Bronchoalveolar Lavage in Malignancy

Venerino Q Poletti, M.D., Giovanni Poletti, M.D., Bruno Murer, M.D., Luca Saragoni, M.D., and Marco Chilosi, M.D.

ABSTRACT

Bronchoalveolar lavage is a useful diagnostic tool in diffuse or disseminated lung malignancies that do not involve the bronchial structures visible by endoscopy. The neoplastic histotype and the intraparenchymal neoplastic growth pattern are good predictors for diagnostic yield; adenocarcinoma, and tumors with lymphangitic or lepidic growth patterns more easily diagnosed by bronchoalveolar lavage; in these cases the diagnostic yield reported is higher than 80%. In hematologic malignancies the diagnostic yield is quite good in secondary diffuse indolent B cell lymphomas and in primary B cell lymphomas of mucosa-associated lymphoid tissue (MALT) type but low in Hodgkin disease. Morphological analysis may be implemented by immunocytochemical or molecular tests to identify the cell lineage and the presence of monoclonality. Disorders in which bronchioloalveolar cell hyperplasia/dysplasia is a significant morphological component may have cytological features in bronchoalveolar lavage fluid that mimic lung neoplasms: acute respiratory distress syndrome (ARDS), acute interstitial pneumonitis (AIP), and acute exacerbation of idiopathic pulmonary fibrosis are the most important clinical entities in this group.

KEYWORDS: Bronchoalveolar lavage, bronchioloalveolar cell carcinoma, lymphoproliferative lung disorders, carcinomatous lymphangitis, lung neoplasms

The diagnosis of diffuse neoplastic involvement is a major and growing diagnostic problem for pulmonologists because such events may mimic nonneoplastic infectious, inflammatory, and metabolic disorders. The problem is compounded in patients with a history of chemotherapy or radiotherapy or in subjects immunocompromised for a variety of causes. In this clinical setting bronchoalveolar lavage (BAL) may have a relevant diagnostic role in detecting neoplastic cells or suggesting an alternative diagnosis. The comprehension of BAL’s role in diagnosis of lung tumors is based upon three points: the growth pattern and cytological characteristics of tumors in the lung parenchyma, correlations between morphology and imaging features, and the diagnostic value added by new investigative techniques such as immunocytochemistry, flow cytometry, and molecular biology techniques.

DIFFUSE LUNG TUMORS: MORPHOLOGICAL AND IMAGING CORRELATIONS

The lungs are the organ system that acquires the most metastases of any system in the entire body. This is related to several unique features of the lungs: they receive the entire cardiac output every minute, they...
have the densest capillary bed in the body, they are the first capillary plexus met after most of the lymphatic drainage enters the venous system, and they consist of delicate membranes that may be beneficial for drawing on nearby oxygenated air for sustenance. The most common situation is presentation in patients with known extrapulmonary solid neoplasm; in this setting metastases are correctly identified in the majority of cases without the need for invasive procedures. However, especially when the primary tumor is still unidentified, or when the clinical features and laboratory findings are atypical or confusing, or the tumors are of mesenchymal origin, disseminated lung neoplasms may imitate other diffuse infiltrative lung diseases either for the clinical profile or for the radiographic findings. Also, multifocal primary lung neoplasms may present clinical and imaging features overlapping those observed in nonmalignant diffuse lung diseases. Tumors infiltrate or grow in the lung parenchyma, giving rise to different histopathological patterns (Table 1). A lepidic pattern is characterized by neoplastic cells growing along alveolar walls replacing the normal lining cells without altering the interstitial tissue. This growth pattern is typically observed in bronchioloalveolar cell carcinoma (BAC) (Fig. 1), but it may also be present in parenchymal lung metastases due to tumors originating in the gastrointestinal tract, pancreas, prostate, breasts, thyroid, ovary, and pleural mesotheliun. When the tumor cells and their secretory products fill the alveolar spaces, histopathology at low power is similar to that observed in infectious lobar pneumonia (pneumonia-like growth pattern) or in desquamative interstitial pneumonitis (DIP pattern). This pattern is typical of epithelial tumors (especially adenocarcinoma) but it is also observed in lymphoproliferative disorders. Lymphangitic spread is characterized by tumor filling of septal and peribronchovascular lymphatics; blood vessels are often coexistent involved and nodular interstitial desmoplasia does occur, mostly in epithelial tumors. Lymphatics are evident and dilated only in lymphangitic spread due to epithelial neoplasms. Lymphangitic carcinomatosis (Fig. 2) is particularly common with carcinomas of the stomach, breast, lung, prostate, pancreas, and ovary. Lymphomas, myeloproliferative disorders, Kaposi sarcoma, and metastatic angiosarcoma may infiltrate or usually infiltrate the lung parenchyma following lymphatic routes. Hematogenous metastases commonly present as parenchymal nodules, eventually with central necrotic areas, favoring the mid- to lower and peripheral

<table>
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<tr>
<td>Lepidic</td>
<td>Lepidic Growth: Ground-glass attenuation, alveolar nodules, &quot;crazy paving&quot; pattern.</td>
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<tr>
<td>Pneumonia-like/desquamative interstitial pneumonia pattern</td>
<td>Pneumonia-like/desquamative interstitial pneumonia: Ground-glass attenuation, alveolar opacification.</td>
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<tr>
<td>Lymphangitic</td>
<td>Lymphangitic: Reticular pattern; tree in bud pattern.</td>
</tr>
<tr>
<td>Hematogenous</td>
<td>Hematogenous: Nodules, randomly distributed.</td>
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<tr>
<td>Intravascular</td>
<td>Intravascular: Tree in bud pattern, mosaic oligemia, pulmonary infarction.</td>
</tr>
<tr>
<td>Interstitial</td>
<td>Interstitial: Ground-glass attenuation; reticular pattern; crazy paving pattern.</td>
</tr>
<tr>
<td>Cystic</td>
<td>Cystic: Cysts ± nodules.</td>
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lung fields. **Pulmonary tumor thrombotic microangiopathy**
(with pulmonary hypertension, acute or subacute cor pulmonale) is a rare clinicopathological entity in which widespread tumor emboli (mainly from the stomach, breast, ovary, gallbladder, liver) along with fibrocellular intimal proliferation and thrombus formation in the small arteries and arterioles of the lung are the histological hallmarks.\(^9\) Intravascular neoplastic growth patterns may involve mainly the capillary bed as typically observed in intravascular lymphomatosis.\(^{20}\) Interstitial interalveolar infiltration by tumor cells (**interstitial pattern**) may closely simulate interstitial inflammation; spindle cell carcinomas, mesenchymal tumors, and malignant thymoma, and lymphoproliferative disorders may present in areas in which the growing pattern is interstitial.\(^{15,21}\) Multiple cystic neoplastic lesions are due to metastatic squamous carcinoma, bronchioloalveolar cell carcinoma, or metastatic papillary tumors; rarely malignant cysts are observed in metastatic low-grade sarcomas,\(^{11}\) low-grade lymphomas, light chain deposition disease,\(^9\) and mesenchymal\(^{20}\) cystic hamartoma.\(^9\)

Histopathological growth patterns have corresponding high-resolution computed tomographic (CT) scan features (Table 1).\(^{22,23}\) However, radiological findings, and sometimes the clinical profile, may also exhibit secondary changes: alveolar hemorrhage, mainly observed in cases of diffuse neoplastic interstitial infiltration or vascular thrombosis,\(^{24,25}\) organizing pneumonia, or eosinophilic pneumonia in the parenchyma surrounding the infiltrating tumor.\(^{21}\)

**BRONCHOALVEOLAR LAVAGE FLUID ANALYSIS: TECHNICAL KEY POINTS AND ADJUVANT TECHNIQUES**

BAL fluid should be processed with the understanding that it is always possible to detect neoplastic cells.\(^{26}\) Simple smears and centrifuged or Millipore-filtered preparations (Millipore, Billerica, MA) are routinely used. However, in recent years liquid-based cytology has emerged as an alternative to conventional cytopreparatory methods. In particular, the ThinPrep system (Cytyc Corporation, Marlborough, MA) has found broad acceptance. Most comparative studies have shown the ThinPrep system to perform as well as or better than conventional preparations in nongynecologic cytology; plus, the residual cells within the vial can be used for DNA analysis or immunohistochemical and other special studies.\(^{27}\)

Besides May–Grünewald-Giemsa or Diff-Quik\(^{21}\) staining methods, Papanicolaou stain should be used routinely for its value in detecting and defining neoplastic epithelial or mesenchymal cells. Diff Quick stain may be used for rapid on-site evaluation, a method already proposed to improve the yield of transbronchial needle aspiration. Preparation of cell blocks has been abandoned by many laboratories. The benefits of this technique (the recognition of histological patterns and the possibility to have unstained slides available for immunocytochemical investigations) are, however, still evident in fine needle aspiration samples, and they may also be appreciated in BAL fluid specimens.\(^{28}\)

Application of immunocytochemistry permits the extension and expansion of morphology by means of increasingly more sensitive and specific markers that can be visualized in single cells. Thyroid transcription factor 1 (TTF-1) along with cytokeratin (CK) 7 and 20 is very helpful in the distinction of primary pulmonary adenocarcinomas from metastatic adenocarcinomas to the lung and mesothelioma; nuclear immunostaining for estrogen and progesterone receptors is used\(^{21}\) to document lung involvement by breast carcinomas. Other markers (an incomplete list includes cytokeratins, vimentin, desmin, CD45, vascular markers - anti CD31 and CD34-\(^{21}\)) may be helpful to distinguish between epithelial tumors and neoplasms of mesenchymal origin. A panel of antibodies directed to vimentin, S-100 protein, HMB\(^{22,23}\), 45, and melanoma-associated antigen recognized by T cells (MART1) is used to establish a diagnosis of melanoma. The oldest markers "carcinoeembryonic antigen (CEA), epithelial membrane antigen (EMA), monoclonal antibodies reacting with glycoprotein antigens –B72.3, Ca 19–9, Ca125, Ca15–3, CD15, MOC-31, and BerEP4" need to be interpreted in the clinical context because they are identified in a variety of neoplastic as well as reactive nonneoplastic epithelial cells.\(^{28,29}\)

The classification of most hematopoietic neoplasms is by a combination of antigen expression and morphology, chromosomal karyotype, and molecular genetics. Flow cytometry is essential for determination of antigen expression in this kind of tumor, allowing one to stain and correctly identify as many as 10 antibodies simultaneously.\(^{30}\)

Molecular technologies are being used with increasing frequency in many areas of diagnostic cytopathology and in an ever-expanding series of research applications utilizing cytological samples. However, to date these techniques have a minor role in the daily practice as far as BAL fluid analysis is concerned.\(^{28}\)

**DIAGNOSTIC VALUE OF BRONCHOALVEOLAR LAVAGE IN LUNG MALIGNANCY**

Application of BAL to the diagnosis of pulmonary malignancy was first reported in the early and mid-1980s,\(^{31–36}\) and the diagnostic role of this tool was confirmed a few years later in numerous articles.\(^{37–45}\) In immunocompromised patients, the diagnostic yield appeared to be less than 50%, and the cells identified were mostly of the hematologic lineage.\(^{32,33}\) Among the early
descriptions of primary lung cancer in BAL specimens were cases of bronchioloalveolar cell carcinoma,\textsuperscript{31,34} probably because of the diffuse pneumonia-like infiltrate with which this neoplasm may manifest. The distinction between localized tumors and diffuse processes was not apparent in the first reports.\textsuperscript{46} However,\textsuperscript{Q24} later, in a prospective study to assess the value of the addition of BAL to the routine bronchoscopic exploration with bronchial washing and postbronchoscopy sputum procedures in the diagnosis of peripheral primary lung cancer not visible through bronchoscopy (39 nodules and 28 infiltrates) being fluorescent guidance was not available, de Garcia et al reported a diagnostic yield of 33% (18/55 patients).\textsuperscript{37} Wongsurakiat et al more recently reported a diagnostic yield of 47% in 55 patients with peripheral lesions on chest radiographs suspected for carcinoma. Eventually, fine needle aspiration biopsy earned the main diagnostic role in the diagnosis of peripheral tumors appearing as solitary nodules.\textsuperscript{49-50} BAL demonstrated and still shows its practical value in the diagnosis of disseminated or diffuse tumors\textsuperscript{51-53} (Table 2).

**Epithelial Tumors**

The histotype was considered an important element to predict the diagnostic yield of BAL in epithelial tumors; adenocarcinoma and BAC are the two forms of tumor in which BAL has the highest diagnostic yield. However, the infiltrative patterns also appeared to be a good predictor\textsuperscript{Q18} being lymphangitic carcinomatosa and tumors with a lepidic growth pattern diagnosed by BAL in the majority of cases\textsuperscript{Q19} (83% and 93% of cases, respectively, in the series reported by Poletti et al).\textsuperscript{52} Levy et al\textsuperscript{Q20} compared the yield of several diagnostic procedures applied to 12 patients with known malignancies (bladder, breast, colon, prostate, and lung) and radiographic evidence of lymphangitic carcinomatosa. Patients with nodular metastases or endobronchial lesions were excluded. BAL was positive in each of the five patients to which it was applied. Bronchial washings were positive in four of seven cases (57%), whereas brushings were positive in two of five cases (40%). Transbronchial lung biopsy was positive in four of nine cases (44%) but was the only positive specimen in one case (8%). The diagnostic yield of BAL remained high (66%) in a more recent paper in which pneumonia-like consolidation due to adenocarcinoma was documented by CT, a more sensitive tool compared with simple radiography.\textsuperscript{54} Because adenocarcinoma (from the lung, breast, gastrointestinal tract, or pancreas) is the usual histological subtype in carcinomatous lymphangitis\textsuperscript{15} and BAC or adenocarcinoma, primary or metastatic, are usually the subtypes causing a lepidic growth pattern or “tumoral pneumonia”\textsuperscript{15} it is clear that adenocarcinoma and BAC may be more easily identified by BAL. Cytological distinction between mucinous and nonmucinous, well-differentiated adenocarcinomas may be based on cytological specimens: in non-mucus-producing tumors variable numbers of well-demarcated rounded or papillary clusters of tumor cells are evident. These clusters are composed of overlapping small, round, or roughly cuboidal cancer cells with scant clear or lightly stained cytoplasm and moderately hyperchromatic nuclei with one or two small nucleoli. In the mucous-producing adenocarcinomas single cells as well as clusters are present. The tumor cells are larger and have abundant mucus-producing cytoplasm. The distinction between different subtypes of BAC and adenocarcinoma with papillary aspects is not feasible by cytology,\textsuperscript{5} (Fig. 3) although the presence of bland neoplastic cells in clusters, cells resembling alveolar macrophages, papillary fronds, occasional fibrovascular septa, depth of focus (three-dimensional clusters), nuclear pseudo-inclusions, and, infrequently, psammoma bodies have been reported as peculiar cytological features of BAC. The expression of TTF-1 and surfactant proteins, and the epidermal growth factor receptor (EGFR) gene mutation that are now part of the diagnostic workup and therapeutic decision steps in subjects with these tumors may be documented on cytological preps\textsuperscript{56} (Fig. 4). In hematogenous metastases with a multinodular pattern the diagnostic yield was lower (45%),\textsuperscript{52} an expected result because, usually, tumor nodules are well demarcated from the surrounding parenchyma with a pushing border.\textsuperscript{15} The analysis of both aliquots (early or “bronchial” and late or “alveolar”) seems to increase the diagnostic yield.\textsuperscript{43} Lymphocytosis or neutrophilic, eosinophilic, or mixed alveolitis may be observed in association with the presence of neoplastic cells.\textsuperscript{57,58} Adenocarcinoma of different subtypes or other common epithelial tumors (squamous, small cell carcinoma) may develop in diverse morphological and clinical settings: idiopathic pulmonary fibrosis, progressive systemic sclerosis, cystic pulmonary airway malformation, Herman-sky-Pudlak syndrome.\textsuperscript{59-62} There are only anecdotal reports of the utility of BAL in the diagnosis of epithelial

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<th>Tumor Histotype</th>
<th>n Cases</th>
<th>Yield</th>
<th>%</th>
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<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>62</td>
<td>48</td>
<td>77</td>
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<tr>
<td>Bronchioloalveolar cell carcinoma</td>
<td>44</td>
<td>41</td>
<td>93</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>10</td>
<td>5</td>
<td>50</td>
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<tr>
<td>Small cell carcinoma</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Ductal breast carcinoma</td>
<td>10</td>
<td>8</td>
<td>80</td>
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<tr>
<td>Renal cell carcinoma</td>
<td>5</td>
<td>3</td>
<td>60</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>15</td>
<td>10</td>
<td>67</td>
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<tr>
<td>Melanoma</td>
<td>2</td>
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Table 2 Diagnostic Yield in Diffuse Malignant Pulmonary Infiltrates\textsuperscript{52}
lung malignancy in this peculiar context; important elements that make cytological analysis of BAL fluid not highly specific and probably not sensitive are the presence of atypical reactive, but not frankly neoplastic, epithelial cells in fibrosing lung disorders and the fact that tumors are not always disseminated at the beginning.

**Malignant Hematologic Disorders**

Pulmonary involvement due to malignant hematologic disorders is not a rare occurrence in patients with a lymph-node-based lymphoma; it is rarer as a clinical event in subjects with myeloid tumors. Primary pulmonary lymphoma occurs in a minority of subjects. The most common type of primary pulmonary lymphoma is extranodal marginal zone B cell lymphoma, which arises from bronchus-associated lymphoid tissue. It represents 70 to 90% of all primary pulmonary lymphomas, 3 to 4% of extranodal non-Hodgkin lymphomas (NHLs), and 0.5 to 1% of primary pulmonary malignancies. An association with collagen vascular diseases, mainly Sjögren syndrome, common variable immunodeficiency, and human immunodeficiency virus (HIV) infection has been reported. The translocation t(11;18)(q21;q21), which results in a fusion of the cIAP2 region on chromosome 11q21 with the MALT1 gene on chromosome 18q21 is documented in more
than one third of cases. Interestingly, this translocation affects the nuclear factor kappa B (NF-kB) pathway. Lymphomatoid granulomatosis is an angiocentric and angiodestructive lymphoproliferative disease involving extranodal sites, consisting of Epstein-Barr virus (EBV)-positive cells admixed with reactive T cells, which usually numerically predominate. The lesion has a spectrum of histological grade and clinical aggressiveness that is related to the proportion of large B cells. The most common site of involvement is lung. The old series probably included cases of extranodal natural killer (NK)/T cell lymphomas primary in the lungs were included. Diffuse large B cell lymphomas make up 10% of cases of primary pulmonary lymphomas. T cell lymphomas and Hodgkin disease rarely occur primarily in the lungs. BAL may contribute to the diagnosis in malignant lymphoproliferative disorders, either primary or secondary in the lungs. In NHL the BAL diagnostic yield reported was 67%. An increased total cell count due mainly to lymphocytosis is evident in the majority of cases. Neoplastic cells in indolent lymphomas of B cell type may have morphological features not easily distinguished from normal or “activated” lymphocytes or form dense aggregates of lymphocytes present in bronchial specimens of patients with follicular bronchiitis/bronchiolitis. Cells with nuclear membrane irregularities and indentations, fine nuclear chromatin, inconspicuous nucleoli, and scant cytoplasm, monocytoïd cells, lymphoplasmacytoid lymphocytes, and plasma cells with occasionally intranuclear inclusions known as Dutcher bodies may be observed, and their presence helps to suggest the diagnosis (Fig. 5). However, the same morphological findings may be present in nonmalignant lymphoproliferative disorders such as lymphocytic interstitial pneumonia or diffuse Castleman diseases. In high-grade B cell lymphomas frankly malignant cells (large cleaved and noncleaved) are the morphological clue for the diagnostic hypothesis; (Fig. ). In T cell lymphomas a spectrum of atypical cells with nuclei varying in shape and ranging from small to large, often with convoluted nuclei, have been described. However, in all these contexts, for a precise diagnostic report, immunocytochemistry and flow cytometry analysis are mandatory to define the cellular lineage; in B cell lymphomas the monoclonality is demonstrated only when a monotypic expression of light chains is evident (Fig. 6). Gene rearrangement analysis has been shown to be useful to document monoclonality of T cell lineage. B-lymphocyte clonality analysis in the setting of a clinical suspicion of both primary and secondary pulmonary lymphoma performed by polymerase chain reaction (PCR) demonstrated a 97% specificity and a 95% negative predictive value because a weak detectable B cell clonality in BAL fluid was observed in 14% of patients with nonlymphomatous lymphocytic pneumonia.
pulmonary lesions, notably autoimmune and chronic infectious diseases. The diagnostic yield of BAL in the diagnosis of parenchymal lung involvement due to Hodgkin disease is low (33%) mainly because diagnostic cells (Reed-Sternberg cells or Hodgkin cells) are scattered in an inflammatory background (Fig. 7A); lymphocytosis in BAL fluid was reported to be more evident in patients in which diagnostic cells were detectable. Reed-Sternberg cells or Hodgkin cells may be better characterized by immunocytochemistry (these cells are, among a variety of markers, positive for CD15, CD30) (Fig. 7B). BAL is useful in the diagnosis of pulmonary involvement due to chronic lymphocytic leukemia and in the differential diagnosis between chronic lymphocytic leukemia in the lung and mimickers such as sarcoidosis. Diagnosis of diffuse myelomatous pulmonary infiltration established by BAL demonstrating the presence of monoclonal plasma cells has been reported. Symptomatic leukemic infiltration of the lung is the least common cause of pulmonary infiltrates in patients with leukemia; only anecdotal reports confirm the BAL utility in the diagnosis of myeloid lung infiltration in these patients. Leukemic pulmonary infiltration causing respiratory failure in ~20% of cases as the first manifestation of acute monocytic leukemia has been reported; in this context BAL was reported to be a very useful diagnostic tool. Because these patients usually have concomitant coagulopathies BAL recovery may be contaminated with blood; up to now there are no studies discussing the criteria useful to differentiate between peripheral blood myeloid cells contaminating BAL fluid and myeloid cells actually infiltrating the alveolar spaces.

Other Malignancies

The reports presenting data on the utility of BAL to detect neoplastic cells from metastatic malignant melanoma and sarcomas are few. Metastases to the lungs from sarcomas occur in patients in which the origin of the tumor is well known. However, the presence of metastases in the lungs due to melanoma in patients in which the history of the primary tumor is remote and forgotten or unknown is a clinical eventuality; cytological BAL features useful to address the correct diagnosis include a variable amount of melanin pigment in the cytoplasm of atypical cells (although in so-called amelanotic melanoma this pigment is absent) (Fig. 8), isolated or loosely cohesive groups of round to oval cells with eccentric nuclei, regular nuclear outlines, the presence of large intranuclear clear zones or “holes” (nuclear cytoplasmic inclusions) (Fig. 8), binucleation and multinucleation, fine chromatin pattern and prominent nucleoli; finally only immunocytochemistry documenting the positivity for HMB, melanoma-associated antigen recognized by T cells (MART1), or SQ proteins confirms the origin of the tumor cells. In our experience BAL was useful to demonstrate diffuse lung infiltration due to epithelial-type malignant...
mesothelioma. The detection of human herpesvirus 8 (HHV8) DNA in BAL is restricted to patients with Kaposi sarcoma and is highly sensitive and specific for pulmonary involvement of Kaposi sarcoma.80

ABNORMALITIES OF NONLYMPHOID CELLS IN BRONCHOALVEOLAR LAVAGE FLUID MIMICKING MALIGNANCIES

In a variety of lung processes the pulmonary alveoli or the cystic airspaces are lined by one or more layers of small cuboidal or columnar cells that are in continuity with and identical or similar to the adjoining bronchioles.80 Furthermore, pneumocytes type II are highly reactive cells that respond to various pathological processes by morphological changes that may perfectly mimic adenocarcinoma or its precursor lesions in cytological samples81; viral infections mainly due to DNA viruses and drugs/radiation may induce on respiratory epithelium marked cellular enlargement associated with nuclear modification that may cause interpretive troubles. Finally, macrophages with large cytoplasmic vacuoles or abundant bubbly or lacy vacuolated cytoplasm characteristically observed in lipoidic pneumonia may be confused with mucus-producing cancer cells.28 In Table 3 the clinical settings in which BAL fluid analysis may present morphological findings suggesting an incorrect diagnosis of lung malignancy are reported.

Linssen et al81 evaluated—only by means of May-Grünwald-Giemsa staining—the prevalence of reactive type II pneumocytes (RPII) in BAL fluid samples obtained from patients with various pulmonary disorders. RPII were generally large cells with a higher nuclear:cytoplasmic ratio and deeply blue-stained vacuolated cytoplasm. Most RPII occurred in cohesive cell groups, and vacuoles tended to be confluent. RPII were present in 22% of cases (94/433 samples), and the highest prevalence was noted in patients with systemic inflammatory response syndrome and alveolar hemorrhage. In addition, RPII tended to occur more frequently in ventilator-associated pneumonia, *Pneumocystis* pneumonia, extrinsic allergic alveolitis, and drug-induced pulmonary disorders in virtually all cases with diffuse alveolar damage or honeycombing as the main morphological feature.

In a study of BAL cytology specimens from a series of 38 patients with acute respiratory distress syndrome (in which the histopathological hallmark is diffuse alveolar damage), Stanley et al82 noted that RPII were transiently present during the early and organizing stages of the disease but did not persist after day 32 following onset of the illness; this temporal span and the clinical setting in which these cytological modifications may characterize BAL profile are per se useful criteria to exclude a diagnosis of malignancy. Furthermore, morphological features in BAL fluid of patients with diffuse alveolar damage have been well defined83: the slides are

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<th>Table 3 Clinical Settings in Which Bronchoalveolar Lavage Findings May Improperly Suggest a Diagnosis of Epithelial Malignancy</th>
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<td><strong>EPITHELIAL ATYPIA SUGGESTING A DIAGNOSIS OF ADENOCARCINOMA</strong></td>
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<tr>
<td>Diffuse alveolar damage of known cause (acute respiratory distress syndrome, severe infections, drugs/radiation82)</td>
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<tr>
<td>Diffuse alveolar damage of unknown cause (Hamman-Rich syndrome, acute exacerbation of idiopathic pulmonary fibrosis)</td>
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<tr>
<td>Viral infections</td>
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<td>Organizing pneumonia</td>
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<tr>
<td>Chronic lung processes with usual interstitial pneumonia as morphological background (idiopathic pulmonary fibrosis, asbestosis, collagen vascular diseases, chronic hypersensitivity pneumonitis, chronic fibrosis due to drugs)</td>
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<td>Chronic pneumonias of varying etiology</td>
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<td>Pulmonary infarction</td>
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<tr>
<td><strong>EPITHELIAL ATYPIA WITH BIZARRE, EOSINOPHILIC CELLS SUGGESTING A DIAGNOSIS OF SQUAMOUS OR GIANT CELL CARCINOMA</strong></td>
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<td>Antineoplastic drugs</td>
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<tr>
<td>Chronic thermal injury</td>
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<tr>
<td><strong>VACUOLATED MACROPHAGES MIMICKING MUCOUS ADENOCARCINOMA</strong></td>
</tr>
<tr>
<td>Lipoidic pneumonia</td>
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<tr>
<td>Organizing pneumonia</td>
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<tr>
<td>Amiodarone lung injury</td>
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<tr>
<td>Metabolic disorders (Gaucher syndrome)</td>
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moderately to highly cellular with a large number of neutrophils and alveolar macrophages. The epithelial component, which may be depicted by monoclonal antibodies against cytokeratins, anti-B7.23 or anti- TTF1, displays various degrees of nuclear atypia28,81; nuclei are large, round to oval, and hyperchromatic, and most have a single nucleolus and irregularities of nuclear contour. Some epithelial clusters are three-dimensional, with peripheral cells showing clear or vacuolated cytoplasm, protruding outward and resembling hobnails. Other aggregates appear two-dimensional, as sheets of cells with flattened and dense cytoplasm (squamotized). Both types of cell clusters may be associated with dense, basophilic, or amphophilic, amorphous extracellular material (the cytological counterpart of hyaline membranes) (Fig. 9A). This cytological pattern mimicking adenocarcinoma has also been recognized in cases of acute interstitial pneumonitis (Hamman-Rich syndrome), acute exacerbation of idiopathic interstitial pneumonitis (Fig. 9B), or along with a huge increase of eosinophils in acute eosinophilic pneumonia. Staining using monoclonal antibodies against cytokeratins may be
useful to document the epithelial nature of these cells (Fig. 9C). Enlarged nuclei, prominent nucleoli, cytoplasmic keratinization, multinucleated bizarre cells indistinguishable from squamous carcinoma or from the uncommon giant cell carcinoma may be observed as an acute effect of irradiation or treatment including cyclophosphamide or other antiblastic drugs on respiratory epithelium. Chronic thermal injury may cause atypical squamous metaplasia of bronchial epithelium. Organizing pneumonia, cryptogenic, due to known causes, or observed in specific clinical contexts, presents a peculiar BAL profile. However, dysplastic type II cells that may be misinterpreted as neoplastic have been described in a minority of cases.

Cytopathic modifications due to viral infections may be troublesome and the differential diagnosis between an infectious process and adenocarcinoma may result difficult. Cluster of nonciliated cells with enlarged nuclei with prominent, occasionally irregular nucleoli accompanied by large single cells with prominent nucleoli may be observed. The presence of ciliocytophoria (partial separation of the ciliated tuft from the nucleated portion of the cell or presence of anucleated tufts of cilia), and of typical inclusions “intranuclear (herpes simplex virus, adenovirus); nuclear and cytoplasmic (cytomegalovirus, measles infection); multiple eosinophilic cytoplasmic inclusions, and multiple deeply basophilic inclusion bodies with clear halos within the degenerated cytoplasm of the multinucleated syncytial giant cells (respectively, in parainfluenza and respiratory syncytial virus infections)” correctly indicate the diagnosis. Furthermore, viruses involved can be specifically identified in cells by immunocytochemistry with monoclonal antibodies and by in situ hybridization with c DNA.

In lipoidic pneumonia, inhaled oils cannot be absorbed and are phagocytized by pulmonary macrophages. The cytoplasm of the enlarged macrophages contains a great many vacuoles, giving it a characteristic lacy appearance. The nuclei are either single or multiple, small, and of normal appearance. Mucus-producing cancer cells, as a rule, display highly abnormal nuclei, and, furthermore, the cytoplasmic mucin in cancer cells is

![Figure 9](image_url) (A) Patient with acute respiratory distress syndrome. A pseudopapillary structure with dysplastic reactive type II cells surrounding amorphous extracellular material (hyaline membrane) (Papanicolaou stain). (B) Patient with acute exacerbation of idiopathic pulmonary fibrosis. A cluster of reactive type II cells surrounds metachromatic extracellular material (Diff-Quik stain). (C) Patient with acute exacerbation of idiopathic pulmonary fibrosis. Dysplastic cells are positive for cytokeratins, demonstrating their epithelial nature (anticytokeratin staining).
almost invariably limited to a single vacuole; however, the definitive diagnosis is based on special stains in unfixed air-dried specimens demonstrating the presence of oil (oil red O, Sudan black)\(^\text{28,53}\) (Fig. 10). Rarely, crowded foamy macrophages, observed mainly in patients with organizing pneumonia and rarely in amiodarone toxicity and Gaucher syndrome, in which macrophages with small eccentric nuclei and abundant striated and finely vacuolated PAS\(^\text{38}\) positive cytoplasm are detectable, may be, at a first glance, difficult to discern from well-differentiated mucus-producing cancer cells.

**BIOMARKERS IN BRONCHOALVEOLAR LAVAGE FLUID AND ITS VALUE IN THE CLINICAL PRACTICE**

Screening programs, including CT, autofluorescent bronchoscopy, biopsies, and bronchial lavage (BL) and BAL collection, and breath condensate analysis, have been initiated with the specific goal of identifying markers for the early detection of cell lung cancer.\(^\text{2,90}\) Gene methylation in BL might help to detect central tumors, but for peripheral cancer detection CT was shown to remain crucial.\(^\text{91}\)

Identification and quantification of exhaled volatile compounds seem to be useful as complementary tests for the early diagnosis of lung cancer\(^\text{2,90}\); however, all these tests are as yet only research projects and not useful in the daily clinical practice.

**REFERENCES**


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Q6: changed as meant? If not, please reword for sense.
Q7: Permission needed to reproduce any of the Tables?
Q8: Dorland’s prefers hypovolemia. Please check and fix if needed
Q9: okay as is?
Q10: please check spelling and hyphenation of this brand name and provide the manufacturer and location.
Q11: changed as meant? if not, please reword for sense.
Q12: please fix placement of hyphens.
Q13: please spell out HMB first use
Q14: This sentence is so long as to be inaccessible. Please reword to simplify or divide.
Q15: is the word Cases necessary here? or simply n?
Q16: should there be a value of 100 in the percent column?
Q17: should there be a value of 50 in the percent column?
Q18: please reword this sentence hereafter for sense.
Q19: does the period belong here?
Q20: please reword for sense and clarity.
Q21: please spell out TTF if possible
Q22: if this is being used as a gene name, please italicize here and in all occurrences, including in refs.
Q23: NK spelled out okay?
Q24: do you mean “from”
Q25: Which Figure does this refer to?
Q26: (MALT spelled out okay?
Q27: please spell out HMB first use
Q28: Please spell out S first use.
Q29: OK to remove asterisks (were they representing bullets? if so, not needed to connote hierarchy)
Q30: Are you adding material in here?
Q31: change semicolon to comma? or something missing?
Q32: changed as meant? or were other list items missing?
Q33: please spell out B and TTF if possible
Q34: not in Dorland's. Please check spelling.
Q35: please fix wording
Q36: okay as is? spell out c?
Q37: do you mean phagocytosed? Please fix as needed
Q38: please spell out
Q39: Edit of legend OK?
Q40: okay to insert “and” here? or something else missing?
Q41: these are editors, correct?
Q42: what does (s) represent? (Suppl)? Please delete or fix as needed.
Q43: is title complete?
Q44: Is the Task Group the author?
Q45: Medline indexes "Eur Respir J" but cannot find a listing for the reference 26 "Technical recommendations and guidelines for, 1989". Please check the reference for accuracy.
Q46: The reference has no authors. Please proof carefully. (in reference 26 "Technical recommendations and guidelines for, 1989").
Q47: Medline indexes "Hematology" but cannot find a listing for the reference 30 "Orfao, Lo'pez, Flores, et al, 2006". Please check the reference for accuracy.
Q49: what does (s) stand for? (Suppl)? please fix as needed
Q51: Medline reports the first author "Pirozynki M" is not correct in the reference 46 "Pirozynki, 1992".
Q52: Medline reports the first author "de Garcia J" is not correct in the reference 47 "de Garcia, Braco, Miravitlles, et al, 1993".
Q53: What is (s)? please fix as needed
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Q56: Please check year and fix vol. no.
Q57: Medline indexes "Haematologica" but cannot find a listing for the reference 74 "Trisolini, Lazzari Agli, Poletti, 2000". Please check the reference for accuracy.
Q58: Reference has only first page number. Please provide the last page number if article is longer than one page. (in reference 74 "Trisolini, Lazzari Agli, Poletti, 2000").
Q59: Medline reports the first author "Tam M" is not correct in the reference 79 "Tam, Reichenberger, McGandy, et al, 1998".
Q60: as spelled in original? please check and fix
Q61: epithelial? endothelial? Please fix
Q62: Medline reports the first author "Walzer T" is not correct in the reference 87 "Walzer, Mukerjee, Levine, 2002".
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