

Evaluation of the Role of Pathology in the Diagnosis and Differential Diagnosis of Pulmonary and Extrapulmonary Sarcoidosis

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SUMMARY

Introduction Sarcoidosis is a multisystem, granulomatous disease of unknown etiology. Sarcoid granulomas appear as immunological response to a particular but still unknown agent of the human body.

Objective The main purpose of this study was to point out the important fact that the exact diagnosis of sarcoidosis must be estimated by clinical and pathological correlation, and team cooperation between the clinician and the pathologist.

Methods Of 751 patients referred with the suspected diagnosis of sarcoidosis, from 1995 to 1999, 663 (431 female and 232 male) were analyzed and confirmed as having sarcoidosis stage I-III based on biopsy findings obtained by bronchoscopy, open lung biopsy, skin biopsy, liver biopsy or splenectomy.

Results Diagnosis of sarcoidosis was made in 663 patients, 431 females and 232 males (ratio 1.9:1). The average age of patients varied from 16 to 67 years, with those below age 50 years being predominant (78.4%). The highest number of patients was diagnosed in stage I of lung sarcoidosis (81.7%). Sarcoidosis was the most common cause of hilar and mediastinal lymphadenopathy (72.2%).

Conclusion Biopsy is a necessary diagnostic procedure for pathological diagnosis of sarcoid granuloma before treatment even in patients where clinical, radiological, biochemical and immunological tests imply the diagnosis of sarcoidosis.

Keywords: granulomatous disease; mediastinal lymph nodes; diagnostic procedures

INTRODUCTION

Sarcoidosis is a multisystem, granulomatous disease of unknown aetiology. Morphology of some granuloma types is characteristic of specific diseases so that this is basis for reaching the final diagnosis. Therefore, this type of inflammation has been defined as specific. [1, 2, 3]. Sarcoidosis belongs to the entities of specific inflammations, because of being characterized by inflammatory lesions, i.e. granulomas. The term "sarcoidosis" originates from the Greek word "sarkodes" meaning "fleshy" and suffix "-osis" meaning "condition" [1].

Sarcoidosis was described for the first time as a skin disease in 1877, in patients with multiple purplish patches on the face and hands. Identical appearance of sarcoid granuloma can be seen regardless of the involved organ [1, 2, 3]. Sarcoidosis mostly develops in younger and middle aged persons, predominantly women, in all geographic areas. It quite frequently occurs presenting severe clinical features in Afro-Americans [1-5].

OBJECTIVE

The important aim of this study was to estimate the role of pathology in the diagnosis and

differential diagnosis of sarcoidosis and to point out the importance of establishing the diagnosis of sarcoidosis only in association of clinical with pathological studies. The purpose was to establish other conditions presenting with sarcoid granulomas and to estimate other conditions where hilar and mediastinal lymphadenopathy could be the major symptom of the disease different from sarcoidosis.

METHODS

Out of the total number of 751 patients, 663 biopsy positive sarcoidosis patients were analyzed in five-year period (1995–1999). Biopsy samples were obtained from different tissues and organs. All obtained tissue samples were routinely fixed and paraffin embedded and hematoxylin-eosin (H&E) stained [6]. The majority of the tissue samples were obtained by transbronchial and bronchial biopsy during bronchoscopy. Open lung biopsy was performed in patients when repeated bronchoscopy did not resolve the etiology of the disease. Buffered formalin instillation with dispersion into the pulmonary parenchyma was made to distend alveolar spaces in their natural size and shape [6, 7]. Sarcoidosis was diagnosed by liver biopsy, splenectomy and skin biopsy.

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Acid-fast staining was performed to exclude or to detect the presence of acid-fast bacilli in granuloma in differential diagnosis to tuberculosis. Phospholipids within the capsule of bacilli were stained red and visible for microscopy.

Periodic Acid Schiff (PAS) staining was performed for distinction of fibrinous necrosis in sarcoidosis from other types of necrosis. Fibrinous necrosis was stained as a clear and lightly red tiny granular mass [7, 8].

Peripheral fibrosis and hyalinization around granulomas was stained by silver impregnation of fibrous fibres.

RESULTS

Sarcoidosis was confirmed in 431 female and 232 male patients, in total ratio 1.9:1. The predominant number of patients (78.4%) was aged below 50 years, with a peak between 41-50 years (Table 1). The youngest patient was a 16-year old girl. The oldest one was a 67-year old man.

Sarcoidosis was most frequently diagnosed in I stage of lung disease by transbronchial biopsy and rarely in II or III stage of lung disease by transbronchial and bronchial biopsy (Table 2).

By liver biopsy and splenectomy the diagnosis of sarcoidosis was confirmed in six patients, also by skin biopsy in six patients. In nine patients the diagnosis of sarcoidosis was established by open lung biopsy.

Sarcoidosis was the leading cause of hilar and mediastinal lymphadenopathy in 72.2% of our patients (Table 3). The diagnosis was made by transbronchial biopsy. Metastatic deposits in lymph node tissue of malignant tumours of other organs were diagnosed also by trans-

bronchial biopsy. The origin of metastasis was accurate as confirmed by immunophenotypization. Mediastinal lymphomas, mostly of Hodgkin type, in 13 patients (1.8%) were the cause of lymphadenopathy.

Tuberculosis was diagnosed in four patients in whom acid-fast bacilli in caseating granulomas was detected.

DISCUSSION

Sarcoidosis is a specific granulomatous disease. It is still a question whether sarcoidosis is a syndrome or an entity [9].

Sarcoidosis can be diagnosed in all ages, but predominantly in young and middle aged persons of both genders, but frequently in women, in all parts of the world and in all races. The incidence is quite high in the Scandinavians. Sarcoidosis is almost unknown disease in South-East Asia and China [4].

Due to the clinical course of sarcoidosis, spontaneous remission is possible. Most often sarcoidosis patients develop symptoms like dry cough, short breath and arthralgia. There are indications that the disease is hereditary [1, 4].

According to the chest radiological findings sarcoidosis is classified in 4 stages: stage 0 – regular chest X-ray; stage I – hilar and mediastinal lymphadenopathy; stage II – hilar and mediastinal lymphadenopathy accompanied with infiltrates in pulmonary parenchyma; stage III – pulmonary infiltrates without hilar and mediastinal lymphadenopathy, with frequent formation of the so-called ground glass effect; stage IV – pulmonary fibrosis [10, 11].

A typical morphological pattern of sarcoidosis is non-caseating granuloma appearing after accumulation of mononuclear inflammatory cells in involved tissue. Accumulation of macrophages transformed to epithelioid cells are surrounded mostly by T cell population of lymphocytes. It is presumed that prolonged antigen stimulation leads to transformation of macrophages to epithelioid cells and appearance of giant, multinuclear cells, specifically of Langhans type [6].

By electron microscopy two types of epithelioid cells are estimated, the first one with the developed endoplasmatic reticulum with a few vacuoles and granules and the second one with a lot of fine granular material. Cytoplasm of epithelioid cells contains enormous amount of dipeptidyl carboxylase enzyme (angiotensin-converting enzyme), so that these cells are responsible for the increasing level of this enzyme in blood serum. The epithelioid cells are specialized for extra cellular secretion, not for phagocytosis [7].

Giant, multinuclear cells arise in fusion of two or more macrophages. Numerous nuclei are pressed to cell membrane, looking as sickle-like or half-moon shaped nuclei (Langhans type cell) or chaotically “thrown” all the way through cell cytoplasm. Sometimes the nuclei are “pushed away” by the Schaumann body. Characteristic cytoplasmatic inclusions are sometimes present, but are not typical only for giant multinuclear cells in sarcoid granuloma. They are asteroid and Schaumann bodies. Asteroid bodies are lipoprotein and acidophilic star shaped inclu-

Table 1. Age of patients with sarcoidosis

| Age (years) | Number of patients |
|-------------|--------------------|
| ≤30 | 148 (22.4%) |
| 31–40 | 162 (24.5%) |
| 41–50 | 210 (31.5%) |
| 51–60 | 117 (17.7%) |
| ≥61 | 26 (3.9%) |
| Total | 663 (100%) |

Table 2. Histological diagnosis of pulmonary sarcoidosis classified by stages

| Stage of pulmonary sarcoidosis | Number of patients |
|--------------------------------|--------------------|
| I | 542 (81.7%) |
| II | 100 (15.1%) |
| III | 21 (3.2%) |
| Total | 663 (100%) |

Table 3. Differential diagnosis: causes of hilar and mediastinal lymphadenopathy

| Differential diagnosis | Number of patients |
|---------------------------------------|--------------------|
| Pulmonary sarcoidosis – stage I | 542 (72.2%) |
| Reactive lymphadenopathy | 160 (21.3%) |
| Metastasis of other malignant tumours | 32 (4.2%) |
| Lymphoma | 13 (1.8%) |
| Tuberculosis | 4 (0.5%) |
| Total | 751 (100%) |

sions. They present amorphous material arising from microfilaments, microtubules, mature and immature centrioles. The Schaumann bodies contain protein matrix impregnated with iron and calcium salts in concentric lamellas and crystals capable for light polarization. This inclusion is formed in epithelioid giant cells or extracellularly, near the peripheral sinus. The granular endoplasmic reticulum is weakly developed in giant cells. Mitochondria and lysosomes show degenerative changes and cytoplasm contains numerous vesicles and different particles [7].

Among epithelioid cell CD4+ lymphocytes and CD8+ lymphocytes are found peripherally. In bronchoalveolar lavage the number of CD4+ lymphocytes is increased compared to CD8+ lymphocytes. A fibrous rim develops around the granuloma spreading to its centre. It is a sign of progression of sarcoidosis and the beginning of the scar arising in the parenchyma probably causing organ's dysfunction and insufficiency. Sometimes granulomas are similar to those in hypersensitive pneumonitis [7, 10, 11].

Necrotizing sarcoid granulomas are described in the literature. They involve pulmonary parenchyma exclusively and are characterized by fibrinous and coagulate necrosis in the central zone of granuloma, without the presence of acid-fast bacilli. Necrosis is clearly red stained by PAS. Necrotizing sarcoid granulomas are the result of vasculitis. Giant cells are more numerous around the necrosis than in sarcoid granulomas. Epithelioid cells are also increased in this type of granuloma. There is no fibrosis and hyalinization around necrotizing sarcoid granulomas. These granulomas resolve after cytostatic and steroid therapy [10].

Differential diagnosis from other granulomas due to their morphology pattern is relatively easy, particularly by open lung biopsy. Pulmonary parenchyma changes are mostly bilateral reticular and reticulonodular infiltrates less than 1cm in size, accompanied with emphysema and "honey comb" lungs. Pleural effusion appears in sarcoidosis very rarely [11, 12]. Sarcoid granulomas are grouped within the parenchyma of the lungs. They can be confluent or non-confluent, separated by fibrous or hyaline thickened septa, and distributed peribronchiolarly, perivascularly, interlobularly or under the pleura. Emphysema or even the "honey comb" pattern can be present in the surrounding pulmonary parenchyma [11, 13, 14].

Stage I of pulmonary sarcoidosis is lymphadenopathy, manifested in hilar and mediastinal lymph nodes enlargement. This stage of the lung disease is diagnosed most frequently, in about 90% of patients. The lymph nodes enlarged size increase from 20mm to 30mm causing dry cough and sensation of the tightness in the chest, but the patient may also be without any of the symptoms. In the lymph node parenchyma numerous, mostly confluent and no confluent granulomas, with or without giant cells are present. Uninvolved lymph node parenchyma is without active follicles [7, 8].

Some conditions (berylliosis), different substances (talc) and lymphomas provoke a lymph node reaction, remaining on sarcoid granulomas [15]. So, they are called sarcoid-like reaction, sarcoid-type reaction or sarcoid-related granu-

lomas. Mostly, they arise in lymph nodes draining organs involved by tumour, called lymph node draining carcinomas. Most frequently they appear in draining lymph nodes in carcinoid tumours, lung and breast cancer and testicular tumours [16, 17].

Vasculitis and systemic fibrous tissue disease, even HIV infection of lymph nodes may provoke a sarcoid reaction. This condition also arises in pulmonary tissues as a sign of rejection after lung transplantation. The sarcoid-related reaction arises as a morphological pattern of post-irradiation pneumonitis with the presence of numerous non-caseating granulomas, surrounded by fibrous tissue. In the sarcoid-like reaction lymph nodes may be increased in measure and in morphology similar to sarcoidosis, so the proper diagnosis is difficult to obtain [6, 8].

Cervical lymph nodes are enlarged in sarcoidosis, as well as in lymphoma. Hodgkin's lymphoma and sarcoidosis arise in younger persons. Almost always Hodgkin's lymphoma is manifested as neck, hilar and mediastinal lymphadenopathy, as well as sarcoidosis [8, 16, 17].

In cases of hilar and mediastinal lymphadenopathy pathological diagnosis of accidentally detected metastasis may not be a surprise. Tumour origin may be from close (lung and breast carcinoma) or distant (intestinal carcinoma) organs [11, 12].

Sarcoidosis often involves skin, eye, liver, spleen, heart, bone marrow, salivary glands, muscles and nervous system [1, 13, 15].

Skin sarcoidosis arises in about 75% of patients with the diagnosis of pulmonary sarcoidosis. Granulomas are often commonly called Boeck sarcoids, arising in the derm and rarely in the hypoderm. They are manifested as soft multiple purplish patches or nodules. Numerous confluent or non-confluent granulomas without necrosis form a skin lesion. Older granulomas contain a high number of giant multinuclear cells and intra-cytoplasmic inclusions. Around the edges of the granuloma there is only a small number of lymphocytes and a rim of dense fibres [3, 13, 14].

Skin changes often occur associated with sarcoidosis, most frequently erythema nodosum. It is localized in the hypoderm manifested as painful red or livid nodules. A non-specific inflammation is histological presentation [1, 13, 14].

Ocular involvement can occur inpatients with sarcoidosis, but the most frequent site is the uvea and iris, causing regional inflammation, glaucoma or blindness. Lachrymal and parotid glands may be involved by sarcoidosis at the same time [3, 13, 14].

The spleen is involved in approximately 75% of patients with pulmonary sarcoidosis, although real splenomegaly is present in about 20%. Sarcoid granulomas are only visible when granulomas are confluent. Numerous tiny granulomas, cellular or hyaline, are diffusely distributed mostly in the white pulp [15, 18, 19].

The liver is also involved but rarely enlarged. Sarcoid granulomas are mostly localized in the portal space, less frequently in the liver acinus. The sarcoid granulomas can be diagnosed by needle biopsy of liver parenchyma [18, 19].

Numerous granulomas are seen in the bone marrow with resorption of bone cortex. Hands and feet are most frequently involved [3, 13, 20, 21].

CONCLUSION

Sarcoidosis is a multisystem disease, with predominance in young persons. Radiological finding of mediastinal and hilar lymphadenopathy is the most frequent sign of pulmonary sarcoidosis. In this stage of the lung disease transbronchial biopsy is most suitable to confirm sarcoido-

sis, but the unexpected diagnosis as lymphoma, metastatic tumour, tuberculosis and reactive lesions could be possible.

By routine microscopic examination, diagnosis of epithelioid granulomas, containing varies numbers of giant, multinuclear cells with or without fibrosis and hyalinization, the diagnosis of sarcoidosis is confirmed. Morphological appearance of sarcoid granuloma is the same despite of the involved organ.

In patients, even when typically clinical, radiological, biochemical and immunological results clearly point to sarcoidosis, biopsy is necessary to confirm the diagnosis.

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Патохистолошка и диференцијална дијагностика плућне и ванплућне саркоидозе

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КРАТАК САДРЖАЈ

Увод Саркоидоза је мултисистемско, грануломатозно обољење непознате етиологије. Саркоидни грануломи настају као имунски одговор на одређени, али још непознати, агенс у организму.

Циљ рада Циљ истраживања био је да се укаже на важну чињеницу да се дијагноза саркоидозе поставља једино у клиничко-патолошкој корелацији, сарадњом клиничара и патолога.

Методе рада Од укупно 751 болесника за којег се сумњало да болује од саркоидозе, анализирана су 663 (431 жена и 232 мушкарца) код којих је током петогодишњег периода постављена дијагноза сва три стадијума овог обољења. Дијагноза болести постављена је на основу биоптичког материјала добијеног током бронхоскопије, налаза хируршке биоп-

сије плућа, биопсије коже и биопсије јетре, као и на основу спленектомије услед спленомегалије непознатог порекла.

Резултати Однос оболелих жена и мушкараца био је 1,9:1. Болесници су имали између 16 и 67 година. Већина болесника (78,4%) била је стара до педесет година. Код највећег броја испитаника установљен је први стадијум плућне саркоидозе (81,7%). Саркоидоза је била најчешћи узрок хиларне и медијастиналне лимфаденопатије (72,2%).

Закључак Пацијенте код којих клинички, биохемијски и имунолошки тестови јасно покажу да болују од саркоидозе потребно је обавезно подвргнути биопсији и патохистолошкој дијагностици узорка узетог ткива пре започињања лечења.

Кључне речи: грануломатозно обољење; медијастинална лимфаденопатија; дијагностика

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