Vol. 95 (2001) 885–890

Quantitative culture of bronchoalveolar lavage fluid in community-acquired lower respiratory tract infections

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Abstract To evaluate the diagnostic value of quantitative bacterial culture of bronchoalveolar lavage (BAL) fluid obtained by fibreoptic bronchoscopy, 67 consecutive immunocompetent adult patients admitted to hospital with community-acquired lower respiratory tract infections from September 1997 to May 1998 were investigated. Results were compared to the findings in eight healthy control persons investigated in February 1998. There was no difference between study patients and control persons when quantitative culture of total cumulative bacterial findings or bacteria categorized as members of the oropharyngeal normal flora were compared. The culture of normal flora in bronchial washings probably reflects contamination of the lower airways with secretions from upper airways by the fibreoptic procedure itself, as fractionated sampling showed a 10-fold reduction in quantitative culture results when a primary bronchial washing was compared to a secondary sampling from the same bronchus in the control group. Twenty-four (36%) of 67 patients were cultured as positive in the study group while all control persons were cultured as negative for bacteria categorized as potential pathogens. With a threshold value for positive culture of 10^4 cfu ml^{-1} the specificity of lavage culture of potential pathogenic bacteria in relation to actual lower airway infection was 100%. Therefore, quantitative bacterial culture of potential pathogenic bacteria in BAL fluid is very specific but only positive in about one-third of unselected immunocompetent adult patients with a lower respiratory tract infection.

doi:10.1053/rmed.2001.1160, available online at http://www.idealibrary.com on IDEAL®

Keywords bacterial colonization; quantitative culture; bronchoscopy; community-acquired pneumonia.

INTRODUCTION

The use of fibreoptic bronchoscopy (FOB) with bronchoalveolar lavage (BAL) as an invasive microbiologic diagnostic tool in hospitalized patients with lower respiratory tract infections (LRTI) have increased in recent years. The method was originally introduced to overcome problems with oropharyngeal contamination of specimens for microbiological culture.

Despite precautions to prevent bacterial contamination with, for example, wedging of the tip of the bronchoscope into an airway lumen, microbiological culture of the BAL fluid almost always contain variable amounts of bacteria thought to be the result of contamination of the bronchoscope by its passage in the upper airways. To partly overcome this problem of interpretation of culture results because of contamination, quantitative culture techniques have been introduced and various thresholds of colony forming units (cfu) per ml of BAL fluid have been evaluated for their sensitivity and specificity when diagnosing pneumonia. Previous studies have focused on very selected groups of patients, most often immunoincompetent patients severely diseased with pneumonia or patients admitted to intensive care because of severe nosocomial respiratory infections (1).

In these highly selected groups of patients it is characteristic that FOB with BAL culture greatly improve microbiological diagnostics, with rates of positive microbiological culture results in patients with LRTI (diagnostic sensitivity) in the range of 85–90% and negative microbiological findings in non-infected subjects (diagnostic specificity) in the range of 97–98% when using cut-off values for BAL fluid of 10^3–10^4 cfu ml^{-1} of bacterial pathogens (2).

In other clinical studies the value of FOB with BAL culture have been evaluated in combination with other invasive and non-invasive techniques in immunocompetent

Received 1 June 2001 and accepted in revised form 15 June 2001
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adult patients with LRTI and in these series the diagnostic sensitivity is reduced to about 50%, where half of the patients, despite invasive diagnostic procedures, remain undiagnosed with respect to microbiological aetiology (3).

In this study we have investigated the value of FOB with quantitative culture of BAL fluid in a group of unselected consecutive adult patients admitted to hospital with community-acquired LRTI and compared microbiological findings with a small group of healthy controls.

MATERIALS AND METHODS

Adult patients admitted to the internal medicine ward at Silkeborg County Hospital with a clinical diagnosis of LRTI were asked to participate in a study of intensified microbiological diagnostics including FOB with BAL culture. The clinical diagnosis of LRTI required acute signs of inflammation with fever (body temperature $>37{\cdot}5^{\circ}C$ once within 48 h of inclusion) and/or increased leucocyte count ($>11\times10^{9}\,l^{-1}$) together with one to three signs of disease in lower airways: newly developed increased coughing, sputum purulence, and/or dyspnoea (4). Patients were included from 1 September 1997 until 31 May 1998. A total of 67 patients were included and all signed informed consent. They were investigated within 24–48 h of admission from their home.

In February 1998 eight healthy subjects agreed to act as controls and were renumerated to participate in FOB with BAL for culture. They also gave written consent.

Methods

Prior to bronchoscopy the mouth and pharynx were anesthesized with a solution of 4% lidocain. The fibrebronchoscope was introduced through the nose or alternatively through the mouth and additional anaesthetics (1% lidocain solutions) were applied as the scope passed the larynx, trachea and bronchial tree. A sterile, thin tube was introduced in the working channel of the bronchoscope; the tip of the scope was wedged in an appropriate segment of the bronchus and thereafter lavage was performed.

In the study group a segment of bronchus affected by a new pulmonary infiltrate as seen by prior chest X-ray was chosen for lavage. Where no infiltrate was apparent on chest X-ray the middle lobe was chosen for lavage. One to three portions of 60 ml isotonic NaCl were used for lavage and the aspirated lavage fluid was collected in one single portion for microbiological culture.

In the eight controls BAL was always performed in the middle lobe with 3×60 ml of isotonic NaCl at body temperature. The first portion of 60 ml was aspirated separately from the two subsequent volumes and the first portion was considered to represent a bronchial fraction. Lavage fluid from the second aspiration was termed the alveolar fraction. The samples from controls were processed for differential cell counts where the BAL fluid was filtered through a nylon filter (Nytal, type 440, Sintab Product AB, Sweden) with a pore size of 100 μm and then centrifuged (Heraeus Sepatech Minifuge T) for 10 min at 250 **g**. The supernatant fluid was decanted and the cell pellet resuspended in PBS-buffer to a volume of approximately 5 ml. Cells were coloured with trypan blue and counted in a Bürker Türk counting chamber with the cell concentration adjusted to approximately 250 000 cells ml^{-1}. For the differential cell counts 0·2 ml of the cell suspension was cytocentrifuged (Shandon Cytospin 2) for 50 min at 72 **g**. The slides were fixed with methanol and stained with May–Grünwald–Giemsa. A minimum of 600 cells were counted for each differential cell count.

To obtain a quantitative culture of bacteria in BAL fluid 1 and 10 μl loops were each plated on 5% horse blood agar and chocolate agar. Plates were incubated in 5% carbon dioxide overnight at 35°C. If growth was too little for isolation of colonies incubation was continued for another 24 h. Counts of different colonies were made and representative colonies were subcultured and subjected to standard procedures for bacterial identification, including Gram-staining, catalase and oxidase reactions. BAL culture proceedings were identical for study and control groups. The sensitivity of the bacterial culture was one cfu with 10 μl BAL fluid cultered corresponding to a minimum finding of 1×10^{2} cfu ml^{-1}.

Isolated bacteria from BAL culture were grouped in three different groups: as cumulative total bacterial count irrespective of family or species, as cumulative bacterial count for bacteria considered to represent the normal respiratory flora (NRF) which included α-haemolytic streptococci (exept *Streptococcus pneumoniae*), coagulase-negative staphylococci, non-haemolytic streptococci, *Corynebacteria*, micrococci and *Neisseria spp*. The last group was bacteria representing the potential pathogenic bacteria (PPB) and comprised any bacterial species not considered a member of the NRF by the clinical microbiologist.

RESULTS

As could be anticipated the study and control groups investigated were very different with respect to basic demographic and clinical characteristics (Table 1). Notably, the median age of patients admitted with LRTI was more than twice the median age of controls. Also, 49% of patients in the study group had signs of varying degrees of chronic obstructive lung disease and 21% had varying signs of other chronic disease compared to none in the control group of healthy persons.

Among the 67 patients in the study group 16 (24%) were culture-negative ($< 10^2$ cfu ml^{-1} BAL fluid) for all bacteria, while 53 patients were culture-positive for one or more bacteria belonging to either the NRF or PPB flora. Two patients in whom only *Candida albicans* were cultured have been considered negative with regard to bacterial BAL culture. One of them was found positive for *Mycoplasma pneumoniae* by PCR and the other had a positive blood culture for *Streptococcus pneumoniae* and was positive for PUT. In comparison all eight control persons were culture-positive (1×10^2 cfu ml^{-1} or larger) on the bronchial BAL fluid for one or more bacteria, but in the control group they were all classified as belonging to the group of NRF and no control persons were cultured positive for any bacteria classified as PPB. When the quantitative culture results for BAL fluid in the 67 patients in the study group were compared with the quantitative culture results for the eight control persons with respect to their comparable bronchial fraction of BAL fluid for the three different groups of bacteria of total cumulative counts, NRF and PPB cfu ml^{-1} (Fig. 1), there was no significant difference between study and control groups with respect to total cumulative counts ($P = 0{\cdot}62$, Mann–Whitney). However, the bronchial fraction of the BAL fluid from the control group had significantly higher counts for NRF ($P < 0{\cdot}02$, Mann–Whitney). All subjects in the control group had no PPB counts. However, also for a substantial number of patients in the study group no PPB were isolated and the difference between the two groups was barely significant (P=0·046, Mann-Whitney).

The range of bacterial species belonging to NRF found in the two groups of subjects was identical. The quantitative culture results in the two groups were compared in relation to a true clinical diagnosis of LRTI in the 67 patients in the study group and no respiratory infections among the eight healthy control persons. Sensitivity, specificity and predictive values were calculated for varying cut-off levels in three groups of bacteria found in BAL culture in relation to clinical diagnosis (Table 2). With increasing cut-off levels for positive culture from $\geq 10^3$ to $\geq 10^5$ cfu ml^{-1} the sensitivity of test falls and the

Table 1. Basic demographic and clinical characteristics of eight persons in the healthy control group and 67 patients in the study group

Group	*n*	Age (years)		Gender (%)		COPD (%)	Other chronic (%)
		Median	Range	Female	Male		
Control	8	30	23–45	38	62	0	0
Study	67	63	31–87	36	64	49	21

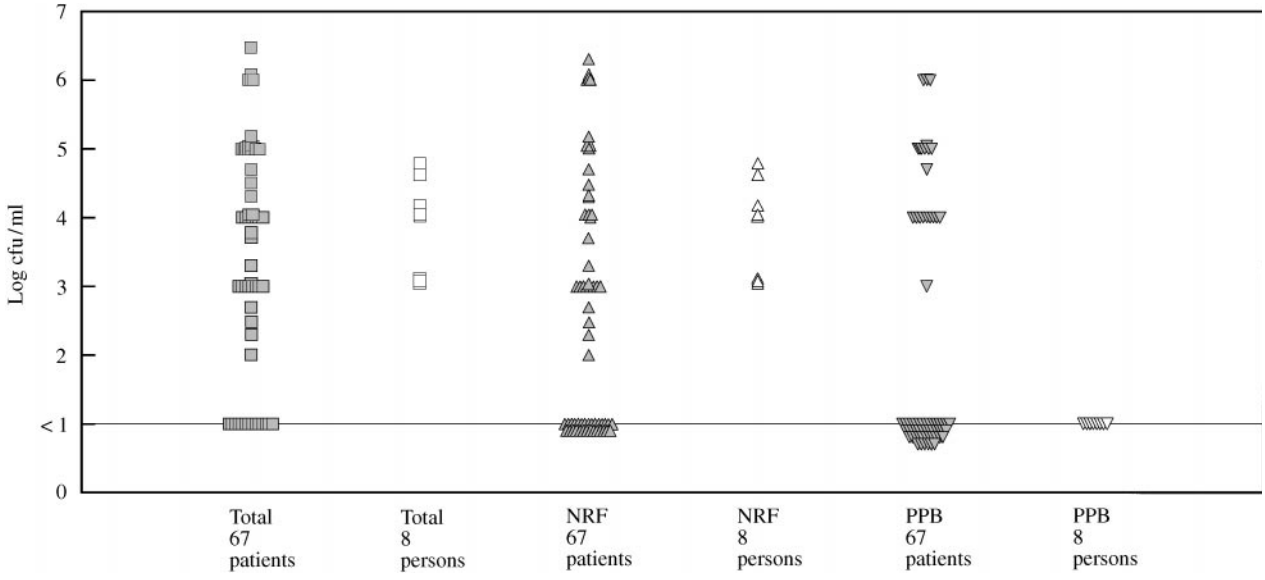

Fig. 1. Scattergram of number of bacteria cultured semiquantitative from BAL fluid in 67 patients admitted to hospital with LRTI and eight healthy control persons. Bacterial numbers were added for each case and grouped into total number of bacteria irrespective of bacterial species, bacteria categorized as respiratory normal flora and bacteria categorized as belonging to potential pathogenic bacteria.

Table 2. Sensitivity, specificity and predictive values of microbiological findings in BAL fluid from FOB in 67 patients with LRTI and eight healthy control persons

Bacterial flora	Cut-off value (cfu ml^{-1})	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Cumulative	$\geq 10^3$	70	0	86	0
	$\geq 10^4$	54	38	88	9
	$\geq 10^5$	30	100	100	15
RNF	$\geq 10^3$	46	0	80	0
	$\geq 10^4$	28	38	79	6
	$\geq 10^5$	15	100	100	12
PPB	$\geq 10^3$	36	100	100	16
	$\geq 10^4$	34	100	100	15
	$\geq 10^5$	16	100	100	13

Table 3. Distribution of bacterial species and concentrations in the bronchial and alveolar fraction of the BAL fluid

Bacteria	Bronchial fraction (cfu ml^{-1}) ($n = 8$)				Alveolar fraction (cfu ml^{-1}) ($n = 8$)			
	$< 10^2$	$10^2 - < 10^3$	$10^3 - < 10^4$	$10^{4-5} \times 10^4$	$< 10^2$	$10^2 - < 10^3$	$10^3 - < 10^4$	$10^{4-5} \times 10^4$
Non-haemolytic streptococci		1	2	5	1	1	3	
Coagulase-negative staphylococci		1	4					
Gram-positive coryneforme rods			1	2				
Micrococci			1					
Neisseria spp.		2						

Table 4. Recovery percentage, total cell counts and percentage of epithelial cells

	Recovery percentage	Total cell count ($\times 10^6$ ml^{-1})	Epithelial cells (%)
Bronchial fraction	28·6	2·8	0–3%
Alveolar fraction	57·7	9·6	0–0%

specificity rises. There appears to be no ideal combination of percentage diseased with a positive test (positive predictive value) on the one hand and the percentage testing positive with disease (sensitivity) on the other. In this respect the highest values are obtained for a bacterial culture of only PPB and cut-off values of either $\geq 10^3$ or $\geq 10^4$ with 100% of patients culture-positive for PPBs actually diseased with infection but only 36% (24 patients) and 34% (23 patients), respectively, of the 67 diseased patients were culture-positive for PPBs. A reliable positive culture test to predict disease thus occurs with 64–66% of diseased patients having negative BAL culture for PPBs. For the study group alone there was a relation between the primary clinical presentation with or without infiltrates on chest X-ray, the presence of chronic obstructive lung disease and a PPB count of $\geq 10^4$ cfu ml^{-1} ($P < 0{\cdot}01$, Fischer's exact test). There was also a significant effect of treatment with any antibiotic within 24 h of BAL sampling with a decreased incidence of positive BAL culture for PPB ($\geq 10^3$ cfu ml^{-1}) with prior antibiotic therapy. Among 31 patients treated with antibiotics within 24 h of sampling, 6/31 (19%) were

culture-positive for PPB while 18/36 (50%) were culture-positive for PPBs among patients not treated with any antibiotic prior to BAL sampling ($P = 0{\cdot}011$, Fischer's exact test). On the contrary, there was no association between counts of NRF and antibiotic treatment prior to sampling of BAL fluid.

In the study group the concentration of NRF varied from $< 10^2$ to more than 10^6 cfu ml^{-1} in BAL fluid culture. When the quantitative occurrence of NRF was associated to clinical variables such as age, pre-existing chronic disease, lung function, prior antibiotic therapy and disease severity there were no significant associations apparent.

When the two different aspirations of BAL fluid from the eight control persons were compared (Table 3) the bronchial fraction had a greater diversity and higher concentrations of bacteria belonging to NRF than the alveolar fraction. All eight were culture-positive with one or more NRF from the bronchial fraction while 3/8 were culture-negative from the alveolar fraction and only non-haemolytic streptococci were found in the alveolar fraction. Overall, the bronchial fraction cultured more than 10-fold more NRF than the alveolar fraction with a median of 10^4 and 3×10^2 total cfu ml^{-1} respectively. When the two fractions are compared with respect to total and differential cell count (Table 4), the alveolar fraction had a three-fold higher total cell count per ml but with no epithelial cells, while the bronchial fraction contained epithelial cells in 2/8 samples (range 1–3%).

DISCUSSION

The first studies (5,6) on the use of quantitative culture of BAL fluid as a diagnostic tool in patients with pneumonia evaluated total cumulative bacterial concentration in BAL fluid as indices of infection. Despite the inclusion of *Neisseria spp*., α-haemolytic streptococci they found impressive diagnostic sensitivities of 13/15 (5) and 13/13 (6) for the culture of any bacteria in a concentration exceeding 10^5 cfu ml^{-1} in patients with pneumonia and in their non-infected control groups they identified 0% (5) or 6/18 (33%) (6) with more than 10^5 cfu ml^{-1}. In both investigations, however, the patients were highly selected with 15 immunocompetent patients with progressive bacterial pneumonia, seven of which showed clinical progression despite antibiotic treatment (5) or 13 immunocompromised patients with pneumonia (6).

In comparison, the present results on cumulative bacterial culture in BAL fluid showed no significant difference between culture results in patients with LRTI and healthy control persons. In patients as well as controls members of the oropharyngeal normal flora were often dominant findings in our BAL culture. Previous studies have made it probable that the culture of NRF represent oropharyngeal contamination introduced by the actual bronchoscopic procedure. In 18 control patients the mean total cfu ml^{-1} was $1{\cdot}1 \times 10^5$ in six persons with more than 1% epithelial cells from upper airways in aspirated BAL fluid, whereas the mean was 4×10^3 cfu ml^{-1} in 12 with less than 1% epithelial cells in BAL fluid (6). When special precautions as intubation are taken (7) to reduce oropharyngeal contamination during bronchoscopy the median concentration of NRF is reduced 10-fold from 2×10^3 cfu ml^{-1} in routine bronchoscopies to $1{\cdot}4 \times 10^2$ cfu ml^{-1} with precautions. The present results supports the view of contamination with NRF during bronchoscopy as we find a 10-fold lower concentration of NRF in the alveolar fraction of BAL fluid as compared to the bronchial fraction of BAL fluid despite a three-fold higher cell count per ml in the alveolar fraction compared to bronchial fraction. Furthermore, we could not associate large quantitative differences in NRF occurrence in the 67 study patients to any clinical correlates such as disease severity or existence of chronic diseases. This was in contrast to the culture of PPB in the patients where the existence of COPD in patients was associated with higher PPB cfu ml^{-1} than in patients with no existing chronic lung disease.

To avoid the influence of oropharyngeal NRF contamination more recent studies have identified relevant PPBs for their study population (2) or discarded the first 20 ml relevant to the BAL procedure (8) before their evaluation of the clinical value of quantitative culture of BAL fluid. As to the cut-off concentration of PPB predictive of LRTI they find that concentrations above 10^3 (2) –10^4 (8) cfu ml^{-1} have a 90% and 81% frequency of positive culture in patients with pneumonia, respectively, while only 2–3% are culture-positive in control groups consisting of healthy volunteers and patients with non-infectious pulmonary diseases. The present small control group with none culture-positive for PPB makes interpretation difficult. However, the present results indirectly supports a cut-off value of $\geq 10^4$ cfu ml^{-1} as only 1/24 PPB isolated from 67 patients with LRTI was below 10^4 cfu ml^{-1}.

With a cut-off concentration of $\geq 10^4$ cfu ml^{-1} for PPB the present results show a diagnostic specificity of 100% but only 34% of patients with clinical infection were culture-positive. This is probably caused by our inclusion of consecutive patients with varying severity of community-acquired LRTI irrespective of recent antibiotic therapy. Aetiologies of community-acquired respiratory infections include significant numbers of atypic aetiologies and viral infections (9), which is not detected by BAL culture for bacteria. Prior antibiotic therapy have been found to reduce the frequency of positive bacterial culture in BAL fluid from 92 to 55% in patients with severe community-acquired pneumonia requiring intensive care (8), just as the frequency of positive bacterial culture of BAL fluid increased with increasing size of infiltrates on chest X-ray (8). Similar associations were also seen in the present study.

Our diagnostic sensitivity of 34–36% compares to previous studies in community-acquired LRTI where diagnostic sensitivities were 46% and includes infections with atypcial and with viral infections (10). Therefore, routine FOB with quantitative BAL culture, as used in this study, is probably only justified in selected groups of immunocompetent patients with severe community-acquired respiratory infections (unpublished data).

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