October 3-7 • San Francisco, CA • www.idweek.org
IDWeek 2018
Advancing Science, Improving Care

POSTER # 356

Bronchoalveolar lavage fluid cytology by GMS stain for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies: analysis of 67 episodes

Fernández-Cruz A.[1,2], Magira E.[2,3], Heo ST [2,4], Evans S. [5], Tarrand J.[6] and Kontoyiannis DP[2]

(1)Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain, (2)Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, Houston, TX, (3)First Critical Care, Evangelismos Hospital, School of Medicine, National University of Athens, Athens, Greece, (4)Internal Medicine, Jeju National University School of Medicine, Jeju Special-Governing Province, Republic of South Korea, (5)Pulmonary Medicine, The University of Texas MD Anderson Cancer center, Houston, TX, (6) Microbiology, The University of Texas, MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Corresponding authors:
DP Kontoyiannis, MD, PhD
dkontoyi@mdanderson.org
Ana Fernández Cruz, MD, PhD
e-mail: afcruz@salud.madrid.org

BACKGROUND

There is a paucity of studies on the yield of Gomori-methenamine-silver (GMS) stain in bronchoalveolar lavage (BAL) cytology and its comparison with fluorescent dyes for the diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematologic malignancies. To that end, we analysed the yield of direct fungal visualization in BAL cytology by GMS stain in a series of culture-positive IPA in patients with hematologic malignancies

PATIENTS & METHODS

We retrospectively analysed all consecutive cases of culture–documented IPA (proven or probable according to the EORTC criteria) in adult patients with hematologic malignancy (September 1999-March 2015) at MD Anderson Cancer Center, a tertiary care cancer center in Houston, TX. All cases had concurrently available BAL cytology GMS.

- BAL (performed by wedging the bronchoscope into a single affected segment or subsegment of the lung, based on radiographic appearance) was aliquoted and 15ml specimens were sent to microbiology, chemistry/hematology, and cytopathology. Bilateral bronchial washings (BW) (retrieved from the major airways without occlusive wedging) were sent for microbiology at the bronchoscopist's discretion.

- The unspun BAL specimen was first divided according to sample volume requirements for all ordered tests:
  - bacteriology culture and Gram stain, Legionella culture, *Aspergillus* galactomannan, *Pneumocystis jiroveci* molecular testing:1 ml each
  - fungal culture (0.5 ml)
  - virology culture (3 ml)
  - AFB decontamination with culture and stains for AFB (6 ml).
  - Calcofluor White™ stain (150 µl). Calcofluor White™ samples were-centrifuged, heat fixed, and directly stained (Alpha Tec Systems, Vancouver, WA).

- GMS was performed in the cytopathology laboratory by centrifuging BAL samples using a high volume cyto-centrifuge. GMS stains were prepared using 5 - 6 ml of BAL per slide. BW samples were infrequently used for GMS staining. Cytopathology also routinely performed two modified Giemsa stains (Papanicolaou) and the Diff-QuiK (Romanowsky stain).

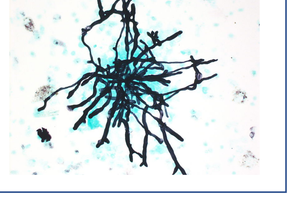

Table 1. Characteristics of cases of pulmonary aspergillosis according to cytology results

	Total	Cytology positive n (%)	Cytology negative n (%)	p
N		28 (41.8%)	39 (58%)	-
Hematologic disease (n, %)				0.898
Acute myeloblastic leukemia/ Myelodysplastic syndrome	28 (39.3%)	13 46.4%)	15 (38.5%)	
Acute lymphoblastic leukemia	3 (4.5%)	2 (7.1%)	1 (2.6%)	
Chronic myeloid leukemia	5 (7.5%)	2 (7.1%)	3 (7.7%)	
Chonic lymphoid leukemia	9 (13.4%)	4 (14.3%)	5 (12.8%)	
Multiple myeloma	5 (7.5%)	2 (7.1%)	3 (7.7%)	
Lymphoma	17 (25.4%)	5 (17.9%)	12 (30.8%)	
SCT	31 (46.3%)	18 (64.3%)	13 (33%)	**0.015**
Recent neutropenia <500 >10 d	15 (22.4%)	7 (25%)	8 (20.5%)	0.769
Prior azole exposure	30 (44.8%)	21 (75%)	16 (41%)	**0.007**
Imaging chest CT or XRay				
Bilateral lesions	50 (74.6%)	22 (78.6%)	28 (71.8%)	0.581
Central lesions	8 (11.9%)	4 (14.3%)	4 (10.3%)	0.711
Cavitary lesions	11 (16.4%)	9 (32.1%)	2 (5.1%)	**0.006**
BAL results				
Aspergillus spp				
fumigatus	34 (50.7%)	16 (57.1%)	18 (46.2%)	0.460
flavus	6 (9%)	3 (10.7%)	3 (7.7%)	0.688
terreus	17 (25.4%)	4 (14.3%)	13 (33.3%)	0.094
niger	4 (6%)	0 (0%)	4 (10.3%)	0.134
other	2 (3%)	1 (3.6%)	1 (2.6%)	0.999
mixed	4 (6%)	4 (14.3%)	0 (0%)	**0.027**
Direct smear (Calcofluor White™ stain)	2/67 (2.9%)	1 (3.6%)	1 (2.6%)	0.999
BAL galactomannan	5/12 (41.7%)	0 (0%)	5 (45.5%)	0.999
BAL culture positive (aimed at lesion)	53/64 (82.8%)	26 (92.9%)	27 (69.2%)	**0.007**
Bronchial washing culture positive (bilateral)	27/39 (69.2%)	9 (32.1%)	18 (46.2%)	0.456
Both BAL & bronchial washing positive	15/36 (41.6%)	9 (32.1%)	6 (15.4%)	**0.006**
Diagnosis of IPA				0.999
Proven IA	2 (3%)	1 (3.6%)	1 (2.6%)	
Probable IA	65 (97%)	27 (96.4%)	38 (97.4%)	
42-day mortality	19 (28.4%)	9 (32.1%)	10 (25.6%)	0.354

RESULTS

- We identified 67 cases of IPA (proven in 2, probable in 65) in 66 patients.
- Most common underlying disease was AML. The majority of patients had active disease.
- 1/3 had a history of corticosteroid use, and recent severe neutropenia was present in 22%.
- Serum galactomannan levels were available in 30 cases and positive in 43%.
- Most (78,7%) patients had CT chest imaging within a week of the BAL date.

- Direct fungal visualization in BAL cytology based on GMS was positive in 41.8% cases, in contrast to only 3.6% positive direct smear Calcofluor White™ stain cases. Of note, one of the 2 cases with positive Calcofluor White™ stain had a negative GMS cytology.
- BAL cytology was diagnostic for co-infections in 7 cases: 2 Pneumocystis j*iroveci* and 5 viral infections (cytopathic changes) (one had both).
- BAL galactomannan was only available in 12 cases (17.6%), and was positive in 41.7%. Of note, GMS cytology was positive in 1 case with negative BAL Aspergillus galactomannan, and Aspergillus galactomannan was positive in BAL in 5 cases with negative cytology.

- Comparison between cases with positive and negative BAL cytology by GMS showed that **cavitary lesions, history of SCT, and IPA caused by >1 *Aspergillus* species** had more often a positive cytology. In addition, BAL GMS cytology was also more often positive when the **positive culture sample was from a BAL aimed at the lesion** compared to bilateral bronchial washing; or when **both cultures were positive**.
- In the multivariate analysis, only cavitary lesions were significantly associated with positive BAL cytology (p=0.045; OR 6.21, 95%CI 1.04-37.11).
- There were no differences in the positivity rate of BAL GMS cytology according to Aspergillus species causing IPA or prior mold-active prophylaxis. No other significant associations were found between cytology and other variables

CONCLUSIONS

- GMS stain in (BAL) was positive in 42% of 67 cases and revealed co-infections in seven. In contrast, only 2/67 (3.6%) of BAL samples were positive by the direct smear fluorescent dye Calcofluor White™ stain.
- Positive GMS was significantly more frequent in IPA with cavitary lesions and IPA caused by >1 *Aspergillus* species, but the proportion of positive cytology among *Aspergillus* species was not different.