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ORIGINAL ARTICLE

Histopathologic Detection and Identification of Infectious Agents in Granulomatous Inflammation: Comparison with Culture

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Abstract

Background: Granulomas in surgical specimens, especially within lung and lymph nodes, commonly have an infectious etiology. Granulomatous tissue is usually sent to both surgical pathology for tissue examination and to clinical microbiology laboratories for culture.

Methods: We performed a retrospective review and comparison of histochemical stains and clinical microbiology culture results in 132 surgical pathology granuloma specimens to determine the utility of tissue morphological examination for detection and identification of fungi and acid-fast bacilli as compared to clinical microbiology culture.

Results: Fungi were detected by histochemical stains in 31 cases. Of these, 21 cases were confirmed by culture, 4 cases were not submitted for fungal culture, and 6 were negative by culture. Morphological examination identification correlated with culture results in 20/21 cases. No fungi were identified by culture alone. Mycobacteria were detected by histochemical stain in 17 cases. 14 of these were confirmed by culture. Culture detected 14 additional cases of mycobacteria that were negative on initial morphological examination.

Conclusions: Surgical pathology tissue examination detected fungi with greater sensitivity than culture. Morphological identification of fungi on histochemical stains is reliable and correlates well with eventual culture results. Histologic detection of mycobacteria is specific but less sensitive and less useful for speciation compared to microbial culture.

Keywords

Granulomatous inflammation, Fungus, Acid-fast bacteria

Introduction

Granulomas are a common finding in surgical specimens. They have a wide variety of etiologies, commonly grouped into infectious causes, non-infectious causes, and unexplained causes. Identifying these by histopathologic means has the advantage of expediency, but the accuracy of this technique remains somewhat unclear.

Several studies have been undertaken to characterize the causes of necrotizing granulomas in tissue. Ulbright and Katzenstein examined 86 necrotizing granulomas and found that 61 (71%) were infectious, 3 (3%) were consistent with granulomatosis with polyangiitis (formerly Wegener's granulomatosis) or hyalinizing granulomas, and 22 (26%) were unexplained [1]. Another study of 190 necrotizing granulomas found that 104 cases (54.7%) were infectious, 51 (26.8%) were non-infectious, and 35 (18.4%) were unexplained [2]. Within the infectious category, the causative agents were divided equally between fungal organisms (52/104) and mycobacteria (52/104) [2].

Although there have been a few studies that have examined this relationship previously, they have focused on only filamentous fungi or *Histoplasma*, while our study was non-filtered institutional inclusive review of fungal and mycobacterial organisms [3-5].

This study retrospectively reviewed five years of granulomas, both necrotizing and non-necrotizing, from various sites to gauge the sensitivity and specificity of histologic examination for the detection of fungi and mycobacteria. This study also examined the concordance rates between fungal morphological diagnosis and culture.

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Materials and Methods

This study was approved by the local institutional review board. Surgical pathology cases with granulomas were collected from 01/01/10 to 11/03/15 after searching the electronic database. Cases were aggregated from the surgical pathology database (McKesson Powerpath) queries using the keywords 'granuloma' and 'granulomatous'.

Autopsy and cytopathology cases were excluded as were surgical pathology specimens from soft tissue, skin, prostate, luminal gastrointestinal tract, and bladder. Specimens were evaluated by examination of standard sections stained with hematoxylin and eosin, Fite modification of the acid-fast bacilli (AFB) technique, and Gomori methenamine silver (GMS). All surgical pathology material was reviewed by a senior surgical pathologist. For each case, the type of granuloma was noted, and, if applicable, the morphology of organism, time to detection, number of forms per 20x field, and the morphological diagnoses (fungus only) were recorded. Each microbial stain was reviewed for 5 minutes before it could be called histologically negative. After surgical pathology review, each patient's corresponding microbiology culture results were recorded. Pertinent clinical data including the patient's immune status was recorded when available.

Results

A total of 132 surgical pathology cases with granulomas were reviewed. 104 were obtained from lung specimens, 25 from lymph nodes, and 3 from liver specimens. Necrotizing granulomas were the most commonly identified (87/132, 66%), followed by non-necrotizing (29/132, 22%), suppurative (11/132, 8%), and plasmacytic/eosinophilic (3/132, 2%). Only 1 case each of pure eosinophilic or sclerosing granuloma (0.7%) was identified. The reason for the higher proportion of necrotizing granulomas versus non-necrotizing granulomas in this study is unclear. It may be related to the defined specimens sites included in this study, whereas other studies have included a wider variety of sites including soft tissue, breast, and salivary gland that may have a higher proportion of non-necrotizing granulomas [6]. Organisms were detected using histochemical stains in 48/132 cases (36%). Of these 48 cases, fungi were seen in 31 specimens and mycobacteria in 17 specimens. Morphological assessments were made for all 31 fungi identified. Culture results were available in 44/48 cases (92%). Cultures confirmed the presence of fungi in 21/31 cases (68%) and mycobacteria in 14/17 cases (82%). In 9 cases (6 fungi and 3 acid-fast bacilli), organisms were identified on the histochemical stains, but the resulting cultures showed no growth. Four cases had fungi detected on histochemical stains, but the specimen was not sent for culture and no comparison result is possible. The fungal morphological assessment made on surgical pathology evaluation was confirmed by culture in 17/18 cases (94%). 3 cases were simply described as branching fungus or mold, without an attempt at speciation. Of these descriptive cases, 2/3 were confirmed by culture and 1/3 showed no growth. One case was incorrectly characterized as *Aspergillus* on morphological examination, but was actually *Coccidioides* on culture.

Table 1: Comparison of fungal morphological diagnosis with clinical microbiology culture results.

Morphology	Type of granuloma	Specimen	Morphological diagnosis	Culture results
1. Small yeasts with central dot	Necrotizing	Lung	*Pneumocystis jiroveci*	No growth
2. Small yeasts with narrow-based budding	Necrotizing	Lung	*Histoplasma*	*Histoplasma*
3. Septate branching hyphae	Plasmacytic/ Eosinophilic	Lung	*Aspergillus*	*Aspergillus niger*
4. Small yeasts with narrow-based budding and halos	Non- necrotizing	Lung	*Cryptococcus neoformans*	*Cryptococcus neoformans*
5. Small yeasts with narrow-based budding and halos	Non- necrotizing	Lung	*Cryptococcus neoformans*	*Cryptococcus neoformans*
6. Spherules	Necrotizing	Lung	*Coccidioides spp*	No fungal culture performed
7. Yeasts with narrow-based budding	Necrotizing	Lung	*Histoplasma capsulatum*	No culture performed
8. Hyaline branching	Plasmacytic/ Eosinophilic	Lung	Mold, not otherwise specified	*Aspergillus fumigatus*
9. Variably-sized (3-10 microns) budding yeasts	Non- necrotizing	Lung	Capsule-poor *Cryptococcus neoformans*	*Cryptococcus neoformans*
10. Spherules	Necrotizing	Lung	*Coccidioides spp*	No fungal culture performed

11. Spherules	Eosinophilic	Lymph node	*Coccidioides spp*	*Coccidioides spp*
12. Branching fungus	Necrotizing	Lung	Branching fungus	*Candida tropicalis*
13. Hyaline hyphae	Necrotizing	Lung	*Aspergillus spp.*	*Coccidioides spp*
14. Spherules	Necrotizing	Lymph node	*Coccidioides spp*	*Coccidioides spp*
15. Septate branching hyphae	Plasmacytic/ Eosinophilic	Lung	*Aspergillus spp.*	*Aspergillus niger*
16. Spherules	Necrotizing	Lung	*Coccidioides spp*	No growth
17. Spherules	Necrotizing	Lung	*Coccidioides spp*	*Coccidioides spp*
18. Small yeasts with narrow-based budding	Necrotizing	Lung	*Histoplasma capsulatum*	*Histoplasma capsulatum*
19. Branching septate hyphae	Necrotizing	Lung	Branching hyphae	No growth
20. Spherules	Necrotizing	Lung	*Coccidioides spp*	*Coccidioides spp*
21. Small yeasts with narrow-based budding	Necrotizing	Lung	*Histoplasma capsulatum*	No growth
22. Spherules	Necrotizing	Lung	*Coccidioides spp*	*Coccidioides spp*
23. Small yeasts with narrow-based budding and halos	Necrotizing	Lung	*Cryptococcus neoformans*	No culture performed
24. Spherules	Necrotizing	Lung	*Coccidioides spp*	No growth
25. Large yeasts with broad-based budding	Necrotizing	Lung	*Blastomyces*	*Blastomycosis*
26. Spherules	Necrotizing	Lung	*Coccidioides spp*	*Coccidioides spp*
27. Spherules	Sclerosing	Lung	*Coccidioides spp*	*Coccidioides spp*
28. Large yeasts with broad-based budding	Necrotizing	Lung	*Blastomyces*	*Blastomycosis*
29. Small yeasts with halos	Non- necrotizing	Lung	*Cryptococcus neoformans*	*Cryptococcus neoformans*
30. Spherules	Necrotizing	Lymph node	*Coccidioides spp*	*Coccidioides spp*
31. Spherules	Necrotizing	Lung	*Coccidioides spp*	No growth

The morphological-culture comparison data are recorded in Table 1. No fungi were identified on culture alone; however, culture detected 14 additional cases of mycobacteria that were not seen on histochemical stains. Table 2 lists the specific organisms identified and the mode of detection. One case was positive for both fungi and mycobacteria. Of the cases with infectious organisms identified, the majority of the granulomas were necrotizing (71% necrotizing for fungus and acid fast bacilli). The majority of the surgical pathology cases were negative for organisms on both histochemical stains and culture. In these cases, the histochemical stains were reviewed for the maximum duration of 5 minutes. The duration and average numbers of organism forms/20x field for the positive cases is recorded in Table 3. Although fungus detection occurred sooner on average than mycobacterial detection (45.9 seconds versus 92.8 seconds), slightly less fungal forms were seen/20x field (19.4 versus 23.4).

Discussion

The results of our study suggest that fungal organisms can be reliably identified using GMS stains on surgical pathology specimens. GMS stains helped to detect 31/132 (23%) specimens with fungal elements. Of those, 21 cases were confirmed with fungal culture growth (68%). Our percentage of culture-confirmed cases is markedly higher than the 39.97% or the 43.4% reported for filamentous fungi in studies by Challa, et al. and Lee, et al. [3,4].

Histology-culture discrepancy may have several possible explanations. GMS staining does not distinguish between viable and non-viable forms. If the organisms are indeed non-viable, detection of fungal elements may be possible on histologic examination, but the resulting cultures will show no growth. The pre-biopsy antifungal treatment was not recorded for the patients in this study, but antifungal therapy may contribute to non-viability of organisms sent for culture. As described by Challa, et al. other causes of discordance could include sampling error of tissues submitted for culture or the handing and processing of tissue within the microbiology laboratory [4]. Our study had no instances of fungi detected on culture that were not seen on surgical pathologic examination. This is similar to the result reported by Weydert, et al. in a smaller study [5].

We found a histopathology-microbial culture diag-

Table 2: Etiological agents in 132 surgical cases with granuloma.

Organism		Number of cases	Method of detection
Fungus (n = 31)	*Pneumocystis jiroveci*	1	Histochemical stain alone: 1 (1 culture-negative)
	Histoplasma	4	Histochemical stain + culture: 2
			Histochemical stain alone: 2 (1 culture-negative, 1 no culture performed)
	Aspergillus	2	Histochemical stain + Culture: 2
	Cryptococcus	5	Histochemical stain + Culture: 4
			Histochemical stain alone: 1 (1 no culture performed)
	Coccidioides	14	Histochemical + Culture: 9 (one incorrectly identified as *Aspergillus* on morphology)
			Histochemical stain alone: 5 (3 culture-negative, 2 no culture performed)
	Blastomyces	2	Histochemical stain + Culture: 2
	Mold, not otherwise specified	1	Histochemical stain + Culture: 1 (culture showed *Aspergillus fumigatus*)
	Branching fungus, not otherwise specified	2	Histochemical stain alone: 1 (culture showed *Candida tropicalis*) (1 culture-negative)
Mycobacteria (n = 31)	*Mycobacterium simiae*	1	Histochemical stain + Culture: 1
	Mycobacterium tuberculosis complex	12	Histochemical stain + Culture: 3
			Culture alone: 9
	Mycobacterium chelonae-abscessus group	3	Histochemical stain + Culture: 1
			Culture alone: 2
	Mycobacterium avium intracellulare (MAI) complex	12	Histochemical stain + Culture: 9
			Culture alone: 3
	Acid-fast bacilli, not otherwise specified	3	Histochemical stain alone: 3 (3 culture-negative)
Negative for organisms		51	
No culture performed **and** no organism identified on special stains		20	

*One case was positive for both mycobacteria and fungi.

Table 3: Average time to detection and number of organisms identified/20x field.

	Average time to detection of first organism	Average number of organism forms/20x field
Fungus	45.9 seconds	19.4
AFB	92.8 seconds	23.4

nosis correlation for fungi in 17/18 cases (94.4%). If the 2 cases that were called "branching hyphae" or "mold" not otherwise specified are included in analysis, the culture correlation increases to 95%. Only 1 of our cases was incorrectly identified on morphology (1/21, 4.8%). It is worth mentioning that previous studies have examined the histological-culture diagnosis correlation for filamentous fungi. Those studies reported greater discordance rates of 16.67%, 17%, and 21% [3,4,7]. However, this discrepancy may be accounted for by the types of fungi included in the respective studies. The most common type of fungi found in our study was *Coccidioides* (Table 2), whereas the most common agent in the filamentous studies was *Aspergillus* [3,4,7].

Based on our results, it would seem that culture has a more significant role in detecting mycobacteria than for fungal organisms within granulomatous specimens. We detected mycobacteria using acid-fast stains in 17/132 (13%) of cases. 14/17 (82%) of those cases were confirmed by microbial culture. The remaining 3 cases did not grow on culture. However, we found an additional 14 cases of mycobacteria on culture when the

corresponding histologic examination had been called negative. This increased the number of total mycobacteria cases to 31/132 (23%). Importantly, 2 of these culture-only cases were *Mycobacterium chelonae-abscessus* group, 3 were *Mycobacterium avium intracellulare* (MAI) complex, and 9 were *Mycobacterium tuberculosis* complex. Although in-depth clinical follow-up was not a feature of our study, it is likely that at least some of these culture-detected cases of mycobacteria were clinically relevant. Our detection rates for histochemical stains and culture are similar to those reported by Nazarullah, et al. [2]. In that study of 52 mycobacterial-positive granulomas, 15 (29%) were visualized by special stains, 6 (12%) were visualized on special stains but culture negative, and 38 (73%) were culture positive [2]. Our study reported 17/31 (55%) cases positive on special stains, 3/31 (10%) positive on special stains but negative on culture, and 28/31 (90%) positive on culture. The sensitivity of histologic examination alone for acid-fast bacteria in our results is only 55%, 95% CI [36%, 73%]. As such, histology should be combined with culture results before reporting any granuloma as negative for acid-fast organisms.

Several authors have suggestions for improving the detection, and therefore the sensitivity, for organisms on special stains. Perhaps intuitively, organisms are most commonly found within the necrosis in cases of necrotizing granulomas. Goodwin, et al. found they were able to increase the sensitivity for *Histoplasma* by 59% by choosing a block with necrosis to be used for the GMS stain [8]. Ulbright and Katzenstein and others reported that the detection of small organisms, like acid-fast bacteria or *Histoplasma*, could increase by 90% by examining special stains at high magnification and re-examining the slide if the first review was negative [1,9,10]. We believe our practice of examining each slide for 5 minutes at high magnification before reporting it as “negative for organisms” contributed to the relatively high sensitivities found in our study. Multiple authors have also noted that performing special stains on more than one granulomatous block can increase the sensitivity of detecting organisms [1,9]. When available, some have resorted to using alternative stains, like the auramine/auramine-rhodamine fluorescence technique, to help identify mycobacteria [9,10]. Immunohistochemistry and in-situ hybridization are possible for the detection and speciation of fungi, respectively, but each have their limitations as described by Aubry [9]. Real-time PCR exists for detection and speciation of mycobacteria, but is most useful when tissue was not submitted for culture or, uncommonly, when cultures are negative [9,10]. It is understood to have excellent specificity, but low sensitivity, so a negative result does not exclude infection [9,10]. Although the presence of granulomatous inflammation is extremely suspicious for infection, it is possible that many cases without organisms represent ‘true negatives’. Although long term clinical follow-up was outside the scope of this study, a percentage of these granulomas may remain idiopathic or be attributable to other causes like vasculitis.

Fungal cultures were ordered in 118/132 (89%) of our specimens and acid-fast bacteria (AFB) cultures were ordered in 112/132 (85%) specimens. Although the majority of the specimens without cultures ordered did not show organisms on histologic examination, there were four cases with fungal elements seen on histology that could not be confirmed by the gold standard. The institutional rate of tissue sent for fungal culture is still much higher than reported in other studies. Lee, et al. Challa, et al. and Weydert, et al. reported fungal culture rates of 31%, 60.8%, and 80%, respectively [3-5].

Conclusion

Surgical pathology tissue examination detected fungi with greater sensitivity than culture. Morphological identification of fungi on histochemical stains is reliable and correlates well with eventual culture results. Histologic detection of mycobacteria is specific but less sensitive and less useful for speciation compared to mycobacterial culture. A minimum 5 minute examination of special stains at high power is recommended before reporting a negative result to maintain the sensitivities reported in this study.

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