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Histopathologic Features of *Pneumocystis carinii* pneumonia in 54 Thai AIDS patients

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Background : *Even though prophylaxis and treatment of PCP have been available, PCP continues to be a major source of morbidity and mortality in AIDS/HIV patients. Its detection is therefore a vital part of current AIDS/HIV management.*

Objective : *To study histopathologic features of PCP in Thai patients with AIDS and address an outstanding issue related to pathologic diagnosis.*

Setting : *Chest Disease Institute, the largest national referral center for the treatment of heart and lung diseases in Thailand.*

Research Design : *Retrospective review*

Materials and Methods : *Microscopic examination of transbronchial lung biopsy (TBLB) with PCP from AIDS/HIV patients. Tissue slides were stained with Hematoxylin & Eosin (H&E), Ziehl-Neelsen stain for acid-fast bacilli and Gomeri's Methenamine Silver.*

Results : *The non-specific findings of interstitial fibrosis (IF) and/or interstitial pneumonitis (IP) were found in all patients. Intra-alveolar foamy exudate was the most common specific change in PCP and was found in 49/54 (90.70 %). Other changes such as diffuse alveolar damage with hyaline membrane and pneumocyte type 2 hyperplasia and concomitant infection with CMV and TB were also observed. Issue related to pathologic diagnosis was addressed.*

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Conclusions : *A spectrum of histopathologic features of PCP is observed in Thai patients with AIDS. Recognition of these features and P. carinii morphology on special stains is very valuable in establishing the diagnosis of PCP and proper management.*

Keywords : *PCP, Pneumonia, HIV, AIDS, Lung infection, Lung pathology.*

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เหตุผลของการทำวิจัย : *ถึงแม้ว่าจะมีวิธีการป้องกันและรักษาโรคปอดนิวโมซิสติสก็ตาม แต่ทว่าโรคนี้ก็ยังคงเป็นสาเหตุต้นตอที่สำคัญที่ทำให้ผู้ป่วยติดเชื้อ AIDS/HIV เกิดอาการป่วยมากขึ้นรวมทั้งเสียชีวิตได้ ดังนั้นการวินิจฉัยโรคนี้จึงเป็นหัวใจสำคัญในการดูแลรักษาผู้ป่วยติดเชื้อ AIDS/HIV ในปัจจุบันนี้*

เป้าหมาย/วัตถุประสงค์ : *เพื่อศึกษาลักษณะพยาธิสภาพของโรคปอดอักเสบนิวโมซิสติส ในผู้ป่วยไทยที่ติดเชื้อ AIDS/HIV และอภิปรายชี้แจงถึงประเด็นที่เด่นชัดอันเกี่ยวข้องกับการวินิจฉัยโรคทางพยาธิวิทยา*

ประเภทโรงพยาบาล : *สถาบันโรคทรวงอกเป็นศูนย์ตติยภูมิที่ให้การรักษาผู้ป่วยโรคหัวใจและโรคปอดที่ใหญ่ที่สุดในประเทศไทย*

รูปแบบการวิจัย : *การตรวจชันสูตรย้อนหลัง*

สิ่งตรวจและวิธีการทำวิจัย : *ใช้กล้องจุลทรรศน์ตรวจชิ้นเนื้อปอดที่มีโรคปอดนิวโมซิสติส ซึ่งได้มาจากคนไข้ติดเชื้อคนไทย สไลด์ได้ถูกย้อมสี Hematoxylin & Eosin (H&E), Ziehl-Neelsen stain และ Gomeri's Methenamine Silver*

ผลการศึกษา : *ลักษณะพยาธิสภาพที่ไม่จำเพาะเจาะจงคือ Interstitial fibrosis (IF) และ/หรือ Interstitial Pneumonitis (IP) นั้นพบได้ในผู้ป่วยทุกราย ส่วนลักษณะที่เฉพาะสำหรับโรคปอดนิวโมซิสติส คือ Intra-alveolar foamy exudate นั้นพบได้ 49/54 ราย (90.70 %) และยังได้พบการเปลี่ยนแปลงอื่น ๆ เช่น diffuse alveolar damage with hyaline membrane and pneumocyte type 2 hyperplasia รวมทั้งการติดเชื้อร่วมกับ CMV และ TB ส่วนประเด็นที่เกี่ยวข้องกับการวินิจฉัยโรคก็ได้อภิปรายไว้ด้วย*

สรุป : *ได้พบลักษณะพยาธิสภาพต่าง ๆ ของโรคปอดอักเสบนิวโมซิสติสในผู้ป่วยไทยที่ติดเชื้อ AIDS การรู้จักลักษณะพยาธิสภาพต่าง ๆ เหล่านี้รวมทั้งการดูลักษณะเชื้อนิวโมซิสติสด้วยการย้อมพิเศษจึงมีประโยชน์อย่างมากในการวินิจฉัยโรคปอดนิวโมซิสติส และการดูแลรักษาที่ถูกต้อง*

คำสำคัญ : *พีซีพี, นิวโมเนีย, เอชไอวี, เอดส์, โรคติดเชื้อในปอด, พยาธิสภาพในปอด*

The first case of *Pneumocystis carinii* pneumonia (PCP) was reported in the United States in 1956. PCP has emerged as a serious opportunistic lung infection in those receiving immunosuppressive treatment for malignant or autoimmune disease or following organ transplantation even prior to the Acquired Immunodeficiency Syndrome (AIDS) epidemic. In fact it was PCP that led to the first discovery of the new deadly disease, AIDS.

The first documented case of AIDS death in the Western world was due to PCP. [1] It was Dr. Grethe Rask, a female Danish surgeon who died of PCP on December 12,1977. [2] In the early 1970's, she had practiced in Democratic Republic of Congo (formerly named Zaire), Africa, performing surgery with minimal personal protective equipment (PPE). She returned to Denmark after being seriously ill for three years, only to discover that even the best medical specialists in her homeland had no idea what went wrong with her. Apparently, she contracted the virus through her occupation.

In the spring of 1981, two homosexual men were diagnosed of having PCP from lung biopsies at Cedars-Sinai Medical Center in Los Angeles, California, U.S.A. At that time, this incident was considered unusual because these patients had no known underlying condition for the opportunistic infection. Shortly thereafter, other similar cases showed up in the community. Following several unusual outbreaks of community-acquired PCP in homosexual men, AIDS was finally discovered in the United States in 1981. Two years later, the actual virus that caused AIDS was isolated and named Human Immunodeficiency Virus (HIV). Within the same year, PCP became the first disease in AIDS-defining illness. [3]

It has been well documented that the lung is the major target of involvement by many infectious diseases, neoplastic and other conditions in AIDS/HIV patients worldwide. Over 80 % of AIDS patients developed lung complications during their disease.[4,5] Data from autopsy studies disclosed lung lesions to be up to 90 % of AIDS patients. [6,7] Reports from the United States indicated PCP was the most common lung infection in AIDS patients and was found up to 80 % of them. [8-10] Publications from Thailand also reported PCP as being the most common lung infection in AIDS patients and the figures were 51/85 (60 %), 36 %, and 41 % respectively. [11-13]

In the past twenty years, a great deal of information on PCP has become available mostly from the United States. However, the data regarding histopathologic features of PCP are limited. In Thailand, there is a lot less information on all aspects of lung complications. This research endeavors to comprehensively review histopathologic features of PCP in Thai patients with AIDS and address an outstanding issue related to pathologic diagnosis.

Materials and Methods

A retrospective review was conducted at Pathology Department, Chest Disease Institute, Department of Medical Services, Ministry of Public Health. Chest Disease Institute is a 500-bed medical center located in Bangkok Metropolitan. It is the largest national referral center for the treatment of heart and lung diseases in Thailand. Thai AIDS/HIV patients with lung complications were identified from our surgical pathology records from the period of January 1, 1993 to December 31, 2001. The total of 161 AIDS/HIV patients underwent fiberoptic bronchoscopy (FOB) with transbronchial lung biopsy (TBLB) and/or

bronchial biopsy (BB) at Chest Disease Institute. The procedures were done by chest physicians as a part of diagnostic modalities of lung lesions. An average of one to six biopsies was obtained from each patient. Although, TBLB is the most common procedure for morphologic diagnosis of lung lesions in AIDS/HIV patients, it is not routinely performed in all of them. TBLB was done only in the complex, recalcitrant or otherwise non-diagnostic cases following routine clinical investigation. None of these patients received antiretroviral (ARV) drugs or prophylactic antibiotics.

All biopsies were processed in tissue processor and multiple serial sections of each patient specimen were routinely prepared for hematoxylin and eosin (H&E) stain and special histochemical stain including Ziehl-Neelsen stain for acid fast bacilli (AFB) and Gomeri's Methenamine Silver (GMS) stain. All slides of H&E stain, AFB and GMS stains of TBLB and BB of these 161 AIDS patients were retrieved for a microscopic examination. In addition to a bright light microscopic examination, all H&E slides were examined under polarized light.

Result

The total number of 304 H&E stained slides, 304 AFB stained slides, and 304 GMS stained slides from 161 Thai AIDS/HIV patients were available for review. Eighteen biopsies of 11 patients were excluded from this analysis due to being inadequate samples. The unsatisfactory specimen was determined by a lack of lung parenchyma, too small of specimen, severe crush artifact, or otherwise non-diagnostic material. The diagnosis of PCP was made in 54 patients from 286 biopsies of 150 Thai AIDS/HIV patients. There were 52 males and 2 females with male to female ratio of 26: 1; and age ranged from 21 to 56 years. The mean age of the patients was 33 years. Their clinical data are shown in Table 1.

Table 1. Clinical Data.

	Number of Patient (N=54)	Percentage
Age range 21 to 56 years		
Mean Age 33 years		
Age Group		
• 20 – 29 years	20	37.04 %
• 30 – 39 years	23	42.59 %
• 40 – 49 years	10	18.52 %
• 50 – 59 years	1	1.85 %
Total	54	100.00 %
Male	52	96.30 %
Female	2	3.70 %
Male / Female Ratio	52/2	26/1

Interstitial fibrosis (IF) and/or interstitial pneumonitis (IP) with a typical intra-alveolar foamy material were seen in 49 cases (90.74 %). *Pneumocystis carinii (P. carinii)* was confirmed with GMS stain in 43 out of 49 cases with these features. The other 6 cases showed no organism on GMS stain because the tissue in the paraffin block was exhausted after a repeat cutting. Concomitant infection with Tuberculosis (TB) was found in 2 patients (3.70 %). Concomitant infection of PCP and Cytomegalovirus (CMV) was found in one patient (1.85 %). Diffuse alveolar damage (DAD) with hyaline membranes was found in 13 out of the 49 cases (24.07 %). Reactive pneumocyte type 2 hyperplasia was found in 10 out of the 49 cases (18.52 %). In addition, there were 5 cases of IF and/or IP without intra-alveolar foamy exudate but *P. carinii* was identified on GMS stain. In conclusion, 48 cases of PCP were confirmed with the identification of *P. carinii* on GMS stain. The detailed histopathologic features of PCP in 54 Thai patients with AIDS are shown in Table 2. Photomicrographs of PCP pathology are illustrated in Figure 1 to 4.

Table 2. Histopathologic Features of PCP.

Histopathologic Features	**Number of Patient (N=54)**
I. IF with variable interstitial inflammation (IF and/or IP)	54 (100.00 %)
II. IF and/or IP with intra-alveolar foamy exudate	49 (90.74 %)
● *P. carinii* organism seen on GMS stain	43 (79.63 %)
● *P. carinii* organism not seen on GMS stain (Tissue blocks were exhausted after a repeated cutting)	6 (11.11 %)
● Co-infection of PCP and TB 2/49	2 (3.70 %)
● With Diffuse Alveolar Damage and Hyaline Membrane Disease 13/49	13 (24.07 %)
● With Pneumocyte Type 2 Hyperplasia 10/49	10 (18.52 %)
II. IF and/or IP without intra-alveolar foamy exudate But *P. carinii* organism was identified on GMS stain	5 (9.26 %)
● Co-infection with PCP and CMV (1/5)	1 (1.85 %)

Abbreviations: IF = Interstitial Fibrosis; IP = Interstitial Pneumonitis; GMS = Gomeri's Methenamine Silver; PCP = Pneumocystis carinii pneumonia; TB = Tuberculosis; CMV = Cytomegalovirus.

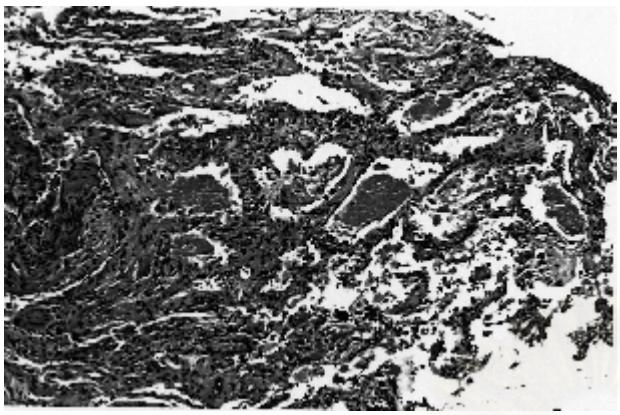

Figure 1. TBLB showing classic features of PCP. Note the characteristic intra-alveolar foamy exudate with granularity, prominent interstitial pneumonitis and patchy foci of fibrosis, (H&E stain X100).

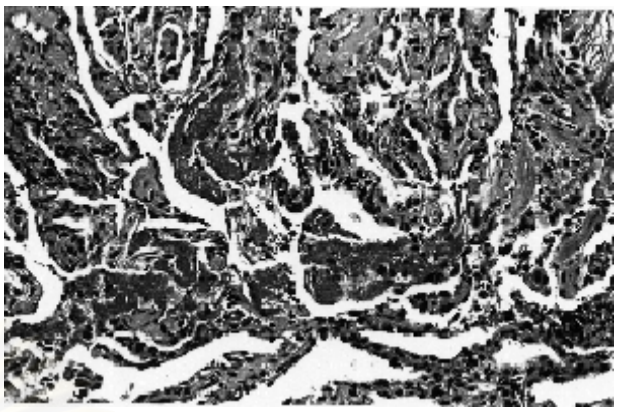

Figure 2. TBLB showing typical intra-alveolar foamy exudate, interstitial pneumonitis and patches of fibrosis. Note the prominent pneumocyte type 2 hyperplasia at the center, (H&E stain X 200).

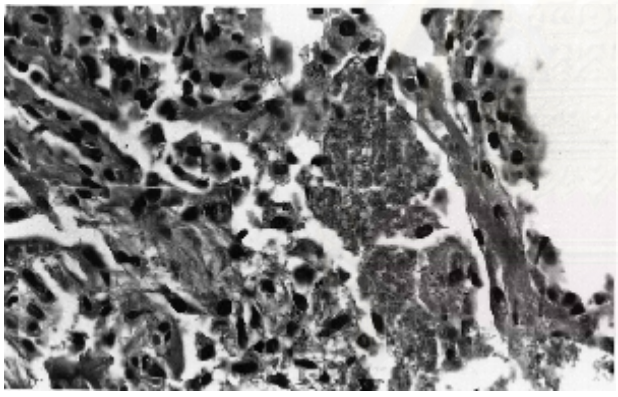

Figure 3. A case of PCP with a typical intra-alveolar foamy exudate containing basophilic dots, diffuse alveolar damage with hyaline membranes, and mononuclear cells infiltrate in the interstitial area, (H&E stain X 400).

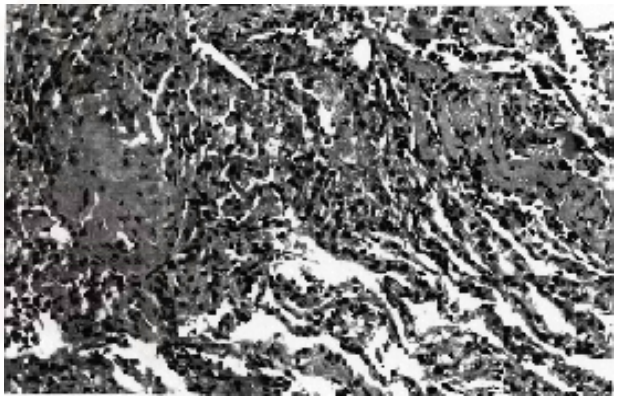

Figure 4. TBLB showing non-specific features of PCP. Note severe interstitial fibrosis and interstitial pneumonitis. Intra-alveolar foamy exudate is hardly seen in this biopsy; however, *P. carinii* organisms are detected on GMS stain, (H&E stain X 200).

Discussion

The population of AIDS/HIV patients who are at highest risk of PCP continues growing. This is in part due to the ongoing AIDS epidemic and prolonged survival of the patients following effective ARV therapy. Despite the availability of prophylaxis and treatment, PCP remains to be a major source of morbidity and mortality in AIDS patients. Thus the accurate diagnosis of PCP is a vital part of current AIDS/HIV management.

PCP is caused by *P. carinii,* an extracellular organism. Morphologically, *P. carinii* resembles a protozoan but it does not have typical protozoan intracellular organelles on electron microscopy. It is also sensitive to antibiotics that normally used for the treatment of protozoa. This property is different from those of most pathogenic fungi. No wonder it was originally classified as a parasite. However, it has recently been re-classified as a fungus based on sequencing analysis of ribosomal RNA and mitochondrial DNA. [14,15] Just like other fungi, it is best demonstrated on GMS stain. β-glucan in the cyst wall of *P. carinii* makes them stain well with periodic acid-Schiff (PAS) and GMS stain.

The diagnosis of PCP is not always easy. In Thailand, it is generally diagnosed based on clinical presentation and Chest X-rays findings. However, there are some complex and recalcitrant cases that require tissue diagnosis. These cases undergo FOB with TBLB in order to establish the definite diagnosis and proper management. FOB with TBLB is the most common procedure used for obtaining lung specimen for pathologic diagnosis of lung lesions in AIDS. In fact, it is done with the hope of finding a treatable disease.

TBLB provides a diagnostic yield for the detection of *P. carinii* of 95 % and can be improved to 98 % when it is used in conjunction with bronchoalveolar lavage (BAL). [16] In the case of PCP, some authors believe the diagnostic yield from BAL alone is good enough to justify the omission of TBLB. [17,18] In general, the diagnostic value of TBLB for other lung lesions is superior to BAL since it provides the tissue necessary for the diagnosis of many infectious diseases, tumors, and other pulmonary complications of AIDS.

The unique feature of this research is none of the patients received ARV drugs or PCP prophylaxis. Therefore, it is very likely that histopathologic changes in this study would reflect the true nature of PCP in AIDS patients. In this study, IF with variable interstitial inflammation were observed in all patients. However, the majority of them showed only minimal inflammatory infiltrates in the interstitial area. The infiltrates were composed of few lymphocytes and rarely plasma cells. In most cases, fibrosis was much more prominent than the features of interstitial inflammation. IF and/or IP are non-specific changes and may be caused by HIV infection or a repeated infection of various organisms or other toxicity. This study also concurred with other reports in that intra-alveolar foamy exudate was the most common specific histopathologic changes of PCP in AIDS patients. In some cases, basophilic dots corresponding to *P. carinii* were visualized in routine H&E stained slides.

Some authors believe that the presence of intra-alveolar foamy exudate with granularity in routine H&E sections is pathognomonic of PCP even in those rare cases when GMS stain is negative. [19] We accept this feature is distinctive enough to be diagnostic in the larger specimen from open lung biopsy or autopsy. In a tiny tissue from TBLB with variable degree of crush artifact, this frothy material is not readily visible on H&E stained slides. We do prefer the diagnosis of PCP to be confirmed with GMS stain which is the most reliable. Other conditions, such as pulmonary alveolar proteinosis (PAP) and pulmonary edema also show foamy material in alveoli. In addition, numerous foamy macrophages in parenchyma distal to bronchial obstruction may mimic frothy exudate seen in PCP.

A meticulous search must be conducted to identify the organism on GMS stain. It would be wise not to quit searching for other organisms after the discovering of *P. carinii* since concomitant infection is relatively common in AIDS.

We found 5 cases of IF and/or IP without intra-alveolar foamy exudate but *P. carinii* was discovered on GMS stain. This observation emphasizes the need to carefully examine special stain for organisms in all TBLB specimens from AIDS patients, even though the histopathologic changes may be non-specific and not suggestive of infection. Presence of *P. carinii* in the lung biopsy is diagnostic for PCP since this organism does not colonize in the lung. Studies using monoclonal antibodies and the molecular biology technique of DNA amplification have failed to demonstrate evidence of *P. carinii* colonization in lung tissue obtained at autopsy or in BAL from immunocompetent individuals. [20-22]

On GMS stain, *P. carinii* can be clearly seen in intra-alveolar foamy exudate as thin-walled, round to oval or crescentic cysts (Figure 5). In addition, small intra-cystic structures of sporozoites may be visualized in the form of rings, dots, or commas. When doing GMS stain, sections containing *P. carinii* rather than other fungal organisms must be used as the positive control in order to avoid false-negatives. [23]

P. carinii cysts need to be differentiated on GMS stain from other fungal spores, particularly those of *Histoplasma capsulatum*, *Candida* species and *Penicillium marneffei*. The spores of *Histoplasma capsulatum* are smaller than *P. carinii* cysts and do not exhibit central clear zones with areas of capsular thickening or crescentic forms but show focal budding forms. In addition, the spores of *Histoplasma*

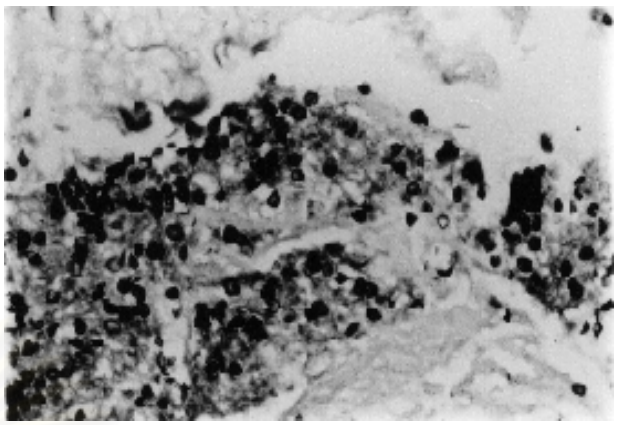

Figure 5. GMS stain showing numerous black staining *P. carinii* organisms. Note that most cysts are round to oval or crescent forms giving the appearance of 'cup and saucer' within the intra-alveolar frothy exudate, (GMS stain X 400).

capsulatum can be found within macrophages and in interstitial area. *Candida* is 3-4 μm yeast with multiple buds and narrow points of attachments. *Candida* yeasts can have elongated bodies, giving the appearance of pseudohyphae. *Penicillium marneffei* is 3-5 μm polymorphic yeast with thick wall and a single transverse septum at its center. Pathologic differentiation of PCP, Histoplasmosis, Candidiasis and Penicillosis are shown in Table 3.

Other chemical stains may be used but the staining quality is generally far inferior to GMS stain. Toluidine blue O and Gram Weigert also stain the cysts of *P. carinii* but it is difficult to interpret since cellular debris and other background materials can simulate the organisms. Giemsa, polychrome methylene blue (Wright's) or Wright-Giemsa (Diff-Quik) stain trophozoites and intracystic forms (sporozoites) of *P. carinii* but not the cysts [24, 25] and it is difficult to interpret on tissue sections. However, they are good

Table 3. Pathologic Differentiation of PCP, Histoplasmosis, Candidiasis and Penicillosis.

Diseases	H&E sections	GMS stain
PCP	Intra-alveolar foamy exudate IF and/or IP Diffuse alveolar damage Pneumocyte type 2 hyperplasia	5-8 μm cysts of *P. carinii* Round to oval cysts or crescent or helmet forms Central clear zone with capsular thickening No budding Mostly seen in foamy exudate
Histoplasmosis	Granulomatous Caseating or non-caseating Suppuro-granulomatous Suppurative, may be necrosis Rapidly calcified granuloma	3-4 μm spores Round to oval spores Solid stain with budding Spores are seen free in the lesion, or interstitium or within macrophages or multinucleated giant cells
Candidiasis	Suppurative Necrosis Granulomatous (less common)	3-4 μm yeasts multiple buds with narrow points of attachments Pseudohyphae Yeasts are seen free in the necrotic area or within macrophages
Penicillosis	Interstitial pneumonitis Suppurative Necrosis Cavitary lesion	3-5 μm yeasts Polymorphic yeasts Round, oval, or sausage forms with single central transverse septa No budding Found free or within macrophages

stains for direct smear samples. Papnicolaou (Pap) stain is a standard cytology stain and it is a reliable rapid stain for *P. carinii* from sputum or BAL but not for tissue section.

We would like to emphasize the importance of tissue processing and preparing extra-slides for AFB, GMS or other special stains for all cases of AIDS. Proper handling and processing of TBLB specimens are crucial to acquiring the best quality slides for microscopic examination. Some spare unstained sections must be properly taken at the time of the first cut for routine H&E sections otherwise the

diagnostic material may be gone for good upon a repeat cutting at a later date. We found 6 cases of classic PCP on H&E slides, unfortunately the organism cannot be discovered on GMS stain because the blocks were exhausted after a repeat cutting.

Diffuse alveolar damage with hyaline membranes and reactive pneumocyte type 2 hyperplasia were observed. However, these findings are not specific changes of PCP. They are pathologic features of acute lung injury caused by a variety of toxic insults such as toxic inhalants, drugs, radiation, sepsis, shock, and infectious agents including infection with *P. carinii.* [26]

Other features such as granulomatous inflammation, necrotizing pneumonia with cavitation, air-filled cysts or pneumatoceles, and lymphocytic interstitial pneumonitis (LIP) like lesion have been described in AIDS patients with PCP as uncommon histopathologic changes. [27- 30] However, these changes were not seen in our study. Vasculitis, with or without parenchymal necrosis, a rare feature of PCP was not observed in this study either. It is possible that all these atypical pathologic features are more common in AIDS patients who have received prophylactic therapy for *P. carinii.* Atypical features may also relate to the improved survival and the influence of ARV therapy. In addition, some atypical features may be seen in a certain age group. Reports indicated that the majority of AIDS patients who were younger than 16 years including infants had the entity LIP. [31, 32]

Conclusion

A spectrum of histopathologic features of PCP is observed in Thai patients with AIDS. Recognition of these features and *P. carinii* morphology on special stains is very valuable in establishing the diagnosis of PCP and its proper management.

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