Does molecular analysis increase the efficacy of bronchoalveolar lavage in the diagnosis and management of respiratory infections in hemato-oncological patients?

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SUMMARY

Objectives: The identification of the specific pathogen responsible for a respiratory infection in patients with hematological malignancies (HM) would ensure relevant treatment and prevent toxicity associated with anti-infective therapy. This large-scale study aimed to explore the clinical impact of fiberoptic bronchoscopy with bronchoalveolar lavage (FOB-BAL) in conjunction with molecular analysis on the diagnosis and management of respiratory infections in hemato-oncological patients.
Methods: All consecutive patients with HM and pulmonary infiltrates, who underwent FOB-BAL between January 2008 and January 2013, were included in the analysis. Clinical characteristics, FOB-BAL results, and treatment adjustments were recorded, and factors predicting a positive BAL were assessed.
Results: Four hundred and twenty-five FOB-BAL procedures were analyzed. BAL revealed a specific diagnosis in 219 (51.5%) patients, 208 of them with a pulmonary infection. Infectious etiological agents found were mainly *Aspergillus spp* (*n* = 142), bacterial species (*n* = 44), and *Pneumocystis jirovecii* (*n* = 34). Multivariate analysis showed that a lymphoproliferative disease, ≥2 symptoms (dyspnea/cough/hemoptysis/pleuritic pain), and less than 4 days between symptom appearance and FOB-BAL, predicted a positive FOB-BAL result. BAL results prompted a treatment modification in 48% of subjects.
Conclusions: FOB-BAL in conjunction with molecular assays is efficient in the rapid detection of life-threatening infections, allowing for adjustment of anti-infective therapy, which may result in better outcomes and reduce treatment-related toxicity.

1. Introduction

Respiratory complications are recorded in about 60% of patients with hematological malignancies (HM) treated with chemotherapy and in 80% of patients undergoing allogeneic stem cell transplantation.[1] Infections appear to be responsible for the majority of these events. Important differential diagnoses include lung involvement by the underlying malignancy, capillary leak syndrome, and lesions caused by chemotherapy or radiation.[2,3] Establishing the specific cause of pulmonary infiltrates would enable the prompt delivery of a specific therapy. In cases of pulmonary infection, identification of the specific etiology would allow the early administration of appropriate therapy, which could improve the outcomes and reduce mortality in this patient population.[4–6] Non-invasive diagnostic tests, such as sputum cultures, blood cultures, and serological tests, have limited diagnostic value in this setting. While open lung biopsy has the

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highest yield, the complication rate limits its use.[7,8] Fiberoptic bronchoscopy with bronchoalveolar lavage (FOB-BAL) enables the selective collection of lung fluid so that a specific diagnosis of infectious, malignant, or hemorrhagic disorders can be achieved with relative ease. As patients with respiratory distress and coagulation disorders can also withstand bronchoscopy, it has become the diagnostic procedure of choice in these individuals.[9–13]

Previous studies,[12–18] including one conducted at our center Rambam Health Care campus,[15] investigating the efficacy of FOB-BAL for the diagnosis of pulmonary infiltrates in patients with HM, have reported a variable diagnostic yield, especially for infectious agents, ranging between 20% and 60%. The introduction of molecular methods has been suggested to improve the diagnostic efficacy of FOB-BAL, particularly in detecting fungal, viral, and *Pneumocystis jirovecii* infections,[19–23] and to promote the tailoring of anti-infectious therapy.

The aim of the present study was to explore the diagnostic yield of FOB-BAL, in the era of molecular techniques, in hemato-oncological patients presenting with pulmonary infiltrates.

2. Materials and methods

This study was approved by the Institutional Review Board of the Rambam Health Care Campus (approval number RMB 0416-12). All consecutive patients with HM and pulmonary infiltrates, who underwent diagnostic FOB-BAL between January 2008 and January 2013, were included in the study.

Bronchoscopies were performed using a flexible fiberoptic bronchoscope (Olympus). Procedures were carried out through the nasal or oral cavity following sedation (5 mg intravenous midazolam) and local anesthesia (lidocaine 2%) and were supported with cardiopulmonary monitoring (continuous assessment of pulse rate, blood pressure, and oxygen saturation). Patients with a platelet count lower than 20×10^9/l received 6 units of platelets within an hour prior to the procedure.

The patients' electronic medical records were reviewed for demographic characteristics, underlying hematological illnesses (type of HM, type of hematopoietic stem cell transplantation (HSCT), graft-versus-host disease (GVHD), neutropenic status), chronic comorbidities (ischemic heart disease, diabetes mellitus, hypertension, chronic lung disease, liver disease, and renal disease), clinical characteristics of the respiratory event (fever, cough, hemoptysis, chest pain, oxygen saturation, respiratory support), chest imaging findings, and in-hospital mortality. Antimicrobial therapy before and after diagnostic bronchoscopy results was also recorded.

The etiological diagnosis of pulmonary infection was established using diagnostic FOB-BAL along with supportive blood and urine microbiological and serological analysis. BAL fluid laboratory analysis included the following: cytological staining for the detection of fungal elements, *P. jirovecii* bodies, and viral inclusion bodies in alveolar cells; bacterial cultures with specific growth media for *Mycobacterium spp* and *Legionella spp*; fungal cultures; viral cultures for herpes simplex virus (HSV) and cytomegalovirus (CMV); PCR for the detection of *Aspergillus spp*, *Legionella spp*, *P. jirovecii*, HSV, CMV, and respiratory viruses DNA. Galactomannan antigen was measured in BAL and serum.

2.1. Definitions

Bacterial pneumonia was defined in the presence of a positive BAL culture for any *Mycobacterium*, *Legionella*, *Nocardia*, or *Actinomyces* species; a positive PCR for *Legionella spp* or *Mycobacterium spp* DNA; a positive BAL culture for pathogenic bacteria (Gram-negative, pneumococci) in the amount of $>10^5$ CFU/ml and in the absence of another pathogen. For any other bacterial species,

a positive blood culture was required, along with a positive BAL culture.

Fungal pneumonia was defined in the presence of a positive BAL culture for any mold or rare yeast. Invasive pulmonary aspergillosis (IPA) was defined according to the modified European Organisation for Research and Treatment of Cancer (EORTC) criteria, accepting a positive galactomannan antigen in blood or BAL samples. A positive PCR in BAL for the detection of *Aspergillus spp* DNA in the presence of a typical chest computed tomography (CT) scan was also considered diagnostic.[24–26] *Candida spp* was considered an etiologic agent only with a concomitant positive blood culture.

Viral pneumonia was defined in the presence of a positive BAL culture and/or PCR for any respiratory virus. For HSV and CMV viruses, there was an additional requirement for the presence of characteristic viral inclusion bodies in the alveolar cells, as detected by direct staining.[27]

P. jirovecii pneumonia (PJP) was defined in the presence of *P. jirovecii* bodies visualized on silver/methenamine stains and/or *P. jirovecii* DNA detected by PCR in BAL fluid obtained from patients with established predisposing factors and typical clinical and imaging features in a high-resolution CT scan.[28]

A polymicrobial pulmonary infection was defined when two or more infectious agents were indentified based on the above criteria. The diagnostic yield of FOB-BAL was considered the proportion of cases with a specific diagnosis. A change in patient management was defined as the addition or subtraction of any anti-infective agent after receiving FOB-BAL results. The anti-infective prophylaxis policy at the center during the study period included the following: ciprofloxacin antibacterial prophylaxis during neutropenia; fluconazole antifungal prophylaxis during neutropenia and for 100 days in allogeneic HSCT recipients (no anti-mold prophylaxis was administered); trimethoprim–sulfamethoxazole anti-*P. jirovecii* prophylaxis in allogeneic HSCT recipients.

2.2. Diagnostic PCR reactions

Aspergillus spp DNA was detected using a two-step (nested) PCR that specifically amplifies the region of the 18S ribosomal RNA gene, which is highly conserved in *Aspergillus* species. The test was performed on extracted DNA using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).[29]

P. jirovecii PCR was used to detect three distinct genes of the pathogen: the mitochondrial large subunit rRNA gene, the major surface glycoprotein,[30] and the T1–T2 region of the large subunit ribosomal RNA gene,[31] aiming to decrease false-positivity. PCR was considered positive if at least two out of the three genes produced a positive signal when amplified.

Legionella DNA was detected using *Legionella* ATCC 33152-specific primers that amplify a portion of the 16S rRNA gene.[32] The primers Leg F2 (5'-GAGGCAGCAGTGGGGAAT) and Leg R2 (5'-CCCAGGCGGTCAACTTAT) were used.

2.3. Statistical analysis

Descriptive statistics included the calculation of the median, standard deviation, and frequency of the analyzed parameters. In cases where the patient underwent more than one bronchoscopy, each procedure was counted individually. Univariate analysis was performed using binary logistic regression, calculating the odds ratios (OR) with 95% confidence intervals (CI) and *p*-values. Multivariable stepwise logistic regression analysis was used to assess the relationship between patient characteristics and positive bronchoscopy. Variables selected for multivariate analysis were those found to be significant in the univariate analysis. Two-

tailed *p*-values of 0.05 or less were considered statistically significant.

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 software (IBM Corp., Armonk, NY, USA).

3. Results

Four hundred and twenty-five FOB-BALs were performed and included in the analysis. All except 13 procedures were performed as part of the investigation of independent respiratory events. The latter 13 procedures were performed in patients with unexplained progressing respiratory infections in whom a prior FOB-BAL had been non-informative. The median age at the time of FOB-BAL was 57 years (range 18–88 years). Forty-one percent (*n* = 175) of FOB-BALs were performed in patients with lymphoproliferative disorders (chronic lymphocytic leukemia (CLL), lymphoma, multiple myeloma (MM)) and 55% (*n* = 235) in patients with acute leukemia. Table 1 presents the characteristics of the patients who underwent FOB-BAL in this study.

3.1. Clinical features and treatment of the respiratory events examined by FOB-BAL

Fever was the most common clinical symptom, recorded in 89% of cases, followed by dyspnea (49%) and cough (36%). In 385 cases (90.5%), high-resolution CT had been performed prior to FOB-BAL. Two hundred and seventy-seven (65%) scans showed diffuse bilateral infiltrates and 148 demonstrated a unilateral involvement.

Anti-infective agents were administered preemptively in 399 cases, before or immediately after the performance of FOB-BAL. These included broad-spectrum antibiotics (*n* = 382, 90%), antifungals (*n* = 303, 71%), antivirals (*n* = 34, 8%), and anti-*P. jirovecii* agents (*n* = 87, 20%).

3.2. FOB-BAL results

FOB-BAL provided a specific diagnosis in 51.5% of cases. FOB-BAL revealed 11 cases of non-infectious etiology (10 bleeding, 1 lymphoma) and 208 (48.9%) cases were diagnosed with a

Table 1
Baseline characteristics of patients who underwent FOB-BAL[a]

Characteristics	
Total number of FOB-BAL procedures, *n* (%)	425 (100%)
Age, years, median (range)	57 (18–88)
Sex, *n* (%)	
Male	242 (57%)
Female	183 (43%)
Hematological malignancy, *n* (%)	
Acute leukemia	235 (55%)
Lymphoproliferative disorder (CLL, lymphoma, MM)	175 (41%)
Other	15 (4%)
Co-morbidities, *n* (%)	
IHD	93 (22%)
Diabetes mellitus	20 (5%)
Chronic lung disease	15 (4%)
Prior transplant[a]	
Allo SCT	108 (25%)
Auto SCT	69 (16%)
GVHD	65 (15%)
Concurrent neutropenia (<0.5 × 10^9 cells/l)	197 (46%)
Thrombocytopenia <20 × 10^9/l prior to FOB-BAL	64 (15%)

FOB-BAL, fiberoptic bronchoscopy with bronchoalveolar lavage; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; IHD, ischemic heart disease; Allo SCT, allogeneic stem cell transplantation; Auto SCT, autologous stem cell transplantation; GVHD, graft-versus-host disease.
  [a] The characteristics are presented per procedure.

Table 2
Distribution of the etiological agents of pulmonary infections in patients with hematological malignancies

Pathogens recovered from BAL samples	Number
Total number of pathogens	259
Fungi, total	188
Molds	147
Aspergillus spp	142
Fusarium	3
Zygomycetes	2
Yeasts	7
Cryptococcus	1
Candida[a]	2
Rare yeasts[b]	4
Pneumocystis jirovecii	34
Bacteria, total	44
Enterobacteriaceae	10
Pseudomonas aeruginosa	10
Stenotrophomonas maltophilia	6
Acinetobacter	2
Legionella pneumophila	10
Nocardia cyriacigeorgica	2
Other[c]	4
Viruses, total	27
Respiratory viruses	23
Influenza	11
Parainfluenza	5
Respiratory syncytial virus	3
Human metapneumovirus	2
Adenovirus	2
Herpes viruses	4
HSV	2
CMV	2

BAL, bronchoalveolar lavage; HSV, herpes simplex virus; CMV, cytomegalovirus.
  [a] These two patients had catheter-related candidemia with multiple Candida pulmonary septic emboli.
  [b] Scopulariopsis (*n* = 2); Geotrichum capitatum (*n* = 2).
  [c] Chrysiomonas (*n* = 1), *Streptococcus pneumoniae* (*n* = 1), undefined Gram-negative bacteria (*n* = 2) .

pulmonary infection. A polymicrobial etiology (two or more infectious agents) was established in 45 (21%) of them. Two hundred and six (48.5%) procedures were non-diagnostic.

The pathogens detected are listed in Table 2. The most common infectious diagnosis was fungal pneumonia (154/208, 74%), with *Aspergillus spp* being the fungal pathogen in 142 (92%) of them. Bacterial pneumonia was diagnosed in 44/208 (21%) cases, caused by Gram-negative aerobic bacilli in 31 (70%) and *Legionella pneumophila* in 10 (23%) of them. PJP was diagnosed in 34/208 (16%) episodes and viral pneumonia in 27/208 (13%) cases, most of them (*n* = 21, 77%) caused by respiratory viruses.

The diagnosis of IPA was 'probable' (according to the criteria of the EORTC/MSG Consensus Group 2008) in 115 cases and 'possible' in 27 cases (all possible IPA cases were supported by positive Aspergillus PCR in BAL). Among the 115 cases of probable IPA, 53 also had a positive PCR for Aspergillus DNA in BAL. Patients with possible IPA, based on the EORTC criteria (and treated for IPA), who had a negative PCR for Aspergillus DNA, were included in the group of 206 patients with a non-diagnostic FOB-BAL. The diagnosis of PJP was confirmed by PCR in 31/34 (91%) cases and in the remaining three cases the organism was also visualized by direct staining. Respiratory viral pneumonia was also diagnosed by PCR.

Among the 45 patients with a polymicrobial infection, 39 had a pulmonary infection caused by two microorganisms and six had an infection caused by three microorganisms. *Aspergillus spp* was the most common co-pathogen in the group of polymicrobial pulmonary infection (39/45, 87%).

3.3. Prediction of a positive FOB-BAL result

Univariate analysis (Table 3) found the diagnosis of a lymphoproliferative disease to be associated with a high likelihood of obtaining a positive FOB-BAL result. A shorter period between the appearance of clinical symptoms and FOB-BAL indicated a greater chance of obtaining a positive result. The more respiratory symptoms were present at the time of FOB-BAL performance, the higher the likelihood of a positive result. There was an association between prolonged neutropenia (mainly occurring in the context of anti-leukemic therapy or stem cell transplantation) and the detection of Aspergillus in BAL, and between the existence of profound lymphopenia and the detection of *P. jirovecii*.

Multivariate analysis confirmed a lymphoproliferative disease (OR 1.67, 95% CI 1.2–2.5, *p* = 0.012), ≥2 respiratory symptoms (OR 2.76, 95% CI 1.2–6.2, *p* = 0.015), and a shorter time (<4 days vs. >4 days) between the first symptom and FOB-BAL (OR 0.61, 95% CI 0.38–0.98, *p* = 0.044) to be associated with a higher likelihood of obtaining a positive FOB-BAL result (**Supplementary Material** Table S1 summarizes the characteristics of patients with a positive vs. a negative BAL result).

3.4. The impact of FOB-BAL results on the management of respiratory infection

As mentioned above, in 94% (*n* = 399) of cases, the patient had received an antibacterial and/or antifungal therapy based on clinical and imaging findings prior to the FOB-BAL being performed.

FOB-BAL results prompted a treatment modification (either cessation or the addition of anti-infectious agents) in 48% (*n* = 205) of cases. The therapy was adjusted in 65% (*n* = 141) of cases where the FOB-BAL result was positive and in 31% (*n* = 64) of cases of a negative test. FOB-BAL results led to the initiation of a new treatment in 89 of 141 cases, including antibacterial (*n* = 16), antifungal (*n* = 43), anti-*P. jirovecii* (*n* = 15), and antiviral therapy (*n* = 15). In the remaining 52 FOB-BAL-positive and 64 FOB-BAL-negative cases (a total of 116 cases), some of the anti-infectious treatment was withdrawn. The discontinued agents included antibacterial (*n* = 75), antifungal (*n* = 58), anti-*P. jirovecii* (*n* = 56), and antiviral drugs (*n* = 9).

Table 3
Univariate analysis of factors predicting a positive FOB-BAL result

Variable	OR	95% CI	*p*-Value[a]
Male	0.995	0.678–1.461	0.979
Lymphoproliferative disorder (lymphoma, MM, CLL)	1.752	1.179–2.604	0.006
Allo SCT	0.803	0.511–1.262	0.342
Auto SCT	0.793	0.465–1.352	0.394
GVHD	1.145	0.832–1.574	0.406
Neutropenia	0.793	0.538–1.168	0.240
Fever	1.060	0.581–1.933	0.849
Cough	1.638	1.098–2.444	0.016
Dyspnea	1.477	1.007–2.164	0.046
Pleuritic pain	2.021	1.009–4.049	0.047
Hemoptysis	2.000	0.944–4.236	0.070
Number of respiratory symptoms			
1	1.473	0.935–2.321	0.095
2	1.932	1.142–3.268	0.014
3	2.991	1.325–6.751	0.008
Time from symptoms to FOB, days			
<4	0.656	0.260–1.653	0.372
≥4	0.500	0.283–0.885	0.017

FOB-BAL, fiberoptic bronchoscopy with bronchoalveolar lavage; OR, odds ratio; CI, confidence interval; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; Allo SCT, allogeneic stem cell transplantation; Auto SCT, autologous stem cell transplantation; GVHD, graft-versus-host disease.
[a] *P*-value for significance <0.05.

3.5. FOB-BAL-related complications

Complications associated with FOB-BAL were rare, amounting to 10 cases (2.4%), and included mild epistaxis (*n* = 2), mild hemoptysis (*n* = 4), non-specific chest pain (*n* = 1), pulmonary edema (*n* = 1), and transient self-limited respiratory decompensation (*n* = 2).

4. Discussion

In this large, single-center study of 425 consecutive patients with HM and pulmonary infiltrates, the diagnostic yield of FOB-BAL was found to be 51.5% (219/425). The vast majority of the diagnosed cases were infectious (208/219, 95%), with IPA being the most common infectious diagnosis (68%, 142/208), followed by bacterial pneumonia (21%, 44/208), PJP (16%, 34/208), and viral pneumonia (13%, 27/208).

The current study demonstrated a very high incidence of IPA in the evaluated cohort compared to previous studies looking at the diagnostic efficacy of FOB-BAL in this patient population.[16–18] There are a number of reasons for this finding. First, during the study period, the Rambam Medical Center was undergoing extensive construction and renovation, which increased the exposure of patients to Aspergillus spp. Moreover, at that time, anti-mold prophylaxis was not given to patients at risk of IPA. Finally, in addition to the standard EORTC criteria, the present study used PCR for Aspergillus DNA detection in BAL, a test that is not employed by most other centers and was not used by the study center 10 years earlier. The detection of Aspergillus DNA in BAL was introduced at the study center in 2002, and its efficacy was assessed in 107 consecutive patients with HM and IPA.[25] The inclusion of PCR in the BAL evaluation for Aspergillus DNA was found to facilitate the rapid diagnosis of IPA and even to improve the patient outcome. This was later confirmed in a meta-analysis published in 2012.[26]

On the other hand, bacterial pneumonia was diagnosed at a lower rate in the present study compared to other trials.[16–18] This could be attributed to the strict definition applied for the diagnosis of these pathogens. Specifically, a 'usual' bacterial isolate from BAL was considered the etiological agent of pneumonia only if it was simultaneously isolated from blood, or no other pathogen was detected. This definition was chosen for several reasons. First, in this study, diagnostic bronchoscopy was mostly performed in patients who had been treated empirically with broad-spectrum antibacterial agents without improvement. Notably, in many of these cases the bacterial isolate was susceptible to the antibiotics the patient received. This fact led to the assumption that the bacterial isolate from BAL was more likely to be a colonizer from the upper airways, rather than the cause of pneumonia, in contrast to the events with concomitant bacteremia, which indicates invasive infection. Another reason is that at the study center, a protective brush is not used when BAL is performed in thrombocytopenic patients,[33,34] which further increases the probability that the bacterial isolate will be a colonizer from the upper airways.

Similar to IPA, PJP was found in a relatively high percentage of the patients. In addition to the gold standard method of direct visualization of the pathogen, PCR was employed in the current study for the detection of *P. jirovecii* DNA in BAL and to establish the diagnosis of PJP. Indeed, the direct visualization was positive only in three out of 34 patients ultimately diagnosed with PJP. The PJP cases with positive PCR and negative smear probably did not represent colonization, since the characteristic clinical and radiological picture of PJP was observed in high-risk patients. Moreover, a previous study by this study group confirmed the PCR assay to be relatively sensitive and highly specific for the detection

of *P. jirovecii* (74% and 95%, respectively), with a low false-positive rate and a positive predictive value of 83%.[28]

In the present study, the diagnosis of CMV pneumonia was established only in the presence of characteristic cytopathic changes in the alveolar cells, given the fact that CMV DNA-positive BAL could simply indicate CMV reactivation in these profoundly immunosuppressed patients. Quantitative PCR was not employed to diagnose pneumonia due to non-availability; this test improves the accuracy of diagnosis and is currently recommended by the German Society of Hematology and Medical Oncology guidelines[35].

While the definitions used in this study could have led to under-diagnosis of bacterial and viral pneumonia, the high percentage of these diagnoses reported in other trials may represent some over-diagnosis.

In the current cohort of 425 consecutive patients with HM, the diagnostic yield of FOB-BAL for the etiological diagnosis of pulmonary infiltrates was 51.5%. Forty-eight percent (*n* = 204) of all the patients, both with positive and negative FOB-BAL results, underwent a modification of the empiric treatment. A similar study was conducted at the present study center between 2000 and 2002.[15] At that time, FOB-BAL provided a specific diagnosis for 30% of the patients and led to a change of therapy in 39% of the study group. The most common infectious etiology in the earlier study was bacterial pneumonia (55%), followed by fungal pneumonia (31%), viral pneumonia (12%), and PJP (2%). These major differences in the incidence of infectious etiologies between the two studies are most likely related to the current implementation of novel molecular and serological diagnostic techniques for the assessment of the BAL fluid. Indeed, nowadays, BAL fluid specimens of HM patients with pulmonary infiltrates are routinely evaluated using PCR for the detection of Aspergillus DNA, *P. jirovecii* DNA, Legionella DNA, respiratory virus DNA, and galactomannan antigen, while none of these was available during the period of the previous study.

The present study did not demonstrate a superior diagnostic yield compared to previously published trials. Hummel et al. analyzed 249 FOB examinations and found 118 (47%) microbiologically documented infectious samples, 40% of which were classified as bacterial pneumonia, and half of the diagnosed fungal pneumonia cases appeared to be caused by *Candida spp*[16]. The high rate of bacterial and Candida pneumonia in the latter trial could be attributed to the liberal definitions implemented by the authors. Shannon et al., assessing 598 infectious episodes in HSCT recipients, reported a diagnostic yield of 55%, with most of the cases being those of bacterial and viral (CMV) pneumonia[17]. Kuehnhardt et al. analyzed 58 episodes of clinically documented pneumonia in patients with HM or solid tumors and found positive FOB results in 67% of cases, most of them defined as Candida pneumonia[36]. These trials were smaller in size, included specific populations of hematological patients, and mainly did not use novel molecular diagnostic techniques. The strict definitions for bacterial, viral, and fungal pneumonia applied could plausibly explain why a higher diagnostic yield was not reached in the present study, despite the use of a wide range of molecular diagnostic tests. However, the comparison of the two trials conducted at the present study center,[15] both using similar definitions but only one employing PCR for the identification of specific pathogens, revealed a significant superiority of the present study in the diagnostic yield reached (51.5% vs. 30.4%, *p* < 0.01).

In the current study, the prompt performance of FOB-BAL after the development of a clinical respiratory picture provided an increased yield of the procedure. This finding is in consensus with prior reports,[17,37] documenting a significantly improved diagnostic yield in FOB-BAL performed within the first 4 days since
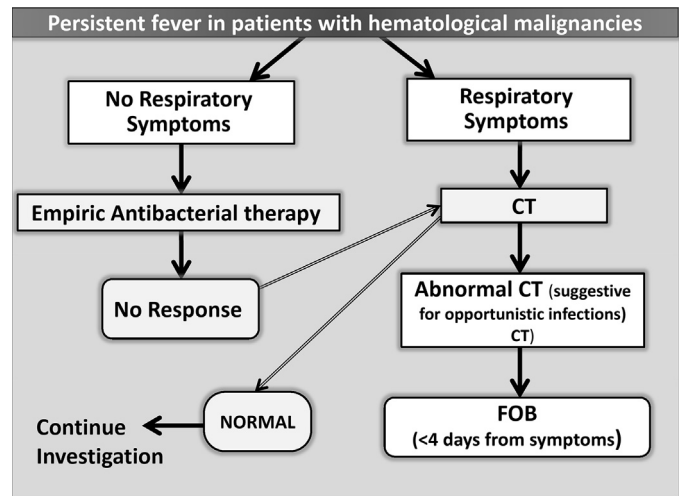

Figure 1. Algorithm for the investigation of patients with hematological malignancies with persistent fever with and without respiratory symptoms.

presentation, compared to the procedures performed at a later time point.

The likelihood of a positive FOB-BAL appears to be higher in HM patients presenting with respiratory symptoms or complaints. This association, also observed in smaller studies,[38] may be explained by a high burden of pathogens responsible for the overwhelming clinical presentation, before broad-spectrum empiric treatment is started. Notably, patients with lymphoproliferative malignancies, often presenting with profound lymphopenia, had a higher likelihood of a positive FOB-BAL result compared to those diagnosed with acute leukemia. This finding may reflect the high proportion of PJP in this population, where the diagnosis is mainly established using molecular techniques.[39] Another explanation could be the fact that the majority of patients with lymphoproliferative malignancies were not exposed to antibiotics (either prophylactic or therapeutic) at the time of developing the infection, whereas patients with acute leukemia had been hospitalized previously and treated with broad-spectrum antibiotics for neutropenic fever.

In summary, this large single-center study confirms FOB-BAL to be safe and effective in determining the etiology of pulmonary infiltrates in HM patients. The routine use of novel molecular and serological techniques in the assessment of BAL fluid (Figure 1), contributes to a rapid and precise detection of life-threatening infections and may allow targeted treatment to be provided to this patient population.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2016.07.011.

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