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Original Article

# Bronchoalveolar Lavage: General Utilization and Correlation between Special Stains and Microbiologic Cultures at a Major Pediatric Institution

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## Abstract

**Background:** Bronchoalveolar lavage (BAL) is a widely used method in the evaluation of lung pathology in adults and children. The main indications in children include confirming suspected infection/inflammation, aspiration pneumonia, and periodic evaluation of lungs in cystic fibrosis patients. **Materials and Methods:** We reviewed a total of 308 consecutive reports from pediatric BAL specimens as a part of quality assurance project aiming to compare morphologic findings of cytological examination with microbiology testing and evaluate impact of reported data at different stages of sample testing process. **Results and Conclusions:** We summarized our findings below and found that in most cases of infections, definitive clinical decisions were taken only after microbiologic culture results were available as compared to morphologic findings.

**Keywords:** Bronchoalveolar lavage, cystic fibrosis, cytological evaluation, lung infection/inflammation, lung pathology, pediatric, quality assurance

## Introduction

Bronchoalveolar lavage (BAL) is a widely used method in the evaluation of lung pathology. The procedure is considered safe and minimally invasive, although the technique used is not completely standardized. Details of the procedure are beyond the scope of this article. Briefly, it generally includes the application of local anesthesia to larynx and bronchial tree, followed by introduction of a bronchoscope transnasally, transorally, or through an in-place tube. After inspection of the airways, a fiberoptic bronchoscope is gently inserted into a segmental or subsegmental bronchus and usually sterile isotonic saline is instilled into the subsegment through the biopsy channel of the bronchoscope and subsequently aspirated and recovered.[1,2] The aspirate is ideally a representative sample, consisting of cell containing solution. BAL fluid obtained at one anatomic site of lung is generally presumed representative of the whole lung, with the exception of localized disease processes. In localized disorders, it is recommended that the area of greatest abnormality demonstrated radiologically is chosen as an appropriate site for BAL. From a specimen quality point view, an adequate specimen should include plentiful lower respiratory tract cells. The latter is morphologically indistinguishable from macrophages.

Clinical indications of BAL in children include establishing the diagnosis of suspected infectious, inflammatory, or rarely neoplastic disorders of the lower respiratory tract, evaluation of suspected aspiration along with periodic monitoring of certain chronic disorders such as cystic fibrosis (CF).

The handling and triage of BAL fluids is not standardized among laboratories, and can vary within the same laboratory based on several factors including the clinical history, differential diagnosis, and specimen volume. At our hospital, samples are initially processed in the cytopathology laboratory where volume is measured, a cell count is obtained, and color and consistency are described. A portion of specimen is then sent to microbiology laboratory for routine staining

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and culture study as ordered by the physician. Cytospin preparations (prepared by centrifuging portions of the specimen and collecting cells on an albumin covered slide) are made for the purpose of routine cytological examination and possible additional stains for organisms as needed.

In this study, we sought to retrospectively review the pathologic and microbiologic results provided for BAL samples collected at our hospital over one calendar year to determine any performance issues for the staining as part of a quality assurance assessment and also to better understand the impact of results on patients' management. In addition, the electronic medical records of one hundred patients were randomly selected from the cohort and reviewed to determine if changes in treatment plan were made based on the staining and/or culture results.

## Materials and Methods

This is a retrospective review of BAL pathology, microbiology reports, and clinical data logged into our laboratory over one calendar year (January 2014 through January 2015). Wright-Giemsa (WG) is the routine stain used for cytology and cell differential, as well as initial detection of intracellular and extracellular bacteria. Subsequently, depending on the clinical history, clinician's request and/or pathologist judgment, additional special stains for organisms may be ordered. The latter include Gram stain (for bacteria), Gomori methenamine silver (GMS, for fungi), Kinyoun's acid-fast stain (for acid-fast bacilli [AFB]), iron stain (to detect hemosiderin), and oil-red-O stain (for intracellular fat). Finally, immunoperoxidase-based stains may be performed to further investigate other potential findings such as viral inclusions, when indicated.

When indicated, a portion of the specimen is sent to microbiology laboratory for culture studies which may include aerobic bacterial, fungal, and acid-fast bacterial cultures. Staining of prepared smears at microbiology laboratory may include Gram stain, Calcofluor fungal stain, and Kinyoun's acid-fast stain. Additional work up at the microbiology laboratory may include cultures for additional specific organisms such as *Legionella* or *Nocardia*, and/or polymerase chain reaction assays for a detection of variety of other pathogens.

## Results

A total of 308 BAL specimens from 261 patients were collected over 1 year and submitted for evaluation in our laboratory. Slightly more than half of the patients (132/261; 50.6%) had the diagnosis of CF, which is typical for a tertiary Pediatric hospital in the United States. The age of patients ranged from 2 months to 41 years with an average of 10.8 years. One hundred and thirty-six samples were collected from male patients and 125 from female patients. All samples had routine pathology testing ordered from and at a minimum aerobic bacterial culture and Gram stain ordered.

### Gram stain

Gram staining was performed on 195 and 292 specimens in the cytology and microbiology laboratories, respectively. In cytology laboratory, 51.8% (101/195) of the specimens were positive for bacteria, while 34.2% (100/292) were positive in microbiology. Bacterial culture was performed on all 308 specimens [Table 1].

### Fungal stain

A fungal stain was performed on 274 and 259 specimens in the cytology and microbiology laboratories, respectively. It was positive for a fungus in 9.1% (25/274) of GMS stains performed in cytology. Of these 25 specimens that were GMS positive, 22 were subsequently confirmed on fungal culture; however, one was negative, and two did not have fungal culture performed. Overall, in microbiologic laboratory, Calcofluor stain was positive in 4.2% (11/259) of BAL specimens. Notably, in the 22 positive fungal culture specimens, Calcofluor stain was positive in only eight of the cases reflecting low specificity.

### Mycobacteria stain

AFB stains were performed on 231 specimens in the cytology laboratory and on 207 specimens in the microbiology laboratory. All AFB stains were negative in both cytology and microbiology laboratories [Table 2].

### Culture

Overall, bacterial culture results were positive in 72% (222/308) of specimens; of which, a pathogen was detected in 78.4% (174/222). For fungal culture, 259 BAL samples had a fungal culture ordered and 76 (29%) had a fungal isolate identified. Mycobacteria culture was performed on 255 specimens and growth of AFB were detected in 2.3% (6/255) of samples.

### Clinical impact

In the randomly selected group of 100 patients, 17% have CF. Review of records showed Gram stain performed in cytology laboratory was positive in 29 cases, negative in 36 cases, and not performed in 35 cases. In all 100 cases, bacterial culture was positive in 65 cases and negative in 35 cases. In these patients, twenty seven had new antibiotics added or changed after culture results were available. GMS stain was positive in 10 of the patients and all of them were confirmed by fungal culture. In eight of the ten patients, antifungal medicine was added based on morphologic findings; however, treatment was further modified in two of these eight patients after culture study results were available [Table 3].

## Discussion

Our study showed that the Gram stain performed and reviewed at cytology laboratory is more sensitive in detecting bacteria than Gram stain reviewed at microbiology laboratory. This may be due to the fact that in the latter, Gram stains is reviewed by technologists while in the former it is read by pathologists. In addition, we noted a strong correlation between the presence

**Table 1: Characteristics and parameters of study participants**

| Parameter | Participants |
|---|---|
| Gender (male/female) | 136/125 |
| Age range | 2 months to 41 years (average 10.8) |
| Cystic fibrosis | 132 |
| Noncystic fibrosis | 129 |

**Table 2: Summary of the cytological special stains for organisms and culture study results**

| Cytological stain | Gram | GMS | AFB |
|---|---|---|---|
| Positive | 101 | 25 | 0 |
| Negative | 94 | 249 | 231 |
| Percentage | 51.8 | 9.1 | 0 |
| **Culture study** | **Bacterial** | **Fungal** | **AFB** |
| Positive | 222 | 76 | 6 |
| Negative | 86 | 183 | 249 |
| Percentage | 72 | 29 | 2.3 |

GMS: Gomori methenamine silver, AFB: Acid-fast bacilli

**Table 3: Clinical impact of special stains for organisms and microbiologic culture studies on bronchoalveolar lavage samples of 100 cases**

| Bacterial study | Gram | Culture | Treatment changed |
|---|---|---|---|
| Positive | 29 | 65 | 27 cases had either a changed or added antibiotic |
| Negative | 36 | 35 | |
| Not performed | 35 | | |
| **Fungal study** | **GMS** | **Culture** | **Treatment changed** |
| Positive | 10 | 10 | Eight cases were added antifungal treatment after GMS stain and two cases had a changed of antifungal after culture results were available |
| Negative | 90 | 90 | |

GMS: Gomori methenamine silver

of intracellular bacteria detected by WG routine stain and presence of pathogenic bacteria by culture.

Confirming the diagnosis of bacterial pneumonia may be achieved based on BAL culture results.[3] This study shows that in most cases where an appropriate antibiotic therapy was added or changed, the decision was based on the culture study results, and these were regardless of what the Gram stain results in the cytology laboratory. Therefore, Gram stain may not be needed in the cytology laboratory since it does not change the treatment plan and the presence of intracellular bacteria can be accurately assessed on routine cytology stains.

Cantral *et al*. reported that bacterial culture study of BAL fluid was sensitive and specific in the diagnosis of bacterial pneumonia but found a poor correlation between the Gram stain of BAL fluid and culture study.[3] Similarly, Allaouchiche *et al*. also found that the Gram stain of BAL correlated poorly with culture study, but they found that Gram stain was useful for rapid diagnosis of ventilator associated pneumonia. However, it is unreliable in the dictation of empiric therapy and better techniques are needed.[4]

GMS is commonly used stain to detect pneumocystis and fungal organisms.[5] We found that GMS stain was sensitive (in correlation to gold standard of microbiologic fungal cultures) and useful in detecting the fungal organisms on the cytospin preparation. Moreover, the morphology of the fungal organisms was reported and most of the time, the type of the fungal species was suggested, in the cytology report.

In regard to Kinyoun's acid-fast stain for AFB, we found that it was not sensitive for detection of mycobacteria in BAL specimens stained in both on cytology and microbiology smears. None of the six cases that had positive AFB culture had AFB reported by cytological examination of AFB stained cytospin slide. These slides were reviewed for a second time and confirmed to be negative. The low sensitivity of the AFB stain may be contributed to sampling, concentration of the fluid, time spent by the examiner on each sample, and the smearing technique.[6,7] It was previously reported that the sensitivity and specificity of AFB stain in lower respiratory specimens was low compared to AFB culture.[8,9]

## Conclusion

BAL is a useful and important key diagnostic procedure in lung diseases of children, especially the evaluation of infectious diseases of the lower respiratory tract. A complete evaluation of the specimen should include morphologic examination of well stained cytospin preparations, utilization of special stains (for organism detection) as well as microbiologic cultures. While each of the above evaluation methods has a role in the diagnosis of infections, clinicians tend to rely more on microbiologic culture results in making definitive treatment decisions.

### Authors' contribution

All authors contributed substantially to the reported work to qualify for authorship and they have all approved the final version of the article.

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### Conflicts of interest

There are no conflicts of interest.

### Compliance with ethical principles

No ethical approval or consent is required for this type of study.

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