THE INDIAN JOURNAL OF CHEST DISEASES AND ALLIED SCIENCES

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Dear Friends,

You are well aware that there is an ever increasing number of patients seeking advice for respiratory allergy and related problems. Many of these conditions can be improved with appropriate advice and treatment reducing unnecessary suffering. Vallabhbhai Patel Chest Institute in collaboration with Institute of Genomics and Integrative Biology is organising the “37th Workshop on Respiratory Allergy: Diagnosis and Management” during April 2-6, 2012. The topics are chosen to give up-to-date basic information about respiratory allergy and related conditions and the practical skills involved in their management. Hands on training in Allergy testing, PFT and laboratory investigations related to respiratory allergy will be provided at the Workshop.

Medical professionals working/with interest in respiratory allergy and applied immunology are the target group of this Workshop.

Eligibility: Medical graduates with PG qualification in Medicine/Pediatrics/ENT/Pulmonary Medicine

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Decoding Population Genetics: Impact on Tuberculosis Control and Treatment

The advent of chemotherapy in the early 1980’s was a boon for tuberculosis (TB) afflicted human race and significantly brought down mortality rate. However, an increase in the number of reported cases of TB in recent times combined with the emergence of human immunodeficiency virus (HIV) and multidrug-resistant TB (MDR-TB) was a serious setback for the efforts to eradicate TB worldwide. Presently, as many as one-third of the global population is latently infected with Mycobacterium tuberculosis and 5% to 10% of them run the risk of developing active disease in their life-time. What could explain this inter-individual variation in susceptibility and proneness to develop active disease? Apart from the environment and socio-economic factors, genetic predisposition of the host could play a significant role in determining the outcome of the infection. It could be possible that wide variation in the genetic make-up of the human population manifested in the form of gene polymorphisms could lead to such inter-individual variation.

The history of the mankind indicates that TB exerts a strong selection pressure on human evolution. Over the Century, TB significantly wiped out the susceptible population from various parts of the world and by the process of natural selection only those who could develop resistance to TB were selected to survive and multiply. For example, European population are generally less susceptible to TB. It is possible that due to their centuries long association with the bacterium, the Europeans have evolved into a more resistant population. In contrast, African sub-Saharan population are highly susceptible to TB probably due to their relatively recent contact with the pathogen. Motulsky suggested that this could have been due to strong selection against susceptibility genes of TB.

Examining the genetic angle of TB susceptibility received impetus from the recent technological advancement in the gene sequencing techniques. Rapid throughput sequencing at a large scale using Sequenom massArray platform (Sequenom Inc., USA) or Illumina platform have resulted in the complete sequencing of the human genome which led to the identification of a large number of sequence polymorphisms that were previously unknown. This information led to the generation of databases such as dbSNP and Hapmap which have facilitated the selection and evaluation of yet unexplored sequence variants in the genes of interest.

Given the complexity of the disease TB, it can be assumed that there would be numerous genetic contributing factors. Protection against TB is determined by the potent immune response in any individual. Therefore, susceptibility or resistance to develop TB may be significantly influenced by the variation in the immune response genes and contribute to a scenario where by virtue of their genetic make-up most of the individuals mount an effective immune response and are able to either clear or contain the mycobacterial infection while a certain few fail to do so. Evidence in support of such a notion comes from clustering of TB disease with higher concordance in monozygotic as against dizygotic twins, the ethnic clustering of the disease with higher prevalence of TB in individuals of recent African descent, as well as the demonstration of both common polymorphisms and rare mutations which confer susceptibility to mycobacterial infection in humans. These studies further supports the view that in addition to unique environment and natural selection ethnically governed host genetic factors may play a part in the susceptibility or resistance to TB.

Since TB is primarily a disease governed by the state of the host immune response, the focus of population genetics rested on the extensive analyses of the genes related to innate and acquired immune response. The technological advances as mentioned above together with the tools of bioinformatics facilitated the identification of a range of genetic variations which include polymorphisms in the innate and acquired immune factor related genes capable of identifying persons who are genetically prone to TB. The studies evaluating the involvement of innate immune response have focused on receptors on macrophages, such as toll-like receptors including TLR1, TLR2, TLR4 and TLR8, VDR, NOS2, P2X7 receptor, SP110, SLC11A (formerly NRAMP1), IRGM and DC-SIGN. On the other hand, the adaptive immune response was characterised mainly by cytokine and chemokine gene polymorphisms. The cytokines of note that have been studied and thought to play a role in susceptibility to TB include variants of interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-1RA, IL-18, IL-8, IL-12, and TNF-β.

The impact of genetic variants has also been exemplified in other associated respiratory ailments, such as asthma, and chronic obstructive pulmonary disease (COPD). In both these conditions, selection of genes studied were those related to inflammatory process and are similar to the genes studied in TB. One possible reason for such overlap might be the involvement of an initial inflammatory phase in all the three diseases. As and when more information
The quest for genetic polymorphisms translating into population genetics have come a long way and still has a longer path to traverse. But the systematic efforts should continue to facilitate better understanding of the genetic basis of resistance or susceptibility to TB which could be translated into targeted immunotherapy as a preventive measure as well as an effective adjunct to multidrug therapy for TB.

**Mridula Bose**

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Role of a Pattern-based Approach in Interpretation of Transbronchoscopic Lung Biopsy and Its Clinical Implications

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ABSTRACT

Background. Transbronchial lung biopsy (TBLB) is commonly performed for confirming the tissue diagnosis of diffuse parenchymal lung diseases (DPLDs). There is an urgent need to establish guidelines for interpretation of TBLB in order to improve its diagnostic utility.

Methods. We retrospectively studied 916 consecutive patients (494 males; mean age 49 years) who underwent TBLB over a 5-year period (July 2005 to July 2010) at Vallabhbhai Patel Chest Institute.

Results. In 615 (67.1%) procedures, material obtained during TBLB was adequate for histopathology interpretation. Pathological features evaluated in each case were: alveolar architecture, inflammatory infiltrate, interstitial fibrosis, atypical cells, pigment deposition, honey-comb change and fibroblast foci. The cases were categorised on the basis of histopathology into six patterns: (1) adequate biopsy without a specific diagnostic abnormality (n=137, 22.3%); (2) acute pneumonitis (n=29, 4.7%); (3) neoplasia (n=109, 17.7%); (4) chronic interstitial inflammation with or without fibrosis (n=138, 22.4%); (5) granulomatous inflammation, (n=186, 30.2%); and (6) other specific causes (n=16, 2.6%). Definitive diagnosis could be made after correlation of TBLB histopathology with clinical and radiological features in 55.3% cases.

Conclusions. TBLB appears to be an important diagnostic tool for the diagnosis of DPLDs. The use of a pattern-based approach to TBLB adds to its diagnostic yield and can be helpful in cases where open lung biopsy is not available.


Key words: TBLB, Diffuse lung disease, Histopathological patterns.

INTRODUCTION

Transbronchial lung biopsy (TBLB) is often employed in the diagnosis of diffuse parenchymal lung diseases (DPLDs). Due to the high morbidity associated with and the non-availability of open lung biopsy (OLB) in many centres, high resolution computed tomography (HRCT) followed by TBLB continue to remain the mainstay of diagnosis of DPLDs. The small size of TBLB specimen makes it a “histopathologist’s nightmare”, leading to difficulty in categorisation within the spectrum of DPLDs. Therefore, TBLB is considered by some as an ‘ailing gold standard’ and is utilised only to exclude diseases, such as, sarcoidosis, lymphangitis carcinomatosis, infection, etc. There is a need for systematic categorisation of the histopathological patterns identified on TBLB for increasing the diagnostic yield and their rigorous correlation with clinical and radiological features for confirming the diagnosis accurately. The present study was undertaken to evaluate the histopathological patterns identified on TBLB and the clinical usefulness of TBLB in the diagnosis of patients with DPLDs presenting to a tertiary care pulmonary centre in North India.

MATERIAL AND METHODS

We retrospectively analysed records of 916 patients who underwent TBLB at the Vallabhbhai Patel Chest Institute over a 5-year period from July 2005 to July 2010. All specimens were stained with haematoxylin-eosin stain; special stains for reticulin and collagen; Gomori silver methenamine; and Masson Trichrome stains. Periodic acid-Schiff stain, Gomori silver methenamine stain, Gram’s stain, Ziehl-Neelsen stain (Z-N) were done to rule out infection. Treatment history was obtained to rule out drug-toxicity.

The number of pieces of alveolated lung parenchyma and bronchial wall were recorded. The
TBLB was considered to be adequately alveolated if more than 20 alveoli were seen. Pathologic features evaluated in each adequate biopsy were: alveolar architecture, inflammatory infiltrate, granulomatous inflammation, atypical cells, interstitial fibrosis, fibroblast foci, vasculopathy, pigment deposition, and honey-comb change. The adequate biopsies were further categorized on the basis of the histopathological patterns into six patterns (Figure 1): adequate biopsy without a specific diagnostic abnormality (pattern 1); acute pneumonitis (pattern 2); neoplasia (pattern 3); chronic interstitial inflammation with or without fibrosis (pattern 4); granulomatous inflammation (pattern 5); and other specific causes (pattern 6).

The cases with chronic interstitial inflammation with or without fibrosis (pattern 4) were further categorized into non-specific interstitial pneumonitis (NSIP), desquamative interstitial pneumonitis (DIP), lipoid interstitial pneumonia (LIP), usual interstitial pneumonia (UIP)-like patterns on the basis of pathological features, which included the anatomic compartment of involvement, the nature of cellular infiltrates, distortion of alveolar architecture, presence of fibroblastic foci and fibrosis and microscopic honey-combing. History of occupational exposure and polarization for identifying dusts/birefringent particles was obtained in all cases. All the cases were then correlated clinically and radiologically to assess the relevance of the pathological diagnosis offered on TBLB.

In cases with acute lung injury (pattern 2), the presence or absence of hyaline membranes, nature of cellular infiltrates, foci of organizing pneumonia (loose fibroblastic proliferation with scattered inflammatory cells and minimal collagen deposition within the interstitium and focally in the alveolar spaces) and type II epithelial cell hyperplasia were noted (Figures 2A and 2B). Clinical correlation revealed non-resolving pneumonia with consolidation; lung opacities with or without cavity formation; and reticulo-nodular opacities suggestive of interstitial lung disease (ILD) to be the three most common clinical-radiological presentations. One case each of actinomycetes and botryomycetes and two cases of nocardiosis were identified. A diagnosis of eosinophilic pneumonia, a histopathologic subtype of acute lung injury, characterized by the triad of reactive type II hyperplasia, eosinophils in alveolar spaces accompanied by densely eosinophilic macrophages and variable amount of fibrin was

Table 1. Pattern based categorisation of histopathological diagnosis on TBLB (n=615)

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Histopathological Diagnosis</th>
<th>No. (%)</th>
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<tr>
<td>1</td>
<td>Normal lung parenchyma</td>
<td>137 (22.3)</td>
</tr>
<tr>
<td>2</td>
<td>Acute pneumonitis</td>
<td>29 (4.7)</td>
</tr>
<tr>
<td>3</td>
<td>Neoplasia</td>
<td>109 (17.7)</td>
</tr>
<tr>
<td>4</td>
<td>Chronic interstitial inflam.</td>
<td>138 (22.4)</td>
</tr>
<tr>
<td>5</td>
<td>Granulomatous inflammation</td>
<td>186 (30.2)</td>
</tr>
<tr>
<td>6</td>
<td>Other specific causes</td>
<td>16 (2.6)</td>
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TBLB=Transbronchial lung biopsy

In the cases with acute lung injury (pattern 2), the presence or absence of hyaline membranes, nature of cellular infiltrates, foci of organizing pneumonia (loose fibroblastic proliferation with scattered inflammatory cells and minimal collagen deposition within the interstitium and focally in the alveolar spaces) and type II epithelial cell hyperplasia were noted (Figures 2A and 2B). Clinical correlation revealed non-resolving pneumonia with consolidation; lung opacities with or without cavity formation; and reticulo-nodular opacities suggestive of interstitial lung disease (ILD) to be the three most common clinical-radiological presentations. One case each of actinomycetes and botryomycetes and two cases of nocardiosis were identified. A diagnosis of eosinophilic pneumonia, a histopathologic subtype of acute lung injury, characterized by the triad of reactive type II hyperplasia, eosinophils in alveolar spaces accompanied by densely eosinophilic macrophages and variable amount of fibrin was
made in 4% cases. In one case with bronchial asthma, the presence of eosinophilic pneumonitis with vasculitis was suggestive of Churg-Strauss syndrome.

Neoplasias (pattern 3) were clinically suspected and sampled by TBLB in 143 cases (15.6%). In 109 of these 143 cases (76.2%), the neoplastic tissue was adequately sampled and the diagnosis of carcinoma was confirmed (Table 2).

Table 2. Neoplasia (n=109) identified by TBLB

<table>
<thead>
<tr>
<th>Type of Neoplasia</th>
<th>No. (%)</th>
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<tr>
<td>Squamous cell carcinoma</td>
<td>66 (60.5)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>14 (12.8)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>14 (12.8)</td>
</tr>
<tr>
<td>Large cell undifferentiated carcinoma</td>
<td>11 (10.1)</td>
</tr>
<tr>
<td>Carcinoid tumour</td>
<td>02 (1.8)</td>
</tr>
<tr>
<td>Lymphangitis carcinomatosa</td>
<td>02 (1.8)</td>
</tr>
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TBLB=Transbronchial lung biopsy

Squamous cell carcinomas were the most common tumours and accounted for 66 cases (60.5%) (Figures 3A, 3B and 3C). Small-cell carcinoma lung (n=14, 12.8%); adenocarcinoma (n=14, 12.8%); large cell undifferentiated carcinoma (n=11, 10.1%); 2 cases (1.8%) each of carcinoid...
tumour and lymphangitis carcinomatosa were identified. Out of 143 cases, TBLB was inadequate in 34 (23.8%) for opinion due to following causes: (a) superficial biopsy taken because of increased vascularity and bleeding tendency; (b) mass lesion obstructing passage of bronchoscope; and (c) sampling of the periphery of the lesion showing pneumonitis with or without overlying epithelial dysplasia and/or carcinoma in-situ.

Among the cases of chronic interstitial inflammation with or without fibrosis (22.4%, pattern 4) (Figures 4A, 4B, 4C, 4D, 4E and 4F), NSIP-like pattern with and without fibrosis was the most common seen in 110 (79.7%) of the 138 cases.

Figure 4. Chronic interstitial inflammation with or without fibrosis (Pattern 4). Photomicrograph showing (A) chronic interstitial inflammation with fibrosis and microscopic honey-combing (Haematoxylin and eosin×40); (B) chronic interstitial inflammation without fibrosis (Haematoxylin and eosin×40); (C) DIP-like pattern without fibrosis (Haematoxylin and eosin×40); (D) high power view of the same (Haematoxylin and eosin×400); (E) intra-alveolar organising pneumonia suggestive of cryptogenic organising pneumonia-like pattern; (Masson Trichrome stain×40); and (F) high power view of the same (Haematoxylin and eosin×400).
On correlation (Table 3) the final diagnosis offered included ILD associated with collagen vascular diseases, tuberculosis fibrocavitary lesions, pneumoconiosis, post-radiation fibrosis and idiopathic interstitial pneumonias. DIP-like pattern, seen in 18 of the 138 (13.0%) cases was next most common pattern and was seen in ILD associated with smokers. Distortion of alveolar architecture with microscopic honey-combing and presence of fibroblastic foci were the criteria used to differentiate UIP-like pattern from fibrosing NSIP and was seen in seven of the 138 (5.2%) cases. These correlated with honey-combing and fibrosis on computed tomography. In three (2.2%) cases with rheumatoid arthritis associated lung disease, interstitial infiltration and widening by lymphocytic infiltrate was seen and categorised as LIP-like pattern. Even though the diagnosis offered by TBLB histopathology alone were not conclusive, the exclusion of the infectious and neoplastic pathologies in these cases, in the absence of OLB, was very helpful in further management of these patients, especially in light of clinical and radiological features and pulmonary function tests.

Table 3. Clinical-radiological-pathological correlation of cases showing interstitial inflammation with or without fibrosis on TBLB (Pattern 4, n=138)

<table>
<thead>
<tr>
<th>Histopathological Features</th>
<th>Clinical Diagnosis</th>
<th>No. (%)</th>
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<tr>
<td>NSIP pattern</td>
<td>Tuberculosis, collagen vascular diseases, sarcoidosis, pneumoconiosis, post-radiation fibrosis, idiopathic ILD</td>
<td>110 (79.7)</td>
</tr>
<tr>
<td>DIP pattern</td>
<td>ILD associated with smokers</td>
<td>18 (13.0)</td>
</tr>
<tr>
<td>UIP pattern</td>
<td>Tuberculosis, collagen vascular diseases, pneumoconiosis, IPF</td>
<td>07 (5.2)</td>
</tr>
<tr>
<td>LIP pattern</td>
<td>Rheumatoid arthritis associated lung disease</td>
<td>03 (2.2)</td>
</tr>
</tbody>
</table>

BLB=Transbronchial lung biopsy; NSIP=Non-specific interstitial pneumonitis; ILD=Interstitial lung disease; DIP=Desquamative interstitial pneumonia; UIP=Usual interstitial pneumonia; IPF=Idiopathic pulmonary fibrosis; LIP=Lipoid interstitial pneumonia

Granulomatous inflammation (pattern 5) was seen in 186 (30.3%) cases. Using the histopathological criteria and correlating with clinical and radiological features, bronchoalveolar lavage fluid analysis and AFB culture, the diagnosis of tuberculosis was confirmed (Figures 5A and 5B) in 121 (65.1%) cases (Table 4). Submucosal non-necrotising granulomas occurring within sclerotic fibrosis, with multinucleated giant cells showing the typical conchoidal (Schaumann) body (Figures 5C and 5D), diagnostic of sarcoidosis were seen in 46 (24.7%) cases. In 19 (10.2%) cases a definitive diagnosis could not be obtained after bronchoscopy and these subjects were referred for surgical biopsy or were empirically started on antituberculosis treatment.

Other specific causes (pattern 6) identified on TBLB accounted for 16 cases (2.6%). These included 3 cases with diffuse alveolar haemorrhage, which were confirmed by the Perl’s Prussian blue stain. Pulmonary vasculitis, characterised by damage to the vessel wall and accompanied by fibrin deposition was seen in two cases. Four cases primarily showed features of pulmonary artery hypertension which were low grade lesions: grade 1 (muscular hypertrophy) and grade 2 (mild intimal proliferation).

An adequate lung parenchymal biopsy without a specific diagnostic abnormality (pattern 1) (Figures 7A and 7B) was identified in 137 (22.3%) cases. These were the cases with radiological abnormalities but had no abnormalities seen in the lung biopsies, signifying them to be a result of either ‘minimal change disease’ or ‘sampling error’. In 301 (32.9%) procedures, tissue was considered inadequate for opinion since it comprised of superficial epithelium only and/or alveolar tissue with less than 20 alveoli. Analysis of these cases revealed various factors predisposing to these failures and included lack of patient co-operation, excessive coughing, bleeding leading to termination of the procedure. These cases were then referred for OLB and/or clinical-radiological correlation.

**DISCUSSION**

The DPLDs comprise of a wide spectrum of over 200 diseases. Many of these diseases have similar clinical presentations with widespread shadowing on the chest radiograph and increasing shortness of breath. Occasionally the radiographic appearances are sufficiently characteristic to enable a specific diagnosis, for example, sarcoidosis, pulmonary eosinophilia, some occupational lung diseases, etc. However, in most patients, chest radiographic patterns are not specific. The final diagnosis can be made from clinical-radiological correlation in about 50% of cases only. Surgical OLB, considered to be the gold standard for the diagnosis of DPLDs, however, is associated with greater morbidity and cost. Moreover, OLB is not available in most centres in the developing countries, such as, India. This has led to a slow but steady increase in the number of TBLBs...
being performed worldwide. Today the most common lung tissue samples seen by pathologists in India and worldwide are those derived using flexible fibreoptic bronchoscopy (FOB).

The usefulness of TBLB for diffuse interstitial pneumonias was first addressed by Andersen in 1978. He stressed on “the importance of an interested and experienced pathologist willing to glean every information from these tiny bits of tissue.” Poletti et al. reported high diagnostic yield of TBLB of 67% and subdivided the results of morphologic features into three groups: (i) specific morphologic diagnosis (29%); (ii) histopathologic changes consistent with the clinical pattern (38%); and (iii) non-specific lesions (33%). With the use of the FOB and multiple biopsy samples, TBLB has been found to achieve a high diagnostic yield in DPLDs with centrilobular accentuation, such as granulomatous and metastatic

<table>
<thead>
<tr>
<th>Histopathological Criteria Used</th>
<th>Tuberculosis</th>
<th>Sarcoïdosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrotising granulomas</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>Submucosal non-necrotising granulomas occurring within sclerotic fibrosis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Langhans type of multinucleated giant cells</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>Multinucleated giant cells showing the typical conchoidal (Schaumann) body</td>
<td>-/+</td>
<td>+/-</td>
</tr>
<tr>
<td>Reticulin stain positive</td>
<td>Within granulomas</td>
<td>Surrounding granuloma</td>
</tr>
<tr>
<td>Acid-fast bacilli stain</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+=Present; -=Absent
diseases. However, over the broad spectrum of DPLDs, the diagnostic information was found to vary from 38% to 79%. In 2002, the American Thoracic Society/European Respiratory Society statement on idiopathic interstitial pneumonias defined a set of histologic patterns that provided the basis for a final clinico-radiologic-pathologic diagnosis. Because the histologic patterns seen by pathologists usually allowed for better separation of these entities than the imaging patterns seen by radiologists, the histologic patterns provided the primary basis for the various categories of idiopathic interstitial pneumonia (IIP) and served as the foundation for the classification. It was recommended that the term *pattern* be added to the IIP designations when referring to the lung biopsy pathologic pattern, to distinguish it from the clinico-radiologic-pathologic diagnosis (e.g., NSIP, DIP, or LIP pattern).

Kitachi *et al.* observed that there was no straightforward consensus of pulmonary pathologists, even on OLB diagnosis of DPLDs and introduced a quantitative diagnostic method in order to systematise the assessment of histopathology of fibrotic interstitial lung lesions. They assessed the alveolar-aeration ratio, the normal alveolar-wall ratio, number and size of lymphoid follicles, number and maximum size of fibrocystic lesions/honey-combing, number of fibroblastic foci, number of granulation tissue formations in terminal air spaces per field, abruptness of transition to fibrosis, smooth muscle proliferation score. The histopathologic summary was then correlated with radiology (CT findings of consolidation, ground-glass opacity, etc.) and clinical course (acute, subacute and chronic process). A similar quantitative assessment of histopathological features needs to be done on TBLB also.
Berbescu et al.\textsuperscript{20} reported that, characteristic histologic features of UIP, a combination of patchwork fibrosis, fibroblast foci, and microscopic honey-combing, could be identified on TBLB specimens. This has lead to renewed interest in role of TBLB in diffuse interstitial lung diseases.\textsuperscript{21} TBLB has also been found to be clinically useful in the diagnosis of 75\% cases of DPLDs;\textsuperscript{21} in the 25\% of TBLBs that were clinically unhelpful, there was failure of the procedure to obtain an adequate quantity of lung parenchyma for a meaningful histological analysis. Leslie et al\textsuperscript{1} have elaborated the most common diagnostic entities and histopathologic patterns seen in TBLB in the setting of diffuse or multifocal lung disease. These included, acute lung injury, eosinophilic pneumonia, diffuse alveolar haemorrhage, chronic cellular infiltrates with or without fibrosis, organising pneumonia, alveolar proteinosis, sarcoidosis, Wegener’s granulomatosis, intravenous drug abuse related microangiopathy, Langerhans cell histiocytosis and lymphangioleiomyomatosis. These were further categorised on the basis of histopathological pattern of lesion into five patterns: (i) acute or subacute injury; (ii) chronic interstitial inflammation with or without fibrosis; (iii) granulomatous inflammation; (iv) vascular diseases (e.g., vasculitis, diffuse alveolar haemorrhage, intravenous drug abuse microangiopathy; and (v) alveolar filling processes (alveolar proteinosis, etc.).

In the present study, we retrospectively analysed the TBLB submitted over 5-year period and used the systematic pattern-based approach described by Leslie et al\textsuperscript{1} to categorise the histopathological features into six histopathological patterns. The three most common diagnostic patterns in our study were granulomatous inflammation, chronic interstitial pneumonitis and carcinoma lung. In 32.9\% procedures, no lung parenchyma was obtained. This was similar to the earlier observations where the problem of inadequate lung tissue from TBLB was observed in up to 20\% of patients.\textsuperscript{22} The pattern-based categorisation added the much needed guidelines to interpretation of TBLB histopathology and provided clarity to clinicians when submitted for correlation with clinical and radiological features.

TBLB showing chronic interstitial pneumonitis, with or without fibrosis was the second commonest finding in our series and the most difficult to interpret. Review of existing literature revealed that previously this finding was considered to be only helpful in supporting a clinical impression of DPLDs or reported as non-diagnostic since the TBLB specimens were generally considered to be too small and non-representative to determine the relative degree of cellularity and fibrosis.\textsuperscript{14,15,23} In the present series too, a confirmatory diagnosis could be given in these cases only after they were correlated clinically and radiologically to assess the relevance of the pathological diagnosis offered on TBLB. Distortion of alveolar architecture with microscopic honey-combing and the presence of fibroblastic foci were the criteria used to differentiate UIP pattern from fibrosing NSIP and these were seen to correlate with honey-combing and fibrosis on CT.

Serious questions on the use of TBLB for the diagnosis of UIP have also been raised,\textsuperscript{24} especially since TBLB samples are insufficient to determine temporal heterogeneity, a critical histologic hallmark. The identification of ‘concordant pattern of UIP’, in which all lobes showed UIP and there is no evidence of intra-patient variation and ‘discordant UIP pattern’ in which intra-patient variation with lung lobes showing a mixture of UIP and NSIP is present\textsuperscript{25} has further compounded the problem of pattern-based diagnosis by TBLB in these two conditions. Therefore, the current assumption is that there is no gold standard for the diagnosis of DPLDs, and clinical, radiologic, and histopathologic evaluation by OLB, have emerged as the silver standard.\textsuperscript{18}

Interstitial lung diseases appear to be under-reported from India. The lack of recognition and inadequate availability of diagnostic facilities, like HRCT are thought to be some of the main reasons for this.\textsuperscript{26} Previously, Ahluwalia et al\textsuperscript{27} have assessed the role of TBLB in ILD and concluded that FOB and TBLB are safe and useful adjuncts to the diagnosis of ILD. The correlation of TBLB histological features with spirometric indices has also been reported in sarcoidosis by Gupta et al.\textsuperscript{28}

TBLB for the diagnosis of lung disease has come a long way from the time these specimens were first obtained via a rigid bronchoscope.\textsuperscript{29} Then, sampling was a problem and the specimens were often too small to enable a definitive diagnosis.\textsuperscript{14,15} With the use of the FOB, advanced radiological guidance and increasing user expertise the diagnostic yield has increased considerably. However, two crucial questions remain. First is the problem of “sampling error”, namely, divergent histopathologic diagnoses in two or more biopsy sites.\textsuperscript{30} This is likely, to be minimised by using HRCT to select multiple biopsy sites representative of the full range of morphologic appearances.\textsuperscript{31} A second crucial consideration is “inter-observer variation” between histopathologists. In a recent study\textsuperscript{32} very significant observer variation was quantified, and the observer agreement was found to be barely clinically acceptable. This is likely to be a result of intermediate histopathologic appearances between two entities in a significant proportion of cases. It is especially because of this scenario that the systematic categorisation of histopathological features seen on TBLB using the pattern-based approach is advocated. These when correlated with clinical and imaging data can be the key determinants of a final consensus diagnosis of DPLDs, especially in patients from developing...
countries such as India, with high burden of chronic respiratory diseases, who are unable to undergo surgical lung biopsy.

REFERENCES

SLEEPCON-2012
NATIONAL CONFERENCE ON SLEEP DISORDERS
(Under the auspicious of Indian Sleep Disorders Association)

April 6 - 8, 2012

April 6, 2012 : Workshop-I — Polysomnography
               : Workshop-II — NIPPV

April 7-8, 2012 : Conference

Abstract Submission Deadline: February 28, 2012
(abstract to be sent by e-mail to: abstractsleep@gmail.com)

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Original Article

Socio-economic Status of Smokers Presenting to the Outpatient Service: Does Chronic Obstructive Pulmonary Disease Make A Difference?

Gulfidan Cakmak, Zuhal Aydan Saglam, Tayyibe Saler, Mustafa Yenigun and Levent Umit Temiz

4th Clinic of Internal Medicine, Haseki Training and Research Hospital, Haseki Milli Caddesi, Aksaray-Istanbul, Turkey

ABSTRACT

Background. Sparse published data are available on the impact of social and personal factors leading to tobacco smoking. Identification of social and economic motives underlying smoking can facilitate the efforts towards control of tobacco smoking.

Methods. A questionnaire was administered to 966 smokers attending the Chest Diseases Out-patient clinic at the Haseki Training and Research Hospital, Istanbul to collect demographic data from the participants. In all of them spirometry was performed.

Results. The participants with chronic obstructive pulmonary diseases (COPD) were less benefiting from social security system and they were less educated. Patients with COPD were generally living in cities. In this group the number of divorced patients were more than the other group.

Conclusions. There are many factors causing individuals to initiate smoking. By eliminating these factors, mortality and morbidity rates caused by smoking will decline dramatically. This study aims to draw attention on personal and social factors for smoking.


Key words: Socio-economic status, COPD, Demographic characteristic, Smoker, Education.

INTRODUCTION

Smoking causes 90% of all lung cancer deaths in men and 80% of all lung cancer deaths in women.1 The number of cigarette smoking individuals is gradually increasing among population, especially the women.2-4 It is important to define factors raising tendency to initiate smoking. This approach will enable to avoid the progression of important disorders associated with smoking.

Smoking affects several organs and systems mainly the heart and the lungs. Lung cancer and chronic obstructive pulmonary disease (COPD) are the most important diseases proven to be associated with cigarette smoking.2,3 COPD is a chronic and progressive inflammatory disease prevailed by environmental and subjective factors which continue to be an important cause of mortality and morbidity.4,5 The prevalence of COPD is reported variously in different age and occupational groups.5-8 This is mainly due to limited number of studies concerning socio-economic and demographic differences of patients, to unreliable statistical figures and different criteria regarding diagnosis of COPD.5,10

Since the prevalence of COPD is still increasing and it is one of the most important causes of mortality, we examined the demographic characteristics of smoker patients recruited from the out-patient clinic of chest diseases in Haseki Training and Research Hospital, Istanbul.

MATERIAL AND METHODS

Nine hundred and sixty-six smokers (733 males) with a history of smoking at least 10 packs per year were recruited from the Chest Diseases Out-patient Clinic at the Haseki Training and Research Hospital during the period 2004-2005. The protocol of the study was approved by the Local Ethics Committee of the hospital and all subjects had given their written informed consent. The patients then were invited to fill a questionnaire consisting of two pages. In the questionnaire, patients were asked about their demographic information (name, age, gender, occupation, marital status); smoking status (duration

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of smoking, pack/years); medical history regarding respiratory complaints; and socio-economic status (education, monthly income, the social insurance system they belong to and residential area).

The participants were excluded from the study if they had undergone thoracic, abdominal or eye surgery within the last three months, hospitalisation because of a cardiac problem, a gastrointestinal bleeding as well as obesity. Patients with active tuberculosis, pregnant women, patients with a neurologic or psychiatric disorder and those who were unable to co-operate were also excluded from the study.

Spirometry testing was performed by a well-trained clinical technologist using a Jaeger 4.0 device according to standardised guidelines. Following 6 to 8 times of forced expiration, best three readings with a difference of maximum 150 mL were recorded and the highest one was accepted. The participants were asked to inhale 200 μg of salbutamol according to pharmacist's instructions and to rest in a sitting position for 20 minutes after inhalation. Then the spirometry testing was repeated. All the tests were performed while the patients were sitting erect with a nose clip applied.

The subjects with forced expiratory volume in the first second (FEV₁) to forced vital capacity (FVC) ratio of 70% or less after bronchodilator administration were diagnosed to have COPD according to Global Initiative for Obstructive Lung Disease (GOLD) criteria. The patients with and without COPD were compared according to demographic characteristics and spirometric parameters.

Subjects were analysed according to their monthly income and divided into three groups, namely, group 1: monthly income below 250€; group 2: monthly income between 250-500€; and group 3: monthly income above 500€. According to the residential area, they were categorised as living in a rural area or a city. As per their educational level, the subjects were divided into being either illiterate; finishing 5 years of school education or 8 years of school education, or being a graduate of high school or faculty. They were also categorised as per their marital status as single, married or widower.

Statistical analysis was performed using one-way analysis of variance (ANOVA), Chi-square tests. Independent groups were analysed using t-test and Pearson-Spearman correlation tests.

RESULTS

In patients with COPD (n=452) the number of males was significantly higher (390 vs 345) compared to non-COPD group (n=574). Patients with COPD were older (mean age 59.8±30.0 vs 49.7±13.0 years; p<0.001) and had a longer duration of smoking (42.7±29.8 vs 31.7±51.9; p<0.001) in comparison with patients without COPD.

Comparison of spirometric values between patients with and without COPD is shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Comparison of COPD and non-COPD groups regarding age, gender, duration of smoking and spirometric values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-COPD Group (n=514)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>49.7±13.0</td>
</tr>
<tr>
<td>Gender (Male)</td>
</tr>
<tr>
<td>n=345 (46%)</td>
</tr>
<tr>
<td>Cigarette smoking (pack years)</td>
</tr>
<tr>
<td>31.7±51.9</td>
</tr>
<tr>
<td>FVC (L)</td>
</tr>
<tr>
<td>3.37±1.03</td>
</tr>
<tr>
<td>FVC (%)</td>
</tr>
<tr>
<td>99.9±20.6</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
</tr>
<tr>
<td>2.81±0.88</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
</tr>
<tr>
<td>94.7±20.9</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
</tr>
<tr>
<td>80.3±41.1</td>
</tr>
<tr>
<td>PEFR (L)</td>
</tr>
<tr>
<td>5.35±1.932</td>
</tr>
<tr>
<td>PEFR₂₅-₇₅ (%)</td>
</tr>
<tr>
<td>81.3±23.5</td>
</tr>
</tbody>
</table>

COPD=Chronic obstructive pulmonary disease; FVC=Forced vital capacity; FEV₁=Forced expiratory volume in first second; PEFR₂₅-₇₅=Forced expiratory flow 25%-75%

There was a negative but statistically insignificant relationship between age and FVC (p=0.167), FEV₁ (%) (p=0.197), FEV₁/FVC (p=0.120), forced expiratory flow 25%-75% (PEFR₂₅-₇₅) (p=0.235); and between cigarette consumption and FVC (%) (p=0.140), FEV₁ (%) (p=0.165), FEV₁/FVC (p=0.084), PEFR₂₅-₇₅ (p=0.167).

The relationship between smoking and education (p=0.230) and the relationship between COPD and social security status (p=0.845) were also statistically insignificant (Table 2). The educational status was lower in patients with COPD but this difference was not statistically significant (p=0.057) (Table 3). While monthly income of subjects without COPD was below 250€ per month, in COPD group the income was between 250-500€ per month. The difference was not statistically significant (p=0.09) (Table 4).

<table>
<thead>
<tr>
<th>Table 2. Social security status and the residential area of subjects with and without COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-COPD Group (n=514)</td>
</tr>
<tr>
<td>Social security</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>Residential area</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>City</td>
</tr>
</tbody>
</table>

COPD=Chronic obstructive pulmonary disease
Table 3. Educational status of subjects with and without COPD

<table>
<thead>
<tr>
<th>Education Level</th>
<th>Non-COPD Group (n=514)</th>
<th>COPD Group (n=452)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illiterate</td>
<td>70 (13.6%)</td>
<td>80 (17.7%)</td>
<td>0.057</td>
</tr>
<tr>
<td>Finished 5 years of school education</td>
<td>166 (32.3%)</td>
<td>163 (36.1%)</td>
<td></td>
</tr>
<tr>
<td>Finished 8 years of school education</td>
<td>126 (24.5%)</td>
<td>110 (24.3%)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>116 (22.6%)</td>
<td>74 (16.4%)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>36 (7.0%)</td>
<td>25 (5.5%)</td>
<td></td>
</tr>
</tbody>
</table>

COPD = Chronic obstructive pulmonary disease

There was no significant difference regarding place of domicile among patients with and without COPD (p=0.431) (Table 2). The number of widowers was higher in COPD group, whereas number of married patients was significantly higher in non-COPD group (p<0.005) (Table 4).

Table 4. Monthly income and marital status of subjects with and without COPD

<table>
<thead>
<tr>
<th>Monthly income (€/month)</th>
<th>Non-COPD Group (n=514)</th>
<th>COPD Group (n=452)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤250</td>
<td>305 (59.3%)</td>
<td>224 (49.6%)</td>
<td>0.09</td>
</tr>
<tr>
<td>250-500</td>
<td>351 (29.4%)</td>
<td>169 (37.4%)</td>
<td></td>
</tr>
<tr>
<td>≥500</td>
<td>58 (11.3%)</td>
<td>59 (13.1%)</td>
<td></td>
</tr>
</tbody>
</table>

Marital status

<table>
<thead>
<tr>
<th>Status</th>
<th>Non-COPD Group (n=514)</th>
<th>COPD Group (n=452)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>16 (0.1%)</td>
<td>2 (0.04%)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Married</td>
<td>419 (81.5%)</td>
<td>317 (70.1%)</td>
<td></td>
</tr>
<tr>
<td>Widower</td>
<td>79 (15.4%)</td>
<td>133 (29.4%)</td>
<td></td>
</tr>
</tbody>
</table>

COPD = Chronic obstructive pulmonary disease

Logistic regression analysis showed significant associations between COPD risk and age, gender, and smoking status (Table 1).

DISCUSSION

Number of smoking individuals is gradually increasing.\textsuperscript{2,4,5} Compared with non-smokers, smoking is estimated to increase the risk of coronary heart disease by 2 to 4 times\textsuperscript{1,12} and stroke by 2 to 4 times.\textsuperscript{1} Smoking is also estimated to increase the risk of developing lung cancer in men and women by 23 times and 13 times, respectively. Smoking also increases the risk of death from COPD by 12 to 13 times.\textsuperscript{1} More deaths are caused each year by tobacco use than by all deaths from human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides, and murders combined.\textsuperscript{1,12}

Smoking is common especially among men in Turkey. In our study, number of males with COPD were significantly higher. However, tobacco consumption is increasing among women. Even though men outnumber women in the work-force, presently, the percent of women working has steadily increased. This increase in consumption of tobacco may be partly due to gradually increasing number of women participating in the work-force. More women are choosing to continue working while also balancing the traditional parenting role. Overburdening heavy and stressful demands of routine life may explain the increase in smoking rate among women. Besides, in some regions of Turkey, smoking is an indicator of maturity among male population. We believe that all these factors are important in high smoking rates. While COPD rate in smoking population is 3.6% in Europe, it varies in different professional groups.\textsuperscript{2,4} We observed a COPD rate of above 40% among smokers in our study.

Life-style, economic and social conditions, rural and urban life have importance in the development of COPD and increase in hospital admission rates of patients with COPD.\textsuperscript{4,13-15} According to a research which has been carried out in China, the prevalence of COPD is high in people who live in rural areas, who have little education, and who have been exposed to biomass and environmental air pollution.\textsuperscript{16} In our study, education levels in COPD group were lower. While the number of individuals who earn less than 250€ per month in the group without COPD was high, the number of individuals in the COPD group with monthly income among 250-500€ was higher. This may be explained by the relatively higher monthly income which may possibly be spent more on cigarette.

Also researches made in Germany and Australia reveal the relationship between low education levels, rural and city life and lung functions. Inevitable exposure to industrialisation and environmental pollution in city life is associated with lung function disorders and COPD.\textsuperscript{4,13} In our study we could not demonstrate a relationship between COPD and domicile in a rural or city area. While smoking and exposure to environmental and technical pollution is a factor in COPD development in cities, smoking and exposure to indoor air pollution may be an indicator in rural areas.

Daily life-style and stress are also found to be related with lung functions.\textsuperscript{17,18} Family life is sacred and essential in Turkish society, so marriage is a very serious and important decision. On the other hand, lots of problems such as economic problems and personal quarrels causes serious stress in marriages. In our society like marriage, also getting divorced may also cause serious stress. We believe this social stress can create functional disorders. The number of married subjects in COPD group was significantly high in our study, but the number of widowers was significantly higher than non-COPD group.
REFERENCES


Pattern of Drug-resistance and Treatment Outcome in Multidrug-resistant Pulmonary Tuberculosis

C. Nagaraja, B.L. Shashibhushan, Mohamed Asif, Manjunath PH and Sagar C

ABSTRACT

Aims and Objectives. To study the pattern of drug-resistance and treatment outcomes among patients with confirmed multidrug-resistant pulmonary tuberculosis (MDR-PTB).

Methods. A prospective study was conducted at Rajiv Gandhi Institute of Chest Diseases, Bengaluru, Karnataka, India. Between January 2005 and December 2008, 224 confirmed MDR-PTB cases were studied for various drug-resistance patterns, and their treatment outcomes were analysed until November 2010. Sputum culture and drug sensitivity tests (DST) were carried out at National Tuberculosis Institute, Bengaluru; DST was done for all first-line drugs except pyrazinamide.

Results. Of the 224 MDR-PTB patients, 146 (65.2%) were resistant to all first-line drugs, 39 (17.4%) to isoniazid, rifampicin and streptomycin; 19 (8.5%) to isoniazid, rifampicin and ethambutol; and 20 (8.9%) to isoniazid and rifampicin. Among them, 145 (64.7%) patients were cured, 5 (2.2%) had treatment-failure, 10 (4.4%) died, and 64 (28.5%) defaulted. Among 145 cured cases, 100 (69%) were resistant to all first-line drugs, 23 (16%) to isoniazid, rifampicin and streptomycin, 11(8%) to isoniazid, rifampicin and ethambutol, and 11(8%) to isoniazid and rifampicin.

Conclusions. The most common pattern observed in this study was resistance to all four first-line drugs followed by resistance to isoniazid, rifampicin and streptomycin. Patients resistant to all first-line drugs had early sputum culture conversion and better cure rate as compared to other resistance patterns.

Key words: MDR-TB, Drug resistance, Treatment outcome.

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality in India. India accounts for 1/5th of global TB incidence. According to the World Health Organization (WHO), each year an estimated 9.4 million new cases of TB are detected leading to nearly 2 million deaths. In India, the numbers of TB patients are 1.96 million per year, and among them 0.8 million are new smear-positive cases comprising of 75 new sputum smear-positive cases per lakh annually with 0.33 million deaths per year. There is a rising trend of drug-resistant TB in different parts of the world, India being next only to China, both contributing more than 50% of global multidrug-resistant (MDR-TB) cases. Frequency of MDR-TB is less than 3% in new cases and 12% to 17% among re-treatment cases as per the recent studies.1, 2 An MDR-TB patient is the one whose sputum culture is positive for Mycobacterium tuberculosis that is resistant in vitro to isoniazid (INH) and rifampicin with or without resistance to other anti-tuberculosis drugs based on drug sensitivity testing.3 Since second-line anti-tuberculosis treatment (ATT) is more toxic and less efficacious than first-line drugs, treatment of MDR-TB is more challenging and requires judicious use of various regimens as per resistance patterns in specialised and designated centers. Hence, this study was done to assess the pattern of drug-resistance among confirmed multidrug-resistant pulmonary tuberculosis (MDR-PTB) patients and their treatment outcomes.

MATERIAL AND METHODS

This study was carried out at the Department of Pulmonary Medicine, Rajiv Gandhi Institute of Chest Diseases, Bengaluru. Between January 2005 and December 2008, 224 confirmed MDR-PTB cases were recruited and their treatment outcomes were analysed until November 2010.

All sputum smear-positive category II failures aged more than 15 years with confirmed reports of MDR-
PTB from the National Tuberculosis Institute (NTI), Bengaluru, were included in the study. Culture and sensitivity reports from other non-accredited laboratories were not considered. Sputum culture for acid-fast bacilli (AFB) and DST were carried out at NTI, Bengaluru, a Revised National Tuberculosis Control Programme (RNTCP)-accredited laboratory. DST was done for all first-line drugs except pyrazinamide (PZA). DST for second-line drugs were carried out in a few MDR failure cases at Tuberculosis Research Centre (TRC), Chennai, the only south Indian centre with DST facility for second-line drugs. Category I and III failures were not included in this study as they were not considered as MDR-suspects when the study started.

Sputum specimens were collected in sterile wide mouthed bottles from sputum smear-positive patients of pulmonary TB. The collected specimens were processed by modified Petroff's method. For each specimen, two Lowenstein-Jensen (LJ) slopes were inoculated each with one 5 mm loopful of the centrifuged sediment, distributed over the surface. All cultures were incubated at 35-37 °C for up to 8 weeks. The tests were done in a biosafety class II cabinet.

Various regimens followed depending on the DST for the treatment of MDR-PTB in this study are given below. The initial regimen contained at least three newer drugs that were not used previously and included at least one injectable aminoglycoside and PZA, as this combination has good bactericidal activity. The number of newer drugs varied in the intensive phase depending on the resistance pattern. One or more drugs, preferably weaker drugs (injectable and cycloserine), were not used in the continuation phase after sputum conversion. The total duration of the treatment was 18 to 24 months.

The patients were followed up every month during the entire period of treatment with clinical assessment and sputum smear examination for AFB. Sputum culture was done thrice during the entire course of treatment — one at sputum smear conversion, second at the end of intensive phase, and third at one month before completion of treatment. Five patients that failed treatment for MDR-PTB were subjected for sputum AFB culture and sensitivity to first- and second-line drugs at TRC, Chennai. Chest radiographs were done once in three months in all the patients. Blood urea and serum creatinine estimation were carried out monthly during the intensive phase in all the patients. Liver function tests were done at baseline in all the patients. Other relevant investigations were carried out based on clinical assessment.

### RESULTS

In total, 224 patients with confirmed MDR-PTB were studied. None of the patients were tested for PZA sensitivity. Of the 224 patients (158 males), 18 patients had diabetes mellitus, 5 had human immunodeficiency virus (HIV) co-infection, 3 had hypertension, 1 had ischaemic heart disease and diabetes mellitus, 2 had chronic obstructive pulmonary disease, and 1 had Hansen’s disease. One hundred and forty-eight patients had cavitary lesions, while the remaining 76 had only infiltrates. Bilateral lesions were seen in 175 patients, right-sided

<table>
<thead>
<tr>
<th>Resistance Pattern</th>
<th>Intensive Phase Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>Streptomycin + Ofloxacin/Levofloxacin + Ethionamide + Ethambutol + Pyrazinamide</td>
</tr>
<tr>
<td>HRS</td>
<td>Kanamycin + Ofloxacin/Levofloxacin + Ethionamide + Ethambutol + Pyrazinamide</td>
</tr>
<tr>
<td>HRE</td>
<td>Streptomycin + Ofloxacin/Levofloxacin + Ethionamide + Cycloserine/Para-aminosalicylic acid + Pyrazinamide</td>
</tr>
<tr>
<td>HRSE</td>
<td>Kanamycin + Ofloxacin/Levofloxacin + Ethionamide + Cycloserine + Pyrazinamide + Para-aminosalicylic acid</td>
</tr>
</tbody>
</table>

H=Isoniazid, R=Rifampicin, S=Streptomycin, E=Ethambutol
lesions in 27 patients and left-sided lesions in the remaining 22 patients.

Table 1 shows the pattern of drug-resistance in the study sample. Majority of them (65%) showed resistance to all the first-line drugs tested. The mean duration of sputum culture conversion according to the resistance pattern is presented in table 2. Treatment outcomes of various MDR-PTB patterns are shown in table 3.

**Table 1. Various resistance patterns seen in the study sample**

<table>
<thead>
<tr>
<th>Resistance Pattern</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRSE</td>
<td>146 (65.2)</td>
</tr>
<tr>
<td>HRS</td>
<td>39 (17.4)</td>
</tr>
<tr>
<td>HR</td>
<td>20 (8.9)</td>
</tr>
<tr>
<td>HRE</td>
<td>19 (8.5)</td>
</tr>
</tbody>
</table>

H=Isoniazid, R=Rifampicin, S=Streptomycin, E=Ethambutol

Figures in parenthesis indicate percentage.

**Table 2. Time to culture conversion in the study sample**

<table>
<thead>
<tr>
<th>Resistance Pattern</th>
<th>Mean Duration of Sputum Culture Conversion (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRSE</td>
<td>40.3</td>
</tr>
<tr>
<td>HRS</td>
<td>48.2</td>
</tr>
<tr>
<td>HRE</td>
<td>51.3</td>
</tr>
<tr>
<td>HR</td>
<td>55.0</td>
</tr>
</tbody>
</table>

**Table 3. Treatment outcomes in 224 patients with MDR-PTB**

<table>
<thead>
<tr>
<th></th>
<th>HRSE</th>
<th>HRE</th>
<th>HRS</th>
<th>HR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured</td>
<td>100</td>
<td>11</td>
<td>23</td>
<td>11</td>
<td>145</td>
</tr>
<tr>
<td>Default</td>
<td>37</td>
<td>5</td>
<td>15</td>
<td>7</td>
<td>64</td>
</tr>
<tr>
<td>Failure</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Death</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>19</td>
<td>39</td>
<td>20</td>
<td>224</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate percentage.

Most common adverse drug reactions observed in this study were gastrointestinal disturbances mainly due to para-aminosalicylic acid (PAS), which subsided with routine management and assurance. Nine patients developed ototoxicity such as tinnitus, hard of hearing, positional imbalance due to streptomycin/kanamycin toxicity (confirmed by audiometry), necessitating us to stop the drug. Five patients complained of acne attributed to ethionamide; but, the drug was not stopped, and eventually the acne subsided in most of them. Nine patients developed cycloserine-induced psychosis requiring anti-psychotic medications. Cycloserine was stopped in two patients. Three patients developed swelling of the thyroid gland. Investigations confirmed hypothyroidism in them, and they improved following replacement therapy. Twelve patients developed arthritis along with elevated serum uric acid levels which warranted us to stop the drug temporarily; they were treated with non-steroidal anti-inflammatory drugs. Uric acid level estimation was done monthly and PZA was re-started once the uric acid levels normalised.

**DISCUSSION**

An appropriate assessment of various patterns of drug-resistance among patients with confirmed MDR-PTB is required to initiate a proper regimen as per DST to improve the treatment outcome. A mere diagnosis of MDR-PTB and initiation of second-line ATT without proper regimens based on DST may not help achieve a good treatment outcome. As there is an increasing trend of MDR-PTB in India, proper formulation of treatment regimens consisting of newer drugs based on various drug resistance patterns in confirmed MDR-PTB cases is very much required as evident from the present study.

In the present study, 65.2% were resistant to all first-line drugs, 91.2% were resistant to at least one other first-line drug apart from INH and rifampicin. Rao et al. showed that of the 577 proven MDR-TB patients, 56.5% had isolates resistant to all first-line drugs, 88% cases had MDR plus resistance to at least another first-line drug. In the present study, MDR plus streptomycin resistance was 17.4% and MDR plus ethambutol resistance was 8.5%, while Kudzawu et al. reported it to be 25% for MDR plus streptomycin resistance and 21.4% for MDR plus ethambutol resistance.

Isolates resistant only to INH and rifampicin were 8.9% in the present study as compared to 10.7% in a study by Chowgule and Dheodhar, 25.2% in a study by Dheodhar et al., 21.4% in a study by Janmeja et al., and 35.7% in the study by Kudzawu et al.

The mean sputum culture conversion was analysed in MDR-PTB patients with various drug-resistance patterns. The patients with resistance to all first-line drugs showed a mean sputum conversion of 40 days and the conversions observed in other resistance patterns were 48 days for MDR plus streptomycin, 51 days for MDR plus ethambutol and 55 days for isolated MDR. In the present study, the mean time to culture conversion observed in all 224 patients was 48.6 days. Joseph et al. found that of the 38 cases, 82% had culture conversion in two months or less. Shin et al. in a study of 230 patients found a culture conversion of 95% after a median period of two months.

The treatment outcomes were also analysed in the present study. In a total of 224 cases, 145 (64.7%) were cured, 5 (2.2%) had treatment failures, 10 (4.4%) patients died, and 64 (28.5%) patients defaulted.
Masjedi et al\(^{11}\) in a study of 43 cases, documented that 29 (67.5%) had a successful outcome (cured), 6 (14%) had treatment failures, and 8 (18.6%) patients died with no defaulters. In the study by Shin et al,\(^{10}\) 77% were cured, 5% died, 7% failed, and 12% defaulted. Patients with resistance to all first-line drugs showed an early sputum culture conversion and a better cure rate compared to other resistance patterns. These observations were probably due to judicious use of more newer drugs in the initial regimen which were not used previously in those patients and is attributed to the bacilli being fully sensitive to these new drugs.

In the present study, 28.5% of patients defaulted despite good pre-treatment counselling and providing the drugs for free under supervision, probably due to a sense of well-being after a few months of treatment or due to social stigma. Inability to collect drugs from the centre due to costs involved in travel and loss of earnings for that particular day was observed more commonly in males as they were the only earning members of the family and also due to broken families. Proper counselling, education, and motivation are needed to improve the adherence to treatment and cure rates. The cure rate (64.7%) in our study was good mainly because of formulation of appropriate treatment regimens based on the various drug-resistance patterns.

In conclusion, close monitoring of drug-resistance patterns in confirmed MDR-PTB isolates is required to formulate different regimens as per the drug-resistance pattern. The commonest pattern observed in this study was resistance to all four first-line drugs followed by resistance to isoniazid plus rifampicin plus streptomycin. Patients resistant to all first-line drugs showed a better cure rate compared to other resistance patterns. Hence, early diagnosis of MDR-PTB and treatment under supervision by formulating appropriate regimens based on resistance pattern are the keys to success in treating MDR-PTB.

REFERENCES

Exhaled Breath Condensate Analysis in Chronic Obstructive Pulmonary Disease

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ABSTRACT

The increasing focus on airway inflammation in the pathogenesis of chronic obstructive pulmonary disease (COPD) has led to development and evolution of tools to measure it. Direct assessment of airway inflammation requires invasive procedures, and hence, has obvious limitations. Non-invasive methods to sample airway secretions and fluids offer exciting prospects. Analysis of exhaled breath condensate (EBC) is rapidly emerging as a novel non-invasive approach for sampling airway epithelial lining fluid and offers a convenient tool to provide biomarkers of inflammation. It has definite advantages that make it an attractive and a feasible option. It is a source of mediators and molecules that are the causes or consequences of the inflammatory process. Measurement of such markers is increasingly being explored for studying airway inflammation qualitatively and quantitatively in research studies and for potential clinical applications. These biomarkers also have the potential to develop into powerful research tools in COPD for identifying various pathways of pathogenesis of COPD that may ultimately provide specific targets for therapeutic intervention. The EBC analysis is still an evolving non-invasive method for monitoring of inflammation and oxidative stress in the airways. The limited number of studies available on EBC analysis in COPD have provided useful information although definite clinical uses are yet to be defined. Evolving technologies of genomics, proteomics, and metabonomics may provide deeper and newer insights into the molecular mechanisms underlying the pathogenesis of COPD. [Indian J Chest Dis Allied Sci 2012;54:27-37]

Key words: Chronic obstructive pulmonary disease, Exhaled breath condensate, Oxidative stress, 8-isoprostane, Hydrogen peroxide.

INTRODUCTION

A major advancement in the knowledge of pathogenesis of chronic obstructive pulmonary disease (COPD) has been the recognition that airway inflammation plays a key and a central role. It is now a part of the current definition of COPD and is believed to be the major underlying process for the altered pathophysiology and clinical manifestations.1 Airflow limitation has long been the pathophysiological characteristic of COPD, and therefore, lung function testing has been considered as the main investigation for diagnosis, assessment of severity, monitoring of response and for following the natural course of the disease. Indeed, spirometry is considered essential to establish permanent airflow limitation and the diagnosis of COPD. However, limitations of lung function tests in the assessment of severity and monitoring of response have been well recognised. The increasing focus on airway inflammation has led to efforts to gain insights into its nature, development and evolution in research studies with an aim to ultimately translate this knowledge into tools for diagnosis, assessment and monitoring of COPD.

Conventionally, drugs in COPD have been evaluated by changes in forced expiratory volume in one second (FEV1) and more recently, using in addition, patient-centered clinical outcome measures. While these tools may be appropriate to evaluate therapeutic interventions such as bronchodilators, these may not serve the purpose for newer drugs that are increasingly targeted at specific pathways of the inflammatory process. Tools to measure airway inflammation are required for the evaluation of such drugs.

Oxidative stress is believed to play a major role in the pathogenesis of COPD and is characterised by an increased oxidant load and a relative or absolute deficiency of antioxidants.2 Oxidative processes and free radical generation lead to increased bronchial hyperresponsiveness and inflammation, apoptosis and destruction of airway epithelial cells, and impair the functions of antiproteases and surfactant.3 The major mediators of oxidative stress and pro-inflammatory molecules include reactive oxygen
species (ROS) such as the superoxides and hydroxyl radicals, reactive nitrogen species (RNS), certain cytokines and eicosanoids, interleukins, tumour necrosis factor-alpha, and activated transcriptional factors such as nuclear factor kappa-B and activator protein 1. These compounds are potential candidates to serve as biomarkers of oxidative stress and airway inflammation. An ideal biomarker for COPD should be stable, be present in sufficient quantities to allow analysis, should have acceptable precision in measurement, should be responsive to change in clinical status, either worsening or improvement, with therapeutic interventions, and should be specific for the disease.

ASSESSMENT OF AIRWAY INFLAMMATION

Direct assessment of airway inflammation requires invasive procedures, such as biopsy and bronchoalveolar lavage that are limited by this disadvantage. These tools provide samples and specimens from limited areas of the lung. Further, these are not feasible options for advanced disease and for serial monitoring. While bronchoalveolar lavage fluid (BALF) has been used in several studies to sample the lower respiratory tract for more than two decades, its invasive nature and a definite, though small, risk of adverse events has prevented it from evolving into an acceptable clinical tool for the assessment of the airway inflammation.

Peripheral blood markers have been used to study airway inflammation but may not be appropriate mirrors of the airway pathology. The increase in the inflammatory cell population and markers in the airway may occur earlier than in the peripheral blood, and reflect the degree of airflow limitation better than do peripheral blood measurements. Non-invasive methods to sample airway secretions and fluids offer exciting prospects. These can provide mediators and molecules that are the causes or consequences of the inflammatory process. Measurement of such markers is increasingly being explored for studying airway inflammation qualitatively and quantitatively in research studies and for potential clinical applications. These biomarkers also have the potential to develop into powerful research tools for identifying the novel pathways of pathogenesis of COPD that may ultimately provide specific targets for therapeutic interventions.

Sputum induction is a semi-invasive technique that not only provides specimens for cytological studies of the airway lining fluid but provides a source of inflammatory mediators that can be measured in the supernatants. However, its application has largely been limited to cytological analysis though it has also been studied as a source of biomarkers reflecting oxidative stress and airway inflammation.

EXHALED BREATH CONDENSATE ANALYSIS

Analysis of exhaled breath is rapidly emerging as a novel and non-invasive approach for sampling airway epithelial lining fluid and offers another convenient tool to provide biomarkers. It has definite advantages that make it an attractive and a feasible option. Besides being completely non-invasive, it is suitable for serial assessments in longitudinal studies, and thus, can be used for monitoring of disease as well as serve as an outcome parameter in clinical drug trials. Other potential uses include measurement of severity of disease, and application as a diagnostic aid. It can be used in stable as well as patients with acute exacerbations and even in patients on a mechanical ventilator. It can be used in the out-patient and occupational settings, and also in field studies for epidemiological purposes. Exhaled breath analysis has been extensively examined as a mirror of the inflammatory processes in the airways in asthma and such studies are now being increasingly carried out in patients with COPD.

Exhaled breath analysis broadly focuses on two areas: measurement of exhaled nitric oxide (ENO) and the detection of biomarkers in exhaled breath condensate (EBC). The use of ENO has found limited success as a potential evaluative tool in COPD compared to its wider acceptability in the management of asthma. This scope of this review is limited to EBC analysis.

The EBC is mainly formed by water vapour but also contains several biomolecules, mainly related to neutrophil-derived products and oxidative stress (leukotrienes, prostaglandins, isoprostanes, hydrogen peroxide), endogenous airway acidification (hydrogen ions), and nitric oxide-derived products (nitrosothiols, and nitrite/nitrate). Its collection simply involves tidal breathing into a chilled collection device. Water vapour in exhaled breath is condensed and collected and various mediators can then be quantified in the condensate. The EBC provides a convenient sample of volatile substances and aerosols and reflects a summation of all areas of the lung that are ventilated.

Exhaled Breath Condensate Analysis in COPD

Several studies have been carried out on EBC obtained from patients with COPD. While several potential clinical applications have been explored including the ability of biomarkers to differentiate between health and disease in smokers, diagnostic specificity
for COPD, assessment of severity, phenotyping, and for evaluation of therapeutic intervention, most of the studies have been limited by small sample sizes and lack of standardised methods of collection of EBC and its analysis. The EBC analysis has the potential to offer clues to diagnosis of infectious agents and may allow risk stratification. Although most of the studies have been in stable patients, acute exacerbations of COPD (AECOPD) are characteristically associated with increased airway inflammation and oxidative stress. Therefore, EBC analysis may also be used for predicting the outcomes and monitoring the course of an exacerbation. However, such studies have been few. Clear clinical indications and utility of EBC analysis in COPD in research studies have not been defined.

EBC studies on some of the more commonly studied biomarkers in patients with COPD are reviewed below.

8-isoprostane

One of the major products of oxidative stress in the lungs is 8-isoprostane. It is a prostaglandin-F(2α) isomer that is formed in vivo by free radical-catalysed peroxidation of arachidonic acid, independent of the cyclooxygenase pathway. This has been one of the most studied biomarker in patients with COPD.

The 8-isoprostane concentrations were observed to be similar in ex-smokers and current smokers with COPD, were increased about 1.8-fold compared to healthy smokers who, in turn, had 2.2-fold higher levels than healthy non-smokers. The 8-isoprostane was similarly reported to be significantly increased in patients with COPD compared to smokers. These results were corroborated in other studies.

While levels of 8-isoprostane in EBC have consistently been found to be increased in patients with COPD, these have usually been lower than those observed in induced sputum or BALF. This was true for all groups of subjects: non-smokers, healthy smokers, symptomatic smokers at risk for COPD and patients with AECOPD. Similarly, in BALF too, 8-isoprostane was found present in significantly higher concentrations than in EBC. Both correlated well with levels of leukotriene B4 (LTB4) in patients with COPD.

The relationship between EBC levels of 8-isoprostane and lung function parameters, and with other measures of severity of COPD has been investigated but found to be inconsistent and variable in different studies. In one study, levels of 8-isoprostane did not differ significantly across GOLD stages of COPD. Further, no correlations were observed between levels of 8-isoprostane and FEV1, neutrophil count, and dyspnoea scores. In another study, the increase in 8-isoprostane levels in EBC was found irrespective of the lung function impairment.

However, there was a significant correlation with emphysema scores on high resolution computed tomography (HRCT) and with Medical Research Council dyspnoea scale scores. Levels of 8-isoprostane in EBC were found to be significantly lower in patients with emphysema than in patients with predominantly chronic bronchitis and correlated significantly with diffusion capacity but not with FEV1. On the other hand, inverse correlations were observed between 8-isoprostane levels and lung function parameters in another study. Again, a recent study did not find any relationship between 8-isoprostane levels and lung function parameters but a significant positive correlation was found with the dyspnoea grade. Severity-related differences in 8-isoprostane were identified according to the body mass index (BMI), obstruction, dyspnoea, and exercise (BODE) index in another recent study. The last study also examined the relationship between oxidative stress and pathophysiology in COPD. In stable patients, blood oxygen levels and dynamic hyperinflation were reported related to airway 8-isoprostane levels in EBC. Concentration of 8-isoprostane were higher in patients who developed dynamic hyperinflation. End-expiratory lung volume change and partial pressure of arterial oxygen (PaO2) independently predicted 8-isoprostane levels.

Phenotyping of COPD offers scope for an individualised approach to treatment besides identifying patients groups with different prognosis and natural history. Till now, the approach to phenotyping has been based mainly on clinical, radiological and physiological characteristics. The EBC analysis also has the potential to serve this purpose. Some studies have evaluated this potential but with opposing results. Levels of 8-isoprostane in EBC were found to be significantly lower in patients with emphysema than in patients with predominantly chronic bronchitis in one study but found to have a significant correlation with HRCT emphysema scores in another study.

The studies examining 8-isoprostane levels in EBC and their significance are summarised in table 1. In general, these studies have found patients with COPD to have higher or similar levels as current smokers and both have higher levels than non-smokers. Thus, it clearly marks oxidative stress in the airways and appears to be a useful parameter. The correlation with spirometry and severity of the disease as assessed by lung function or symptoms is modest or inconsistent. This is not surprising considering that COPD is a disease with multiple dimensions and lung function has usually been found to have modest or poor relationship with other measures of severity. Whether the oxidative stress has a consistent and predictable relationship with clinical severity is yet to be established and requires
further investigation. No study has evaluated changes in response to any therapeutic intervention. Thus, 8-isoprostane remains a promising biomarker in EBC in COPD but requires further evaluation before its utility as a clinical tool can be established.

Hydrogen Peroxide (H$_2$O$_2$)

In COPD patients, ROS may be produced endogenously by activated inflammatory cells including neutrophils, macrophages, and eosinophils, or exogenously, by exposures to air pollutants or cigarette smoke that act directly or by causing neutrophil influx. Even a single cigarette has been shown to cause a significant increase in EBC H$_2$O$_2$ in healthy non-smokers. Airway H$_2$O$_2$ is produced by superoxide dismutase-mediated conversion of superoxide anions. Being soluble, it appears in the exhaled breath. Therefore, H$_2$O$_2$ can serve as a direct marker of oxidative stress. The H$_2$O$_2$ measurements in EBC have been carried out in several studies in COPD.

Levels of H$_2$O$_2$ were found increased in EBC of stable patients with COPD compared to healthy controls. Patients with AECOPD had even greater levels than stable patients suggesting that oxidant production was increased further during exacerbations. While confirming increased H$_2$O$_2$ in stable COPD, it was found that patients who were current smokers did not exhale more H$_2$O$_2$ than those who were ex-smokers or those who had non-smoking COPD. Both these studies show that cigarette smoking made no difference to the EBC H$_2$O$_2$ levels and the oxidative stress was a feature intrinsic to COPD irrespective of the cause.

In another study, the increased levels of EBC H$_2$O$_2$ showed a positive correlation with the levels of sputum neutrophils, indicating a neutrophil-dependent mechanism for its production. The levels of H$_2$O$_2$ in severe and moderate COPD were significantly higher than in mild disease. H$_2$O$_2$ had significant correlations with FEV$_1$ and with dyspnoea scores in patients with moderate and severe disease. Smokers and patients with COPD

### Table 1. Summary of studies on 8-isoprostane in EBC in patients with COPD

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Study Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montuschi et al $^{15}$ 2000</td>
<td>COPD ex-smokers (n=25) COPD current smokers (n=15) Control non-smokers (n=10) Smokers (n=12)</td>
<td>COPD ex-smokers and current smokers did not differ; Levels in patient with COPD increased 1.8-fold over healthy smokers; smokers had 2.2-fold higher levels than non-smokers; Smoking caused an acute increase in exhaled 8-isoprostane by about 50%; No correlation with severity of airways obstruction or dyspnoea</td>
</tr>
<tr>
<td>Biernacki et al $^{14}$ 2003</td>
<td>COPD acute exacerbation (n=21) before and after treatment</td>
<td>Levels were increased during exacerbation, decreased on treatment at 2 weeks and further at 2 months</td>
</tr>
<tr>
<td>Kostikas et al $^{19}$ 2003</td>
<td>COPD (n=30) Smokers, Stage 0 (n=10)</td>
<td>Levels were significantly elevated in patients with COPD; No significant difference across severity groups; No correlation with lung function and dyspnoea grades</td>
</tr>
<tr>
<td>Izquierdo et al $^{10}$ 2006</td>
<td>COPD (n=39) Control smokers (n=15)</td>
<td>Levels were significantly lower in patients with emphysema than in patients with chronic bronchitis or in the controls; correlated significantly with DLCO/VA but not with FEV$_1$</td>
</tr>
<tr>
<td>Ko et al $^{26}$ 2006</td>
<td>COPD ex-smokers (n=32) Control non-smokers (n=17)</td>
<td>COPD patients had higher levels compared to controls; Levels increased across the groups with worsening FEV$_1$</td>
</tr>
<tr>
<td>Makris et al $^{14}$ 2008</td>
<td>COPD patients (n=18) Control ex-smokers (n=5) Non-smokers (n=7)</td>
<td>Levels were significantly elevated in COPD; Levels correlated with emphysema score in HRCT and with dyspnoea scores</td>
</tr>
<tr>
<td>Mazur et al $^{22}$ 2009</td>
<td>COPD acute exacerbation (n=10) Control non-smokers (n=14) Healthy smokers (n=17) Symptomatic smokers (n=9)</td>
<td>In induced sputum, levels were at least 10-fold higher compared to EBC levels; Healthy non-smokers had the lowest levels and patients with AECOPD, the highest levels in EBC; Inverse correlations with lung function parameters</td>
</tr>
<tr>
<td>Inonu et al $^{11}$ 2011</td>
<td>COPD (n=25) Control smokers (n=26) Non-smokers (n=29)</td>
<td>COPD and smokers did not differ; Both had significantly higher levels than non-smokers; No correlation with lung function parameters but significant positive correlation with dyspnoea grade</td>
</tr>
<tr>
<td>García-Rio et al $^{15}$ 2011</td>
<td>COPD (n=76)</td>
<td>Significant severity-related differences in levels according to the BODE index; Levels were higher in those who developed dynamic hyperinflation; Hyperinflation and PaO$_2$ predicted levels on multivariate regression</td>
</tr>
</tbody>
</table>

EBC=Exhaled breath condensate; COPD=Chronic obstructive pulmonary disease; FEV$_1$=Forced expiratory volume in one second; HRCT=High resolution computed tomography; AECOPD=Acute exacerbations of COPD; BODE Index=BMI, obstruction, dyspnoea, and exercise index; PaO$_2$=Partial pressure of arterial oxygen
were observed to have similar levels of $H_2O_2$ in EBC. There was no correlation between $H_2O_2$ levels and lung function parameters.\textsuperscript{11}

There are uncertainties about the origin of $H_2O_2$ released in the lung. Fractionated samples of EBC from the airways and from the lung periphery revealed 2.6 times higher $H_2O_2$ in the former that was two-fold higher in smokers and five-fold higher in patients with COPD compared to non-smokers, suggesting that airways may be the dominant location of $H_2O_2$ production.\textsuperscript{20}

Some studies have looked at the effect of therapeutic interventions on $H_2O_2$ levels in EBC. A two-week treatment in stable patients with COPD with inhaled beclomethasone was not found to change EBC $H_2O_2$ significantly although exhaled NO was reduced.\textsuperscript{21} N-acetylcysteine, a precursor of reduced glutathione that together with glutathione peroxidase may remove $H_2O_2$, given for 12 months in stable COPD patients had no effect in the first six months but later reduced EBC $H_2O_2$ by about 2.5 fold.\textsuperscript{22} Patients with lower respiratory tract infection showed no significant changes in $H_2O_2$ concentrations in EBC on recovery and there were no significant correlations between with spirometry and serum inflammatory parameters. In contrast, several serum inflammatory markers did decrease during hospitalisation.\textsuperscript{23} On the other hand, patients with moderate to severe COPD during an exacerbation showed significant decrease in $H_2O_2$ concentrations in EBC suggesting that the oxidative stress markers like $H_2O_2$ are suitable in monitoring exacerbated COPD.\textsuperscript{7}

The studies examining $H_2O_2$ levels in EBC and their significance are summarised in table 2. From these studies, it may be concluded that $H_2O_2$ is a reliable marker of oxidative stress in patients with COPD and tends to reflect the severity of airway inflammation and clinical manifestations. Therefore, it has the potential to serve as a tool for assessment and monitoring of disease. Responsiveness to therapeutic intervention is not established.

### Cytokines and Eicosanoids

Considering the central role of airway inflammation in the pathobiology of COPD, cytokines that play a role in recruiting inflammatory cells, especially neutrophils and in activation of inflammatory cells and perpetuation of airway inflammation may be useful biomarkers in monitoring its severity. In

<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Year</th>
<th>Study Population</th>
<th>Results</th>
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<tbody>
<tr>
<td>Dekhuijzen et al.\textsuperscript{17}</td>
<td>1996</td>
<td>COPD (n=12), COPD acute exacerbation (n=19), Controls (n=10)</td>
<td>Levels were highest in acute exacerbation followed by stable COPD, followed by controls</td>
</tr>
<tr>
<td>Nowak et al.\textsuperscript{18}</td>
<td>1998</td>
<td>COPD ex-smokers/smokers/non-smokers (n=32), Control non-smokers (n=17)</td>
<td>Levels were higher in COPD than controls; No difference between smoker and non-smoker COPD patients</td>
</tr>
<tr>
<td>Nowak et al.\textsuperscript{19}</td>
<td>1999</td>
<td>COPD ex-smokers/smokers/non-smokers (n=44), Control non-smokers (n=17)</td>
<td>Levels were higher in COPD than controls; No difference between smoker and non-smoker COPD patients</td>
</tr>
<tr>
<td>Ferreira et al.\textsuperscript{21}</td>
<td>2001</td>
<td>COPD non-smokers (n=20), before and after 2-week treatment with inhaled beclomethasone 500 μg twice daily</td>
<td>2-week treatment with inhaled beclomethasone did not change $H_2O_2$ significantly</td>
</tr>
<tr>
<td>Kasielski and Nowak\textsuperscript{22}</td>
<td>2001</td>
<td>COPD (n=22), before and after N-acetylcysteine, 600 mg once a day for 12 months</td>
<td>No effect of N-acetylcysteine in the first six months but later reduced $H_2O_2$ by about 2.5 fold</td>
</tr>
<tr>
<td>van Beurden et al.\textsuperscript{23}</td>
<td>2003</td>
<td>COPD (n=25) with lower respiratory tract infection</td>
<td>No significant changes in $H_2O_2$ concentration after treatment of infection</td>
</tr>
<tr>
<td>Kostikas et al.\textsuperscript{24}</td>
<td>2003</td>
<td>COPD (n=30), Smokers, Stage 0 (n=10)</td>
<td>Levels were significantly elevated in COPD patients; severe and moderate COPD had significantly higher levels than those with mild disease; significant correlation with lung function, induced sputum neutrophil counts and dyspnoea scores in moderate and severe COPD</td>
</tr>
<tr>
<td>Inou et al.\textsuperscript{21}</td>
<td>2011</td>
<td>COPD (n=25), Healthy smokers (n=26), Non-smokers (n=29)</td>
<td>COPD and smokers did not differ; Both had significantly higher levels than non-smokers; no correlation with lung function parameters or dyspnoea</td>
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infective exacerbations of COPD may be associated with an increase in inflammation in the airways. Therefore, levels in EBC may be assumed to reflect the intensity of the inflammatory processes in the airways.

LTB4, a potent and selective chemoattractant of neutrophils, is released from macrophages and epithelial cells as well as from activated neutrophils. Exhaled LTB4 may be useful in monitoring of exacerbation in patients with COPD and has been investigated in a few studies. The increased concentration of LTB4 in EBC of patients with AECOPD and its subsequent decline towards normal levels may position it as a marker of resolution or control of the inflammatory process and thus may have a role in monitoring of response. The LTB4 levels in EBC were reported increased in patients with COPD compared to controls. Patients with airflow reversibility had values higher than those without airflow reversibility. In another study, EBC LTB4 correlated significantly with diffusion capacity but not with FEV1. Levels of interleukin (IL)-8 were observed to be lower in patients with emphysema than in patients with predominantly chronic bronchitis.

The EBC profiles of other exhaled prostaglandins and leukotrienes have been studied to differentiate the inflammatory response in asthma and COPD. Prostaglandin E2 and prostaglandin F2α were found markedly increased in EBC from patients with COPD but not in asthmatics. In contrast, leukotriene E4 was increased in asthma but is not detectable in normal subjects or in patients with COPD. Whether EBC is the appropriate source for these biomarkers is not clear. In one study, IL-8 was mostly undetectable in EBC in patients with AECOPD and stable COPD as well as in healthy controls. In other studies, though levels in EBC were much less than those observed in induced sputum, IL-8 was increased in healthy smokers, symptomatic smokers at risk for COPD and patients with acute exacerbation. Inverse correlations with lung function parameters were observed.

Similarly, concentrations of cys-LTs and LTB4 were found to be significantly higher in BALF compared to EBC in patients with COPD. No significant differences were found between smokers and ex-smokers serum or EBC levels of IL-8 and LTB4 in EBC.

Exacerbations in COPD are most frequently caused by infections which are also responsible for an increase in inflammation in the airways. Therefore, infective exacerbations of COPD may be associated with the increased production of other inflammatory mediators, for example, IL-8, IL-6, endothelin-1 and tumour necrosis factor-alpha (TNF-α) levels in EBC during these exacerbations.

Chemokines related to neutrophil and monocyte inflammation (growth-related oncogene alpha [GROα] and monocyte chemoattractant protein-1 [MCP-1]) have been studied in EBC of COPD patients but found to have no utility in diagnosis or assessment of the severity.

EBC Acidification

Studies in asthma have pointed to the role of airway acidification in the airway pathophysiology. It may cause bronchoconstriction, impaired ciliary motility, increased mucus production and viscosity, and airway epithelial damage. The EBC pH is easy to measure and therefore is a potential biomarker in airway inflammatory diseases. Exhaled carbon dioxide may be a potential confounder especially in patients with acute or chronic hypercapnoic respiratory failure in AECOPD and stable COPD, respectively.

A comparison of asthmatics and mild COPD patients with healthy controls did not reveal significant differences. However, another study found lower pH in more severe COPD patients. Other workers reported that EBC pH was lower in COPD patients compared to asymptomatic normal smokers. It was related to disease severity, and to parameters reflecting airflow limitation, hyperinflation and air trapping. It was also observed that EBC pH was lower in ex-smokers than current smokers suggesting a probability of the presence of more severe disease in the former.

In a more recent study, EBC pH was found to be lower in patients with COPD and in smoking controls compared with non-smoking controls but was not different between COPD and smoking controls. It was not related to severity of airways obstruction or to airway inflammation assessed by sputum leukocyte counts, and was not responsive to corticosteroids. Its utility as a biomarker was questioned.

Other Biomarkers

Besides the biomarkers discussed above, other workers have measured other compounds in EBC with a view to understand the nature of the inflammatory process and identify clinically useful substances.

Malondialdehyde (MDA) is a product of lipid peroxidation and has long been considered as a marker of oxidative stress. It is measured as thiobarbituric acid-reactive substances (TBARs), which are the end-products of lipid peroxidation.
Patients with stable COPD exhibited increased lipid peroxidation, measured as TBARs, that were not found to correlate with cigarette smoking status. Two recent studies have differed in their observations. Levels of MDA were not found to be different in patients with COPD and non-smokers in one study. However, in the other, MDA was found to be significantly higher in patients with COPD compared to asthmatics and healthy controls. Further, COPD patients showed an inverse correlation between MDA concentrations and FEV₁. These were found to be increased in EBC from patients with COPD, correlating with inflammation. These were found to be significantly higher in patients with COPD compared to asthmatics and healthy controls. Further, corticosteroid treatment was not demonstrated in EBC of patients with COPD. 40

Among other biomarkers under investigation in COPD are purines that are present on airway surfaces in physiologically significant concentrations and are proposed to play a role in airway inflammation. These were found to be increased in EBC from patients with COPD, correlating with GOLD severity and FEV₁. % predicted. 37

**Products of Nitric Oxide**

Nitric oxide (NO) is perhaps the most extensively studied marker of airway inflammation in exhaled air. Its utility is fairly well established in asthma. However, in COPD, its status is still evolving. While larger airways are likely the primary source of exhaled NO in asthma, that in COPD appears to originate in the peripheral airways. NO lead to formation of nitrite, nitrate, and S-nitrosothiol in the epithelial lining fluid. The compounds have been measured in EBC in a few studies.

Increased levels of S-nitrosothiols have been demonstrated in EBC of patients with COPD. Liu et al. measured nitrite/nitrates (NOx) levels in EBC in COPD patients and reported no significant differences in comparison with healthy non-smoking controls. Further, corticosteroid treatment was not found to have any effect on the levels.

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**TECHNICAL ISSUES IN EXHALED BREATH CONDENSATE ANALYSIS**

The EBC is usually collected by asking the subject to breathe tidally using a mouthpiece and a non-rebreathing valve to separate inspiratory and expiratory air streams. The expired air is passed through a condenser, which is cooled to 0 °C or −20 °C in a refrigerated circuit. The resultant condensate is collected into a cooled vessel. It usually takes between 10 and 15 minutes to obtain 1 to 3 mL of condensate. The EBC is then stored at −20 °C or −80 °C till further analysis of the biomolecules.

Methodological issues that may influence results are important and were recently reviewed. These include techniques and procedures of collection and storage, analytical methods, inherent variation of EBC biomarkers over time, and other factors affecting EBC composition, such as smoking and demographics. These contribute to the differences and variations among different studies reviewed above. The lack of standardisation of the EBC analysis in the earlier studies makes comparison of studies difficult. Analytical methods are being improved to increase their sensitivity. To address the need for standardised methodologies of EBC analysis, American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force has published methodological recommendations regarding the use of EBC.

Besides self-fabricated collection systems, the most commonly used commercially available devices for EBC collection are the portable R Tube (Charlottesville, Virginia, USA) and non-portable EcoScreen (Jaeger, Wuerzburg Germany). pH measurements in EBC collected by these two systems are repeatable and reproducible.

Use of different methods of collection has been found to give different results that do not follow a uniform pattern. From the comparative studies so far, it appears that the possible differences between devices appear to vary with the biomarker measured and with the presence or absence of the disease. The physical and chemical properties of the surface of the collecting device may affect the yield by different capacities for adsorption.

The effect of differing expiratory flow rates has not been examined in patients with COPD. The differences between normals and patients may arise because patients have a different breathing pattern. Whether expiratory flow rate and tidal volume need to be controlled has not been addressed in studies in COPD. Theoretically, hyperventilation, forced exhalation, greater turbulence in more severe airways obstruction or larger tidal volumes should yield higher concentrations of the EBC markers by favouring greater aerosolisation of airway lining fluid. In one study in patients with asthma, EBC H₂O₂ was shown to vary inversely with the expiratory flow rate during collection. However, the pattern of breathing had no significant effect on concentrations of LTB₄, LTE₄, prostaglandin (PG) E₂, pH, NO₃⁻ or total protein. The EBC collection is invariably done using oral breathing. The nasal and oral contamination while collection may change the pH, NO metabolites and leukotrienes like LTB₄. One concern has been oral contamination with ammonia that can affect EBC pH measurements. However, studies have shown that this is unlikely to have any significant effect.

Oral contamination may also affect EBC NO metabolites. Nitrate levels in EBC are influenced by dietary intake. Nitrate is reduced to nitrite by bacterial activity that takes place primarily in the oropharyngeal tract of healthy subjects.
Oropharyngeal nitrate possibly contributes to exhaled NO in non-inflamed airways. The LTB4 levels have been reported to be raised in EBC samples that contained salivary amylase. Therefore, the contribution of oral contamination should be investigated with respect to each specific EBC mediator.

The concentrations of mediators measured in the EBC are likely to be affected by the water vapour content and variable degrees of aerosolisation. These may not reflect the true levels in the airway lining fluid. These factors may explain the differing levels reported for different mediators even in normal non-smoking controls as well as the fairly wide within-session variations. Possible solutions to such sources of error include corrections by dilutional factors estimated from another consistent constituent of EBC or report ratios of mediators rather than absolute levels. These approaches need validation. As most specimens are stored and kept frozen at –20 ºC or –80 ºC till analysis, the levels of markers may be affected. However, this has not been found to be true for most markers for storage periods upto 2 or 3 months.

The extent of nasal contamination as well as the effect of using a nose-clip to occlude the nose is largely unknown in patients with COPD. Whether a nose clip should be worn is not certain. However, the reproducibility of EBC pH (see below) was similar, with and without a nose clip.

The acute effect of smoking is an increase in the oxidative stress. Smoking was shown to cause an acute increase in exhaled 8-isoprostane in COPD patients by about 50%. In normal subjects, acute increase in exhaled 8-isoprostane in COPD smoking was found to increase levels of H2O2. On the other hand, the acute effect of smoking a single cigarette on other EBC compounds such as NO3, nitrosothiols, nitrotyrosine and certain cytokines is variable or inconsistent.

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The ATS/ERS Task Force methodological recommendations on EBC include the following general suggestions to ensure uniformity in methodology among studies: collect during tidal breathing using a nose clip and a saliva trap; define cooling temperature and collection time (10 min is generally sufficient to obtain 1-2 mL of sample and is well tolerated by patients); use inert material for condenser; do not use resistor and do not use a filter between the subject and the condenser. In addition, specific recommendations on methodology of collection, storage and analysis have been made for individual biomarkers.

Several other potential confounders including age, sex, diet, drugs and race as well as other biological factors such as circadian fluctuations may influence results. The available information on the effect of these factors on EBC composition is scanty. The EBC pH was found not to be influenced by age. Nowak et al collected EBC seven times every 4 hour during 24 hours and three times every 7 days during two consecutive weeks and observed diurnal variation in H2O2 levels with two-peak values at 12:00 and 24:00 hour. The mean H2O2 concentration estimated over the whole two-week period was higher in patients above 40 years regardless of smoking habit and it positively correlated with age in never-smoked subjects. Neither moderate exercise nor one puff of salbutamol nor ipratropium influenced significantly the concentration of H2O2 and TBARs in EBC. The mean H2O2 concentration was found to be increased significantly during the day in both the COPD patients and controls. The effect of diet on nitrate levels was discussed above.

The large number of measurable biomarkers and the diversity of the methodologies hamper exploration of wide clinical application and development of any consensus. Koutsokea et al have summarised the ranges of concentrations of different biomarkers include H2O2, NO-related products, arachidonic acid metabolites and pH in the studies that have been carried out so far. The only one marker with established reference values in healthy subjects is EBC pH, whereas the others need further refinement and standardisation of the methodologies. Until the technical aspects of measurements are sorted out, EBC analysis is likely to remain confined to research studies. Even though it appears to be a very promising tool, its adoption as a clinical tool in near future is unlikely.

**Reproducibility of Exhaled Breath Condensate**

Variations on repeated measurements are an important consideration in deciding the utility of an assay. These variations may be natural or biological or may simply reflect the effect of technical factors reviewed above. Variations in any assay must be known before significance can be assigned to changes observed in response to changed clinical status or therapeutic intervention. A change with any intervention should be shown to be greater than spontaneous variability to attribute it to the beneficial effect of treatment. Variations may be within-assay (several measurements performed during the single assay) or short term (within a day) or over a longer term (over a few days) in a stable condition. As airway inflammation is likely to be variable over time due to variations in environmental exposures, infections and disease-related factors, normal subjects can be expected to show much greater stability compared to patients and any variability in the former is likely to reflect the imprecision in the assay that may be an inherent limitation in the method or may be influenced by other technical factors.

Van Hoydonck et al concluded that levels of 8-isoprostanate and H2O2 cannot be reproducibly
assessed in EBC from healthy smokers because of their low concentration and/or the lack of sensitivity of the available assays. Within-assay coefficient of variation of EBC 8-isoprostane was 29.2% that was lower than the corresponding values for within-day (65.3%) and between-day (79.1%) variations. Using the Bland Altman method, there were wide limits of agreement for within-day and between-day reproducibility. While within-assay variability and group mean changes were small, considerable within-day and between-day variability raises questions about its suitability for monitoring and assessment of response to therapeutic interventions. The results were similar with LTB4. In contrast, pH was reported to have a much better stability—within-assay, within-day, and between-day. The EBC pH was observed to be robust and reproducible and not affected by several patient-related and technical factors in health. The H2O2 has been shown to have had a better stability and repeatability compared to 8-isoprostane in patients with COPD. However, it needs to be noted that all the studies looking at precision of measurements over time had small sample sizes. An important consideration in looking at repeatability and reproducibility of measurements is the statistical method used. Inappropriate methods may underestimate variation. Correlations and comparisons of group means can hide vital differences and agreement analysis is the preferred method to look at repeatability. The issue of short-term and long-term stability needs to be addressed for several other mediators, in health and disease, and the minimal clinically important change remains to be defined.

Safety Issues

The studies in patients with COPD have used different EBC collection devices and in none of these any adverse effects have been reported. A major concern is the possibility of transmission of infection between individuals. Filters cannot be used in the inlets of the collecting devices as these may absorb some of exhaled substances. Use of disposable systems is certainly an effective way of preventing any such transmission of infection between patients but this adds to the total costs. Use of non-disposable EBC collection systems, however, was not shown to facilitate transmission of infection in EBC studies in patients with cystic fibrosis.

NOVEL METHODS FOR EXHALED BREATH CONDENSATE ANALYSIS

Application of newer technologies offer exciting avenues for exploring unknown biomarkers. The tools of genomics, proteomics, and metabonomics, that investigate gene expression, protein expression, and metabolic regulation in the pathogenesis of disease, respectively, offer an entirely different approach to the characterisation of EBC in COPD than what has been followed so far.

The development of proteomic techniques such as liquid chromatography-mass spectrometry and gel electrophoresis-mass spectrometry have been applied to investigate the proteome of lung specimens such as sputum, BALF, EBC, cells and biopsies from COPD patients. These may provide a better understanding of the proteome differentially expressed by COPD patients in the course of the disease. The proteins recovered in EBC might be used to monitor respiratory diseases including COPD non-invasively in the future.

Metabonomics may be another important tool that can provide qualitative and quantitative information on low-molecular weight metabolites present in cells, tissues, and fluids. The EBC may provide a suitable matrix for metabonomic studies to investigate biochemical profiles of metabolites using nuclear magnetic resonance (NMR) spectroscopy. The use of nuclear magnetic resonance profiling of EBC may allow hypothesis-free profiling of biomarkers, rather than the traditional hypothesis-driven approach. It may now be possible to separate specific EBC profiles, with implication in disease phenotyping and personalised therapy. The application of NMR spectroscopy in study of EBC in COPD is going to evolve in future and at present very little published work is there.

CONCLUSIONS

Exhaled breath condensate analysis is an evolving non-invasive tool for monitoring of inflammation and oxidative stress in COPD. It may provide an insight into the complex pathways of the origin and perpetuation of airway inflammation in COPD. Potential clinical applications include diagnosis, monitoring of severity and evaluation of therapeutic interventions, phenotyping and prognostication. While studies so far have focused on measurements of levels of markers such as 8-isoprostane, hydrogen peroxide, products of NO, lipid mediators, eicosanoids and airway fluid acidification, this method of studying airway inflammation is still in its infancy. Evolving technologies of genomics, proteomics, and metabonomics may provide deeper and newer insights into the molecular mechanisms underlying the pathogenesis of COPD and identify targets for therapeutic interventions permitting specific interventions unlike the present treatment strategies that are largely symptomatic and supportive.


41. Liu J, Sandrini A, Thurston MC, Yates DH, Thomas PS. Exhaled breath condensate as a marker of oxidative stress in different exhalation flows may differentiate between bronchial and alveolar inflammation in patients with asthma and COPD. *Eur Respir J* 2002;20:174a.


Tracheal Diverticulum

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CLINICAL SUMMARY

A 26-year-old male, an occasional smoker without any preceding significant medical or surgical illness, presented to us with a one-year history of symptoms that were suggestive of recurrent respiratory tract infections associated with intermittent episodes of streaky haemoptysis. On physical examination, he was overweight with a body mass index (BMI) of 28 Kg/m² but no other remarkable findings.

INVESTIGATIONS

The haemogram and serum biochemistry parameters were in the normal range. Sputum smear examination for acid-fast bacilli was negative. Chest radiograph showed no abnormality. High resolution computed tomography (HRCT) of the chest (Figure 1) showed few areas of fibrosis in the left lower lobe and a diverticulum arising from the right posterolateral wall of upper trachea at the level of second/third dorsal vertebra. Fibreoptic bronchoscopy showed an opening in the right posterior wall of trachea (Figure 2). Two-dimensional echocardiography was within normal limits. Spirometry showed normal lung functions.

DIAGNOSIS

Tracheal diverticulum causing recurrent respiratory tract infections.

DISCUSSION

Paratracheal air cyst, as an entity, was first described by Rokitansky in 1838.1 Tracheal diverticulum is one of the differential diagnosis, others being a laryngocoele, a pharyngocoele, a Zenker’s diverticulum, an apical hernia of the lungs and apical bullae/blebs.2 Tracheal diverticulum is rare and usually found post-mortem.3 Katz et al4 described
four types of tracheal diverticuli-rudimentary bronchus, cystic dilatation of mucus gland duct, tracheocele and diverticulum associated with tracheobronchomegaly. Tracheal diverticuli may be congenital or acquired. Both the varieties are lined by ciliated columnar epithelium. The congenital variety, which is thought to represent a malformed supernumary branch of the trachea, has cartilaginous rings in its wall that are similar to the tracheal wall. These usually arise 4 to 5 centimeters below the true vocal cords, are relatively small and narrow-mouthed and may occur in isolation or in association with other congenital anomalies within the tracheobronchial tree. In the acquired variety, that is thought to be due to increased intra-luminal pressure causing out-bulging of a weak part in the tracheal wall, the wall is devoid of any cartilaginous rings. Another mechanism that has been proposed for its development is cystic distension of the mucous gland ducts. It can arise at any level and is wide-mouthed and larger in size. Majority of the tracheal diverticuli arise at the D2 vertebral level. The supportive presence of oesophagus and aortic arch on the left side of the tracheal wall makes it less susceptible to the development of diverticula explaining the preponderant right sided location of the diverticula. These patients are commonly asymptomatic. However, they may present with chronic productive cough, dyspnoea, stridor, haemoptysis and repeated episodes of tracheobronchitis. A small air cyst orifice is usually difficult to visualise on bronchoscopy. In previous studies, even in surgically proven tracheal diverticula, no mucosal orifices were detected on pre-operative bronchoscopy. Hence, a computed tomography of the trachea with reconstruction in the coronal plane is considered the needed modality for diagnosis. Since most of the tracheal diverticula are either asymptomatic or do not cause significant symptoms, a conservative symptomatic medical treatment with antibiotics, mucolytics and physiotherapy usually suffices and very rarely a surgical resection is required.

The duration of symptoms in our patient was only one year and no other anomaly of the tracheobronchial tree was found. Thus, it is most likely that the tracheal diverticulum was of an acquired variety. The patient was managed conservatively with physiotherapy and vaccinations to reduce the frequency of infections and prevent them.

REFERENCES

Bronchoscopic Management of Benign Bronchial Stenosis by Electrocautery and Balloon Dilatation

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ABSTRACT

Benign bronchial stenosis is managed by surgical or bronchoscopic methods. Although surgical approach is definitive, it is technically demanding and is costlier than bronchoscopic treatment. Here, we report the case of a 27-year-old female patient with symptomatic benign bronchial stenosis of the left main bronchus. The stenosis was dilated successfully through a fibreoptic bronchoscope by electrocautery followed by balloon bronchoplasty and application of mitomycin-C. On follow up, there was no evidence of re-stenosis. [Indian J Chest Dis Allied Sci 2012;54:41-43]

Key words: Flexible bronchoscope, Benign bronchial stenosis, Electrocautery, Balloon dilatation.

INTRODUCTION

Benign tracheobronchial stenosis in adults can be a complication of a variety of diseases/interventions, including tuberculosis (TB), sarcoidosis, Wegener’s granulomatosis, trauma, endotracheal intubation, tracheostomy, bronchial sleeve resection, irradiation, and fibrosing mediastinitis. Depending on the site of the lesion and severity of the narrowing, the stenosis may cause symptoms of dyspnoea, stridor, wheeze, cough, or recurrent respiratory tract infections. Definitive treatment of such stenoses is surgical resection and re-anastomosis. However, this may not be a feasible option in many patients because of poor general condition, compromised pulmonary functions, or technical difficulties.

A variety of bronchoscopic techniques, such as bougie or balloon dilatation (bronchoplasty), Nd-YAG laser resection, cryotherapy, electrocautery, and stent placement have been used as an alternative, if a surgical approach is not possible. In some cases, successful management of a strictured segment may require more than one modality or technique through the bronchoscope.

In the present report, we describe the use of a multi-modality treatment consisting of electrocautery, balloon bronchoplasty, and subsequent local application of mitomycin-C for the dilatation of a benign tracheobronchial stenosis.

CASE REPORT

A 27-year-old woman presented with complaints of recurrent episodes of cough with expectoration during the last four years. She had been hospitalised several times with complaints of high grade fever, productive cough, and breathlessness and treated each time with intravenous antibiotics and bronchodilators, to which she responded. She denied any history of TB, surgical intervention in the chest, or any other significant medical illness in the past.

On examination of the chest, breath sounds on the left side were reduced with localised wheeze on the same side. Chest radiograph showed deviation of the mediastinum to the left with crowding of ribs, indicating a loss of volume. The left hemithorax was hazy and showed multiple soft, nodular opacities with small calcific foci and thickening of the left apical pleura. A contrast-enhanced computed tomography (CT) of the thorax confirmed loss of lung volume on the left side with fibrosis and bronchiectasis of the left upper lobe. The left lower lobe was largely normal with minimal dilatation of a few bronchi. A reconstructed coronal image of the CT showed that the left main bronchus was narrowed with a 2.5cm long, narrow stricture; however, the distal bronchi were patent as shown in figure 1.

Routine investigations including complete blood counts, renal and liver function tests, and urine examination were in the normal range. Sputum smears for acid-fast bacilli, and smears and cultures for pyogenic organisms and fungi were also negative. An autoimmune screen for collagen vascular diseases...
and anti-neutrophil cytoplasmic antibodies were also negative.

This resulted in a significant dilatation of the stenosis to about 4mm. Linear patency of the distal bronchial tree was then checked by inserting a guide wire. The stricture was then dilated using a 6mm × 4cm long balloon (microinvasive mf/8-3/5/180). The balloon was passed through the suction channel of the bronchoscope. Correct placement of the balloon in the stricture segment was done under direct vision (Figure 3). The balloon was then inflated in the stricture segment with normal saline for one minute and this procedure was repeated once. Further dilatation was then done using a 10mm balloon for one minute.

A fibreoptic bronchoscopy was performed under sedation and local anaesthesia that revealed a very narrow opening (about 2mm) in the left main bronchus about 3cm from the carina. The bronchoscope could not be passed beyond the stenosis. The right sided bronchial segments were essentially normal. Dilatation of the stricture was planned during the same sitting. Initially, an electocautery probe was passed through the suction channel of the fibreoptic bronchoscope (Figure 2). Linear cuts were made on the walls of the stricture at 10 O’clock, 2 O’clock, and 6 O’clock positions with this probe under vision through the bronchoscope, and the stenotic opening was enlarged.

Following this a dilatation of about 9mm was achieved, allowing easy passage of the bronchoscope whose outer tip diameter was 5.8mm (Figure 4). The distal bronchi were found to be patent, though full of mucoid secretions. Bronchial secretions were sent for microbiological examination including direct smear and culture for Mycobacterium tuberculosis, and these were reported negative subsequently.
To prevent re-stenosis due to fibrosis, 3mL of 0.2mg/mL mitomycin-C was sprayed at the stricture site. After dilatation and thorough suctioning, the upper lobe, lingular lobe, and lower lobe openings were clearly visible. The upper lobe opening was also found to be narrowed, and it was dilated using a 6mm balloon. The patient tolerated the procedure well and was discharged the same day after a few hours of observation.

A second bronchoscopy done after a week confirmed that the patency of the dilated bronchi was maintained. Follow-up bronchoscopies were done at 3 and 7 months after the original procedure. These showed a well dilated left main bronchus with no signs of re-stenosis. The patient had mild cough and expectoration for a few days after the procedure, that responded to symptomatic treatment.

The cause of the bronchial stricture could not be ascertained in this patient where investigations failed to show any definitive aetiology.

**DISCUSSION**

Tracheobronchial strictures can be managed either by surgery or by employing a variety of endoscopic techniques. Surgical resection of the stenosis is an option but the associated risks, and the technical limitations of surgical resection and reconstruction warrant the need for other therapeutic options in many patients. Serial dilatation of the stenosis with a blunt-tipped rigid bronchoscope can be performed; but, initial dilatation of a tight stenosis as in our patient can prove difficult, especially if there is a mismatch between the stenosis and the diameter of the rigid bronchoscope. Moreover, the procedure requires general anaesthesia.5,10

Among the treatment options reported, treating such strictures using a flexible bronchoscope along with cautery/laser and balloon dilatation appears to be least invasive, quick, safe, and inexpensive, and it is associated with a low risk of complications. It can be done under local anaesthesia with conscious sedation. Post-procedure stay in the hospital is short and the patient can be discharged on the same day. The only drawback is a possible need for multiple sittings as serial dilatations may be required. Various studies have shown that more than 50% of patients treated with balloon dilatation may not need any other form of therapeutic intervention. Hence, it may reasonably be considered as the preferred option to restore the airway lumen in benign stenosis.1,2

In the case described here, re-stenosis did not occur, possibly due to the short length of the stricture as well as the use of mitomycin-C to decrease the fibrosis after the dilatation. Various studies have shown that topical application of mitomycin-C, a potent inhibitor of fibroblasts, reduces granulation tissue formation and prevents restenosis.11

Complications associated with dilatation are tearing of the bronchial wall due to excessive stretching, resulting in pneumothorax, pneumomediastinum, and subcutaneous emphysema. These complications can be avoided using cautery/Nd:YAG laser for cutting open the fibrotic stricture prior to balloon dilatation, as it avoids the need for excessively high pressures for dilating the balloon.12 This was done successfully in the present case.

To conclude, balloon dilatation along with electrocautery via a flexible bronchoscope is an effective and safe multi-modality approach for benign tracheal stenosis. It may be used as a preferred option to surgery or use of a rigid bronchoscope because of lower costs, avoidance of general anaesthesia and fewer complications. The application of mitomycin-C to the dilated segment immediately after the procedure appears to decrease the chances of re-stenosis.

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Tracheobronchial Compression by Right-sided Aortic Arch in a Middle Aged Male

Manohar Lal Gupta, Chand Bhandari, Mahesh Mishra and Jyotsna Sinha

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ABSTRACT

Exertional dyspnoea is a common symptom among middle-aged population. Diagnostic evaluation of such patients is often challenging and confusing. We report a patient presenting with exertional dyspnoea and an obstructive ventilatory defect on spirometry that was refractory to bronchodilator therapy. Careful review of the chest radiograph and spirometry pointed towards variable intra-thoracic airways obstruction as a cause of dyspnoea. Contrast enhanced computed tomography (CECT) of the thorax and bronchoscopy established the diagnosis of a right-sided aortic arch resulting in tracheobronchial compression and tracheomalacia. [Indian J Chest Dis Allied Sci 2012;54:45-47]

Key words: Tracheomalacia, Exertional dyspnoea, Variable intrathoracic airway obstruction, Right side aortic arch.

INTRODUCTION

Right-sided aortic arch with an aberrant origin of great vessels is a rare, but well recognised cause of tracheobronchial/oesophageal compression in infants and children. Such an anomaly is usually associated with some or other congenital cardiac defect(s) also. We encountered tracheobronchial compression secondary to a right-sided aortic arch in a middle-aged male who did not have other anomalies, such as, vascular ring, aberrant origin of great vessels, or congenital cardiac defect(s). Considering its rarity of occurrence and similarity of presentation of this condition with other common respiratory disorders, this case is being reported.

CASE REPORT

A 55-year-old, non-smoker male presented with progressive exertional dyspnoea for the last four years. Patient denied any history of fever, expectoration, haemoptysis, and/or chest pain. Patient had received inhaled corticosteroids, short-acting/long-acting beta-2 agonists with oral corticosteroids on few occasions during this period. None of these measures resulted in a significant relief in his symptoms.

Vital signs and general physical examination were within normal limits. Chest was barrel shaped with bilateral expiratory wheezing. Cardiac and abdominal examination were normal.

Routine blood counts, serum biochemistry and urine analysis were within normal limits. Arterial blood gas analysis (on room air) showed partial pressure of arterial oxygen: 68 mmHg; partial pressure of arterial carbon dioxide: 23.6 mm Hg; pH: 7.46; bicarbonate: 18.8 meq/L suggestive of compensated respiratory alkalosis. Chest radiograph postero-anterior view (Figure 1) showed absence of left aortic knuckle and narrowing of lower tracheal air column from the level of clavicle downwards. Both the lung fields showed evidence of over-inflation. Electrocardiogram and two dimensional echocardiography were within normal limits.

Figure 1. Chest radiograph (postero-anterior view) showing (A) absent left aortic knuckle shadow and narrowing of lower tracheal air column; and (B) left-sided aortic arch and normal tracheal lucency in a healthy adult.

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Spirometry (Figure 2) showed forced vital capacity (FVC) 2.07 L (90% of predicted), forced expiratory volume in first second (FEV1) 0.75 L (43% of predicted) with a percentage of FVC expired in first second (FEV1%) of 36.23. The configuration of the flow-volume loop, e.g., absence of sharp peak in expiratory loop and a squared-off appearance with various other indices of flow-volume graph, e.g., FEV1/ peak expiratory flow rate (PEFR) greater than 10 ml/L/min, forced inspiratory flow at the 50% forced vital capacity (FIF50) less than 100 L/min, FEV1/FIF50 (percentage of FVC expired in first 0.5 seconds) greater than 1.5 and FEF50/FIF50 of 0.4, favoured the diagnosis of variable intrathoracic airway obstruction.

Bronchoscopy was performed to visualise the extent and nature of intrathoracic airway obstruction. It was found that lower tracheal lumen including carina and the right main bronchus were narrowed due to posterolateral compression. During expiration a near complete occlusion was seen, suggestive of dynamic compression and tracheomalacia.

DISCUSSION

Clinico-radiological findings of this patient are similar to any patient with chronic obstructive airways disease (COAD). Severe airway obstruction on spirometry further supports the diagnosis of obstructive airways disease. In the present case, compression by the right-sided aortic arch and right descending aorta resulted in tracheomalacia of lower part of trachea resulting in variable intrathoracic airway obstruction. Right-sided aortic arch is widely recognised as a cause of tracheobronchial and oesophageal obstruction in infants and children, when it forms a complete vascular ring.1,2 Occasionally this can also be seen in adult patients.3,4 However, right-sided aortic arch resulting in tracheobronchial obstruction in the absence of the vascular ring is exceedingly rare. Isolated case reports of the right-sided aortic arch with aberrant anomalous left innominate artery5 or an aberrant left subclavian artery6 causing airways obstruction have been reported. Gidding et al7 have reported airway compression by right aortic arch in the absence of vascular ring in a six-month old infant with tetralogy of Fallot. In our patient, compression of lower part of the trachea and the right main bronchus occurred due to aortic arch descending aorta and there were no aberrant arteries and /or a vascular rings to cause
airways obstruction. This case highlights the need for critical analysis of chest radiograph and flow-volume curve in a patient presenting with exertional dyspnoea. Diagnosis of COAD should not be considered on volume-time graph alone. A careful analysis of both volume-time graph and flow-volume curve are needed to exclude intra-thoracic airway obstruction mimicking COAD.

To the best of our knowledge, this is the first case of its kind documenting a right-sided aortic arch without vascular ring as a cause of airways compression in a middle-aged patient. Possible mechanisms of delayed onset of symptoms in our patient include atherosclerotic changes in vessel with advancing age\(^5\) and progressive tracheomalacia due to prolonged compression.\(^8\)

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Giant Solitary Fibrous Tumour of the Pleura

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ABSTRACT

Solitary fibrous tumour of the pleura is a rare primary pleural neoplasm. These tumours are usually asymptomatic and are incidentally detected. Majority of these neoplasms are benign and surgical excision provides excellent results. With the widespread use of imaging and better diagnostic criteria, this tumour is likely to be detected more frequently. We encountered a patient with a giant solitary fibrous tumour of the pleura. In this report, we describe the case of a patient with a giant solitary fibrous tumour of the pleura, review the literature and present the details of management of this patient. [Indian J Chest Dis Allied Sci 2012;54:49-52]

Key words: Pleural, Localised mesothelioma, Solitary fibrous tumour.

INTRODUCTION

Solitary fibrous tumours of the pleura (SFTP) are rare neoplasms of mesenchymal origin. Most of the tumours are slow growing, benign, pedunculated and arise from the visceral pleura.¹ Most of the patients are asymptomatic and the presence of the tumour is usually incidentally detected. Though the number of cases reported is limited, it appears that surgical excision provides good results.² We present our experience with a giant SFTP, outline our management and review the literature.

CASE REPORT

A 39-year-old female presented with a swelling in her neck. She was diagnosed to have a diffuse colloid goiter. However, during work-up, her chest radiograph showed a large mass lesion in the right chest. She was referred to our institute for further investigation and management.

On evaluation, the patient had no respiratory symptoms or significant past illness. She had no pallor, clubbing, pedal oedema or lymphadenopathy. Respiratory examination revealed dullness in the right lower hemithorax with decreased air entry. Haemogram and liver function tests were normal. Sputum examination for acid-fast bacilli was negative. The chest radiograph (Figure 1) showed a large homogeneous mass lesion in the right lower zone.

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vascularity (Figures 2 and 3). An image guided fine needle aspiration cytology of the thoracic mass showed a few neoplastic round to spindle cells with marked nuclear atypia and scanty cytoplasm. An image guided core-needle biopsy showed predominantly fibrovascular connective tissue with one of the cores showing atypical oval to spindle cells with vesicular nuclei arranged in a diffuse manner.

Giant Solitary Fibrous Tumour of the Pleura R. Burrah et al was arising from the visceral pleura and partly adherent to the right lower lobe. Complete surgical excision along with wedge resection of the right lower lobe was done. The patient had an uneventful recovery and was discharged on the ninth post-operative day.

No native lung parenchyma was seen. On immunohistochemistry (IHC), the neoplastic cells were positive for calretinin and CD34, and negative for cytokeratin and smooth muscle antigen (SMA). A provisional pre-operative diagnosis of SFTP was considered and the patient was worked up for surgery. A right thoracotomy showed a solid, well-encapsulated, non-homogeneous and pedunculated tumour measuring 25cm × 15cm (Figure 4). The mass was arising from the visceral pleura and partly adherent to the right lower lobe. Complete surgical excision along with wedge resection of the right lower lobe was done. The patient had an uneventful recovery and was discharged on the ninth post-operative day.

The histopathological examination revealed the tumour to be mostly composed of spindle cells in the background of dense collagenous stroma (Figure 5). There were no significant nuclear pleomorphisms or mitosis. These features suggested that the mass was of mesenchymal origin and was benign in nature. The adjacent lung parenchyma was compressed but free of tumour. The IHC of the specimen was positive for calretinin, CD34, CD99 and BCL-2 and negative for cytokeratin and SMA (Figure 5).

Figure 4. Intra-operative picture showing a large lobulated vascular mass adherent to the lower lobe of the right lung.

Figures 2 and 3. Computed tomography of chest showing a heterogeneous soft tissue mass in the right hemithorax.

Figure 5. Photomicrograph of the solitary fibrous tumour showing: (A) typical architecture composed of alternating hypocellular (upper left) and hypercellular (lower right) areas (Haematoxylin and eosin ×40); (B) benign spindle cells separated by thick bands of keloid like collagen (Haematoxylin and eosin ×100); (C) diffuse positivity for CD34, DAB (×40); and (D) positivity for MIC2/CD99, DAB (×200).
Diagnosis of an SFTP was offered and a desmoplastic mesothelioma was suggested as a differential diagnosis. The patient has been on regular follow-up for the past two years and has no evidence of recurrence (Figure 6).

Figure 6. Follow-up chest radiograph (postero-anterior view) showing no evidence of recurrence.

DISCUSSION

Primary tumours of the pleura can broadly be divided into diffuse and localised types. Diffuse tumours are usually associated with asbestos exposure, are commonly malignant, and hence, carry a poor prognosis. On the contrary, the localised pleural tumours now also known as SFTP, have no association with environmental factors, are usually benign and carry a good prognosis. SFTPs are rare, accounting for 5% of all pleural neoplasms, and only about 900 cases have been reported in the literature.

SFTP was first described as a separate pathological entity by Wagner in 1870, but it was Klemperer and Rabin in 1931 who suggested that these tumours were of a submesothelial rather than of mesothelial origin. SFTP in the past has been referred to by various terms including localised mesothelioma, fibrous mesothelioma, solitary fibrous mesothelioma, submesothelial fibroma etc to name a few.

SFTP commonly presents in the sixth decade of life and most series suggest an equal predilection between the genders. All large series in the literature report that up to 50% of patients were asymptomatic and diagnosed incidentally. Patients usually become symptomatic because of large tumours or malignant neoplasms. Imaging investigation of choice appears to be contrast enhanced CT of the thorax. Though Cardillo et al suggest that benign nature of these tumours can be made out on CT, this is not accepted in the published reports. Image guided FNAC has low sensitivity in most series and certain centres do not recommend their use in operable lesions. The objective method of differentiating SFTP from diffuse tumours is by IHC. SFTP stains positive for CD34, CD99, BCL-2 and stains negative for cytokeratin.

The mainstay of treatment appears to be surgical resection with preservation of as much lung parenchyma as possible. Frozen section examination is recommended to ensure the completeness of the resection. Smaller lesions are amenable to resection by video assisted thoracoscopic surgery (VATS). Giant SFTPs are usually vascular, with adhesions and requires a thoracotomy to assist their removal. Guo et al have reported their experience with pre-operative embolisation of these lesions to ensure decreased bleeding during their extirpation and were successful in all their five cases. Though there have been reports of adjuvant therapies for malignant and recurrent tumours their role remains undefined because of the rarity of these tumours. Benign tumours have a very good outcome with recurrence rates of less than 8%. The malignant tumours, on the other hand, have a high recurrence rate with most deaths occurring within 24 months.

Our patient was asymptomatic and incidentally detected to have a large thoracic mass. The pre-operative core biopsy of the lesion was suggestive of SFTP. Because of the large size and increased vascularity, a conventional thoracotomy and excision of the mass was performed. The pathological examination was suggestive of a benign mesenchymal lesion and the IHC confirmed the diagnosis to be SFTP.

SFTPs are rare primary localised neoplasm of the pleura. Majority of the lesions are benign. These are usually asymptomatic and definitive pre-operative diagnosis may not be possible in most of the patients. Surgery appears to be the best treatment modality and can result in cure in majority of the patients. With the widespread use of imaging and better investigational modalities, such as, IHC and application of diagnostic criteria, these tumours are likely to be detected more frequently. Therefore, the thoracic surgeon should be aware about SFTPs and consider them in the differential diagnosis of soft tissue tumours of the thorax.

REFERENCES


Pulmonary Disease due to *Mycobacterium massiliense*

Subhra Mitra¹, S. Roy Tapadar¹, D. Banerjee¹, S. Bhattacharjee², Sunanda Dey³ and S. Kundu²

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**ABSTRACT**

We report a case of a patient suffering from multidrug-resistant pulmonary tuberculosis (MDR-PTB) who later developed an invasive infection of the respiratory tract with a rapidly growing non-tuberculous mycobacteria (NTM), recently identified as *Mycobacterium massiliense*, closely related to *M. abscessus*. To the best of our knowledge, this is the first case of *M. massiliense* infection being reported from India. [Indian J Chest Dis Allied Sci 2011;53:53-57]

Key words: *Mycobacterium massiliense*, Non-tuberculous mycobacteria, Rapidly growing mycobacteria.

**INTRODUCTION**

*Mycobacterium massiliense*, a newly-described species of rapidly growing mycobacteria (RGM), is closely related to the *Mycobacterium chelonae–Mycobacterium abscessus* group.¹ First identified by Adekambi *et al*,¹ the name pertaining to Massilia, the Latin name of Marseille, where the organism was isolated. Since its first recovery from lung secretions in 2004,¹ it has been isolated from blood,²,³ intramuscular injection sites⁴ and surgical wounds.²,⁵ It has been implicated as an invasive pathogen, a claim also supported by its close relation to *M. abscessus*, a known pulmonary pathogen.

**CASE REPORT**

A 60-year-old woman presented with a 10-year history of cough and mucoid/mucopurulent sputum along with occasional haemoptysis. Shortness of breath had developed over the last two years. She received anti-tuberculosis treatment (ATT) in various courses in 2000, 2002 and 2003. She stayed in a Sanatorium from November 2004 to November 2006 where she was empirically treated with second-line anti-tuberculosis drugs.

Past history showed sputum-smear positive for acid-fast bacilli (AFB) in 2000, 2002, 2003 and 2004 and culture for *Mycobacterium tuberculosis* positive in 2002 and 2003. Details of mycobacterial isolation and treatments are presented in table 1. From January 2007 onwards, she was empirically treated with second-line anti-tuberculosis drugs.

This time, she was again found to be sputum-smear positive and culture positive for *M. tuberculosis* by BACTEC 460 TB method. Drug sensitivity testing showed the growth to be resistant to rifampicin, isoniazid and pyrazinamide. She was prescribed injection kanamycin, together with ofloxacin, ethionamide, ethambutol, cycloserine and PAS according to the drug susceptibility report and was admitted for the initial period of therapy. She was found culture negative for *M. tuberculosis* in September 2007. Kanamycin was stopped after eight months of therapy and oral anti-tuberculosis drugs were continued. She remained sputum-smear negative for AFB, attended the outpatients less regularly but reportedly continued her anti-tuberculosis drugs.

A re-evaluation in April 2009, prompted by recurrent symptoms, found her to be sputum-smear positive for AFB on two occasions. Culture for *M. tuberculosis* by the BACTEC 460 radioactive method grew non-tuberculous mycobacteria (NTM). *Mycobacterium* speciation by deoxyribonucleic acid (DNA) sequencing yielded *Mycobacterium massiliense*. Anti-tuberculosis drugs were stopped (her last anti-tuberculosis drugs course ran for two years and two months) and she was kept under observation. She remained sputum-smear positive for AFB and a sputum culture for mycobacteria grew NTM again in November 2009.

A repeat culture in April 2010 by BACTEC MGIT 960 (Fluorescent technology) grew RGM and *Mycobacterium* speciation yielded *M. massiliense*. This time a drug sensitivity study was done (Table 2). BACTEC drug susceptibility for *M. tuberculosis* was determined by following the modified proportion method. The critical proportion for resistance was taken as 1% for all the anti-tuberculosis drugs.

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The identification of isolate as NTM in April 2009 was done by BACTEC 460 and PNB method (sensitive/resistant to p-nitrobenzoic acid). Speciation of NTM was done by DNA sequencing utilising 16S rRNA and hsp65 gene targets, and the bacteria was identified as *Mycobacterium massiliense*. Routine susceptibility testing for treatment of RGM is recommended.

Drug susceptibility of NTM was done by broth microdilution method (MIC testing). Susceptibility testing and subsequent result interpretations are as per CLSI guidelines M24-Aa.

There was a weight loss of 10 Kg since the start of her illness. Her nutritional status was poor with a body mass index of 10 (weight 24 Kg). She was pale and had clubbing with bilateral, scattered, coarse, mid-to-late inspiratory crackles on chest examination. On investigation, haemoglobin was 10.9 g/dL and erythrocyte sedimentation rate was 22mm in the first hour and white blood cell count was 7900/mm³. Blood sugar, urea, serum creatinine, liver function and thyroid function tests were in the normal range. Serology for human immunodeficiency virus (HIV) was negative and CD4+ count was 253/mL.
A recent chest radiograph (postero-anterior view, Figure 1) (May 2010) showed fibrotic lesions with thin-walled cavities in both the lung fields. Compared with the radiograph done a year earlier, there was some increase in the size of the cavities. High resolution computed tomography of the thorax (Figure 2) in November 2009 showed bilateral tubular bronchiectasis and scattered fibrosis with thin-walled cavities in the right upper and middle and left lower lobes.

From July 2010 onwards, the patient was treated with injection amikacin and oral doxycycline, ofloxacin, clarithromycin (based on drug susceptibility report) on a body weight basis. After institution of therapy, there was a decrease in the volume, purulence and blood streaking of the sputum. Temperature remained in normal range but she developed tinnitus and vertigo one month later, requiring withdrawal of amikacin. Sputum-smear for AFB was negative at the end of the first and second months of therapy. On a review in March 2011, after 9 months of therapy, she had minimal symptoms, an increase in weight of 7 Kg, and was sputum-smear negative for AFB. Chest radiograph (PA view, Figure 3) showed fewer infiltrations and diminished size of the cavities in both the lung fields.

**DISCUSSION**

Currently, more than 125 NTM species have been catalogued, an increase from approximately 50 in 1997. The partial 16S rRNA gene sequence analysis is the most widely-used method for identification of NTM and has led to the description of 40 new species since 1992.

In a developing country like India, tuberculosis (TB) is a major health problem though NTMs are also being increasingly reported as causative agents for human infections. While Jesudason and Gladstone from south India reported an NTM isolation rate of 3.9%, Chakrabarthy et al from Chandigarh documented an NTM isolation rate of 7.4% from various clinical specimens. *M. fortuitum* and
M. chelonae were the commonest isolates in these studies.

Chronic pulmonary disease is the most common clinical manifestation of NTM and it reflects the form of disease and co-morbidities rather than the species of NTM involved.15 Our patient, an elderly thin-built woman, had prior TB, bronchiectasis and possibly gastro-oesophageal reflux disease as facilitating co-morbidities and presented clinico-radiologically as chronic pulmonary TB.

The radiographic features of NTM may be primarily fibrocavitary, similar to pulmonary TB, or characterised by nodules and bronchiectasis.8 The cavities are thin-walled with greater pleural reaction and lesser surrounding parenchymal opacities than that seen in classic pulmonary TB.9,13

Isolation of NTM in culture is essential for the diagnosis of NTM lung disease. However, since the NTM are ubiquitous and may contaminate respiratory specimens, two or more positive culture results for NTM from separate expectorated sputum samples are required to make the microbiologic diagnosis clinically significant as per the American Thoracic Society guidelines.9 In our patient, sputum culture was thrice positive for NTM and DNA speciation done twice yielded M. massiliense.

Colonisation without infection (i.e., no tissue invasion) is an unproven condition for NTM,9 some preferring the term ‘indolent disease’ in that it may not be evident that NTM are causing progressive disease unless the patient is followed up for several years. The significance of NTM isolation from a patient during therapy for pulmonary TB is uncertain.9 Co-existence of M. tuberculosis and NTM has been reported in both HIV-1 infected and non-infected patients.14 In these situations NTM may be present only as a saprophyte (coloniser),14 but both may also simultaneously cause disease requiring specific treatments for the two mycobacteria.15

Persistent symptoms with repeated isolation of the same pathogenic NTM species from respiratory specimens and progressive radiologic changes suggested disease rather than colonisation,16 prompting us to treat our patient after a year of observation.

Rapidly growing mycobacteria are uniformly resistant to the “standard” anti-tuberculosis drugs and antibiotic susceptibility testing is, therefore, recommended for all clinically significant isolates. The treatment of the present patient was based on the drug-susceptibility report.

Koh et al17 compared the clinical features and treatment outcomes between patients with M. abscessus and those with M. massiliense lung disease. The clinical and radiographic manifestations of disease caused by both species were similar. Response rates to combination antibiotic therapy including clarithromycin were higher in patients with M. massiliense than in those with M. abscessus lung disease.17 Inducible resistance to clarithromycin found in M. abscessus isolates may explain this lack of efficacy.17

In our patient, sputum-smear conversion occurred after one month of therapy and has remained negative at eight months follow-up with a significant clinico-radiologic improvement.

Non-tuberculosis mycobacteria may be an under-recognised cause of AFB sputum positivity, especially in patients who have received treatment several times or have developed MDR-TB.

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Regression Equations for Spirometry in Children Aged 6 to 17 Years in Delhi Region

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Departments of Cardio-respiratory Physiology1, Former Director2 and Biostatistics3, Viswanathan Chest Hospital, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

ABSTRACT

Background. Most of the studies carried out in India to develop regression equations for spirometry in children are now several years-to-decades old and had used equipment and measurement protocols that have since changed. Prediction equations using the current standardisation protocols for spirometry are not available. The lung health of the population may have changed too.

Objective. To develop regression equations for spirometry for children aged 6 to 17 years of north Indian origin in Delhi region.

Methods. School children of north Indian origin, as determined by mother tongue and parentage, aged 6 to 17 years were screened by a health questionnaire and physical examination and those found “normal” underwent spirometry according to the standardised procedure recommended by the American Thoracic Society/ European Respiratory Society (ATS/ERS) task force in 2005. Pearson’s correlation analysis was carried out to identify the predictor variables for spirometric parameters. Prediction equations were developed using the multiple linear regression procedure. The independent variables were entered in sequence of height, age and weight. R2, adjusted R2 and R2 change, standard errors of the estimate (SEE), and estimates of regression coefficients were obtained and the goodness of fit was examined.

Results. Data was obtained in 365 boys and 305 girls. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), peak expiratory flow rate (PEFR), forced expiratory flow rate at 50% and 75% exhalation of vial capacity (F50 and F75) and mean forced expiratory flow rate over the middle 50% of the vital capacity (F25-75) showed moderate to strong correlations with age, height and weight in both boys and girls. In both genders, the equations explained very high variability of FVC, FEV1 and PEFR as shown by the R2 values. The explained variability for flow rates was lesser, with that for F75 being the least.

Conclusions. Regression equations for spirometry variables for children of north Indian origin in Delhi region have been developed. These represent the first such effort from India after the publication of the ATS/ERS task force 2005 guidelines on standardisation of spirometry. [Indian J Chest Dis Allied Sci 2012;54:59-63]

Key words: Pulmonary function, Spirometry, Normals, Children, Delhi, Regression equations.
use of locally developed prediction equations. Equations using the current standardisation protocols for spirometry have not been developed in India. Ethnic variations in pulmonary function are well known. This precludes development of a single equation for all Indians. There is an urgent need to develop these equations for different regions of the country. Delhi is a cosmopolitan city in the northern region of the country. We, therefore, carried out a study to develop regression equations for spirometry in children aged 6 to 17 years of north Indian origin in Delhi region. We present here a brief communication of the study carried so that these can be applied in practice at the earliest. A detailed evaluation and comparative analysis with previous Indian studies as well as with equations from other countries will be presented subsequently.

MATERIAL AND METHODS

The study was approved by the Institutional Ethics Committee. It was carried out in children in a school selected randomly from a list of schools in Delhi. The school caters to a wide area of Delhi and the children are drawn from a very wide socio-economic spectrum. Hence, it approximates a representative sample. All the children from ages 6 to 17 years were eligible for inclusion provided they had been in Delhi for more than half of their life. Additionally, the mother tongue was required to be Hindi, Urdu or Punjabi, the predominant languages in this region, and both parents had to be of north Indian origin to limit the confounding effects of ethnic variation. A demonstration of the testing procedure was given to familiarise the children with pulmonary function testing. For each age, a target of 15 to 20 boys and girls with technically acceptable tests was set and consecutive eligible children were included till the sample requirement was met. This number was based on statistical considerations of numbers required for linear regression procedures, previously published studies1-9 and feasibility.

A questionnaire was designed in English and Hindi was answered by either parent. It had several sections as follows: cover letter from Principal, information sheet, consent form and questionnaire about the health status. Children with completely filled-up questionnaires with written consent for participation were included. Those judged to be “normal” from the questionnaire responses were again examined by the investigators in the school to confirm that they were eligible for inclusion. The exclusion criteria included current or past history of any chronic respiratory, cardiac or other system disease, thoracic cage abnormality, any current acute illness, recent chest infection (within 6 weeks), current smokers or with environmental tobacco smoke exposure, and unwilling parents/children.

Age in completed years, gender, standing height to the nearest centimeter without shoes and weight rounded off to the nearest kilogram were recorded at the time of the testing. Weight was measured wearing light clothing and no footwear to the nearest 0.5 Kg using an electronic scale that was calibrated on a weekly basis with known weights. Height was measured with the children standing erect with head in the Frankfurt plane and ankles pressed against a wall on which a measuring tape had been fixed.

Spirometry was carried out in accordance with the recommendations of the ATS/ERS task force.10 The spirometer with a heated Lily screen pneumotach (Medisoft Micro 5000, Belgium) was calibrated daily. The manoeuvres were performed in the sitting position with a nose-clip applied. The children was asked to inhale completely and rapidly with a pause of <1 s at total lung capacity (TLC) and exhale with maximum force until no more air was expelled out while maintaining an upright posture. At the completion of expiration and on signal from the technician, the subject was asked to inhale completely. The manoeuvres was monitored on the computer screen. Throughout the procedure, loud verbal encouragement was given to obtain the expiratory and inspiratory manoeuvres completely with maximal force. The procedure was monitored for compliance with the acceptability and repeatability criteria recommended by the ATS/ERS task Force.10 At least three acceptable and two repeatable efforts were obtained.

The highest values of FVC, FEV1, and PEFR were selected. The flow rates, forced expiratory flow rate at 50% and 75% exhalation of vital capacity (F50 and F75) and mean forced expiratory flow rates over the middle 50% of the vital capacity(F25-75) were obtained from the “best” curve, i.e. the one with the highest sum of FVC and FEV1.

Statistical Analysis

Statistical analysis was carried out using statistical package for the social sciences (SPSS) 16.0 and GraphPad Prism 4.01 softwares. Analysis was carried out separately in boys and girls. In the present study, the dependent variables were FVC, FEV1, PEFR, F50, F75, and F25-75. Pearson’s correlation analysis was carried out to identify the predictor variables. Prediction equations were developed using the multiple linear regression procedure. Linear and non-linear models were developed and the former was selected based on criteria of simplicity and ease of clinical application, high predictive capability (R2) and yield of smallest residuals.

The independent variables were entered in the sequence of height, age and weight. Models were constructed including one (height), two (height and
age) and three predictor variables (height, age and weight) and \( R^2 \), adjusted \( R^2 \) and \( R^2 \) change on entering each predictor variable were calculated for each model. If the \( R^2 \) change at each step was significant and there was a substantial improvement in the predictive ability, the model was accepted. If not, the model at the previous step was accepted. Standard errors of the estimate (SEE) were calculated and analysis of variance was carried out for each model to evaluate the significance of the regression equation. Estimates of regression coefficients were obtained and their significance was determined by student’s ‘t’ test.

The goodness of fit was examined by testing for independence of predictor variables (by examining the tolerance statistics and VIF values), normality and equal variances of the residuals (by residuals normality plot), and linear type relationship between the predictors and the outcome variable (by Q-Q plots). Unusual and influential observations were examined. These included outliers, points with high leverage and high influence. Analysis was repeated excluding these observations to determine their impact on the models and the original models were retained if the effect on the equations was small and inconsequential.

**RESULTS**

Acceptable data was obtained in 365 boys and 305 girls. The ages and the anthropometric data (mean±SD) in the two genders were respectively: age (years), 11.53±3.37 and 11.74±3.23 (p>0.05); height (m), 1.49±0.18 and 1.45±0.14 (p<0.01); weight (Kg), 44.56±18.42 and 40.97±13.82 (p<0.01); and body mass index (Kg/m²) 19.04±4.43 and 18.75±3.91 (p>0.05). The age distribution is shown in table 1. The median, (range) and IQR in boys were as follows: age (years): 11, (6 to 17), IQR: 9 to 14; height (m): 1.5, (1.09 to 1.91), IQR: 1.35 to 1.65; weight (Kg): 44, (15 to 98), IQR: 28 to 58. The median, (range) and IQR in girls were as follows: age (years): 11, (6 to 17), IQR: 9 to 15; height (m): 1.48, (1.09 to 1.7), IQR: 1.35 to 1.58; weight (Kg): 41, (15 to 78), IQR: 30 to 51.

The pulmonary functions of boys and girls were compared for the entire sample (Table 2). FVC, FEV\(_1\) and PEFR were significantly higher among boys. However, the flow rates, F\(_{50}\), F\(_{75}\) and F\(_{25-75}\) were not significantly different. Table 3 shows the correlations of FVC and FEV\(_1\) with age, height and weight in boys and girls. Table 4 shows the correlations of PEFR, F\(_{25-75}\), F\(_{50}\) and F\(_{75}\) with age, height and weight in boys and girls. The regression equations developed are given in table 5. The equation is expressed as follows:

\[
\text{Spirometric parameter} = \text{Constant} + (\beta \text{ Coefficient for height x height in cm}) + (\beta \text{ Coefficient for age x age in years}) + (\beta \text{ Coefficient for weight x weight in Kg})
\]

The SEE is provided for calculation of the lower limits of normal. As FEV\(_1\)/FVC was found to be independent of age and height in boys, and had only a weak correlation with age in girls, no equations were developed for this parameter.

### Table 1. Age distribution of the children

<table>
<thead>
<tr>
<th>Age (in years) (n)</th>
<th>Boys (n=365)</th>
<th>Girls (n=305)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>26</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>17</td>
<td>24</td>
<td>22</td>
</tr>
</tbody>
</table>

### Table 2. Pulmonary function distribution in boys and girls for the whole sample

<table>
<thead>
<tr>
<th>Spirometry Parameter</th>
<th>Gender</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Range</th>
<th>Interquartile Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>Boys</td>
<td>2.88±1.09*</td>
<td>2.61</td>
<td>1.09 to 6.40</td>
<td>2.03 to 3.71</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>2.42±0.72</td>
<td>2.41</td>
<td>0.83 to 4.320</td>
<td>1.84 to 2.93</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>Boys</td>
<td>2.43±0.94*</td>
<td>2.24</td>
<td>0.92 to 5.24</td>
<td>1.66 to 3.19</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>2.14±0.65</td>
<td>2.14</td>
<td>0.81 to 3.96</td>
<td>1.60 to 2.64</td>
</tr>
<tr>
<td>PEFR</td>
<td>Boys</td>
<td>5.47±2.02*</td>
<td>5.04</td>
<td>1.75 to 12.00</td>
<td>3.90 to 6.86</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>5.00±1.46</td>
<td>4.97</td>
<td>2.05 to 9.92</td>
<td>3.94 to 6.05</td>
</tr>
<tr>
<td>F(_{25-75})</td>
<td>Boys</td>
<td>3.04±1.10*</td>
<td>2.82</td>
<td>0.97 to 6.60</td>
<td>2.21 to 3.70</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>2.97±0.91</td>
<td>2.89</td>
<td>1.24 to 5.46</td>
<td>2.29 to 3.56</td>
</tr>
<tr>
<td>F(_{50})</td>
<td>Boys</td>
<td>3.22±1.24*</td>
<td>2.86</td>
<td>1.04 to 7.13</td>
<td>2.32 to 3.99</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>3.17±1.04</td>
<td>3.02</td>
<td>1.00 to 5.82</td>
<td>2.33 to 3.99</td>
</tr>
<tr>
<td>F(_{75})</td>
<td>Boys</td>
<td>1.45±0.75*</td>
<td>1.22</td>
<td>0.34 to 4.43</td>
<td>0.92 to 1.84</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>1.52±0.66</td>
<td>1.41</td>
<td>0.33 to 3.86</td>
<td>1.00 to 1.92</td>
</tr>
</tbody>
</table>

FVC=Forced vital capacity; FEV\(_1\)=Forced expiratory volume in one second; PEFR=Peak expiratory flow rate; F\(_{25-75}\)=Mean forced expiratory flow rate over the middle 50% of the vital capacity; F\(_{50}\) and F\(_{75}\)=Forced expiratory flow rates at 50% and 75% exhalation of vital capacity; *=p<0.001; **=p<0.01; ns=Not significant, p>0.05
Table 3. Pearson’s correlation coefficients (r) for FVC and FEV₁ with age, height and weight

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FVC</td>
<td>FEV₁</td>
<td>FVC</td>
<td>FEV₁</td>
</tr>
<tr>
<td>Age</td>
<td>r=0.89</td>
<td>p&lt;0.0001</td>
<td>r=0.85</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Height</td>
<td>r=0.92</td>
<td>p&lt;0.0001</td>
<td>r=0.88</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Weight</td>
<td>r=0.86</td>
<td>p&lt;0.0001</td>
<td>r=0.84</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4. Pearson’s correlation coefficients (r) for PEFR, F₂5-75, F₅₀ and F₇₅ with age, height and weight

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEFR</td>
<td>F₂5-75</td>
<td>F₅₀</td>
<td>F₇₅</td>
</tr>
<tr>
<td>Age</td>
<td>r=0.87</td>
<td>p&lt;0.0001</td>
<td>r=0.77</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Height</td>
<td>r=0.87</td>
<td>p&lt;0.0001</td>
<td>r=0.69</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Weight</td>
<td>r=0.78</td>
<td>p&lt;0.0001</td>
<td>r=0.60</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 5. Regression equations for spirometry parameters

<table>
<thead>
<tr>
<th>Spirometry Parameter</th>
<th>Constant</th>
<th>β Coefficient for Height</th>
<th>β Coefficient for Age</th>
<th>β Coefficient for Weight</th>
<th>Standard Error of Estimate</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>-3.226</td>
<td>0.032</td>
<td>0.066</td>
<td>0.014</td>
<td>0.394</td>
<td>0.872</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-2.763</td>
<td>0.026</td>
<td>0.080</td>
<td>0.008</td>
<td>0.360</td>
<td>0.856</td>
</tr>
<tr>
<td>PEFR</td>
<td>-5.043</td>
<td>0.050</td>
<td>0.267</td>
<td>0.926</td>
<td>0.790</td>
<td></td>
</tr>
<tr>
<td>F₂5-75</td>
<td>-2.704</td>
<td>0.030</td>
<td>0.111</td>
<td>0.620</td>
<td>0.686</td>
<td></td>
</tr>
<tr>
<td>F₅₀</td>
<td>-2.540</td>
<td>0.027</td>
<td>0.145</td>
<td>0.772</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td>F₇₅</td>
<td>-1.617</td>
<td>0.014</td>
<td>0.081</td>
<td>0.537</td>
<td>0.491</td>
<td></td>
</tr>
<tr>
<td><strong>Girls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>-1.733</td>
<td>0.019</td>
<td>0.073</td>
<td>0.015</td>
<td>0.291</td>
<td>0.840</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-1.633</td>
<td>0.017</td>
<td>0.078</td>
<td>0.010</td>
<td>0.256</td>
<td>0.847</td>
</tr>
<tr>
<td>PEFR</td>
<td>-2.474</td>
<td>0.030</td>
<td>0.211</td>
<td>0.014</td>
<td>0.771</td>
<td>0.725</td>
</tr>
<tr>
<td>F₂5-75</td>
<td>-2.662</td>
<td>0.032</td>
<td>0.082</td>
<td>0.573</td>
<td>0.603</td>
<td></td>
</tr>
<tr>
<td>F₅₀</td>
<td>-1.229</td>
<td>0.017</td>
<td>0.118</td>
<td>0.708</td>
<td>0.544</td>
<td></td>
</tr>
<tr>
<td>F₇₅</td>
<td>-1.791</td>
<td>0.018</td>
<td>0.060</td>
<td>0.504</td>
<td>0.431</td>
<td></td>
</tr>
</tbody>
</table>

Equation: Spirometric parameter = Constant + (β Coefficient for height x height in cm) + (β Coefficient for age x age in years) + (β Coefficient for weight x weight in Kg)

Descriptive statistics for FEV₁/FVC is presented in table 6. The 5th percentile can be used to define the lower limit of normal.

Table 6. Descriptive statistics for FEV₁/FVC ratio

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean±SD</th>
<th>5th percentile</th>
<th>50th percentile</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>84.41±5.86</td>
<td>73.96</td>
<td>84.57</td>
<td>94.11</td>
</tr>
<tr>
<td>Girls</td>
<td>88.45±5.86</td>
<td>79.24</td>
<td>88.48</td>
<td>97.32</td>
</tr>
</tbody>
</table>

DISCUSSION

We have presented prediction equations for various spirometry parameters for children of north Indian origin in Delhi region between the ages of 6 to 17 years. These are derived from spirometry carried out as per the current standardised protocol recommended by the ATS/ERS task force. The FVC, FEV₁, PEFR, F₁₀, F₇₅, and F₂₅-₇₅ showed moderate to strong correlations with age, height and weight in both boys and girls. The FEV₁/FVC was not correlated with age or height in boys, and with height in girls, though it showed a weak correlation with age in the latter.
In both boys and girls, the equations explained very high variability of FVC, FEV1 and PEFR as shown by the $R^2$ values. However, the explained variability for flow rates was lesser, with that for $F_{50}$ being the least. The very high values for $R^2$ for FVC and FEV1 are also the reason why the interpretation algorithms for spirometry are based exclusively on these two parameters. The FVC and FEV1 are the only parameters for which reproducibility criteria have been defined and the flow rates have less precision. Effects of environmental exposures including environmental tobacco smoke and outdoor air pollution, physical activity and conditioning, nutritional factors, genetics and respiratory infections are extremely difficult to capture and therefore never factored in any model. In addition, pulmonary function has inherent variability on repeated testing. Therefore, pulmonary function parameters usually have a high standard deviation and consequently wide range of 95% confidence limits for defining normalcy.

It is remarkable that among all the predictors, height alone explained a major part of variability. Inclusion of age, and weight in some equations, explained a small additional variability as shown by the $R^2$ change. Thus, in boys, for FVC and FEV1, all the three predictor variables were retained in the equations while for PEFR, $F_{50}$, $F_{75}$ and $F_{25-75}$ only height and age were included. For girls, weight was retained in the equations for FVC, FEV1, PEFR and $F_{50}$. For all the parameters, the adjusted $R^2$ values were very close to $R^2$ implying that if the equation was derived from the population rather than the sample, we would get similar results.

These equations are the first effort from India after the recent standardisation of spirometry and indeed one of the few available globally. Revision of prediction equations was long overdue as technology and measurement protocols have changed and also to determine if lung health in population is changing due to changes in nutritional status, environmental exposures and rates of early childhood respiratory infections. The previous studies from India, with city and year, were: Bhattacharya and Banerjee (Delhi, 1966), Jain and Ramiah (Delhi, 1967), Vohra et al (Ahmedabad, 1984), Malik and Jindal (Chandigarh, 1985), Kumar et al (Patiala, 1992), Gupta et al (Delhi, 1993), Chowgule et al (Mumbai, 1995), RajKappor et al (Rohtak, 1997) and Vijayan et al (Chennai, 2000).

The present study has a few limitations. A wider selection from several schools would have provided a more representative sample. However, the selected school caters to a very wide socio-economic spectrum, and therefore, may be viewed as being fairly representative of Delhi. In order to ensure ethnic homogeneity, we included only children whose parents were of north Indian origin. A multicentric study with participation of other cities of the region was not feasible because of limited resources. Therefore, while the equations predict pulmonary function of children of north Indian origin, we suggest that the equations may also be validated in other north Indian states in further studies. A chest radiograph would have confirmed normalcy. However, this was not feasible in a field study. A careful and detailed history and examination by the physicians, however, ensured that only normal children were included.

These equations should be helpful in interpretation of spirometric data, and thus, in the management of respiratory diseases such as asthma in children. Equipments used in most pulmonary clinics use softwares that carry western prediction equations and are not valid for Indian population. These equations would allow manufacturers to provide Indian equations in the softwares. The equations described by us have been developed for northern parts of the country. As ethnic differences are important, each region needs to develop such equations following the standardised protocols and strict quality control.

Acknowledgements

The project titled “Pulmonary Function in Normal Children in Delhi Region: Development of Reference Standards for Spirometry” was approved and funded by the Indian Council of Medical Research. The support of the Council is gratefully acknowledged.

References

To the Editor: I read with interest the article by Mukherjee A et al.1 High sputum grade indicate that the patient is harboring large bacillary load which also indicate severity of the disease and presence of natural drug resistant strain. The authors had added all outcomes (i.e., default, failure and death) to compare the unfavourable outcome between high and low grade sputum positive patients. However, the risk for death and default rate are not related to category of patients or their treatment.2 In the present study, no significant difference in failure rate among low-grade and high-grade sputum positive patient was observed (2.1% vs 2.5%, p>0.37). So the authors’ advocacy to add another anti-tuberculosis drug in the current regimen of Revised National Tuberculosis Control Programme (RNTCP) for initial higher grade of sputum positive patient is not justifiable. Other than fluoroquinolone, we have no other effective and less toxic oral medicine to add in the current regimen. However, fluoroquinolones are rampantly used for various common diseases and the high prevalence of resistance to ofloxacin has been reported from India.3 The authors have not discussed the reason behind relatively high death rate among scanty positive patients.

Irrespective of smear report, RNTCP guidelines have recommended to start continuation phase after completion of prolongation of intensive phase of the treatment. As per Directly Observed Treatment, short course-Plus (DOTS-PLUS) guidelines, a multidrug-resistant tuberculosis (MDR) suspect is a new smear positive patient who remain smear positive at the end of five months of the treatment. The outcome analysis of new smear positive patients who remained positive at the end of prolongation of intensive phase of the treatment may be helpful to recommend sputum culture for the early detection of MDR.

REFERENCES


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The Authors’ Reply: We would like to thank the reader for showing interest in our article.1 Death, default and failure have been included under unfavourable outcomes in several studies on the RNTCP in the past,2 and hence were used by the authors.

Rajpal et al3 in their study on patients notified under the RNTCP found that the proportion of defaulters was higher in 3+ patients. The authors go on to say that from the practical point of view, it would appear that grading of sputum need not be merely an academic exercise but instead help pinpoint a group of patients who are likely to default oftener than others.3 Even studies from Gambia4 have recommended that patients with high bacterial load in initial sputum smears need to be closely supervised, as they are more likely to default from treatment.

Singla et al5 have shown significantly high failure rates among patients with 3+ TB patients. The present study also shows a higher failure rate (2.1% vs 2.5%) among the groups although the difference is not statistically significant. Given the large number of patients suffering from tuberculosis even a small decrease in the rates of failure will have a huge impact in terms of the absolute number of patients with failure. Studies with a larger sample may be able to bring out the statistical significance.

The authors would like to suggest the use of streptomycin and not fluoroquinolones as the additional drug for the patients with higher grades of initial sputum positivity. A regimen similar to Category II may be used in patients with 3+ initial sputum smear grades.

REFERENCES


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Analyzing Policy Transfer: Perspectives for Operational Research

K. Bissell, K. Lee and R. Freeman

International Journal of Tuberculosis and Lung Disease 2011;15:1140-1148

Policy transfer occurs regularly. In essence, a strategy developed elsewhere is taken up and applied in another policy context. Yet what precisely is policy transfer and, more importantly, under what conditions does it occur? This paper describes policy transfer and addresses three main questions, exploring what perspectives of policy transfer might contribute to operational research (OR) efforts. First, what facilitates the transfer of OR results into policy and practice? Second, what facilitates effective lesson-drawing about OR results and processes between and within countries? And third, what would increase the amount of OR being carried out by low- and middle-income countries and used to inform policy and practice at local and global levels?

Mexico’s adoption and adaptation of the DOTS strategy is used here as an example of policy transfer.

Evaluation of a Simple Loop-mediated Isothermal Amplification Test Kit for the Diagnosis of Tuberculosis


International Journal of Tuberculosis and Lung Disease 2011;15:1211-1217

Objective. A new loop-mediated isothermal amplification (LAMP) test kit, including a simple DNA extraction device for the detection of Mycobacterium tuberculosis complex, was developed for commercial use and evaluated for its usefulness in diagnosing tuberculosis (TB).

Design. The LAMP test was performed using untreated and N-acetyl-L-cysteine (NALC) NaOH-treated sputum specimen. The efficiency of the kit was compared with other conventional laboratory examinations, including other nucleic acid amplification (NAA) tests.

Results. The sensitivity of LAMP using raw sputum (direct LAMP) in smear- and culture-positive specimens was 98.2% (95% CI 94.9-99.4), while the sensitivity in smear-negative, culture-positive specimens was 55.6% (95% CI 43.4-68.0). The diagnostic sensitivity of direct LAMP for the diagnosis of individuals with TB was 88.2% (95% CI 81.4-92.7). The sensitivity values of direct LAMP were slightly, but not statistically significantly lower than those of Cobas Amplicor MTB and TRC Rapid MTB, while the sensitivity of the LAMP test using NALC-NaOH treated sputum was significantly lower than other NAA tests (P<0.05) for smear-negative, culture-positive specimens. The new commercial version of the LAMP kit was easy to handle and yielded results within 1 h of receiving sputum specimens.

Conclusions. This test is considered a promising diagnostic tool for TB, even for peripheral laboratories with limited equipment, such as those in developing countries.
Grading the Severity of Obstruction in Mixed Obstructive-Restrictive Lung Disease

Zechariah S. Gardner, Gregg L. Ruppel and David A. Kaminsky

Chest 2011;140:598-603

Background. The severity of obstructive pulmonary disease is determined by the FEV₁ % predicted based on the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. In patients with coexisting restrictive lung disease, the decrease in FEV₁ can overestimate the degree of obstruction. We hypothesize that adjusting the FEV₁ for the decrease in total lung capacity (TLC) results in a more appropriate grading of the severity of obstruction.

Methods. We examined a large pulmonary function test database and identified patients with both restrictive (TLC <80% predicted) and obstructive (FEV₁/FVC < the lower limit of normal) lung disease. FEV₁ % predicted was adjusted for the degree of restriction by dividing it by TLC % predicted. We compared the distribution of severity grading between adjusted and unadjusted values according to ATS/ERS criteria and determined how the distribution of severity would change based on asthma and COPD guidelines.

Results. We identified 199 patients with coexisting restrictive and obstructive lung disease. By ATS/ERS grading, the unadjusted data categorized 76% of patients as having severe or very severe obstruction and 11% as having mild or moderate obstruction. The adjusted data classified 33% with severe or very severe obstruction and 44% with mild or moderate obstruction. Of the corrected values, 83% resulted in a change to less severe obstruction by ATS/ERS guidelines, and 44% and 70% of patients, respectively, would be reclassified as having less severe obstruction by current asthma and COPD guidelines.

Conclusions. This method results in a more appropriate distribution of severity of obstruction, which should lead to more accurate treatment of obstruction in these patients.

Association of Occult Metastases in Sentinel Lymph Nodes and Bone Marrow With Survival Among Women With Early-Stage Invasive Breast Cancer

Armando E. Giuliano, Debra Hawes, Karla V. Ballman, Pat W. Whitworth, Peter W. Blumencranz, Douglas S. Reintgen, Monica Morrow, A. Marilyn Leitch, Kelly K. Hunt, Linda M. McCall, Andrea Abati and Richard Cote

JAMA 2011;306:385-393

Context. Immunochemical staining of sentinel lymph nodes (SLNs) and bone marrow identifies breast cancer metastases not seen with routine pathological or clinical examination.

Objective. To determine the association between survival and metastases detected by immunochemical staining of SLNs and bone marrow specimens from patients with early-stage breast cancer.

Design, Setting, and Patients. From May 1999 to May 2003, 126 sites in the American College of Surgeons Oncology Group Z0010 trial enrolled women with clinical T1 to T2N0M0 invasive breast carcinoma in a prospective observational study.

Interventions. All 5210 patients underwent breast-conserving surgery and SLN dissection. Bone marrow aspiration at the time of operation was initially optional and subsequently mandatory (March 2001). Sentinel lymph node specimens (hematoxylin-eosin negative) and bone marrow specimens were sent to a central laboratory for immunochemical staining; treating clinicians were blinded to results.

Main Outcome Measures. Overall survival (primary end point) and disease-free survival (a secondary end point).

Results. Of 5119 SLN specimens (98.3%), 3904 (76.3%) were tumor-negative by hematoxylin-eosin staining. Of 3326 SLN specimens examined by immunohistochemistry, 349 (10.5%) were positive for tumor. Of 3413 bone marrow specimens examined by immunocytchemistry, 104 (3.0%) were positive for tumors. At a median follow-up of 6.3 years (through April 2010), 435 patients had died and 376 had disease recurrence. Immunohistochemical evidence of SLN metastases was not significantly associated with overall survival (5-year rates: 95.7%; 95% confidence interval [CI], 95.0%-96.5% for immunohistochemical negative and 95.1
The safety of talc pleurodesis is under dispute following reports of talc-induced acute respiratory distress syndrome (ARDS) and death. We investigated the safety of large-particle talc for thoracoscopic pleurodesis to prevent recurrence of primary spontaneous pneumothorax (PSP).

418 patients with recurrent PSP were enrolled between 2002 and 2008 in nine centres in Europe and South Africa. The main exclusion criteria were infection, heart disease and coagulation disorders. Serious adverse events (ARDS, death or other) were recorded up to 30 days after the procedure. Oxygen saturation, supplemental oxygen use and temperature were recorded daily at baseline and after thoracoscopic pleurodesis (2 g graded talc).

During the 30-day observation period following talc poudrage, no ARDS (95% CI 0.0-0.9%), intensive care unit admission or death were recorded. Seven patients presented with minor complications (1.7%, 95% CI 0.7-3.4%). After pleurodesis, mean body temperature increased by 0.41°C (95% CI 0.33-0.48 °C; p<0.001) at day 1 and returned to baseline value at day 5. Pleural drains were removed after day 4 in 80% of patients.

Serious adverse events, including ARDS or death, did not occur in this large, multicentre cohort. Thoracoscopic talc poudrage using larger particle talc to prevent recurrence of PSPs can be considered safe.

Effect of a Dietary Portfolio of Cholesterol-Lowering Foods Given at 2 Levels of Intensity of Dietary Advice on Serum Lipids in Hyperlipidemia: A Randomized Controlled Trial

David J.A. Jenkins, Peter J.H. Jones, Benoit Lamarche, Cyril W.C. Kendall, Dorothea Faulkner, Luba Cermakova, Iris Gigleux, Vanu Ramprasath, Russell de Souza, Chris Ireland, Darshna Patel, Korbua Srichaikul, Shahad Abdulnour, Balachandran Bashyam, Cheryl Collier, Sandy Hoshizaki, Robert G. Josse, Lawrence A. Leiter, Philip W. Connelly and Jiri Frohlich

JAMA 2011;306:831-839

Context. Combining foods with recognized cholesterol-lowering properties (dietary portfolio) has proven highly effective in lowering serum cholesterol under metabolically controlled conditions.

Objective. To assess the effect of a dietary portfolio administered at 2 levels of intensity on percentage change in low-density lipoprotein cholesterol (LDLC) among participants following self-selected diets.

Design, Setting, and Participants. A parallel-design study of 351 participants with hyperlipidemia from 4 participating academic centers across Canada (Quebec City, Toronto, Winnipeg, and Vancouver) randomized between June 25, 2007, and February 19, 2009, to 1 of 3 treatments lasting 6 months.
Intervention. Participants received dietary advice for 6 months on either a low-saturated fat therapeutic diet (control) or a dietary portfolio, for which counseling was delivered at different frequencies, that emphasized dietary incorporation of plant sterols, soy protein, viscous fibers, and nuts. Routine dietary portfolio involved 2 clinic visits over 6 months and intensive dietary portfolio involved 7 clinic visits over 6 months.

Main Outcome Measures. Percentage change in serum LDL-C.

Results. In the modified intention-to-treat analysis of 345 participants, the overall attrition rate was not significantly different between treatments (18% for intensive dietary portfolio, 23% for routine dietary portfolio, and 26% for control; Fisher exact test, P=.33). The LDL-C reductions from an overall mean of 171 mg/dL (95% confidence interval [CI], 168-174 mg/dL) were –13.8% (95% CI, –17.2% to –10.3%; P<.001) or –26 mg/dL (95% CI, –31 to –21 mg/dL; P<.001) for the intensive dietary portfolio; –13.1% (95% CI, –16.7% to –9.5%; P<.001) or –24 mg/dL (95% CI, –30 to –19 mg/dL; P<.001) for the routine dietary portfolio; and –3.0% (95% 0, –6.1% to 0.1%; P=.06) or –8 mg/dL (95% CI, –13 to –3 mg/dL; P=.002) for the control diet. Percentage LDL-C reductions for each dietary portfolio were significantly more than the control diet (P<.001, respectively). The 2 dietary portfolio interventions did not differ significantly (P=.66).

Among participants randomized to one of the dietary portfolio interventions, percentage reduction in LDL-C on the dietary portfolio was associated with dietary adherence (r=–0.34, n=157, P<.001).

Conclusion. Use of a dietary portfolio compared with the low-saturated fat dietary advice resulted in greater LDL-C lowering during 6 months of follow-up.

Trial Registration clinicaltrials.gov Identifier: NCT00438425
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