INTRODUCTION

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A comprehensive melding of the fields of dermatology and infectious diseases is long overdue. It is the skin that is often the first sign of infection and the easiest organ to quickly access with an educated eye, culture, scraping, and histopathologic evaluation. It is the observation of the skin that can hold the chance for the earliest diagnosis and thus the most timely attempts at therapy. This same observation can guide the clinician through the maze of enumerable, often confusing, and sometimes costly follow-up confirmatory tests. Let us now, in these pages, take this opportunity the integument has given us to lead us through the ever-increasingly important field of infectious diseases.

TECHNIQUES IN DIAGNOSING DERMATOLOGIC MANIFESTATIONS OF INFECTIOUS DISEASES

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GETTING THE SAMPLE

A vital step toward making the right diagnosis when dealing with infectious diseases is ordering the appropriate test. That implies having a certain idea of the range of possible organisms involved and directing your workup toward ruling in or out a specific agent. Of course, there will be cases where a more blind approach is in order and a large range of diagnostic possibilities should be considered. In those situations, smears and cultures for bacterial, mycobacterial, and fungal microorganisms are indicated. Also viral diseases should be considered in specific situations, such as febrile patients with disseminated maculopapular or vesicular rashes. However, just for practical purposes, it is better to take a syndromic approach, considering a range of possibilities regarding the etiology of the lesions and then, selecting the appropriate test. Let us take an example such as a patient with a sporotrichoid pattern of lesions. If the diagnosis to confirm is sporotrichosis, a fungal culture will be very sensitive and very specific. Pyogenic bacteria such as *Staphylococcal aureus* can also produce such a pattern. In these cases, a Gram stain and routine culture will be helpful. But, if the patient likes fishing, swimming, or diving besides gardening an atypical mycobacterial infection (*M. marinum*) also has to be listed in the differential. In such cases, a biopsy, acid-fast stain, and mycobacterial culture should also be considered, although recognizing this is a difficult diagnosis to make because of the low sensitivity of each individual test. In the same line of thought, the same patient just came back from a trip to the Amazon: leishmaniasis is then another possibility. In leishmaniasis, there is no test with high sensitivity, so a panel approach is indicated (direct exam, culture, intradermal reaction, histopathology, and PCR, when available). Nocardia, another disease capable of giving such a pattern, can only be detected if the laboratory takes special precautions while culturing. Then, it is better to direct our workup toward a specific diagnosis. Of course, that also implies having some knowledge regarding the sensitivity and specificity of each test for a specific etiological agent.

Nobody other than the clinician will know best where to take the sample from. Unfortunately for regulatory or administrative reasons, the task is commonly left to a technician.

As a rule, purulent or oozing secretions are considered excellent samples and should be regularly submitted for direct examination with Gram, fungal, and acid-fast staining. Abscesses should be punctured and the pus submitted under sterile conditions. Taking a biopsy of an abscess is usually not rewarding, but clinically if there is a thick wall surrounding the cavity, it may reveal a granulomatous infiltrate when biopsied.

Solid lesions, such as those suspicious for granulomatous diseases, are better studied submitting the tissue for culture; even then, the appropriate area should be sampled. In mycetoma, for example, unless the biopsy is taken from areas containing granules, the yield of histology and culture will be very low.

When dealing with dry, scaly lesions, such as in tinea cases, the scraping is very sensitive. However, in hairy areas (scalp, beard), getting some hairs may reveal the presence of spores in the absence of superficial hyphae. This is usually the case when a tinea barbae has been previously treated blindly with a topical antifungal. In cases where there is a possibility of tinea incognita, it is advisable to microscopically examine the proximal portion of the hairs. When dealing with white onychomycosis, scrape the surface. If the subungeal area is affected, the detritus under the nail are most likely to reveal the hypha or spore. Nail clippings are considered good samples, even suitable for histological study. The diagnosis of microscopic ectoparasites, such as scabies, requires taking the sample from the most commonly affected areas. Blindly scrapping off different areas is not very rewarding. In contrast, scrapping a whole scabies burrow will frequently reveal the presence of the mites, eggs, or feces.

Moist ulcers can be swabbed and the secretions submitted for direct examination and culture; dry ulcers can be sampled by touch preparation. Aspirating the fluid under the border of the ulceration with a micropipette is useful for leishmania; in leprosy, examining the fluid obtained by slitting the ear lobe under pressure is an excellent method to visualize the mycobacteria.

TECHNIQUES

Smears

Direct examination of material obtained from lesions is a vital first step to orient the clinician toward a specific etiology. Gram and acid-fast stains are now routinely performed by laboratory technicians, and the techniques themselves are beyond the scope of this book. However, the results provided are of vital importance. Gram stain is regularly done in urethral secretions to look for *Neisseria gonorrhoeae*; its absence in the presence of neutrophiles is indicative of nongonococcal chlamydial urethritis. Acid-fast staining of smears from leprosy patients may help establish the bacterial load.

Some tests are more easily done, on a daily basis, at dermatology offices around the world. Examples of such tests are potassium hydroxide (KOH) preparations and the Tzanck test. The KOH preparation is usually done using a 5% to 40% concentration. The idea is that the reaction will dissolve most of the normal host cells, sparing the infectious agent. The condenser of

the microscope is lowered, to facilitate observation by light contrast. The test is done on skin scrapings to detect the presence of hyphae in dermatophytes or candida. Yeast of invasive fungi such as *Blastomyces* and *Paracoccidioides* can also be detected by this method in purulent secretions. Hairs and nails can also be examined under the microscope in a similar manner. The same preparation can be used to examine scrapings while looking for scabies mites and *Demodex*. A variation on the theme is adding colored stains to facilitate viewing of fungal structures. Using mineral oil instead of KOH may allow the assessment of viability and motility of ectoparasitic mites. Instead of regular scraping, one can use adhesive tape to take the sample, a technique especially useful when dealing with rapidly moving targets, such as the face of a small child. Surprisingly, a similar technique will allow detection of large fungal structures, such as the agents of chromoblastomycosis and lobomycosis when the tape is applied on top of the clinical lesion. This is possible because of the phenomenon of transepidermal elimination of the microorganisms. Tzanck test implies the examination of cells at the base of an unroofed blister in suspected cases of Herpes simplex or Herpes Zoster infection. Once air dried, the slide is stained with Wright, Giemsa, or methylene blue. The goal is to detect multinucleated giant keratinocytes that are indicative of herpetic infections. A more sophisticated technique utilizes an immunofluorescent antibody against the virus, allowing species-specific identification.

Touch preparation of a genital ulcer can be examined under dark field for the presence of treponemal spirochetes. The same preparations, if stained as a PAP smear may allow detection of the presence of multiple intracellular bacteria in cases of granuloma inguinale. Touch preparation of the bottom of a large ulcer with undermined borders and acid-fast staining will be extremely useful in detecting large amount of mycobacterium in a Buruli ulcer (Table T-1).

Table T-1: Selected Microorganism with Special Culture Requirements

H. influenza	Chocolate agar with factors V and X
N. gonorrhea	Thayer-Martin media
B. pertussis	Bordet-Gengou agar
C. diphtheriae	Tellurite plate, Loffler's medium, blood agar
M. tuberculosis	Lowenstein-Jensen agar
Lactose-fermenting enterics	MacConkey's agar (pink colonies)
Legionella	Buffered charcoal yeast extract agar
F. tularensis	Blood or chocolate cystine agar
Leptospira	Fletcher's or Stuart's medium with rabbit serum
Fungi	Sabouraud's agar

Table T-2: Special stains

Giemsa	*Borrelia, Plasmodium*, trypanosomes, *Chlamydia, Leishmania*
PAS	Stains glycogen, mucopolysaccharides; good for fungi
Ziehl-Neelsen	Acid-fast bacteria and *Nocardia*
India ink	*Cryptococcus neoformans*
Silver stain	*Fungi, PCP, Legionella, Bartonella, Klebsiella granulomatis*

Culture

The purpose of cultures is isolation of the infectious agent to comply with one of the Koch postulates. If the diagnosis is uncertain, samples should be sent for bacterial, fungal, viral and acid-fast bacterial cultures. Culturing requires special media, depending on the microorganism suspected (see Table T-2). One has to keep in mind that certain areas of the body are heavily contaminated, such as the mouth and perianal region. The skin, by no means an aseptic organ, can be colonized by different bacteria and fungi. The result of cultures should be interpreted appropriately, with correlation to the clinical lesion. Some culture media are designed to facilitate the growth of the microorganism (such as the Thayer-Martin media for gonococcal infection or oxygen depleted systems for anerobes). Others, such as the Mycosel, will restrict the growth of saprophytic fungi while allowing the growth of dermatophytes. Most bacteria will grow in a matter of days; some, such as Brucella, may require weeks in specially designed media (such as Ruiz Castañeda). *Candida* will also grow fast, even on bacterial culture media, in days. Regular fungi, cultured in Sabouraud's agar may grow as fast as in 1 week (*Sporothrix*), 2 weeks (dermatophytes), or 4 weeks (*Histoplasma* and *Actinomyces*). Mycobacteria may be fast growers (*M. fortuitum, M. chelonae*, or *M. abscessus*) or take several weeks (*M. tuberculosis* and *M. ulcerans*), with additional specific temperature requirements.

Surprisingly enough, even in the 21st century, some famous pathogens are still unable to be isolated in culture media. *Treponema pallidum, Mycobacterium leprae*, and *Loboa Loboi* are three examples. The isolation of virus by culture is not routinely done (except for herpes simplex and varicella-zoster viruses). Modern diagnosis relies more on molecular techniques or serologic assays.

Intradermal reactions

Intradermal reactions are widely used to support the diagnosis of some dermatological and nondermatological diseases. They are mainly indicated for the detection of type I (immediate hypersensitivity) and type IV (delayed hypersensitivity) reactions toward exogenous or endogenous antigens. Intradermal reactions for the diagnosis of infectious diseases are indicated to detect previous contact with the agent as revealed by delayed hypersensitivity to the whole organisms or their antigens.

The intradermal reaction is a localized inflammatory reaction with marked proliferation of lymphocytes, monocytes, and small numbers of neutrophiles, with a tendency toward cellular accumulations arround small vessels. The induration results from fibrin formation.

The principle of an intradermal reaction is the inoculation of an antigen into the superficial layer of the dermis through a

fine-bore needle with its bevel pointing upward. The quantity injected may vary from 0.01 to 0.1 mL, but 0.1 mL is universally used. Although the test could be done at any site, the proximal part of the flexor aspect of the forearm is conventionally used. Corticosteroids or immunosuppressive agents should be discontinued before testing for intradermal reactions because they may inhibit the delayed hypersensitivity reaction. Intradermal reactions for the detection of delayed hypersensitivity are read at 48 hours, although they can be read as early as 12 hours and as late as 4 days. The size of the induration is more important than erythema when interpreting delayed hypersensitivity reactions.

The tuberculin test (also called PPD [purified protein derivative], Pirquet test, or Mantoux test) is a diagnostic tool used to detect latent infection or recent infection (as shown by conversion from negative to positive) and as part of the diagnosis of tuberculous disease. A standard dose of tuberculin is injected intradermally on the flexor aspect of the forearm and a reading is taken after 48 hours. The reaction is read by measuring the diameter of induration in millimeters. The interpretation of the test result will depend on all relevant clinical circumstances. An induration measuring more than 10 mm in diameter is considered to be a positive response while that measuring less than 5 mm is considered negative. A positive test indicates past or present infection with *M. tuberculosis* or vaccination with bacillus Calmette-Guérin (BCG). An induration of more than 15 mm is unlikely to be due to BCG vaccination and is strong evidence in favor of active tuberculosis. In the absence of specific risk factors for tuberculosis, an induration between 6 and 10 mm is more likely to be due to previous BCG vaccination or infection with environmental mycobacteria than to tuberculosis infection. When there is a higher probability of tuberculosis, such as recent contact with an infectious case, a high occupational risk or residence in a high prevalence country, an induration of 6 mm or more is more likely to be due to tuberculosis. Anergy is present in AIDS patients. Other factors that can weaken the reaction include severe tuberculous disease, renal failure, diabetes, immunosuppressive drugs, and old age. Initial skin tests may have a booster effect on reactions to subsequent doses. More sophisticated tests based on interferon production by stimulated cells are also available. Intradermal reactions for atypical mycobacteria have also been prepared. They include PPD-Y for *Mycobacterium kansasii*, Scrofulin for *Mycobacterium scrofulaceum*, and Burulin for *Mycobacterium ulcerans*.

The leishmanin test was first done by Montenegro in 1926, in Brazil. This test (also called the Montenegro test) is indicative of the delayed hypersensitivity reaction to leishmania, which plays a major role in disease resolution and wound healing. It usually becomes positive early in the course of cutaneous or mucocutaneous leishmaniasis (except in diffuse cutaneous leishmaniasis) and only after recovery from visceral leishmaniasis. It is highly sensitive for cutaneous leishmaniasis. The test is considered positive when induration is more than 5 mm in diameter after 48 to 72 hours. The test is not species specific. A negative test may be attributed to an anergic state, decreased cell mediated immunity, early treatment, or presence of an unusual serotype of leishmania, whereas a positive test favors active disease if the patient is not a resident of the area. The same positive reaction does not have the same relevance for natives and current residents.

The lepromin test classifies the stage of leprosy based on the reaction and differentiates tuberculoid leprosy, (in which there is a positive delayed reaction at the injection site) from lepromatous leprosy (in which there is no reaction despite the active infection). The test is not diagnostic since normal uninfected persons may react. Two types of antigens are available: Mitsuda lepromin, an autoclaved suspension of tissue (whole bacilli) obtained from experimentally infected armadillos; and Dharmendra lepromin, a purified chloroform-ether extracted suspension of *M. leprae* (fractionated bacilli with a soluble protein component). The response after intradermal injection is typically biphasic, with an early Fernandez reaction (in the form of a tuberculin reaction with Dharmendra antigen) and a late Mitsuda reaction (in the form of erythematous, papular nodules with Mitsuda antigen).

Other important tests used for diagnostic aid, or to evaluate the cellular immune response in patients suspected of having reduced cell-mediated immunity, or in epidemiological studies include the anthraxin test, the onchocerca skin test, the candidin test, the coccidioidin or spherulin test, the histoplasmin test, and the trichophytin test. Finally, there are some tests of historical importance only, as they are no longer used for diagnostic purposes: Lymphogranuloma venereum (Frei's test), Chancroid (Ito Reenstierna test), Bartonellosis (Foshay test) and Scarlet fever (Dick's test).

Serology

Some tests are based on the detection of the infectious agent antigens in serum or by the detection of the circulating antibodies generated by the host. Agglutination tests (latex agglutination test) are based on the capturing the antibody from a suspected patient with whole bacteria or antigen absorbed to latex particles. The presence of circulating antibodies will then be detected by the agglutination phenomenon. *N. meningitidis* and *Cryptococcus* can be detected by latex agglutination. The complement fixation (CF) test measures complement-consuming (complement-fixing) antibody in the serum or CSF of the patient. The serum to be tested is mixed with known quantities of complement plus the antigen targeted by the antibodies to be measured. The degree of complement fixation indicates the amount of antibody in the specimen. CF is used for diagnosis of some viral and fungal infections, particularly coccidioidomycosis.

Enzyme immunoassays are based on detection of antibody binding to a substrate linked to an enzyme. They are very sensitive and for that reason, commonly used for screening. They include the enzyme immunoassay (EIA) and the enzyme-linked immunosorbent assay (ELISA). ELISA is available for *Chlamydia* infections, herpes virus, and human immunodeficiency virus (HIV). On the other hand, Western blot test detects the specific antibodies by measuring its union to antigens fixed to a membrane by blotting. It is quite specific and commonly used as a confirmatory test in tandem with ELISA as the screening test (i.e. in HIV, HTLV-1, and *Borrelia burgdorferi*).

Other examples of humoral responses that can be tested include the treponemal serology test (including VDRL, RPR, and the most specific FTA-Abs), as well as antibodies against *Borrelia, Legionella, Bartonella*, and *Leptospira*. Also many viral infections, such as hepatitis A, B and C, Epstein-Barr virus (EBV), dengue, CMV, coxsackie, and parvovirus can be tested. Rising titers of four times the normal baseline over a 2-week period are especially useful in viral diseases with occasional skin involvement. Even parasitic diseases such as enteric amebiasis, cysticercosis,

and fasciolasis, as well as toxocariasis and toxoplasmosis can be studied via serology.

Molecular biology

The current concept in the use of molecular biology techniques is based on the detection of DNA and RNA material from specific organisms, providing an extremely reliable method of diagnosis with high specificity and sensitivity. The current method can rely either on the amplification of the material and posterior identification (polymerase chain reaction) or on the direct detection of the material in tissues (in situ hybridization).

Polymerase chain reaction (PCR) consists of denaturalization of nuclear material (DNA), with posterior addition of complementary primers and synthesis of new chains by adding an enzyme such as a polymerase. By using repeated cycles of high and low temperatures one can obtain an amplification of nuclear material (amplicon) until reaching amounts detectable by gel eletrophoresis or enzyme assay base using color detection. Real time PCR is a more sophisticated method, whereas the newly synthesized amplicons can be detected as they are produced by using immunofluorescent methods. Ligase-based method (LCR) amplifies the probe rather than the microorganism's nuclear material. Some methods, like transcription-mediated amplification (TMA) and nucleid acid sequence-based amplification (NASBA), rely on the amplification of RNA material, which is usually more abundant than DNA. The current application for those techniques includes several pathogens listed in Table T-3. For example, both PCR and TMA are FDA approved for the diagnosis of pulmonary and extrapulmonary tuberculosis. By this technique, some researchers have been able to demostrate the presence of bacillus in cutaneous tuberculosis and tuberculids. These findings are, however, not consistent with other similar studies. The variable sensitivity and specificity of the method may be related to tissue inhibitors or fixation methods. PCR is also available for the second and third most common mycobacteriosis around the world, which are, *Mycobacterium leprae* and *Mycobacterium ulcerans*. The PCR technique has been very helpful in the study and discovery of new bartonella species as well.

Several viruses are suitable for PCR testing, with some variations in methodology such as the multiplex reverse transcriptase PCR used in varicella zoster infections. The cause–effect relation between HHV-8 and Kaposi's sarcoma has been demostrated by PCR. The role of different HPV in cutaneous neoplasia, such as HPV-16 in verrucous carcinoma of the foot and HPV-6 and -11 in anogenital verrucous carcinoma have been possible by this amplification method. PCR allows not only identification but also quantification of viral loads in in HIV infections. In some parasitic diseases such as leishmaniasis, the PCR techniques are considered quite sensitive and quite specific. The gene targets include 18S-rRNA, small subunit rRNA, mini-exon gene repeat, β-tubulin gene, transcriber spacer regions, and microsatellite DNA of the kinetoplast. The sensitivity is so high that it may allow detection of as little as half a parasite. Many fungal infections can now be precisely identified, as is the case for mycetomas by *Madurella mycetomatis*.

Another useful technique now available is in situ hybridization (ISH). It is based on the detection of nucleic acid material from different microorganisms directly in tissues, even if they are fixed in formalin. Fluorescent ISH (FISH) is a more sophisticated

Table T-3: Skin infectious diseases where PCR can be a useful diagnostic technique

Bacterial pathogens
Mycobacterium tuberculosis
M. leprae
M. ulcerans
Rickettsia rickettsii
R. prowazekii
Baronella henselae
Spirochetes
Treponema pallidum
Borrelia burgdorferi
Virus
Herpes simplex 1 and Herpes simplex 2
Varicela zoster virus
Human herpesvirus 8
Human papillomavirus
HIV
Hepatitis C virus
Parvovirus B19
Epstein-Barr virus
Parasites
Leishmania infantum
Leishmania braziliensis
Balamuthia mandrillaris
Fungi
Aspergillus fumigatus
Aspergillus versicolor
Aspergillus flavus
Blastomyces dermatitides
Cryptococcus neoformans
Candida albicans
Candida dubliniensis
Coccidioides immitis
Histoplasma capsulatum
Sporothrix schenckii
Madurella mycetomatis
Various dermatophytes

Modified from Sra KK, Torres G, Rady P, Hughes TK, Payne DA, Tyring SK. Molecular diagnosis of infectious diseases in dermatology. J Am Acad Dermatol. 2005 Nov;53(5):749–65.

Table T-4: Infectious Agents that can be Identified by ISH

Varicella Zoster
Molluscum contagiosum
HIV
HPV
EBV
Hepatitis C virus
HHV-8
Mycobacterium tuberculosis
Mycobacterium leprae
Leishmania spp.
Candida albicans
Cryptococcus neoformans

Modified from Sra KK, Torres G, Rady P, Hughes TK, Payne DA, Tyring SK. Molecular diagnosis of infectious diseases in dermatology. *J Am Acad Dermatol* 2005;53(5):749–765.

method that is used not only to identify infectious agents but also in gene mapping and chromosome analysis. ISH has been used to confirm the presence of HPV in epidermodysplasia verruciformis and EBV in hydroa-like lymphomas. Table T-4 details infectious agents that can be identified by ISH.

The development of DNA microarray technology, also known as DNA chip technology, will allow testing for multiple microorganisms or genetic defects, all at once. In this method, DNA from a clinical sample is first amplified by PCR, converted to RNA or more DNA, mixed with fluorescent dyes, and then applied over a plate containing many different oligonucleotides or cDNA libraries. After the hybridization between sample material and fixed probes on the plates takes place, stimulation by laser will light up the hybridized labeled probes. The total image then obtained will reflect the DNA or RNA contents in the original sample and compare them to control arrays. This technique has allowed identification of M. tuberculosis variants with gene mutations conferring resistance to isoniazid and rifampin. As more of these sophisticated methods become available, our understanding of disease mechanisms and the interactions between humans and pathogens will be complemented by knowledge about microorganism genetic variations, drug resistance, and disease epidemiology.

Skin biopsy as a diagnostic technique

While dealing with cutaneous infectious diseases the accessibility of skin as a source of sampling makes the biopsy an important diagnostic tool. Like any other method, one can determine the sensitivity and specificity of the biopsy for diagnostic purposes. Specificity can be very high, as in a case of molluscum contagiosum or rhinoscleroma, or very low, such as in a grossly superinfected ulcer, where one can see many bacterial colonies on the surface. The sensitivity can be very high, in a case of South American blastomycosis (paracoccidioidomycosis), or very low in a case of sporotrichosis.

Biopsies can be considered suggestive of, compatible with, or diagnostic of a specific infectious disease. How we use these terms, although subjective, may be based on the frequency of specific histological patterns seen associated with a specific agent, or the findings of the microorganism itself in the histological cuts. We think the term "suggestive" should be used if the histological pattern is commonly seen in a condition unsuspected by the clinician. The term "compatible with" should be used if the pattern seen coincides with one of the entities considered in the differential listed by the clinician, although the microorganism itself is not seen. The term "diagnostic of" should imply visualizing the agent itself, and carries the most certainty. Biopsies also allow establishing the inflammatory or neoplastic nature of a lesion.

The prevalence of a specific cell in a pattern will also direct our diagnosis:. For example a plasma cell–rich liquenoid infiltrate with histiocytes will be in favor of a spirochete-induced disease (either syphilis or borreliosis). Vacuolated histiocytes accompanied by a lymphoplasmacytic infiltrate are commonly seen in leishmaniasis, rhinoscleroma, and granuloma inguinale. Suppurative granulomas are indicative of mycobacteria and deep fungal infection. A dense perivascular and interstitial, superficial and deep inflammatory infiltrate rich in eosinophils, with extension into the subcutaneous tissue, is suggestive of a deep larva migrans or gnathostomiasis.

An important fact obtained from the skin biopsy is the granulomatous nature of an infiltrate. Many chronic infectious diseases are characteristically granulomatous. Granulomas can be divided into five types: tuberculous, suppurative, foreign body, palisaded, or sarcoidal. It should be mentioned that tuberculous granulomas are not exclusively seen in tuberculosis but in several other entities. Caseation necrosis is a more reliable sign of tuberculosis, but in its absence, other diagnostic possibilities for tuberculoid granulomas may include leishmaniasis, tuberculoid leprosy, and sporotrichosis. Even caseation necrosis may not be that specific as it can be seen in nontuberculous processes such as rosacea.

Suppurative granulomas, containing neutrophils in the center, are commonly seen in deep fungal infections such as North American blastomycosis, paracoccidioidomycosis, chromoblastomycosis, and sporotrichosis, as well as in some atypical mycobacterioses. A pseudocarcinomatous hyperplasia may be seen on top of the granuloma. Stellate necrosis in a granuloma is suggestive of cat scratch disease. Sarcoidal granulomas can be seen in sarcoidosis but also as a reaction to foreign materials such as silica and beryllium. Rarely, leprosy and paracoccidioidomycosis can be quite sarcoidal in appearance.

The diagnosis by histological patterns of the inflammatory diseases of the skin, as was outlined by Dr. Ackerman, can also be applied to these infections. There are some patterns that are quite specific for certain diseases like rhinoscleroma, bartonellosis, lepromatous leprosy, and Buruli ulcer.

In rhinoscleroma there is a diffuse infiltrate that occupies all of the dermis. The infiltrate is made up of foamy histiocytes, arranged in mantles. Plasma cells are intermixed in the infiltrates. Some of these cells accumulate such an amount of immunoglobulin within their cytoplasm that they develop into eosinophilic globules, so-called Russell bodies. Occasionally, rod-shaped

bacteria will be seen within some of the histiocytes. These represent the causal agent, *Klebsiella rhinoscleromatis*.

The cutaneous lesions of bartonellosis (including verruga peruana and bacillary angiomatosis) are characterized by a dome-shaped silhouette. Below a flattened epidermis there is a capillary vascular proliferation, with a background of histiocytes intermingled with small neutrophilic abscesses. Occasionally in cases of bacillary angiomatosis, there is a purple material in the intercellular space representing aggregates of bartonellas. The combination of histiocytes, vascular proliferation, and neutrophilic abscesses should be considered suggestive of infection by this group of bacteria.

Lepromatous leprosy is characterized by a proliferation of foamy histiocytes at the level of the reticular dermis, either in a diffuse or linear form, following vascular or neural structures. Commonly a zone of uninvolved papillary dermis separated from the epidermis may be seen (which is referred to as the Grenz Zone). Basophilic globi represent clumps of intracellular mycobacteria. Buruli ulcer, an infection induced by *M. ulcerans*, will produce a pattern of necrotizing panniculitis with a sparse or absent inflammatory infiltrate, and innumerable acid-fast bacilli will be present as well. This pattern of bacteria-rich necrotizing panniculitis is not seen in any other mycobacterioses like tuberculosis or leprosy.

Depending on how easily the causative microorganism can be seen, skin biopsies can be divided into three categories: biopsies of high, medium, and low sensitivity.

The first category is those biopsies that have a high diagnostic sensitivity. They are diagnostic when the causal agent is seen either in routine (H&E) or slides stained for microorganisms. Examples in this category include molluscum contagiosum, *K. rhinoescleromatis*, and deep fungal agents such as *P. braziliensis* (South American blastomycosis), *L. loboi* (lobomycosis), *C. neoformans*, *C. immitis*, *B. dermatitides*, *H. capsulatum*, and the agents of chromoblastomycosis. Easily detectable as well are the sulphur grains of actinomycosis, eumycetomas, and actinomycetomas. The acid-fast stain allows the detection of innumerable bacilli in lepromatous leprosy and Buruli ulcer. PAS and Gomori stains typically reveal large amounts of hyphae in mucormycosis or in immunosuppresed patients with invasive aspergillosis.

The second category includes entities with biopsies of medium diagnostic sensitivity. In such cases the infectious agent is not always visible in the routine cuts. Agents occasionally seen in skin biopsies include leishmaniasis, free living amebas, bartonellas in cases of bacillary angiomatosis (with Warthin-Starry stain), and dermatophytes. In leishmaniasis the number of visible parasites is related to the different evolutionary stages and the degree of immunity developed by the host. In early lesions the *Leishmania* spp. are seen intracellulary inside macrophages close to the epidermis. In cases of poor immune response (the so-called diffuse cutaneous leishmaniasis), the parasites are observed in great numbers as intracellular forms. In long-standing cases, the infiltrate is made of lymphocytes and plasma cells, and the possibility of finding parasites is small. If present, they are scarce, and they can easily be confused with fragments of plasma cells. So, in leishmania cases the specificity of the biopsy diminishes as a method of diagnosis. It is better to take a panel approach, including intradermal leishmanin tests, direct examination and culture, and where available, PCR studies. In syphilis cases, an inmunoperoxidase stain is now available that can facilitate the identification of treponemes in tissue cuts.

The last category is of those biopsies with very little diagnostic sensitivity, where the microorganism is particularly difficult to visualize, even with special stains. This includes parasites causing cutaneous larvae migrans, gnathostomiasis, fungi such as *Sporotrichum*, and bacteria such as the bartonellas of verruga peruana. Also difficult to see are *M. tuberculosis, M. marinum*, and the *M. leprae* in cases of tuberculoid leprosy. In sporotrichosis, diagnosis is based on culture isolation, which is by far the method of greatest sensitivity. In tuberculosis, the direct examination and culture also have a low sensitivity. Commonly, just a tuberculous granulomatous inflammation is seen. It is in this group of diseases that PCR technology appears very promising. Special stains such as rodamine or immunoperoxidases such as anti-BCG may allow better detection.

In summary, the biopsy of skin is of variable utility from a diagnostic point of view. The diagnosis can be suggested by a specific pattern, but absolute certainty is only obtained when the causal agent can be seen. The clinician should consider these limitations. Rather than depending only on special stains, the pathologist should familiarize himself or herself with the histological pattern of the disease, as well as with the frequency the causal organisms are seen in routine cuts. The new techniques of immunoperoxidase and PCR will indeed play a role in those entities where routine H&E-stained biopsies have the lowest sensitivity.

SUGGESTED READINGS

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PRINCIPLES OF MANAGEMENT OF DERMATOLOGIC INFECTIONS IN THE SKIN

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As with all of medicine, correct therapy depends on a correct diagnosis, which is established through clinical and laboratory (see chapter on *techniques in diagnosing manifestations of infections*) observations.

The progress of an infection can be more carefully monitored in the skin than in any other organ due to ease of clinical observation. The four primary signs of acute infection are color, warmth, tenderness (and/or pain) and swelling. Additional signs include induration, erosion or ulceration, vesiculation, pustulation, linear streaking along lymphatics, lymphadenopathy, asymmetry, vasculitic appearance, necrosis, and progression. This progression can be visually monitored and marking the edge with an indelible marker can help demonstrate the spread or regression of the edge of the infection. These clinical signs can all be less apparent in the growing population of immunocompromised patients who do not mount the typical immune response. On the contrary, immunocompromised patients may have worse clinical signs that appear at a faster rate than those seen in nonimmunocompromised individuals.

Probably the best example of the importance of these principles in all of medicine is illustrated in the case of necrotizing fasciitis, the sine qua non of importance for the skin and infectious diseases. Each chapter will have a brief history about the disease or diseases covered at the beginning as well as a reminder of potential pitfalls and myths at the end. The pitfalls and myths section will help to serve as a summary of where it is easy to go wrong clinically and where you want to go right. Necrotizing fasciitis will be used as a brief sample of how each chapter will be laid out in the book.

HISTORY

Necrotizing fasciitis is thought to be first named and described in detail by Joseph Jones, who was a historian and leading medical officer in the Confederate army during the Civil War of the United States. He kept copious records of hundreds of patients seen during the civil war including many injured soldiers at Andersonville prison in Georgia, which was ill equipped in resources and physical room to keep the overabundance of captured Union troops who often died of malnutrition, disease, and starvation. The original designation for this illness was "hospital gangrene." Fournier, a respected French dermatologist was credited with describing a group of patients with this form of cellulitis in the groin, mainly in men, 1883 and hence the designation "Fournier's gangrene." In 1924, Meleny ("Meleney's ulcer") recognized that this condition rapidly became a fatal systemic disease. Wilson coined the term "necrotizing fasciitis" in 1952. Five cases were reported in Gloucestershire, England, between June 1 and June 30, 1994 and two of theses cases were seen in patients who had an operation in the same operating theater. This led to the idea that it was a highly contagious "flesh-eating" bacterial infection. No other such cluster of patients has ever been reported, however.

CLINICAL DIAGNOSIS

The commonest predisposing condition is diabetes (seen in 40 to 50%), especially when associated with dialysis. Other associated underlying diseases are traumatic wounds, AIDS, metastatic cancer (with or without chemotherapy), varicella (especially in children which is sometimes called varicella gangrenosum), postoperatively and rarely post delivery. The abdomen, perineum, and extremities are the commonest areas affected.

Fever is present in at least two-thirds of patients with hypotension and in at least a third of cases. Altered sensorium is present in approximately one-fourth of patients and crepitus in 10%.

Pain and anesthesia have both been reported at the site of erythema, which rapidly becomes edematous and bluish. Gangrene develops within 5 days or less and may or may not be covered with a bulla. Mortality can range from 25% to 50%, especially if treatment is delayed. An unexplained association with the oral use of nonsteroidal anti-inflammatory agents has been reported in some cases.

LABORATORY DIAGNOSIS

A several fold increase in creatinine phosphokinase can be helpful in differentiating necrotizing fasciitis from erysipelas or other more benign types of cellulitis. Culture most often shows group A β-hemolytic streptococci (including strains causing toxic shock) when there is a single agent present. *Klebsiella* is probably the second most common. Community-acquired methicillin-resistant *Staphylococcus aureus* has also been reported as a pathogen. There is a trend toward more polymicrobic infections. Necrotic tissue for culture may be helpful in determining the causative bacteria, but awaiting this report can be fatal for the patient.

TREATMENT

Poor prognosis is associated with increased age (especially greater than 60 years old), female gender, delay of first debridement, extent of infection, increased creatinine, increased lactate, anemia, thrombocytopenia, and extent of other organ system involvement.

Early extensive surgical debridement is mandatory (magnetic resonance imaging may help tell the extent of debridement necessary) and may have to be repeated multiple times.

Immediate intravenous broad-spectrum antibiotic therapy with adequate coverage for streptococci, staphylococci, as well as gram-negative organisms is also important. This can be modified when cultures are obtained. Hyperbaric oxygenation and high-dose intravenous IgG immunoglobulin have been advocated as being beneficial by some authors.

PITFALLS AND MYTHS

This skin infection illustrates the importance of early decision making based on clinical data. Laboratory data may be confirmatory but may be acquired too late to save the patient's life. Overtreatment of a less serious infection is preferable to undertreatment of necrotizing fasciitis. The difficult patient is the one with a paucity of symptoms, (i.e. patients exhibiting no pain and little generalized toxicity). Do not assume that a relatively asymptomatic patient is not gravely ill and will stay symptom free for very long. Hospitalization is quite helpful since significant changes in the patient's condition can occur over hours. This disease is usually more extensive than first suspected (see Fig. Principles.1).

Do not let cultures that do not show streptococci or are polymicrobic dissuade you from the diagnosis. When reviewing the medical literature remember that multiple names may have been used for the same condition. "Progressive ischemic gangrene" is one example.

Clinical judgment and close observation are of no small importance. Remember that in any skin infection the great advantage is that the skin can be easily seen and followed as no other organ. Use your skill of careful surveillance.

While awaiting laboratory confirmation of a clinical diagnosis there are a subgroup of patients (such as those discussed already with necrotizing fasciitis) that are so ill that empiric therapy needs to be started before actual identification of the offending organism is established (see Table Principles.1).

With infections in dermatology, unlike many other specialties, there is often an opportunity to use topical instead of oral, intramuscular, subcutaneous, or intravenous therapy. The advantages of topical versus systemic treatment of cutaneous infections is discussed in Table Principles.2. Combination therapy in serious disease is often the best choice.

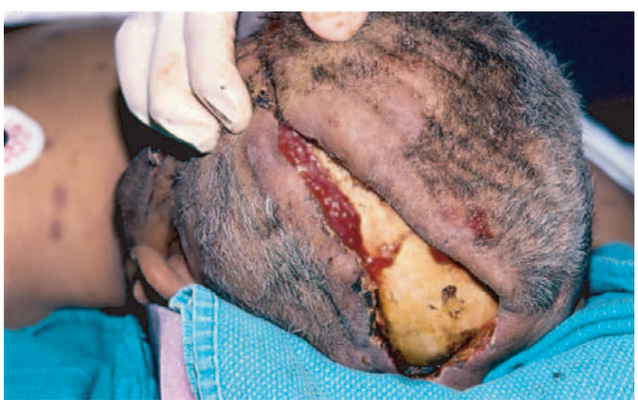

Figure Principles.1 Necrotizing fasciitis on the scalp of an elderly man with MRI showing extension well into the neck and shoulders. Culture was positive for β-hemolytic streptococci. The patient survived in large part due to repeated aggressive surgeries.

Table Principles.1 Conditions Where Empiric Therapy Is Needed

Blueberry muffin baby with generalized papules, pustules, or purpura at birth (always cover for herpes simplex virus until diagnosis is confirmed) and consider the TORCH (toxoplasmosis, others {HIV, syphilis, varicella}, rubella, cytomegalovirus, herpes simplex) complex
Necrotizing Fasciitis (always cover for streptococci)
Cellulitis (always cover for staphylococcus and β-hemolytic streptococci since a positive culture is often difficult and may only be obtainable from a blood culture if patient is septic)
Cutaneous necrosis (cover for staphylococcus, β-hemolytic streptococci and if indicated by history for *vibrio vulnificus* infection or if indicated by clinical setting for sepsis and immunocompromise, pseudomonas, candidiasis, or aspergillosis)
Vasculitis (cover for Rocky Mountain spotted fever and meningococcus)
Staphylococcal scalded skin syndrome (cover for staphylococci including methicillin-resistant staphylococci, which may now be community acquired and streptococci)
Atypical mycobacteria (histopathology and culture may be falsely negative, so may have to treat based on clinical impression alone)

Table Principles.2 Topical vs Systemic Therapy for Skin Infections

	Topical Therapy	*Systemic Therapy*
Pro	1. Less systemic side effects	1. More efficacious, faster
	2. No access site needed	2. Treats concomitant disease in other organs
	3. Gastrointestinal absorption not essential	3. Can monitor blood levels
	4. No gastrointestinal symptoms	
	5. Less chance of sensitization	
	6. Healing benefit of a base as well as an anti-infectious agent	
Con	1. Topical therapy may obscure disease progression	1. Serious allergic reaction more likely
	2. Contact sensitization can occur	2. May need access site
	3. Less efficacious, slower	3. May be much more expensive
	4. Systemic absorption can still result in systemic side effects	4. May not be available
		5. Absorption of oral drugs may be erratic
		6. Drug interactions are an important consideration

Table Principles.3 General Guidelines for Topical Therapy of Skin Infections

1. Ointments' (hydrophobic) bases repel water and tend to hold the product on the skin longer and increase penetration. Ointments also protect the skin better and facilitate re-epitheliazation.
2. If a skin condition is weeping or oozing then an initial period of 2 to 5 days may be needed when the exudate is debrided chemically or surgically or the wound is dried with water, saline, Aveeno (oatmeal), or Domeboro compresses. A cream (hydrophilic base) may be used if more drying is felt to be indicated.
3. A thick adherent eschar may need surgical debridement to allow healing and treatment of the underlying infection.
4. Incision and drainage may be as important or more important (as shown in the recent outbreak of MRSA skin infections) than any topical or even systemic therapy.
5. Restoration of the epidermal barrier (such as in eczema) is necessary to prevent the need for continual therapy.
6. Signs of increased heat, spreading redness, regional lymphadenopathy, fever, and increased tenderness may indicate that systemic therapy needs to be added to topical therapy.
7. Allergic contact dermatitis due most commonly to antibiotics, antifungals, antiyeast, preservatives, and adhesives in tape and other surgical dressings needs to be considered in the differential diagnosis of any worsening skin infection. Clues to this include itching, blistering, and sudden increased redness without fever or regional lymphadenopathy. Unless accurately diagnosed, the condition will continue to worsen.
8. Systemic absorption of the drug is always a consideration. This is especially a concern when the epidermal barrier is compromised, in intertriginous areas where absorption is increased, large areas are treated, and in infants where the body surface area is increased, when large areas compared to the body mass.

Table Principles.4 Topical Therapy for Skin Infections

Antibacterial	*Bacitracin*
	Neosporin cream and ointment contains polymyxin B, Neomycin, bacitracin; neomycin allergy 1%
	Polysporin cream and ointment contains polymyxin B and bacitracin; can use if neomycin allergy
	Centany (Bactroban) cream and ointment contains mupirocin, and is effective against MRSA in addition to the same coverage as polysporin and neosporin. Resistance by some staphylococci is reported
	Silver-containing compounds used in chronic ulcers, burn patients, and patients with toxic epidermal necrolysis
	0.5 % silver nitrate solution poured over dressing
	Silvadene Cream (cannot use if sulfa allergy is present)
	Aquacel Ag Hydrofiber
	Acticoat Absorbent Fiber
	Silvercel
	Contreet Foam
	Polymem Silver Foam
	Urgotal S. Ag
	Silvasorb Hydrogel
	Iodine-containing products
	Betadine solution, surgical scrub, hand cleanser, and ointment
	Tincture of iodine
	Lugol's solution
	Altabax (retapamulin) ointment
	Gentamicin cream and ointment