Incorporating Pathology in the Practice of Infectious Disease: Myths and Reality

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The role pathology plays in establishing or excluding infectious diseases has been established. However, as the practice of pathology has become subspecialized, there is not enough infectious disease specimen volume to have a pathologist dedicated full time to this crosscutting subspecialty. So, what are the myths and realities of a practicing infectious disease pathologist in the hospital setting? Infectious disease clinicians tend to consult pathologists when there are questions regarding terminology used in pathology reports; when there is the need to perform additional studies on formalin-fixed, paraffin-embedded tissues; and when there is an interest in seeing biopsies or resections obtained from patients and in obtaining photographs for presentations. Pathologists consult infectious disease pathologists when there is a need to review diverse inflammatory reactions; for identification of fungi, parasites, or unknown structures; to define the need to use special stains and other techniques in order to identify organisms in tissues that have been formalin fixed; and to help with terminology to be used in reports. This review explores in more detail why and how these consultations occur.

Keywords. infectious diseases; myths; pathology; practice; reality.

The practice of pathology is becoming subspecialized as there are very specific nuances to each area (for example, gastrointestinal pathology, pulmonary pathology, etc) [1, 2]. Pathology subspecialties are driven by the site where the specimen originates (ie, respiratory, gastrointestinal), and specimen volume justifies the number of pathologists dedicated to a particular subspecialty. Although the role pathology plays in the establishment or exclusion of infectious diseases has been established [3], this crosscutting subspecialty has low specimen volume in developed countries and, thus, is frequently considered nonessential. There are only a few training programs in infectious disease pathology, no fellowships accredited by the Council for Graduate Medical Education, and no certification boards. Consequently, most infectious disease pathologists develop their expertise based on their interest in the field. In order to be an asset to a pathology department, infectious disease pathologists must have another pathology subspecialty (eg, gastrointestinal, respiratory, or other) or manage a clinical laboratory, frequently a microbiology laboratory. They become consultants to other pathologists as they are “the person to go to when something odd is found.”

Pathologists spend most of their time determining if a case has a neoplasia. In some subspecialties, the pathological diagnosis integrates other laboratory parameters. For example, hematopathologists use the World Health Organization classification of leukemias and lymphomas, incorporating molecular markers present in tissue into their reports [4]. In subspecialties where inflammatory processes are frequent, such as dermatopathology, the correlation with clinical features is paramount, and dermatologists are trained to perform pathological examinations. However, when dealing with infectious diseases, pathologists do not feel the need to incorporate the genus and species of the organism that has caused the inflammatory process in their reports. This is likely due to the pressure to release pathology results in a predetermined time that does not allow for correlation with microbiology results, which often takes time, and to the fact that formalin fixation is not conducive to identification.
of microorganisms. Viewed in this context, why is a pathologist with infectious disease expertise needed?

DEFINITIONS

The Merriam-Webster dictionary defines pathology as “the study of diseases and of the changes that they cause” [5]. If we apply this definition to infectious diseases, it will include the inflammatory reaction that microorganisms produce. However, host immune competency determines the presence of and type of inflammatory reaction to the microbe. Similarly, visualization of the causative organism may be possible using different techniques, although, on some occasions, the organism may not be present in the sampled area or it may have been destroyed by the inflammatory reaction or therapy.

The Merriam-Webster dictionary defines microbiology as “a branch of biology dealing with microscopic forms of life” [5]. It differs from infectious disease pathology in that it is only concerned with the microorganism. In practice, infectious disease clinicians solve the majority of their cases by integrating culture, serology, or polymerase chain reaction (PCR) results with clinical findings. Integration of pathology into the infectious disease practice adds the host reaction dimension and possible identification of an organism when results from microbiology, serology, and other studies have been unsuccessful, as well as other directions that may be pursued to determine a diagnosis in unsolved cases. Here, we review the myths and realities of a practicing infectious disease pathologist in the hospital setting.

CONSULTATIONS BY INFECTIOUS DISEASE PHYSICIANS

Physicians trained in infectious diseases require knowledge of microbiology [6–8]. This training is traditionally obtained when the trainee spends time in the microbiology laboratory. The training is reinforced during microbiology rounds, where interesting microbiological findings are shown to the infectious diseases team and the team inquires about their patients’ results [9]. However, time constraints and off-site microbiology laboratories have led to the disappearance of microbiology rounds at many institutions [10]. This has generated the need to create alternative methodologies for teaching microbiology. These methods range from websites that have microbiology content to mixed training models with online material for theoretical content complemented with a practical laboratory phase [11, 12].

At our institution, microbiology rounds are the venue where we integrate pathology, microbiology, and infectious diseases. The teams that take care of patients frequently inquire about the terminology used in pathology reports and the possibility and need of performing additional studies on formalin-fixed, paraffin-embedded tissues. Also, they are interested in seeing biopsies or resections obtained from their patients and in obtaining photographs for presentations (Table 1).

Questions that are frequently asked regarding pathology reports relate to when fungi are present in a specimen and the inappropriate use of terminology by pathologists who have incomplete knowledge of mycology or are too concise in their diagnoses. The presence of septated hyaline molds frequently triggers the pathologist to diagnose Aspergillus. Pathologists do not realize that many molds, including Fusarium spp. (Figure 1), Scedosporium spp., Paecilomyces spp., Acremonium spp., and even Candida spp., have the same morphologic features; these yeasts will form pseudohyphae that appear as septated hyaline hyphae [13–16]. From the infectious disease perspective, identification of Aspergillus in the pathology report may trigger treatment that will not cover molds that are intrinsically resistant to certain antifungals. Similar to what happens with molds, the correlation of what is found in cultures with what pathologists diagnose in tissues regarding a specific yeast genus is not always concordant [17–19]. Knowing the limitations, some pathologist only state that there are fungal elements present in a specimen; this does not help the infectious disease specialist as there is no guiding description. An ideal pathology report should include a description of the yeasts (approximate size, budding

| Table 1: Consultations by Infectious Disease Physicians and Pathologists |
|---------------------------------|-----------------------------------|
| Consultation by Infectious Disease Physicians | Example |
| Pathology reports | Inadequate terminology (fungi, filamentous bacteria), incomplete information (fungi) |
| Additional studies | Different inflammatory response in immunocompromised patients, thus clinical and epidemiologic data should guide studies; special stains and molecular studies in patients with endocarditis and osteomyelitis |
| Unexplained cases with a possible infectious cause that have pathology specimens | Etiologic agent found in approximately 30% of cases, usually using serology; identify what specimens and tests to obtain |
| Photographs | To be used for teaching and case presentations |
| Consultations by pathologists | Granulomas, acute inflammation, plasma cells |
| Inflammatory reactions | Actinomyces, Nocardia, partially treated bacteria |
| Filamentous bacteria | Fungi and parasites |
| Identification of organisms | Special stains, molecular methods |
| Use of other techniques | Terminology used in reports |

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pattern, and other characteristics) or hyphae (septated vs pauciseptated, hyaline vs pigmented, and other morphologic features), structures the hyphae invade, and the host reaction (inflammation, necrosis, hemorrhage) [15, 16]. This descriptive diagnosis should be followed by a comment that indicates the different genera that can have the particular fungal morphology that is present.

Another frequent use of inappropriate terminology by pathologists occurs when filamentous bacteria are present in the specimen. Most pathologists associate microcolonies of filamentous bacteria, known as sulfur granules, to the diagnosis of actinomycosis [20]. Pathologists may order a Gram or bacterial silver stain (Steiner or Warthin–Sarry) to highlight the bacteria but may fail to order a modified acid-fast stain that would help differentiate from *Nocardia* infections, which may also contain sulfur granules [21]. It is also important to know that although sulfur granules can be present in the tissue specimen, neither *Actinomyces* nor *Nocardia* may be recovered in the culture [22]. This creates confusion as the pathologist diagnosed a specific bacterium that was not recovered. By understanding the pitfalls that occur in pathology reports, infectious disease physicians can communicate the background microbiology and indicate the need to perform all special stains required.

Immunosuppressed patients are at risk of many unusual as well as common infections, and the type of immune compromise will define which infections they may have and the type of inflammatory response that may be present [23]. The inflammatory reaction in tissue specimens guides the pathologist in the use of different techniques for identifying organisms. For example, if granulomas are seen, the pathologist will order acid-fast stains for mycobacteria and silver stains for fungi [24–26]; if suspect nuclear changes are noted, they will order immunohistochemistry for cytomegalovirus, herpes, or other viruses [27, 28]; if epithelial and endothelial changes are noted together with an abundance of plasma cells, they will perform immunohistochemistry for syphilis [29, 30]. However, if the inflammatory response is modified, the correct technique for identifying the microorganism may not be performed. To compound the problem, the tissue specimen is usually obtained by a non–infectious disease specialist and the requisition may not provide adequate history and the potential suspect microorganism(s).

In these instances, direct communication between the infectious disease physician and pathologist is imperative so that clinical and epidemiologic data can guide the use of different techniques in tissue sections (Figure 2) [31]. These communications should preferably be done in real time, thus expediting patient treatment [32]. Conversely, in some instances, the results obtained in pathology may guide tests done in microbiology. For instance, a skin lesion with granulomas and presence of acid-fast organisms in a patient who received a transplant may suggest the need to look for *Mycobacterium haemophilum* [33]. Here, communication with the microbiology laboratory is paramount so that the specimen is placed in culture media that contains hemin or ferric ammonium citrate and is incubated at lower temperatures (30°C–32°C). The literature is peppered with examples of immunosuppressed patients presenting with unexpected tissue reactions. An example of this is *Clostridium difficile*–associated disease in patients who have undergone hematopoietic stem cell transplant and show nonspecific findings with the absence of pseudomembranes or ulcers [34]. Several infectious agents, including *Histoplasma* sp., *Cryptococcus*, and nontuberculous

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**Figure 1.** Neutropenic patient with refractory acute lymphoblastic leukemia presented with fever and erythematous skin lesions. A biopsy of 1 of the lesions demonstrated hyaline hyphae inside the blood vessels of the superficial dermis (A, hematoxylin and eosin stain, original magnification 100×). A distinct septae is marked with an arrow. Minimal inflammation or necrosis is noted. In this case, the hyaline hyphae could be confused with pauciseptated molds of the *Mucorales* genera or any of the hyaline septated molds such as *Aspergillus*. Blood cultures grew *Fusarium* (B, Gram stain from blood culture bottle; C, lactophenol cotton blue preparation; original magnification 100×), indicating that the mold present in the blood vessels was not *Aspergillus* or a *Mucorales*. 

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**Table: Normal**

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mycobacteria, can cause inflammatory pseudotumors that are composed of abundant spindle cells accompanied by some necrosis and rare multinucleated giant cells [35–37]. These inflammatory pseudotumors have been described in patients with solid organ transplants and patients with human immunodeficiency virus (HIV; Figure 3). Accumulation of Pneumocystis jirovecii in the alveolar spaces with minimal inflammatory response is the expected pathology in immunosuppressed individuals; however, some patients with HIV or malignancies can produce granulomatous inflammation. Some have suggested that a B-cell deficiency is responsible for the granulomatous response to P. jirovecii as a patient receiving anti-CD20 antibody (rituximab) for refractory nephrotic syndrome had this response pattern [38].

Defining which host or microorganism factors contribute to the different inflammatory responses is not always easy, and additional studies in this area are often required.

The perception of what pathologists do regarding diagnoses and additional studies is highlighted in cases of endocarditis. Both the Duke criteria and European Society of Cardiology state that a vegetation or abscess needs to be confirmed by histopathological examination as a major criteria or the gold standard for diagnosis [39, 40]. Pathologists classify native valvular...

Figure 2. Patient with polycythemia vera presented with fatigue and fevers; the hematologists obtained a bone marrow biopsy, expecting transformation. The bone marrow was hypercellular. No inflammatory foci or granulomas were noted, so no further studies were performed (A, hematoxylin and eosin stain, original magnification 10×). The infectious disease consultant noted that the patient kept chickens in his house and liked exploring caves. With this information, a Grocott methenamine silver stain was performed. Small yeasts with narrow base budding (arrow) were found in the bone marrow biopsy (B, original magnification 100×). Histoplasma urine antigen and cultures were positive.

Figure 3. Patient after a renal transplant was found to have a mass in the forearm. A biopsy of the lesion was obtained and showed abundant spindle cells (A, hematoxylin and eosin stain; original magnification 10×) in a mucoid background, rare small giant cells, and minimal necrosis. A Grocott methenamine silver stain was performed and showed clusters of budding yeasts (arrow; B, original magnification 100×). The possibility of Histoplasma was suggested because the small yeast appeared to be in clusters. The Histoplasma urine antigen was negative, but the Cryptococcus serum antigen was positive. Hence, a mucicarmine stain was performed and showed faint positivity (arrow C, original magnification 100×).

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lesions as active if there is acute inflammation, valve destruction, or granulation tissue or healing in the absence of inflammation or thrombi [41]. When prosthetic valves are removed because of endocarditis, the pathological examination may not include histopathology as there may not be material that can be scraped off and sent for study (gross only) [42]. However, lesions are focal, thus the specimen sent to pathology may not have the features that trigger the use of special stains for detecting bacteria or fungi. In addition, some hospital practices may not include use of these stains, even when active inflammation is present. It should be noted that studies that have assessed the use of histopathological examination of resected valves in patients with endocarditis have not demonstrated that histopathology adds value. These authors propose that the tissue specimen is better used for cultures and PCR testing [43, 44]. Several authors have studied formalin-fixed valves in patients with endocarditis using both broad-spectrum 16S rRNA probes and probes specific for organisms that are difficult to culture, such as *Tropheryma whipplei* and *Coxiella burnetii*, in the correct epidemiological context [45, 46]. The histopathological diagnosis of osteomyelitis presents challenges that are similar to those for endocarditis. Infectious disease guidelines state that the definitive diagnosis of osteomyelitis is provided by use of histopathology [47, 48]. In the case of diabetic foot infections, histopathological study of a bone biopsy provides the diagnosis, while culture identifies an organism that ultimately defines treatment [49]. The Italian guidelines on prosthetic joint infections specify the number of neutrophils per high-power field a pathologist should find in frozen sections of tissues that surround the prosthesis in order to make the diagnosis of osteomyelitis. It is unclear why the guidelines advocate the use of frozen sections because there was a 100%

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**Figure 4.** A patient presented to her primary care physician with fever, chills, arthralgias, and fatigue. She had a history of mitral valve prolapse. She was prescribed clarithromycin for 1 week. However, after the antibiotic treatment was completed, symptoms returned. She was found to have a holosystolic murmur, and a transthoracic echocardiography demonstrated a mobile vegetation on the mitral valve. Multiple blood cultures were negative. The patient was taken to surgery. The valve demonstrated abundant acute inflammatory infiltrate, necrosis, and thrombus formation (A, hematoxylin and eosin stain; original magnification 4×). Culture of the valve was also negative. A Steiner silver stain (B, original magnification 100×) demonstrated small particles that were suggestive of bacteria (circled), while the Gram stain (C, Lillie–Twort Gram stain; original magnification 100×) was noncontributory. *Haemophilus parainfluenza* was found by using 16S rRNA in the formalin-fixed, paraffin-embedded heart valve.
correlation with permanent sections in the studies cited in addition to a good correlation with cultures [50, 51]. A recent study that compared histology with culture demonstrated that both perform similarly when determining the presence of osteomyelitis. However, it should be noted that histology detected the tissue reaction (inflammation and necrosis or sequestrum), while culture identified the organism [52]. The lack of understanding of pathological procedures (frozen sections vs permanent slides) and the features that pathologist use to make the diagnosis (tissue reaction not presence of bacteria) create a false sense of security and may result in potential difficulties when implementing guidelines adequately.

In general, formalin-fixed tissues are not considered optimal for microbiological testing. However, formalin-fixed, paraffin-embedded valves from patients with endocarditis have been tested using PCR electrospray ionization mass spectrometry, yielding up to 55% positive identification of organisms [53]. Studies such as this provide proof that in sequential sections of formalin-fixed specimens, it is possible to identify the inflammatory response using histology and to identify a specific organism using modern molecular techniques (Figure 4). In the case of bone samples, the additional decalcification step with strong acid solutions on top of the formalin fixation poses added problems when obtaining adequate material for molecular testing. Nonetheless, a case report in which the authors used a specific probe for Brucella and determined that B. abortus was the cause of a culture-negative osteomyelitis in a paraffin block is encouraging [54]. Now the question is, when should molecular methods be applied [55, 56]? Should they be performed routinely or just for those cases where cultures are negative?

Infectious disease physicians consult pathologists when they have a case in which an infection is suspected or for a differential diagnosis where a pathogen has not been found and there are tissue specimens. As part of the Emerging Infections Program conducted by the Centers for Disease Control and Prevention (CDC), a study of unexplained cases of possible infectious origin found an etiologic agent in 28% of such cases [57]. Clinical presentation and pathology helped define a syndrome (neurologic, respiratory, or cardiac); however, reference serological tests solved almost 70% of the cases, with fewer than 30% being solved by the use of molecular testing. There are 2 questions that frequently arise in these situations: what specimen is best for the diagnosis of these unexplained cases and what studies should be pursued? If a syndrome is defined, most physicians will start with noninvasive tests and resort to biopsies only if no answer is obtained. When an autopsy is to be performed, communications between the infectious disease clinician, microbiologist, infectious disease pathologist, and prosector can best identify what tissues should be obtained and how these tissues should be handled. For example, for patients in whom influenza virus is suspected to be the cause of death, it is important to sample the larger bronchioles, bronchi, and trachea during the autopsy, rather than peripheral lung tissues [58]. These specimens can be studied using culture, immunohistochemistry, and PCR assays [59].

**CONSULTATIONS BY PATHOLOGISTS**

Pathologists who have an interest in infectious diseases are frequently consulted by other pathologists for a variety of reasons, including to review granulomatous and other inflammatory reactions, find filamentous bacteria, identify fungi and parasites, use special stains and other techniques to identify organisms in formalin-fixed tissues, and identify appropriate terminology for use in reports (Table 1). Consultations by pathologists tend to be more frequent than those by infectious disease physicians. However, many of the cases that pathologists encounter are referred to the infectious disease physician for treatment.

The inflammatory reactions that frequently trigger the need for assistance by an infectious disease pathologist include finding a granuloma, chronic inflammatory infiltrates that contain increased numbers of plasma cells, and abscess formation. Granulomas are usually studied using acid-fast stains and silver

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**Figure 5.** An immigrant from Malaysia presented with hemiparesis. A mass was noted in the spinal cord at the L4 level. The mass was excised and found to be composed of necrotic material. The Gram stain demonstrated fine, faintly purple filaments (A, Brown–Brenn Gram stain, arrows marking the filaments; original magnification 100×). An acid-fast stain showed that these filaments were positive (B, original magnification 100×). The neuropathologist diagnosed Nocardia. A purified protein derivative placed on the patient was positive. There was no culture of the material from the surgery; however, 3 weeks later, Mycobacterium tuberculosis grew from the patient’s sputum. In this case, the routine use of “modified (weak acid) acid-fast stains” should have prompted either the use of a regular acid-fast stain or a comment stating that the organisms could be mycobacteria or Nocardia.
stains for fungi; the infectious disease pathologist is consulted in order to confirm a particular finding (are these true acid-fast bacilli or artifacts? are these yeasts? are they hyphae? can these be identified further?) [26]. Both pathologists and infectious disease physicians need to recognize that *Mycobacteria* are acid fast but also Gram positive and should not be confused with *Nocardia* (Figure 5). Knowledge of which acid-fast stain is used routinely in the laboratory can help in the differentiation of these bacteria. If the laboratory uses a modified acid-fast stain (using weak acid to decolorize) routinely, both *Mycobacteria* and *Nocardia* will stain; however, if the acid-fast stain used is a regular acid-fast stain, then it is likely *Mycobacteria*.

The presence of inflammatory infiltrates with abundant plasma cells suggests infection with spirochetes, in particular, *T. pallidum* or *Borrelia* spp. Silver stains for bacteria (such as Steiner) and immunohistochemistry using antitreponemal antibodies may be useful in finding these organisms in tissues. However, both techniques have pitfalls as the silver stains highlight all spirochetes and there may be cross-reactivity of polyclonal antibodies between these spirochetes [60]. In these cases, the epidemiological setting will drive further serological testing.

A phenomenon well known to microbiologists regarding the formation of long filaments when bacteria are inadequately treated is rarely known by pathologists [61]. Most frequently, when a silver stain (usually for fungi) is ordered and pathologists see these long filaments (Figure 6), they confuse them with either fungal elements or the filamentous bacteria *Actinomyces* and *Nocardia*. Here, knowledge of the size and width of the filaments is useful when determining if these are hyphae; the clinical history and cultures will confirm that the patient has been taking antibiotics in suboptimal doses to be bactericidal.

Pathologists in developed countries see few parasites in tissues, though parasites are occasionally seen in patients who have lived or traveled overseas. Probably the most common parasite seen in tissues is *Toxoplasma* as it has a worldwide distribution and is frequently found in immunocompromised hosts. Pathologists routinely use immunohistochemical assays to determine the presence of this protozoan [62]. Leishmaniasis should be considered in American troops and contractors who have been stationed in the Middle East. In skin and other tissue biopsies, the kinetoplast can be difficult to distinguish. When pathologists encounter parasites or structures that could be parasites, they usually consult the pathologist with infectious diseases expertise or the microbiologist in their hospital. The following CDC resources can be used when an institution does not have an infectious disease

**Figure 6.** A neutropenic patient with acute myelogenous leukemia presented with fever and cough. He was admitted and found to have *Pseudomonas* in blood cultures. The susceptibility testing showed resistance to multiple antibiotics. Further complications included pneumonia and hepatic and renal failure. After 1 week in the intensive care unit, the patient expired, and an autopsy was performed. Hematoxylin and eosin stains of the lungs demonstrated multiple foci with mucous, necrosis, and inflammation (circled; A, original magnification 10×). A Gram stain demonstrated abundant filamentous bacteria consistent with inadequately treated *Pseudomonas* (the arrow points to 1 of the filamentous bacteria; B, Lillie–Twort Gram stain; original magnification 100×).
CONCLUSIONS

Having a pathologist who has a special interest in infectious diseases is an asset for patient care as it provides consultation to both infectious disease physicians and pathologists. This subspecialty is particularly needed in tertiary care facilities where complex immunosuppressed patients who are at risk for infections are treated. Practitioners can use regular meetings, such as microbiology rounds, to discuss patients’ infections. Practitioners can use regular meetings, such as microbiology rounds, to discuss patients’ infections.

Note

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