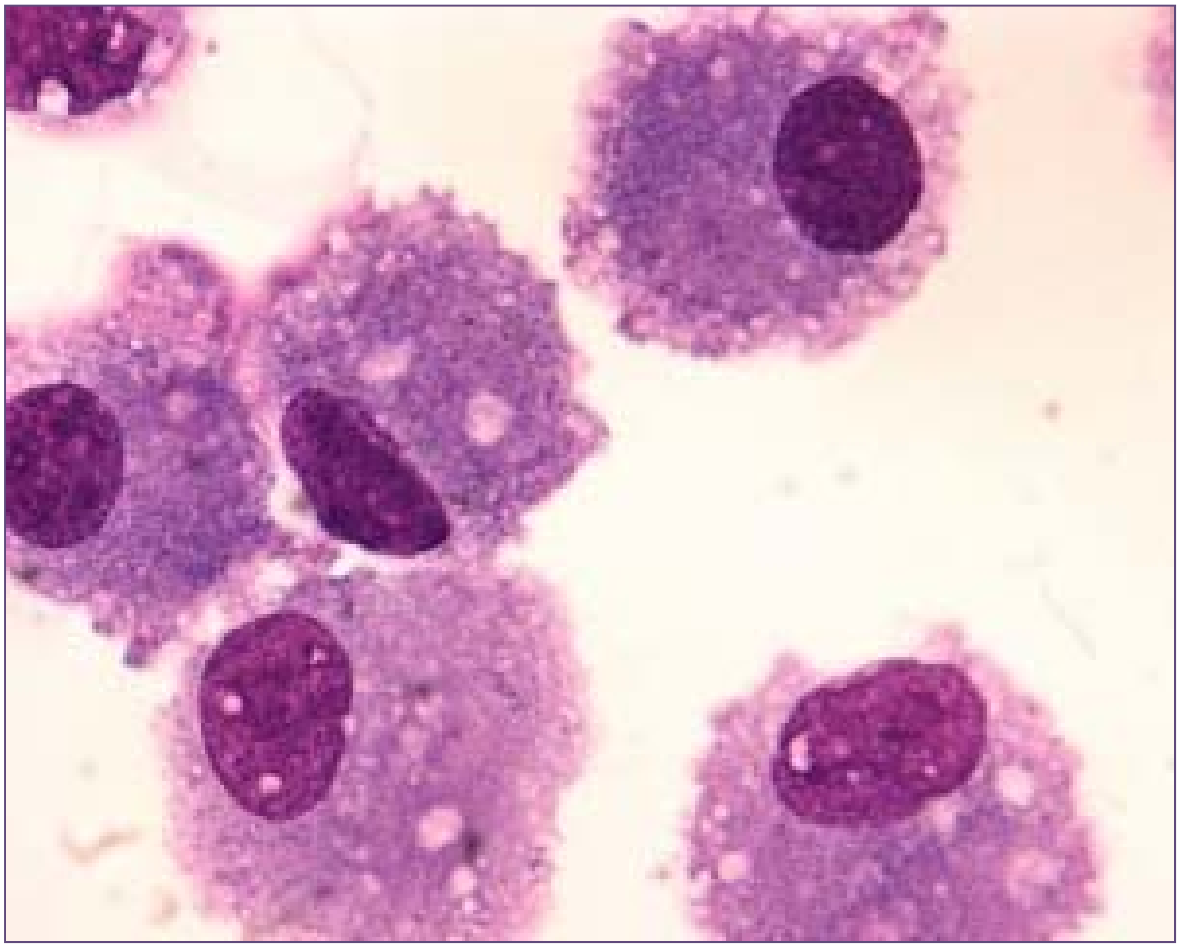
Bronchoalveolar Lavage (BAL)

Training Module

Created 10/31/13

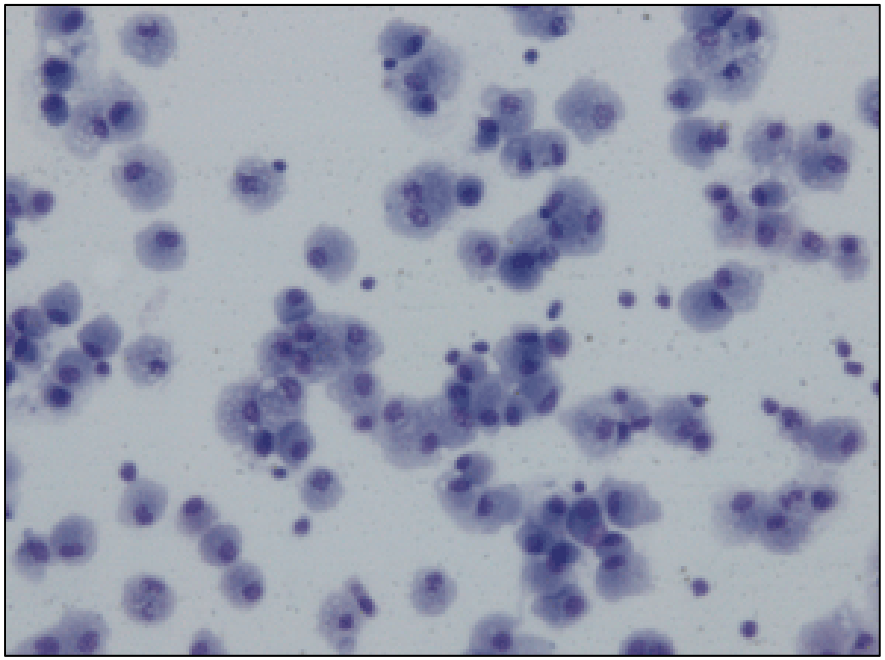
Cells normally present in BAL fluid

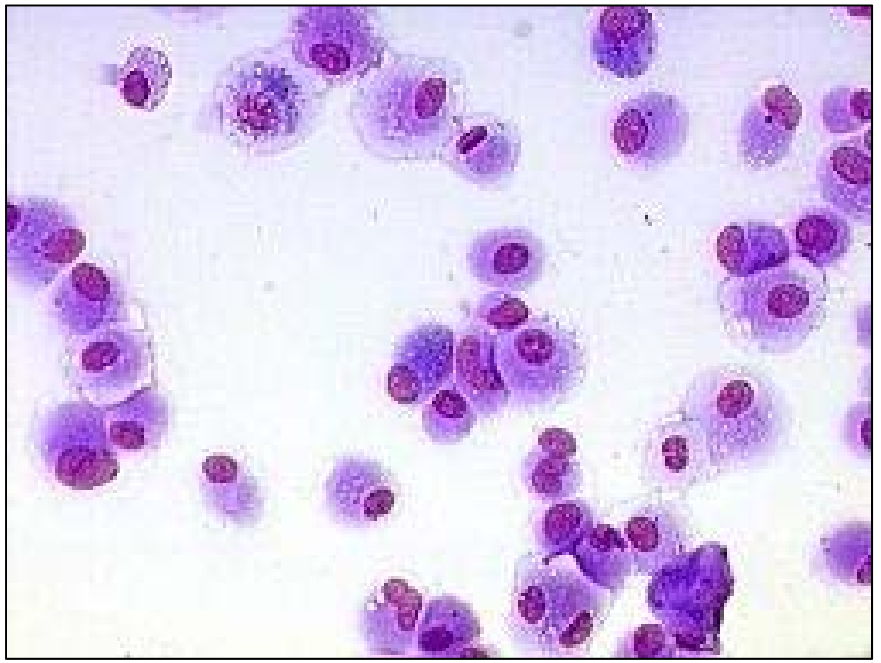
Alveolar Macrophages



Alveolar macrophages are mononucleated cells ranging in diameter from 10 µm to 40 µm. They have pale and abundant cytoplasm, which is equally proportioned around the nucleus. The nucleus is round to oval. The cytoplasm can contain all sorts of phagocytized material such as hemosiderin, carbon, micro-organisms and debris. Giemsa stain.

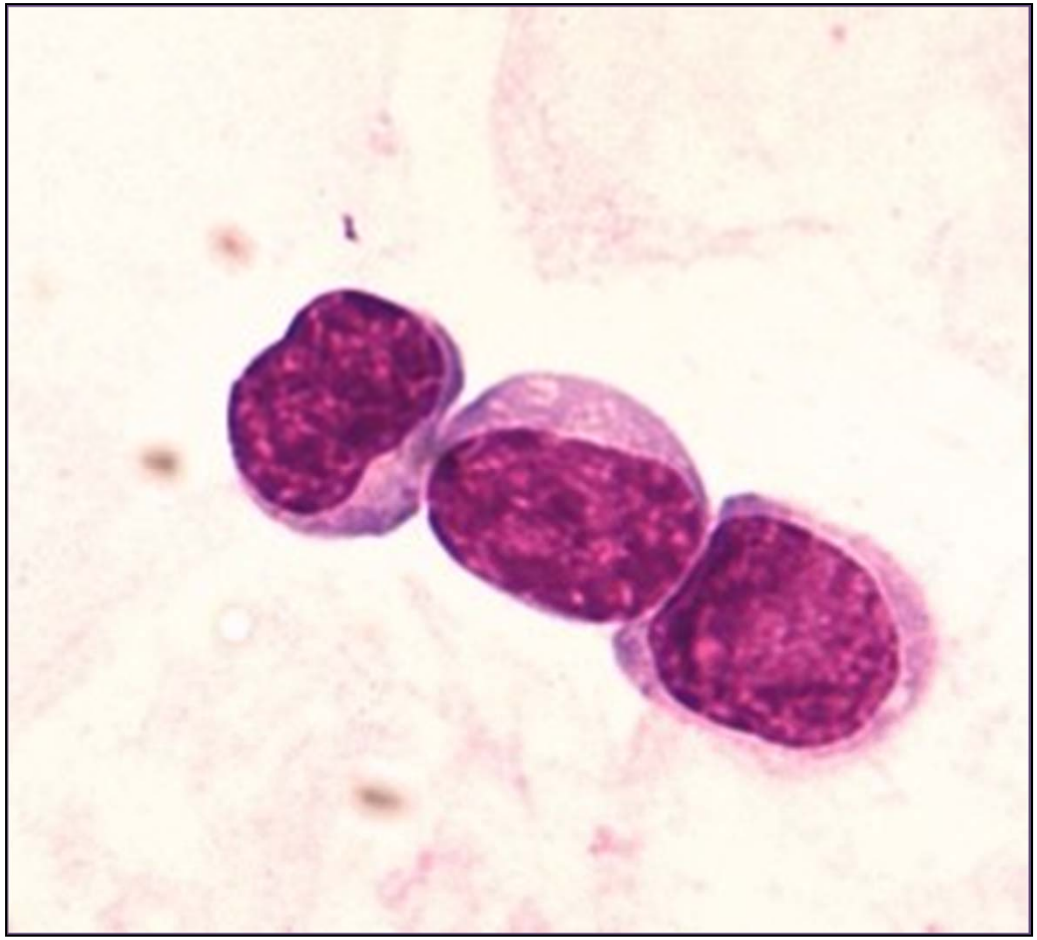
Low power BAL (cytospin)





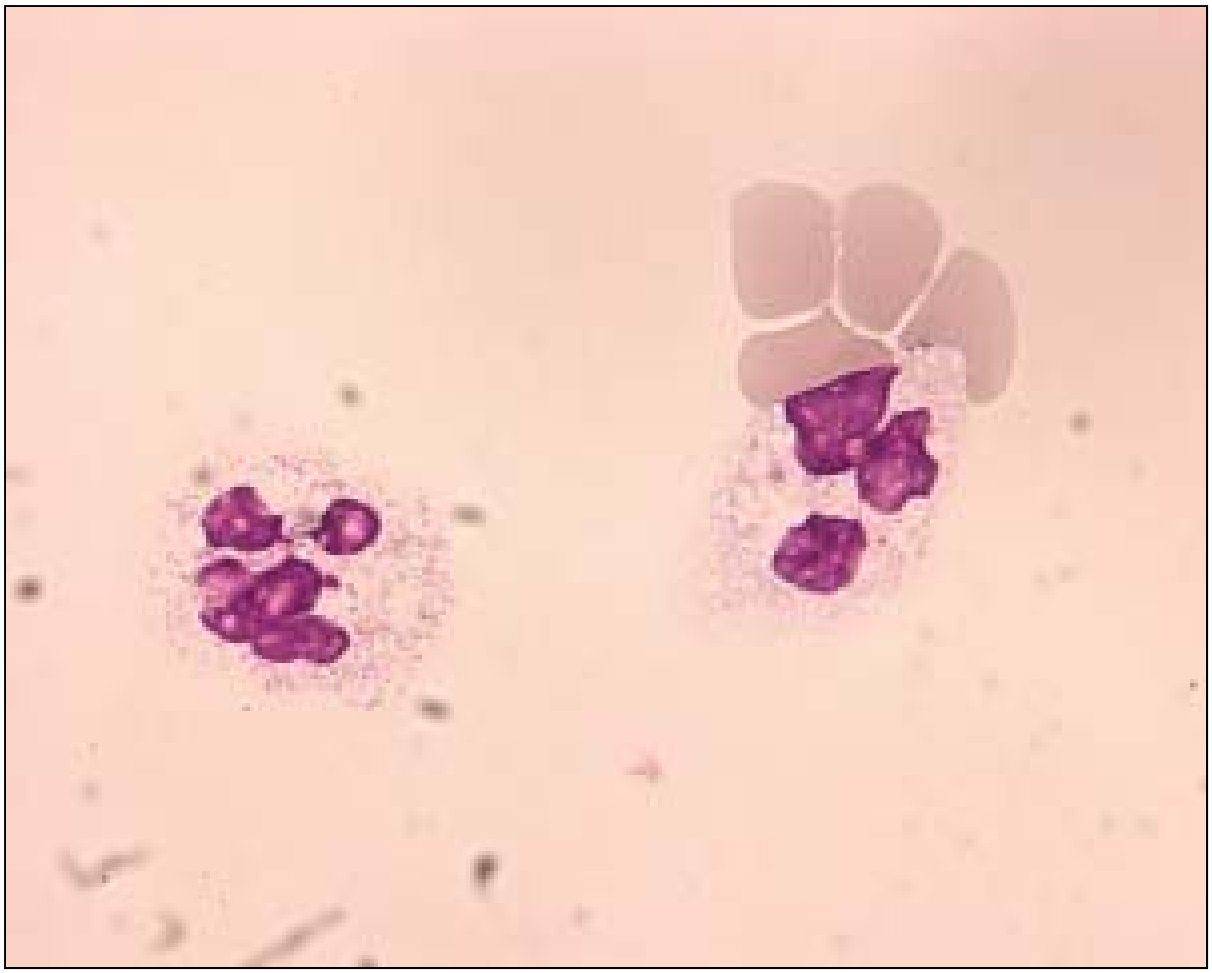
An adequate BAL fluid specimen should have abundant alveolar macrophages as seen here.

Lymphocytes



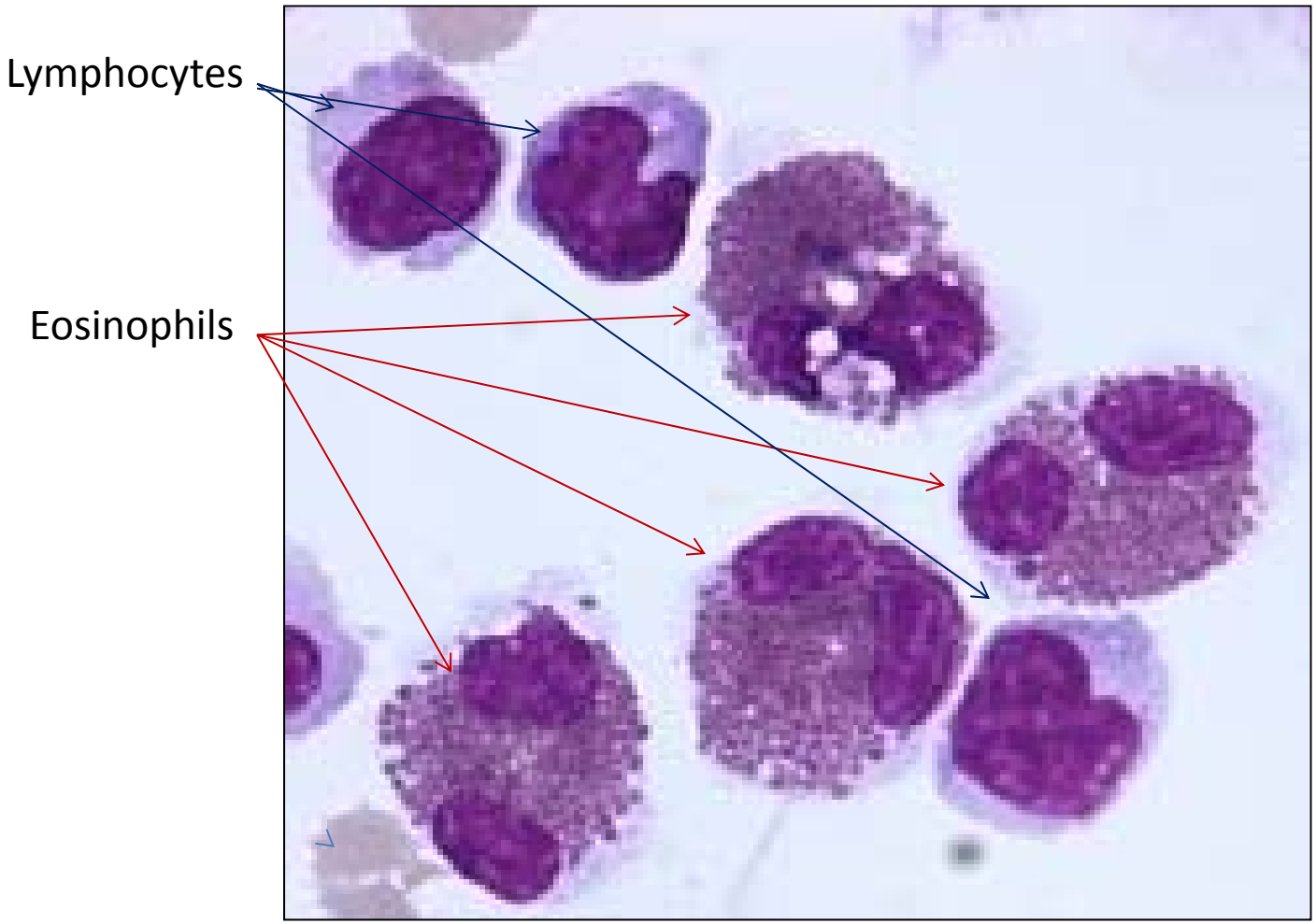
Three mature lymphocytes are visible. Lymphocytes are the smallest nucleated cells present in BAL fluid. They have a relatively large nucleus compared to the amount of cytoplasm. Sometimes only a nucleus is visible with a small rim of cytoplasm. Increased number of lymphocytes are seen in BALF samples of patients with sarcoidosis, PCP, Extrinsic allergic alveolitis and drug induced pneumonitis. Giemsa stain.

Neutrophils



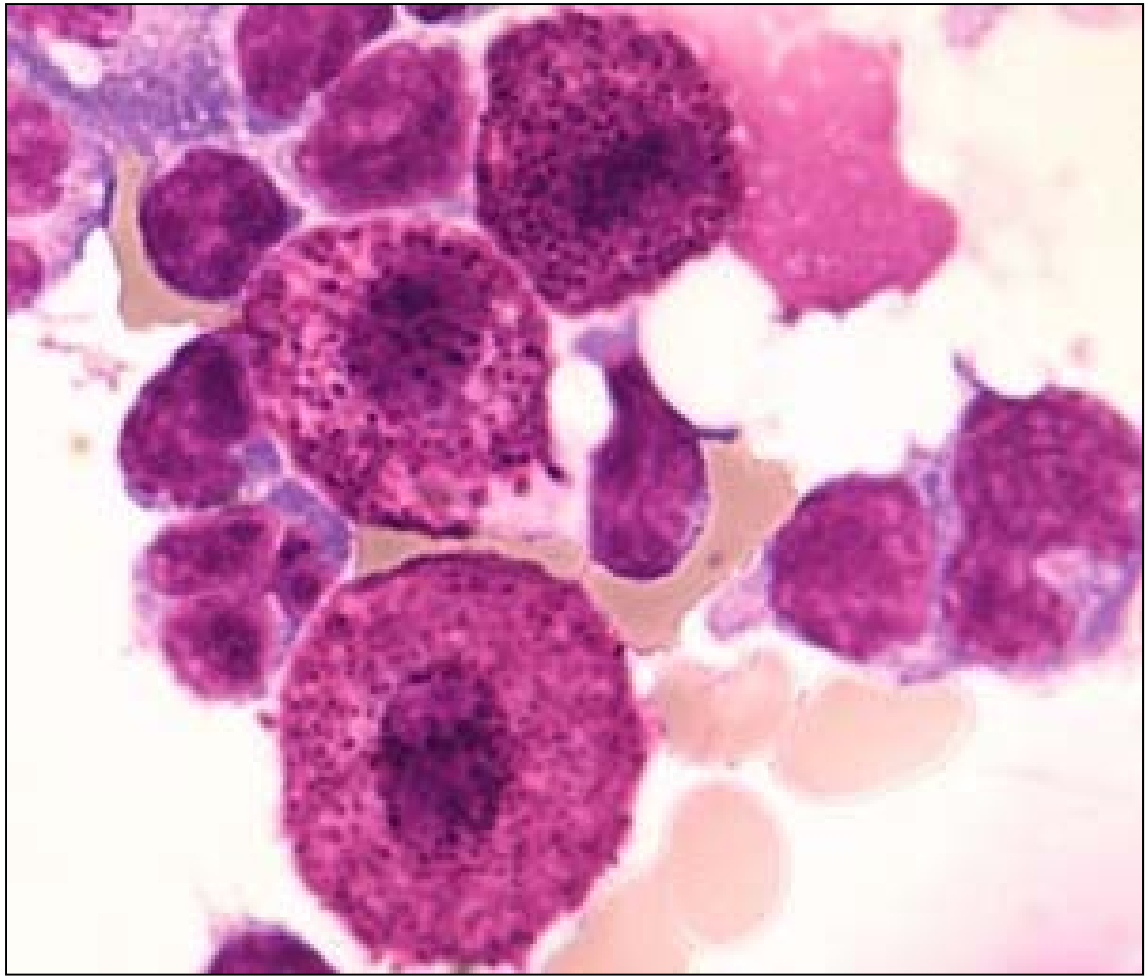
Polymorphonuclear neutrophils (PMN) have an average diameter of 12-15 µm. They have an irregular nucleus which consists of two to five lobes which are connected by delicate filaments. The cytoplasm is colorless and includes many small granules which stain pink to red in a MGG stain. Giemsa stain.

Eosinophils



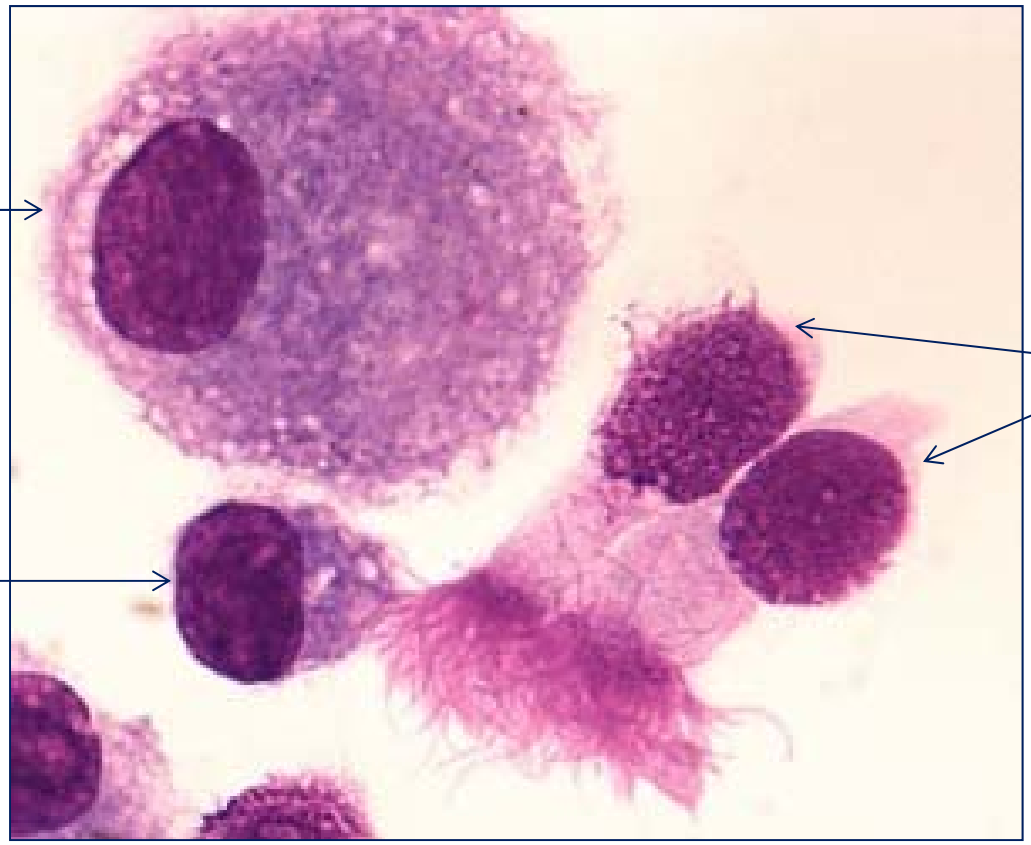
Eosinophils have nuclei with two lobes. Their cytoplasm contains large bright pink granules. The number of eosinophils in BALF may be increased in patients with asthma, drug-induced lung disease, acute or chronic eosinophilic pneumonia, extrinsic allergic alveolitis, idiopathic pulmonary fibrosis and pneumocystis pneumonia (PCP). Giemsa stain.

Mast Cells



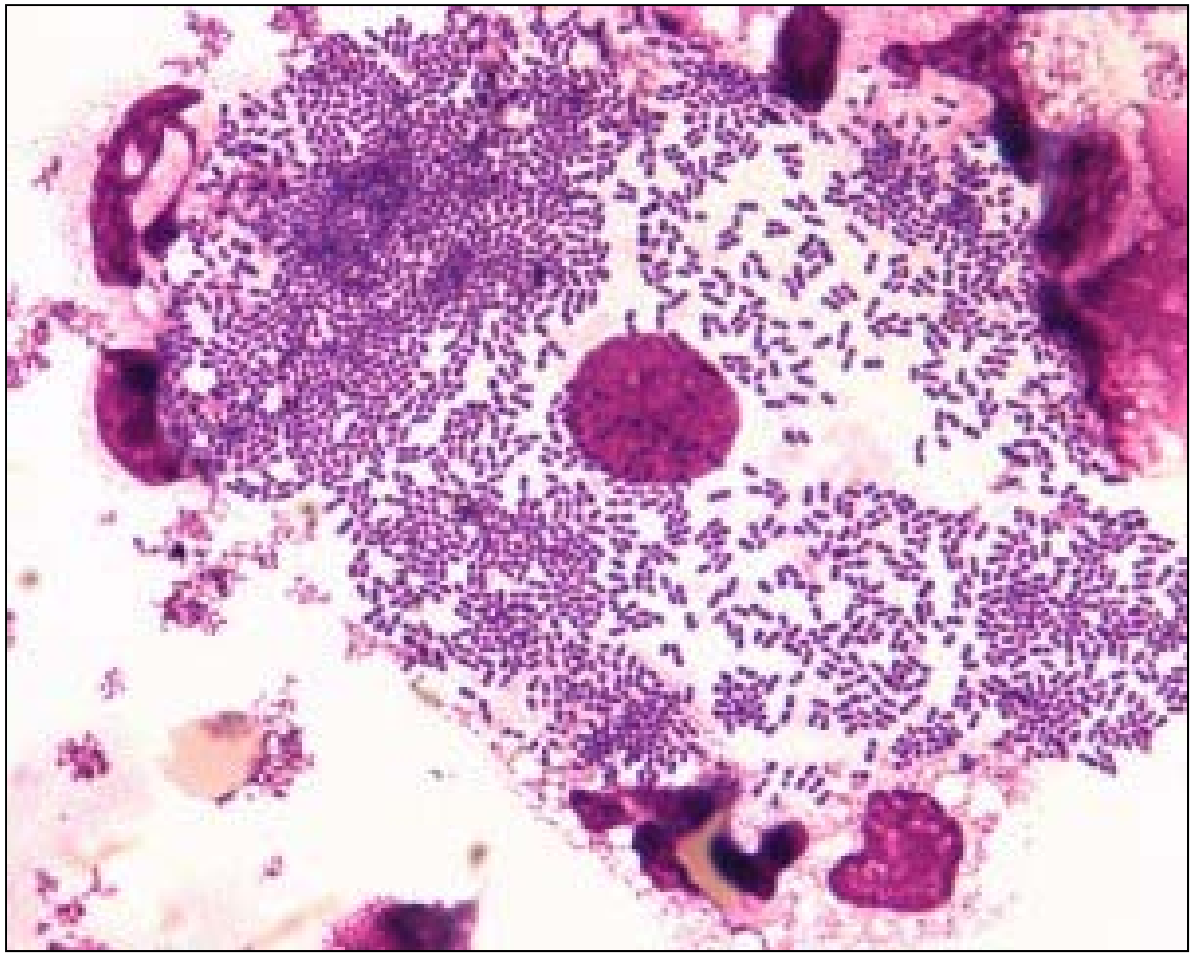
Three Mast cells surrounded by lymphocytes. The cytoplasm of mast cells is filled with small purple granules that often hide the oval, centrally located nucleus. These cells are rarely seen in BALF. An increased number of mast cells may be found in BALF of patients suffering from asthma, bronchiolitis obliterans or farmer's lung disease. Giemsa Stain

Bronchial Epithelial Cells



The presence of bronchial epithelial cells (ciliated epithelial cells and goblet cells) indicates contamination from the higher bronchial tract. The ciliated epithelial cells are easily noted columnar cells with a basal nucleus, a pale cytoplasm and a distinctive endplate with a bunch of cilia. Loose cilia can sometimes be confused with slim Gram-negative rods. The mucus secreting Goblet cells are more difficult to recognize. They are also pale, elongated cells with a nucleus at the base. The cytoplasm extends above the nucleus in a shape like that of a wine goblet. Giemsa Stain

Squamous Epithelial Cell

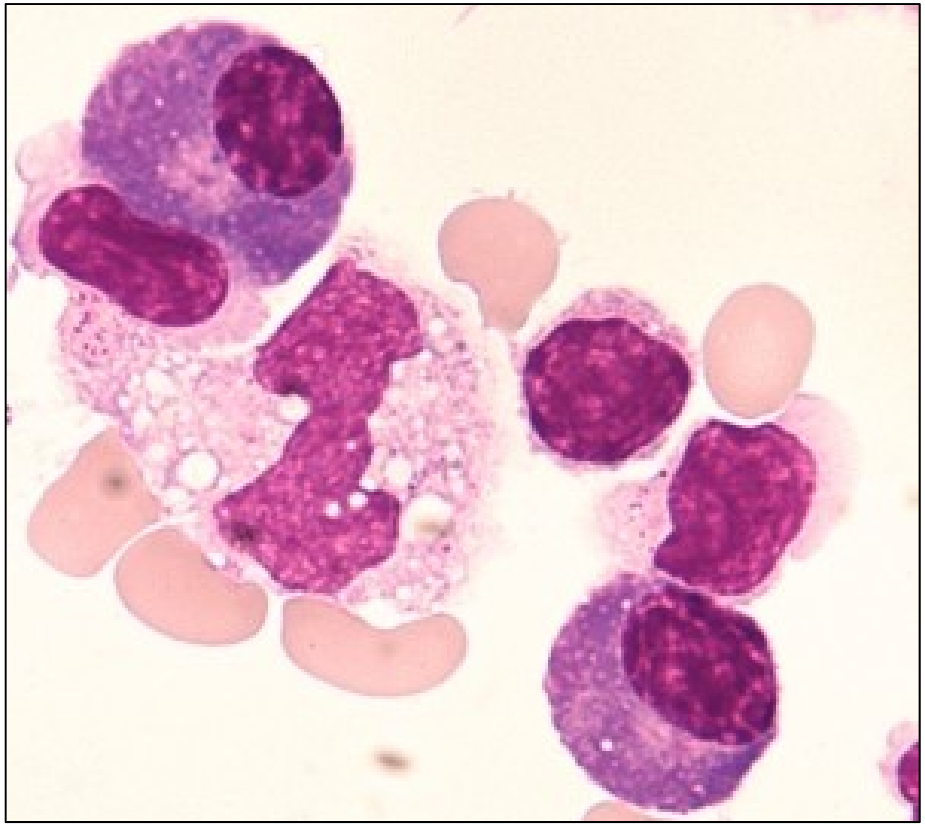


Presence of squamous epithelial cells in BAL fluid points to oropharyngeal contamination of the BAL fluid. They are large, square, flat, angular cells with a dark colored, relatively small nucleus. Often they are covered with bacteria (as in the image above) and can therefore influence the specificity of the quantitative culture in a negative way. Giemsa Stain

Cells not normally present in BAL fluid

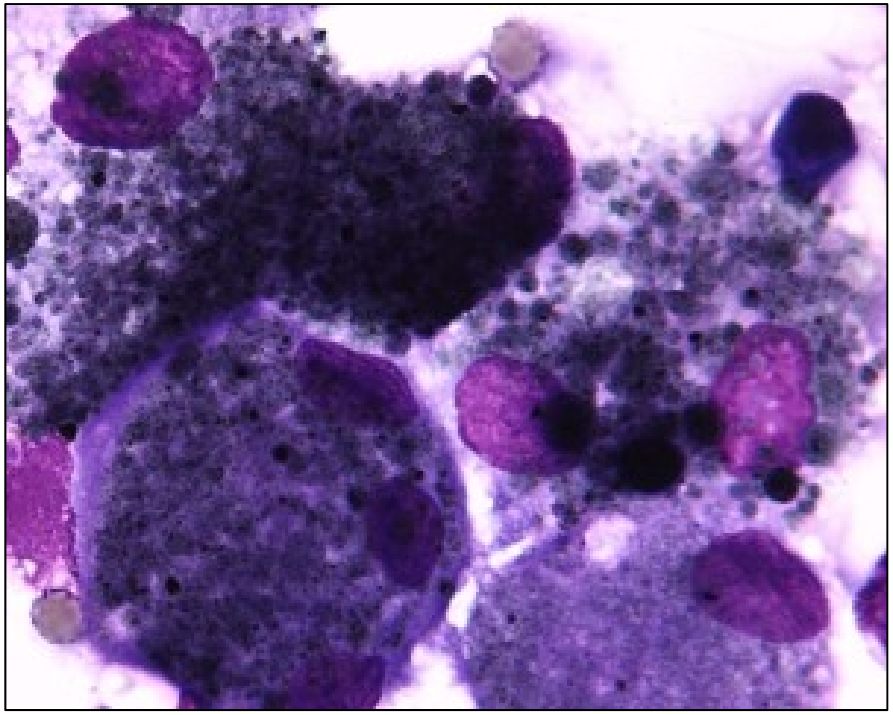
(Helpful hint: You should never see a meso in a BAL)

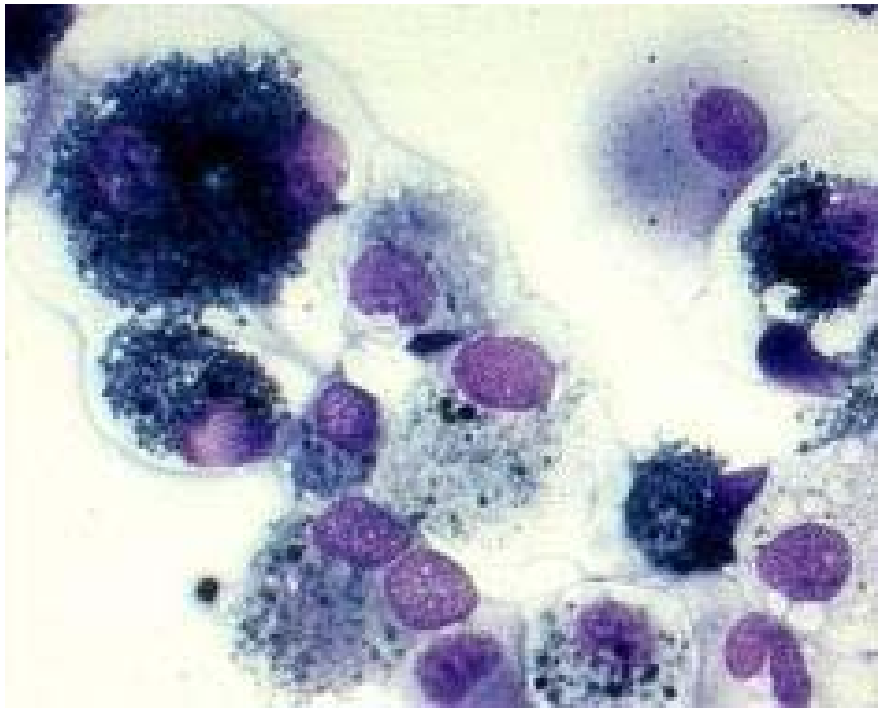
Plasma Cell



In the upper left corner and the lower right corner Plasma cells are present. Plasma cells are cells with a diameter of 8 to 20 µm, they have a similar appearance as lymphocytes, but with a round to oval nucleus which lies eccentric. Their cytoplasm is stained intense blue with a clear zone in the vicinity of the nucleus. Plasma cells can be seen in BAL fluid of patients with *P. jiroveci* pneumonia, drug-induced pulmonary disease, extrinsic allergic alveolitis and malignant lymphomas. Giemsa Stain

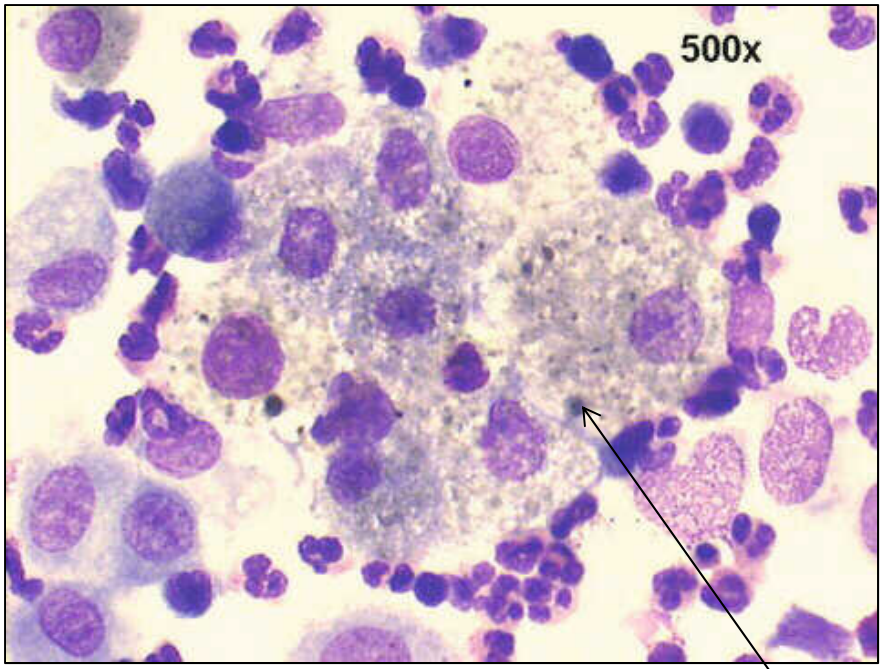
Macrophage with intracellular debris

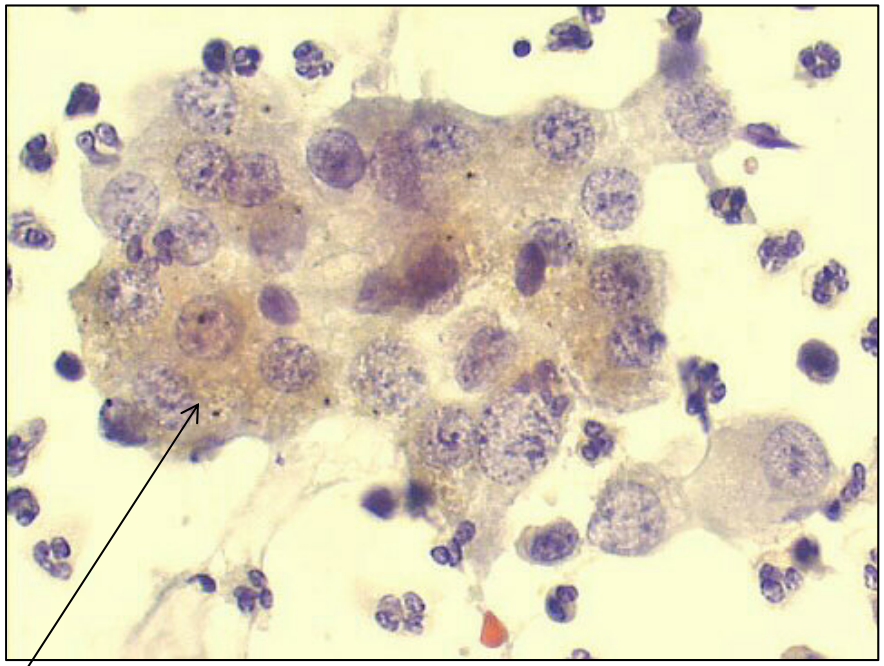




The cytoplasm of alveolar macrophages can contain all sorts of material which they have phagocytised, such as carbon, haemosiderin, cell fragments, foreign bodies or debris. In the Giemsa stain (left) this can be seen as dark blue to black granules within the cytoplasm of the alveolar macrophage. These particles are particularly prominent in the lungs of smokers.

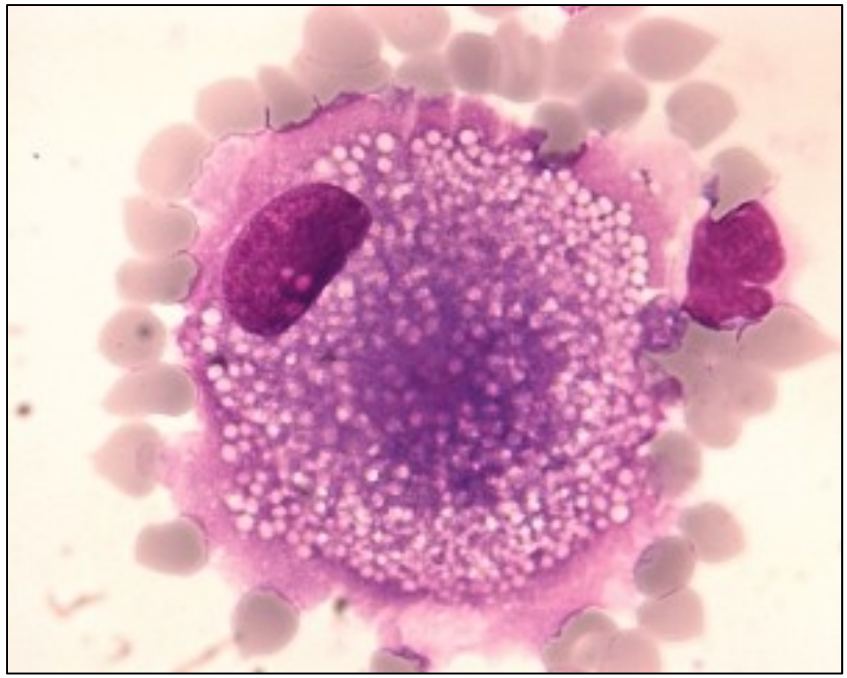
Macrophage with intracellular debris

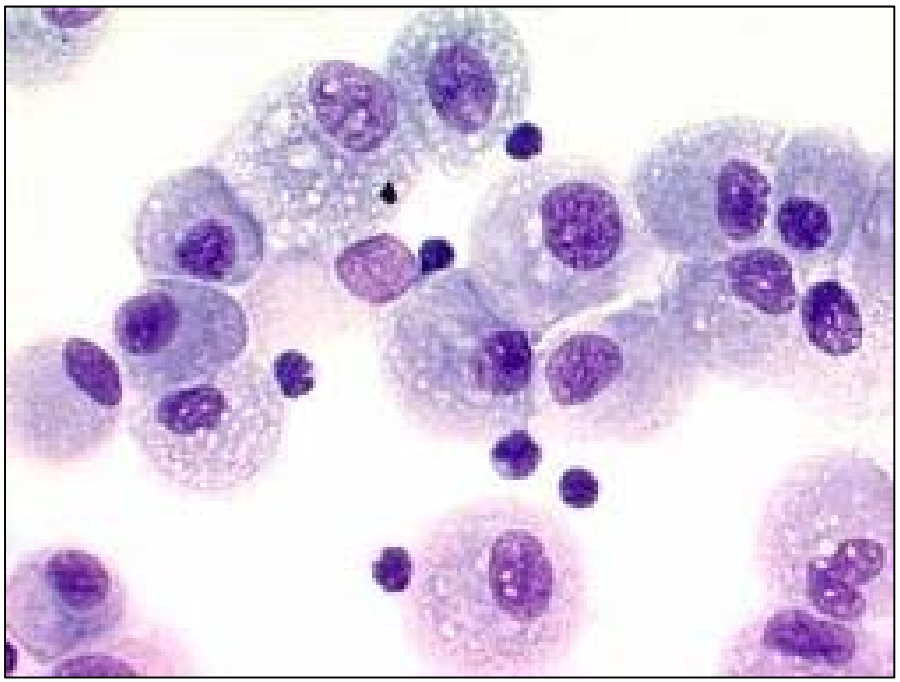




The BAL cytology smears show many pigmented alveolar macrophages. The question: is this melanin, carbon or hemosiderin? The answer lies in the staining for hemosiderin (Prussian Blue).

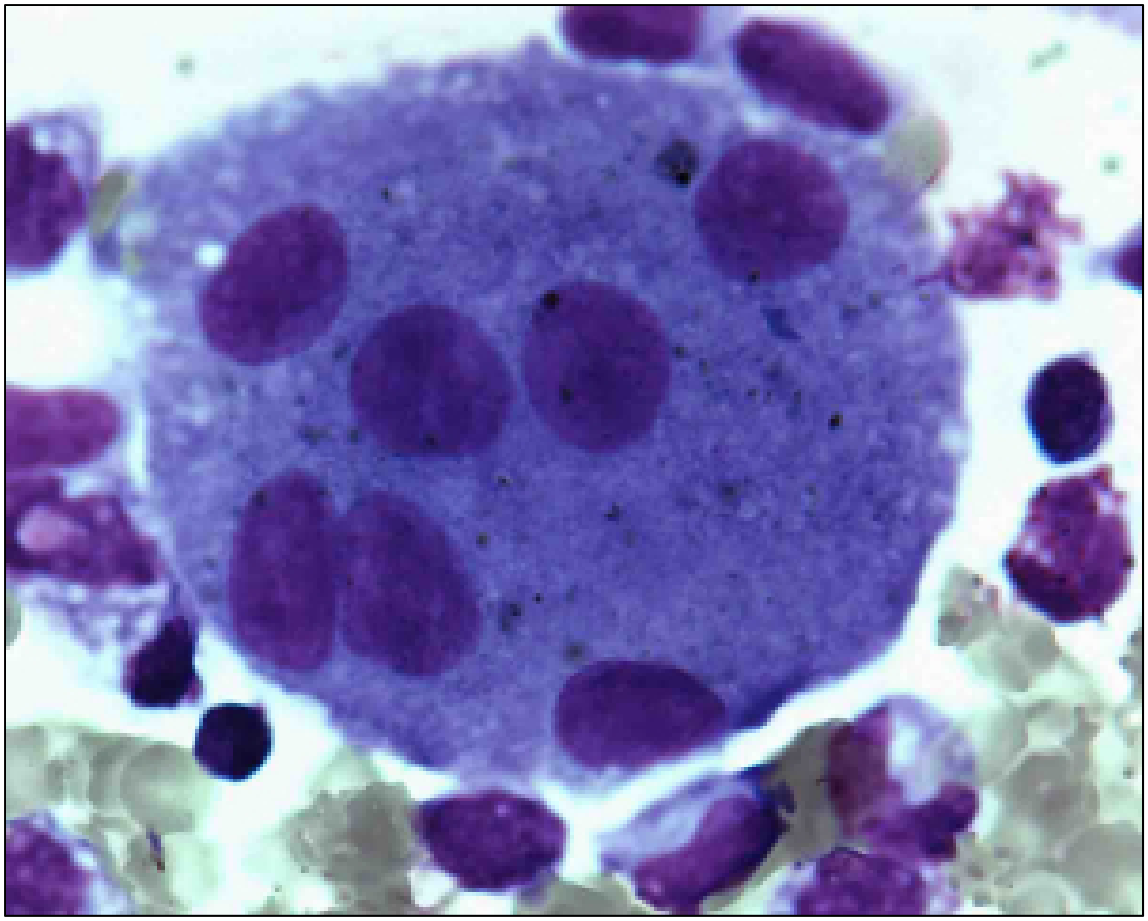
Foamy Alveolar Macrophage





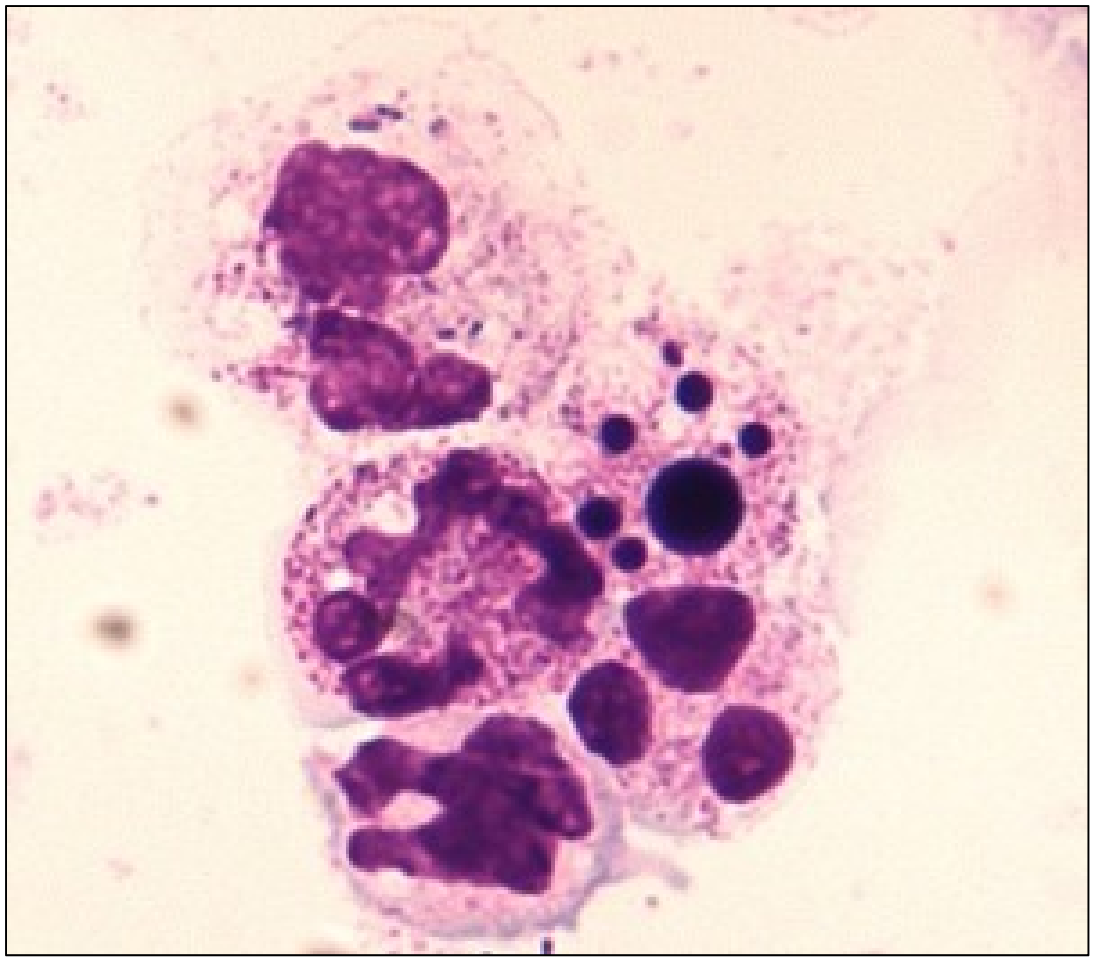
Foamy alveolar macrophages. The cytoplasm of these macrophages show complete vacuolisation. The presence of these foamy alveolar macrophages is usually non-specific, however, it can be seen in aspiration pneumonia or drug-induced pulmonary disease. Giemsa Stain

Multinucleated Alveolar Macrophage



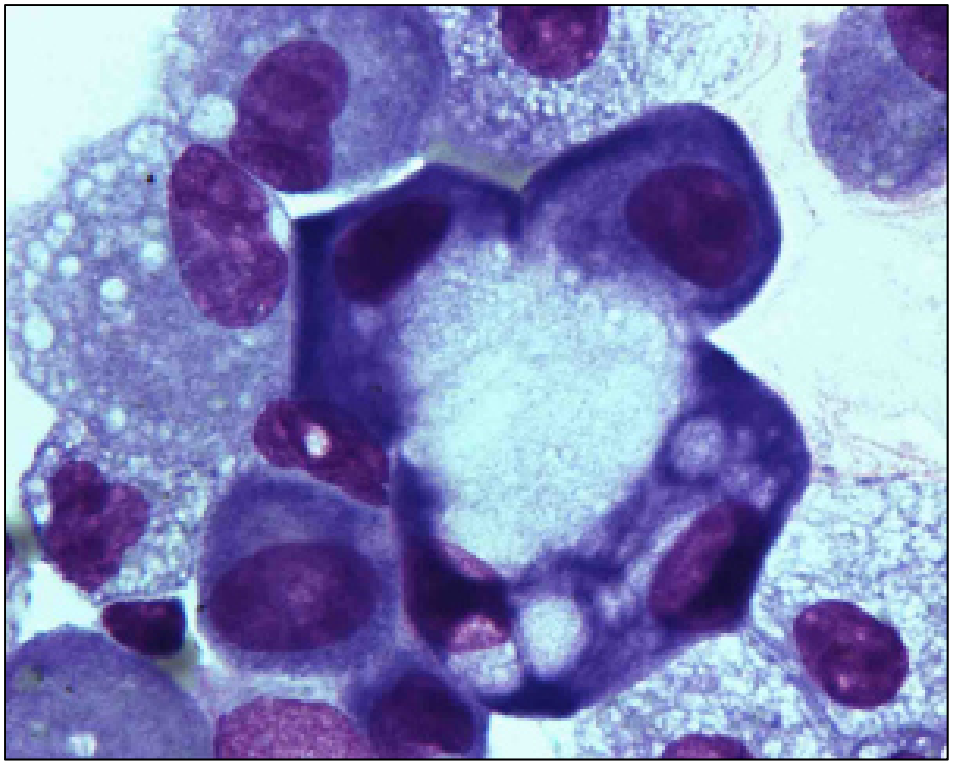
Multinucleated alveolar macrophages can occasionally been seen, their presence is non-specific and does not imply infection or foreign body reaction. Giemsa Stain

Necrobiotic PMN



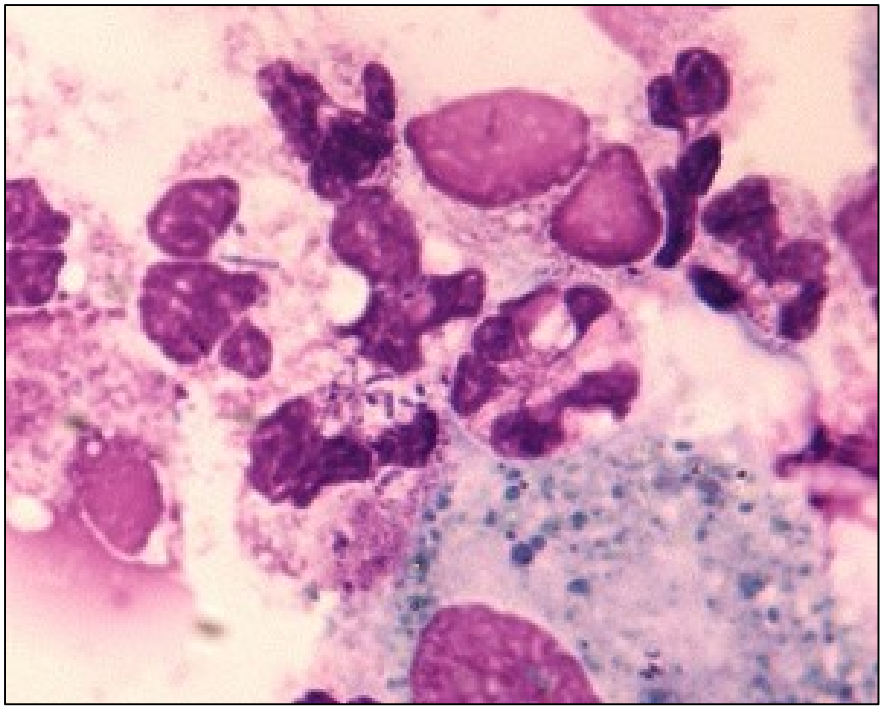
A necrobiotic neutrophil surrounded by three PMNs (of which one contains phagocytised bacteria). Necrobiotic neutrophils are cells that show loss of the nuclear chromatin pattern. The nucleus in reduced to a dingle or multiple small droplets that stain intense dark blue. These type of cells can be seen in longstanding inflammation. Giemsa Stain

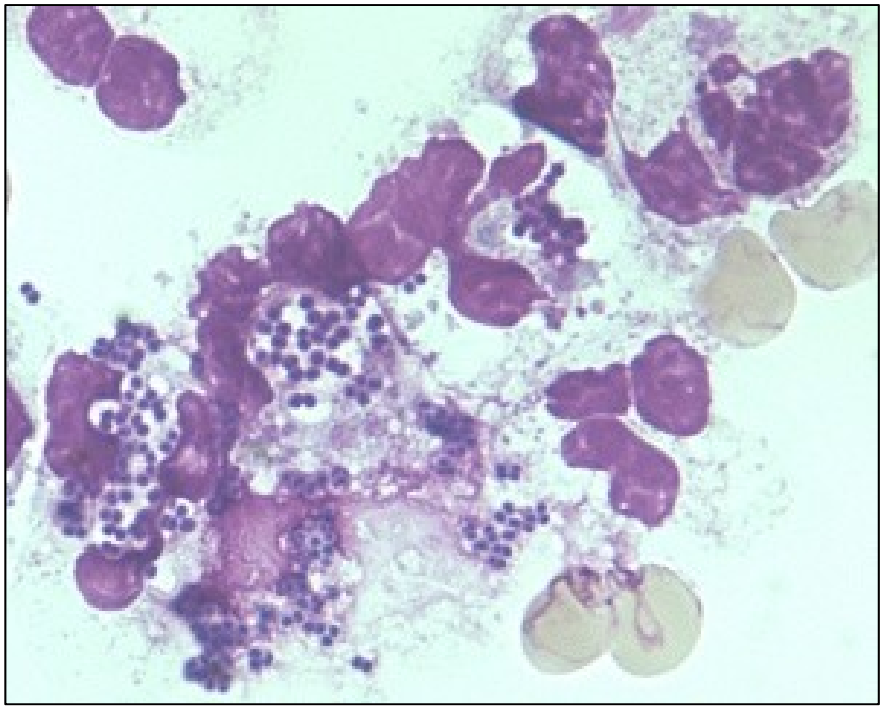
Reactive type II pneumocyte



Type II pneumocytes are surfactant producing cells present at the inside of the alveoli (alveolar lining cells). In normal circumstances these cells are not seen in BAL fluid, or are indistinguishable from alveolar macrophages. However, in case of serious pulmonary damage, they can be found in BAL as reactive type II pneumocytes (RPII). RPII cells are large cells which can be easily spotted, even at a low magnification. They have a small, dark colored nucleus which sometimes is irregularly shaped, due to the vacuolated cytoplasma, the nucleus is often pushed towards the cell border. They have a deeply blue stained cytoplasma, intercytoplasmic connections are often present and many RPII show large vacuoles that tend to confluent. In a minority of cases RPII cells are present as singly lying cells with small vacuoles. Reactive type II pneumocytes may resemble malignant cells. RPII cells can be seen in patients with, for instance, acute respiratory distress syndrome (ARDS) or *Pneumocystis jiroveci pneumonia*. Giemsa Stain

Infected Cells



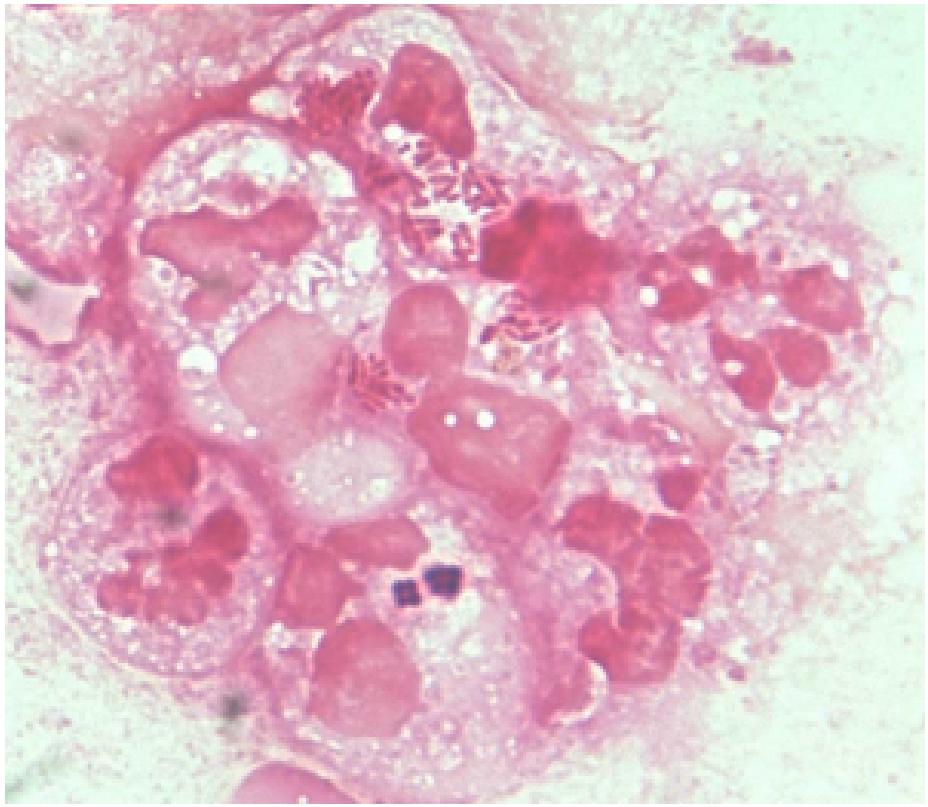


Infected cells (IC) are cells which have phagocytized micro-organisms. The majority of these cells are PMNs, however in a small percentage of cases alveolar macrophages are also involved. Different stains can be used for the evaluation of the percentage of IC in BAL fluid, the Giemsa stain was found superior over the Gram-stain and the acridine-orange stain, resulting in the highest reliability. Left - In the center are PMNs visible which have phagocytized rod-shaped bacteria. Several PMNs which have phagocytized round bacteria (cocci).

Infected Cells

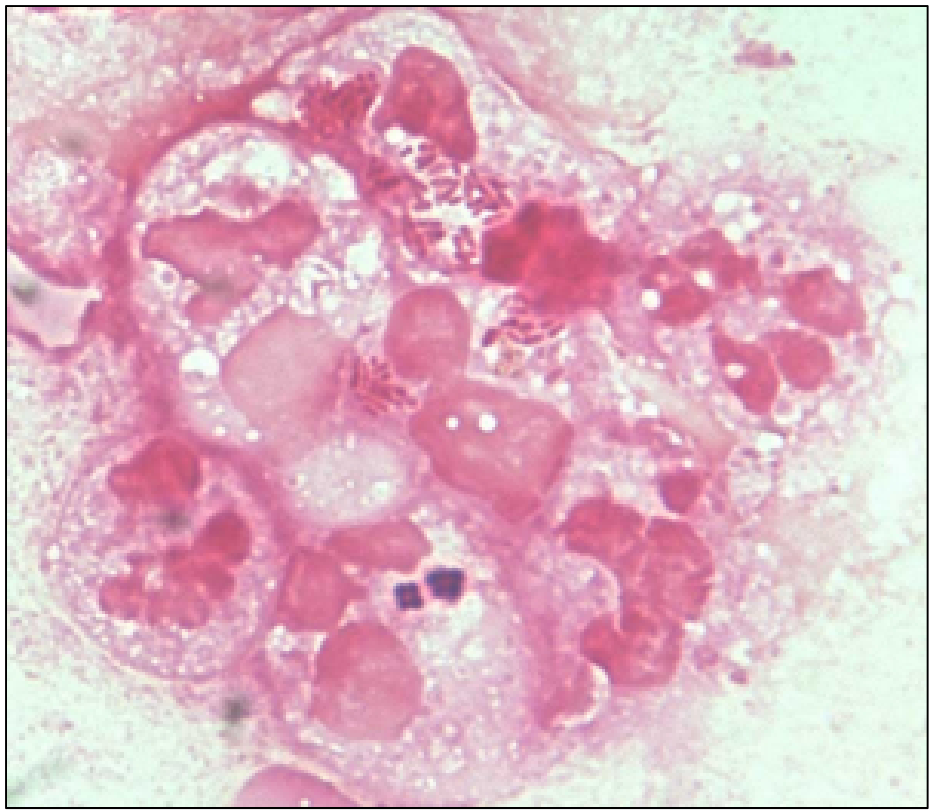
Left - Gram stain. PMN which has phagocytized Gram-positive cocci. Vacuoles surrounding the bacteria are clearly present. PMN which has phagocytized Gram-positive cocci and Gram-negative rods. Vacuoles surrounding the bacteria are clearly present.

Infected Cells



Gram stain: three polymorphonuclear neutrophils (PMNs) that have phagocytized Gram negative rods whilst one PMN has phagocytized Gram positive cocci. PMNs that have phagocytized are called infected cells (IC). In ventilated patients the presence of ≥ 2% IC is indicative for the diagnosis Ventilator-associated pneumonia (VAP).

Infected Cells



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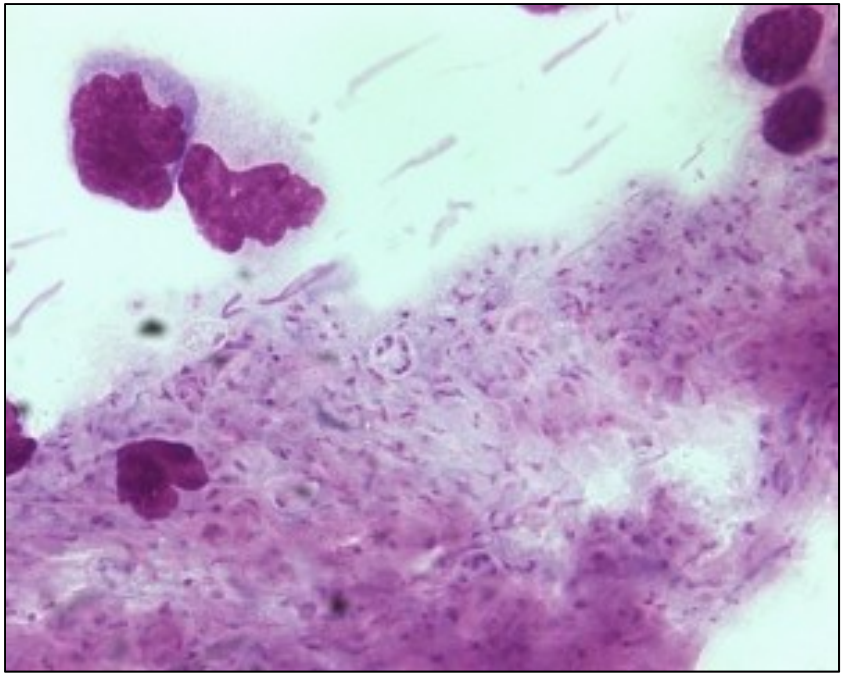
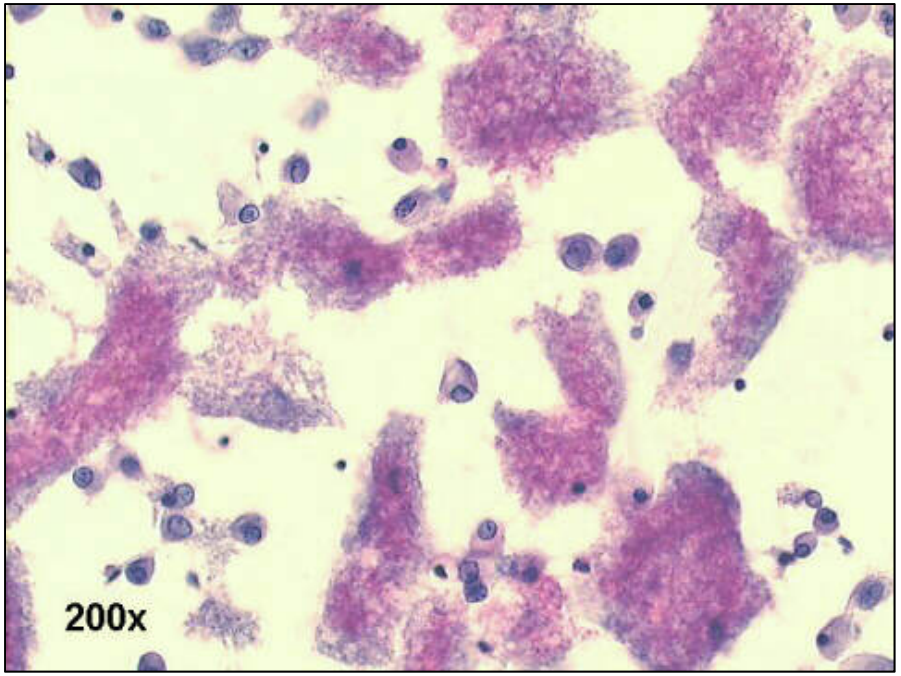
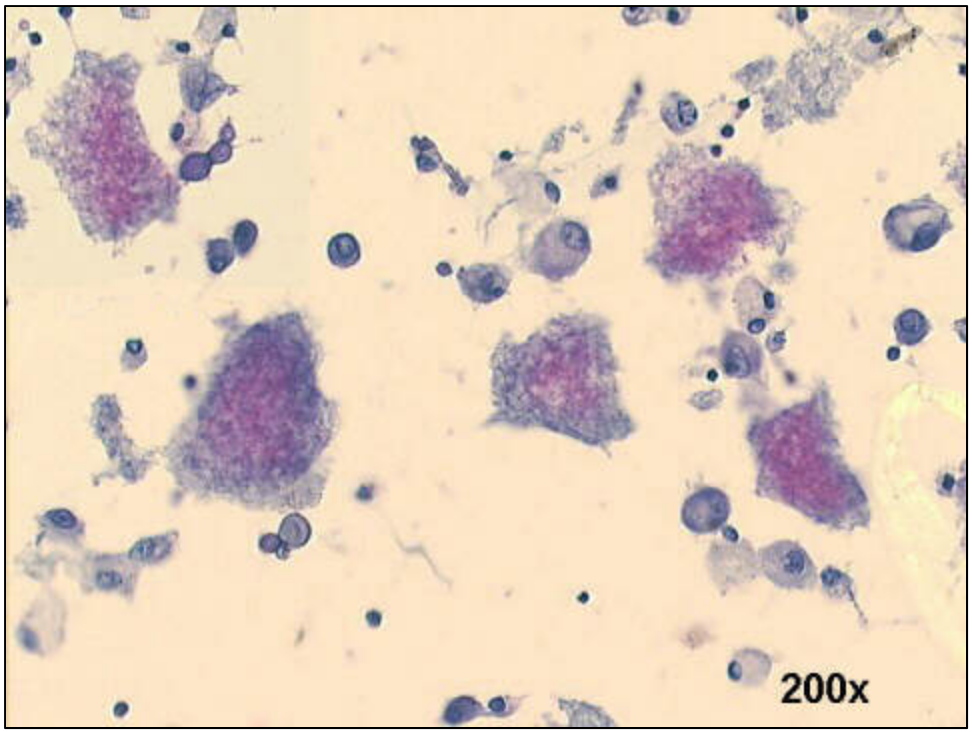
Infected Cells

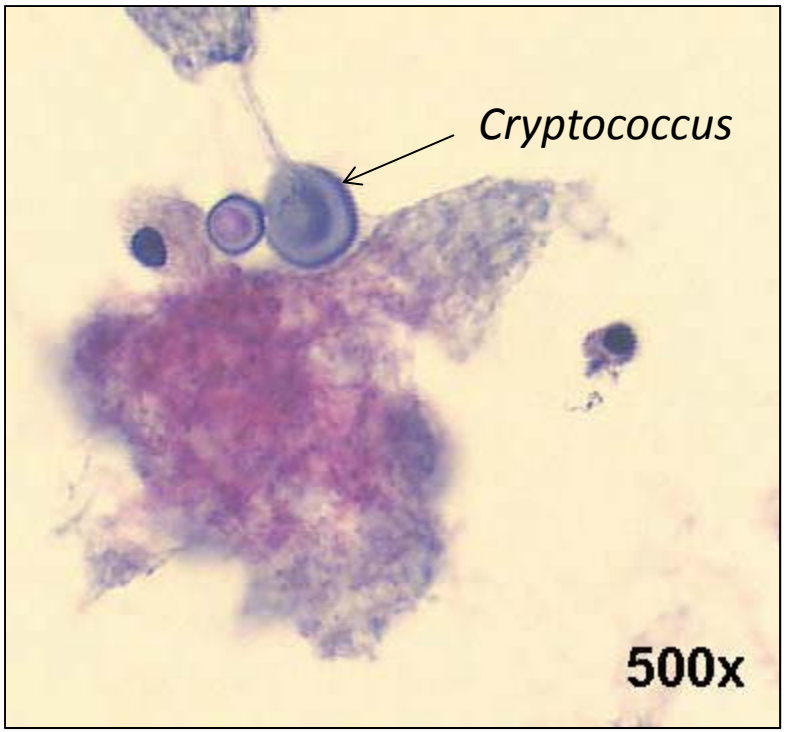




*Pneumocystis jiroveci*is an opportunist, causing pneumocystis pneumonia (PCP) in immunocompromised patients. However, the microscopic evaluation of BAL fluid samples for the presence of *P. jiroveci* is cumbersome and requires specialised microscopists. Especially in BAL fluid samples containing a low burden of *P. jiroveci*, establishing the diagnosis is often difficult and time consuming. Left - Giemsa stain. Showing *P. jiroveci* trophozoits present in BAL fluid. Right – This patient actually has both *Pneumocystus* and *Cryptococcus* (shown on higher magnification in the next slides)

Infected Cells

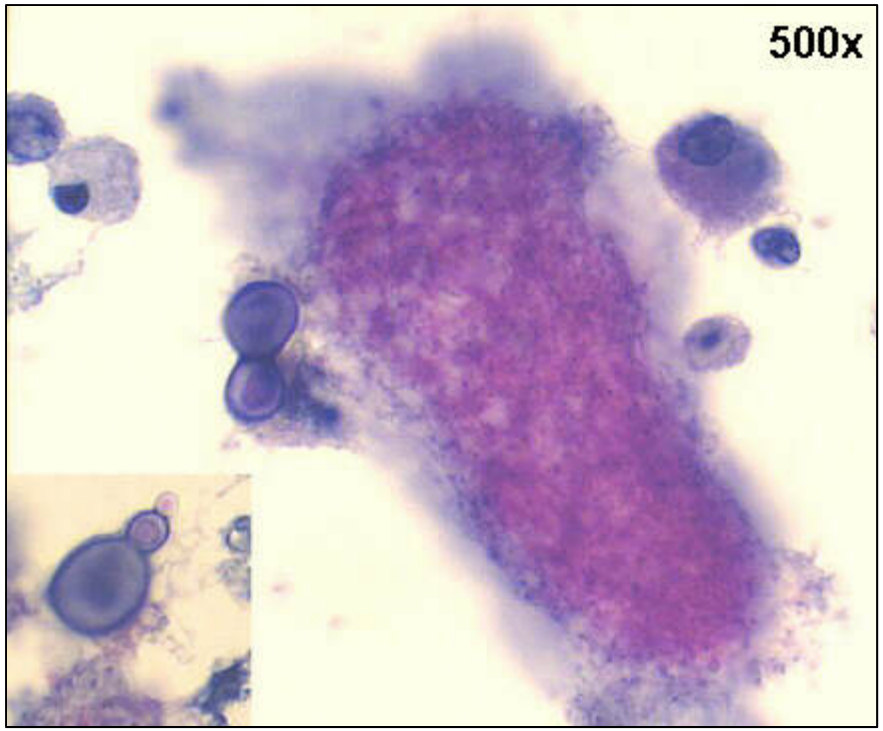


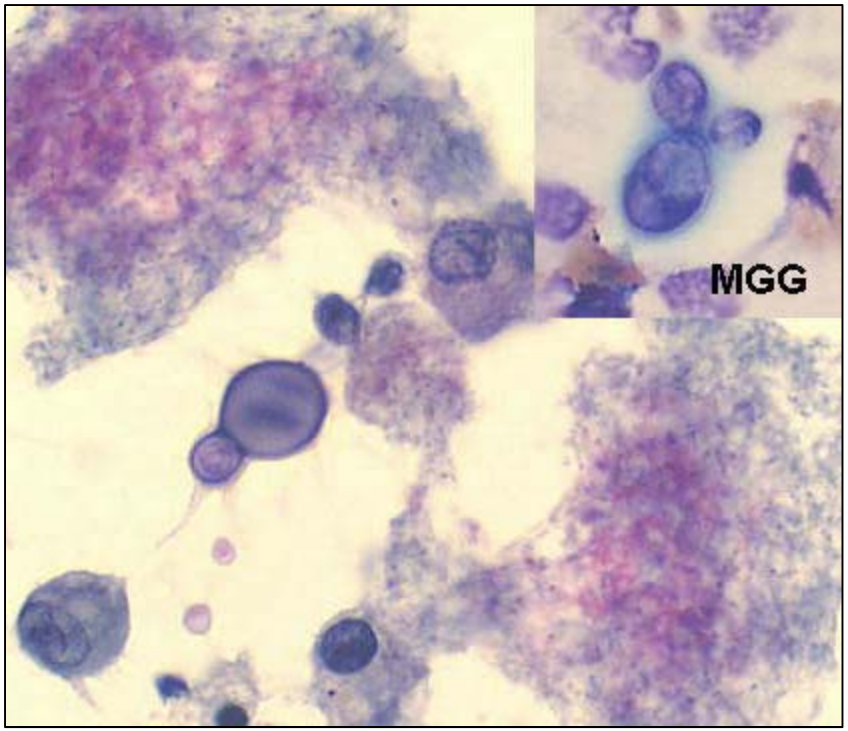


This patient has both *Pneumocystus* and *Cryptococcus.*

Cryptococcus neoformans. The fungus is spherical, and produces a single teardrop shaped bud. The thick mucoid capsule produces an unstained halo around the organism, in Papanicolaou stained smears. In Giemsa-stained smears the capsule may stain in pink color, due to its high sugar content, and the fungi are more easily seen, even if in low numbers.

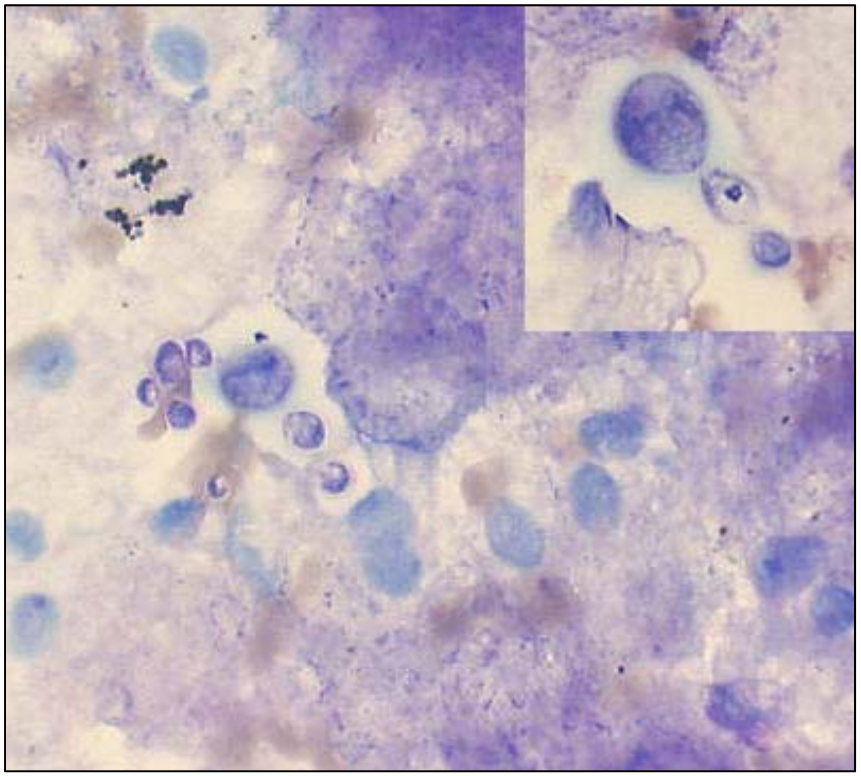
Infected Cells

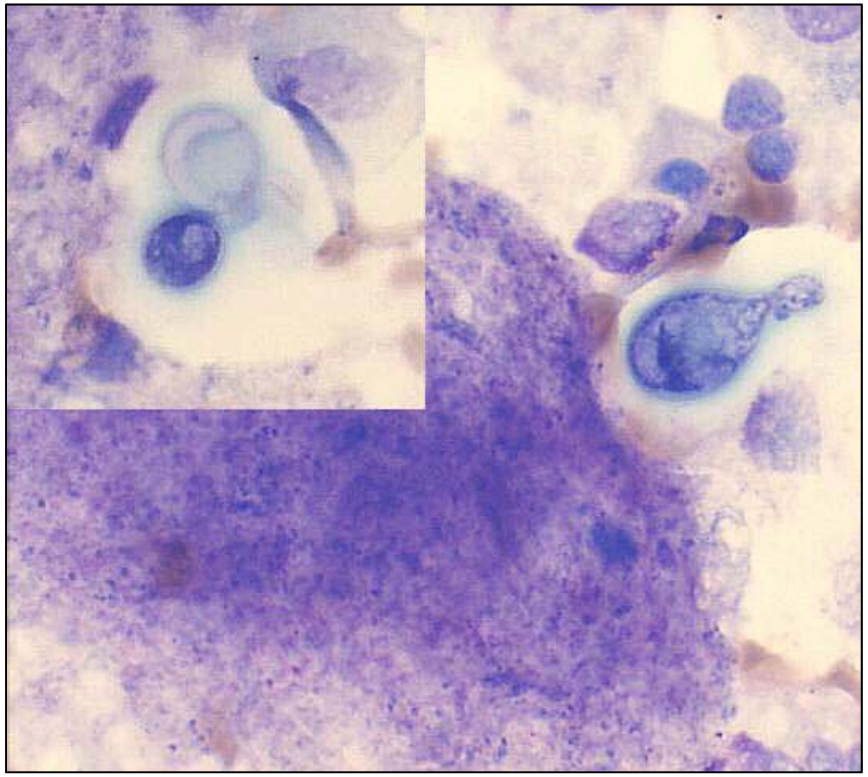




This patient has both *Pneumocystus* and *Cryptococcus.*

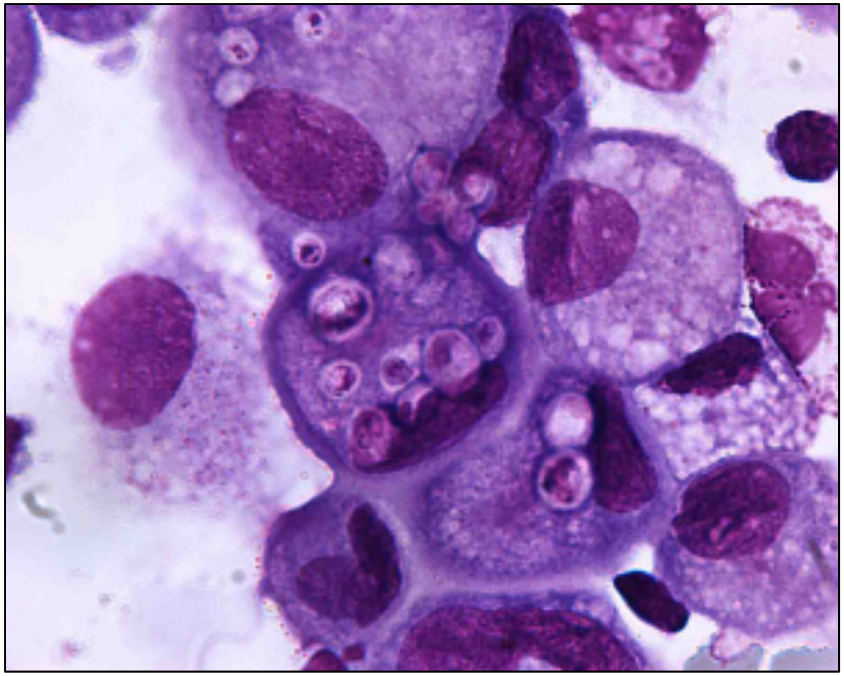
Infected Cells

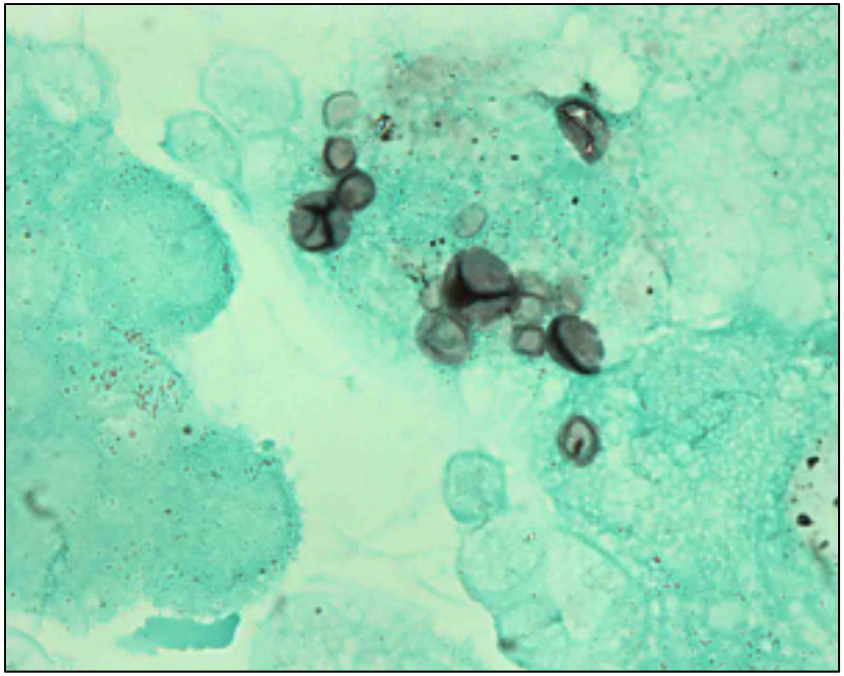




This patient has both *Pneumocystus* and *Cryptococcus.*

Infected Cells – *Cryptococcus neoformans*

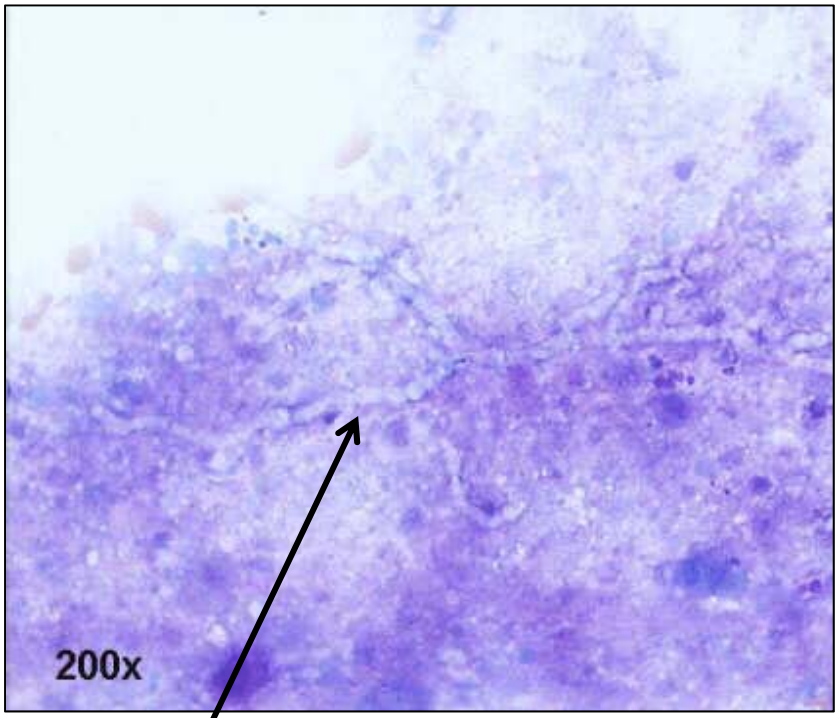


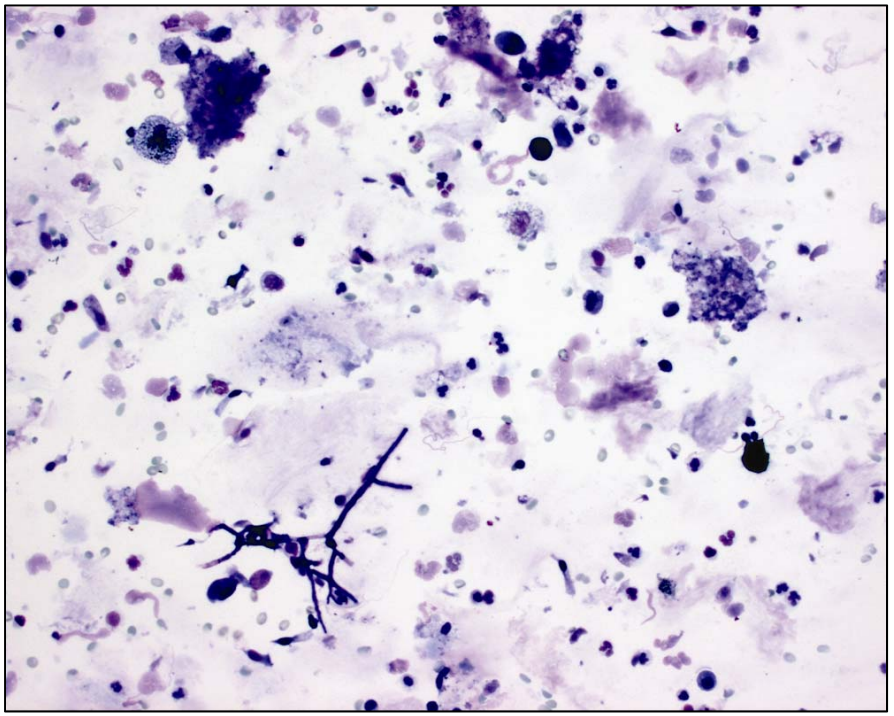


Left - Cytocentrifuge preparation of BAL fluid showing multiple yeast like structures. A thickened cell wall is clearly present in a number of these structures. Giemsa stain.

Right - Grocott stained cytocentrifuge preparation of BAL fluid showing multiple yeast like structures which vary in size. This image may be mistaken for *Pneumocystis jiroveci*, however, no wall structures are present (which would be present in *P. jiroveci*).

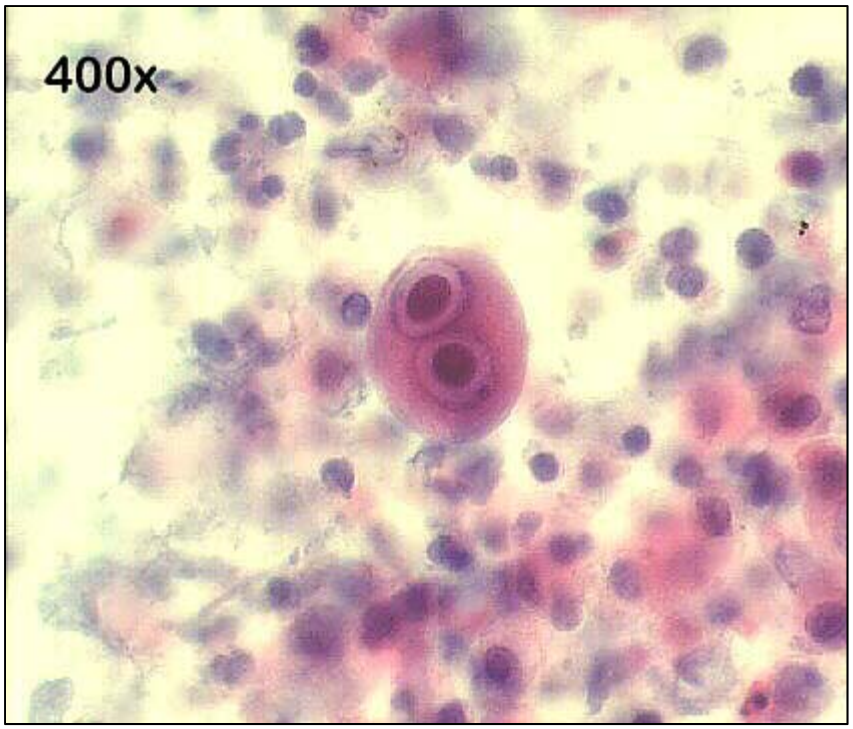
Infections – *Aspergillus fumigatus*





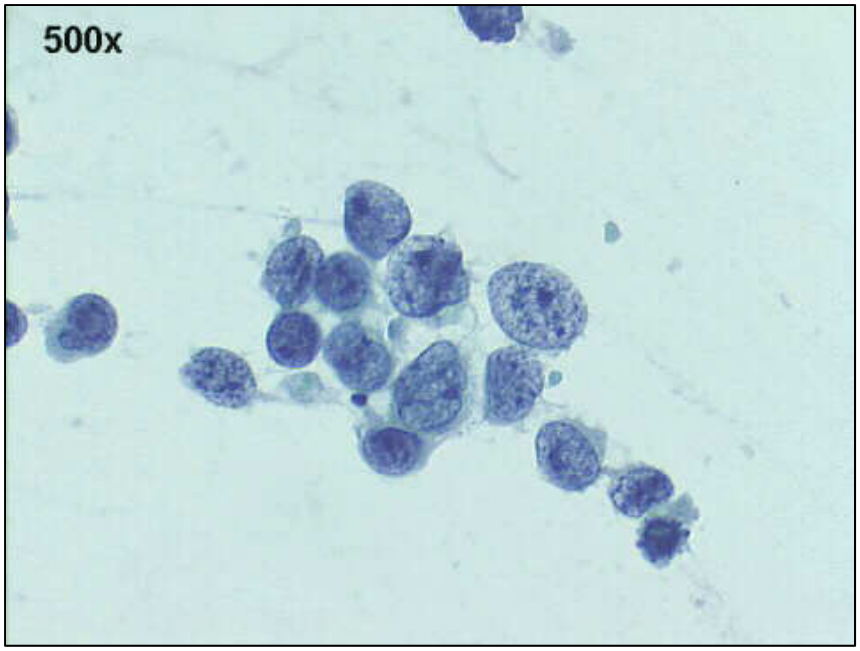
Aspergillus fumigatus is a fungus notorious for causing life-threatening pulmonary infections in immunocompromised patients. Cultures of BALF or biopsies are very specific, however the sensitivity is low (30-50%). Giemsa stained cytocentrifuge preparation of BAL fluid showing segmented hyphae with 45º branching suspect of *Aspergillus fumigatus*. Culture of BAL fluid yielded *Aspergillus fumigatus*. The photo on the right actually shows both *Aspergillus* (lower right) and *Pneumocystis* (tufts of foamy material in the upper portion of the photo).

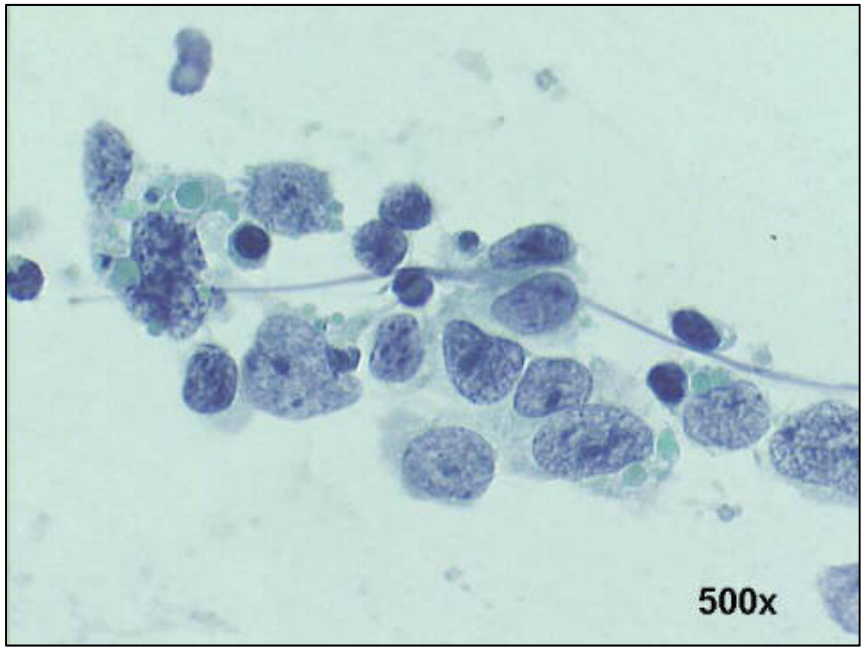
Infections – CMV



The presence of many cells with intranuclear huge pink inclusions surrounded by a clear halo ("owl's eye" appearance) is highly suggestive of CMV infection. The nuclei in these cells are several times larger than the nuclei of the pulmonary alveolar macrophages.

Malignant Cells

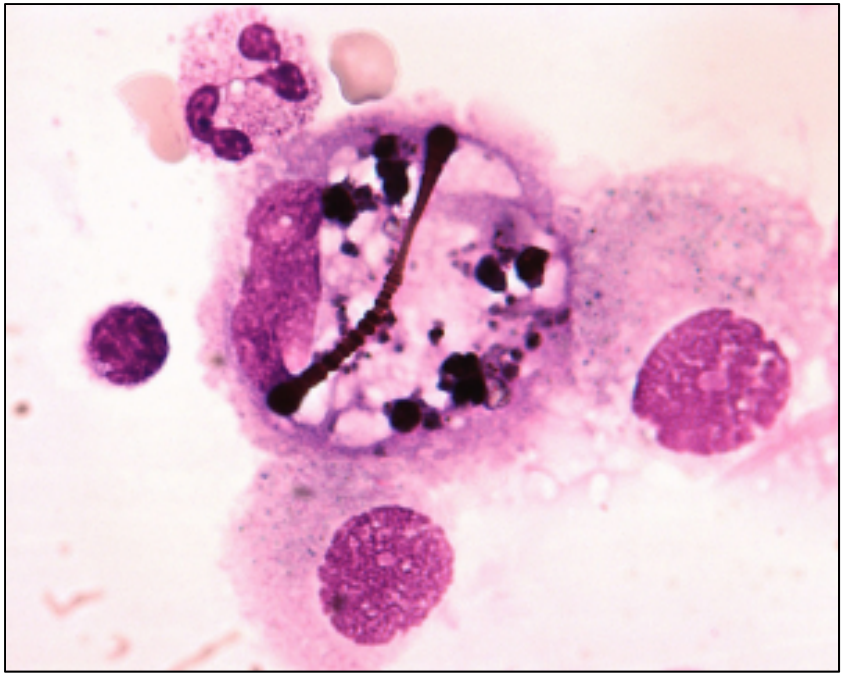


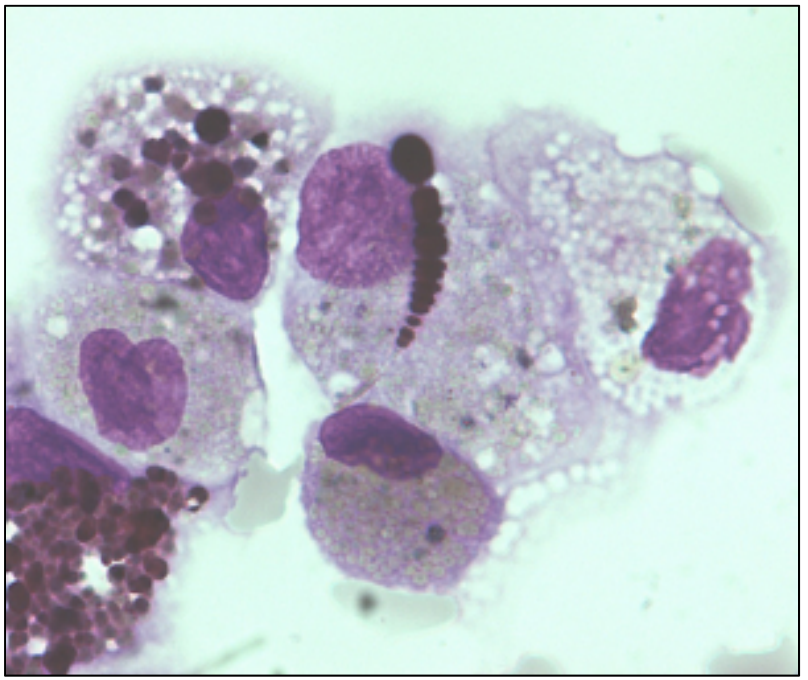


The smears show the typical features of small cell carcinoma: single small cells, with some loose groups showing nuclear molding, with scant or absent cytoplasm. The nuclei are oval or angulated, with "salt and pepper" chromatin, small or absent nucleoli (a few nuclei have larger nucleoli). The main differential diagnosis is malignant lymphoma.

Mast Cells (MC) are generally seen in early SAID. AM is an alveolar macrophage

Foreign bodies – asbestos fiber





Left - Asbestos fiber phagocytized by an alveolar macrophage. Giemsa stain.

Right - Asbestos fiber in BAL fluid. The asbestos fiber is phagocytized by an alveolar macrophage. Giemsa stain