out by photolyases which specifically recognize and repair photoproducts.[9] The potential of DNA photolyase in skin cancer prevention has been increasingly recognized. Eryfotona AK-NMSC is a film-forming medical device in cream or fluid formulation containing the DNA-repair enzyme photolyase and high-protection UV filters (Repairsomes™). Eryfotona AK-

Corresponding author: M. Milani, Direzione Medica ISDIN Italia, viale Abruzzi, 320131 Milan, Italy.

NMSC is indicated in the treatment of cancerization field in patients with AK or non-melanoma skin cancer. Clinical studies evaluating the histological and cellular effect of Eryfotona have shown a potential benefit in the treatment of the cancerization field in AK patients.[10] In particular the use of Eryfotona AK-NMSC improves the confocal microscopic appearance of cancerization field, improving also p53 gene expression at keratinocyte level.[11] Very recently Puig *et al*.[12] have shown that in patients with AK the use of Eryfotona AK-NMSC treatment improved cancerization field, restoring normal cellular phenotype through CPI-17 gene up-regulation, a gene involved in the normal phenotype recovering. In patients with AK treated with PDT, in comparison with a simple sunscreen cream, treatment with Eryfotona AK-NMSC was associated with a significant lower recurrence rate of new AK lesions.[13] In this study, partially reported elsewhere,[14] we describe a series of case reports of patients with AK or NMSC treated with Eryfotona AK-NMSC, both as coadjuvant and single treatment, applied twice daily in cream or fluid formulation with imaging documentation.

Clinical series

Eryfotona AK-NMSC in the cancerization field treatment in subjects with SCC

Case 1.—This was a 71-year old man with a type II Fitzpatrick skin type with a large (>10 cm in diameter) SCC localized in the scalp. In the area surrounding the lesion several AK lesions (>12) could be observed. In October 2012, after the surgical removal of the SCC (Figure 1), the subject was treated with Eryfotona AK-NMSC, fluid, applied twice daily in the cancerization field area. After 2-month treatment the majority of AK lesions were clinically disappeared (Figure 2). The product was well tolerated.

Case 2.—This was a 65-year old man with an AK lesion on the helix of the right ear. The subject was treated as single therapy with Eryfotona AK-NMSC in fluid formulation, applied twice daily in the affected area. After 2-month treatment the AK lesion was clinically disappeared. The product was well tolerated.

Eryfotona AK-NMSC in the treatment of cancerization field and AK lesion

Case 3.—This was a 74-year-old man, with a Fitz-Patrick prototype II and with AK lesions located mainly

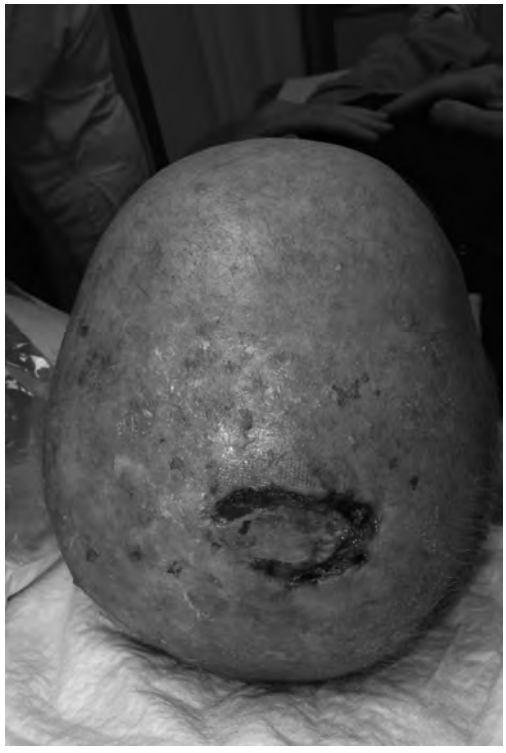

Figure 1.—Case 1, after the surgical removal of the SCC.

Figure 2.—Case 1, after 2-month treatment.

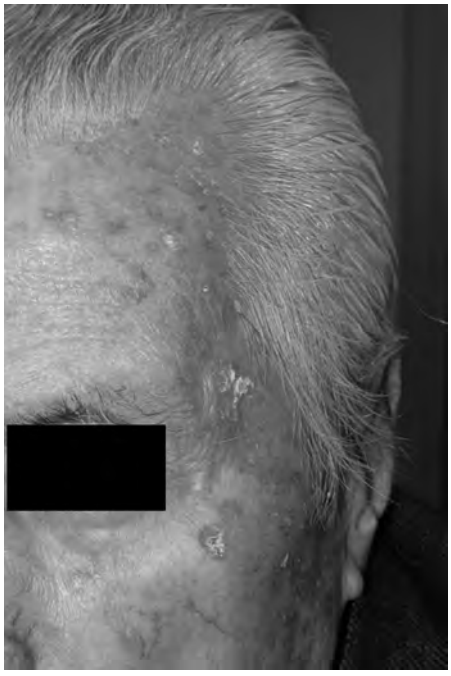

Figure 3.—Case 3, patient with a Fitz-Patrick prototype II and with AK lesions.

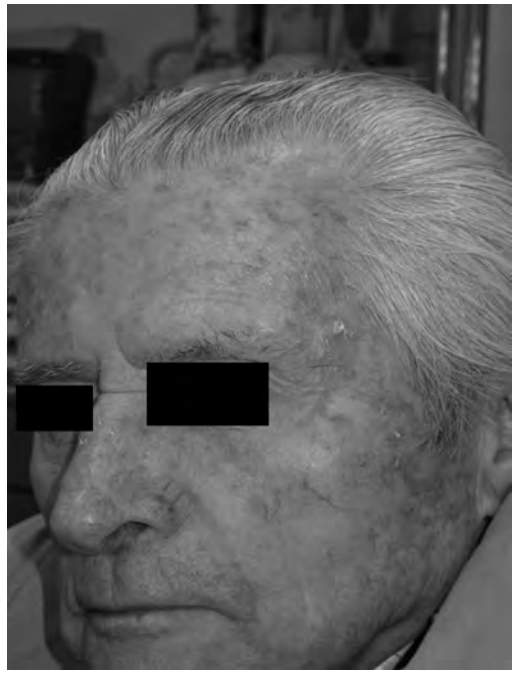

Figure 4.—Case 3, after 2-month treatment. The majority of AK lesions were clinically disappeared.

in the front, check, temple and nose (Figure 3). The subject was treated, as the only therapy, with Eryfotona AK-NMSC, fluid, applied twice daily in the cancerization field area. After 2-month treatment the majority of AK lesions were clinically disappeared (Figure 4). The product was well tolerated.

Case 4.—This was a 65-year-old man, with a Fitzpatrick skin type II with AK lesions located mainly in the, temple. Before treatment 13 clinical visible AK were present. The subject was treated, as unique therapy, with Eryfotona AK-NMSC, fluid, applied twice daily on the lesions area and on the cancerization field area. After 2-month treatment the majority of AK lesions were clinically disappeared. The product was well tolerated.

Case 5.—This was a 65-year-old man with a Fitzpatrick skin type II with multiple) AK lesions located in the scalp. The subject was treated, as unique therapy with Eryfotona AK-NMSC in fluid formulation, applied twice daily in the lesions area and at the cancerization field area. After treatment there was a clinical improvement of the appearance of AK lesions. Treatment duration was 6 weeks. There were no local side effects.

Case 6.—This was a 65-year-old man with a Fitzpatrick skin type II with multiple AK lesions located in the front and scalp area (Figure 5). The subject was treated, as single therapy with Eryfotona AK-NMSC, fluid, applied twice daily in the lesions area and at the cancerization field area. Also in this case Eryfotona AK-NMSC induced a significant improvement of the treated area (Figure 6). Treatment duration was 4 weeks. No tolerability and safety problems were observed during the treatment.

Table I summarizes main characteristics of this clinical cases series and the clinical evolution of AK lesions after Eryfotona AK-NMSK treatment.

Discussion

Chronic sun exposure triggers several inflammatory pathways in the skin favoring keratinocytes genetic modification.[15] One skin lesion AK can present as a discrete well-defined lesion;[16] however, multiple less-defined subclinical lesions over large areas of skin are more common, and additional lesions often become evident over time.[17] Subclinical AK lesions are common, particularly in patients with sun-damaged skin.[18] Subclinical lesions have the same histopathology features as clinically visible AK, which includes focal areas of abnormal keratinocyte proliferation and differentiation, but are not yet discernible on the skin surface.[19] Subclinical AK may ex-

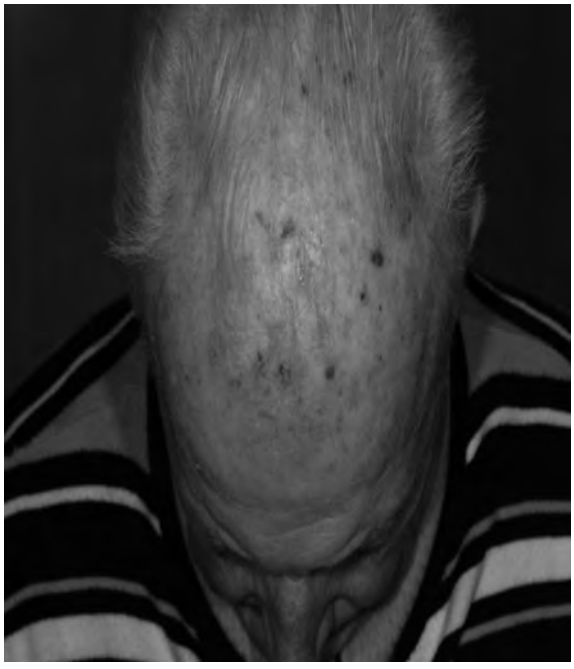

Figure 5.—Case 6, a 65-year-old man with a Fitzpatrick skin type II with multiple AK lesions located in the front and scalp area.

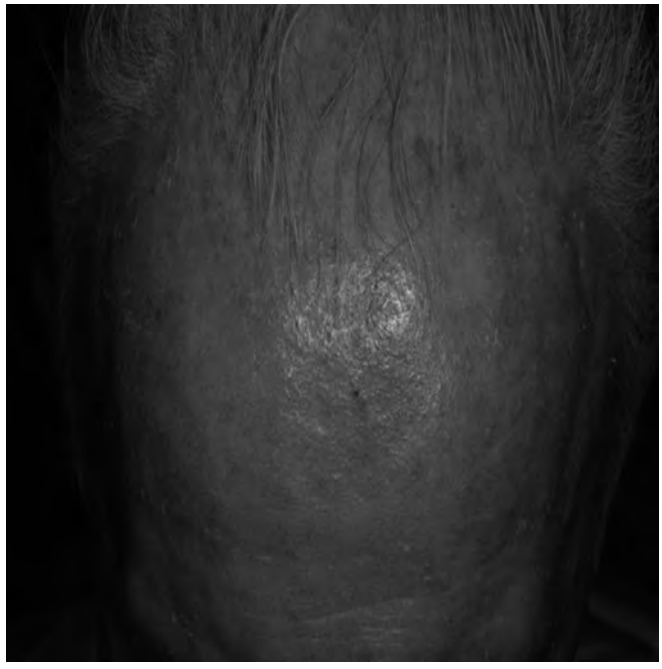

Figure 6.—Case 6, visible improvement of the treated area.

TABLE I.—*Main characteristics of this clinical cases series and the clinical evolution of AK lesions after Eryfotona AK-NMSK treatment.*

Case	Sex	Age years	Number of clinical visible lesions at baseline	Clinical improvement*
1	Man	71	15	++
2	Man	65	1	+++
3	Man	74	14	++
4	Man	65	3	+++
5	Man	65	12	++
6	Man	65	25	+++

*: Legend: +++ = >75% clearing; ++ = 50-75% clearing; + = <50% clearing.

ceed the number of visible lesions by 10-fold,[20] and the emergence of untreated subclinical lesions over time may account for many apparent recurrences, as they visibly manifest as clinically new lesions. The concept of "field cancerization" was first introduced by Slaughter *et al*.[21] and refers to the histologically and genomic cell alteration in the region surrounding a tumor. This concept suggests that subclinical preneoplastic changes are frequently present in skin site surrounding AK or NMSC lesions.[22] The goals of treatment are to eliminate the AKs, minimizing their risk of progression to invasive SCC. The concept of cancerization field has also therapeutic implications. Lesion-directed and field-directed are the two treatment approaches of AK.[23] Lesion-directed treatment modalities include cryotherapy, surgery and electrodessication with or without curettage. Field-directed treatment modalities include no ablative and ablative laser resurfacing, dermabrasion, chemical peels, topical immunomodulators (imiquimod, 5-fluorouracil and diclofenac) and photodynamic therapy.[24] Eryfotona AK-NMSC is a film-forming medical device in cream or fluid formulation containing the DNA-repair enzyme photolyase and high-protection UV filters (repairsomes). Eryfotona AK-NMSC is indicated in the treatment of canceri-

zation field in patients with AK or NMSC. Clinical studies have shown that this topical product can, in patients with AK, normalize p53 genetic expression both in the short-term and after 12-month treatment at the cancerization field level. Very recently Puig *et al*. have shown that in patients with AK the use of Eryfotona AK-NMSC treatment improved the field of cancerization, restoring normal phenotype through CPI-17 up-regulation, a gene involved in the normal phenotype recovering of keratinocytes. In addition Eryfotona AK-NMSC improves the confocal microscopic appearance of the AK lesions and the surrounding skin. In patients with AK treated with PDT, in comparison with a simple sunscreen cream, treatment with Eryfotona AK-NMSC was associated with a significant lower recurrency rate of new AK lesions.

Conclusions

These case-report series support the clinical efficacy of the use of photolyase and high-protection UV filters (Repairsomes™; Eryfotona AK-NMSC) in the treatment of cancerization field and AK lesions. These case reports are in agreement with clinical studies evaluating the effects of Eryfotona AK-NMSC on cancerization field levels and in the prevention of AK recurrence after specific treatments such as photodynamic therapy or other field-directed or lesion-directed treatments for AK or NMSC. Further clinical controlled studies are warranted in order to fully evaluate the potential of this therapeutic approach in the treatment of AK and NMSC.

Riassunto

Efficacia di un dispositivo medico a base di fotoliasi nel trattamento di cancerizzazione in pazienti affetti da cheratosi attinica o tumore della pelle non melanoma

Eryfotona AK-NMSC (ISDIN Spain) è un dispositivo medico disponibile in formulazione crema o fluido e contiene fotoliasi, un enzima con azione di riparazione del DNA e filtri UV ad alta protezione veicolati in liposomi (Repairsome). Eryfotona AK-NMSC è indicato nel trattamento del campo di cancerizzazione in pazienti con cheratosi attinica (*actinic keratosis*, AK) o tumore della pelle non-melanoma (*non-melanoma skin cancer*, NMSC). La fotoliasi è un enzima che riconosce e ripara direttamente il danno indotto dai raggi UV al DNA. Il più comune danno UV indotto al DNA è la formazione di dimeri ciclobutano pirimidina (CPD). Studi clinici, che hanno valutato l'effetto sia a livello istologico che di espressione genica di Eryfotona AK-NMSC, hanno mostrato un potenziale beneficio nel trattamento del campo di cancerizzazione in pazienti affetti da AK. In particolare l'utilizzo di Eryfotona AK-NMSC migliora l'aspetto del campo di cancerizzazione valutato tramite microscopia cionfocale. Eryfotona AK-NMSC migliora l'espressione del gene p53 a livello dei cheratinociti presenti nel campo di cancerizzazione. In questo studio abbiamo riportato una serie di 6 casi di pazienti con lesioni da AK o NMSC trattati con Eryfotona AK-NMSC, sia come trattamento coadiuvante o come unico trattamento, Eryfotona AK-NMSC veniva applicato due volte al giorno (in formulazione fluida). I casi valutati hanno tutti una documentazione fotografica. La documentazione clinica fotografica delle lesioni cutanee al basale e dopo il trattamento Eryfotona è stata ottenuta in tutti i casi con una fotocamera ad alta definizione digitale. Lo studio ha incluso 6 pazienti, con più lesioni da AK del cuoio capelluto o del viso con o senza NMSC i quali sono stati trattati per una media di 1-3 mesi con Eryfotona AK-NMSC in formulazione fluido. Le immagini prima e dopo il trattamento di questa serie di casi clinici mostrano un notevole miglioramento del campo di cancerizzazione e delle lesioni della cheratosi attinica. Questo serie di casi clinici supporta l'efficacia clinica dell'uso di Eryfotona AK-NMSC (filtri UV ad alta protezione e fotoliasi) nel trattamento del campo di cancerizzazione e delle lesioni AK in pazienti con danno attinico.

PAROLE CHIAVE: Cheratosi attinica - Fotoliasi - Sole, protezione.

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Transient symptomatic zinc deficiency resembling acrodermatitis enteropathica in a breast-fed premature infant: case report and brief review of the literature

E. ZATTRA, A. BELLONI FORTINA

Dermatology Unit, Department of Medicine University of Padua, Padua, Italy

Transient symptomatic zinc deficiency is a rare disorder clinically indistinguishable from acrodermatitis enteropathica characterized by periorificial and acral dermatitis that usually occurs in exclusively breast-fed infant especially if preterm. We describe a three-month-old breast-fed preterm boy who developed the typical skin lesions. Maternal breast milk zinc was lower than the levels from other 2 mothers of infants at the same gestational age. The disease improved and serum zinc level became normal with oral supplementation of zinc. No recurrence of the dermatosis was observed when the treatment was stopped after weaning.

KEY WORDS: Zinc deficiency - Acrodermatitis enteropathica - Infant, premature - Milk, human.

Zinc is an essential oligometal for the optimum growth and development of humans.[1] A zinc deficiency may lead to dermatitis, diarrhea, alopecia, neurologic symptoms and growth limitation.[1] Zinc deficiency can be caused by a genetic or an acquired transient defect, clinically indistinguishable.[2] Both term and preterm infants may be in negative zinc balance.[2]

The genetic form is a rare autosomal recessive condition known as Acrodermatitis enterophatica (AE; OMIM 201100), first described by Danbolt in 1943,[3] which usually starts after weaning.[4]

The acquired form is due to nutritional zinc deficiency which may be seen in infants with severe malabsorption, in premature infants who receive prolonged parenteral alimentation and in breast-fed infants when the zinc level in the mother's milk is abnormally low.[5]

Corresponding author: A. Belloni Fortina, MD, Dermatology Unit, Department of Pediatrics, University of Padua, via Giustiniani 3, 35128 Padua, Italy. E-mail: belloni@pediatria.unipd.it

We report a case of a transient symptomatic zinc deficiency (TSZD) in a breast-fed premature infant, with the measurements of the maternal serum zinc levels.

Case report

A three-month-old Caucasian male, first child of healthy non consanguineous parents, was born prematurely at 27 weeks' gestation weighing 1065 g.

His neonatal course was complicated by respiratory distress syndrome and patent ductus arteriosus. Nutritional support initially consisted of total parenteral nutrition which provided trace elements including zinc and was stopped after 15 days.

He was then exclusively breastfed. At three months of age he developed an eruption consisting of erythematous and scaling plaques with pustolar lesions, erosions and crusts initially located on his face, predominantly perioral-ly and perinasally (Figures 1, 2), associated with intermittent diarrhea A diagnosis of impetigo was considered and he was treated orally with amoxicillin and clavulanic acid and topically with 2% mupirocin cream with only partial improvement.

A week later the eruption spread to the scalp and the perianal region (Figure 3) and the patients was sent for a dermatology consultation. The clinical features, the prematurity of the child and the exclusively breast-fed nutrition led us to consider a diagnosis of zinc deficiency, later confirmed by an undetectable serum zinc level. All of the routine laboratory investigations gave nega-

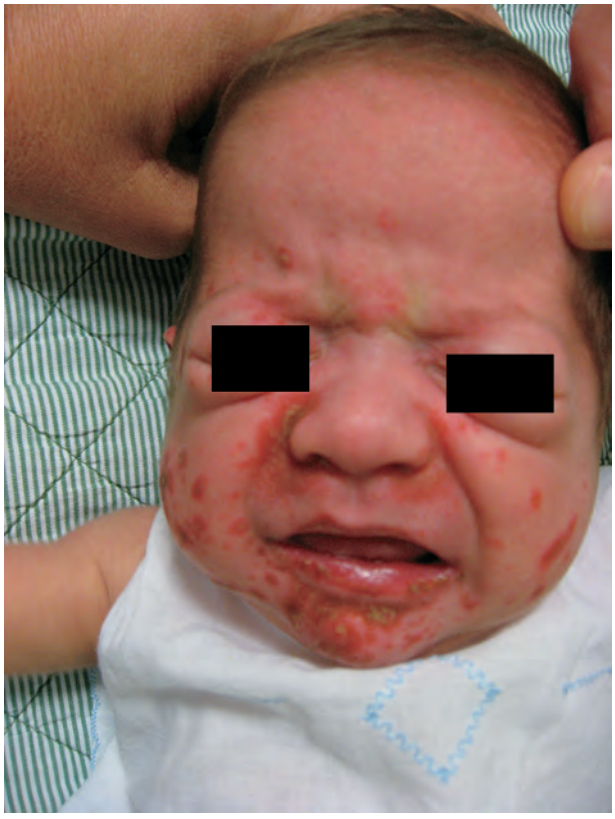

Figure 1.—Skin lesions on presentation, showing erythematous papules and plaques with crusts and pustules on the face, predominantly in the perioral and perinasal regions.

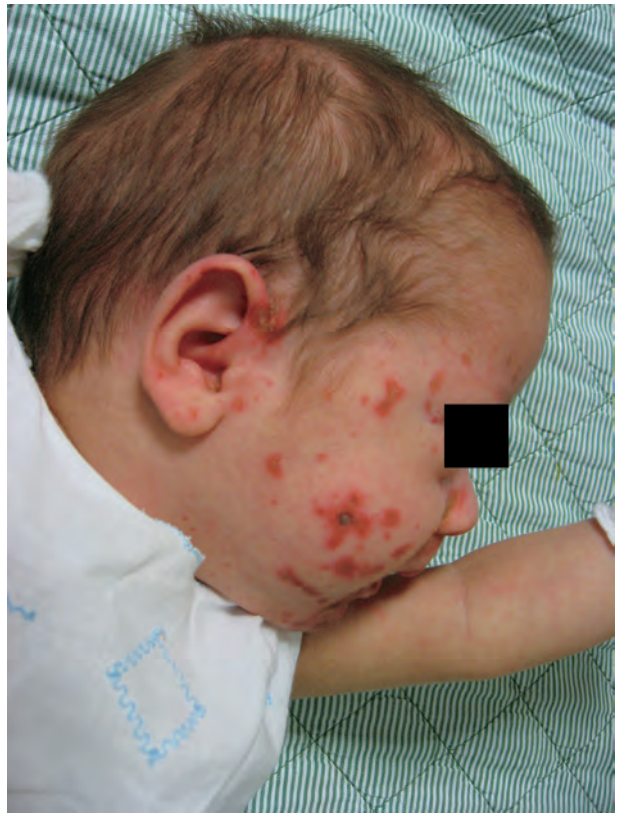

Figure 2.—Erythematous and pustular lesions on the face.

tive or normal results. We measured the maternal serum zinc level which resulted within the normal range (18.3 umol/L; normal range: 11.5-22.2 umol/L), but maternal breast milk zinc level was lower (0.46 mg/L; normal range: 0.62 mg/L-15 mg/L) than the breast milk zinc level of two mothers of infants at the same gestational age (1.87 mg/L and 2.42 mg/L, normal range 0.62 mg/L-15 mg/L).

The clinical suspicion of acrodermatitis enteropathica-like eruption was confirmed and a treatment with oral zinc sulphate at a dosage of 10 mg/day was issued. In a few days we obtained a rapid improvement of the dermatitis (Figure 4) and the normalization of serum zinc level at 17.3 umol/L (normal zinc level for infant aged 1-3 months is >7.6 umol/L [6]).

At the same time we noticed a regularization of the alvus.

Oral zinc supplementation was continued and the child remained asymptomatic, with normal serum zinc levels. After weaning, during the follow-up the treatment was stopped with no recurrence of the dermatosis (Figure 5).

Discussion

TSZD is characterized by erythematous, scaly, thin papules and plaques or vesiculobullous and pustular lesions distributed predominantly in acral and periorificial regions. Such eruption may occasionally also spread to the trunk.[5]

Nail changes such as onychodystrophy, onycholisis and paronychia, and oral stomatitis, perlèche, blepharitis, conjunctivitis and photophobia may also occur. Other clinical manifestations are retardation of growth, impairment of wound healing, hypogonadism and neuropsychiatric features.[5]

TSZD and acrodermatitis enteropathica are clinically indistinguishable although the pathologic mechanisms are different.[6]

TSZD usually appears between 8 and 24 weeks of age and occurs in exclusively breast-fed infants especially if preterm, as they are more susceptible

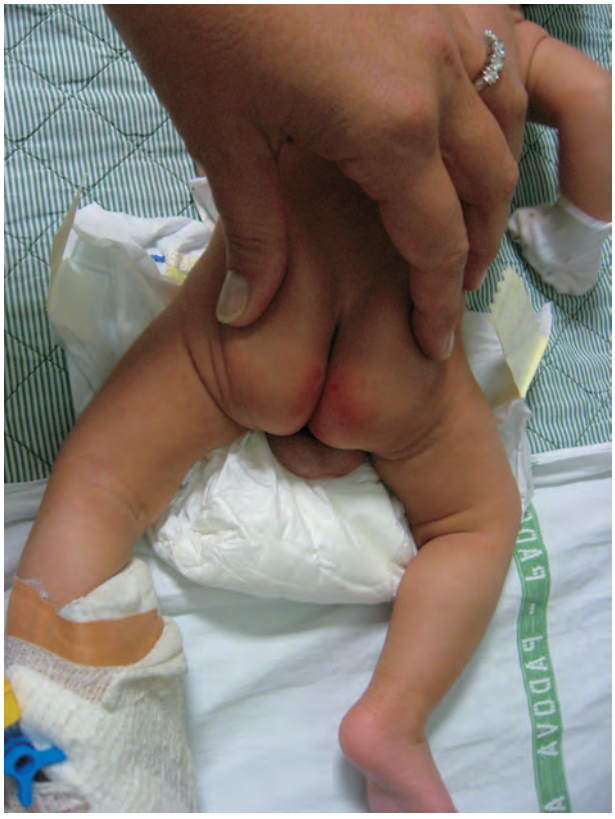

Figure 3.—Erythematous lesions around the anal region and buttocks.

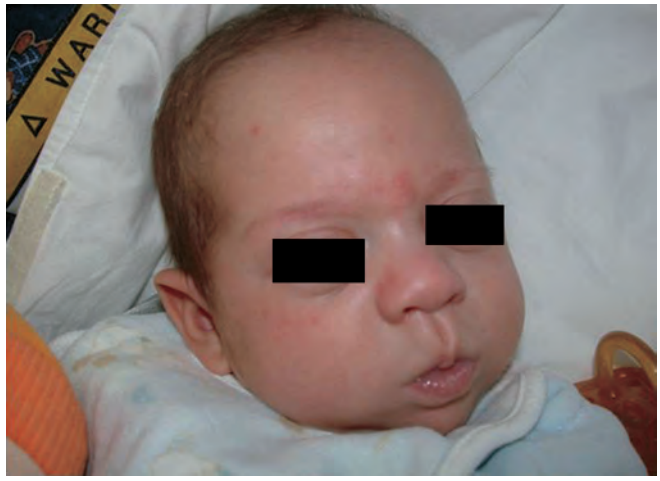

Figure 4.—Skin lesions 8 days after the start of the zinc supplementation, showing complete resolution of lesions without scarring on the face.

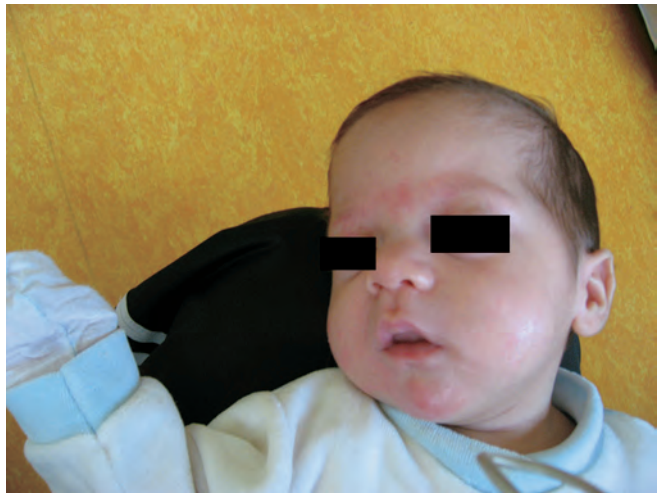

Figure 5.—Resolution of the cutaneous lesions on the face during the treatment.

to a negative zinc balance. In these patients, the enteric absorption of zinc is normal and zinc deficiency seems to be caused by a defective transfer of zinc from serum to breast milk, due to a mutation of zinc transporter gene SLC30A2 (ZnT-2).[7, 8] This disease improves with oral supplementation of zinc or after weaning.[9] Zinc can be administered as acetate, gluconate, sulphate or amino acid chelates. The recommended dose is 2-4 mg/kg for infants between 0 and 6 months. The clinical remission and the normalization of serum zinc levels are generally noted within 4-28 days.[9]

Acrodermatitis enteropathica is an autosomal recessively inherited disease caused by genetic defect in the production, structure or function of a zinc-binding protein low molecular weight secreted by the pancreas.[4] This ligand is necessary for zinc absorption from bovine milk. Mutations in the SLC39A4 gene located on chromosome 8q24.3 have been found to be responsible for this disease.[10] Maternal milk has a protective role during infancy because its zinc-binding ligand is different, so manifestations of zinc deficiency begin after weaning. Affected patients then require life-long zinc replacement.[4]

There are other numerous skin conditions which can mimic the zinc deficiency eruption such as atopic dermatitis, impetigo, diaper dermatitis, contact dermatitis, perioral dermatitis, cronic mucocutane-

ous candidiasis, metabolic and genetic disorders (biotin and decarboxylase deficiencies, essential fatty disorders, cystic fibrosis), epidermolisis bullosa, seborrheic dermatitis.[11]

Conclusions

In conclusion, TSZD is a rare pathology but we have to keep this diagnosis in mind when an eruption mainly localized in acral and periorificial regions occurs in premature infants.

Riassunto

"Transient symptomatic zinc deficiency" indistinguibile da "acrodermatitis enteropathica" in un bambino pretermine allattato al seno: caso clinico e breve review della letteratura

La *transient symptomatic zinc deficiency* è una malattia rara clinicamente indistinguibile dalla acrodermatitis enteropatica, caratterizzata da una dermatite periorifiziale ed acrale che solitamente si verifica nei bambini esclusivamente allattati al seno in particolar modo se prematuri. Descriviamo il caso di un bambino di tre mesi di età, pretermine, che ha sviluppato le tipiche lesioni. I livelli di zinco nel latte materno erano più bassi dei livelli di altre due madri di bambine alla stessa età gestazionale. La malattia è migliorata e la zinchemia si è normalizzata con supplementazione orale di zinco. La malattia non ha recidivato dopo lo svezzamento.

PAROLE CHIAVE: Zinco, carenza neonatale - Acrodermatitis enteropatica - Neonato prematuro - Latte umano.

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Juvenile ulcerated necrobiosis lipoidica successfully treated with oral cyclosporin A

TO THE EDITOR: Necrobiosis lipoidica (NL) is an inflammatory granulomatous disease of the skin, that is usually considered a striking feature of diabetes mellitus yet it may also be present in nondiabetic patients. In fact NL, first decribed by Oppenheim in 1929,[1] developes in approximately 0.3% of all diabetic patients, with a median age of onset of 30 years.[2] A retrospective review [3] has found that 90% patients with NL are diabetic, will develop diabetes or have a family history of diabetes. There are few reported cases of ulcerated NL in children. We report a 14 year-old girl with ulcerative NL, who was successfully treated with oral cyclosporin A.

A 14-year-old girl presented at the outpatient clinic with a 3-year history of plaques on legs, abdomen and mammary region. The plaques on the anterior left leg were ulcerated (Figure 1). Biopsy of an ulcerated lesion was performed, and NL was histologically confirmed. The patient had an 8-year history of insulin-dependent diabetes mellitus. The glycosylated haemoglobin was 14 % (normal range less than 8%), indicating poor glicemic control. Treatment with topical and intralesional corticosteroids was uneffective. Written informed consent was obtained and the patient started with oral cyclosporine A (CsA) 3 mg/kg per day after appropriate baseline investigations. The areas of ulceration improved very rapidly, with a dramatic re-epitheliation of 40% after 2 weeks and a complete wounds healing after 5 months (Figure 2). The treatment was continued for 3 month at the starting dosage, then it was reduced in parallel with the association of local tacrolimus ointment 0,01% twice a day. After nine months of therapy, CsA was discontinued, with no relapse at 4-month ~~of~~ follow-up. No side-effects were recorded during the course of treatment.

There are few cases reports of NL in children, and ulceration is exceptional. The prognostic significance of ulceration in children is currently unknown, yet we must keep in mind that in adults it is associated with a long-term risk of malignant transformation to squamous cell carcinoma. As well as pathogenesis of NL, including ulcerative lesions, is still largely to be elucidated, a general agreement on the optimal safe and effective treatment option has not been found so far. Focusing our attention on ulcerated NL of adults, a variety of treatments [4] have been found useful in small cohorts of patients such as potent topical steroids, tacrolimus, corticosteroids injected into the active border, PUVA, oral pentoxifylline, surgical treatment, nicotinamide, antiplatelet agents, mycophenalate mofetil, anti-TNFalfa agents, and oral CsA.[5, 6] The reported case was re-

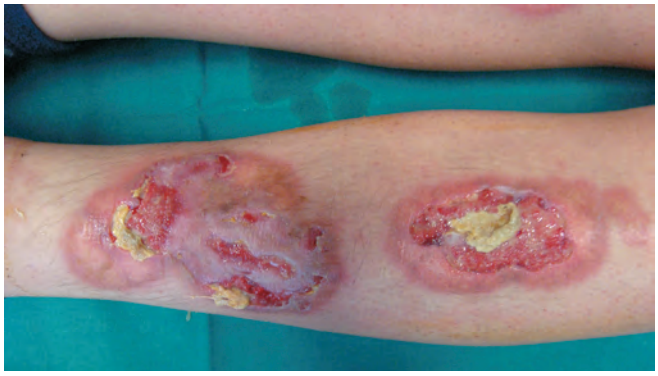

Figure 1.—Ulcerated plaques surrounded by an extensive area of erythema on the anterior surfaces of her left leg.

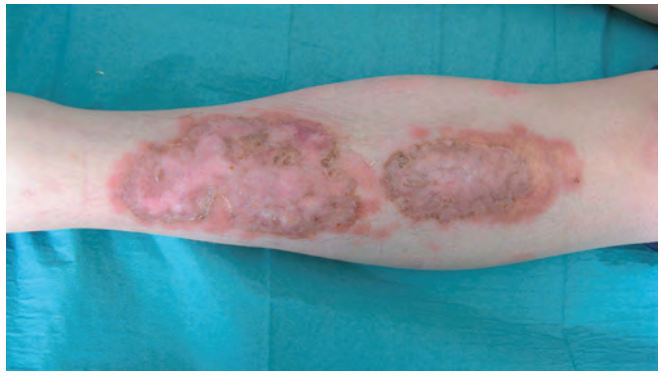

Figure 2.—Complete wounds healing after 5 months.

sistant to topical and intralesional corticosteroids, and was successfully treated with CsA. The use of CsA is supported by the hypothesis that cell-mediated immunity, including inhibition of production and release of lymphokines *e.g.* interleukin 2 and prevention of clonal proliferation of T lymphocytes in response to specific antigens, may be involved in the pathogenesis of ulcerated NL In conclusion, we reported a case of severe, widespread and ulcerative NL of the childhood, in which a cycle of CsA treatment proved effective, safe and well tolerated.

G. GUALDI
Department of Dermatology, University of Brescia, A.O. Spedali Civili, Brescia, Italy
giuliogualdi@libero.it

P. MONARI
Department of Dermatology, University of Brescia, A.O. Spedali Civili, Brescia, Italy

C. FARISOGLIO
Department of Dermatology, University of Brescia, A.O. Spedali Civili, Brescia, Italy

P. CALZAVARA-PINTON
Department of Dermatology, University of Brescia, A.O. Spedali Civili, Brescia, Italy

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Treatment of pilonidal sinus disease with autologous platelet-rich plasma

TO THE EDITOR: The sacrococcygeal region is an area with rather frequently occurring suppurating sinus tract, called "pilonidal sinus disease", with orifice on the cutis and extending through subcutaneous tissue. The term pilonidal is derived from the Latin pilus, hair, for the presence of hair in a cystic mass that causes a foreign body reaction usually leading to the formation of an abscess cavity.[1] Over time, with the movements of the muscle planes during physical activities, the cyst can become inflamed and infected with the risk of draining to the skin through sinus tracts and fistulae, which represents a complication of this disease.

A 53-year-old male patient, office clerk, body mass index (BMI) of 28, underwent surgical excision of pilonidal sinus disease with fistulectomy in 2007, and wound healing was induced by secondary intention.

Four years later the patient continued to present a painful bleeding ovoidal ulcer, with raised edges about two inches in diameter in the natal cleft (Figure 1). He complained of psychological distress for the continuous secretions of serous exudate and blood leading to continuously soiled and ill-smelling clothing. The lesion had been subjected to several cycles of medical therapy with topical application of antiseptic solutions (povidone-iodine), without any benefit. Thus, it was decided to start a treatment of the ulcer with platelet-rich plasma (PRP) preparations with the aim to induce the healing process. The patient was informed about the type of proposed treatment, he showed no notable medical history items contraindicating the therapy and the blood count was within normal limits. During the initial assessment, two specific questionnaires were administered: the Numeric Rating Scale (NRS) scale [2] for the measurement of subjective pain and the SF12,[3] consisting of 12 questions that investigate two synthetic health status indices, the Physical Component Summary (PCS-12) related to patient physical state and Mental Component Summary (MCS-12) for his state of mind. These assessments were repeated at the end of treatment and at follow-up 6 months later.

After informed consent was obtained, 10 applications of platelet gel were performed on a weekly basis. Each preparation was obtained from 8 cc of whole blood drawn by venupuncture into a test tube (Regen ® Fibrin Polymer 2). The blood was centrifuged at 3100 rpm for eight minutes thereby obtaining PRP. Calcium gluconate 10% was added to the PRP, which was further centrifuged for 15 min. A sterile field was set up around the ulcer after having thorough cleansed the skin area with sterile normal saline and local anesthesia with 4 cc of 2% lidocaine was injected

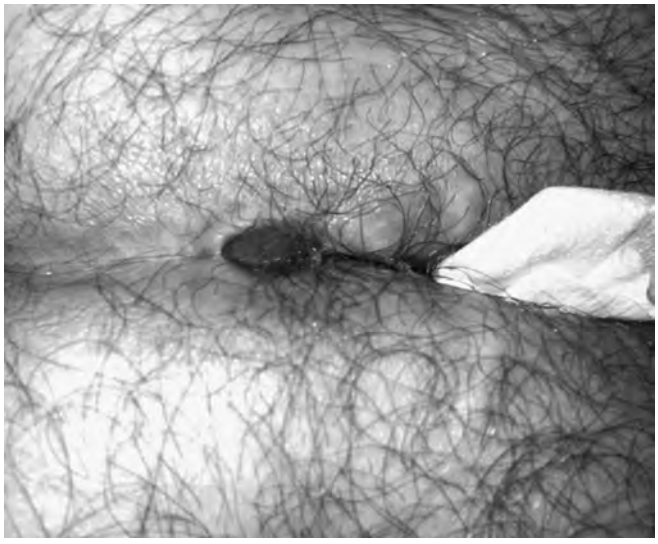

Figure 1.—Lesion before treatment.

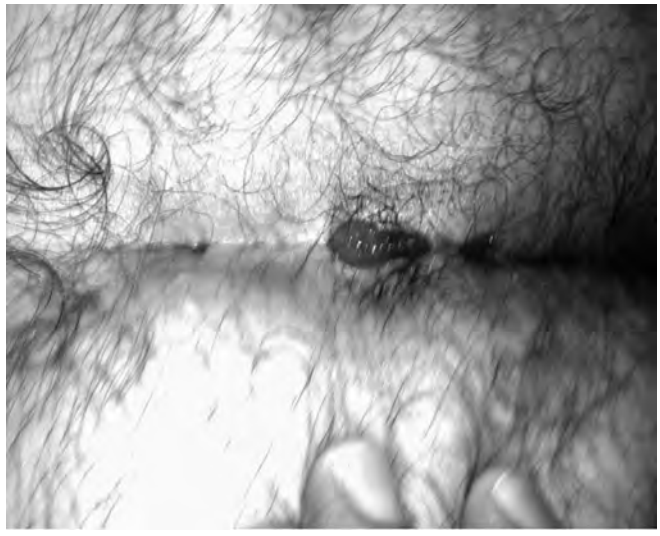

Figure 2.—Initial healing of the lesion after three weekly platelet-rich plasma applications.

subcutaneously, and necrotic tissue was removed. After the second centrifugation was completed the platelet gel was immediately applied to the cleansed and bleeding lesion, with sterile surgical instruments. The procedure was completed with the application of conventional gauze dressings. The quality checks performed on 1 mL PRP showed a platelet concentration factor of 2, while the percentage of platelet recovery was 25% less than whole blood. During the treatment period from March to June 2011, the patient was prohibited from taking NSAIDs and advised to avoid heavy physical activity. After the third application, photographs of the lesion area showed a slightly reduced size, the edges were less raised, the granulation tissue was well represented (Figure 2), the pain was no longer present and the patient reported also less serum and blood discharge from the wound. At the end of the procedure we achieved *a restitutio ad integrum* (Figure 3), and the pain had completely subsided and since the ulcer was healed it is evident that secretions were no longer present.

An evaluation of pain symptoms showed a pretreatment value of 8 and a value of 2 at the end of the therapy and finally the complete remission of pain symptoms at follow-up six months later; the self-administered SF12 questionnaire showed the following scores: 28 before starting treatment, 34 at the end of the procedure, and that value remained unchanged even at follow-up 180 days after.

The radical treatment of pilonidal sinus disease can be obtained by the large bloc excision of all the involved skin and subcutaneous tissue. This technique, if implemented properly, causes moderate pain and should bring to relapse into no more than 2-3% of cases; it is indicated in cases of extensive disease, with suppuration in progress and especially in recurrences. The operation involves creating a large surgical wound that extends in depth to the presacral plane. Healing occurs by secondary intention with granulation tissue formation, which closes the wound completely within 30-40 days depending on the extent of the disease.

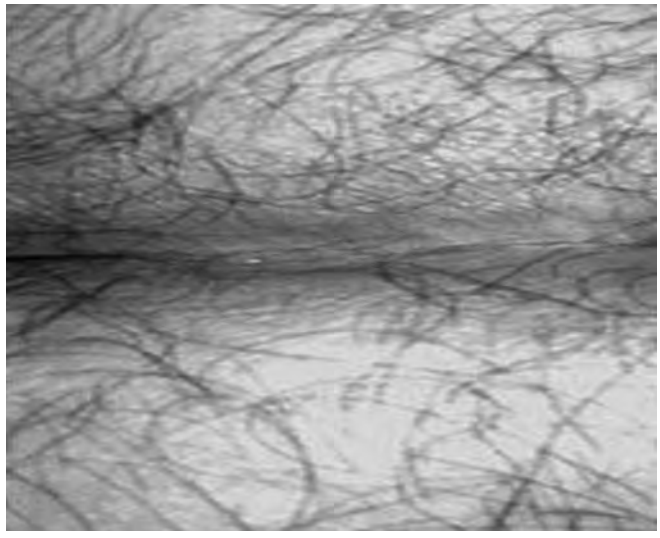

Figure 3.—Healed wound at the end of treatment (10 weekly platelet-rich plasma applications).

In the case described, despite the careful observation of the surgical technique and postoperative course, wound healing was never achieved, so we resorted to the application of platelet gel, an autologous blood component that accelerates the normal mechanisms which lead to tissue

regeneration. Due to the degranulation of platelets, a variety of growth factors are released, such as transforming growth factor-β, platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor, insulin-like growth factor which promote the synthesis of extracellular matrix proteins and release other cytokines. The latter in turn include hepatocyte growth factor and basic fibroblast growth factor, that are chemotactic and mitogenic for endothelial cells and promote angiogenesis and revascularization, key steps of tissue regeneration.[4]

The platelet gel is a simple therapy, an inexpensive and minimally invasive procedure that provides a concentrate of autologous growth factors, which can be used to accelerate the physiological processes of healing, it has a significant analgesic effect too.[5]

Our work represents the first case in the treatment of a chronic wound dehiscence of a surgical excision of pilonidal sinus with platelet-derived autologous growth factors. All of the proposed treatment sessions were performed on an outpatient basis, at no time there were adverse effects, the patient was able to resume normal social activities, emotional and sentimental relationships, overcoming a psychological problem that was triggering a pathological substrate.

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D. FILOMIA
Operative Unit of Immunohematology and Transfusional Medicine
Castrovillari Hospital, Cosenza, Italy

C. VENTURA
Operative Unit of Surgery
Castrovillari Hospital, Cosenza, Italy

A. CRESCIBENE
Operative Unit of Orthopedy and Traumatology
Paola Hospital, Cosenza, Italy

J. ALMOLLA
Operative Unit of Radiology,
Castrovillari Hospital, Cosenza, Italy

M. NAPOLITANO
Operative Unit of Immunohematology and Transfusional Medicine
Cosenza Hospital, Cosenza, Italy

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Segmental neurofibromatosis type 1: a frequently underestimated disease

TO THE EDITOR: A 65 year old woman was referred to our outpatient service due to the presence of several asymptomatic nodules localized on her left hand and wrist. The patient reported that these lesions were present since birth and had been slowly growing over the years. The patient denied a positive family history for similar lesions. Upon physical examination, about 30 flesh-colored or violaceous nodules were observed on the dorsal side of her left hand and wrist, ranging in size from 2 mm to 1 cm (Figure 1). The nodules did not itch, burn, or bleed and were soft-domed, easily manually invaginated into the skin with pressure (button-hole sign). No similar lesions were found elsewhere. Café-au-lait macules, plexiform neurofibromas, Lisch nodules and axillary freckling were not present. The histopathologic evaluation of a nodule confirmed the diagnosis of neurofibroma. Thus, considering the lack of other cutaneous findings, the negative family history for neurofibromatosis, and the dermatomal distribution of the neurofibromas, a diagnosis of segmental neurofibromatosis type 1 was made.

Neurofibromatosis type 1 (NF1) is an autosomal dominant, multisystem disorders in which the typical features are café au lait macules (CALMs), freckling, multiple peripheral neurofibromas and Lisch nodules. The last ones

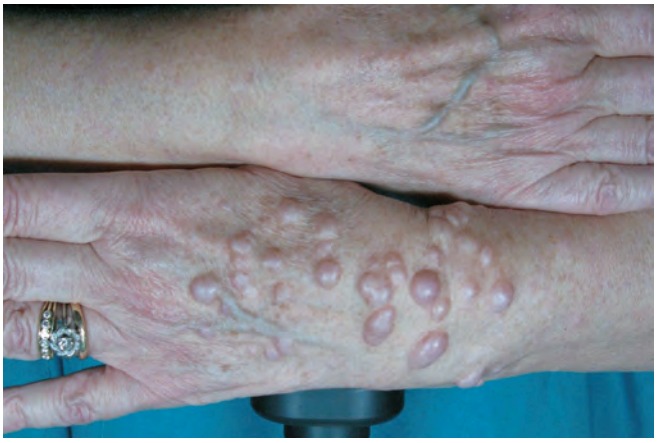

Figure 1.—Neurofibromas on dorsal side of left hand and wrist.

are pigmented iris hamartomas, but have also demonstrated to be neurofibromas of the iris.[1] These patients may also develop skeletal abnormalities, vascular disease and learning disability.

The term segmental neurofibromatosis type 1 (SNF1) refers to those patients who have skin manifestations of NF1 localized to a particular area of the skin. In these patients disease features are restricted to one or more body segments, which varies from a narrow strip to one quadrant or one half of the body.[2] The prevalence of SNF1 in the general population is estimated approximately to be 0.002%.[3] SNF1 does not represent a separate type of NF, therefore the term "NF5" taken from Riccardi's classification should be definitely dropped.[4]

Ruggieri and Huson [3] have proposed the use of the term "mosaic localized NF1" for these individuals, as this condition is due to a late post-zygotic mutation in the NF1 gene leading to somatic mosaicism. They have also classified the various types of mosaic NF1 into pigmentary changes alone (background hyperpigmentation, CALMs, and freckling), neurofibromas alone, both pigmentary and neurofibromas, and isolated plexiform neurofibromas.[3]

Neurofibromas, benign peripheral nerve sheath tumors, are the most frequent manifestations of SNF1 (84/150 patients; 56%) [3] and in more than half of patients are the sole sign of the disease, as observed in our patient. The appearance of the individual neurofibromas is exactly the same as when they occur in the generalized disease. Neurofibromas can occur anywhere on the body and usually range in size from 0.1 cm to several centimeters in diameter. They are almost always asymptomatic; painful neurofibromas, mostly related with the involvement of a nerve or nerve plexus, and itching neurofibromas have rarely been described. Neurofibromas tend to follow a dermatomal distribution, most commonly cervical, followed by thoracic, lumbar, and sacral. Patients with SNF1 may present with systemic involvement. It has recently been demostrated that the incidence of malignancy in patients with segmental neurofibromatosis may approach that of patients with neurofibromatosis type 1.[5]

Because of generally limited and asymptomatic clinical manifestations and the overall benignity of SNF1, it may be easily ignored by the patient and passes unnoticed by the physicians. Therefore new reports may help physicians become more aware of this condition, frequently underestimated or misdiagnosed in the clinical practice and in the general population.

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F. DRAGONI
Division of Dermatology, Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy
federicadragoni@yahoo.it

A. BASSI
Division of Dermatology, Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy

R. CONTI
Division of Dermatology, Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy

S. MORETTI
Division of Dermatology, Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy

P. CAMPOLMI
Division of Dermatology, Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy

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Chronic keratosis lichenoides: rare and elusive

TO THE EDITOR: Keratosis lichenoides chronica (KLC) is very rare and elusive disease, with approximately a few as 70 cases, reported in literature.[1] It was originally described by Kaposi in 1895, but was named after Nekam who reported a typical case in 1938. In 1972, Margolis coined the name, keratosis lichenoides chronica, and this has been widely accepted for the past 30 years,[2] except in France, where it is named "lichenoid tri-keratosis".

KLC is typically presented in adolescents or young adults as a widespread symmetrical

lichenoid eruption formed by hyperkeratotic papules that adopt a reticulate, striated or linear

pattern, but in literature are described patients with KLC of pediatric onset.[3] There are no known differences in prevalence between genders or races. The aetiopathogenesis is unknown. A mode of inheritance or influence of any other genetic alteration, or relationship with any drug or infection have not been defined.[4]

KLC is sporadically associated with systemic diseases, most importantly glomerulonephritis and lymphoma.

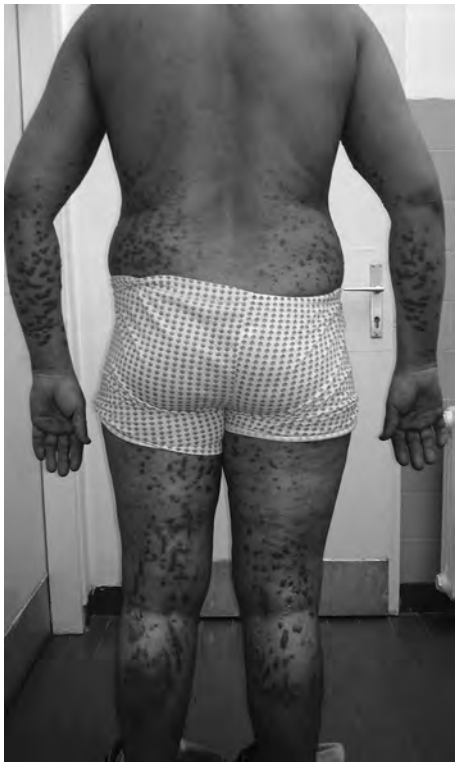
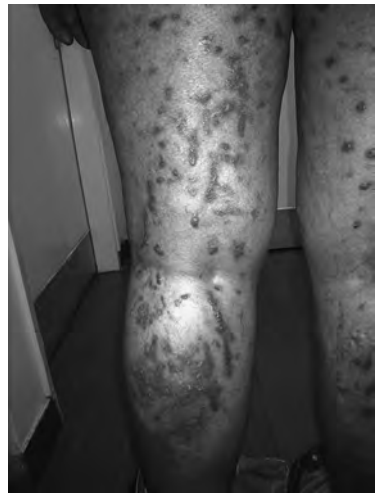


Figure 1.

KLC is commonly considered either as a distinct entity, an another unusual inflammatory disease or simply a manifestation of rubbing and scratching persistently.

The two diseases that are most similar to KLC, both clinically and histologically, are lichen planus and chronic cutaneous lupus erythematosus, but it would seem that under the name KLC different entities may be hidden. It is well known that more than 10 diseases have been published under the title of KLC, among them lichen simplex chronicus, lichen planus, lupus erythematosus, and epider-



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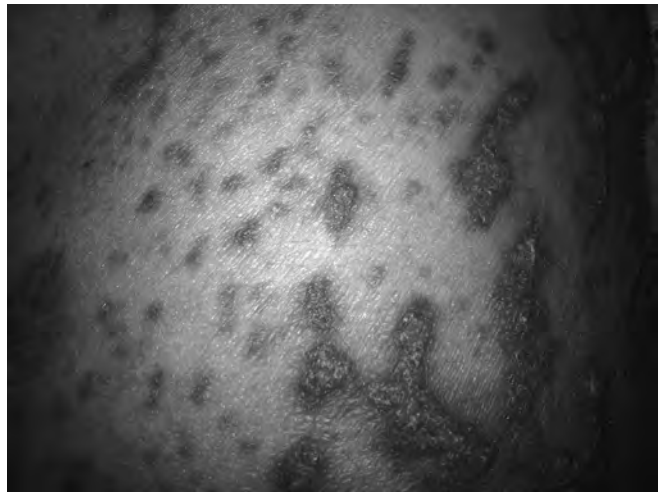

Figure 3.

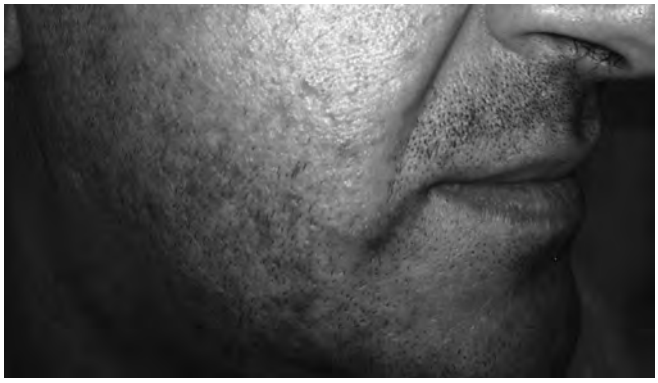

Figure 4.

molysis bullosa dystrophica, not to mention linear psoriasis and inflammatory linear verrucous epidermal nevus.

Recently proposed hypothesis of antigen mimicry and epitope spreading could be the possible pathogenic inductor in cases of KLC and other lichenoid dermatoses.[5]

Boer A. in his detailed article published 2006 [4] proposed a concept of diagnosing KLC based on common clinical and histopathologic findings (lichenoid interface dermatitis affiliated with numerous necrotic keratocytes and covered by parakeratosis housing neutrophils in staggered fashion.[4]

The treatment of KLC is challenging because the disease is resistant to many different therapies, including topical and systemic corticosteroids, methotrexate, cyclosporine, antibiotics, antimalarial agents, phototherapy and biologics.

We present young male patient (33 of age) with violaceous hyperkeratotic papules arranged in a bilaterally symmetrical reticular and linear pattern on the skin of the trunk and extremities (Figures 1-3). On the skin of his face were present uneven scaly, erythematous papules (Figure 4). Mucosal membranes, palms, soles and nails were unaffected.

Skin lesions appeared ten years ago and spread with slow progression. Lesions were asymptomatic and never regressed spontaneously. During last few years, this therapy included broadband UVB therapy, topical and oral corticosteroids and acitretin, with only minimal (acitretin) or no success.

Laboratory findings were with no abnormalities, and general health of our patient was good.

Serology tests to HIV, Hepatitis and TPHA were all negative.

Histopathological examination of biopsy specimen revealed hyperkeratosis, infundibular keratosis, irregular papilomathosis and acanthosis, increased number of capillaries, and perivascular chronic inflammatory infiltrate (Figures 5, 6).

We diagnosed this patient as having Keratosis lichenoides chronica, and proposed treatment with calcipotriol ointment.

Our patient has skin lesions typical for the diagnosis

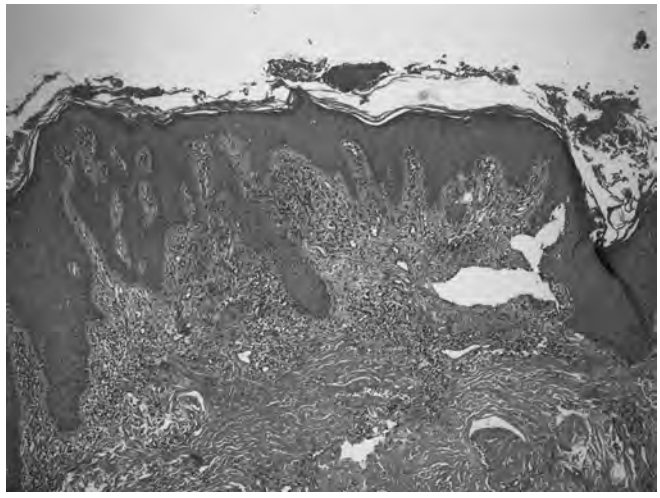

Figure 5.

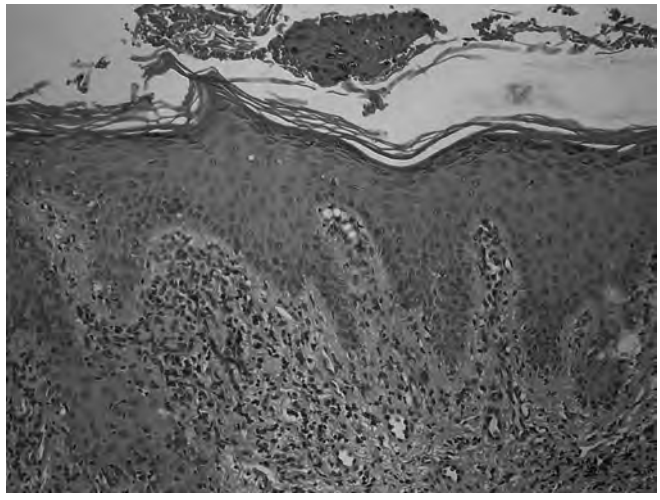

Figure 6.

(keratotic papules, linear arrangement, seborrhoeic – like lesions on the face, duration of lesions). His lesions never improve with natural sunlight and there is distinctive absence of reaction to therapy. His general health is good, but he became slightly depressed and disappointed by the failure of the therapy.

His histopathologic findings do not meet completely criteria listed by A. Boer,[4] but elements for lichen or lupus erythematosus are also absent. His treatment included successively topical glucocorticoids, broadband UVB therapy, oral glucocorticoids and acitretin during six months, with minimal improvement with retinoid.

We diagnosed this patient with the KLC, and tried to

treat him as above mentioned, but his diagnosis stil need (at least) histopathologic confirmation.

The success of his treatment is a goal that is more difficult to achieve.

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M. LJUBENOVIC
Clinic of Dermatology and Venereology, Clinical Centre Nis, Nis, Serbia

D. LJUBENOVIC
Department of Maxilofacial Surgery, Clinic of Stomatology, Nis, Serbia
milanka_ljubenovic@yahoo.com

D. MIHAILOVIC
Clinic of Dermatology and Venereology, Clinical Centre Nis, Nis, Serbia

V. LAZAREVIC
Clinic of Dermatology and Venereology, Clinical Centre Nis, Nis, Serbia

I. BINIC
Clinic of Dermatology and Venereology, Clinical Centre Nis, Nis, Serbia

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A case report of fibrosing alopecia in a female pattern distribution

TO THE EDITOR: Cicatricial alopecias encompass a spectrum of conditions characterized by inflammation and subsequent destruction of the hair follicle, causing irreversible hair loss. Examples of cicatricial alopecia include lichen planopilaris, frontal fibrosing alopecia, follicular degeneration syndrome and pseudopelade of Brocq. Here we describe a female patient with an unusual presentation of cicatricial alopecia.

A 78-year old lady presented with worsening hair thinning over the crown area of the scalp, associated with mild pruritus, occurring gradually over several years (Figure 1A). She had taken tamoxifen for breast cancer for seven years and stopped it one year before presentation. Her regular medications were simvastatin and bendroflumethazide for six years. There was a family history of male pattern alopecia. Full blood count, iron studies and thyroid function tests were within the normal range and an antinuclear antibody titre was negative.

On examination, she had alopecia in a female pattern distribution, predominantly involving the crown (Figure 1A), with preservation of the frontal hairline. On close inspection of the affected area, there was peri-follicular inflammation, scaling and scarring (Figure 1B). There were no associated cutaneous, nail or mucous membrane features of lichen planus.

Histology of a scalp biopsy showed vertical scarring in the dermis with loss of hair follicles and significant focal perifollicular fibrosis associated with a lymphocytic infiltrate. The majority of hair follicles were terminal. Although no miniaturization of hair follicles was evident, an element

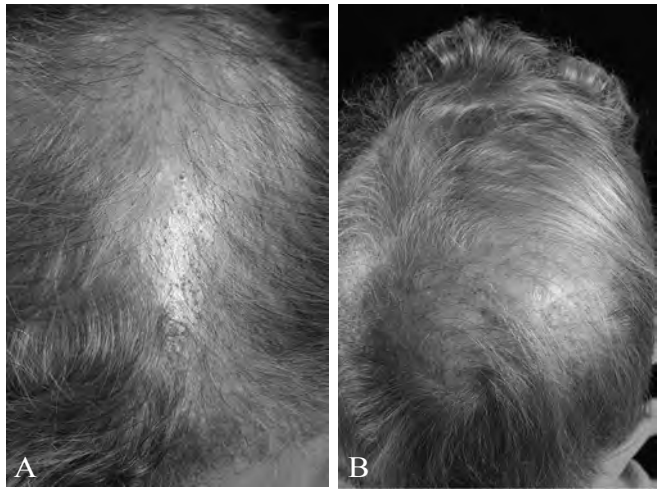

Figure 1.—Alopecia in a female pattern distribution, predominantly involving the crown.

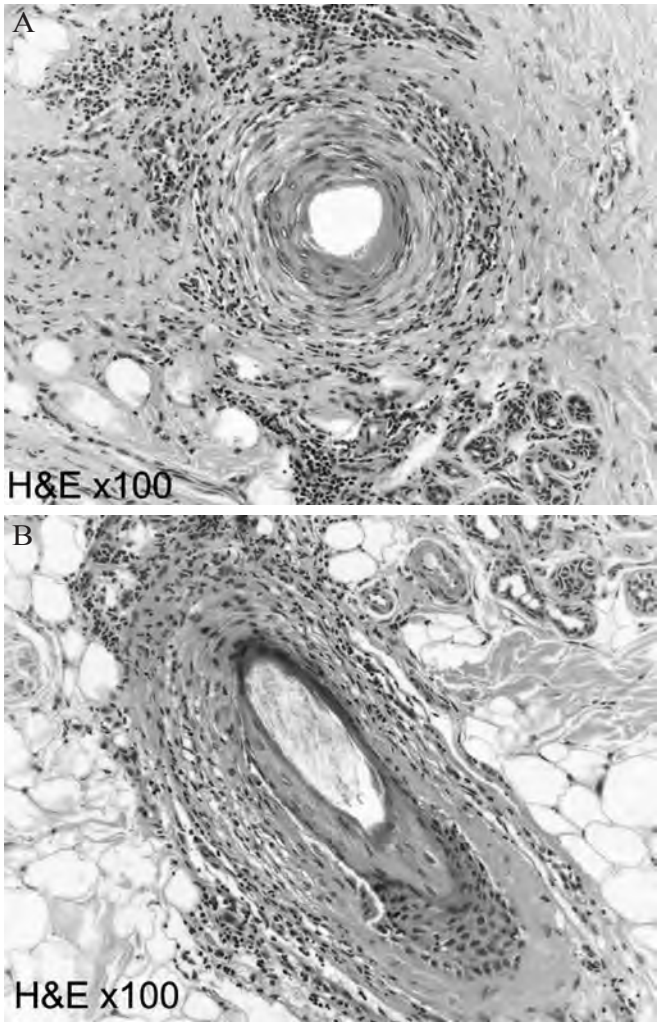

Figure 2.—Scalp biopsy: horizontal section at the isthmus showing perifollicular lymphocytic inflammation with concentric fibrosis and eccentric epithelial atrophy (haematoxylin and eosin, original magnification x100).

of female pattern of hair loss could not be excluded due to the lack of a control sample from the occiput.

She was treated with topical clobetasol propionate twice weekly. When reviewed two months later, she reported some hair thinning on the eyebrows, which was clinically associated with perifollicular erythema. Management with topical clobetasol 0.05% alternate days to the scalp and twice weekly to the eyebrows led to satisfactory control of the inflammation and to date her alopecia has been stable for over twelve months.

Fibrosing alopecia in a pattern distribution (FAPD) is a distinct and rare subset of cicatricial alopecia, where inflammation and fibrosis are confined to the area of pattern hair loss. Zinkernagel and Trueb first described in 2000 a series of 19 patients with this type of alopecia.[1]

Clinically, there is perifollicular erythema, hyperkeratosis and scarring with a female pattern distribution, in contrast with LPP where the findings are rather patchy and irregularly distributed.

The histological features of early lesions overlap with those seen in lichen planopillaris (LPP). FAPD has therefore been classified by some authors as a subtype of LPP and also called patterned LPP [2, 3] although the nosologic relationship between FAPD and LPP is not fully understood.

The typical histological findings include a lymphocytic lichenoid inflammatory infiltrate around the isthmus and the infundibular region in the early stage, with miniaturization of hair follicles, concentric perifollicular lamellar fibrosis and fibrosed follicular tracts in advanced cases.[1, 2]

The histology of FAPD shares histological features with other scarring alopecias such as frontal fibrosing alopecia, follicular degeneration syndrome and pseudopelade of Brocq, suggesting a common background and pathogenetic mechanism.[4, 5] The aetiology of the inflammation and subsequent fibrosis in a specific pattern, similar to that of pattern alopecia, is yet to be elucidated, but is likely to include genetic, environmental and hormonal factors. In support of the latter, it has been reported that antiandrogens may halt the progression of hair loss in FAPD.[1, 4]

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P. DE MOZZI
Dematology Department, University Hospitals of Leicester, Leicester Royal Infirmary, Leicester, UK
paola.demozzi@uhl-tr.nhs.uk

S. M. CRICHLOW
Dematology Department, Luton and Dunstable Hospital, Luton, UK

P. D. DA FORNO
Histopathology Department, University Hospitals of Leicester, Leicester, UK

B. ALEXANDROFF
Dematology Department, University Hospitals of Leicester, Leicester Royal Infirmary, Leicester, UK

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