Cytological and Immunocytochemical Study of Bronchial Wash in Bronchogenic Carcinoma

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Summary:

**Background:** Carcinoma of the lung has become the most common type of cancer since 1985 & the most common cause of cancer death in both males & females.

**Aim of study:** To assess the diagnostic accuracy of bronchial wash cytology & application of immunocytochemical methods, using two tumor markers (low molecular weight cytokeratin & epithelial membrane antigen) for more accurate & precise diagnosis of lung tumors.

**Patients, materials and methods:** Fifty five suspected lung cancer cases according to their clinicoradiological examinations were included in this study.

Bronchial wash cytology was performed for all the 55 patients. Smears were stained by conventional cytological stain in addition to immunocytochemical staining using low molecular weight cytokeratin & epithelial membrane antigen. The final results of bronchial wash were compared to histopathological results & final clinical diagnosis as.

**Results:** Cytological smears of bronchial wash revealed the presence of malignant cells in 33 cases (60%).

The sensitivity of bronchial wash cytology was 82.5%; the specificity was 100.0%, with overall accuracy of 87.3%.

Using cytokeratin staining, 26 cases (47%) were positive for malignant cells, & 29 were negative. The sensitivity, specificity & overall accuracy were 65.0%, 100.0%, & 74.5% respectively.

With EMA staining, 19 cases (27%) were positive for malignant cells & 36 were negative. The sensitivity, specificity & overall accuracy were 47.5%, 100.0%, & 61.8% respectively.

Combined use of CK & EMA raised the sensitivity to 72.5%, specificity to 100% & overall accuracy of 80%.

**Conclusions:** Using more than one monoclonal antibody, or using combined conventional cytology & immunocytochemistry increase the sensitivity for detection of malignant cells in bronchial wash smears.

Introduction:

Bronchogenic carcinoma is the most insidious neoplasm in the whole realm of oncology, & it is usually discovered by the age of fifties. Lung cancer is the second most common type of cancer in Iraq in both males and females by the year 1998, 1999, 2000. Bronchogenic carcinoma is presently the most common malignant disease in the industrial world and it become increasingly frequent during the past 50 years, it account for 19% of all cancer and 27% of cancer death.

Bronchoscope and bronchial wash were introduced by Ikeda (1964) since that; flexible fiberoptic bronchoscope has an important role in both diagnosis and staging of lung cancer.

Patients, Materials, and Methods:

During the period from the first of September 2004 to the first of June 2005, fifty five patients suspected to have lung cancer according to their clinico-radiological examinations, attending the Cardiothoracic Department at AL-Kadhimya Teaching Hospital underwent fiberoptic bronchoscopic examination, with collection of bronchial wash and brush samples from all the patients and transbronchial biopsy for 29 patients with visible bronchoscopic findings. These samples were processed in the pathology lab of college of medicine of AL-Nahrain University.

Bronchial wash:

With the use of FOB, bronchial wash was performed by installation of 10 nil. of saline through the accessory port of the bronchoscope into the suspected area and reaspirated and collected into can tube, then centrifugation smearing of the sediment then immediately fixed in 95% ethanol alcohol .. at least. Eight slides were prepared, four of them in an ordinary glass slides (two for H&E stain, and two for pap stain) for...
conventional cytological examination. The other four slides are positive charged slides (two for low molecular weight CK, and the other two for EMA) for immunocytochemical staining.

**Bronchia brush:**
Bronchial brush is done to the suspected area or to the viewed mass by FOB by a special brush device which is introduced through the accessory port of the FOB. Brush result in higher yield of cellular materials. Brushing specimens should be handled immediately to reduce the artifact in the specimens. After removal of the FOB and the brush, the brush materials are smeared directly on glass slides with circular movement about 0.5 to 1 cm. in diameter and immediately fixed in 95% alcohol for 30 min.

**Statistical analysis:**
The test of significance used in this work is the "difference between two proportions"; these differences were calculated using the Chi-square analysis, the difference is regarded as significance if the P value is less than 0.05.

The suspicious cases were regarded as false negative for statistical analysis.

**Results:**
Total of 55 patients presented with clinical and radiological criteria of pulmonary tumors including cough, haemoptysis, repeated chest infection and dyspnea with CXR and/or CT findings. Of 55 patients, According to the final clinical and histopathological diagnosis, 40 patients were diagnosed as having bronchogenic carcinoma. Of these 40 positive patients, there were 28 (50.9%) male patients and 12 (21.8%) female patients (table 1).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>28</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>15</td>
<td>55</td>
</tr>
</tbody>
</table>

SQCA found to be the most common type of pulmonary tumors according to the final clinical and histopathological diagnosis and representing 26/40 (65%) cases (table 2).
Immunocytochemical results:
All the 55 samples of Bronchial wash studied by immunocytochemical techniques using LMW cytokeratin (CK) and epithelial membrane Antigen (EMA).

CY results:
Staining with CK were positive in 26/55 cases (47%) and were negative in 15 cases with 14 false negative cases which proved to be malignant by other methods of diagnosis (table 4).

Table 4 Immunocytochemical stain (CK) of the 55 bronchial wash smears.

<table>
<thead>
<tr>
<th>CK response</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive CK</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>Negative CK</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>False negative CK</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100</td>
</tr>
</tbody>
</table>

The most identifiable tumor was SQCA with 65.5% while small cell carcinoma shows negative staining with 0%.

The sensitivity of CK staining was 65.0%, specificity 100%, PPV 100%, NPV 51.7%, and over all accuracy 74.5%.

EMA Results:
Table 5 show that 19/55 cases (35%) were stained positively with EMA while 15/55 cases were negative and 21/55 cases were false negative, the results of immunocytochemical staining was compared with the final clinical and histopathological diagnosis (table 5).

Table 5 Immunocytochemical stain (EMA) of the 55 bronchial wash smears.

<table>
<thead>
<tr>
<th>EMA response</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive EMA</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>Negative EMA</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>False negative EMA</td>
<td>21</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100</td>
</tr>
</tbody>
</table>

The most common type of cancer encountered was SQCA with 63% while small cell carcinoma also shows negative staining. The sensitivity, specificity, PPV, NPV and accuracy were, 47.5%, 100%, 100%, 41.7%, and 61.8% respectively.

Table (6, 7) show the distribution of cases in response to positive cytology, CK and EMA staining, there is significant correlation between the immunocytochemical staining in both markers and the positive cytological results.

Table 6 Distribution of cases in response to cytology and immunocytochemical staining of cytokeratin.

<table>
<thead>
<tr>
<th>Immunocytochemistry stain</th>
<th>Cytology result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive CK</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Negative CK</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

P=0.0011

Table 7 Distribution of cases in response to cytology and immunocytochemical staining of EMA.

<table>
<thead>
<tr>
<th>Immunocytochemistry stain</th>
<th>Cytology result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive EMA</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Negative EMA</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

P=0.0176

When both cytological results and immunocytochemical staining were combined there was much more increase in the accuracy and sensitivity. The sensitivity was 95%, specificity 100%, PPV 100%, NPV 88.2%, Overall accuracy 96.3%
FIGURE 3 Bronchial wash smear showing SQCA with intense homogenous cytoplasm staining of EMA stain. (400X)

Discussion: -
Lung Tumors frequently exhibit; multiple pathogenesis of cellular differentiation, making them difficult to be diagnosed and classified by routine light microscopic examination (112). Immunocytochemical techniques have become widely used in cytopathology by using multiple tumor markers to aid in the diagnosis of different types of cancers and to increase the sensitivity of various diagnostic methods.

From total of 55 cases, most of the cases were among males 40/55 (72.7%), the rest 12/55 (27.3%) were among females. This result is similar to studies of Vincent et al "3j, and Santose et al 2005’14).

Forty cases were proved to have malignancy by final clinical and histopathological diagnosis. Most of the malignant cases were between the age of 60-69 years old with 18/40 and representing 47.3% of the total cases. This results is similar to another Iraqi studies done in Mousl and Baghdad ‘1’ - ‘G’ As reported in the literature, lung cancer is relatively uncommon before the age of 50 years with rate increasing rapidly after the age of 50 years (‘). The most common type of carcinoma diagnosed in our study was squamous cell carcinoma (65%), this results is similar to finding of Santose et al ‘14), this may be because that most of the patients selected in this study found to have squamous cell carcinoma or because over 90% of the patient were smokers and as we know the ultimate relationship between smoking and its role in the pathogenesis of SQCA.

The sensitivity of Bronchial wash cytology was 82.5%; this finding is similar to the finding of Al-Rawi. FA., a study which gives sensitivity of 82% but differ from the results of Biob et al giving 76%, Troung et al giving 66%, Bederssian et al 76% (" Ahmed A. et al who gives sensitivity of 94.5% (8).

The preparation of cytological specimens to be examined immunocytochemically was the same procedure of conventional cytology. Ideally it is preferable to use the cytocentrifuge (cytospin) apparatus which gives circles with concentrated cells on the slide rendering the further steps of application of immunocytochemical materials much easier and cost effective, the fixation of the smear in 95% ethanol alcohol. Because the cytological smears are not routinely subjected to formalin, so one could conclude that epitope retrieval technique are unnecessary for immunocytochemical staining of these specimens. Because alcohol fixed tissue by dehydration and removed of water molecule that are non covalently bound to the side chain of amino acid, not by induction of molecular cross links in protein, which change the nature three dimensional protein configuration; there by altering the normal 3-dimensional structure of epitope, making it more difficult for the antibody to bind to its target as a formalin did (9).

By the use low molecular weight cytokeratin, 26/55 cases were positive (47%). The most common type of cancer encounter with CK staining was squamous cell carcinoma, because of its high percentage in the selected cases, increase expression in that type of tumor (2w) or due to the sensitivity of the markers for SQCA as reported by Chu PG, et al (2”). Many studies concentrate on the usefulness of antikeratin antibodies in the differentiating small cell carcinoma of the lung from other histological types of lungs cancers, (22,23,24) small cell carcinoma of the lung had no reactivity for keratin; whereas all others types of lung carcinoma did have reactivity (”). So the use of antikeratin antibody is important at least to differentiate small from non-small cell lung cancer which is an important point for treatment especially that SCCA is metastatic at diagnosis and responsive to cytotoxic chemotherapy and radiotherapy, therefore inorder to improve the dismal prognosis of patient suffering from SCCA. It is of great important to be able to recognize accurately the SCCA in cytological preparation by rapid and reliable cyto diagnostic tests (’). According to EMA staining, 35% of the cases were positive. The most common type present with EMA staining was squamous cell carcinoma again. The sensitivity of EMA was 47.5%; this result is close to the finding of Domagala-Kulawik et al on Bronchial wash cytology (251).

The result, show that combination of both cytology and immunocytochemical techniques will increase the sensitivity of bronchial wash cytology from 82.5% to 95%, this results similar to Weinberg et al (2004) and Rosell et al (1998) (26,27).
Combinations of both markers increase the sensitivity to 72.5%. There was highly significant correlation between cytokeratin positive staining and EMA positive staining.

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