**Lecture :4**

**Pneumocystis carinii**

*Pneumocystis carinii* is a eukaryotic microorganism that is found worldwide. Its host range is wide and includes humans and other mammals such as rabbits, dogs, goats, swine, cats, chimpanzees, owl monkeys, and horses. *Pneumocystis carinii* was first described as a developmental stage in the life cycle of *Trypanosoma cruzi* by Chagas in 1909 but was later recognized as a separate entity.

It was first shown to cause human disease in 1951. In Europe, an epidemic of interstitial plasma cell pneumonia occurred in premature and malnourished children, particularly those in orphanages in the late 1930s and early 1940s. A new epidemic of *P. carinii* pneumonia was described in the 1960s in children with congenital defects of the immune system and in children and adults with acquired defects secondary to malignancy or its treatment.

The relationship of *P. carinii* pneumonia with immunosuppression was established in organ transplant cases. In the *P. carinii* pneumonia was detected in previously
healthy men, which led to a search for an underlying cause of immunosuppression, and the pandemic known as acquired immunodeficiency syndrome (AIDS) was defined.

Those most at risk from *P carinii* pneumonia were identified on the basis of the degree of human immunodeficiency virus (HIV)–induced immunosuppression as measured by a CD4 T lymphocyte count less than 200. In the United States, Australia, and Europe, *P carinii* pneumonia in HIV-infected patients is seen largely in those unaware of their HIV serostatus at presentation or in those noncompliant with or intolerant of prophylaxis and antiretroviral therapy.
Morphology:

The morphological characteristics of *P. carinii* are quite constant in all mammalian species. Generally, 4 morphological forms are identified:

trophozoites, cysts, precysts, and sporozoites (also known as intracystic bodies). The trophozoites are pleomorphic & measure 2 to 4 under electron microscopy. Trophozoites appear as unicellular structures with a thin wall or pellicle and sometimes have 2 or more nuclei. The cyst is the diagnostic form of *P. carinii* & Giemsa, Papanicolaou, & Grocott methenamine silver nitrate stains & immunocytochemical techniques using monoclonal antibodies. Giemsa and Papanicolaou stained smears show an indirect evidence of *P. carinii* infection by the demonstration of foamy exudate in the form of alveolar casts as shown in fig. 1 and 2. This makes the Grocott methenamine silver nitrate
&immunocytochemical stains mandatory to confirm the cysts of *P. carinii* (fig. 3,4,5). In light microscope, the cyst appears as a spherical, cup-shaped or crescent-shaped object measuring 4 to 8µm in diameter. Some cysts are empty & collapsed. Other contains dark bodies or dots in silver stained preparation (fig.3). The dark bodies or dots are focal thickening of the cyst wall.(fig.6). Phase contrast & electron microscopy have revealed up to 8 sporozoites in the cyst. In transmission electron microscopy the exhibit various shapes depending on whether or not they contain Sporozoite.

The *Pneumocystis* organism causing disease in *Pneumocystis carinii* is seen in stain with two forms that may be in the alveolar walls as well as in the alveoli. The trophozoites range from 1-5µm and consist of a small nucleus surrounded by a mass of protoplasm that is variable in size. These forms are best seen in Giesma-stained impression smears of lung but can also be identified in Giesma or hematoxvlin-stained tissue sections. Electron microscopy shows filopodia or pili projecting from the amoeba like trophozoites, the cyst are about 5µm in diameter and are usually present among the uninucleated forms. In Giesma stained touch imprints of lung or smear prepared from bronchial washings, the cysts appear as round masses of unstained cytoplasm containing 2-8 purple stained nuclei presumably. The life cycle consists of asexual multiplication in the trophozoite stage and also in the cyst stage. However, the proposed life cycles are based primarily upon observations of material obtained from experimentally induced disease in immunosuppressed rates, and further work is needed to complete details of the life cycle, it is not known whether transmission occurs during close contact between hosts or by means of a stage capable of surviving in the outside environment for a significant period. Experimental infections in rats are not initiated by introducing *Pneumocystis*, it is either already presents in the lungs or enters from the environment during immunosuppression treatment.

Pathogenesis and Symptoms:
The presence of large numbers of Pneumocystis in the lungs results in intra-alveolar aggregates of serous exudates, various stages of the parasite, histiocytes, lymphocytes, plasma cells and cellular microbial debris. In advanced cases, most of the alveoli are partially or completely filled and the septa are thickened.

The material in the alveoli persists with little phagocytosis, or other inflammation, and there is deficient expectoration. At autopsy the lungs are dense in areas where the parasites are most numerous and the alveoli arc mostly filled; grossly, such areas look and feel like pancreas.
Microscopically, the alveoli have a honeycomb appearance especially in tissue sections stained by Warthin-Starry method. The septa are thickened by an infiltrate of lymphocytes, histocytes and also plasma cells. Deficiency of immunoglobulin production, parasites may not be numerous in the thickened septa even though many are in alveoli.

Incubation period of Pneumocystis is at least 6 weeks but may be much longer depending on the factors affecting the individual resistance, patients on high doses of corticosteroids or other immunosuppressive drugs develops symptoms in 6 to 8 weeks, the infection may be suspected in any patient with a diffuse or nodular bilateral pulmonary infiltrate that is disproportionate with the minimal physical findings consisting of malaise, anorexia, slight fever, dyspnea or non productive cough and with lungs usually clear to percussion and auscultation except for scattered rales. The WBCs may be normal or slight elevated and occasionally there is an eosinophilia.

Prognosis:
Increased dyspnea and cyanosis until death occurs by asphyxia.
Diagnosis:
Diagnosis have been made on the basis of clinical observations, every effort should be made to demonstrate Pneumocystis in material from the patient. Bronchial aspirates, transbronchial biopsy and open lung biopsy and percutaneous pulmonary needle biopsy have been successfully employed.

Ordinary cough specimens are usually unsatisfactory.

Animal inoculation is not useful because natural infection is almost universal and it required 6 to 8 weeks.

Although Pneumocystis has been cultured ,the technique is primarily maturation of uninucleated forms present in the inoculums and not analogous to bacterial cultures in which large numbers of organisms are derived from a few; therefore it is impractical.

Giesma staining of impression smears of tissue or smears of the sediment from bronchial aspirates reveals the uninucleated trophozoites as well as the organism within the cysts.

Grocott's methenamine silver nitrate stain of the impression smears as well as of tissue sections will stain the cyst wall and the distinctive crescent shaped thickenings in the cyst wall which resemble a pair of parentheses.

The specialized techniques such as immune-cytochemistry &polymerase chain reaction (PCR) are also used with slightly more promising results

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