Diagnostic Value of Epithelioid Cell Granulomas in Bronchoscopic Biopsies

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Abstract

Background The granulomatous inflammatory response is a manifestation of many lung diseases.

Objective To evaluate the diagnostic value of epithelioid cell granulomas in bronchoscopic biopsies in daily clinical practice.

Methods The data of 105 patients with epithelioid cell granulomas in biopsy tissue who had undergone the bronchoscopic lung biopsy or bronchial biopsy at the Centre of Pulmonology and Allergology of Vilnius University Hospital Santariskiu klinikos (Vilnius, Lithuania) were examined. All cases were divided into non-necrotizing epithelioid cell granulomas and epithelioid cell granulomas with necrosis.

Results Of all the cases 66% had non-necrotizing epithelioid cell granulomas and 34% had epithelioid cell granulomas with necrosis. Without respect to the presence of necrosis in granulomas, the majority of the patients (79%) had sarcoidosis or tuberculosis; 94% of the patients with sarcoidosis had non-necrotizing epithelioid cell granulomas and the remaining 6% had granulomas with necrosis. The sensitivity of non-necrotizing epithelioid cell granuloma for the diagnosis of sarcoidosis was 94% and specificity 60%. The positive and negative predictive values were 68% and 92%, respectively.

Of the patients with tuberculosis 76% had epithelioid cell granulomas with necrosis and 24% had non-necrotizing epithelioid cell granulomas. The sensitivity of epithelioid cell granuloma with necrosis for the diagnosis of tuberculosis was 76% and specificity 85%. The positive and negative predictive values were 69%, and 88%, respectively.

Conclusion A significant overlap in types of granulomatous inflammation between tuberculosis and sarcoidosis was found. The type of epithelioid cell granuloma alone was not sufficient for the final clinical diagnosis.

Key words: biopsy, bronchoscopy, epithelioid cell granuloma, granulomatous inflammation, sarcoidosis, tuberculosis

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Introduction

The granulomatous inflammatory response is ubiquitous in pathology, being a manifestation of many infective, toxic, allergic, autoimmune and neoplastic diseases and also conditions of unknown etiology. Granulomatous diseases and reactions are a regular occurrence in everyday clinical histopathology (1). Granulomas are structured masses composed of lymphocytes and macrophage-derived cells, which assume an epithelioid aspect. They may occur in any tissue or organ (2). The granulomas in tuberculosis, extrinsic allergic alveolitis (hypersensitivity pneumonitis), and chronic beryllium disease are often identical to those of sarcoidosis, and historically have been called “classical sarcoid granulomas” (3). Observations that granulomas are of differing morphology led to numerous attempts to classify granulomatous inflammation, either to help in the diagnosis of granulomatous dis-
ease or to further the understanding of the granulomatous process. Granulomas have been divided into “foreign body” and “epithelioid”, “low turnover” and “high turnover”, “immunological type” and “non-immunological type”. However, no classification has been very successful (1).

Pathologists usually differentiate granulomatoses by their morphologic appearance into epithelioid cell granulomas with necrosis and non-necrotizing granulomas. The distribution pattern of the lung granulomas may assist in sorting out specific diseases. However, the distribution pattern may not be assessed in bronchoscopic lung biopsies (4).

Usually biopsy data are a basis for clinical diagnosis and direct therapy method. The aim of this study was to evaluate the diagnostic value of the epithelioid cell granulomas in the bronchoscopic bronchial and lung biopsies in a daily clinical practice.

### Materials and Methods

The data obtained from the case records of all 105 patients with epithelioid cell granulomas in biopsy tissue who had undergone the bronchoscopic lung or bronchial biopsy since January 2001 to December 2007 at the Centre of Pulmonology and Allergology of Vilnius University Hospital Santariškių klinikos (Vilnius, Lithuania) were examined. Material of the study consisted of 85 (81%) patients with granulomas in lung biopsies and 20 (19%) patients with granulomas in the bronchial biopsies.

All cases were divided into non-necrotizing epithelioid cell granulomas and epithelioid cell granulomas with necrosis. Final clinical diagnosis was established according to the data of histological examination and the results of other examinations (e.g. bronchoalveolar lavage fluid findings, the specific immune test). All cases of tuberculosis were culture-positive (data were available later than the data of histological examination). In Lithuania, tuberculin skin test does not require the approval or a signed patient's informed consent form for the retrospective study of case records. The bronchoscopic lung and bronchial biopsies were performed after applying local anesthesia. The lung biopsies were performed under fluoroscopic control; from 4-12 (average 8) biopsies were done. Most of the samples were 1-3 mm in diameter.

In this study we did not subdivide bronchial and lung biopsy material into separate groups as granulomatous inflammation in sarcoidosis, tuberculosis and Wegener's granulomatosis involves both airways and lung parenchyma (5-9). Microscopic sections were routinely stained with Hematoxylin and Eosin (HE), and the Ziehl-Neelsen stain for acid-fast bacilli. The PAS and Gomori methenamine silver stains for fungi was also performed in some cases. Non-necrotizing epithelioid cell granulomas were diagnosed when tightly packed; well formed noncaseating granulomas consisting of a cluster of epithelioid cells and giant cells were found (Figs. 1A, B, C and D). Epithelioid cell granulomas with necrosis were diagnosed when the zone of necrosis bordered by palisading histiocytes and multinucleated giant cells was seen. Epithelioid granulomas with necrosis contain centrally located amorphous, eosinophilic granular tissue debris. Necrotic epithelioid cells with condensed, hyperchromatic and fragmented nuclei and acidophilic cytoplasm (apoptotic bodies) were seen in the necrosis area (Figs. 2A, B and C). Small necrobiotic foci or few apoptotic cells were not regarded as necrosis. The biopsy samples were examined at the Lithuanian National Centre of Pathology (Vilnius, Lithuania). The Centre is accredited by the College of American Pathologists.

### Results

Of all 105 cases, 69 (66%) patients had non-necrotizing epithelioid cell granulomas and 36 (34%) patients had epithelioid cell granulomas with necrosis. The data of the final clinical diagnosis of the patients with epithelioid cell granulomas are shown in Tables 1, 2.

As indicated in Tables 1, 2, without respect to the presence of necrosis in granulomas, the majority of the patients had sarcoidosis or tuberculosis (79% of all cases). So it was possible to calculate the diagnostic value of non-necrotizing epithelioid cell granulomas for sarcoidosis and the diagnostic value of epithelioid cell granulomas with necrosis for tuberculosis.

Of 50 patients with sarcoidosis, 94% had non-necrotizing epithelioid cell granulomas; 6% of them had granulomas with necrosis. In our cases, sensitivity of non-necrotizing epithelioid cell granuloma in bronchoscopic biopsy for the diagnosis of sarcoidosis was 94% and the specificity 60%. The positive and negative predictive values were 68%, and 92%, respectively. Of 33 patients with tuberculosis, 76% had epithelioid cell granulomas with necrosis and 24% of them had non-necrotizing epithelioid cell granulomas. The sensitivity of epithelioid cell granuloma with necrosis for the diagnosis of tuberculosis was 76% and the specificity 85%. The positive and negative predictive values were 69%, and 88%, respectively.

It should be noted that in all 3 cases of carcinoma of lung-related sarcoïd reaction (not true sarcoidosis), the histological type of lung cancer was adenocarcinoma. Unfortunately, in 8 (8%) of the cases the pathological findings and data of other examinations were insufficiently specific for the precise disease diagnosis (to establish granuloma etiology). So the final clinical diagnosis remained only syndromic (granulomatous pneumonitis, alveolitis and alike).

### Discussion

The data of 105 cases of epithelioid cell granulomas in bronchoscopically obtained tissue were evaluated retrospectively. The major findings were: 1) granulomatous inflam
Figure 1. A) Sarcoidosis. The multiple noncaseating granulomas situated around the bronchiole (arrow) with marked narrowing of the airway lumen. Granulomas are surrounded by concentric fibrosis (Hematoxylin and Eosin staining, ×100). B) Sarcoidosis. The concentric laminated calcification (arrow) within giant cell is a Schaumann body (Hematoxylin and Eosin staining, ×200). C) Hypersensitivity pneumonitis. Non-necrotizing epithelioid cell granuloma with Langhans cell (Hematoxylin and Eosin staining, ×100). D) Non-necrotizing epithelioid cell granulomas (arrows) in a case of lung adenocarcinoma (upper left side) (Hematoxylin and Eosin staining, ×100).

Figure 2. A) Tuberculosis. Multiple necrotizing (arrow) epithelioid cell granulomas with Langhans cells (Hematoxylin and Eosin staining, ×40). B) Tuberculosis. Langhans type giant cell (arrow) (Hematoxylin and Eosin staining, ×200). C) Tuberculosis. A large zone of necrosis (on right) is bordered by palisading histocytes (arrow) and inflammatory infiltration (on left) (Hematoxylin and Eosin staining, ×100).

Our findings show that the sensitivity of non-necrotizing epithelioid cell granuloma in bronchoscopic biopsy for the diagnosis of sarcoidosis is high, as well as the negative predictive value of this type of epithelioid cell granuloma for the exclusion of sarcoidosis. However, the specificity of epithelioid cell granuloma without necrosis in our group was relatively low-only 60%. This result was significantly lower than that described by Winterbauer et al (10). They found that noncaseating epithelioid cell granuloma in transbronchial biopsy had a specificity of 89% for the distinction between sarcoidosis and other forms of diffuse lung disease. Nevertheless we have to note that there are differences in our patients’ population. Patients with tuberculosis were the
Table 1. Final Clinical Diagnosis Established in Cases of Non-necrotizing Epithelioid Cell Granulomas

<table>
<thead>
<tr>
<th>Final clinical diagnosis</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Sarcoidosis</td>
<td>47</td>
<td>68</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Carcinoma of lung</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Unknown aetiology</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>100</td>
</tr>
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</table>

Table 2. Final Clinical Diagnosis Established in Cases of Epithelioid Cell Granulomas with Necrosis

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>25</td>
<td>69.2</td>
</tr>
<tr>
<td>Fungal infection</td>
<td>4</td>
<td>11.2</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>3</td>
<td>8.4</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>2</td>
<td>5.6</td>
</tr>
<tr>
<td>Necrotizing sarcoid granulomatosis</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>Unknown aetiology</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>100</td>
</tr>
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</table>

next largest group after sarcoidosis in our study population. Lithuania is a moderate tuberculosis-burden country. The incidence of lung tuberculosis in Lithuania is about 50 cases per 100,000 population (11). These facts may influence our results significantly.

In tuberculosis, histologically the sites of active involvement are marked by a characteristic granulomatous inflammatory reaction that forms both caseating and noncaseating tubercles (12). The basic histopathological lesion in sarcoidosis is the noncaseating epithelioid cell granuloma (13). But noncaseating epithelioid cell granuloma was found in 24% of our patients with tuberculosis. Recently Ergete and Bekele (14) demonstrated that the frequency of epithelioid cell granuloma without necrosis in Ethiopian patients with tuberculosis is 3.4%. Fenholds et al (15) published data about examination of surgical lung tissue obtained from seven tuberculosis patients from South Africa. All patients were undergoing surgery for a severe bleeding from lungs. In all cases both necrotic as well as nonnecrotic granulomas in lung tissue sections were found. However, both types of granulomas from all patients were positive for M. tuberculosis DNA. We can speculate that these differences in our results are related to the different health status of our patients as well as various stages of the disease (as necrosis is the next step after the granuloma formation). For clinical practice it means that even in cases of advanced tuberculosis in bronchoscopic biopsy (when much more limited amount of material is available) only nonnecrotic granulomas may be found. Surely clinical diagnosis is based not only on biopsy data, but in daily clinical practice in a significant number of cases it is difficult to distinguish Stages II and III sarcoidosis from focal non-destructive tuberculosis.

Protective immunity is characterized by the formation of granulomas at the site of infection (16). Clinical experience indicates that host immunity plays an important role in the host-pathogen interaction occurring in persons exposed to Mycobacterium tuberculosis. There is compelling clinical evidence that, in addition to the innate virulence of the M. tuberculosis itself, the host response plays a major role in determining the clinical manifestations and ultimate outcome of persons who encounter this pathogen (17). We thought that we investigated our patients rather soon after the onset of the active tuberculosis. The explanation for this phenomenon could be simple; a great part of the population of Lithuania undergoes an annual chest X-ray or once in two years.

In our study, 4% of our non-necrotizing epithelioid cell granuloma cases were related to carcinoma of the lung and 3% cases were related to other malignant diseases. These results are in line with those described by others authors (18-20). Sarcoid reactions in malignant disease appear in close association with tumors, in regional lymph nodes, or in more distant locations. They have been reported to occur in a variety of malignant diseases, with particularly high incidences in lymphoproliferative disorders. The cause of sarcoid reactions has remained unclear (21).

Only 2 of 69 non-necrotizing granuloma cases in our
group were caused by hypersensitivity pneumonitis. Our suggestion is that the overall incidence of hypersensitivity pneumonitis in our country due to the relatively cold climate is low. However this disease may be under diagnosed especially in rural regions where specialized pulmonological service is not always available. Equally, the diagnostic accuracy of bronchoscopic biopsy in hypersensitivity pneumonitis may be low (22). In our department for the majority of patients with hypersensitivity pneumonitis the final diagnosis is confirmed by the typical clinical signs, diffuse ground glass pattern on lung computed tomography scan, the bronchoalveolar lavage fluid findings (low CD4/CD8 ratio), and low diffusing capacity carbon monoxide.

Fungal infection (all cases of invasive and semi-invasive aspergillosis) was rare in our study group. This finding is in concordance with very low incidence of fungal infection in the whole Lithuanian population. All of our study patients were ill with chronic hematological disorder. The major factors associated with the development of these forms of aspergillosis include hematological malignancy and chronic pulmonary disease (23, 24). The incidence of pulmonary vasculitis was the fourth in the group of diseases of epithelioid cell granuloma with necrosis, but the portion was small. These disorders as such are rare. Most often in our practice Wegener's granulomatosis is seen. Pulmonary vasculitis is characterized by necrotizing vasculitis and granulomatous inflammation. However, manifestation of diseases is determined by the size of vessels involved, relative amounts of inflammation and tissue necrosis (25). Thus, transbronchial or endobronchial biopsy may not reflect the true pathological changes.

Granulomatous inflammation is best defined as a special variety of chronic inflammation in which cells of the mononuclear phagocyte system are predominant and take the form of macrophages, epithelioid cells and multinucleated giant cells. In most instances these cells are aggregated into well demarcated focal lesions called granulomas, although a looser, more diffuse arrangement may be found. In addition there is usually an admixture of other cells, especially lymphocytes, plasma cells and fibroblasts (1). Different stages of granuloma formation can be encountered: first-a loose aggregation of macrophages, histiocytes, lymphocytes and even neutrophils. During each step the granuloma becomes more compact, and the margins are better circumscribed. With aging, epithelioid cell granula might undergo fibrosis and hyalinization. However, in some diseases, like hypersensitivity pneumonitis, the granulomas remain less well delineated. A very important finding is central necrosis, defining the necrotizing epithelioid cell granuloma, though small necrobiotic foci or a few apoptotic cells are not regarded as necrosis (4).

It is very important to remember these formation stages of granuloma when biopsy results are assessed. Rosen et al (26) found that nongranulomatous, nonspecific interstitial pneumonitis were predominant or prominent histopathologic findings in 62% of 128 granuloma-containing specimens from open lung biopsies obtained from patients with sarcoidosis. Their data strongly suggest that interstitial pneumonitis represents a very early lesion in pulmonary sarcoidosis, antedating the appearance of the characteristic epithelioid granulomas (26). If diagnosis requires pulmonary tissue, multiple lung segments may need to be sampled to achieve at least 85% of diagnostic yield of transbronchial biopsy for sarcoidosis (27), though data from open lung biopsy show the occurrence of pulmonary granulomas in 100% of the patients with Stage I sarcoidosis (28).

Despite the importance of the granulomatous lung diseases as a group, progress has been slow in our understanding of their pathogenesis, predicting their clinical course, and preventing their associated morbidity and mortality. To assist these goals, expression profiling has been performed using samples from patients with these diseases. The number of studies using gene or protein expression profiling of patients with granulomatous lung disease is limited. The reported data produced with these technologies do not allow any consensus conclusions (29).

In the present study, a potential limitation was its retrospective nature. To perform a prospective study for the subject of our study is not realistic in a small country. A prospective study of even up to three years will lose most rare cases of the granulomas. It would not be possible to calculate the real sensitivity and specificity, so the aim of the study (to evaluate the diagnostic value of the granulomas in a daily clinical practice) would not be achieved.

The second limitation to this study is the large difference in number of tuberculosis and sarcoidosis cases. The comparison would be much stronger if the two diseases were more equal in number. This difference is due to different diagnostic ways of tuberculosis and sarcoidosis that are approved in our department. In most cases of tuberculosis the diagnosis is confirmed by sputum microscopy and culture (data become available later). Only patients with negative sputum microscopy and high tuberculosis suspicion underwent bronchoscopy with bronchoalveolar lavage and biopsy. Thus, in daily clinical practice relatively more patients with biopsy proven sarcoidosis than tuberculosis patients with biopsy are seen in our clinic.

In some cases the etiology of granulomatous inflammation was not specified, though the authors have followed up most of these patients for at least one year. We can not exclude the possibility of a drug-induced reaction, occupational or environmental granulomatous reaction against metal compounds, or rare infection. We have not compared bronchoscopic biopsies with surgical lung biopsy as we do not perform it routinely for tuberculosis and most interstitial lung diseases. Due to a small number of cases (as population of the country is quite small), it was not possible to calculate the sensitivity and specificity of epithelioid cell granulomas for diagnosis of diseases other than sarcoidosis and tuberculosis.

To summarize, our results confirmed that the etiology of epithelioid cell granuloma is a miscellaneous one. Epithe-
lioid cell granuloma with or without necrosis in bronchoscopic biopsy alone does not confirm a specific disease. A significant overlap in types of granulomatous inflammation between tuberculosis and sarcoidosis was found. In our population, the specificity of non-necrotizing epithelioid cell granuloma for diagnosis of sarcoidosis is relatively low. Epithelioid cell granuloma with or without necrosis in broncho-


does not exclude tuberculosis. An additional examination for tuberculosis and/or follow-up of the patient in such a case is strongly recommended.

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References