Mycobacterial Diseases
ISSN: 2161-1068

Tamura et al., J Mycobac Dis 2014, 4:4
DOI: 10.4172/2161-1068.1000158

Research Article Open Access

Epithelioid Cell Granuloma Detection in the Sputum of Patients with Pulmonary Tuberculosis

Atsuhisa Tamura[1,2*], **Akira Hebisawa**[2], **Masahiro Shimada**[2], **Junko Suzuki**[1], **Akira Yamane**[1], **Hideaki Nagai**[1] **and Ken Ohta**[1]

[1]*Center for Pulmonary Diseases and Respiratory Disease Division, Japan*

[2]*Clinical Research Center and Pathology Division, National Hospital Organization Tokyo National Hospital, Kiyose-shi, Tokyo, Japan*

[*]**Corresponding author:** Atsuhisa Tamura, Center for Pulmonary Diseases, National Hospital Organization Tokyo National Hospital, 3-1-1 Takeoka, Kiyose-shi, Tokyo 204-8585, Japan, Tel: +81-424-91-2111; Fax: +81-424-94-2168; E-mail: tamura-in@tokyo-hosp.jp

Rec date: Apr 28, 2014; **Acc date:** Jun 24, 2014; **Pub date:** Jun 28, 2014

Abstract

Background: Epithelioid cell granulomas (ECG) are evident in the histology of various pulmonary diseases, including pulmonary tuberculosis (PTB); however, the diagnostic value of ECG in sputum cytology for patients with granulomatous lung diseases is unclear.

Methods: To evaluate the value of identifying ECG in the sputum of patients with PTB and other respiratory diseases, we retrospectively reviewed the cytology database and clinical profiles of patients admitted to our department between 2003 and 2011.

Results: Of the 11,782 patients examined by sputum cytology, ECG was detected in the cytology specimens of 32 (0.3%) patients. Thirty-one of these patients had PTB, and the remaining patient had pulmonary nontuberculous mycobacteriosis (PNTM). There were no ECG-positive cases among patients with other respiratory diseases, with the exception of one patient with comorbid lung cancer and PTB. The incidence of ECG was significantly higher in PTB (0.8%, 31/3716 cases) than in PNTM (0.09%, 1/1117 cases, P>0.05). Langhans giant cells with (2 cases) and without (5 cases) necrosis was detected only in PTB specimens. Clinical findings in the PTB cases included cavities (68%) on radiography and positive sputum smear for acid-fast bacilli (90%); the PNTM case also showed cavity and smear-positive sputum.

Conclusion: In our population of patients with various respiratory diseases, the existence of ECG in sputum cytology was rare and limited to pulmonary mycobacteriosis, particularly PTB. We suggest ECG detection is a useful aid for diagnosing unsuspected and infective PTB, and is associated with disease severity.

Keywords: Epithelioid cell granuloma; Langhans giant cell; Necrosis; Sputum; Cytology; Pulmonary tuberculosis

Introduction

Granulomatous inflammation appears in the histology of various pulmonary settings, including infectious diseases such as pulmonary tuberculosis (PTB) and non-infectious lung diseases such as sarcoidosis [1,2]. Epithelioid Cell Granulomas (ECG), the hallmark of tuberculous disease representing the host-defense mechanism against the inciting agent, consists of blood-derived macrophages, epithelioid cells (differentiated from macrophages/histiocytes by their elongated, distorted nuclei and indistinct cytoplasm) and multinucleated giant cells (Langhans giant cells), surrounded by T-lymphocytes [3]. Histological ECG detection in bronchoscopic biopsy samples may be used for early clinical diagnosis of PTB [4,5]. Recent findings have shown that a cytological approach is useful for non-neoplastic lung diseases [6], and cytologic detection of ECG obtained by fine-needle aspiration is useful for diagnosing unusual types of extrapulmonary tuberculosis such as tuberculous lymphadenitis [7], cutaneous tuberculosis [8], and bone tuberculosis [9]. In contrast, the existence of ECG in sputum cytology is quite rare; there are case reports of ECG detection in the sputum of PTB patients [10,11]. The use of non-invasive sputum cytology for detecting ECG in PTB has not been discussed. The aim of this study was to determine the diagnostic value of ECG in the sputum cytology of patients with PTB and other granulomatous lung diseases.

Materials and Methods

The Center for Pulmonary Diseases at Tokyo National Hospital has the largest number of beds for tuberculosis in Tokyo Metropolitan City, with 300 beds in the respiratory disease division (including 100 beds in the tuberculosis ward and 200 beds in the ward for other respiratory diseases). If sputum cytology and culture examinations are ordered, the fresh sputum samples in the laboratory are divided into bacteriological and pathological specimens. For pathology, the sputum materials are prepared as smears on glass slides, immediately fixed in 95% alcohol, and Papanicolaou-stained [12] with hematoxylin, OG-6, and EA-50 (eosin-Y and light green). Cytology specimens were reviewed and diagnosed by agreement between a cytologist and a pathologist; the presence and absence of malignant findings were evaluated, and the presence of non-malignant findings such as epithelioid cells, ECG, necrosis, fungus, and foreign bodies was routinely noted. Spontaneous sputum samples were generally used in the laboratory; however, when difficulties were encountered, induced sputum sampling was usually used.

From 2003 to 2011, 11,782 admitted patients underwent sputum cytology examinations in our department. The whole disease population characterized by granulomatous lung diseases (>10 cases) was as follows: PTB (3716 patients, 32%), pulmonary non-tuberculous mycobacteriosis (PNTM, 1117 patients, 9%), chronic pulmonary aspergillosis (629 cases, often encountered as sequelae of PTB, 5%), sarcoidosis (135 patients, 1%), hypersensitivity pneumonitis (77 patients, 0.7%), pneumoconiosis (72 patients, 0.6%), pulmonary cryptococcosis (12 patients, 0.1%), and Wegener's granulomatosis (12 patients, 0.1%). In Japan, approximately 1-2% of active pulmonary mycobacteriosis cases are complications of lung cancer [13]; in this study, 35 (0.9%) of 3716 PTB patients and 10 (0.9%) PNTM patients also had lung cancer. Lung cancer without pulmonary mycobacteriosis (1345 patients, 11%), a non-granulomatous lung disease, was also listed, because tumor-associated sarcoid reactions are sometimes present in the lymph nodes or in the parenchyma around the tumor [14,15].

Sputum cytology identified 32 ECG-positive cases (0.3%). The background factors, examination findings, bacterial findings, radiographic findings, and pathophysiology were analyzed in these selected cases.

Chi-square test or Fisher's exact test was used for statistical analyses of various parameters. Differences were considered significant at $P<0.05$.

This study was approved by the institutional review board and required no informed consent.

Results

Thirty-two ECG-positive cases (Figure 1) identified by sputum cytology were diagnosed with pulmonary mycobacterioses, including PTB (31 cases) and PNTM (one case). ECG was not detected in other granulomatous or non-granulomatous lung diseases such as chronic pulmonary aspergillosis, sarcoidosis, hypersensitivity pneumonitis, pneumoconiosis, and lung cancer without complicating pulmonary mycobacteriosis. The frequency of ECG was significantly higher in PTB (0.8%) than in PNTM (0.09%) ($P<0.05$). In all 32 cases, ECG were present in a background of inflammatory cells; Langhans giant cells were detected in seven cases, two of which exhibited necrosis (PTB only; Figure 2A and 2B). Of the 35 cases with PTB and lung cancer, only one (2.9%) was ECG-positive according to sputum cytology results.

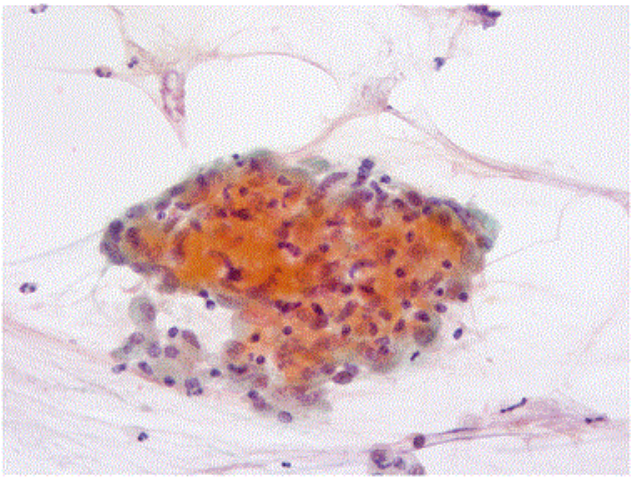

Figure 1: Epithelioid cell granuloma (a cluster of epithelioid cells showing elongated and distorted nuclei and indistinct cytoplasm) without necrosis in patients with sputum smear-positive pulmonary tuberculosis (sputum cytology, Papanicolaou-stain, original magnification 40×).

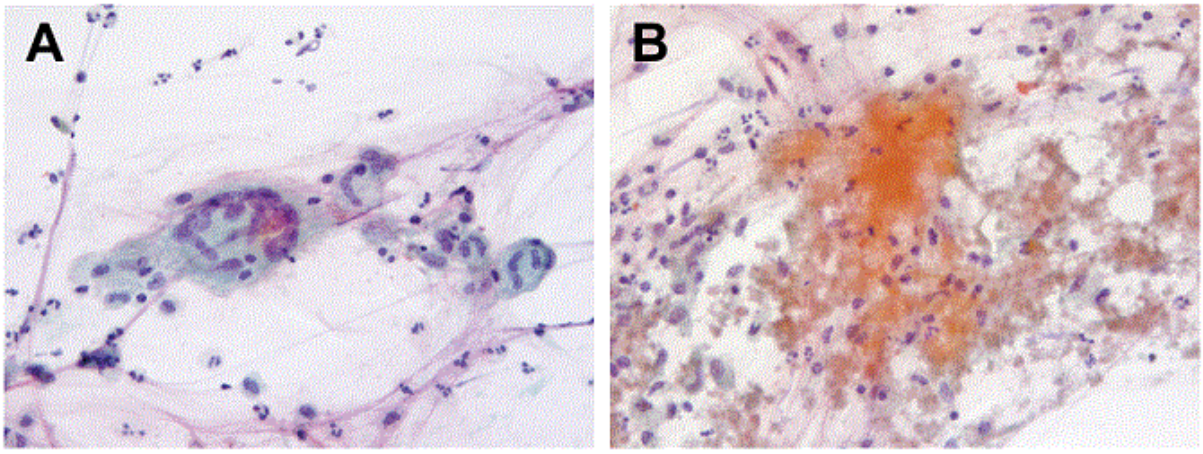

Figure 2: (A) Giant Langhans cells with peripherally oriented and overlapped nuclei in patients with sputum smear-positive pulmonary tuberculosis (sputum cytology, Papanicolaou-stain, original magnification 40×). (B) Necrosis and scattered epithelioid cells in patients with sputum smear-positive pulmonary tuberculosis (sputum cytology, Papanicolaou-stain, original magnification 40×).

Clinicopathological features of the ECG positive PTB cases (n=31) are shown in Table 1. The PTB cases included 23 men with a median age of 70 years. Sixteen patients (approximately 50% of the subjects) were aged over 70 years. Underlying diseases included diabetes in four cases, malignant tumors after surgery in two cases, and human immunodeficiency virus positivity in one case; no other significant

findings were observed. Radiography revealed 21 cases with a cavitary shadow (68%) and extensive spreading of the disease (exceeding hemi-thorax) in nine cases (29%). In 2 of 31 cases, endobronchial tuberculosis was suspected on radiography and computed tomography.

Factors	Number of cases (%)
Sex	
Male	23 (74%)
Median age [range], years	70 (28–86)
Underlying diseases	
Absent	24(77%)
Radiographic findings	
Cavity	21 (68%)
Extensive spread exceeding hemi-thorax	9 (29%)
Bacteriological findings	
Sputum culture-positive	29 (94%)
Sputum smear-positive	28 (90%)
1+/2+/3+	07-10-2011
ECG: Epithelioid Cell Granulomas; PTB: pulmonary tuberculosis	

Table 1: Clinicopathological features of the ECG-positive cases in PTB (n=31)

Twenty-nine cases (94%) showed positive sputum culture for *M. tuberculosis* consistent with sputum cytology, and the remaining cases were also culture-positive with other sputum samples. Twenty-eight cases (90%) were sputum smear-positive for acid-fast bacilli, and 11 (35%) also had a large (3+) amount of mycobacteria on the sputum smear. The frequency of ECG positivity in smear-positive PTB cases was 1.1% (28/2521 cases).

There was one case of ECG positivity in PNTM (*Mycobacterium avium* complex lung disease (MACLD). Radiography did not show nodular bronchiectasis, a typical radiographic pattern of MACLD [16], but fibrocavitary MACLD, with extensive spreading of the disease and upper lobe cavitary shadow on radiography, similar to PTB in this study. Bacteriological examination of the smear and culture of PNTM sputum were also positive, like most TB cases in this study. Thus, no notable differences in clinical features between PTB and PNTM were observed.

Discussion

The significance of sputum cytology in patients with respiratory diseases, especially PTB, has been discussed by Nasiell et al. [17] in 1972; 0.2% of epithelioid cells and/or multinucleated giant cells were observed in smear cytological specimens of sputum and/or bronchial secretions. Of the 66 patients with epithelioid cells and/or multinucleated giant cells, a majority had PTB (40 patients), followed by lung cancer (11 patients), and sarcoidosis (three patients). Roger et al. [18] reported sputum cytology in PTB patients revealed an extremely high incidence of epithelioid cells (28%) and Langhans giant cells (5%). Further, Tani et al. [19] reported a high frequency of epithelioid cells (56%) in PTB patients using cell-block preparations. These reports indicate that the presence of epithelioid cells and Langhans giant cells in sputum and/or bronchial secretions implies a strong indication of PTB; however, they do not describe the clinical characteristics of these PTB patients.

Recently, Bhatia and Singh [6] reviewed cytological diagnoses of granulomatous inflammation and noted ECG has been observed in tuberculosis, sarcoidosis, occupational lung diseases, and fungal infections such as aspergillosis if polymorphous exudates are also present. They also indicated that the presence of necrosis with ECG is suggestive of tuberculosis.

In this study, ECG positivity on sputum cytology was 0.3% among patients with respiratory diseases and, unlike Nasiell's report [17], ECG positivity was restricted to pulmonary mycobacteriosis, especially PTB (0.8% in PTB and 0.09% in PNTM), whereas in cases with other granulomatous respiratory diseases such as fungal diseases, sarcoidosis, and cases with lung cancer, ECG were absent. Concomitant findings of Langhans giant cells with or without necrosis were specific findings of PTB.

Previous studies have described histological ECG-positive rates of 61% in sputum-smear negative PTB [4] and 66% in sputum-smear negative PNTM [20] by transbronchial biopsy. Cytological ECG-positive rates obtained by fine-needle aspiration in patients with extrapulmonary tuberculosis were higher; 20% showing ECG without necrosis and 62% showing ECG with necrosis/abscesses in tuberculous lymphadenitis [7]; 89% of lupus vulgaris showing cohesive ECG in cutaneous tuberculosis [8]; 85% with ECG in tuberculosis of the bone [9]. Although the ECG-positive rate was extremely low in comparison to these invasive and expensive examinations, sputum cytology is non-invasive and low-cost, and is routinely performed to rule out lung cancer in patients admitted with respiratory diseases. Through this analysis of a large sputum cytology database, we believe that although sputum cytology is not a primary procedure, it could be a useful aid for the diagnosis of pulmonary mycobacteriosis, especially PTB.

This study revealed the clinical features of ECG-positive cases; patients had PTB with cavity (68%) on radiographs and were positive for acid-fast bacilli in sputum smear (90%). Furthermore, the ECG-positive rate in sputum smear-positive PTB (1.1%) was greater than the rate in culture-positive PTB (0.8%). These findings suggest ECG in sputum cytology is associated with PTB severity.

Although there was only a single case of PNTM (MACLD) with ECG-positive sputum, the clinical portrait was atypical and severe, similar to the ECG-positive PTB cases, in its radiographic and bacteriological characteristics. ECG in sputum cytology may be associated with the clinical situation rather than the species of Mycobacterium.

Since detecting *M. tuberculosis* is the gold standard for the diagnosis of tuberculosis, ECG-positivity in sputum cytology is of course no more important than bacteriological examination for PTB diagnosis; however, sputum cytology is a non-invasive, cost-effective, and routine examination, so ECG-detection in sputum cytology may be a useful aid to cope with unsuspected, but highly infective PTB patients. Furthermore, the coexistence of necrosis and Langhans giant cells are highly indicative of PTB diagnosis.

Acknowledgments

This work was supported in part by a grant from Research in the Policy Based Medical Service Network for Chest Diseases in Japan. The authors thank Mr. Kenshi Urata, Pathology Division, National Hospital Organization Tokyo National Hospital, for technical assistance.

References

1. Sandor M, Weinstock JV, Wynn TA (2003) Granulomas in schistosome and mycobacterial infections: a model of local immune responses. Trends Immunol 24: 44-52.
2. El-Zammar OA, Katzenstein AL (2007) Pathological diagnosis of granulomatous lung disease: a review. Histopathology 50: 289-310.
3. Gonzalez-Juarrero M, Turner OC, Turner J, Marietta P, Brooks JV, et al. (2001) Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with *Mycobacterium tuberculosis*. Infect Immun 69: 1722-1728.
4. Tamura A, Shimada M, Matsui Y, Kawashima M, Suzuki J, et al. (2010) The value of fiberoptic bronchoscopy in culture-positive pulmonary tuberculosis patients whose pre-bronchoscopic sputum specimens were negative both for smear and PCR analyses. Intern Med 49: 95-102.
5. Danila E, Zurauskas E (2008) Diagnostic value of epithelioid cell granulomas in bronchoscopic biopsies. Intern Med 47: 2121-2126.
6. Bhatia A, Singh N (2008) Tissue reaction patterns in respiratory infections: a practical approach to a cytological diagnosis. Indian J Chest Dis Allied Sci 50: 33-38.
7. Bezabih M, Mariam DW, Selassie SG (2002) Fine needle aspiration cytology of suspected tuberculous lymphadenitis. Cytopathology 13: 284-290.
8. Kathuria P, Agarwal K, Koranne RV (2006) The role of fine-needle aspiration cytology and Ziehl Neelsen staining in the diagnosis of cutaneous tuberculosis. Diagn Cytopathol 34: 826-829.
9. Handa U, Garg S, Mohan H, Garg SK (2010) Role of fine-needle aspiration cytology in tuberculosis of bone. Diagn Cytopathol 38: 1-4.
10. Gupta KB, Tandon S, Kalra R, Parkash P (1999) Cytologic findings in sputum indicating pulmonary tuberculosis. Ind J Tub 46: 49-50.
11. Kumar B (2009) Granuloma in sputum cytology of pulmonary tuberculosis: a case report. Acta Cytol 53: 341-343.
12. PAPANICOLAOU GN, CROMWELL HA (1949) Diagnosis of cancer of the lung by the cytologic method. Dis Chest 15: 412-421.
13. Tamura A, Hebisawa A, Masuda K, Shimada M, Ichikawa M, et al. (2007) Coexisting lung cancer and active pulmonary mycobacteriosis. Nihon Kokyuki Gakkai Zasshi 45: 382-393.
14. Laurberg P (1975) Sarcoid reactions in pulmonary neoplasms. Scand J Respir Dis 56: 20-27.
15. Segawa Y, Takigawa N, Okahara M, Maeda Y, Takata I, et al. (1996) Primary lung cancer associated with diffuse granulomatous lesions in the pulmonary parenchyma. Intern Med 35: 728-731.
16. Wallace RJ Jr, Zhang Y, Brown BA, Dawson D, Murphy DT, et al. (1998) Polyclonal Mycobacterium avium complex infections in patients with nodular bronchiectasis. Am J Respir Crit Care Med 158: 1235-1244.
17. Nasiell M, Roger V, Nasiell K, Enstad I, Vogel B, et al. (1972) Cytologic findings indicating pulmonary tuberculosis. I. The diagnostic significance of epithelioid cells and Langhans' giant cells found in sputum or bronchial secretions. Acta Cytol 16: 146-151.
18. Roger V, Nasiell M, Nasiell K, Hjerpe A, Enstad I, et al. (1972) Cytologic findings indicating pulmonary tuberculosis. II. The occurrence in sputum of epithelioid cells and multinucleated giant cells in pulmonary tuberculosis, chronic non-tuberculous inflammatory lung disease and bronchogenic carcinoma. Acta Cytol 16: 538-542.
19. Tani EM, Schmitt FC, Oliveira ML, Gobetti SM, Decarlis RM (1987) Pulmonary cytology in tuberculosis. Acta Cytol 31: 460-463.
20. Tamura A, Muraki K, Shimada M, Suzuki J, Kashizaki F, et al. (2008) Usefulness of bronchofiberscopy for the diagnosis of pulmonary non-tuberculous mycobacteriosis-an analysis mainly on pulmonary M. avium complex disease. Kekkaku 83: 785-791.