Cytologic Study of Expectoration from Commited Patietns in a Pneumology Department (2001-2007)
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ABSTRACT This study was made in the Municipal Hospital of Caracal’s Pneumology section during the period 2001 – 2007, while the section was running 45 beds, with occupancy of 115%. During this period a number of 4862 patients were investigated from which 3120 had had their expectoration taken for the cytological exam. The cytological exam is advised in case of suspecting of the pleura lung even though no obvious modifications during the previous radiological exam were present. The exam is advised not only for suspicion of the pleura lung but also for determining the diagnosis of the pulmonary affliction and also in evaluation of the evolution of the affection. It was noticed an increased number of epithelial cells and cylindrical cells malpighienne, this percentage being higher in the chronic lung diseases, train and in the lung cancer. Red blood cells and eosinophils have been found in very small percentage without being able to determin cause behavior therapy. Cytology performed at cough showed a more frequent hyperplasia than metaplasia, the latter being more frequent in broncho-pulmonary disease-cancer. Obtained microscopy images have exemplified our survey data.

KEY WORDS Malpighian cells, cylindrical epithelium cells, sangvin origin cells, hyperplasia, metaplasia, dysplasia

Material and method
For the cytological diagnosis of the lung cancer, one can examine: the expectoration, the bronchi aspiration and the washes and the puncture products. (Bercea O 1983)

In practice, cytological examination of sputum allows obtaining very good results without excessive cost, without being invasive and with great certainty. This kind of exam does not imply any inconvenience for ill: can be repeated whenever necessary and can be performed in patients who do not support bronchoscopic examination. Ineffectiveness of sputum examination in patients suspected of bronchopulmonary neoplasia require cytological examination of bronchial aspirate and washes, which could not be done in our department because the bronchoscope was not available.

Harvesting the expectoration
Irrespective of further processing, for sputum collection the following are recommended:
− To harvest the sputum with coughing efforts powerful as possible, so as to eliminate bronchial secretions in the smallest bronchi and thus leading to a proper result;
− To collect, if possible, only the product of expectoration, with a minimum of saliva;
− Patient to follow a perfect hygiene for the mouth, to avoid mixing sputum with peeled detris or possible food scraps.

For patients without or with very low expectoration were recommended various ways to stimulate the production of bronchial secretion and cough reflux, but the real contribution of most of those methods remains doubtful (Kossut and collaborators. 1964). We obtained satisfactory results using saline spray, at a convenient temperature. (Cooney W, Dzuria B. 1972).

If sputum smears are to be processed, harvested should be done in clean Petri dishes for 1-2 hours, after which after which it is brought in the shortest possible amount of time to the laboratory. Being given the rapid alteration of tumor cells, it is preferable the harvesting do be done in the laboratory or to be collected in containers containing alcohol 70 degrees. (Allegra S, Broderick PA, 1973)

The technique of performing the smearing
After exhibiting biological product on the glass slide and drying it, the fixing in alcohol was done followed by staining the dried smear with May-Grunwald Giemsa method and with the hematoxylin and eosin method.

The May-Grunwald-Giemsa technique of stainig
− staining with pure May-Grunwald solution (12-15 drops), 3 minutes
− distilled water (12-15 drops) for a minute
− Giemsa solution (10 ml dilution solution + 10 drops distilled water) for 15-30 minutes
− washing with tap water
− air-drying (Diaconita Gh, Eskenasy 1963)

The staining technique with hematoxylin eosin (for smirring)
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- Mayer hematoxylin: 2-3 minutes
- washing with tap water
- lithium carbonate-turns: 1-2 seconds
- washing with tap water
- eosin: 1 minute
- 3 alcohol washes (1-2 minutes each)
- 4 Xilol washes (2 seconds each of the first 3, 10 minutes for the last one)
- mounting the smear in Canada balsam.

(Diaconita Gh., Eskenasy 1963)

**Achieving iconography**

The most significant images were photographed using Nikon-Microflex AFXDX system with a Nikon FX35DX photography box.

35 mm Kodak Gold 200 and 400 ASA were used.

The photographs were scanned with an AGFA SNAPSCAN 1236 scanner and processed using image analysis software Olympus Micro Image 3.0-32 bit.

**Discussions**

Cytological examination was performed in all patients with cough, thereby harvesting heir expectoration.

The percentage of patients who were able to harvest sputum was different, depending on the disease studied, being only 35.3 for pleurisy and reaching nearly 86.3 in case of the group "bronchopulmonary suppurations and abscesses.

And for the group that dominated the category in number, namely that secondary pulmonary tuberculosis, the percentage was high (65.3%), mainly due to support of the patient understanding the necessity of this act and by medical staff who helped in patient education and harvesting sputum.

The data obtained in shown in Table 1. Similar results were reported by Erozan YS, Frost JK 1970.

**Table 1:** Cytological exams harvested by disease groups.

<table>
<thead>
<tr>
<th>Disease</th>
<th>TBC</th>
<th>Abscesses and LANP</th>
<th>BPCO + CPC</th>
<th>Lung cancer</th>
<th>Other disease</th>
<th>Pleurisy</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr</td>
<td>1520</td>
<td>370</td>
<td>466</td>
<td>413</td>
<td>69</td>
<td>101</td>
<td>2726</td>
</tr>
<tr>
<td>Percentage</td>
<td>65.3%</td>
<td>93%</td>
<td>83%</td>
<td>98%</td>
<td>79%</td>
<td>88%</td>
<td>49%</td>
</tr>
</tbody>
</table>

**Table 2 - Malpighiene epithelial cells in the studied diseases.**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>TBC</th>
<th>Abscesses and LANP</th>
<th>BPCO + CPC</th>
<th>Lung cancer</th>
<th>Other disease</th>
<th>Pleurisy</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr</td>
<td>1307</td>
<td>370</td>
<td>466</td>
<td>413</td>
<td>69</td>
<td>101</td>
<td>2726</td>
</tr>
<tr>
<td>Percentage</td>
<td>86%</td>
<td>93%</td>
<td>83%</td>
<td>98%</td>
<td>79%</td>
<td>88%</td>
<td>49%</td>
</tr>
</tbody>
</table>

**As absolute number, followed the cells that are usually rhombic or polygonal. In this case the nucleus is being picnotic, being also tachycromatic and more abundant in cytoplasm.** (Korompoi 1979)

These two types of cells originate from oral epithelium for superficial cells, and from throat mucus and respiratory tree, including the bronchial tree for keratinized cells. (FUNKHOUSER JW 1972)

Parabazale and intermediate epithelial cells are rarer in our expectorations: 49% and 61% respectively, being found in a much higher percentage in case of lung cancer 83 and 76 respectively and much lower for secondary pulmonary tuberculosis and pleurisy by different etiologies. (Sassy-Dobray, 1970).

These cells originate from deeper epithelial layers of the upper airway; as observed in this study, the importance of these cells was much higher than in the first two but was not an indicator of certainty. They are present in this percentage as most studied are chronically ill, with several sharp spikes in history and secondary...
pulmonary tuberculosis patients with new case have at least two months of clinical evolution.

The study of cells from cylindrical epithelium illustrated in Table 3. and fig 2.

**Table 3 Cells from the cylindrical epithelium for the studied diseases.**

<table>
<thead>
<tr>
<th></th>
<th>Ciliate</th>
<th>Mucus</th>
<th>Replacing</th>
<th>Bazal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nr %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBC</td>
<td>745</td>
<td>49</td>
<td>623</td>
<td>41</td>
</tr>
<tr>
<td>Abscesses and LANP</td>
<td>218</td>
<td>53</td>
<td>197</td>
<td>48</td>
</tr>
<tr>
<td>BPCO + CPC</td>
<td>264</td>
<td>47</td>
<td>236</td>
<td>42</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>328</td>
<td>76</td>
<td>257</td>
<td>61</td>
</tr>
<tr>
<td>Other disease</td>
<td>37</td>
<td>48</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>50</td>
<td>39</td>
<td>37</td>
<td>14</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>1642</td>
<td>52</td>
<td>1379</td>
<td>44</td>
</tr>
</tbody>
</table>

Thus ciliate cells were present in the studied expectorations in a percentage of 52. This cells showed the presence of airway inflammation but as we can see they were more frequent in lung cancer. (Fishman A.P. 1989)

Replacement cells and with mucus were present in a much smaller percentage, 25 and 44 respectively, which are also present in a chronic inflammatory process (chronic bronchitis, asthma and bronchiectasis). (Nasiell 1968)

It was found an over average percentage 61 and 51 for lung cancers, considering as hypothesis the presence of airway inflammation, but as it can be seen it was more present in case of lung cancer. (Hattori, 1971: Greenberg, 1975).

For cylindrical basal epithelium, it was the rarest percentage, representing 12% with an increase of 41% for patients with lung cancer, which represents in our conception a persistent chronic inflammation or irritation, such as in heavy smokers. (Korompai, 1979)

Another aspect of our study was to histiocitary elements (macrophage) and those of blood origins (in Table 4. and fig. 3.)

So we got this abundance of macrophage involved in chronic infections processes in patients studied (82%) as that of the neutrophil (84%). (Kierzenbaum, 1965)

**Table 4 Histicitare elements and of blood origins in studied diseases.**

<table>
<thead>
<tr>
<th></th>
<th>Macrophages</th>
<th>Neutrophil</th>
<th>Erythrocyte</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr %</td>
<td>Nr %</td>
<td>Nr %</td>
<td>Nr %</td>
<td>Nr %</td>
</tr>
<tr>
<td>TBC</td>
<td>1398</td>
<td>92</td>
<td>1459</td>
<td>96</td>
</tr>
<tr>
<td>Abscesses and LANP</td>
<td>399</td>
<td>97</td>
<td>403</td>
<td>98</td>
</tr>
<tr>
<td>BPCO + CPC</td>
<td>506</td>
<td>90</td>
<td>528</td>
<td>94</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>392</td>
<td>93</td>
<td>375</td>
<td>89</td>
</tr>
<tr>
<td>Other disease</td>
<td>61</td>
<td>78</td>
<td>65</td>
<td>84</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>104</td>
<td>81</td>
<td>105</td>
<td>82</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2860</td>
<td>82</td>
<td>2935</td>
<td>84</td>
</tr>
</tbody>
</table>

Instead this red cells and eosinophils was a valuable indicator when studied diseases such as: number of red blood cells was increased in the bronchopulmonary neoplasms (31% compared to the average of 9%) actually representing repeated bleeding from the tumor, obtaining also an important increase of eosinophils in the patients with asthma, being in the chronic obstructive pulmonary disease group (Roger Nasiell, 1976).

Table 5. shows cellular changes nonmalignant of the bronchial epithelium and sometimes their relationship with bronchial carcinoma.

**Table 5 - Cytology (hyperplasia, metaplasia and dysplasia) in studied diseases.**

<table>
<thead>
<tr>
<th></th>
<th>Hyperplasia</th>
<th>Metaplasia</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr %</td>
<td>Nr %</td>
<td>Nr %</td>
<td>Nr %</td>
</tr>
<tr>
<td>TBC</td>
<td>350</td>
<td>23</td>
<td>167</td>
</tr>
<tr>
<td>Abscesses and LANP</td>
<td>103</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>BPCO + CPC</td>
<td>101</td>
<td>18</td>
<td>51</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>177</td>
<td>42</td>
<td>214</td>
</tr>
<tr>
<td>Other disease</td>
<td>16</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>18</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>765</td>
<td>24</td>
<td>500</td>
</tr>
</tbody>
</table>

A. HYPERPLAZIA was found at about 24% of subjects who were able to harvest expectoration, is much higher: 177 cases out of 421 (or 42%) if bronchopulmonary neoplasms.
that basal hyperplasia is more common in heavy smokers and patients with carcinoma bronchial by default.

It should be stressed that from the group that we had studied most patients were heavy smokers (more than 20-40 cigarettes / day for over 20-30 years).

Black and Ackermann (1967) on a study of 60 cases of epidermoid and undifferentiated carcinomas found 13% basal hyperplasias.

Carroll on 92 cases of primary lung carcinoma, finds a hyperplasia around 50% of epidermoid cancers.

After a report by the U.S. Department of Public Health (1964), bronchial epithelial hyperplasia lesions of smokers associated with atypical cells are considered precancerous lesions.

And the study that we conducted showed data similar to those existing in literature.

B. EPIDERMOID METAPLASIA. The replacement of respiratory epithelium by an epidermoid metaplasia epithelial is the result of chronic irritation of the bronchial tree (tobacco, air pollution, carcinogenic substances sometimes allergens) plus various lung diseases such as the following: asthma, tuberculosis, bronchiectasis, abcess lung, chronic pneumonia, chronic bronchitis.

This is demonstrated in this paper as well where metaplasia was found in a proportion of 16 of the cases studied, being present in 11% secondary pulmonary tuberculosis and in 12% abcesses and bronchopulmonary suppurations and only 9% in chronic obstructive pulmonary disease.

Niskanen (1970) found an epidermoid metaplasia in 50% cases of chronic lung disease.

Metaplasia has dominated in our lung cancer study and was found in a proportion of 52 (respectively 214 cases), more than in other diseases.

Nasiell (1968) found this metaplasia in 60% of lung cancer cases and 21.4% in cases without cancer.

Nasiell (1968) found in another study in epidermoid carcinoma and undifferentiated squamous metaplasia in 88% and 80% histological material in showing the importance of sputum cytologic material for a full diagnosis and much cheaper. In adenocarcinomas the percentage is 57% histologically and 48% smear.

Black and Ackermann (1967) found the same figures as Nasiell (1968) involving metaplasia with bronchial carcinoma (88%).

Sanderund found a higher incidence of metaplasia in smokers (80%) then to nonsmokers (54%).

Niskanen, Weller (1970) found a relationship between epidermoid metaplasia and invasive carcinoma of the lung or of small cell considered as a generalized cancer.

C. DYSPLASIA. In epidermoid carcinoma well differentiated or differentiated, Nasiell (1968) found dysplasia in 50% histological and 46% cytology. And in the control group without cancer, dysplasia was found in 4% histological and 22% cytological.

In this study, dysplastic cells were present in 29% of lung cancer cases, 119 cases respectively and in cancerous diseases dominated "absecesses and bronchopulmonary suppurations" 5%, then those of pulmonary tuberculosis, was 4 percent, compared to the total disease where the percentage was 7. We have to mention the large number of patients.

D. BRONCHIAL CARCINOMA. Regarding bronchial carcinoma, of the 421 cases studied we found only 68 to expectorate tumor cells, which are derived from: epidermoid carcinoma, anaplastic carcinoma with small cell and big and adenocarcinomas cells, these results were very disappointing.

In terms of imagery acquired for the chapter on cytology of sputum:

Photo 1. Posters superficial cell Staining M.G.G. 40 Lens

Photo 2. Superficial cells in the posters (cylindrical cells) and diskariotic cells Staining M.G.G. 40 Lens
In these images can be observed:

A. Ciliated cylindrical cells are easily recognized by the presence of a ribbed plate, frequently descuamating in
small plates, nucleus being round or oval with a diameter of maximum 7-15 microns when sometimes is bigger has protoplasm which leads to bomb. There are also, in this study, non-ciliated degenerated cell which are hard to distinguish from cancer cells; presence of multinucleated ciliated cells may be an indicator of chronic inflammations.

B. Calciforme cells are mucosecretante cells without cylinder; they are in smaller number. They have eccentric nucleus, but with an abundant cytoplasmic secretion as small vacuoles, the nucleus is usually round or oval shape

C. Diskariotic cells meet especially epithelial dysplasia defending a pavement floor, but with the disappearance of cell polarity, with architectural changes, with signs of maturation of cells from deeper layers, presence of cells with nuclear atypia, leading to appearing of cells whose cytoplasm shows signs more or less obvious aging, but whose core is frequently tachromatic: having ranging shape and size.

D. Neoplastic cells, where cells have various shapes and nucleus is hyperchrome, irregular in size and shape, able to appear as dense and pictonic masses. Citoplasma is well conserved, eosinophilia, with varying degrees of keratinisation, is birefringenta, making the cellular limits appear clear. Some cells possess many nuclei, while others are have non. (epidermoid carcinoma). Also in the obtained images have appeared cubical or cylindrical cells with glandular appearance, with clear cytoplasm, with secretory vacuoles and large eccentric,regular, rounded nuclei,ular or policromatofilic. The cytoplasm is basophilic or cytoplasmatic. The desquamation is done in posters or in the form of isolated cells (adenocarcinoma).

Conclusions
1. Cytological examination of sputum was performed in a total of 3120 patients, could see the increased number of epithelial cells and cylindrical cells malpighiene. This percentage was higher in the chronic lung diseases, train and in the lung cancer.
2. Regarding histiocitar cells of blood origin, this showed a marked increase in macrophages and neutrophils; red blood cells and eosinophils have been found in very small percentage without being able to determin cause behavior therapy.
3. Cytology performed at cough showed a more frequent hyperplasia than metaplasia, the latter being more frequent in broncho-pulmonary disease-cancer. Also dysplasia was more common in patients with lung cancer, being useful in the positive diagnosing this disease.

Obtained microscopy images have examplified our survey data, thanks to the collaboration with the pathological anatomy service of Medicine, University of Craiova

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