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ORIGINAL ARTICLE

Granulomatous inflammation diagnosed by fine-needle aspiration biopsy

Dianna L. Ng, MD*, Ronald Balassanian, MD

Department of Pathology, University of California, San Francisco, California

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KEYWORDS
Granuloma;
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Mycobacteria

Introduction Fine-needle aspiration biopsy (FNAB) is a minimally invasive biopsy technique and an important tool for diagnosing infectious diseases. Rapid onsite evaluation allows for triage for ancillary testing, including microbiologic cultures. We aimed to determine the etiology of granulomatous inflammation diagnosed by FNAB by correlating with culture results and clinical history.

Materials and methods A 16-year retrospective review of cases diagnosed as "granulomatous inflammation" or "granuloma" was performed at the Departments of Pathology at the Zuckerberg San Francisco General Hospital and Trauma Center and University of California, San Francisco.

Results A total of 339 FNABs diagnosed as granulomatous inflammation were identified. Necrotizing granulomatous inflammation was present in 117 of 339 cases (34.5%) and non-necrotizing granulomatous inflammation was present in 222 of 339 cases (65.5%). A pathogen was detected in 100 of 339 (29.5%) FNABs by either cytomorphology, special stains, or culture, or a combination of more than one test. Of the 100 pathogen-positive cases, necrotizing granulomatous inflammation was seen in 50 of 100 (50%) and non-necrotizing granulomatous inflammation was identified in 50 of 100 (50%) cases. Culture results were available in 239 cases and positive in 70 (29%). Positive culture results included 40 of 239 (17%) cases with *Mycobacterium tuberculosis* complex, 15 of 239 (6.3%) with atypical mycobacterial species, 6 of 239 (3%) with *Coccidioides immitis*, 2 of 239 (<1%) with *Histoplasma capsulatum*, and 2 of 239 with *Talaromyces marneffei* (<1%).

Conclusions Granulomatous inflammation is a nonspecific finding and suggests a broad range of disease processes, ranging from infection to malignancy. FNAB is an excellent minimally invasive technique that allows for ancillary testing critical for definitive diagnosis.

Introduction

Fine-needle aspiration biopsy (FNAB) is a useful tool for the diagnosis of infectious disease. Cytologic diagnosis can also be quicker than conventional culture and microbiologic

*Corresponding author: Dianna L. Ng, MD; Department of Pathology, UCSF Medical Center, 1001 Potrero Ave, Building 3, Room 104, San Francisco, CA 94110; Tel.: (628) 206-8216; Fax: (628) 206-5988.
E-mail address: dianna.ng@ucsf.edu (D.L. Ng).

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testing, especially for fastidious organisms such as fungi and mycobacteria, which may take days to weeks before yielding a culture result. In some cases, cultures are negative and morphology provides the only means for diagnosis and guidance of therapy.[1,2] In regions with endemic tuberculosis, the presence of granulomatous inflammation alone is considered diagnostic of mycobacterial infection. In these settings, FNAB has been shown to be an effective tool in diagnosing tuberculosis, particularly when paired with special stains, culture, and molecular studies.[3-11] In populations with a low prevalence of tuberculosis, however, granulomatous inflammation is a nonspecific finding and may be seen in the setting of a broad range of diagnoses including mycobacterial and fungal infections, sarcoidosis, foreign body reaction, and malignancy.[12] In this study, we performed a retrospective review of FNABs with a diagnosis of granulomatous inflammation and correlated the results with clinical history and ancillary testing to identify the etiology of the granulomatous inflammation.

Material and methods

FNAB cases from January 1, 2000, to March 1, 2016, with diagnoses of "granulomatous inflammation" or "granuloma" were identified in the pathology databases at Zuckerberg San Francisco General Hospital and Trauma Center and University of California, San Francisco (UCSF). Routine cytology stains included May-Grünwald Giemsa and Papanicolaou stains. Cases were then classified as necrotizing or non-necrotizing and correlated with clinical history and special stains (Kinyoun acid-fast stain, Grocott's methenamine silver stain [GMS], and periodic acid−Schiff), which were all performed on the FNAB cell block material. The cases were also correlated with microbiology culture studies including bacterial, mycobacterial, and fungal culture results, when available. The study received institutional review board approval by the UCSF Committee for Human Research (#16-20124).

Results

A total of 339 FNABs with diagnoses of granulomatous inflammation were identified, representing 160 women and 146 men for a total of 306 patients, with a median age of 47 years (range: 1-90 years). There were 124 of 306 (40.5%) patients who were seronegative for human immunodeficiency virus (HIV), 41 of 306 (13.4%) were seropositive for HIV, and 141 of 306 (46.1%) did not have documented

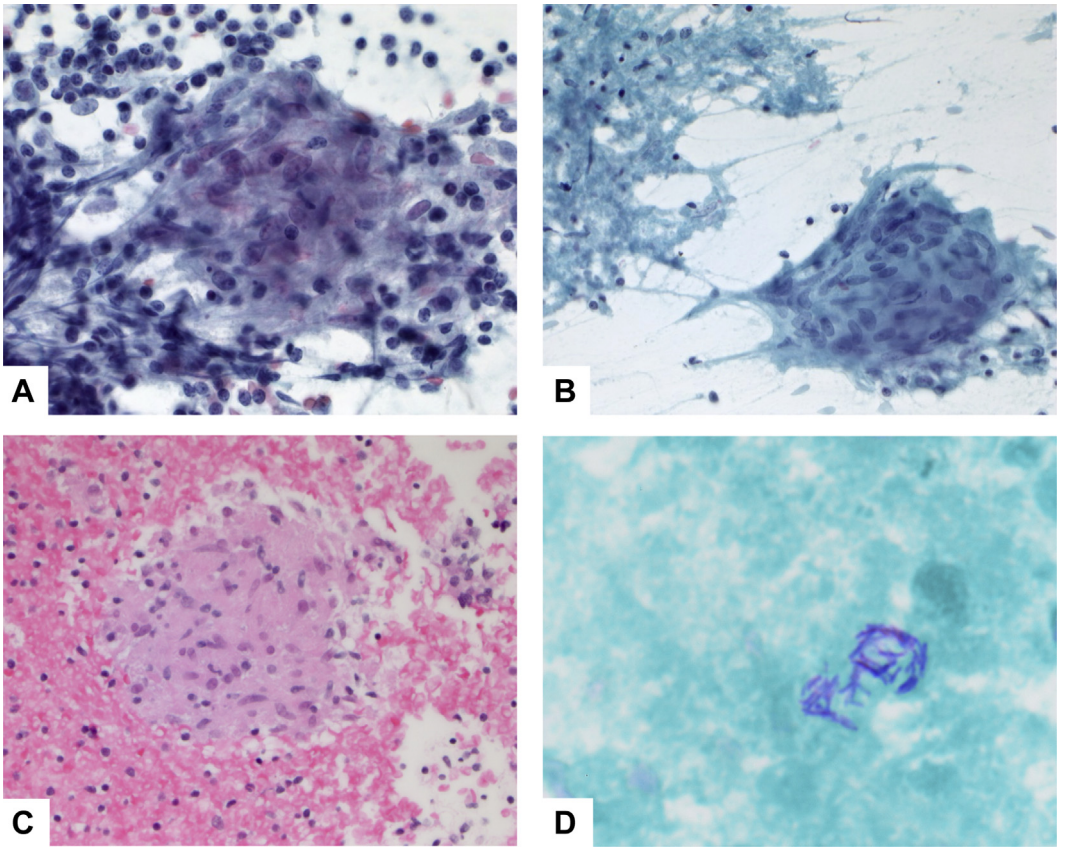

Figure 1 Fine-needle aspirate biopsy specimens with granulomatous inflammation. A, Non-necrotizing granulomatous inflammation. B, Necrotizing granulomatous inflammation. C, Non-necrotizing granuloma on cell block. D, Acid fast bacilli on direct smear (A: Papanicolaou stain, 60×; B: Papanicolaou stain, 40×; C: Hematoxylin and eosin, 40×; D: Kinyoun stain, 100×).

Table 1 Fine needle aspiration biopsy cases with positive culture results and association with necrosis.

Organism (n = 70)	Number of cases (%)	Number of cases with necrosis (%)
Mycobacterium tuberculosis complex	40/70 (57)	24/40 (60)
Atypical mycobacteria	15/70 (21)	6/15 (40)
Coccidioides immitis	6/70 (9)	1/6 (17)
Histoplasma capsulatum	2/70 (3)	1/2 (50)
Talaromyces marneffei	2/70 (3)	0/2 (0)
Aspergillus species	1/70 (1)	0/1 (0)
Nocardia species	1/70 (1)	0/1 (0)
Unclassified acid-fast bacilli	1/70 (1)	1/1 (100)
Staphylococcus aureus	1/70 (1)	0/1 (0)
Escherichia coli	1/70 (1)	0/1 (0)

testing. One patient had a history of renal transplantation and 1 had a history of Crohn's disease.

The sites sampled by FNAB included lymph nodes (200; 59%), lung (56; 17%), soft tissue (44; 13%), breast (12; 35%), salivary gland (10; 29%), liver (8; 2.3%), thyroid (4; 1.1%), kidney (2; 0.6%), pancreas (1; 0.3%), spleen (1; 0.3%), and brain (1; 0.3%). Microorganism stains were performed in 140 (41.3%) cases and microbiology culture results were available for 239 (70.5%) FNABs. However, only 109 (32.2%) cases had a complete workup for infection, which includes GMS and acid-fast stain stains as well as microbiology culture results for mycobacteria, bacteria, and fungus.

Necrotizing granulomatous inflammation was present in 117 (34.5%) (Fig. 1A) cases and non-necrotizing granulomatous inflammation was present in 222 (65.5%) cases. A specific pathogen was detected in 100 of 339 (29.5%) FNABs by either cytomorphology, special stains, or microbiology culture, or a combination of more than 1 test.

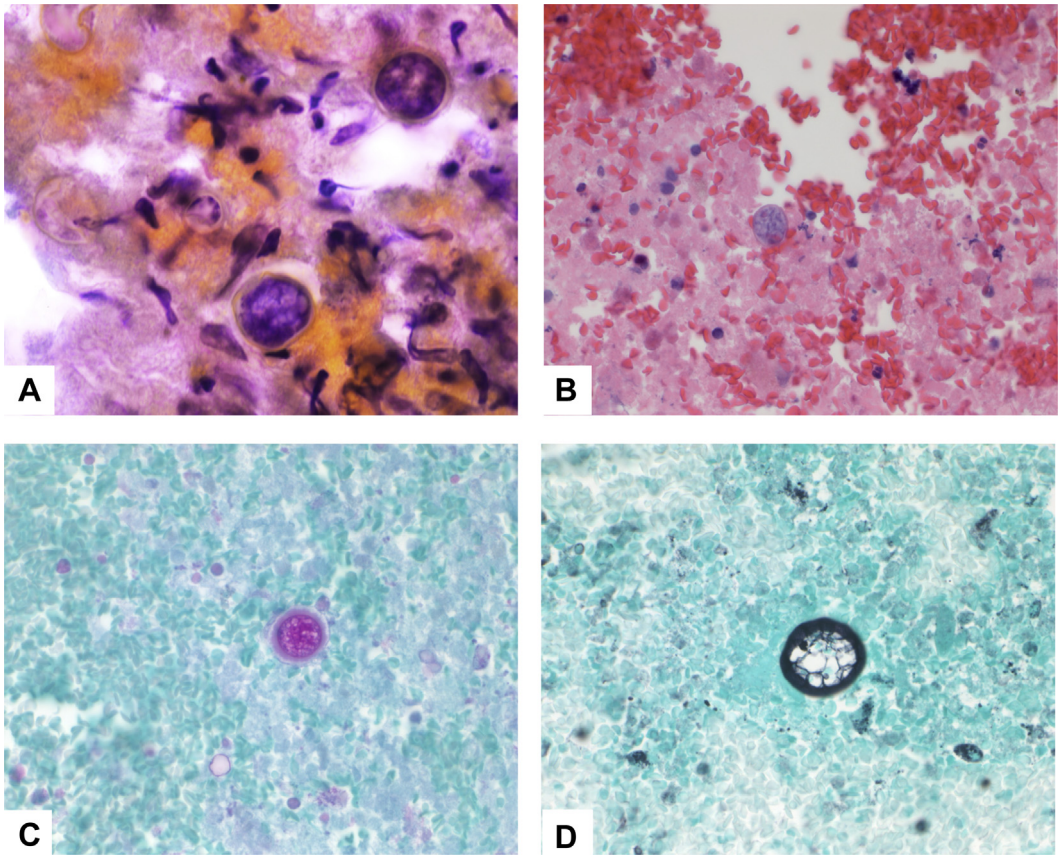

Figure 2 *Coccidioides immitis*. A, Spherules with endospores. B-D, Cell block with *Coccidioides* spherules (A: Papanicolaou stain, 40×; B: hematoxylin and eosin stain, 60×; C: periodic acid–Schiff stain, 60×; D: Grocott's methenamine silver stain, 60×).

Of the 100 pathogen-positive cases, necrotizing granulomatous inflammation was seen in 50 of 100 (50%) and non-necrotizing granulomatous inflammation was identified in 50 of 100 (50%) cases. Microbiology culture results were positive in 70 of 239 cases (29%). Positive culture results revealed: 40 of 239 (17%) cases with *Mycobacterium tuberculosis* complex, 15 of 239 (6.3%) with atypical mycobacterial species, 6 of 239 (3%) with *Coccidioides immitis*, 2 of 239 (<1%) with *Histoplasma capsulatum*, 2 of 239 with *Talaromyces marneffei* (<1%) and 1 case each of *Aspergillus species, Escherichia coli, Nocardia species, Staphylococcus aureus,* and unclassified *Mycobacteria* species (Table 1, Figs. 2-5).

Fungal organisms were detected in 20 of 100 (20%) cases by cytology, special stains, and/or culture. Fungal organisms were morphologically identified in 18 of 20 (90%) cases, either on routine Papanicolaou or May-Grünwald Giemsa stain or by evaluation microorganism stains (Fig. 1B-D). Of these cases, 9 had a full workup with cytology, special stains, and culture performed, of which 3 of 9 (33%) were detected using all testing methods, 1 of 9 (11%) by culture only, and 5 of 9 (56%) on both cytology and microorganism stains. Two of the cases were morphologically consistent with *Candida* species, 2 with *Coccidioides immitis*, 1 with *Cryptococcus* species, and 1 with *Pneumocystis jiroveci*. Mycobacteria were detected in 77 of 100 (77%) cases by special stain and/or culture (Fig. 1E). Of these cases, 32 had cytology, special stains, and culture performed, of which 6 of 32 (19%) were identified on all testing methods, 18 of 32 (56%) by culture only, and 8 of 32 (25%) with special stains only. Three cases of bacterial infection were detected by culture. There was 1 case in which fungal organisms were seen on the direct smears and confirmed by GMS on cell block, but the culture was positive for *Mycobacterium tuberculosis* complex.

Mycobacteria accounted for the majority cases of necrotizing granulomatous inflammation, 43 of 77 (56%), with tuberculosis representing 24 of the 77 cases (31%). Necrosis was seen in only a subset of fungal cases (3 of 20, 15%) and none of the bacterial cases (Table 2). No infectious etiology was identified in 62 of 117 (53%) of cases with necrosis. Of the total 339 cases of granulomatous inflammation, carcinoma was present in 3 of 339 (0.9%) cases (Fig. 1F), acute lymphoblastic lymphoma/leukemia in 1 of 339 (0.3%), and foreign body giant cell reaction in 16 of 339 (4.7%) FNAB cases. Sarcoidosis was suspected in 17

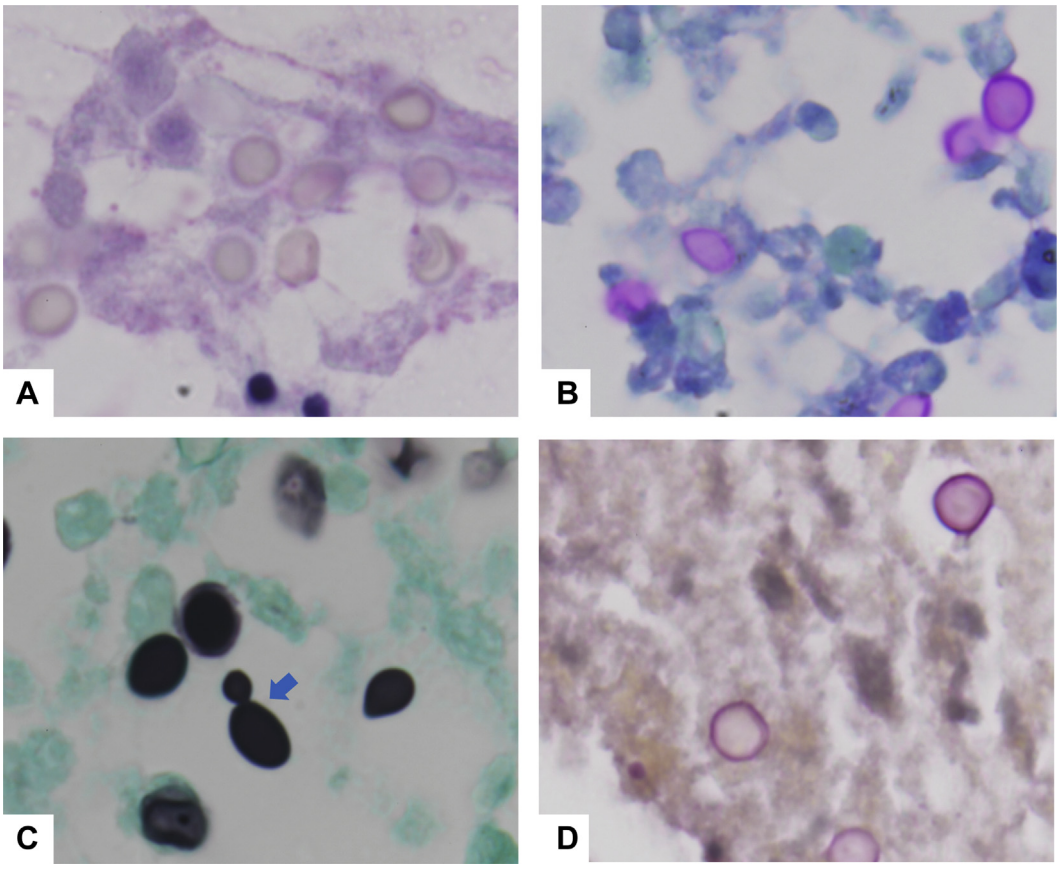

Figure 3 *Cryptococcus neoformans*. A, Spherical encapsulated yeast. B-D, Cell block with spherical and narrow-based budding yeast (arrow). (A: Papanicolaou stain, 100×; B: periodic acid–Schiff stain, 100×; C: Grocott's methenamine silver stain, 100×; D: mucicarmine stain, 100×).

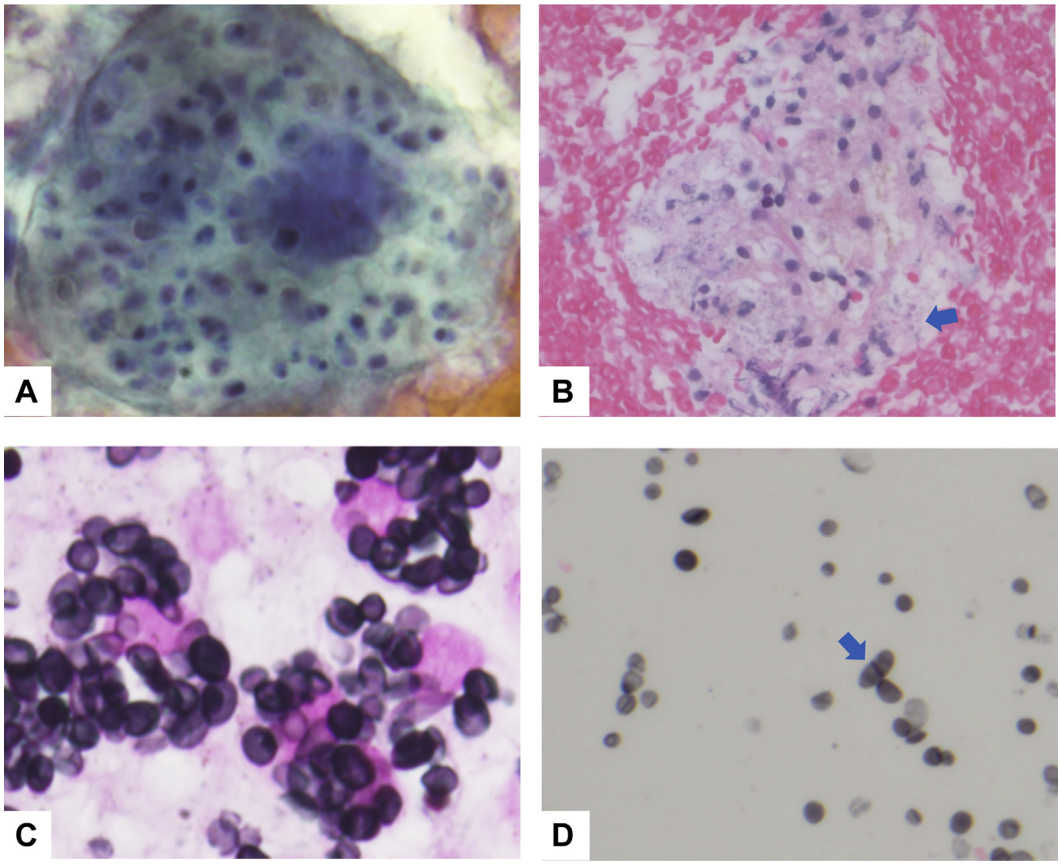

Figure 4 Comparison of *Histoplasma capsulatum and Talaromyces marneffei*. A-C, Histoplasmosis with intracellular yeast filling macrophages (arrow). D, Talaromycosis with oval yeast and central septa (arrow). (A: Papanicolaou stain, 100×; B: H&E stain, 60×; C: Grocott's methenamine silver stain, 100×; D: Grocott's methenamine silver stain, 100×).

of 306 (5.6%) patients, and 1 patient had granulomatosis with polyangiitis, 1 patient had chronic granulomatous disease, and 1 patient had chronic sialadenitis. Therefore, the etiology of the granulomatous inflammation was not determined in 199 of 339 (59%) cases, through cytology, microorganism stains, microbiology cultures, as well as correlation with clinical history.

Discussion

FNAB has become increasingly important for the diagnosis of infections. As a minimally invasive technique, FNAB allows for assessment of the cytomorphology as well as rapid onsite evaluation and triage of the sample at the time of biopsy for ancillary studies such as microorganism stains,

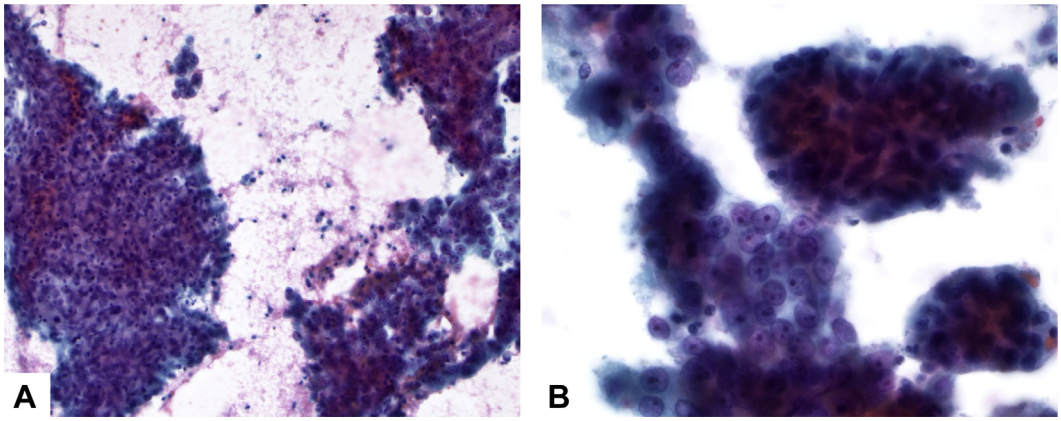

Figure 5 Adenocarcinoma. A and B, Adenocarcinoma in association with granulomatous inflammation (A: Papanicolaou stain, 40×; B: Papanicolaou stain, 60×).

Table 2 Etiology of granulomatous inflammation and association with cytology, special stain, and culture results.

Final diagnosis (N = 339)	NGI	NNGI	Culture positive	Positive cytology/ special stains
Mycobacterial	43	34	56	22
Fungal	3	17	11	7
Bacterial	0	3	3	1
Sarcoid	0	20	0	0
Foreign body	9	0	0	0
Neoplasm	0	6	0	0
Autoimmune/chronic inflammatory	2	4	0	0
Unknown	60	138		
Total	117	222	70	30

culture, and molecular diagnostics. Granulomatous inflammation has traditionally been associated with mycobacterial infections.[9] Indeed, in highly endemic settings, granulomatous inflammation alone may be enough for a presumptive diagnosis of tuberculosis. In low prevalence regions, however, the data are more limited. One study evaluating the performance of FNAB in diagnosing tuberculosis in the United States noted a sensitivity, specificity, negative predictive value, and positive predictive value of 46%, 100%, 94%, and 100%, respectively.[12] It is important to note that the cytomorphology of tuberculosis depends on the extent and duration of the disease as well as the immune status of the patient. In immunocompromised patients, granulomas may be less well-formed or entirely absent and the inflammation may be mixed, consisting of neutrophils, lymphocytes, and/or macrophages.[10,13] Acid-fast bacilli are often difficult to identify and have been noted in only 20% to 62% of culture positive cases.[10] In fact, granulomatous inflammation is relatively infrequent in culture-positive patients and was present in only in 25% of tuberculosis cases in 1 study evaluating cases in the United States.[12] Granuloma formation is also less frequent in HIV-positive than HIV-negative patients (13% versus 28%).[12] The literature has described a wide array of fungi that have been diagnosed by FNAB, including *Talaromyces marneffei,* (formerly *Penicillium marneffei*), *Aspergillus species, Cryptococcus neoformans, Mucorales species, Candida* species, *Phialophora parasitica, Sporothrix schenckii,* and *Cladosporium* species.[14-16] In 1 case series of FNAB of fungal infections, a majority of cases revealed a mixed inflammatory pattern, consisting of lymphocytes, neutrophils, eosinophils, macrophages, and multinucleated giant cells (75%). Epithelioid granulomatous inflammation was present in only in 30% of biopsies.[14] Studies have also attempted to distinguish sarcoidosis from tuberculosis based on patterns of granulomatous inflammation but have not been able to identify any distinguishing characteristics.[17,18]

Although the etiology of the granulomatous inflammation was not identified in a majority of the total cases (199 of 339; 59%), of the 100 cases in which a pathogen was detected, mycobacteria was the most commonly identified microorganism (77%). Necrosis was seen only in a minority of fungal infections (15%), and approximately half (56%) of the mycobacterial cases demonstrated necrosis. Conversely, no pathogen was identified in about half (53%) of the cases with necrotizing granulomatous inflammation. One case of necrotizing granulomatous inflammation was attributable to granulomatosis with polyangiitis. Therefore, necrosis is not a reliable diagnostic indicator of a particular pathogen or that there is an ongoing infectious process.

Therefore, it is important to recognize granulomatous inflammation at the time of rapid assessment of an FNAB. Although culture is a critical and essential adjunct for diagnosis, our study found that only 30% of cases with granulomatous inflammation that had also been sent for microbiology cultures were positive. Overall, 29.5% of all cases had a pathogen detected by cytomorphology, special stains on cell block, or culture. Correlation with clinical history is also important and revealed a non-infectious etiology, such as sarcoidosis or granulomatosis with polyangiitis, in another 12% (40 of 339) of cases.

In conclusion, this study emphasizes the critical importance of clinical history, ancillary testing, and radiologic findings in determining the cause of granulomatous inflammation in settings that are non-endemic for tuberculosis. Although culture is the gold standard for the diagnosis of infectious disease, FNAB specimens are often the only available diagnostic material. As ancillary testing for infectious diseases continues to evolve, including molecular techniques and mass spectrometry, we may be able to better elucidate the etiology of granulomatous inflammation on minimally invasive FNAB samples.

Declarations of interest

None.

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References

1. Clement CG, Williams-Bouyer NM, Nawgiri RS, Schnadig VJ. Correlation of microbiologic culture and fine-needle aspiration cytology: a 14-year experience at a single institution. *Cancer Cytopathol*. 2015;123:612–619.
2. Allison DB, Simner PJ, Ali SZ. Identification of infectious organisms in cytopathology: a review of ancillary diagnostic techniques. *Cancer Cytopathol*. 2018;126(Suppl 8):643–653.
3. Nataraj G, Kurup S, Pandit A, Mehta P. Correlation of fine needle aspiration cytology, smear and culture in tuberculous lymphadenitis: a prospective study. *J Postgrad Med*. 2002;48:113–116.
4. Abdissa K, Tadesse M, Bezabih M, et al. Bacteriological methods as add on tests to fine-needle aspiration cytology in diagnosis of tuberculous lymphadenitis: can they reduce the diagnostic dilemma? *BMC Infect Dis*. 2014;14:720.
5. Aljafari AS, Khalil EA, Elsiddig KE, et al. Diagnosis of tuberculous lymphadenitis by FNAC, microbiological methods and PCR: a comparative study. *Cytopathology*. 2004;15:44–48.
6. Wright CA, van der Burg M, Geiger D, Noordzij JG, Burgess SM, Marais BJ. Diagnosing mycobacterial lymphadenitis in children using fine needle aspiration biopsy: cytomorphology, ZN staining and autofluorescence—making more of less. *Diagn Cytopathol*. 2008;36:245–251.
7. Rajwanshi A, Bhambhani S, Das DK. Fine-needle aspiration cytology diagnosis of tuberculosis. *Diagn Cytopathol*. 1987;3:13–16.
8. Radhika S, Gupta SK, Chakrabarti A, Rajwanshi A, Joshi K. Role of culture for mycobacteria in fine-needle aspiration diagnosis of tuberculous lymphadenitis. *Diagn Cytopathol*. 1989;5:260–262.
9. Field AS, Geddie WR. Role of fine needle aspiration biopsy cytology in the diagnosis of infections. *Diagn Cytopathol*. 2016;44:1024–1038.
10. Michelow P, Omar T, Field A, Wright C. The cytopathology of mycobacterial infection. *Diagn Cytopathol*. 2016;44:255–262.
11. Goel MM, Ranjan V, Dhole TN, et al. Polymerase chain reaction vs. conventional diagnosis in fine needle aspirates of tuberculous lymph nodes. *Acta Cytol*. 2001;45:333–340.
12. Ellison E, Lapuerta P, Martin SE. Fine needle aspiration diagnosis of mycobacterial lymphadenitis. Sensitivity and predictive value in the United States. *Acta Cytol*. 1999;43:153–157.
13. Rao JS, Kumari SJ, Kini U. Correlation of CD4 counts with the FNAC patterns of tubercular lymphadenitis in patients with HIV: a cross sectional pilot study. *Diagn Cytopathol*. 2015;43:16–20.
14. Das R, Dey P, Chakrabarti A, Ray P. Fine-needle aspiration biopsy in fungal infections. *Diagn Cytopathol*. 1997;16:31–34.
15. Chaiwun B, Khunamornpong S, Sirivanichai C, et al. Lymphadenopathy due to Penicillium marneffei infection: diagnosis by fine needle aspiration cytology. *Mod Pathol*. 2002;15:939–943.
16. Lim D, Lee YS, Chang AR. Rapid diagnosis of Penicillium marneffei infection by fine needle aspiration cytology. *J Clin Pathol*. 2006;59:443–444.
17. Kaur G, Dhamija A, Augustine J, Bakshi P, Verma K. Can cytomorphology of granulomas distinguish sarcoidosis from tuberculosis? Retrospective study of endobronchial ultrasound guided transbronchial needle aspirate of 49 granulomatous lymph nodes. *Cytojournal*. 2013;10:19.
18. Fritscher-Ravens A, Ghanbari A, Topalidis T, et al. Granulomatous mediastinal adenopathy: can endoscopic ultrasound-guided fine-needle aspiration differentiate between tuberculosis and sarcoidosis? *Endoscopy*. 2011;43:955–961.