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ORIGINAL ARTICLE

DISTAL AIRWAYS BACTERIAL COLONIZATION AND INFLAMMATORY PATTERN IN CHILDREN WITH DIFFERENT CHRONIC LUNG DISEASES: A BRONCHOSCOPIC STUDY

By

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Background: ***Children with chronic lung diseases are vulnerable to develop bacterial colonization of their distal airways. However, this diagnosis is based usually on nonspecific samples, such as gastric lavage or sputum cultures.***

The aim of this study: ***was to assess applicability of fibro-optic bronchoscope technique to determine distal airway microbial colonization and inflammation in children with chronic lung diseases compared to conventional methods.***

Methods: ***Bronchoscopic bronchoalveolar lavage fluid (BALF), sputum and blood samples were collected from 10 healthy children and 30 patients with chronic lung diseases (with different diagnoses). All samples were subjected to microbiological assessment (direct films, cultures, and antibiogram test), cytology assessment and biochemical assessment of Lactic dehydrogenase enzyme (LDH) and Alkaline phosphatase enzyme (ALP) levels as indicators for ongoing pulmonary damage.***

Results: ***Distal airway bacterial colonization by potentially pathogenic microorganisms (PPMs) was defined in 73% of children with the most common organism Staphylococcus aureus followed by E. coli, Pseudomonas aeruginosa, and Citrobacter freundii. BALF analysis was superior to sputum (OR=5.5; 95%CI: 1.6-19.7, P=0.004) and blood samples (OR=38.5; 95%CI: 6.4-302, P=0.0001) analyses as they missed detection of many organisms. Antibiogram analysis revealed that most of gram negative organisms were highly sensitive to imepenem, tobramycin, ceftriaxone, garamycin, and amikin. Most of gram positive organisms were highly sensitive to vancomycin , dalacin and oxacillin.***

All patients (even if not colonized) visualized ongoing distal airways inflammation and had higher levels of inflammatory markers and cellular loads in comparison to healthy controls.

Conclusion: ***BAL fluid sampling is a safe, technically simple procedure in children and has a significant diagnostic value compared to sputum or blood markers for distal airways bacterial colonization and inflammation.***

Keywords: ***bronchoalveolar lavage fluid, children, chronic lung disease, colonization.***

INTRODUCTION

Children are vulnerable to many risk factors which may precipitate chronic lung disease either prenatally, natally, or postnatally.[1] The terminology of pediatric "chronic lung diseases" include abnormalities in airways, lung parenchyma, blood vessels or pleura. These pathologies may result from congenital parenchymal lung defects (such as congenital lobar emphysema, congenital cystic lung, sequestrated lobes…), airway disease (such as bronchiectasis, primary ciliary dyskinesia, cystic fibrosis…) or acquired interstitial lung diseases.[2]

In contrast to the healthy population, distal airway bacterial colonization may occur in patients with chronic lung diseases, who often have altered pulmonary defenses. Persistence of Potential Pathogenic Microorganisms (PPMs) in the airways because of impairment in mucus clearance may lead to a vicious circle characterized by chronic bacterial colonization, persistent inflammatory reaction, and progressive tissue damage.[3]

Over the years, new diagnostic techniques such as bronchoscopic bronchoalveolar lavage (BAL) and use of a protected specimen brush have been studied and are now widely used in adults.[4] The applicability of these bronchoscopic methods is reasonable in adults[5] and they are now considered to be the best methods to diagnose distal airways bacterial colonization in this population. In children, these bronchoscopic methods are not easily applicable, mainly because of the small size of the airways and because of complications associated with these procedures.[6] Moreover, experience with these methods in children is still limited and their validity remains to be determined.[4]

The primary objective of this study was to demonstrate frequency of microbial colonization and ongoing cell damage in distal airways of children with stable chronic lung diseases using bronchoscopic bronchoalveolar lavage (BAL) fluid compared to sputum and blood samples. The second objective was to evaluate the safety and feasibility of bronchoscopic technique in the pediatric population.

PATIENT AND METHODS

Patients: This cross sectional descriptive study was performed over 17 months, with patients 13 years old or younger (age ranged between 8 months and 13 years, median 5.25 years, mean 6.36+5 years) in the period from July 2004 till November 2005. The study included 40 children who were divided into 2 groups:

A. Thirty patients with chronic pulmonary diseases, sixteen (53.3%) were females and fourteen (46.7%) were males, following up at the Pediatric Chest Clinic, Children's Hospital, Ain Shams University defined as chronic lung disease while they were free of any exacerbation and off antibiotics for at least 2 weeks. A sample size of 30 patients was considered appropriate (confidence level of study power more than 85% - calculated using Epi Info software for windows, version 3.3.2; Atlanta, CDCP; 2005). Inclusion criteria for the study: 1- Persistent chest symptoms for more than 6 weeks as; chronic cough, dyspnea, excess sputum or wheezes. 2- Abnormal and persistent chest X ray findings for more than 6 weeks as; persistent lobe collapse, miliary appearance, unresolving pneumonia, persistent consolidation, honey combing, ground glass appearance. Exclusion criteria were as follows: recent pulmonary infection, recent antibiotic intake within 2 weeks, known asthmatic or tuberculous children, contraindication to perform a bronchoscopic BAL (severe bronchospasm, pulmonary hypertension, cardiopulmonary instability, severe hypoxemia), refusal from parents to participate, loss of specimen, and impossibility to complete BAL.

B. Control group (Group III); Ten clinically healthy children (with no history or manifestations of any chest illness) were randomly selected from the same general population to which the patients belong. These ten were operated upon due to elective non pulmonary surgical causes (inguinoscrotal

hernia, lymph node biopsy....). BALF was collected from them while they were operated upon and under general anaesthesia after their parents' consent.

All studied patients were subjected to full medical history, thorough clinical examination, plain chest X rays and sample collections. Informed consent was obtained from the parents. This study was approved by the Research Ethics Committee of Ain Shams University Hospital.

Specimen collection:

- ***Blood:*** Five ml of sterile blood collection was done from a peripheral vein.
- ***Sputum:*** Sputum was collected in the form of spontaneous sputum or gastric lavage. Older and cooperative children were asked to expectorate immediately before bronchoscopy. Gastric lavage was done for young children - who can't expectorate- in the early morning (fasting) French catheter (Size 8) was introduced into stomach through the nose or mouth. Stomach was lavaged by 1 cc NaHCOs 5% + 9cc saline while patient is under anaesthesia before the bronchoscopy procedure.[7]

  Processing of sputum samples: The collected sputum was divided into two parts. The first part was used for cytologic examination. Briefly, sputum was treated by adding 2 mL of 0.1% dithiothreitol, then vortexed for 2 to 3 min to homogenize the sample, 2 mL of phosphate-buffered solution was added to stop the action of dithiothreitol, then the suspension was centrifuged at 300 rpm for 10 min and separated into supernatant and precipitant. The sediment was stained by Geimsa stain to be examined microscopically to evaluate cell type and estimate lymphocyte and neutrophil ratio to the total cell count. The different cells were counted per 300 cells on various fields throughout the slide. The differential cell count was reported as percentages. The second part was sent for microbiological cultures and biochemical assessment.[8]

- ***Bronchoalveolar Lavage:*** *All BALs were done by the same operating team, using a Pantex fiberoptic bronchoscope system (PENTAX FB-10X bronchoscope with; external diameter of 3.4 mm, working channel diameter = 1.2 mm, working length = 600 mm and total length = 900 mm).*

  Pre-bronchoscopy evaluation of the cardiac condition, blood pressure and arterial blood gases was done. All subjects studied were fasting for at least 6 hrs before bronchoscopy. All subjects studied underwent bronchoscopy under general anaesthesia using deep Halothane inhalation, monitored by ECG, and pulse oximetry and non invasive blood pressure monitor.[3,6]

  Flexible bronchoscopy was introduced via endotracheal tube, laryngeal mask, or through face mask using Swivel connector to maintain anaesthesia. The tip of the bronchoscope was wedged into the diseased segment seen in chest x-ray, or in the right middle lobe and lingula when pathology is generalized. One to three ml/kg aliquot of pre-warmed sterile normal saline 0.9% (maximum of 3 aliquots) was used for lavage and rapidly aspirated through the suction port of the bronchoscope.[4]

Specimen Evaluation: Samples were rapidly transferred to the laboratory. Macroscopic examination: of BALF and sputum/gastric lavage for caseous, purulent or bloody areas, and necrotic material. Sputum and BALF samples were pooled and centrifuged at 300 rpm for 15 minutes and separated into supernatant and precipitant. The supernatant portion was sent immediately for biochemical assessment of ALP & LDH. The sediment was used for microbiological assessment. Blood samples were divided into 2 parts; the first part is used as whole blood for microbiological assessment and the second part is centrifuged for serum collection used for biochemical markers.

(I) Microbiology:

A) *Microscopic examination:* The sediment was spread on slides and stained by Geimsa stain to

be examined microscopically to evaluate cellular pattern. The differential cell count was performed by counting 300 non squamous cells on various fields throughout the slide. The differential cell count was reported as percentages of the total cell count.

B) *Quantitative bacteriologic cultures: on suitable media including blood agar, MacConckey agar and choclate agar medium were performed. All cultures were incubated at* 37°C under aerobic and anaerobic conditions and in a CO2-enriched atmosphere. Cultures were evaluated for growth after 24 and 48 h and discarded, if negative, after 5 days. Bacterial growth was identified by:

- Appearance of growth.
- Rate of growth.
- Colony pigmentation.
- Type of media.
- Microscopic examination.

Bacterial agents were classified into potential pathogenic microorganisms (PPMs) or non- PPMs. PPMs were those microorganisms recognized as agents causing respiratory infections, whether or not belonging to the gastrointestinal or oropharyngeal flora; Gram-ve rods, such as Pseudomonas aeruginosa, Enterobacteriaceae and Haemophilus spp.; Gram-+ve cocci, such as Staphylococcus aureus, Streptococcus pneumoniae. Non-PPMs were those microorganisms belonging to the oropharyngeal or gastrointestinal flora that are not usually involved in respiratory infections in non-immunocompromised patients (Streptococcus viridans group, Neisseria spp., Corynebacterium spp., Candida spp., and others). Colonization of lower airways cultures was considered if BAL cultures yielded ≥103 colony-forming units (cfu)/mL, and were considered negative if they were sterile or yielded <103 cfu/mL.

Bacterial sensitivity: antimicrobial susceptibility of the isolated organism was done.

(II) Cell damage and inflammatory markers:

Biochemical assessment of serum and the supernatant Lactate dehydrogenase enzyme (LDH) and Alkaline phosphatase enzymes (ALP) levels, in IU/L using enzyme kinetic assay on automated Hitachi 917.[9]

All samples were analyzed by the same microbiologist and the same hematologist, who were blinded as to the origin of the samples.

Statistical Analysis: Descriptive data are expressed as means ± SD. Continuous data were compared by the Student paired t test or Wilcoxon signed-rank test. Categorical data were compared by χ^2 test analysis. Receiver operating curve (ROC) was done to calculate cut off values. Kappa test was performed for diagnostic agreement. Significant results were expressed as p value, OR and 95% CI. Statistical significance was established at $p < 0.05$. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 11.5; SPSS Inc., Chicago, IL, USA; 2006).

RESULTS

Demographic Data: Thirty patients were included in the study. Demographic data for the 30 patients are presented in Table 1.

Table 1. Demographic data of the patients.

Variable	Value
Patients, n	30
Sex, male: female	14: 16
Age, year*	6.4± 5
Diagnosis of chronic lung disease, n:	
Bronchiectasis, n (%)	13(43%)
Postpneumonic	6
GERD	1
Primary ciliary dyskinesia	1
Congenital cystic lung	1
Cystic fibrosis	2
Congenital Immune defficiency	5
Interstitial pulmonary fibrosis, n (%)	6 (20%)
Others, n (%)	11 (37%)
Repeated bronchopneumonia	4
Unresolved bronchopneumonia	7

Definition of abbreviations: GERD=Gastro-esophageal reflux disease. * = Mean ± standard deviation.

Distal airways bacterial colonization: The overall colonization rate among patient group was 73.3% (22 out of 30). BALF culture resulted in growth of 32 PPMs from 22 patients. Gram negative organisms were the predominating organisms detected in the airways of clinically stable patients with chronic lung diseases. Sputum culture resulted in growth of only 14 different PPMs from 10 patients. This means that BALF is evident to be superior in detection of colonization (OR=5.5; 95%CI: 1.6-19.7, P=0.004).

Colonizing organisms detected by BALF culture; total gram –ve (n=22) (68.8%) and total Gram +ve (n=10) (31.2%). Isolated gram –ve organisms; E.coli (n=6), Pseudomonas Aeruginosa (n=5), Citrobacter Freundii (n=4), Serratia Odoriferra (n=2), Hemophilus Influenza(n=1), Klebsiella Species (n=1), Enterobacter Cloaca (n=1) and Enterobacter Agglomerans (n=2). Isolated gram +ve organisms; Staphylococus aureus (n=6), Methicillin resistant staph aureus (MRSA) (n=2) and Pneumoccocus pneumonae (n=2).

Types of colonizing organisms detected by sputum culture; total gram –ve (n=5) (35.7%) and total gram +ve (n=9) (64.3%). Isolated gram –ve organisms; E.coli (n=1), Pseudomonas Aeruginosa (n=2) and Serratia Odoriferra (n=2). Isolated gram +ve organisms; Staphylococus aureus (n=6) and Pneumoccocus pneumonae (n=3).

Blood cultures showed poor results regarding airways colonization, with BALF much superior and specific (OR=38.5; 95%CI: 6.4-302, P=0.0001).

Table 2. Colonization rate according to sputum and BALF analysis in the patients group.

	Patients without pulmonary colonization	Patients with pulmonary colonization
According to sputum	20 (66.7%)	10 (33.3%)
According to BALF	8 (26.7%)	22(73.3%)

(OR=5.5; 95%CI: 1.6-19.7, P=0.004)

The colonization rate was highest between patients with bronchiectasis (92.3%), high in patients with persistent or recurrent pneumonia and/or collapse (63.3%), and lowest in patients with interstitial lung disease (50%).

Antibiotic sensitivity tests for BALF culture; for gram –ve organisms showed that the best sensitivity for antibiotics was detected with imepenem (16/22), tobramycin (11/22), ceftriaxone (10/22), garamycin (9/22), and amikin (9/22), while the highest resistance was with; cefoperazon (16/22), cefotax (15/22) and ampicillin/sulbactam (14/22). For gram +ve orgamisms; the best sensitivity for antibiotics was detected with vancomycin (7/10), dalacin (5/10) and oxacillin (5/10), while the highest resistance was with; penicillin (5/10), and erythromycin (4/10).

After acquisition of the microbiological results patient group was divided into 2 subgroups; group I with bacterial colonization and group II without bacterial colonization (Fig. 1).

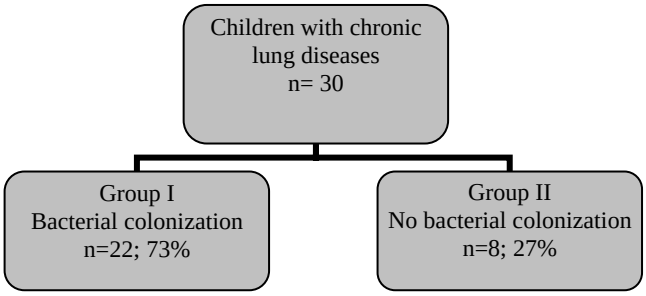

Fig 1. Classification of the children according to bacterial colonization.

Stastical comparison between group I and group II regarding; clinical symptoms (OR=0.85; 95%CI: 0.34-2.09, P=0.07), weight of the children (OR=0.99; 95%CI: 0.93-1.07, P=0.08), height of the children (OR=0.98; 95%CI: 0.94-1.02, P=0.1), finger clubbing (OR=0.85; 95%CI: 0.34-2.09, P=0.2), blood cell counts total and differential (OR=0.9; 95%CI: 0.4-1.3, P=0.07) and frequency of chest radiological abnormalities (P > 0.05); all showed statistically non significant difference.

Bronchoscopic Data: Bronchoscopic visualisation of the airways manifested, different degrees of inflamed mucosa, contained different morphology of pathologic secretions and these findings directed the BAL samples. Adverse events during the procedure were benign, transient, and did not require any treatment: lowest oxygen saturation noted was 80% and lasted for less than 2 minutes; bradycardia and a drop in blood pressure occurred in 2 patients but resolved spontaneously within seconds.

Table 3. Complications during bronchoalveolar lavage.

Complications	(n)
Pneumothorax	0
Hemoptysis	5
Decrease in SaO2 (< 92%)	10
New radiological opacity	1
Decrease in Pa (> 8 mm Hg)	1
Bradycardia	2

Definition of abbreviations: N = number of cases; Pa = mean arterial pressure; SaO2 = arterial oxygen saturation.

BALF Data: Forty samples of BAL were analyzed. All specimens were considered satisfactory (< 1% squamous epithelial cells). The amount of fluid injected was (18.1 ± 5.4 ml), the amount of aspirated fluid (7.2 ± 2.5 ml) and the ratio of fluid aspirated to fluid injected was appropriate (40 ± 12%).

Table 4. Results of brochoalveolar lavage.

BALF markers	Patients group (n=30) Mean+SD	Control group (n=10) Mean+SD	P	
LDH (IU/L)	536+169	21.8±4.5	P<0.05	S
ALP(IU/L)	64.6+22.6	9.8±3.6	P<0.05	S
Total leucocytic count (103cells/ml)	3123.9±448	670+20.6	P<0.001	HS
Neutrophil% (absolute count)	64.2+4.4 (2005.5+20)	6.4±0.7 (42.9+0.15)	P<0.05	S
Lymphocyte% (absolute count)	20.5+2.02 (658.6+9)	6±1.6 (42+0.31)	P<0.05	S
Macrophage% (absolute count)	15.4+5.07 (495+22.4)	87.6±1.5 (586.9+0.3)	P<0.05	S

S=Significant HS=Highly Significant

In the current study; levels of inflammatory markers (LDH and ALP) in brochoalveolar lavage were found to be significantly higher in patients than in the control group (P<0.05). Mean BALF (ALP) level in group I (68.95+7 IU/L) was statistically higher than group II mean level (52+12 IU/L) with P<0.05. Similarly; mean BALF (LDH) level in group I (639.4+188 IU/L) was statistically higher than group II mean level (226.6+132 IU/L) with P<0.05. However, being non specific, their local collection suggests significant distal airway ongoing inflammation and cell damage in patients group. Nevertheless, these markers showed lower levels in patients' sera with the lack of statistically significant correlation between the levels of inflammatory markers in the BAL samples and those in the serum. This suggests that these markers are locally produced and not simply derived from the blood by simple diffusion.

Table 5. Comparison of the results of level of total leucocytic count in brochoalveolar lavage (BAL) between groups I, II and control.

TLC (103cells/ml)	Group I (n=22)	Group II (n=8)	Control (n=10)
Mean+ SD	4572.7±808	2850±835.1	670+20.6
Group I (n=22)		z=2.47 P<0.05 S	z=2.93 P<0.001 HS
Group II (n=8)			z =3.44 P< 0.001 HS

In the present work, when we compared the levels of total leucocytic count in BALF in the three groups, it was found that BALF of group I and II contained significantly higher levels than that of control group (P<0.001) and BALF of group I contained significantly higher levels than that of group II (P<0.05).

On drawing receiver operating curve (ROC) for total leuckocytic counts in BAL, area under the curve (AUC) = 0.798, with best cut off level = 1250x 103 cell /ml BALF, giving sensitivity (to detect colonization) = 95.5%, specificity = 75%, Positive Predictive Value (PPV) = 77.8% and Negative Predictive Value (NPV) = 66.7%. This means that, it is a good positive test to prove colonization and fair enough to exclude it.

In the sputum of our patients, there was no significant difference between group I and group II regarding the mean values of total and differential cells (p >0.05). However, sputum total leuckocytic cell count showed significant higher values in patients compared to healthy controls. On drawing receiver operating curve (ROC) for total leuckocytic counts in sputum, area under the curve (AUC) = 0.679, with best cut off level = 1350x 103 cell /ml sputum, giving sensitivity (to detect airway inflammation) = 86.4%, specificity = 37.5%, Positive Predictive Value (PPV) = 79.2% and Negative Predictive Value (NPV) = 50%.

Nevertheless, levels of BALF both neutrophil % and lymphocyte % in patients group were significantly higher than that of control group, their counts in BALF of group I was almost the same as group II, with no significant difference.

On the other hand, BALF macrophage % was found to be significantly higher in healthy control compared to patients group (and without any statistical difference between group I and group II)..

Agreement between sputum and BAL results of pathogens.

Table 6. Agreement between sputum and bronchoalveolar lavage bacterial colonization results.

		BAL		
		No Growth	**Gram +ve**	**Gram -ve**
Sputum	No growth	8	1	17
	Gram +ve		9	
	Gram -ve			5

Kappa test value = 0.07 P >0.05 NS

Key for Kappa test; Kappa value (strength of agreement); <0.2 (Poor), 0.21-0.4 (Fair), 0.41-0.6 (Moderate), 0.61-0.8 (Good), 0.81-1.00 (very good).

This indicates that there was a poor diagnostic agreement between BALF and sputum for detection of bacterial colonization in children with chronic lung diseases (Kappa value= 0.07, P >0.05).

DISCUSSION

BAL diagnostic specimen: In adults, bronchoscopic BAL is considered one of the most reliable diagnostic tests for respiratory infections and is used to obtain quantitative cultures and cytologic results.[4,6] Non bronchoscopic BAL has also been used for children in the past 10 years with good results. The advantages of doing a blind (non bronchoscopic) BAL include ease of performance at the bedside, feasibility through small endotracheal tubes (< 4.0 mm), less discomfort for the patient, and

low cost. The disadvantage is that, without bronchoscopy, it is difficult to predict in which part of the lung the sample will be taken.[2]

Some authors consider that a bronchial aspirate is reliable only if directed toward the affected lung region and this is only feasible with visualisation by a bronchoscope.[8,9] In this study, bronchoscopic BAL showed a significant more diagnostic yield than sputum and blood samples.

Therefore, bronchoscopic BAL speciments seems ideal for the pediatric population, regarding reproducibility, safety and costs. Even though bronchoscopic BAL is considered fairly safe, complications of BAL were frequent (in 50% of children) in this study, but most were transitory and benign. Meticulous monitoring of the patients' hemodynamics while performing the bronchoscopic lavage, together with shortening the length of the procedure and the use of the appropriately sized scope are important precautions for performing a safe BAL for pediatric patients. Complications could probably be totally avoided if the procedure is done by a qualified operator and the children be monitored carefully during and after the procedure.[6,8]

Distal airways bacterial colonization: The lower respiratory tract of a healthy child is sterile. In contrast, patients with bronchiectasis and chronic pulmonary disease are often colonized with Potentially Pathogenic Microorganisms (PPMs).[10]

Scientific evidence suggests that colonization of distal airways by PPMs may be specifically harmful to this group of patients. These microorganisms represent a potential risk for lung infections and may secrete several inflammatory mediators that cause progressive tissue damage and airway obstruction.[11]

The phenomenon of chronic pulmonary colonization, secondary inflammatory reaction, and progressive lung injury is a "vicious cycle" and is the reason why appropriate evaluation of distal airway colonization is needed. In order to break this vicious cycle, it is necessary to identify the colonizing bacteria and to know which antibiotic to be administered.[1,12]

The idea of using antibiotics for long periods of time in patients with chronic pulmonary disease was then introduced in a trial to break this vicious circle.[13] Many protocols were studied to see the effect of these medications on pulmonary conditions as reflected by patient's symptoms, radiological scoring, pulmonary function testing and inflammatory cells and markers in lung fluids and secretions. These antibiotic courses- termed chronic suppressive antibiotic therapy- are chosen according to cultures and proper isolation of the colonizing bacteria.[10,14,15]

Accurate knowledge of the type of colonizing agents may be important to standardize empirical antibiotic strategies for those patients.[16]

In the present study, BALF samples and sputum samples were used to study the rate and pattern of bacterial colonization and to study the types of the most predominant organisms.

It was proved by using BALF culture that; among 30 patients with different chronic pulmonary disorders, 22 patients (73.3%) were colonized by different types of potentially pathogenic organisms (PPM). While on using sputum as a sample for culture, it yielded colonization in only 10 children with a rate 33.3 % Table 2.

This result was in concordance with the study done by Angrill and coworkers, 2001 who studied bronchial colonization by PPMs in 60 patients with bronchiectasis in a stable clinical situation using BALF culture and found that colonization rate was 60%.[8]

In this study, BALF culture resulted in growth of 32 PPMs from 22 patients. Gram negative organisms were the predominating organisms detected in the airways of clinically stable patients with chronic lung diseases. Sputum culture resulted in growth of only 14 different PPMs from 10 patients. Also, Staphylococcus aureus was the most common organism in BALF cultures followed by E. coli, pseudomonas aeruginosa, citrobacter freundii, other Enterobacteriacae,

pneumococcus pneumonae, H. influenzae and lastly Klebsiella species. Two BALF samples resulted in heavy growth of MRSA.

Indicators of bacterial colonization: In the present study, colonization rate was highest between patients with bronchiectasis (92.3%), high in patients with persistent or recurrent pneumonia and/or collapse (63.3%), and lowest in patients with interstitial pulmonary fibrosis (50%).

These data came in concordance with the study done by Cabello and coworkers 1997. When they studied BALF samples from the airways of 100 patients with different chronic pulmonary problems, they found the highest colonization rate in patients with bronchiectasis.[3] So, category of chronic lung disease in children may point to higher or lower risk of distal airway bacterial colonization.

Cellular enzymes in the extra cellular space, although of no further metabolic function in this space, are still of benefit because they serve as indicators suggestive of disturbances of the cellular integrity induced by pathological conditions. Lactate dehydrogenase (LDH) and Alkaline Phosphatase (ALP) are cytoplasmatic enzymes present in essentially all major organ systems. The extracellular appearance of LDH or ALP is used to detect cell damage or cell death.[9]

LDH or ALP are released into the peripheral blood after cell death caused by e.g. ischaemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings. Therefore, the total serum LDH or ALP are highly sensitive, but nonspecific as inflammatory markers.[15]

In the current study; levels of inflammatory markers (LDH and ALP) in brochoalveolar lavage were found to be significantly higher in patients than in the control group and in group I (colonized)were significantly higher than in group II (non colonized). Nevertheless, the lack of statistically significant correlation between the levels of inflammatory markers in the BAL samples and those in the serum suggests that these markers are locally produced and not simply derived from the blood by simple diffusion.

The relatively low levels of serum ALP and LDH compared with their levels in BALF and the poor correlations between them suggest that the inflammatory process in the airways is mostly compartmentalized and that serum enzymes can not be used as a reflection for the ongoing airway inflammatory damage.

In 2002 Angrill and coworkers, had studied the levels of some inflammatory markers in BALF samples from patients with bronchiectasis and compared them to the serum levels. They found that patients showed a slight systemic inflammatory response. The levels of the different markers in BALF were significantly higher than in serum.[5] They concluded that some increase in LDH is caused by transudation of serum, but to a minor component. The LDH in BALF appeared to originate from lung cells, probably Alveolar Macrophages (AMs) or Polymorphnuclear cells (PMNs).

Similarly, in the study of Cobben and coworkers in 1999, results of ALP and LDH levels in BALF of children with chronic lung diseases were very similar to that found by the present study.[9]

These significantly high levels of chemical markers in children with chronic lung diseases may reflect the aggressive ongoing tissue damage and cell death -even in absence of exacerbation- that may eventually lead to progressive nonstop impairment and deterioration of pulmonary functions. So analysis of LDH level in BALF is a potentially useful tool for evaluating local lung tissue damage.[16]

Cytological assessment; showed that total leuckocytic counts in BALF may has a specific diagnostic value for colonization. In this study, cut off level = 1250x 103 cell /ml BALF, giving sensitivity (to detect colonization) = 95.5%, specificity = 75% for detection of distal airways bacterial colonization.

Levels of BALF neutrophil % and lymphocyte % in all patients were significantly higher than that of the control group. This observation- again- reflects the tissue inflammation that is going on even without clinical exacerbation or colonization.

The BALF of group I contained more neutrophil and lymphocytes counts than group II, however this difference was not statistically significant.

Interestingly, our findings suggest that airway inflammation may occur even in the absence of colonization as demonstrated by the significant increase in levels of the different inflammatory mediators and cells among patients with negative BAL cultures, and may be intensified by the presence of colonizing microorganisms.

Angrill and co workers, 2001 studied the bronchial inflammatory reaction in patients with bronchiectasis. Patients with bronchiectasis and negative cultures for PPM in the BALF had a more intense inflammatory reaction than did control subjects, with a higher percentage of neutrophils and higher concentrations of inflammatory markers. The group of patients with PPM in the airways had a higher BAL neutrophil count, higher BALF concentrations of inflammatory markers. So they concluded that inflammatory reaction may be intensified by the presence of PPM colonizing the lower respiratory tract.[8]

Further studies are needed to prove the correlation of the intensity of the cellular infiltrate to the intensity of the bacterial load.

In this study, BALF macrophage % was found to be significantly higher in controls than in patients Table 3. This suggests that the differential leukocytic count in BALF of healthy children is relatively made by macrophages. This could be explained by the immunological theory of self protection during active inflammatory processes (like chronic lung diseases). As activation of proinflammatory cytokins (including macrophage inhibitory factor) are potent down regulators for macrophage production and activity.[3]

These findings were in concordance with the results of the ERS task force 2000 on bronchoalveolar lavage in children.[4]

Ratjen and coworkers, 1994 studied the differential cytology of BAL fluid in 48 children aged 3–16 years (mean age ± SD 7.9±3.5 yrs) undergoing elective surgery for non pulmonary illnesses in a trial to put standard levels for pediatric population.[17] Macrophages were the relative predominant cell type, with a mean percentage of 81.2±12.7 percent, and this goes well with the results of our control group.

Promising diagnostic tool: Microbiological evaluation of the distal airways in patients with stable chronic pulmonary diseases may aid in determining the role of colonization in disease progression.[16]

Although different studies[3,5,10] had used sputum bacteriology as a diagnostic tool to evaluate the presence of bronchial infection during exacerbations in children with bronchiectasis, very few have evaluated the pattern of bronchial colonization in clinically stable children (while not in exacerbation) and fewer studies dealed with BALF samples from children.

In our study, BAL cultures and sputum cultures gave different results. Sputum cultures did not reliably reflect conditions in the lower airways and have limited value. The results of BAL and sputum analysis agreed in 8 patients (26.7%), in whom both techniques resulted in negative cultures. Also they agreed in detection of 9 gram positive strains and 5 gram negative strains.

Sputum culture had missed 18 organisms in 12 patients. The defect was so evident in detection of the gram negative PPMs as it detected only 5 PPMs out of totally detected 22 PPMs. This poor agreement with the results of BALF culture may be explained by the fact that children can not produce adequate amount of sputum and sputum arises from upper respiratory tract and oropharyngeal flora contaminate it, so it does not reflect the condition in the peripheral airways.

Management of bacterial colonization: Our results showed that, Imepenem, tobramycin, ceftriaxone, amikin and garamycin were the most efficient drugs against the isolated strains of the gram negative pathogens. Vancomycin, dalacin, oxacillin and garamycin were the most efficient drugs against the isolated strains of the gram positive pathogens.

Antibiotic resistance has increased in all the major pathogens. It is more difficult to decide, on the available evidence, whether patient characteristics and the risk of antibiotic resistance should influence choice of empiric antibiotic treatment.(1) Most new antibiotics are modifications of existing structures, suggesting that every effort should be made to conserve the sensitivity of current antibiotics by using them appropriately.(18)

Inhaled antibiotics, in particular aminoglycosides, have attracted interest for many years because of the greater therapeutic index achieved by direct delivery of high-dose to the endobronchial space with limited systemic absorption and toxicity.(19) Moreover, the high prevalence of gram negative organisms inhabiting the airways of the chronically ill children is added to this interest.(17)

Polypeptide antibiotics of the polymyxin class delivered by aerosol have been widely used in Europe, with reports of uncontrolled clinical studies using doses of 500,000 to 1 million IU of colistin twice daily (potency of 30,000 IU/mg) for several months. Such therapy has been associated with decreases in isolation of P. aeruginosa and possible slowing of decline in FVC.(19,20)

Nevertheless, its use parenterally has been limited by systemic toxicity, giving rise to increasing interest in administering these agents by inhalation.(18)

Fluoroquinolones have several characteristics that have made them appealing for oral maintenance therapy in children older than 8 years. Ciprofloxacin, the most commonly used quinolone in CF, possesses a broad-spectrum antibacterial activity with excellent bacteriocidal activity against P. aeruginosa strains isolated from individuals with CF and may be additive with aminoglycosides. Administration of ciprofloxacin to over 3,000 children has been associated with a very low incidence of arthropathy.(21)

Macrolides such as erythromycin, clarithromycin, and azithromycin have been effective in treatment of chronic airway infections with P. aeruginosa.(22) On the basis of in vitro studies, several anti-infective and anti- inflammatory modes of action have been proposed to account for the efficacy of macrolides in this setting. Antimicrobial effects are augmented by excellent biofilm penetration and intracellular accumulation in P. aeruginosa.(1)

In conclusion: E.coli, Pseudomonas aeruginosa and Staphylococus aureus were the most common organisms in these patients. Knowledge of the type of colonizing agents may be important for future antibiotic prophylactic strategies and for the empirical antibiotic regimens when exacerbations occur in these patients.

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REFERENCES

1. Evans DJ, Bara AI, Greenstone M. Prolonged antibiotics for purulent bronchiectasis in children and adults. The Cochrane Database of Systematic Reviews. 2007;DOI:10.1002/14651858.CD001392.pub2.

2. Morillas HN, Zariwala M, Knowles MR. Genetic Causes of Bronchiectasis: Primary Ciliary Dyskinesia. Respiration 72. 2007;3:252-63.

3. Cabello H, Torres A, Celis R. Bacterial colonization of distal airways in healthy subjects and chronic lung disease: a bronchoscopic study. Eur Respir J. 1997;10:1137–44.

4. De Blic J, Midulla F, Barbato A, et al. Bronchoalveolar lavage in children. Eur Respir J. 2000;15:217–31.

5. Angrill J, Agusti C, de Celis R. Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors. Thorax. 2002;57:15-19.

6. Midulla F, de Blic J, Barbato A, et al. Flexible endoscopy of paediatric airways. Eur Respir J. 2003;22:698–708.

7. Wickremasinghe M, Ozerovitch LJ, Davies G: Non-tuberculous mycobacteria in patients with bronchiectasis. Thorax. 2005;60:1045-51.

8. Angrill J, Agust C, de Celis R, et al. Bronchial Inflammation and Colonization in Patients with Clinically Stable Bronchiectasis. Am. J. Respir. Crit. Care Med. 2001;164:1628-32.

9. Cobben NAM, Drent M, Jacobs JA, et al. Relationship between enzymatic markers of pulmonary cell damage and cellular profile: a study in bronchoalveolar lavage fluid. Exp Lung Res. 1999;25:99-111.

10. Onen ZP, Eris Gulbay B, Sen E, et al. Analysis of the factors related to mortality in patients with bronchiectasis. Respir Med. 101. 2007;7:1390-97.

11. Notarangelo LD, Plebani A, Mazzolari E, et al. Genetic causes of bronchiectasis: primary immune deficiencies and the lung. Respiration 74. 2007;3:264-75.

12. Davies JC. Pseudomonas aeruginosa in cystic fibrosis: pathogenesis and persistence. Paediatr Respir Rev. 2002;3:128-34.

13. Sanata MA, Matos E, do-Socorro Fontoura. Prevalence of pathogens in cystic fibrosis patients in Bahia, Brazil. Braz. J .Infect. Dis. 2003;7:69-72.

14. Gibson R, Emerson J, McNamara S. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. Am J Respir Crit Care Med. 2003;167:841–49.

15. Koh WJ, Kwon OJ: Bronchiectasis and non-tuberculous mycobacterial pulmonary infection. Thorax. 2006;61:458-60.

16. Hill A, Campbell EJ, Hill SL, et al. Association between airway bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. Am J Med. 2000;109:288–95.

17. Ratjen F, Bredendiek M, Brendel M, et al. Differential cytology of bronchoalveolar lavage fluid in normal children Eur Respir J. 1994;7:1865–70.

18. King P, Holdsworth S, Freezer N: Bronchiectasis. Intern Med J. 2006; 36:729-37.

19. Hodson ME, Gallagher CG, Govan JR. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. Eur Respir J. 2002;20:658–664.

20. Katz SL, Ho SL, Coates AL. Nebulizer Choice for Inhaled Colistin Treatment in Cystic Fibrosis. Chest. 2004;119:250-255.

21. Prober CG, Walson PD, Jones J. Committee on Infectious Diseases and Committee on Drugs: technical report: precautions regarding the use of aerosolized antibiotics. Pediatrics. 2000;106:89-92.

22. Jaffe A and Bush A. Anti-inflammatory effects of macrolides in lung disease. Pediatr Pulmonol. 2001;31:464–73.