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	Imaging and pathological findings of AIDS complicated by pulmonary rhodococcus equi infection: a comparative study			
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2008年北京市"十百千"百层次人才资助 项目,2009年获北京市高层【详情】	<b>Objective</b> To investigate the imaging features and pathological basis of AIDS complicated by pulmonary			

rhodococcus equi infection.

## 问题解答

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**Methods** A total of 13 cases of AIDS complicated by pulmonary rhodococcus equi infection was retrospectively analyzed based on their imaging, bacterial culture and pathological data, including 10 cases by chest CT scanning and X-ray radiology and 3 cases by X-ray radiology. All 13 cases were definitely diagnosed by bacterial culture, including 1 by CT-guided pulmonary puncture with following H&E staining and PAS staining for diagnostic biopsy and another 1 by bronchial biopsy with following H&E staining and PAS staining for pathological diagnosis. The imaging findings and the pathological findings of AIDS complicated by pulmonary rhodococcus equi infection were compared and evaluated. **Results:** Totally 9 subjects (70%) had radiological demonstrations of central ball liked high density shadows in unilateral pulmonary hilus areas; 10 (77%), cavities and liquefied levels; 3 (23%), pleural

effussion. The foci were found in pulmonary inner zone in 10 subjects (77%); in pulmonary outer zone, 1 (7%). The pathological findings included intra-alveolar hemorrhage, lymphocyte infiltration and granulation tissue proliferation, which were in line with the pathological process of necrotic pneumonia. After 8-month following-up of anti-rhodococcus equi therapy of these 13 cases, 9 cases had obviously decreased or shrunk pulmonary cavities, 1 death, 1 missed following-up, 1 completely absorbed foci and 1 not receiving reexaminations.

**Conclusions:** The radiological demonstrations of AIDS complicated by pulmonary rhodococcus equi infection are central ball liked high density areas in unilateral pulmonary hilus area, parenchymal changes, secondary cavities, ground glass liked changes in the lung fields, nodules and treeinbuds sign, which are characteristic rather than specific.

Key Words: AIDS, rhodococcus equi, pulmonary infection, radiological demonstrations, pathology

Rhodococcus equi infection, commonly occurring in grazing areas, especially in patients with AIDS or with T-lymphocyte immonodeficiency of various causes. Literature reviews reveal that comparitive studies of imaging and pathological findings of AIDS complicated by rhodococcus equi infection are rare. In this paper, a total of 13 cases of AIDS complicated by pulmonary rhodococcus equi infection was retrospectively analyzed based on their data of diagnostic radiology, bacterial culture and pathological findings to enrich knowledge of such an AIDS complication and thus to improve its diagnosis.

# Materials and methods

## Subjects and their recruitment

A total of 13 cases, admitted during the preriod from the year of 2003 to the year of 2009 to hospitals in Beijing (patients from Second People's Hospital of Lincang City, Yunnan Province; Longtan Hospital of Guangxi Zhuang Autonomous Area; People's Hospital of Hezhou, Guangxi Zhuang Autonomous Area; and Fourth People's Hospital of Nanning, Guangxi Zhuang Autonomous Area) and definitely diagnosed to have AIDS complicated by rhodococcus equi infection through bacterial culture and pathological biopsy or confirmatively diagnosed by local Center for Disease Control were recruited as the subjects of the study.

## **Reagents sources**

The bacteria identification was performed with Apicoryne manufactured by Biomerieux and culture medium from Oxvoid in Britain. The bacterial culture was rountinely conducted for pure breeding, with following Apicoryne indentification with additional catalase experiment.

### Identification of bacteria strains

All samples were inoculated into a blood plate at temperature of 35°C for 18 to 24 hours inoculation. The 18 strains bacteria growing in culture were characterized by following features: about 0.5mm in diameter, being opaque in slightly yellowish bacterial colony. After 48 to 72 hours inoculation, their diameter increased to 1 to 2mm and was susceptible to emulsification. The bacterial colony was mucus liked, generating orange-pink pigment and salmon-pink pigment with possible growth of plain agar. Gram staining of fresh bacterial colony was positive. The thallus was polymorphistic in blunt ended rod shape with a few in globular shape, oval shape, branch liked shape and paliform arrangements. Globular shape dominated the old cultures. All bacteria strains were positive in catalase test and the total 23 strains of bacteria were identified as rhodococcus equi with API CORYNE system.

## X-ray radiology

Chest standard lateral DR X-ray was performed with GEDR8000 type radiology system. CT scanning was performed with GE single-row CT system. Totally 13 cases received X-ray radiology and 10, helical chest CT scanning with single-row detector. Pitch, milliampere second and maximum kilovoltage was variable with mucus. The scanning slice thickness was 5mm for 4 cases and 10mm for 6 cases. Three senior radiologists discussed all the film findings by CT scanning and X-ray radiology for agreement of the diagnosis. The pulmonary tissue (Window width of 1,000-1,500 H and Window level of -500-600 H) and the soft tissue (Window width of 35-450H and Window level of 30-50H) were set. Contrast medium was not applied in any case. The clinical symptoms of each patient as well as their immunie level, diagnostic laboratory findings and their therapeutic process were recorded.

#### **Pathological examinations**

CT-guided pulmonary puncture and bronchial biopsy were conducted to harvest tissues, with following H&E staining for2 cases and PAS staining for 2 cases for their pathological diagnosis. **Clinical data** 

The total 13 cases included 8 males and 5 females aged from 20 to 55 years (with a mean of 31 years), with occupations of 5 city workers, 3 famers, 2 free lances and 3 unemployed. Of the total, 10 were married and 3 remained single, 2 with a history of drug abuse, 2 with a history of sleep walking and 3 with a history of blood transfusion. Fever was the common symptom of these 13 cases with a body temperature ranging from 38 °C to 40 °C. Coughing up reddish yellow phlegm was found in 10 cases, couphing up blood in 4, dyspnea in 11, pulmonary wet rales in 13; emaciation in 6, poor appetite in 6 and diarrhea in 2; arthralgia in 1; oral candida infection in all 13 and oral herpes in 4; chest pain in 4 and hepatitis B in 3 with 1 case having no obvious symptom. Their CD4+ lymphocyte count was 50 cells per  $\mu$ l.

The study was permitted by the Ethics Committee of Affiliated Beijing You'an Hospital of Capital Medical University and abnormalities demonstrated by chest X-ray or CT scanning, such as globular tumor, parenchymal changes, pulmonary atelectasis and pleural effussion were classified by senior chest radiologists based on their shapes and locations. The radiolucent shadow and liquified level at the center of globular mass shadows was taken as the critria for the judgement of tumor formation period. Enlargment of lymph nodes were defined as the short diameter being less than 10 mm. Of the total 13 cases, 12 had focal pulmonary lesions and 1 unilateral invasive lesions. The confirmative diagnosis was made by bacteria culture for the total 13 cases, with 1 by CT guided pulmonary puncture biopsy and H&E, PAS staining and 1 by bronchial biopsy with following H&E, PAS staining. The anti-bacterial therapies were administered for the 13 cases for 16 ~200 days and the anti-viral therapies for 30~355 days.

# Results

#### **Distribution of bacterial strains**

Of 23 strains of rhodococcus equi, 13 strains were found in sputum, which had been cultured for successive 3 times indicating no possibly polluted bacteria, 4 in urine samples, 1 in blood samples and 5 in pulmonary tissues.

#### **Radiological demonstrations**

Totally 9 cases (70%) of AIDS complicated by pulmonary rhodococcus equi infection had radiological demonstrations of central ball liked high density shadows in unilateral pulmonary hilar areas; 5 had effusive infiltration, large flaky or ball liked tumor shadow in right pulmonary hilar areas; 4 had effusive infiltration and large flaky or ball liked lymph node shadows in left pulmonary hilar areas. The foci was patchy or flaky, radiating from the hilar areas to lung fields with blurred edges illustrated in Figs A, B, C and D. There was also 1 case of atelectasis of the left lung as showing in Figs E and F, large amount of inflammatory exudates filling in the alveola and bronchi demonstrated as parenchymal changes, X-ray radiology demonstrating invasive dense shadows in the left lung, and CT scanning demonstrating shrinkage of pulmonary tissues with surrounding strip liked liquid density shadow with air bronchogram of major bronchi in parenchymal areas. In addition, partial atelectasis was found in 5 cases with lateral chest X-ray demonstrating strip liked increased density shadows in pulmonary segments, its top pointing to the hilus. Accompanying pulmonary cavities and liquified level were found in 10 cases (77%) including 6 cases of right cavity shadows with 4 cases of accompanying liquified level, 3 cases of left cavity shadows with 1

case of accompanying liquified level and 1 case of honeycomb cavity in superior, middle and inferior lobes of the left lung, demonstrated as having thickened cavity walls gradually thinner with increasing tensions of the cavity alongwith the progression of the complications and thus uneven thickness of the cavity walls (Figs. G and H). One case was found to have bilateral pulmonary hilar enlargement and dearrangement; 2 cases have mediastinal lymphadenectasis and 3 cases (23%) have pleural effussion. The foci were found in the inner area of lungs in 10 cases (77%); in the outer area of lungs in 1 case (7%). The 8-month following-up found obviously shrunk foci in all 13 cases after anti-rhodococcus equi therapies, with 9 cases having obviously decreased or shrunk pulmonary cavities, 1 case of death, 1 case of missed following-up, 1 case of completely absorbed foci and 1 case not receiving reexaminations.

Typical histopathology found necrotic cavity and soft tissue tumor and effusion after rhodococcus equi infection with a great amount of effusion of eosinophile granulocyte (Von Hansemann cell)<sup>[1]</sup>. H&E stainning revealed intra-alveolar hemorrhage with great amount of epithelial cells or collagenoblasts, parenchymal changes of the pulmonary tissues and thickened alveolar septum (Fig. I). PAS stainning demonstrated diffusive distribution or tribe–liked distribution of rod shaped pink or prunosus rhodococcus equi (Fig. J). The typical pathological demonstrations were necrosis, dense histocytic infiltration and accompanying cytoplasm particles ingurgitating cocci.

#### Discussions

Rhodococcus equi was firstly found in the year of 1923 and was known as corynebacterium equi. Subsequent findings of its substantial difference from corynebacteria equi by cytic wall structrual analysis contributed to its present categorization into rhodococcus equi [2], which have been commonly believed as pathogenic bacteria of horses, pigs and cattles, with few infections found in human being. In recent years, due to the increased occurrence of human immunodeficiencies, reports on human repiratory diseases and septicemia caused by rhodococcus equi has increased <sup>[3]</sup>. Rhodococcus equi, a kind of facultative parasites in cells, optimaly grow at the temperature of 30 °C, having a favorable growth temperature of  $10 \sim 40$  °C <sup>[4]</sup>. Rhodococcus equi was often misconsiderred as diphtheroid bacilla, sporeforming bacillus and micrococcus due to its uncertain findings from acid-fast staining and its polymorphism. In sheep blood agar, they can have synergistic hemolysis with staphylococcus aureus, monocytogenes listeria and corynebacterium pseudotuberculosis<sup>[5]</sup>, which is the characteristic features of rhodococcus equi. The toxicity properties of rhodococcus equi has been in speculation until recent findings of poisonous plasmids <sup>[6, 7]</sup>, which enlightens further study of its pathogenic mechanism.

E MARCHIORI et al. (2005) reported their findings of 1~2 months couphing and fever history with accompanying tachypnes and chest pain in 5 cases of AIDS complicated by pulmonary rhodococcus equi infection [8]. In this group of 18 such cases, there were fever with a body temperature of 38~40°C, coughing with reddish yellow phlegm in 10 cases and coughing up blood in 4 cases, dyspnea in 11 cases and wet rales of lungs in 13 cases, emaciation in 6 cases, poor appetite in 6 cases and diarrhea in 2 cases, arthralgia in 1 case, oral candida infection in all 13 cases and oral herpes in 4 cases, chest pain in 4 cases, hepatitis B in 3 cases and 1 case having no obvious symptom. The typical clinical manifestaions are fever, cough, dyspnea and chest pain, while emaciation, diarrhea and arthralgia are not characteristic. The commonest lesion of rhodococcus equi infection is chronic suppurative bronchopneumonia and extensive pulmonary abscess <sup>[8]</sup> with imaging demonstrations of sub-acute pneumonia with lung cavities.

Fig. A	Fig. B
Fig. C	Fig. D
Fig. E	Fig. F
Fig. G	Fig. H

Fig. I

Fig. J

Figs. A and B. AIDS patient definitely diagnosed by CDC, male, aged 30 years, having symptoms of cough, reddish yellow sputum, fever, chest pain, dull percussion note of the right lung and diminished respiration by ausculation; ball shaped tumor shadow with blurred edge in the right lung; sputum and blood culture with findings of rhodococcus equi; bronchial and alveolar irrigating solution culture with findings of

rhodococcus equi.

Figs. C and D. AIDS patient definitely diagnosed by CDC, male, aged 28 years, having symptoms of cough, reddish yellow sputum, fever, chest pain, dull percussion note in the left lung and disminished respiration by ausculation; ball shaped tumor shadow with blurred edge in the left lung; rhodococcus equi findings in sputum and blood cultures as well as in bronchial and alveolar irrigating solution cultures; bronchoscopic biopsy confirming rhodococcus equi infection after H&E and PAS staining. Figs. E and F. AIDS patient definitely diagnosed by CDC, male, aged 36 years, having symptoms of cough, reddish yellow sputum, fever, chest pain, dull percussion note and diminished respiration by ausculation of the left lung; X-ray radiology demonstrating diffusive dense shadow in the left lung; CT scanning revealed shrunk pulmonary tissue with surrounding strip liked liquefied density shadows; air bronchogram of major bronchi in parenchymal areas.

Figs. G and H. AIDS patient definitely diagnosed by CDC, having cavities in the right pulmonary fileds with variable wall thickness and liquefied-air level; surrounding nodules ground glass liked tissue changes. Fig. I. H&E staining demonstrating intra-alveolar massive hemorrhage, a great amount of red blood cells, intact cell walls, dominant amount of epithelial cells or collagenoblasts, parenchymal changes of pulmonary tissues, thickened alveolar septum.

Fig. J. PAS staining revealing cumulating piled strip shaped prunosus rhodococcus equi.

Prof. Chen Shi'en investigated infant horse infected with rhodococcus equi and found that the X-ray characteristic features of rhodococcus equi infection are different sized abscesses of the lungs and tendencies of alveloar fusion in foci locations. In present study, there were 9 cases of ball shaped mass shadows around pulmonary hilus, 4 cases of exudative infiltration or ball shaped mass shadows in the right pulmonary hilar areas, 1 case of infiltration and large flaky or ball shaped mass shadows in out area of the right lung, 4 cases of exudative infiltration and large flaky or ball shaped mass shadows in the left pulmonary hilar area, 1 case of diffusive atelectasis of the left lung, 5 cases of partial atelectasis, 9 cases of singular vomica, 1 case of multiple honeycomb liked cavities, 6 cases of the right cavity shadows, 4 cases of accompanying liquified level, 3 cases of the left cavities with 1 case of accompanying liquified level. There was also 1 case of bilateral flaky shadows in surrounding areas of bilateral pulmonary hilus, 3 cases of left pleural effusion, 2 cases of mediastinal lymphadenectasis, with exudative inflammation in surrounding areas of vomica, a little pleural effussion and/or pachynsis pleural adhesion. Due to the impaired immunodeficiency of the patients, rhodoccoccus equi may excessively proliferate in the alveoli to cause parenchymal changes of pulmonary tissues like ball shaped nodules and mass foci [9]. This group mainly has manifestations of central ball liked high density shadows in lateral pulmonary hilar areas, taking up 70 per cent (9 cases), with the maximal size of 5cm×6cm, being centrally dense and blurred edges as halo sign, whose imaging demonstrations being similar to those of pulmonary tumors.

E MARCHIORI and his colleagues studied 5 cases of AIDS complicated by pulmonary rhodococcus equi infection with CT scanning demonstrations of central lobular nodes infusion and tree bud signs in 3 cases distributing in the surrounding areas of parenchymal changes. Of the 3 cases, one had scanning demonstrations of the signs in the inferior lobes of both lungs. Rhodococcus equi has the ability to continuously impair alveolar macrophages, which constitutes its pathogenic basis, whose invasion of alveolar wall and interlobular septum causes pulmonary interstitial pneumonia and thickened interlobular septum. In this group, there was 1 case of exudative grid liked changes of both lungs, which may be related to the early course of the disease. CAPDEVILA and his colleages (1997) reported a group of cases suffering from AIDS complicated by pulmonary rhodococcus equi infection, the death rate being 15.4%. Donisi <sup>[10]</sup> investigated 12 cases of positive HIV complicated by rhodococcus equi infection, the average CD4+ lymphocyte count being 47 cells per ul, the blood culture being positive in 83% cases and the death rate being 58%. Harvey <sup>[11]</sup> investigated 11 cases of positive HIV complicated by rhodococcus equi infection, death occurring in 6 cases with a death rate of 54.5 %.

The death rate of this group is 7%, which is significantly different from other studies, the reasons of such differences possibly being directed related to occurrence regions, course of disease and availability of anti-bacterial and HAART therapies. E MARCHIORI et al (2005) studied AIDS complicated by pulmonary rhodococcus equi infection and they found their subjects' CD4+ lymphocyte count being less than 50 cells per ul, while in this study the average immunity of the subjects were lower than the level of 49 cells per ul, which is in line with findings of former research. Rhodococcus equi infection complicating AIDS commonly has singular lesion in surrounding areas of pulmonary hilus with local foci and

accompanying mediastinal lymphadenectasis of the pulmonary hilus. Wicky and his colleagues [12] analyzed retrospectively chest X-ray radiology and routine CT scanning of 9 cases of AIDS complicated by pulmonary rhodococcus equi infection with findings of 8 out of 9 cases having mediastinal lymphadenectasis. Due to insusceptibility of AIDS patients to various infections like mycobacteria buberculosis, parasites, bacteria, virus and fungi, its imaging demonstrations are diverse and nonspecific, therefore, its diagnosis should combine clinical examination findings and pathological, laboratory test results [13].

AIDS complicated by pulmonary rhodococcus equi infection is clinically rare, whose diagnosis may be restricted to common bacterial infection and thus resulting in missed diagnosis and misdiagnosis. The presnt golden criteria for the diagnosis of rhodococcus equi infection is the results by bacterial culture and/or molecular diagnostic techniques, such as polymerase chain reaction (PCR). The Gram staining identifying small Gram positive bacteria facilitates its early diagnosis [14]. PCR techniques have been successfully applied into detection of rhodococcus equi [15] with favorable sensitivity and specificity, which is more accurate than bacteria culture [14].

As the developing of radiological techniques, it has been an important way for the early diagnosis of rhodococcus equi infection. However, due to its multiple and complex imaging demonstrations, the comparison to pathological findings is especially important. The imaging demonstrations should be differentiated from pneumocystis carinii pneumonia, pulmonary tuberculosis, staphylococcus aureus pneumonia, and central type lung cancer. Pneumoncystis carinii pneumonia has imaging demonstrations of ground glass liked changes in pulmonary fields with visible parenchymal changes and lobular central nodules.

Pulmonary tuberculosis commonly has imaging demonstrations of enlarged lymph nodes, large tubercles and parenchymal changes while staphylococcus aureus pneumonia has characteristic pathological findings and imaging demonstrations similar to those of bronchial pneumonia, especially lobular pneumonia [16] with tubercles of blurred edges and in diameter of 4-10mm. Staphylococcus aureus pneumonia develops rapindly while pulmonary rhodococcus equi infection is chronic, with the former having HRCT scanning demonstrations of lobular central tubercles and branch thread liked shadows (tree buds sign), which occurs in 40% patients [17]. Pulmonary abscess occurs in 15~30% patients with rhodococcus equi infection, which is commonly sigular with chest CT scanning demonstrations of oval shaped vomica with thick wall, possible intra-vomica liquifed level, irregular shaped inner wall of vomica constantly changing in short peirod of time.

In this study, 10 (77%) cases of AIDS complicated by pulmonary rhodococcus equi infection have pulmonary cavity and liquefied level, with thickened wall of the cavity, which becomes thinner alongwith the increasing tension inside the cavity. Central type lung cancer commonly demonstrated as unilateral oval shadows in the pulmonary hilus, rough edges, sometimes with sublobes or bronchostenosis, while AIDS complicated by pulmonary rhodococcus equi infection has ball shaped mass in the pulmonary hilus, commonly unilateral central ball liked high density shadows in the pulmonary hilar areas with dense centre and blurred edges without bronchostenosis. In addition, parenchymal changes have been rarely reported in cases of invasive pulmonary aspergillomycosis. This retrospective study has limits in limited case numbers and extensive imaging technique indeces. Further study should enlarge the samples to summarize the pathogenic basis and imaging characteristics of AIDS complicated by pulmonary rhodococcus equi infection.

#### References

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