

# Pathology of Sarcoidosis

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## ABSTRACT

The role of pathology in the diagnosis of sarcoidosis is identification of granulomas in tissue specimens and performance of studies to exclude known causes of granulomatous inflammation. The granulomas of sarcoidosis are nonspecific lesions that, by themselves and in the absence of an identifiable etiologic agent, are not diagnostic of sarcoidosis or any other specific disease.

Among the diseases to be excluded are mycobacterial, fungal, and parasitic infections, chronic beryllium disease and other pneumoconiosis, hypersensitivity pneumonitis, and Wegener's granulomatosis. Even after extensive workup a substantial number of granulomas will remain unclassified. Not every disease that features nonnecrotizing granulomas of undetermined etiology is sarcoidosis.

The granulomas of sarcoidosis may exhibit focal necrosis of minimal amount. In cases with granulomas that exhibit a greater degree of necrosis an infectious or other nonsarcoid etiology should be strongly suspected.

Strict clinical, radiological, and pathological criteria must be used for diagnosis. In cases that exhibit necrotizing granulomas with more than minimal, focal necrosis, extrathoracic involvement only, and/or incompatible clinical and radiological findings, the diagnosis of sarcoidosis should be approached with great caution. The diagnosis is most secure when compatible clinical and radiological findings are supported by the demonstration of microorganism-negative, nonnecrotizing granulomas in a biopsy specimen accompanied by biopsy evidence or strong clinical evidence of multisystem involvement, and negative cultures for bacteria, mycobacteria, and fungi. A positive Kveim-Siltzbach test provides strong support for the diagnosis of sarcoidosis.

**KEYWORDS:** Sarcoidosis, pathology, granuloma, biopsy, diagnosis

The current definition of sarcoidosis includes the following: "Sarcoidosis is a multisystem disorder of unknown cause(s)...The diagnosis is established when clinico-radiological findings are supported by histologic evidence of noncaseating epithelioid cell granulomas. Granulomas of known causes and local sarcoid reactions must be excluded."<sup>1</sup>

Because the identification and evaluation of granulomas are the province of pathology and are key to the diagnosis, the following discussion focuses on the sarcoid granuloma, its differential diagnosis, and the role of

pathology in the diagnosis of sarcoidosis. Lung involvement, biopsy procedures, biopsy sites, and pathological evaluation of biopsy specimens will also be considered in detail.

## THE SARCOID GRANULOMA

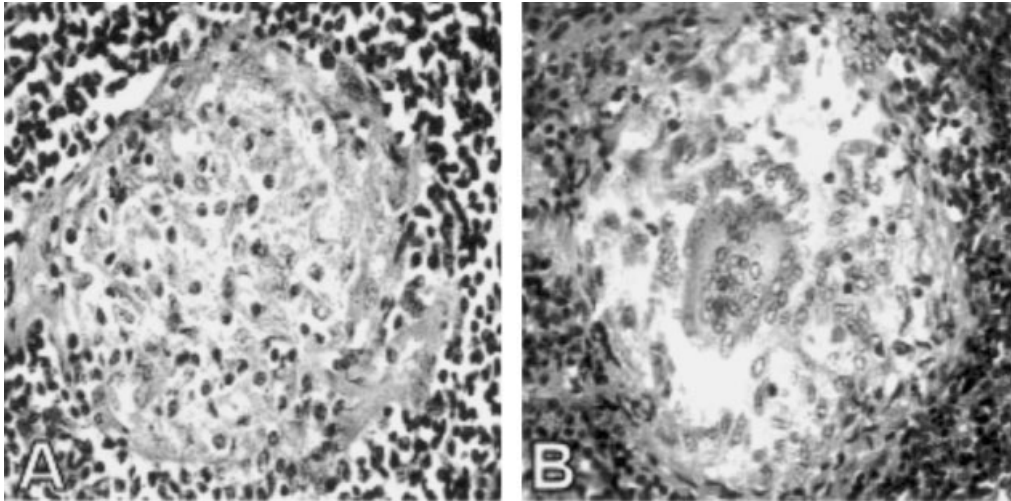
In almost all cases the demonstration of granuloma(s) is necessary to establish the diagnosis of sarcoidosis. However, it is important to recognize that the granulomas

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Sarcoidosis: Evolving Concepts and Controversies; Guest Editors, Marc A. Judson, M.D., Michael C. Iannuzzi, M.D.

Semin Respir Crit Care Med 2007;28:36-52. Copyright © 2007 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662. DOI 10.1055/s-2007-970332. ISSN 1069-3424.

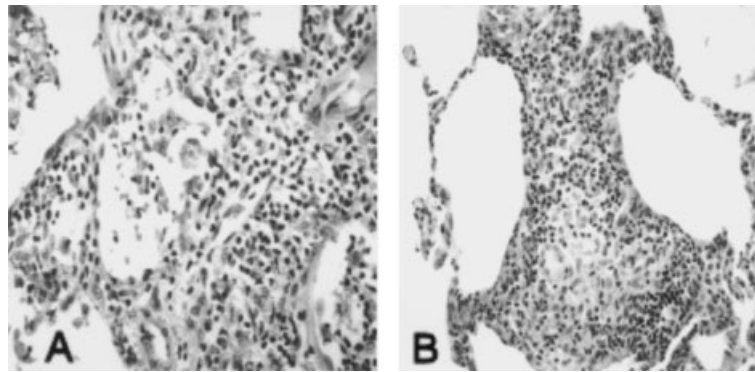


**Figure 1** (A) Nonnecrotizing granuloma. (B) Nonnecrotizing granuloma with giant cell.

in sarcoidosis (Fig. 1) are nonspecific inflammatory lesions that, in the absence of a demonstrable etiologic agent, are not diagnostic of sarcoidosis or any other granulomatous disease. Although investigations into the immunopathogenesis of sarcoidosis have increasingly elucidated the identity and complex interactions of inflammatory mediators that result in granuloma formation, the nature of the agent(s) triggering granuloma formation in sarcoidosis remains unknown. It is generally accepted that the granulomas in sarcoidosis develop as a response to the presence of a persistent and poorly degradable antigen(s) of undetermined nature that induces a local T helper cell-mediated immune response. The fact that the lungs and intrathoracic lymph nodes are involved in most, if not all, patients with intrathoracic sarcoidosis strongly suggests that the triggering agent(s) are minute respirable atmospheric particles of antigenic material that enter the body via inhalation. No living or inert agent has yet been conclusively proven to cause sarcoidosis. The possible roles of mycobacteria, propionibacteria, mycoplasma, and other microorganisms as etiologic agents have been extensively explored but there has been no definitive evidence that any of them cause sarcoidosis. The likelihood that sarcoidosis results from a defective cellular immune response to a variety of antigenic triggering agents is currently under investigation.<sup>2,3</sup> Implicit in this concept is that sarcoidosis is not caused by a single antigenic triggering agent and that its development is directly related to the idiosyncratic characteristic of the host's genetically determined exaggerated immune response.

Granuloma formation is initiated by antigen presentation by macrophages to lymphocytes. This initiates a complex series of lymphocyte-macrophage interactions resulting in the production of a large number of lymphokines and cytokines that cause the migration of macrophages, mostly bone marrow-derived, to the areas of antigen localization. They ultimately become ar-

ranged in the compact groupings that we recognize as granulomas. A granuloma may be defined as a "compact (organized) collection of mononuclear phagocytes (macrophages or epithelioid cells) which may or may not be accompanied by accessory features such as necrosis or the infiltration of inflammatory leukocytes."<sup>4</sup> In sarcoidosis, cellular infiltrates consisting predominantly of Th1 helper lymphocytes are present at sites of disease activity prior to the emergence of granulomas. This is the alveolitis of sarcoidosis that is considered to be a precursor of granuloma formation in the lungs (Fig. 2). In sarcoidosis these precursor lesions have been reported to be present in 62% of open-lung biopsy specimens.<sup>5</sup> Although seen mostly in open-lung biopsy specimens, the finding of alveolitis has also been reported in transbronchial biopsy specimens.<sup>6</sup> All stages of granuloma formation from small lymphocytic infiltrates to the emergence of fully formed granulomas are demonstrable. The alveolitis of sarcoidosis is also reflected in bronchoalveolar lavage (BAL) specimens demonstrating a marked Th1 helper cell lymphocytosis.<sup>7</sup> In the process of granuloma formation macrophages undergo maturation characterized by functional changes, including increased secretory capability and decreased phagocytic ability as well as morphological changes, resulting in their transformation into epithelioid cells. Granulomas are usually surrounded by a peripheral mantle of lymphocytes but this mantle may be absent in "naked granulomas." Small numbers of lymphocytes are also scattered throughout granulomas among the epithelioid cells. Immunohistochemical studies have demonstrated that, in sarcoidosis as well as in tuberculosis and tuberculoid leprosy, T8 suppressor lymphocytes are restricted to the outer peripheral mantle, whereas T4 helper lymphocytes are present throughout the granuloma admixed with epithelioid cells.<sup>8-11</sup> Fusion of epithelioid cells results in the formation of multinucleate giant cells that may be either of Langhans or foreign body types. The number and size



**Figure 2** (A) Alveolitis; no granuloma evident. (B) Small emerging granuloma (bottom) with surrounding alveolitis.

of giant cells vary, and they may be absent entirely. The factors influencing giant cell formation are not well understood. The sarcoid granuloma is typically non-necrotizing, (Fig. 1) but small to moderate amounts of central granular necrosis may be present (Fig. 3A–C). This type of necrosis has been reported in up to one third of granuloma-containing biopsy specimens from patients with sarcoidosis.<sup>12,13</sup> Apoptotic nuclei are often seen within and adjacent to these small foci of necrosis<sup>14,15</sup> (Fig. 3C,D). Suppurative necrosis (Fig. 3E) and large, confluent foci of necrosis (Fig. 3F) may occur but are extremely rare. Although granulomas are often classified as being either caseating or noncaseating, in the author's opinion it is preferable to refer to them as necrotizing or nonnecrotizing. Caseation refers to the nonspecific, cheeselike *gross* appearance that may be seen in mycobacterial and fungal infections, necrotic neoplasms, syphilis, typhoid, tularemia, lipid aspiration, and others. The *gross* appearance of caseation is due to incomplete proteolytic enzyme digestion and liquefaction of necrotic cells. Caseation does not accurately describe a microscopic appearance. The granulomas may exhibit a uniform appearance suggesting origin at the same point in time. However, in many cases emerging and young granulomas may coexist with older granulomas exhibiting partial or complete fibrosis.

## INCLUSIONS

A variety of inclusions may be present, including Schaumann's bodies, asteroid bodies, birefringent crystals, and Hamazaki-Wesenberg bodies (Fig. 4). These inclusions are nonspecific and are not diagnostic of sarcoidosis.

### Schaumann's Bodies (Conchoidal Bodies) and Birefringent Crystals

These are large, concentrically lamellated, calcified structures that are usually present within the cytoplasm of giant cells (Fig. 4A), mostly in sarcoidosis and, to a lesser extent, in chronic beryllium disease, tuberculosis, hypersensitivity pneumonitis, and other granulomatous

conditions. They are very common in sarcoidosis, having been reported in up to 88% of cases.<sup>16</sup> Rupture of the cell membranes of Schaumann's body-containing giant cells may result in their extrusion into the extracellular space. The majority of Schaumann's bodies have birefringent crystals, mostly composed of calcium oxalate, associated with them (Fig. 4B,C). It has been suggested that these crystals may serve as a nidus for their formation.<sup>17</sup> Birefringent crystals without associated Schaumann's bodies (Fig. 4B) have been reported in 41% of cases of sarcoidosis and to a lesser extent in other granulomatous conditions.<sup>16</sup>

### Asteroid Bodies

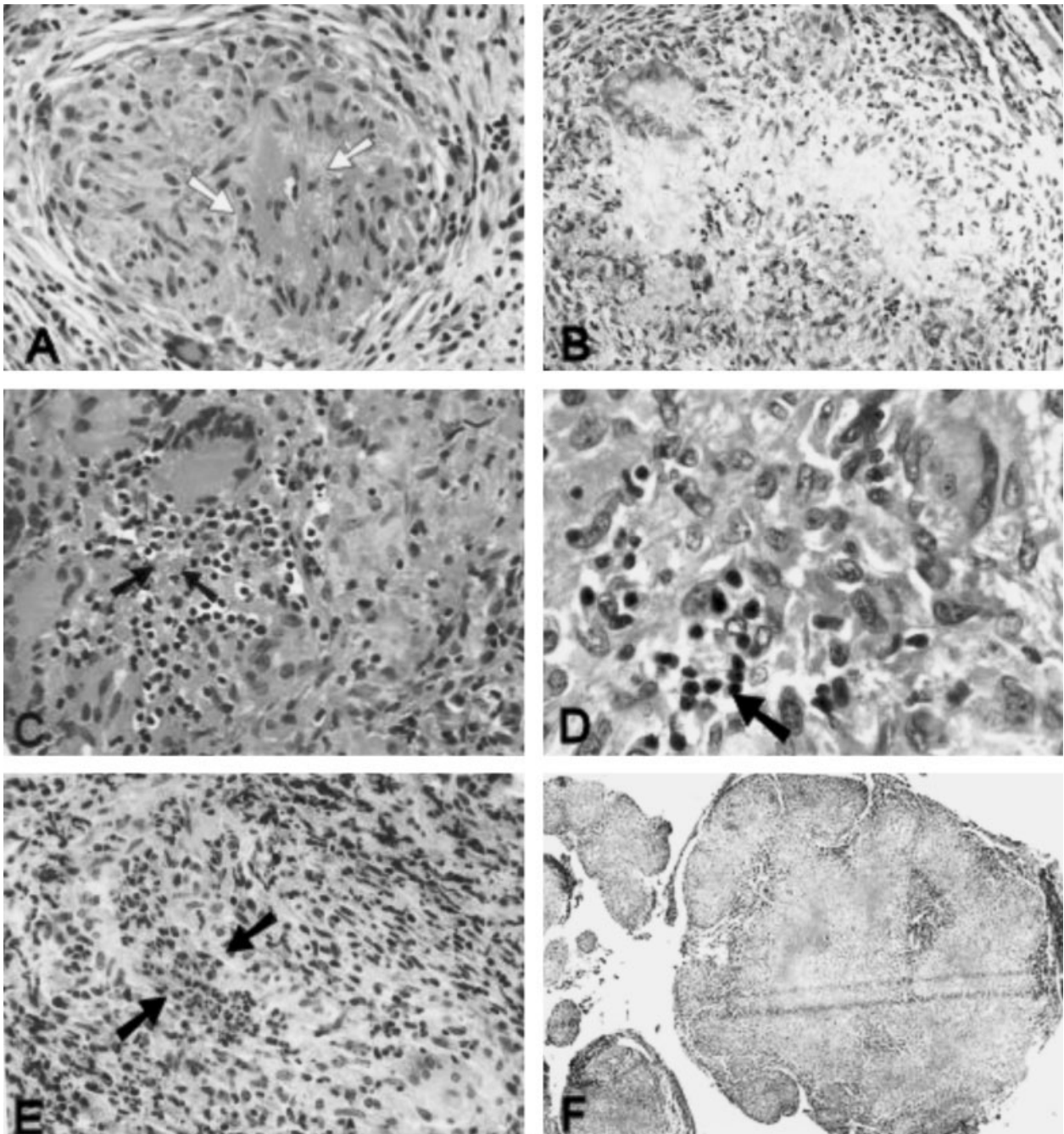
Asteroid bodies are intracytoplasmic stellate inclusions within giant cells exhibiting 30 or more rays radiating from a central core (Fig. 4D). They probably represent functionally obsolescent cell organelles. Asteroid bodies have been reported in from 2 to 9% of tissues from patients with sarcoidosis.<sup>12,13</sup> They may also be encountered in foreign body granulomas and rarely in other granulomatous conditions.

### Hamazaki-Wesenberg Bodies

Hamazaki-Wesenberg bodies, also known as yellow-brown bodies, yellow bodies, spindle bodies, and chromogenic bodies, are giant extracellular and intracellular lysosomes. They may be seen with light microscopy in granulomatous and nongranulomatous lymph nodes from patients with sarcoidosis and a variety of other disorders<sup>13,18–21</sup> (Fig. 4E,F). They are oval or spindle-shaped, range in size from 0.5  $\mu\text{m}$  to 0.8  $\mu\text{m}$ , and often exhibit a yellow-brown color in slides stained with hematoxylin and eosin. Because they may exhibit an appearance that is similar to yeastlike budding they may be easily mistaken for fungal organisms<sup>21</sup> (Fig. 4F).

In the past various morphological features of granulomas have at one time or another been considered to be diagnostic of sarcoidosis. These include uniformity of appearance, absence of a peripheral rim





**Figure 3** (A) Granuloma with minute focus of granular necrosis (between arrows). (B) Granuloma with moderate amount of necrosis (unusual finding). (C) Granuloma with numerous apoptotic cells and a minute focus of granular necrosis (between arrows). (D) Apoptotic cells within granuloma (at arrow). (E) Granuloma with suppurative necrosis (between arrows, very rare finding). (F) Lymph node replaced by granulomas; massive necrosis (very rare finding).

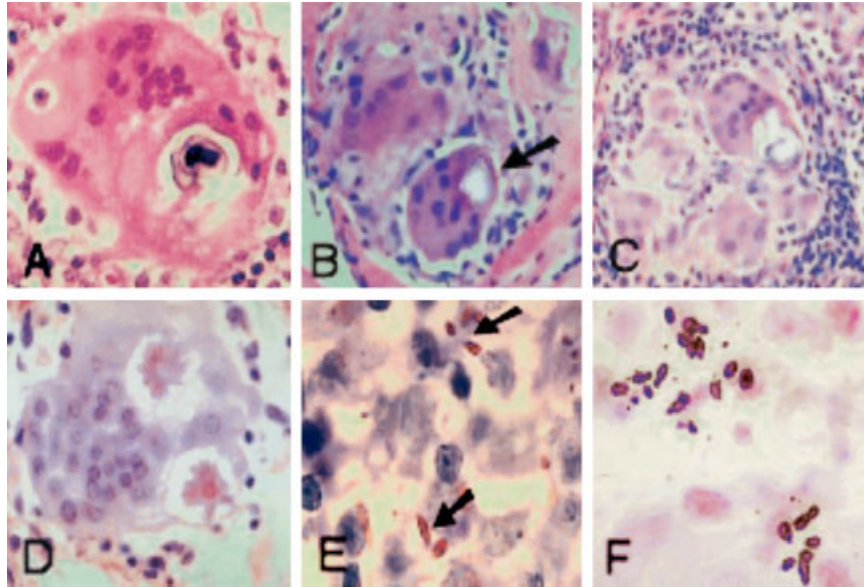
of lymphocytes (naked granulomas), an intact fine reticulum network, and presence of inclusions such as Schaumann's bodies and asteroid bodies. However, there is no morphological feature of the granulomas that is specific for or diagnostic of sarcoidosis.

Over time granulomas may resolve or they may undergo healing by fibrosis. Fibrosis usually begins at the periphery and may extend centrally until the entire granuloma is replaced by fibrous tissue (Fig. 5). Nodular

fibrous lesions representing healed granulomas (Fig. 5) may be encountered in lymph nodes. Calcification may accompany extensive fibrosis (Fig. 5).

### LUNG INVOLVEMENT IN SARCOIDOSIS

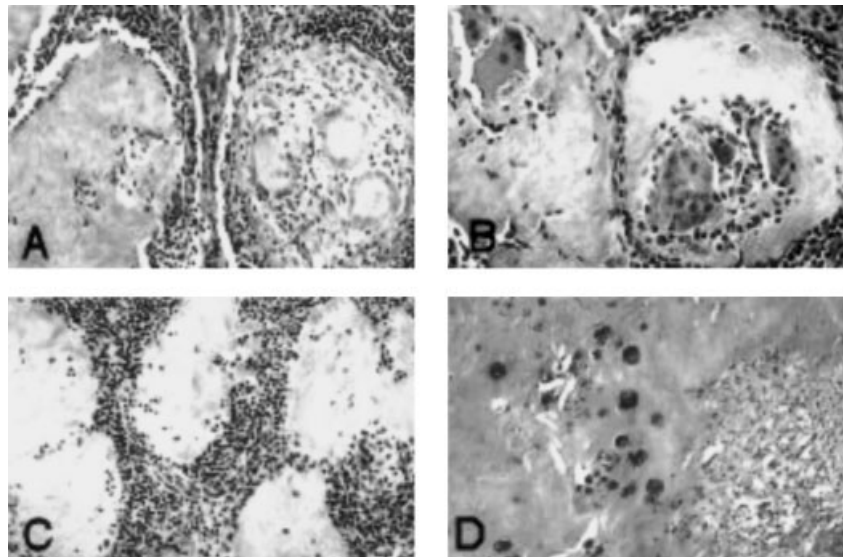
Open-lung biopsy specimens obtained from patients with hilar lymphadenopathy (stage 1 disease) are reported to demonstrate the presence of granulomas in



**Figure 4** (A) Schaumann's body within giant cell. (B) Calcium oxalate crystal within giant cell; polarized light (at arrow). (C) Early Schaumann's body forming around calcium oxalate crystals within giant cell; polarized light. (D) Asteroid bodies within giant cell. (E) Hamazaki-Wesenberg bodies in lymph node; oil immersion (at arrows). (F) Hamazaki-Wesenberg bodies in lymph node, methenamine silver stain; oil immersion. Note resemblance of these structures to budding yeasts. (Panels C and F reprinted from Rosen Y. Sarcoidosis. In: Dail DH, Hammar SP, eds. Pulmonary Pathology. New York: Springer-Verlag; 1994:620. With kind permission of Springer Science and Business Media.)

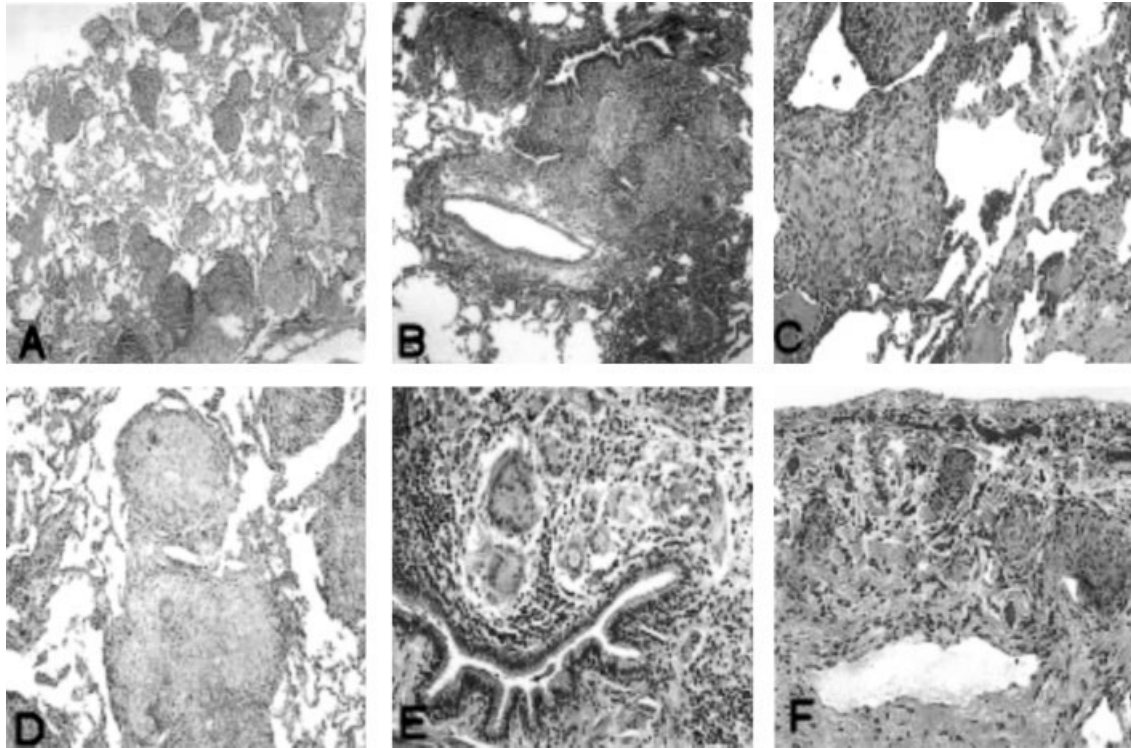
100% of specimens.<sup>22</sup> This strongly suggests that lung involvement is present in all patients with intrathoracic sarcoidosis. Nongranulomatous interstitial pneumonitis/alveolitis is the precursor of lung parenchymal granulomas. Granulomas tend to be most prevalent around bronchovascular bundles and the fibrous septae containing pulmonary veins (Fig. 6). These septae also contain the pulmonary lymphatics through which the

granuloma-inciting agent(s) are presumed to be transported from the lung periphery to the hilar and mediastinal lymph nodes. This "lymphangitic distribution" is very characteristic of sarcoidosis. Granulomas may also be present throughout the lung parenchyma either as single discrete lesions or as confluent masses (Fig. 6). Confluence of granulomas may result in large, single or multiple, radiographically



**Figure 5** (A) Granuloma with early peripheral fibrosis (right) and completely fibrotic granuloma (left). (B) Granuloma with extensive peripheral fibrosis (right) and subtotally fibrosed granuloma (left). (C) Lymph node containing only completely fibrosed granulomas. (D) Massive confluent fibrosis with focal calcification. Residual granulomas present (right). (Panels B and C reprinted from Rosen Y. Sarcoidosis. In: Dail DH, Hammar SP, eds. Pulmonary Pathology. New York: Springer-Verlag; 1994:617. With kind permission of Springer Science and Business Media.)





**Figure 6** Lung involvement in sarcoidosis. (A) Numerous discrete parenchymal granulomas. (B) Localization of granulomas around bronchovascular bundle. (C) Interstitial granulomas. (D) Confluent granulomas forming a small nodule. (E) Bronchiolar granulomas. (F) Visceral pleural granulomas. (Panel E reprinted from Rosen Y. Sarcoidosis. In: Dail DH, Hammar SP, eds. Pulmonary Pathology. New York: Springer-Verlag; 1994:624. With kind permission of Springer Science and Business Media.)

demonstrable nodules. Nodular sarcoidosis (NS) has been reported to be a presenting feature in ~5% of patients<sup>23</sup>; its radiographic appearance may suggest metastatic or primary neoplasm.

Clinically significant extrapulmonary sarcoidosis involving the heart, central nervous system, liver, and other sites occurs in 4–7% of patients at presentation with increasing incidence as the disease evolves.<sup>1</sup>

### Airways Involvement

Granulomatous involvement of large and small airways is frequent<sup>24</sup> (Fig. 6). It has been detected by endobronchial biopsy (EBB) in 40 to 71% of patients.<sup>25–27</sup> The bronchoscopic appearance of the bronchial mucosa is reported to be abnormal (erythema, mucosal nodules, plaques, and cobblestoning<sup>27</sup>) in up to 55% of patients. Biopsy of abnormal-appearing bronchial mucosa is twice as likely to yield granulomas as is biopsy of normal-appearing mucosa.<sup>28</sup> The yield of granulomas with EBB is reported to be significantly greater in African Americans than in white Americans.<sup>27</sup> Evidence of airways obstruction obtained from pulmonary function studies is frequent in all stages of sarcoidosis<sup>29</sup> and may be present in up to 75% of patients exhibiting radiographic evidence of pulmonary fibrosis.<sup>30</sup> Bronchostenosis is an unusual complication of granulomatous airways involvement, usually presenting as multifocal lesions in patients with

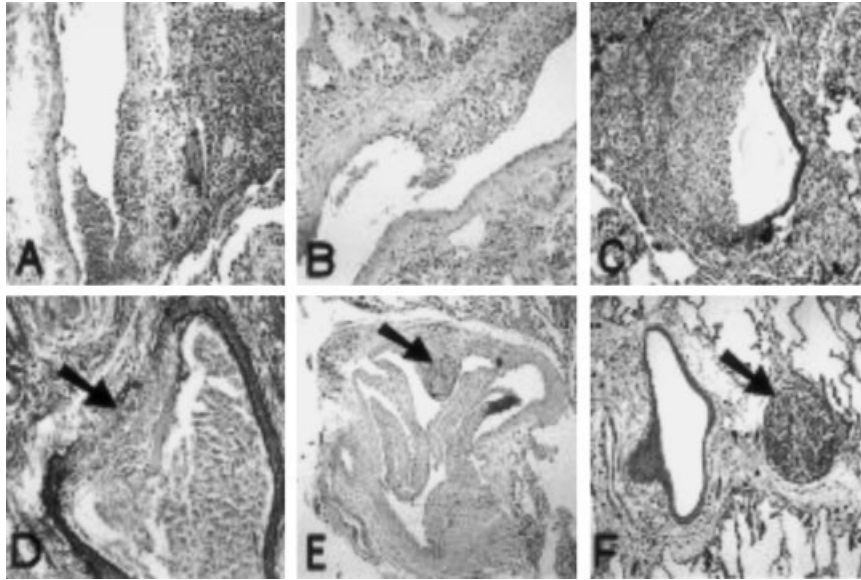
radiographic evidence of pulmonary fibrosis.<sup>31,32</sup> Endobronchial mass lesions presenting as a manifestation of airways involvement are rare.<sup>33</sup> Bronchiectasis, either saccular or cylindrical, results from bronchial wall injury by granulomas, superimposed bronchial infection, and radial traction by peribronchial scar tissue in individuals with advanced progressive pulmonary fibrosis.

### Pleural Involvement

Although granulomatous involvement of the visceral pleura may be seen in up to 35% of open-lung biopsy specimens<sup>34</sup> (Fig. 6) radiographic evidence of pleural effusion or thickening has been reported in only 10% of patients.<sup>35,36</sup> There are rare case reports of presentation with chylothorax<sup>37</sup> and a discrete pleural mass.<sup>38</sup>

### Vascular Involvement, Granulomatous Pulmonary Angiitis

Granulomatous angiitis/vasculitis is a very frequent finding in pulmonary sarcoidosis<sup>39–41</sup> (Fig. 7) and may also occur in extrapulmonary sites. In a study of 128 open-lung biopsy specimens from patients with sarcoidosis<sup>40</sup> granulomatous angiitis was found in 69% of the specimens. Venous involvement (92%) was more prevalent than arterial involvement (39%). Sixty-one percent of the biopsy specimens showed both venous and arterial



**Figure 7** Granulomatous pulmonary angiitis. (A) Transmural involvement of pulmonary vein. (B) Intimal granuloma in pulmonary vein. (C) Transmural involvement with elastic tissue destruction, pulmonary vein; elastic tissue stain. (D) Transmural with elastic tissue destruction (at arrow), pulmonary artery; elastic tissue stain. (E) Intimal granuloma (at arrow) in pulmonary artery. (F) Granuloma within septal lymphatic vessel (at arrow).

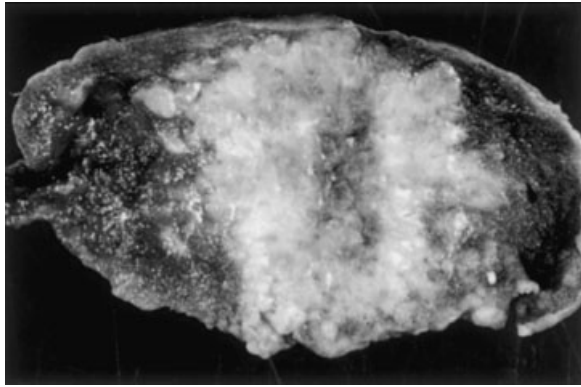
involvement. Arterial involvement alone was seen in only 9% of the specimens. The granulomas are located mostly in the media and adventitia but intimal granulomas also occur. The presence and extent of granulomatous angiitis varies directly with the number of extravascular granulomas. These vascular lesions can usually be identified with routine H&E staining. However, in some cases, especially when the involved vessel is located in the midst of confluent granulomas, the lesions only become evident with elastic tissue stains. Elastic stains show focal destruction of elastic tissue. A subsequent autopsy study of 40 patients with sarcoidosis<sup>41</sup> documented granulomatous angiitis in 100% of the cases. Granulomatous angiitis, both active and healed, was identified in elastic and muscular pulmonary arteries, arterioles, venules, interlobar veins, bronchial arteries, and lymphatic vessels. Venous involvement was more prevalent than arterial involvement and lymphatics were involved in 70% of the cases. In the author's experience it is unusual to encounter granulomatous angiitis in transbronchial biopsy specimens. However, there are reports of granulomatous angiitis seen in from 12.2%<sup>42</sup> to 53%<sup>43</sup> of transbronchial biopsies from cases of sarcoidosis. Granulomatous angiitis of sarcoidosis may produce marked vascular narrowing and stenosis; thrombosis, infarction, and aneurysm formation have not been reported.

Sarcoidosis is an uncommon cause of pulmonary hypertension, with an overall incidence of up to 5% and a higher incidence in advanced fibrotic disease.<sup>44</sup> Causes of pulmonary hypertension in sarcoidosis include destruction of the distal capillary bed accompanying advanced fibrosis leading to hypoxemia, external compression of large pulmonary veins or arteries by

enlarged mediastinal or hilar lymph nodes, pulmonary vasoconstriction by vasoactive factors, portal hypertension complicating hepatic sarcoidosis, and granulomatous angiitis sometimes simulating pulmonary veno-occlusive disease (PVOD).<sup>44</sup> A small number of case reports strongly suggest that in some sarcoidosis patients marked narrowing of pulmonary veins secondary to granulomatous angiitis may be the cause pulmonary hypertension.<sup>45-48</sup> In a study of pulmonary hypertension in patients with sarcoidosis, explanted lungs from four of five patients with stage IV disease who underwent lung transplantation had granulomatous pulmonary phlebitis; all five lung specimens exhibited an occlusive venopathy consisting of obliterative intimal fibrosis and recanalization resembling the findings in PVOD.<sup>44</sup> In the same study there was a group of seven patients with pulmonary hypertension and nonfibrotic pulmonary sarcoidosis. Because no other cause of pulmonary hypertension was found in this group the possibility of a specific sarcoidosis vasculopathy was suggested. Unfortunately there was no tissue examination to support this hypothesis.

Granulomatous pulmonary angiitis is a nonspecific lesion whose presence is not diagnostic of sarcoidosis. It may be seen in a variety of conditions, including tuberculosis, Wegener's granulomatosis, necrotizing sarcoid granulomatosis, chronic beryllium disease, foreign body embolization in drug abusers, following cardiac catheterization, and schistosomiasis.

A "microangiopathy" involving arterioles, venules, and capillaries, characterized by endothelial cell abnormalities and basal lamina layering, has been reported to be a frequent finding in tissues from various body sites involved by sarcoidosis.<sup>49</sup> It has



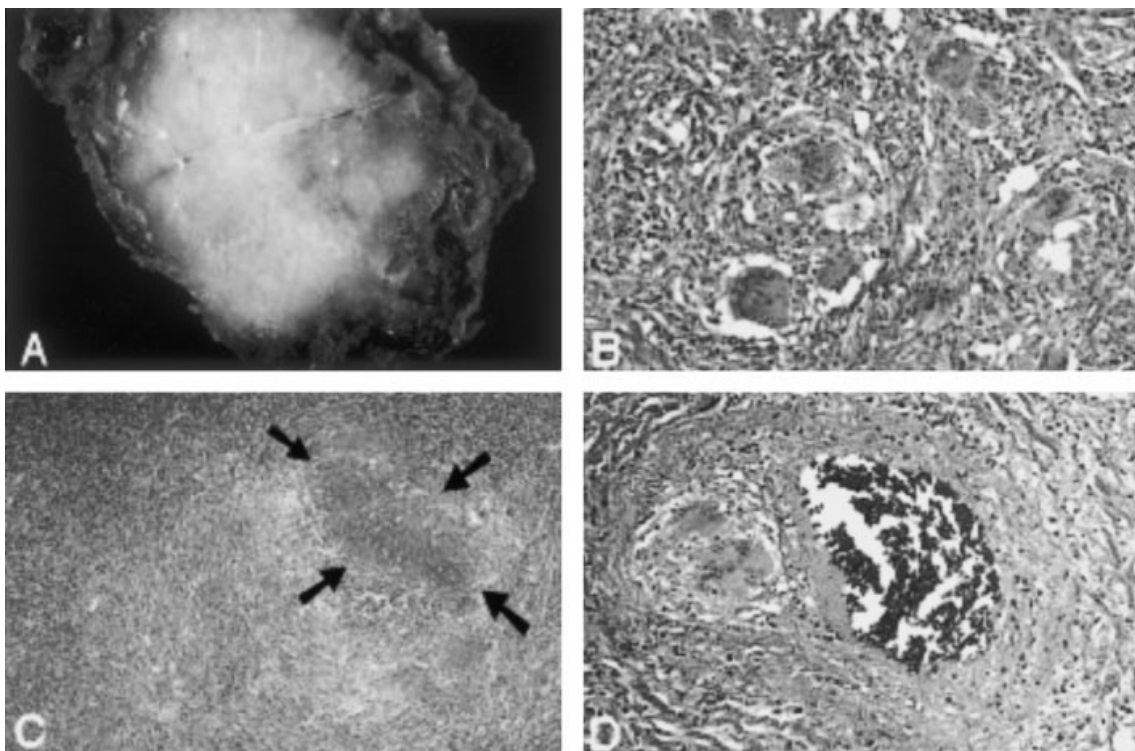
**Figure 8** Nodular sarcoidosis.

been suggested that this “microangiopathy” is responsible for some of the manifestations of sarcoidosis in the eye, kidney, skeletal muscle, cardiac muscle, and other locations and that it has a significant role in progression of sarcoidosis.

A variety of systemic vasculitides involving small, medium, and large blood vessels mimicking hypersensitivity vasculitis, polyarteritis nodosa, microscopic polyangiitis, and Takayasu’s disease in patients with sarcoidosis have been the subject of several reports encompassing a small number of patients.<sup>50</sup> More confirmational data are needed because neither the diagnosis of sarcoidosis nor vasculitis is well documented in some of the cases.

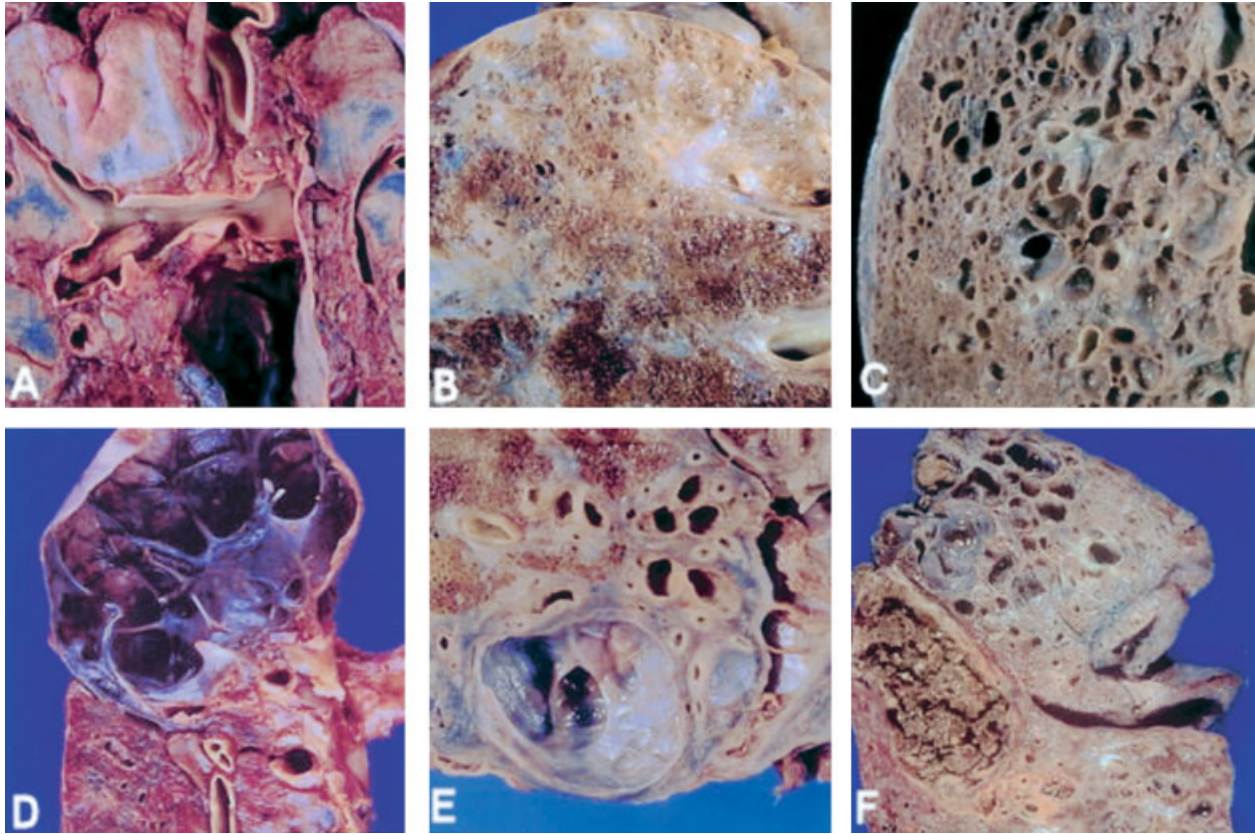
### Nodular Sarcoidosis and Necrotizing Sarcoid Granulomatosis

Sarcoidosis presenting with one or more nodular lung lesions, sometimes simulating the appearance of metastatic or primary lung neoplasm, has been reported to be a presenting radiographic finding in 1.5 to 4% of patients<sup>51–55</sup> (Fig. 8). Although NS is well recognized in the radiological literature, its pathological features have not been well characterized. Necrotizing sarcoid granulomatosis (NSG) was the name given by Liebow in 1973<sup>56</sup> to an unusual condition of the lungs characterized by nodular foci of granulomatous inflammation, granulomatous angiitis, and foci of necrosis (Fig. 9). His question as to whether NSG represented a distinctive type of vasculitis with a sarcoid reaction or a variant of sarcoidosis still remains unanswered. In the years following Liebow’s original publication reports of individual cases and a small number of series of patients with NSG<sup>57–59</sup> failed to definitively resolve the nature of this condition. Although lung involvement predominates, a small number of cases of extrapulmonary disease have been reported. The morphological resemblance of the NSG lesions to sarcoidosis has led some authors to suggest that NSG is a variant of sarcoidosis.<sup>60</sup> Confluent granulomas forming nodules, granulomatous angiitis, and necrosis have been observed in six of 128 (4.7%) open-lung biopsy specimens from patients with well-documented sarcoidosis.<sup>34</sup> This approximates the 1.5 to 4% incidence of NS reported in the radiological



**Figure 9** Necrotizing sarcoid granulomatosis versus nodular sarcoidosis. (A) Large lung nodule. (B) Nonnecrotizing granulomas. (C) Confluent granulomas with large area of necrosis (between arrows). (D) Granulomatous angiitis, pulmonary arteriole.





**Figure 10** (A) Bilateral hilar lymphadenopathy. (B) Subpleural pulmonary fibrosis. (C) Diffuse pulmonary fibrosis with honeycombing. (D) Bullous emphysema involving upper lobe of lung. (E) Saccular bronchiectasis forming a "cystic" lesion and several cylindrically ectatic bronchi above the "cyst." (F) Aspergilloma, occupying an ectatic bronchus, in a lung with advanced sarcoidosis.

literature. Based upon the close morphological resemblance of NSG to NS and the apparent similar incidence of the two, the conclusion that NSG is identical to or a variant of NS appears reasonable. However, there also appear to be differences, including, in NSG, the low incidence of hilar lymphadenopathy and other clinical features of sarcoidosis, and apparent rarity of extrapulmonary involvement. In addition there are reports of failure to demonstrate serum angiotensin-converting enzyme (SACE) elevation<sup>58,61,62</sup> or presence of angiotensin-converting enzyme (ACE) or one of its reaction products in granulomas, and negative Kveim tests<sup>63</sup> in the very small number of patients in whom these tests were performed.

### Progression and Advanced Lung Involvement

In the majority of individuals with pulmonary sarcoidosis, the granulomas either resolve or heal by fibrosis of individual granulomas or small groups of confluent granulomas. In 10 to 30% of cases the lungs undergo progressive fibrosis, which, in some cases, results in end-stage "honeycomb lung" (Fig. 10). Fibrosis is mediated by macrophage cytokines such as fibronectin and alveolar macrophage-derived growth factor.<sup>64,65</sup> Honeycomb lung is a nonspecific end stage of a variety

of chronic interstitial lung diseases, including sarcoidosis. It is characterized by parenchymal fibrosis, bronchiolectasis, and enlarged, dilated air spaces (Fig. 10). The remodeled honeycomb lung exhibits marked impairment of both its diffusion and its ventilatory functions. In sarcoidosis honeycombing tends to be most pronounced in the upper regions and beneath the pleura. Once the stage of honeycombing is reached there may be very few or no granulomas present. Honeycombing may be accompanied by pulmonary hypertensive arteriopathy.

Emphysema, sometimes bullous, may develop in individuals with advanced pulmonary sarcoidosis (Fig. 10). Possible pathogenetic mechanisms include airspace dilatation and rupture secondary to granulomatous bronchostenosis, airspace dilatation and rupture at the periphery of fibrotic and collapsed lung tissue, and destruction of alveolar walls by alveolitis.<sup>66</sup> Emphysema encountered in elderly smokers with sarcoidosis is unlikely to be caused by sarcoidosis.

Cavitation rarely, if ever, occurs in sarcoidosis. The radiographic cystic changes seen in very advanced pulmonary sarcoidosis, sometimes interpreted as cavitation, usually reflect the presence of either or both saccular bronchiectasis (Fig. 10) and bullous emphysema (Fig. 10).

**Table 1 Autopsy Studies of Sarcoidosis**

Author	Country	Data Collection Dates	# Patients	Death due to Sarcoidosis (%)	Death due to Other Cause (%)	Antemortem Diagnosis of Sarcoidosis (%)
Huang <sup>73</sup>	US	1960–76	23	61	39	NS
Iwai <sup>74</sup>	Japan	1974–85	143	57	43	37
Perry <sup>75</sup>	US	1958–92	38	67	33	45

NS, not stated.

**Table 2 Causes of Death in Fatal Cases of Sarcoidosis: Autopsy Data**

Author	Country	Cardiac Sarcoidosis	Pulmonary Sarcoidosis	Other Organ Involvement
Huang <sup>73</sup>	US	14%	64%	22%
Iwai <sup>74</sup>	Japan	85%	14%	1%
Perry <sup>75</sup>	US	50%	43%	7%

Aspergilloma, a fungus ball or mycetoma due to saprophytic colonization of foci of saccular bronchiectasis by *Aspergillus* spp., may occur in advanced pulmonary sarcoidosis as a unilateral or bilateral lesion (Fig. 10). Hemoptysis is a major symptom in the majority and is sometimes fatal.<sup>67</sup> Patients with aspergillomas complicating sarcoidosis tend to have advanced and diffuse lung disease and may not be suitable candidates for surgery. The prognosis for sarcoidosis patients with aspergilloma(s) appears to be poor, with mortality of 58% reported over a two to 11 year follow-up period.<sup>67</sup> Although mycetomas are usually the result of colonization by *Aspergillus* spp. they may occasionally be produced by other fungi, including *Candida*, *Pseudoallescheria*, *Scedosporium*, *Coccidioides*, and *Monosporium* as well as *Nocardia*, a bacterial microorganism.

### Amyloidosis

There are a small number of individual case reports of amyloidosis associated with sarcoidosis.<sup>68,69</sup> Most of these are of the AA type. It is uncertain whether the occurrence of amyloidosis is directly related to sarcoidosis or coincidental.<sup>70</sup>

### Lung Cancer

There is currently insufficient evidence to support the existence of an increased risk of developing lung cancer in individuals with sarcoid-related pulmonary fibrosis.<sup>71</sup>

### Mortality and Autopsy Findings

Mortality in sarcoidosis varies between 1 and 5%.<sup>1</sup> A meta-analysis of published data showed a mortality rate of 4.8% for patients in a referral setting compared with 0.5% in a population-based setting<sup>72</sup> (i.e., the population of patients likely to be encountered by community

physicians in Western countries). Death caused by sarcoidosis in Western countries is most often the result of advanced pulmonary disease with cardiopulmonary failure, whereas deaths from cardiac sarcoidosis in Japan far outnumber those from pulmonary sarcoidosis.<sup>73</sup> Data from three autopsy studies of patients with sarcoidosis<sup>73–75</sup> (Tables 1–3) in the United States and Japan show a high percentage of cases with cardiac involvement as the cause of death (Tables 1 and 2). In approximately one third of the cases death was due to a cause other than sarcoidosis (Table 1). Antemortem diagnosis of pulmonary sarcoidosis is significantly more accurate than it is for extrapulmonary sarcoidosis (Table 3). The fact that sudden death is often the first clinical manifestation of cardiac sarcoidosis accounts for much of the difficulty in making the diagnosis antemortem.

### THE DIAGNOSIS OF SARCOIDOSIS

Demonstration of the presence of nonnecrotizing granulomas in a biopsy specimen is usually required to establish the diagnosis of sarcoidosis. Although clinical criteria are generally unreliable, Winterbauer and Moore emphasized their usefulness for the diagnosis of stage 1 sarcoidosis.<sup>76</sup> In their study of 100 patients with bilateral hilar lymphadenopathy, all 30 patients who were asymptomatic and 50 of 52 patients with completely negative physical examinations had sarcoidosis. They

**Table 3 Percent Agreement of Antemortem and Postmortem Diagnoses in Cases of Fatal Sarcoidosis: Autopsy Data**

Author	Cardiac Sarcoidosis (%)	Pulmonary Sarcoidosis (%)	Other Organ Involvement (%)
Huang <sup>73</sup>	0	100	33
Perry <sup>75</sup>	29	75	NS

NS, not stated.

recommended that "bilateral hilar lymphadenopathy in asymptomatic patients with negative physical examinations or in association with erythema nodosum or uveitis should be considered a priori evidence of sarcoidosis and biopsy confirmation of the diagnosis is not necessary." Biopsy confirmation may not be mandatory in patients presenting with Löfgren's syndrome consisting of erythema nodosum, bilateral hilar lymphadenopathy, fever, and arthralgia.

Although the main purpose of obtaining a biopsy specimen is to demonstrate the presence of granulomas and to establish the presence or absence of microorganisms using acid-fast and fungal stains, the biopsy may serve other useful purposes, including provision of lesional tissue for (1) culture for microorganisms, and (2) additional diagnostic and research procedures, including immunohistochemical staining, electron microscopy, enzyme determinations, and chemical analysis.

Biopsy specimens should be cultured for mycobacteria, fungi, and aerobic and anaerobic bacteria if possible. However, there is evidence that in cases of mycobacterial and fungal infections, significantly more microorganisms are identified in tissues by acid-fast bacilli (AFB) and fungal stains than by culture.<sup>77</sup> Culture of small biopsy specimens is usually not possible but should be done when larger specimens are obtained via thoracoscopy or open-lung procedures.

In a study of 736 patients with sarcoidosis, 95% had intrathoracic disease.<sup>78</sup> Lung and mediastinal lymph nodes were the most common intrathoracic, and skin and peripheral lymph nodes the most common extrathoracic, biopsy sites. Due to the fact that hepatic granulomas may be present in a variety of nonsarcoid conditions, liver biopsy by itself is not reliable for diagnosis and should be supplemented by biopsy of another site, if possible.

### Transbronchial Lung Biopsy

Because sarcoidosis involves the lungs in almost all patients, transbronchial lung biopsy (TBLB) with the fiberoptic bronchoscope is the initial biopsy procedure of choice unless there are obvious lesions in more easily accessible sites such as the skin or conjunctiva. Studies that appeared in the 1970s and 1980s established the usefulness of TBLB in sarcoidosis demonstrating an overall sensitivity ranging from ~50 to over 90%.<sup>79-83</sup> The yield of granulomas is highest in those patients who have radiographic evidence of lung involvement and is directly proportional to the number of biopsy specimens obtained until a plateau is reached. Four to six specimens appear to be adequate for stage 2 disease and as many as 10 may be needed in stage 1 disease.<sup>84</sup> Other factors that correlate with the yield of granulomas include biopsies taken from more than one lobe in all patients and biopsy samples obtained from the most

involved areas in patients with stages 2 and 3 disease.<sup>85</sup> Biopsy fragments that do not float in water contain little or no alveolated tissue and are, therefore, less likely to contain granulomas than specimens that float and are composed mostly of alveolated tissue.<sup>86</sup>

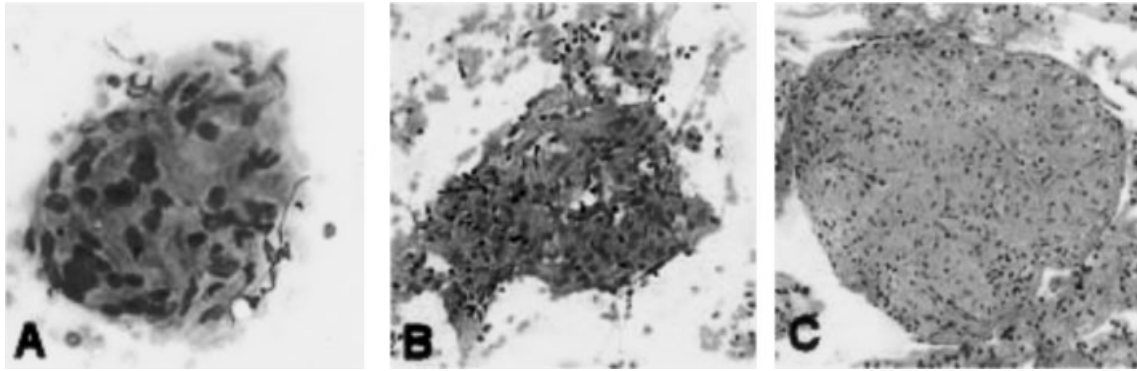
### Endobronchial Biopsy

Endobronchial involvement is common in sarcoidosis, and endobronchial biopsy (EBB) can significantly increase the yield of granulomas and provides an alternative to mediastinoscopy. In a prospective study of 34 patients with sarcoidosis EBB (six specimens) detected granulomas in 61.8% of patients and TBLB (six specimens) detected granulomas in 58.8%.<sup>87</sup> Seventy-five percent of biopsy specimens obtained from abnormal-appearing mucosa contained granulomas compared with 30% of specimens obtained from normal-appearing mucosa. Fifty percent of the patients with a negative TBLB had a positive EBB. The addition of EBB increased the granuloma yield of TBLB by 20%.

### Transbronchial Needle Aspiration

Transbronchial needle aspiration (TBNA) of mediastinal and/or hilar lymph nodes concurrent with or subsequent to TBLB in selected cases of suspected sarcoidosis may significantly increase the diagnostic yield, particularly in individuals with stage 1 and stage 2 disease.<sup>88,89</sup> Fine needle aspiration (FNA) performed with a 21 or 22 gauge needle<sup>89-91</sup> or core biopsy performed with an 18 or 19 gauge needle<sup>92-94</sup> both provide specimens that are suitable for identification of granulomas. Cytology specimens obtained by FNA are evaluated for the presence of granulomas with no or minimal necrosis, epithelioid histiocytes occurring in clusters, and multinucleate giant cells<sup>92</sup> (Fig. 11). TBNA utilizing a 22 gauge cytology needle was reported to yield specimens with features consistent with sarcoidosis in 16 of 21 (76%) patients with sarcoidosis.<sup>92</sup> In a study of 51 consecutive patients suspected of having sarcoidosis the combined use of TBLB and TBNA with a cytology needle increased the diagnostic yield in stage 1 patients from 60% with TBLB alone to 83%.<sup>89</sup> The yield in stage 2 patients increased from 76% with TBLB alone to 86%. In another study the combination of TBLB and TBNA resulted in a diagnostic yield of 93.7% for stages 1 and 2 combined compared with 62.5% for TBLB alone and 65.6% for TBNA alone.<sup>90</sup> The reported diagnostic yield for biopsy specimens obtained with an 18 or 19 gauge histology needle ranges from 55 to 87.5%.<sup>94,95</sup> Transesophageal endoscopic ultrasound-guided FNA of mediastinal lymph nodes has been reported to yield 82% positive biopsies<sup>95</sup> in cases of sarcoidosis. Needle biopsies of mediastinal and/or hilar lymph nodes obtained by either the transbronchial or



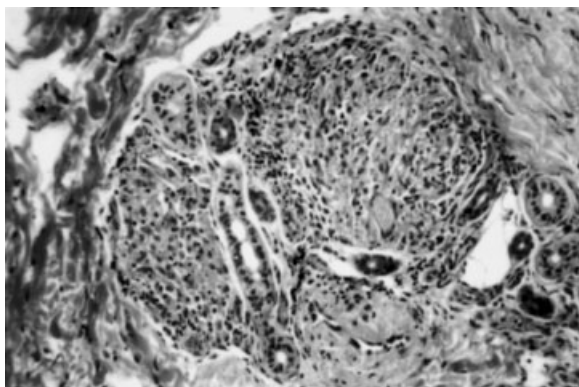


**Figure 11** Granulomas obtained by transbronchial needle aspiration. (A) Dif-Quick stain of smear. (B) Papanicolaou stain of smear. (C) Cell block; hematoxylin & eosin. (Courtesy of Dr. Mala Gupta.)

transesophageal routes promise to significantly reduce the need for mediastinoscopy for the diagnosis of sarcoidosis.

### The Kveim-Siltzbach Test (KST)

The Kveim-Siltzbach test (KST) involves the intradermal injection of a suspension of granuloma-containing spleen or lymph node obtained from a patient with sarcoidosis.<sup>96-99</sup> Each batch of test material must be validated by administration to patients with sarcoidosis and nonsarcoidosis controls to ensure a sensitivity of at least 60% and no more than 2 to 3% false-positive reactions.<sup>99</sup> The test is positive when a papule develops at the injection site and a biopsy of the papule performed 4 to 6 weeks following injection demonstrates non-necrotizing granulomas that are not caused by injected foreign bodies (Fig. 12). A positive test using properly validated test material has a specificity of 97 to 98% for the diagnosis of sarcoidosis.<sup>99</sup> The nature of the antigen in the test material that induces granuloma formation is unknown and the mechanism of the test is poorly understood. Because of the difficulties in preparation and validation of the test material, the nonavailability of commercial test material, and the need to wait 4 to 6



**Figure 12** Positive Kveim skin biopsy; nonnecrotizing granulomas.

weeks for a result, the KST is rarely performed for diagnosis, and very few centers worldwide have the capability to perform the test. Transbronchial lung biopsy with its high yield of granulomas has essentially replaced the KST for the diagnosis of sarcoidosis. However, it remains a valuable investigative tool.

### The Role of Pathology in Establishing the Diagnosis of Sarcoidosis

The diagnosis of sarcoidosis is made by the patient's physician based upon a synthesis of clinical, radiological, histological, and clinical laboratory information. Because the granulomas that are seen in sarcoidosis are non-specific lesions, the pathologist is almost never able to suggest the diagnosis of sarcoidosis based solely upon examination of a biopsy specimen. The primary role of the pathologist is (1) to identify and characterize granulomas or to document their absence and (2) to exclude, insofar as possible, known causes of granulomas, primarily infections. Diagnoses other than sarcoidosis that the pathologist must consider in evaluating granuloma-containing tissue specimens are outlined in the next section.

### INFECTIOUS CAUSES

Mycobacterial and fungal infections and, to a lesser extent, parasitic infections and nonmycobacterial bacterial infections need to be excluded.

#### Mycobacterial and Fungal Infection

Staining with the Ziehl-Neelsen or Kinyoun acid-fast stains or fluorochrome staining using auramine O with or without rhodamine (AR) are routinely used in attempts to identify AFB in tissue. The fluorochrome stains are technically simpler than the Ziehl-Neelsen and Kinyoun stains, enabling more rapid screening at lower magnification and generally exhibiting greater sensitivity and greater predictive value of a negative

result for both *Mycobacterium tuberculosis* and nontuberculous mycobacteria.<sup>100-102</sup> Although the fluorochrome stains are generally thought to have less specificity than the Ziehl-Neelsen and Kinyoun stains this is not well documented. In some laboratories the fluorochrome stain is used for initial screening with positive specimens then examined with Ziehl-Neelsen or Kinyoun stains for confirmation. AFB are most likely to be identified in necrotizing granulomas,<sup>103</sup> with the highest yield within the necrotic centers of the granulomas.<sup>78</sup> Unfortunately the sensitivity of acid-fast stains in tissue and in clinical specimens is low. Published reports indicate a sensitivity ranging from 8.3 to 60% for the Ziehl-Neelsen stain in tissues with positive mycobacterial cultures.<sup>104-107</sup> The specificity of the Ziehl-Neelsen stain is reported to be greater than 95% in almost all studies. The sensitivity of the AR stain for detection of mycobacteria in tissue is reported to range from 31 to 85%.<sup>107,108</sup> The significance of the relatively low sensitivity of traditional staining methods for AFB is that a negative AFB stain does not exclude mycobacterial infection, particularly when evaluating small biopsy specimens containing only small amounts of lesional tissue.

Polymerase chain reaction (PCR) is a sensitive and rapid method for the diagnosis of mycobacterial infection in formalin-fixed, paraffin-embedded tissues.<sup>106,108</sup> However, the interpretation of the significance of finding mycobacterial DNA sequences by PCR in cases of suspected sarcoidosis should be approached with great caution. Several studies have reported the presence of mycobacterial DNA in granuloma-bearing tissues of patients with sarcoidosis.<sup>109-113</sup> However, other investigators have failed to confirm these findings.<sup>114-116</sup> The significance of the presence of mycobacterial DNA in the granulomas of sarcoidosis is undetermined and, by itself, does not provide evidence to establish a diagnosis of either mycobacterial infection or sarcoidosis.

In the case of fungal infections the organisms are far more likely to be detected with fungal stains than are mycobacteria with acid-fast stains. Therefore, negative fungal stains, particularly in the presence of abundant lesional tissue, permit exclusion of fungal infection with a higher degree of confidence than a negative AFB stain would permit exclusion of mycobacterial infection. With both types of infection cultures should supplement staining when possible.

An important study that sought to determine the frequency of positive microbiological cultures in 92 adult patients with transbronchial biopsy specimens exhibiting epithelioid granulomas and negative histochemical stains for microorganisms found positive cultures for mycobacteria and fungi in specimens from 10 patients (11%); mycobacteria were cultured in nine and fungus (*Histoplasma*) in one.<sup>42</sup> Positive cultures were obtained from sputum, bronchial or alveolar washings, and tissue samples. The remaining 82 subjects (89%) had sarcoi-

dosis. It was determined that a high clinical suspicion of sarcoidosis, numerous granulomas, and presence of Schaumann's bodies were significantly correlated with the diagnosis of sarcoidosis. The infectious cases exhibited fewer granulomas. Necrosis was more prevalent in the infectious granulomas (40% vs 19.5%) but the difference was not statistically significant. The findings from this study emphasize that, although clinicopathological assessment of transbronchial biopsy specimens is useful in predicting the diagnosis of sarcoidosis, a significant number of infectious granulomas can be missed in the absence of cultures for microorganisms.

### Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis (HP)/extrinsic allergic alveolitis is caused by hypersensitivity to inhaled organic antigens. Because the characteristic microscopic appearance consists of interstitial chronic inflammation and a variable number of granulomas it may overlap the microscopic appearance of sarcoidosis. In the absence of a relevant exposure history morphological features may be very helpful in distinguishing between HP and sarcoidosis. In sarcoidosis chronic interstitial inflammation (alveolitis) is usually a minor component and numerous granulomas are usually present and dominate the microscopic appearance. In HP the opposite is generally true (i.e., alveolitis is the predominating feature with relatively few or even rare granulomas present). Schaumann's bodies may be prominent in both conditions. Bronchoalveolar lavage may be helpful in the differential diagnosis because the alveolitis in sarcoidosis contains predominantly T4 helper cells, whereas in HP the interstitial inflammatory cells are predominantly T8 suppressor cells.<sup>117</sup>

### Chronic Beryllium Disease

There is no difference in the appearance of the granulomas and granulomatous lung involvement in chronic beryllium disease (CBD) and sarcoidosis. The diagnosis of CBD is highly dependent upon an occupational exposure history. The diagnosis can be confirmed by demonstration of the presence of beryllium in body fluids and/or tissues and sensitization of the patient's lymphocytes to beryllium. Other metallic dusts or fumes such as aluminum, titanium, zirconium, and others may also induce pulmonary granulomas.

### Wegener's Granulomatosis

Although Wegener's granulomatosis (WG) is characterized by vasculitis and its name implies the presence of granulomas, its distinction from sarcoidosis is usually not difficult. The vasculitis of WG is usually nongranulomatous compared with the granulomatous vasculitis in

sarcoidosis. Epithelioid granulomas are rare and, if present, are usually not as well formed as they are in sarcoidosis. Single or small groups of multinucleate giant cells are more likely to be seen in WG than are discrete granulomas. The usual case of WG exhibits large geographic areas of basophilic necrosis, a feature that by itself should exclude sarcoidosis.

The etiology of a significant percentage of granulomas cannot be determined utilizing currently available diagnostic methodology. In a report of 303 granulomatous conditions encountered in routine surgical pathology practice, 185 (61%) were stated to be of undetermined origin.<sup>107</sup> In a study of solitary necrotizing granulomas of the lung, their etiology in 22 of 86 (26%) cases was undetermined following extensive workup.<sup>77</sup> Another report indicates that the etiology of 21% of lung granulomas cannot be determined.<sup>118</sup> A syndrome characterized by granulomas of undetermined etiology occurring in liver, lymph node, bone marrow, spleen, and other sites in patients presenting with prolonged fever, hepatomegaly, splenomegaly, a benign course, and tendency to recur has been called the GLUS syndrome (granulomatous lesions of unknown significance).<sup>118</sup> The granulomas may be necrotizing or nonnecrotizing. They have been shown to contain B lymphocytes and natural killer cells that are not found in the granulomas of sarcoidosis. It has been estimated that 15 to 20% of granulomas identified in liver, bone marrow, and lung may fit into the GLUS syndrome. Although the features of GLUS suggest a DNA-viral etiology such as cytomegalovirus, Epstein-Barr virus, or other unidentified DNA virus, it is likely that GLUS cases represent a variety of etiologies.

There are no morphological features that enable the pathologist to make a diagnosis of sarcoidosis. Statements such as "consistent with sarcoidosis" or "suggestive of sarcoidosis" are not helpful and may be misleading. Nonnecrotizing granulomas have been reported to be the only biopsy finding in up to 40% of cases of tuberculosis.<sup>107</sup> The appearance of the granulomas in those cases of tuberculosis was certainly consistent with sarcoidosis but a statement to that effect in the pathology report could have been very misleading to the physicians managing the patients. The pathology report is but one of many sources of data that the clinician uses to make the diagnosis of sarcoidosis.

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