



RESEARCH ARTICLE

Cytopathologic studies in sheep pneumonias

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Özet

Oruç E. Koyun pnömonilerinde sitopatolojik çalışmalar.
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Amaç: Pnömonili koyun akciğerlerinden hazırlanmış sitolojik preparatlarla, aynı akciğerlerin histopatolojik kesitleri karşılaştırılarak, farklı lezyonlara sahip akciğerlerin eksfoliyasyon özelliklerinin belirlenmesidir.

Gereç ve Yöntem: Kataral, irinli, fibrinli, intersitisyel ve verminöz lezyonlara sahip 65 hasta ve 5 sağlıklı akciğerden, ince iğne aspirasyonu ile alınarak hazırlanmış, Papanicolau EA 65 ve May-Grunwald Giemsa ile boyanmış sitolojik preparatlar histopatolojik kesitlerle karşılaştırıldı.

Bulgular: Sitolojik ve histopatolojik bulgular karşılaştırıldığında, makrofaj ve nötrofil lökositlerin kataral, irinli fibrinli ve verminöz pnömonilerde; eozinofil lökositlerin, nötrofillerle birlikte verminöz pnömonilerde ve lenfositlerin de intersitisyel pnömonilerde, kontrol grubuna göre önemli oranda artış gösterdiği tespit edildi.

Öneri: Lezyonlu koyun akciğerlerinden hazırlanan sitolojik preparatların, özellikle verminöz ve intersitisyel pnömonilerde histopatolojik kesitlere uyumlu bir eksfoliyasyon gösterdiği, nötrofil lökosit ve makrofajların artışı ise pnömoninin karakteri hakkında kesin bir belirleyici olmamakla birlikte, kataral, irinli, fibrinli ve verminöz pnömonilerde değişken bir artış gösterdiği kanısına varıldı.

Abstract

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Aim: Aim was to investigate the exfoliation characters of sheep pneumonias by the comparison of histopathologic and cytopathologic results.

Materials and Methods: Fine needle aspiration smears obtained from 5 healthy and 65 pneumonic sheep lungs showing catarrhal, purulent, fibrinous, interstitial and verminous lesions were stained with Papanicolau EA 65 and May-Grunwald Giemsa and compared to histopathologic sections.

Results: When compared cytopathologic and histopathologic findings, it was observed that macrophages and neutrophile leucocytes increased in catarrhal, purulent, verminous and fibrinous pneumonias; eosinophile leucocytes increased in verminous pneumonias together with neutrophils and lymphocytes increased in interstitial pneumonia.

Conclusions: Cytopathologic examinations of lung smears prepared from pneumonic lungs were found remarkable for the prediagnosis of sheep pneumonias, especially, in verminous and interstitial, and the increased macrophages and neutrophile leucocytes counts were found considerable but not determinative for the differantiation of pneumonia type in sheep.

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Keywords: Sheep, pneumonia, cytology, histopathology.

► Introduction

Pneumonias are an important disease group caused by some infective and environmental factors together in many times. Different etiologic agents cause lesions in different characters (Oruç et al. 2003, Oruç 2006). Antibiotic using, virologic isolation difficulties and need some complex diagnostic procedures make difficult the diagnosis of the cause of pneumonia. Histopathological changes indicate to etiologic agent due to show specific changes and pneumonias are diagnosed according to histopathological findings in many times. However, a histopathologic procedure needs running of a period and delays the diagnosis. Cytology is a valuable pre-test using in many pathologic processes for giving some hints which shed light on histopathologic character of the disease (Cowell ve Tyler 1985). With the examination of a cytologic sample prepared duly suggests the character of the lesion, tumoral (Kıran et al 2000, Birdane et al 2004, Oruç 2006) or inflammatory (Dawson et al 2005, Oruç ve Uslu 2006). Moreover, malign or benign tumors or the character of inflammation can be recognised (Cowell ve Tyler 1985, Teske 2008) in many times by this technique. In previous cytological studies, some tumoral cells from pulmonary adenomatosis (Rakich and Latimer 1989, Kıran et al 2000, Dawson et al 2005) and increasing of some inflammatory cells (macrophage, neutrophile leucocyte, eosinophile leucocyte and lymphocyte) were reported (Kıran et al 2000, Mauchline et al 2005).

In this study, it was aimed to determine the exfoliation characters of some pneumonia types in sheep, compare cytologic and histopathologic findings and finally, to build up a rapid prediagnostic method for sheep pneumonia by the examination of the cytological smears prepared from diseased lungs.

► Materials and methods

Total 70 lungs (65 pneumonic and 5 healthy) were used as study material. For this aim, fresh lung samples were obtained from slaughterhouse and slaughterhouse visited daily for a week (4 times in total).

• Preparing the cytology slides

After the gross examination, fine needle aspiration (FNA) smears were directly prepared from the diseased (pneumonic) and healthy lungs. Air dried smears were stained with Papanicolau EA 65 (Bio-Optica, Catalogue number: 05-12017) and May-Grunwald Giemsa (Bio-Optica Catalogue number: 04-080802) according to manual. Cytopathological examinations were semiquantitatively assessed under the light microscope with an ocular grid and 4X, 10X, 40X objective, respectively. A total of 10 high-power (100X) fields were randomly chosen and 200 cells were counted. Erythrocytes and deformed cellular materials were not evaluated.

• Preparing histopathological slides

For histopathologic examination, lung samples were taken and fixed in 10% neutral buffered formalin solution. After the routine histopathological processing, tissue samples were embedded in paraffin wax and sectioned at 5 µm. All sections were stained with Hematoxylin and Eosine (H-E).

• Statistical analysis

Differences between the groups were tested by the analysis of variance (ANOVA) and Duncan test using SPSS for Windows version 10.0 for the statistical analysis. The data were displayed as means ± standard deviation, and p values less than 0.05 were considered significant.

► Results

• Cytological findings

Macrophages: Macrophages were considerably large and nucleus/cytoplasm rate were 1/3 in some macrophages. In many slides, macrophages contain stained and unstained vacuoles and phagocytic materials. Macrophages were polygonal shaped, and nucleus was generally central, sometimes marginally located because of cytoplasmic materials (Fig 1-2). **Neutrophile leucocytes:** These cells have multi-segmented (2-5 segment) nucleus and were easily recognisable in slides. Cytoplasm of neutrophiles were pale stained with both May-Grunwald Giemsa and Papanicolau EA 65 stains (Fig 1, 3, 4). **Eosinophile leucocytes:** These cells were rarely encountered on cytologic slides and similar to neutrophile leucocyte other than eosinophilic cytoplasmic granules (Fig 4). **Lymphocytic cells:** Lymphocytes have minimal or without (not recognisable) cytoplasm and dark-blue stained nuclei (Fig 5). **Ciliated epithelial cells:** Polygonal or prismatic shaped cells have marked cilia along an edge. Nucleus

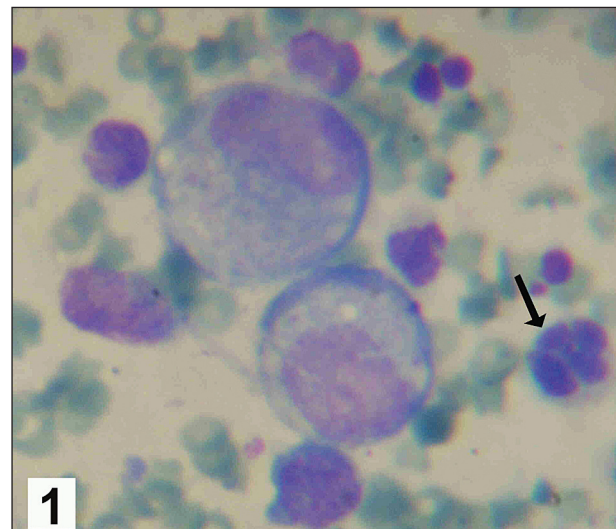


Figure 1. FNA smear of lung from purulent bronchopneumonia. Large macrophages with phagocytic materials and neutrophile leucocyte with segmented nuclei (arrow). May-Grunwald Giemsa stain. Original magnification: X1000

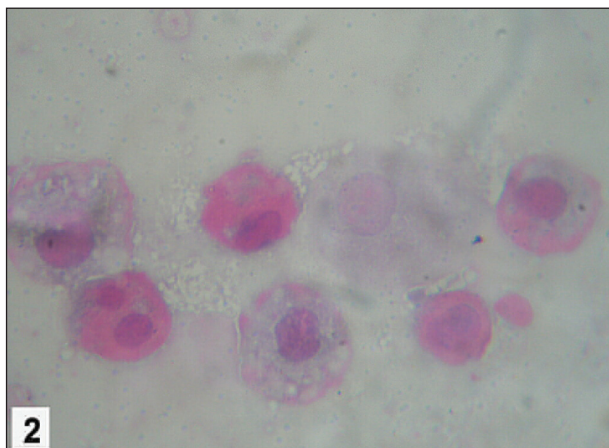


Figure 2. FNA smear of lung from catarrhal bronchopneumonia. Macrophages with variable cytoplasm-nucleus rate. Papanicolau stain. Original magnification: X1000.

were oval-cubic and generally located reverse side of cilia. These epithelial cells exfoliated as clusters and have intracytoplasmic secretion materials (Fig 6).

• Histopathological findings

After microscopic examination, histopathologic lesions were described as catarrhal, purulent, fibrinous, interstitial and verminous.



Figure 3. FNA smear of lung from purulent pneumonia. Increase in neutrophil leucocytes. Papanicolau stain. Original magnification: X1000.

► Discussion

It was shown that lung cytology given compatible results in some disease of cat (Greenlee 1984), dog (De Mello 2005), horse (Derksen 1989, McKane 1993) and sheep (Kiran et al 2000, Mauchline et al 2005). It was reported that lung aspirates or cytological slides commonly contain many erythrocytes, less respiratoric epithelial cells clusters and less alveolar macrophages (Padrid et al 1991, Barton 2004, Oruç 2005). Similarly, many erythrocytes were observed in cytological slides but these cells could not mask the examination of other inflammatory cells. However, haemorrhagic pneumonic lesions were not commented because of this erythrocytic content. Alveolar macrophages, neutrophile leucocyte, eosinophile leu-

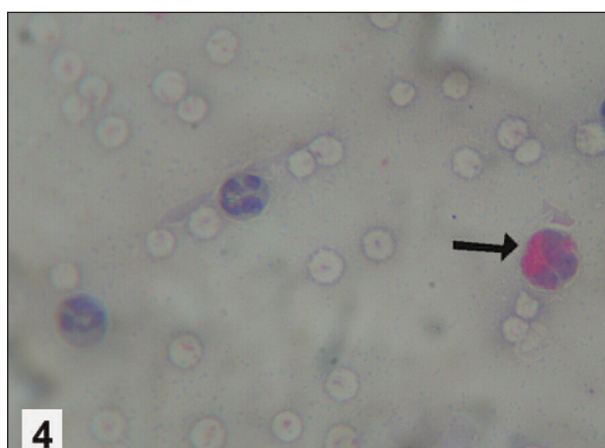


Figure 4. FNA smear of lung from verminous pneumonia. Eosinophil leucocyte (arrow). Papanicolau stain. Original magnification: X1000.

cocytes lymphocyte. Mast cells and epithelial cells can generally be observable cells in diseased or healthy lung smears (Collie et al 1999, Mauchline et al 2005, Oruç 2005, De Mello 2005).

In present study, cytological exfoliations were broadly found compatible with histopathological sections. Statistically, there was no marked difference regarding the macrophages between control and interstitial

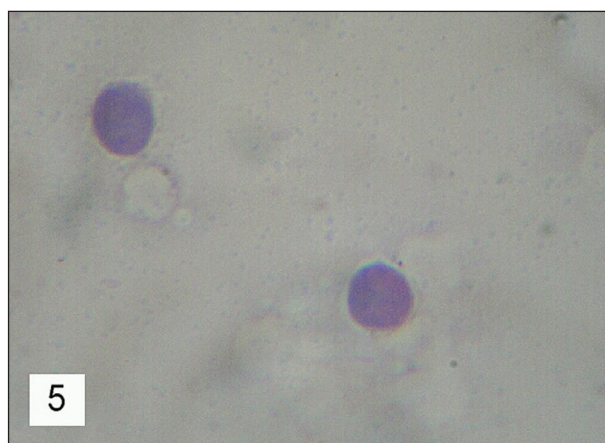


Figure 5. FNA smear of lung from interstitial pneumonia. Increasing in lymphocytes Papanicolau stain. Original magnification: X1000.

pneumonias ($p < 0.05$). The frequency of macrophages increased in fibrinous and verminous pneumonias than healthy lungs ($p < 0.05$). Macrophages were significantly higher also, in catarrhal and purulent pneumonias when compared to control group ($p < 0.05$). This shows that increasing of macrophages in cytological smears is an important finding of inflammatory reaction. However, an increasing of only macrophages is not sufficient for the diagnosis of catarrhal, purulent, fibrinous and verminous pneumonias. Similarly, neutrophile leucocyte population was not determinative for this differentiation of the pneumonia types. Significant differences were found among neutrophile leucocyte in each group ($p < 0.05$). It is possible to say, the increase of these cells (macrophage and neutrophile leucocyte) in cytologic smears signs to an

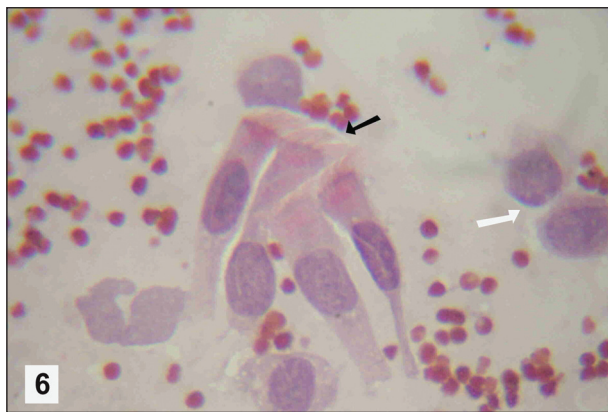


Figure 6. FNA smear of lung from control group. Ciliated epithelial cells (black arrow) and other epithelial cells (white arrow). Papanicolaou stain. Original magnification: X1000.

inflammatory cell types can easily be recognisable in cytological smears by the microscopic examination because of specific morphology. It is concluded that cytopathologic examination of lung smears prepared from pneumonic lungs were found remarkable for the prediagnosis of sheep pneumonias, especially, in verminous and interstitial pneumonias. The increase of macrophages and neutrophile leucocyte were found considerable regarding the inflammatory reaction, but not determinative for the differentiation of pneumonia types. In addition, both May-Grunwald Giemsa and Papanicolaou EA 65 result a good staining. Notwithstanding, it is thought that similar cytological slides prepared from alive animals by bronchoalveolar lavage or other techniques will be give helpful results for early diagnosis of sheep pneumonias.

Table 1. The evaluation of cell types numbered in different pneumonia

Cell type	Group I Control n=5	Group II Catarrhal pneumonia n=15	Group III Purulent pneumonia n=14	Group IV Fibrinous pneumonia n=24	Group V Interstitial pneumonia n=9	Group VI Verminous pneumonia n=3
Macrophage	24.0±1.64 ^f	55.8±1.10 ^a	56.2±1.31 ^a	44.6±0.92 ^b	23.0±0.97 ^c	42.3±0.88 ^b
Neutrophile	3.60±0.93 ^f	52.4±0.96 ^c	92.1±1.38 ^a	87.0±0.79 ^b	17.3±0.87 ^e	38.6±1.45 ^d
Eosinophile	1.80±0.37 ^c	1.53±0.31 ^c	3.14±0.38 ^{bc}	4.71±0.56 ^b	2.22±0.36 ^c	70.0±0.58 ^a
Lymphocyte	14.6±0.81 ^e	19.9±0.64 ^d	34.5±1.15 ^{bc}	39.5±1.14 ^b	111±2.64 ^a	31.6±0.87 ^c
Ciliated epithel	57.0±2.41 ^a	25.2±0.61 ^b	3.57±0.33 ^d	4.88±0.37 ^d	10.3±0.67 ^c	2.67±0.88 ^d

a, b, c, d, e, f: Different letters in the same line are significantly different (Duncan test, $p < 0.05$).

inflammatory reaction against bacterial or parasitic agent. But not determinative for the diagnosis of specific pneumonia type when compared to histopathologic sections, unlike neutrophiles and macrophages. The frequency of eosinophiles increased in verminous pneumonias than the others ($p < 0.05$). This increase was found important for verminous pneumonia when this increase were seen together neutrophiles in cytological smears. Besides, the increase of lymphocyte in cytological smears was determinative for interstitial pneumonias. The frequency of lymphocytes increased in interstitial pneumonias more than the other groups ($p < 0.05$).

May-Grunwald Giemsa and Papanicolaou EA 65 stains were used in this study for the examination of cellular details. Both stains result a good staining for the microscopic examinations. It was observed that Papanicolaou EA 65 was more effective for nuclear details and May-Grunwald Giemsa was more effective for cytoplasmic content, especially in phagocytic materials.

► Conclusions

The results obtained from this study confirmed that

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