Short Report

Bronchoalveolar lavage to evaluate new pulmonary infiltrates in allogeneic hematopoietic stem cell transplant recipients: impact on antimicrobial optimization

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SUMMARY

Background: Pulmonary complications cause significant morbidity and mortality after allogeneic hematopoietic stem cell transplant (AHSCT). Bronchoscopy with targeted bronchoalveolar lavage (BAL) is often used in AHSCT patients with suspected lower respiratory tract infection (LRTI) to help guide management.

Aim: To evaluate how positive BAL results change antimicrobial management of AHSCT recipients with suspected LRTI.

Methods: We performed a retrospective review of BAL results from January 2014 to July 2016 for 54 AHSCT recipients. A positive BAL was determined by culture, multiplex polymerase chain reaction (PCR), Aspergillus galactomannan antigen (AGA), and cytology.

Findings: BAL was positive for infectious etiologies in 63%, and antimicrobials were adjusted in 48/54 (89%) of patients. Antibacterial escalation was predicted by a positive BAL bacterial culture (OR 7.61, P=0.017). Antibiotic de-escalation was more likely with an elevated AGA (OR 3.86, P=0.035). Antiviral initiation was more likely with positive BAL multiplex PCR (OR 17.33, P=0.010). Antifungals were more likely to be escalated or...
Introduction

Bronchoscopy with bronchoalveolar lavage (BAL) is an important diagnostic tool to evaluate AHSCT recipients with new pulmonary infiltrates or without clinical response to empiric therapy [1,2]. Its utility is dependent on culture and molecular diagnostic results and the capability of changing medical management [3]. In studies prior to the availability of polymerase chain reaction (PCR), antibiotics were withdrawn in up to 50% of AHSCT recipients [4,5]. This study aims to determine how Aspergillus galactomanan antigen (AGA) and multiplex PCR added to traditional BAL testing affects antimicrobial treatment in AHSCT recipients with new pulmonary infiltrates.

Methods

The first BAL completed after AHSCT in patients over age 18 between January 2014 and July 2016 at the authors’ institution were included. The decision to perform a BAL was at the discretion of the attending physician caring for the patient. BAL fluid was submitted for gram stain, bacterial, mycobacterial and fungal cultures, multiplex PCR, AGA, and cytology. This study was approved by Virginia Commonwealth University’s Institutional Review Board.

A positive BAL was defined by a positive culture, multiplex PCR, elevated AGA (optic density (OD) >0.49), and/or cytology (which includes detection of Pneumocystis jirovecii). A positive bacterial culture required species isolation, excluding coagulase-negative Staphylococcus and mixed respiratory flora. A positive fungal culture required the presence of fungal colonies, except non-cryptococcus yeast. The multiplex PCR used was the commercial kit BioFire FilmArray® that can detect adenosvirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza B, parainfluenza (subtypes 1–4), respiratory syncytial virus (RSV), Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae. Diffuse alveolar hemorrhage (DAH) was diagnosed per guidelines [6]. A change in management was defined by escalation or de-escalation of antibiotics, antifungals, or antimicrobials, adaptation of antifungals, or initiation of prednisone within 7 days after BAL since this is the time interval to obtain AGA results at the authors’ institution. De-escalation was defined as cessation of one or multiple agents, conversion to oral therapy, or transition to a more narrow spectrum. Escalation was defined as adding a new antimicrobial agent or transitioning to a broader spectrum. Fungal adaptation was a transition to an alternative agent within the same class of antifungals. Antimicrobial use within 7 days of BAL was considered prior therapy.

AHSCT was performed on the Bone Marrow Transplant Unit at the authors’ institution. The conditioning regimen, immunosuppressive, and supportive therapies followed internationally accepted protocols. Levofloxacin and fluconazole were used as anti-infective prophylaxis and prophylaxis for cytomegalovirus (CMV) and Pneumocystis jirovecii pneumonia were used according to guidelines [7].

A descriptive analysis performed using medians and inter-quartile ranges (IQR) for continuous variables due to non-normal distributions and frequencies and percentages for categorical variables. We used a Fisher’s exact test to compare categorical variables and ANOVA for continuous variables. A P-value of less than 0.05 was considered statistically significant. All analysis was performed using JMP Pro 12 [SAS Institute, Cary, NC, USA].

Results

BAL was performed in 120 AHSCT recipients during the study period. 66 procedures were excluded for patient age<18 (n=1), subsequent procedures on the same patient (n=34), and BAL performed before transplant (n=31). 54 patients who had a BAL after AHSCT were included.

Patient demographics, antimicrobials at bronchoscopy, and BAL results are described in Table I. Most antimicrobial therapy was started within two days (n=11) or over 7 days (n=18) before BAL. Eleven patients were not receiving antimicrobials. BAL was positive for infectious etiologies in 63%, mostly with elevated AGA (17/54), followed by multiplex PCR (13/54), positive bacterial (8/54), fungal (4/54) and AFB culture (1/54). None of the patients with a positive bacterial culture were on levofloxacin prophylaxis. Of the patients with a positive fungal result, 14/17 were on antifungal prophylaxis. Only 2/17 patients with a positive BAL AGA also had a positive serum AGA. All of the positive multiplex PCR were for viruses. Nine patients had multiple infectious etiologies, all with a positive multiplex PCR.

Antimicrobials were adjusted in 48/54 (89%) of patients (Table II). Overall antibiotic escalation occurred in 19/54, and was associated with a positive BAL bacterial culture (OR 7.61, P=0.017) (Table II). Antibiotic de-escalation was more likely with an elevated AGA (OR 3.86, P=0.035) (Table II). Antiviral initiation was more likely with positive BAL multiplex PCR (OR 17.33, P=0.010) (Table II). Antifungals were more likely to be escalated or changed with an elevated AGA (OR 4.33, P=0.020) (Table II). The patients with a negative BAL were more likely to be started on steroids (OR 0.19, P=0.043).
Table I
Patient characteristics summarized by frequencies and percentages or medians and interquartile range (n=54)

| Characteristic | Transplant | Preceding autologous SCT 4 (7%) | AHSCT | Related 16 (30%) | Unrelated 38 (70%) |
|----------------|------------|---------------------------------|-------|------------------|-------------------|
| Hematologic Disease | AML 14 (26%) | MDS/Myelofibrosis 13 (24%) | ALL 8 (15%) | CML 6 (11%) | Non-Hodgkin Lymphoma 6 (11%) | CLL 1 (2%) | Other 6 (11%) |
| Months since AHSCT | 3.5 (1–9.25) | | | | | | |
| Prior GVHD requiring treatment | 24 (44%) | | | | | | |
| Current GVHD Treatment | 37 (69%) | | | | | | |
| Immunosuppression at BAL | Tacrolimus 26 (49%) | Prednisone 21 (39%) | Cyclosporine 9 (7%) | Sirolimus 4 (7%) | | | |
| Neutrophil <1000 (cells/µL) | 18 (33%) | | | | | | |
| Age at Bronchoscopy (years) | 56.2 (41.1–62.9) | | | | | | |
| O2 requirement (L/min) | 32 (59%) | | | | | | |
| Antimicrobial prophylaxis at time of bronchoscopy | Cytomegalovirus prophylaxis 51 (94%) | Pneumocystis jirovecii prophylaxis 27 (50%) | Bactrim 4 (8%) | Atovaquone 5 (9%) | Pentamidine 18 (34%) | | |
| Fungal prophylaxis | Voriconazole 21 (39%) | Fluconazole 12 (22%) | Micafungin 7 (13%) | Posaconazole 3 (6%) | | | |
| Levofoxacin prophylaxis | 16 (30%) | | | | | | |
| Antimicrobials at bronchoscopy | 43 (80%) | | | | | | |
| No antimicrobials | 11 (20%) | | | | | | |
| Antibacterials | 40 (74%) | Vancomycin 22 (41%) | Meropenem 15 (28%) | Cefepime 14 (26%) | Levofoxacin 7 (13%) | Other 8 (15%) | |
| Antifungal | 26 (48%) | Micafungin 10 (18%) | Voriconazole 10 (18%) | | | | |

Table I (continued)

| Characteristic | Posaconazole | Amphotericin B | Antiviral | Ribavirin | Foscarnet | Tamiflu | Ganciclovir | Cidofovir |
|----------------|--------------|----------------|-----------|-----------|-----------|---------|------------|----------|
| Duration of antimicrobials prior to BAL | 0 days 11 (20%) | 1–2 days 11 (20%) | 3–4 days 8 (15%) | 5–7 days 7 (13%) | >7 days 18 (33%) | | | |
| Nasopharyngeal PCR Testing (n=46) | Positive 12 (26%) | Influenza 2 (4%) | Parainfluenza 3 (7%) | Rhinovirus/enterovirus 2 (4%) | RSV 4 (9%) | RSV + rhinovirus/enterovirus 1 (2%) | | |
| Serum AGA >0.49 | 2 (4%) | | | | | | | |
| Overall BAL results | Positive 34 (63%) | Bacterial Culture 8 (15%) | Fungal Culture 4 (8%) | Fungal hyphae on cytology 6 (11%) | AGA >0.49 17 (31%) | Viral multiplex PCR 13 (24%) | AFB Culture 2 (4%) | DAH 2 (4%) | Negative 20 (37%) |
| Culture Results | Bacterial culture 8 (15%) | Pseudomonas aeruginosa 2 (4%) | Haemophilus influenzae 1 (2%) | Stenotrophomonas maltophilia 1 (2%) | MRSA 1 (2%) | VRE 1 (2%) | Nocardia nova complex 1 (2%) | Streptococcus agalactiae 1 (2%) | Fungal culture 4 (8%) | Aspergillus fumigatus 2 (4%) | Rhizomucor spp. 1 (2%) | One fungal colony 1 (2%) | AFB culture 1 (2%) | Mycobacterium avium complex 1 (2%) | Viral multiplex PCR | Positive 13 (24%) | Influenza 2 (4%) | Parainfluenza 4 (8%) | Rhinovirus/enterovirus 4 (8%) | (continued on next page)
**Discussion**

BAL was helpful to determine the etiology of pulmonary complications and optimize antimicrobials. This included escalating antibiotics to target organisms that were isolated. We believe it changes practice as BAL is not only valuable when results are positive, but when an aggregate negative BAL work up offers assurance for providers to address noninfectious etiologies. This is critical in intensive care settings when antimicrobial prophylaxis effectively limited bacterial infections due to susceptible organisms, but may have added to the selection of more resistant bacteria such as *Stenotrophomonas* and *Nocardia spp.*

BAL was critical to recognize and treat invasive fungal infections, prompting antifungal escalation in 33% and initiation of targeted treatment for isolated *Rhizomucor spp.* Only two of the patients with an elevated BAL AGA also had an elevated serum AGA. BAL assisted in the diagnosis of these infections. This finding is important in that delays in treatment carry the potential for increased mortality [8]. This is recognized in the 2019 American Thoracic Society guidelines, which suggest moving to BAL AGA and requesting BAL Aspergillus PCR when serum AGA is negative. At a cutoff of 0.5, the serum AGA sensitivity is approximately 74–88% and at 1.0, it is 79–88%. At a cutoff of 1.5, sensitivity reduces to 59% and specificity increases to 95%. For BAL AGA with a cutoff of 1.0, the sensitivity and specificity increases to 90% and 94% respectively [9]. In addition, *Aspergillus spp.* accounts for only 70% of invasive fungal infections in AHSCT, and the remaining 30% includes deadly pathogens including zygomycetes (6–8%), fusarium (6–7% in USA), dematiaceous, and other rare molds that may target therapy for a microorganism isolated on bacterial culture that was not previously covered, including virulent organisms such as *Nocardia nova complex, Pseudomonas aeruginosa, Haemophilus influenzae, and Stenotrophomonas maltophilia.* Antibiotics were also escalated due to severity of illness despite negative culture data (n=8) and to cover an alternative infection (n=6), such as endocarditis. Likely the use of antimicrobial prophylaxis effectively limited bacterial infections due to susceptible organisms, but may have added to the selection of more resistant bacteria such as *Stenotrophomonas* and *Nocardia spp.*

**Table II**

| Positive BAL result (N) | Outcome | N | OR (95% CI) | P Value |
|------------------------|---------|---|-------------|---------|
| **Overall (35/54 positive)** | Escalation/Addition Antibiotics | 19/54 | 2.10 (0.62, 7.12) | 0.257 |
| De-escalation Antibiotics | 20/54 | 2.37 (0.70, 8.00) | 0.245 |
| Escalation/Addition Antifungals | 18/54 | 2.03 (0.77, 10.18) | 0.142 |
| Adaptation Antifungals | 5/54 | 2.33 (0.26, 24.41) | 0.641 |
| De-escalation Antifungals | 5/54 | 0.35 (0.05, 2.33) | 0.347 |
| Steroid initiation | 7/54 | 0.19 (0.03, 1.08) | 0.043 |
| **Bacterial (8/54)** | Escalation/Addition Antibiotics | 6/8 | 7.61 (1.36, 42.71) | 0.017 |
| De-Escalation Antibiotics | 1/8 | 0.20 (0.02, 1.79) | 0.234 |
| Escalation/Adaptation Antifungal | 1/8 | 0.17 (0.02, 1.50) | 0.122 |
| De-escalation Antifungal | 1/8 | 1.5 (0.14, 15.46) | 0.567 |
| Escalation/Addition Antiviral | 0/8 | 0 | 1.000 |
| **Aspergillus/AGA (17/54)** | Escalation/Addition Antibiotics | 5/17 | 0.68 (0.20, 2.36) | 0.760 |
| De-Escalation Antibiotics | 10/17 | 3.86 (1.15, 12.90) | 0.035 |
| Escalation/Adaptation Antifungal | 11/17 | 4.33 (1.28, 14.67) | 0.020 |
| De-escalation Antifungal | 0/17 | 0 | 0.168 |
| Escalation/Addition Antiviral | 3/17 | 3.54 (0.53, 23.53) | 0.315 |
| **Viral (13/54)** | Escalation/Addition Antibiotics | 7/13 | 2.82 (0.78, 10.15) | 0.181 |
| De-Escalation Antibiotics | 5/13 | 1.08 (0.30, 3.92) | 1.000 |
| Escalation/Adaptation Antifungal | 6/13 | 1.34 (0.38, 4.71) | 0.750 |
| De-escalation Antifungal | 1/13 | 0.27 (0.08, 7.58) | 1.000 |
| Escalation/Addition Antiviral | 4/13 | 17.33 (1.72, 174.29) | 0.010 |

BAL = bronchoalveolar lavage, AGA = *Aspergillus* galactomannan antigen.
The bolded areas represent statistically significant p values < 0.05.

* Number of patients with each outcome out of those with the same etiology on BAL. These were the numbers used to develop odds ratios.
require culture or tissue biopsy diagnosis. Endemic mycoses only account for 1% of invasive fungal infections in AH SCT and may be amenable to serologic or antigen testing without invasive procedures [8].

A positive multiplex PCR was associated with antiviral initiation (ribavirin for parainfluenza or peramivir for influenzae), (Table II). However, the results were similar on nasopharyngeal and BAL multiplex PCR, as noted in prior studies [10]. It is unclear if this multiplex PCR test is adequate for diagnosing LRTI and more studies will need to be done to evaluate this. BAL remained necessary to detect coinfections, as 9/13 patients with a positive multiplex PCR also had a positive bacterial culture (n=2) or elevated AGA (n=7) (Table I). Reports of viral and bacterial coinfection, particularly with adenovirus or rhinovirus have been associated with higher morbidity/mortality but the understanding of this is limited [11,12]. It would be interesting to further investigate with larger studies the effect of viral illnesses on bacterial or fungal coinfections and how they affect treatment outcomes to determine if these more aggressive prophylaxis and diagnostic testing would benefit these patients.

BAL also had a major role in antimicrobial de-escalation. Antibiotics were either stopped or converted to oral in 20 patients with a negative BAL bacterial culture. An elevated AGA was predictive of antibiotic de-escalation (Table II), which highlights a new role for BAL in antibiotic optimization [13].

A negative BAL was associated with initiation of steroids for treatment of other conditions including graft versus host disease, pneumonitis, DAH, and complications of engraftment (Table II). Even though the BAL did not aid in these diagnosis (except DAH), it mitigated the risk of starting steroids by lowering suspicion for pulmonary infections.

Study limitations include potential selection bias (only patients who had a BAL were included) and the fact our results are from a single hospital. BAL was performed at the discretion of the attending physician, and therefore the patient group only includes those deemed eligible for the procedure, which was likely urged by clinical status. A control group comparison (with or without available sputum cultures, nasal multiplex respiratory pathogen panel, serum AGA and fungal urine antigen testing) would be likely biased to include those who were deemed less sick as well as those considered too high risk to be eligible for bronchoscopy, due to high oxygen requirement or bleeding risk. It would be unethical to randomly assign patients to bronchoalveolar lavage due to known risks and benefits. Additional studies across multiple hospitals would be valuable to confirm our results. A multivariate regression analysis could not be performed due to low sample size.

In summary, our study found that BAL is a beneficial diagnostic tool to evaluate new pulmonary infiltrates in AH SCT recipients. BAL permitted targeted antimicrobial treatment and positively affected antimicrobial de-escalation and guided management of non-infectious diagnostic considerations. These data will inform local antimicrobial stewardship efforts. Additional studies are needed to confirm our findings and to identify how BAL can be used to improve antimicrobial prescribing.

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