Infectious Agents Associated with Cylindrical Bronchiectasis*


Great Lakes, Illinois

Cylindrical bronchiectasis is a rather frequent complication of bronchopneumonias occurring in Naval recruits at the Great Lakes Naval Training Center. In 1962, 137 cases of cylindrical bronchiectasis were treated at the Naval Hospital, which represents an incidence of bronchopneumonia complications of 4.5 per cent.

The factors involved in the pathogenesis of bronchiectasis are complex. The host, the infectious agent, and the environmental setting of their encounter, play a role. It is well-recognized, however, that the sequence of events leading to bronchiectasis is initiated by an infectious process, resulting in inflammation, obstruction and finally destructive changes in the bronchus. The present study was limited to identification of the infectious agents associated with cylindrical bronchiectasis. Both isolation from specimens obtained by bronchoscopy and immunologic studies on serum samples were employed.

Materials and Methods

Experimental Design

The study population consisted of 173 patients who had been referred by their ward physicians to the Ear, Nose and Throat Department of the Naval Hospital for bronchoscopy and bronchography, from November, 1961 to April of 1962. The criteria for referral (in cases of suspected bronchiectasis) were persistence of respiratory signs and symptoms, and/or lack of resolution of pulmonary infiltrates for approximately six weeks following the onset of the acute respiratory process. In addition, non-bronchiectatic and non-pulmonary cases have been included in this study for comparative purposes.

The Technique of Bronchoscopy and Specimen Collection

Standard Jackson bronchoscopes (usually of the 7 mm. x 40 cm. or 8 mm. x 40 cm. size) were used throughout. All endoscopic equipment was sterilized by means of prolonged exposure to formaldehyde gas in a specially designed cabinet. Sterility was verified by means of weekly cultures. Each patient was examined by previously selected completely separate sets of instruments. Telescopes were decontaminated between successive procedures by wiping with 95 per cent ethanol. Pre-medication was considered particularly important because of the young age group. Unless specific contraindications existed, all patients received a combination of pentobarbital (Nembutal), morphine sulfate, and atropine (with dosage adjusted to weight), given approximately 90 minutes before anticipated time of bronchoscopy.

Local anesthetics was obtained in all cases by means of topical cocaine, which was sprayed into the oro-pharynx in 1 per cent concentration and was followed by 10 per cent cocaine directly into the larynx with a Leukens syringe under mirror control. The total dosage did not exceed 250 mg. of cocaine. Anesthesia was administered over a period of at least 15 minutes. No reactions of any kind were observed.

In most instances, the bronchoscope was passed directly through the glottis into the trachea without use of the slide laryngo-
scope. Trachea and bronchi were examined in a systematic manner. First, the character of the trachea and its mucosa was noted. Then, the carina was examined with emphasis on its relative sharpness and mobility. The right upper lobe orifices were studied next by means of the right angle telescope. The orifices of the right middle and right lower lobes were visualized directly through the bronchoscope. The left lung was then examined in a similar fashion. Careful inspection was made of the bronchial mucosa for edema or infection. The character and origin of the bronchial exudate was noted.

An attempt was made in all cases to obtain specimens from the particular bronchus involved. If sufficient material was not available, bronchial washings with normal sterile saline were carried out. Collections were obtained with a Holinger specimen collector by aspiration into sterile 15 ml. centrifuge tubes. Bronchial epithelium was sampled by superficial abrasion of the bronchial mucosa with an open-end aspirator. Currettings were aspirated into similar centrifuge tubes, which contained 5 ml. of antibiotic-free lactalbumin hydrolysate broth with 0.5 per cent gelatin (pH 7.2). All specimens were immediately capped with sterile cotton plugs, and taken to the Naval Medical Research Unit No. 4 for analysis. Bronchograms were done either immediately following specimen collection or several days afterwards.

Specimens were promptly cultured for bacteria and mycoplasma. Portions for viral isolations from both specimens were thoroughly ground in small tissue grinders, placed in 4 ml. screw-cap vials, quick-frozen, and stored at −70°F. for subsequent inoculation into tissue cultures. In addition, a blood specimen was obtained at the time of bronchoscopy and two weeks later for serological studies. Sera were stored at −20°F.

**Bacterial Isolation Techniques**

A loopful of the bronchial exudate was inoculated onto the surface of a sheep blood agar plate for isolation and identification of beta-hemolytic streptococci, pneumococci, and *Hemophilus influenzae*. In the case of Hemophilus, satellite formation along a streak of Staphylococcus on the blood agar plate, was used for identification. Staphylococci were isolated on Difco's staphylococcal 110 medium, and colonial color was recorded after several days of incubation at room temperature (22-25°C.). Suspicious colonies were subcultured onto mannitol-tellurite agar (Vogel-Johnson medium, Difco). Mannitol fermentation was considered a positive test for coagulase production. *Streptococcus mitis* was differentiated from *Streptococcus salivarius* on Difco's Mitis-Salivarius Agar. Lancefield's streptococcus group K organisms were isolated on a modified Litsky's group D medium containing 1 per cent 2, 3, 5 triphenyl-tetrazolium-chloride. All beta-hemolytic streptococci and group K streptococci were identified by the acid extraction method of Swift, Wilson and Lancefield. Enterococci were isolated and differentiated on Levine's eosin-methylene blue agar. Anaerobic incubation was not done routinely. When performed, it was carried out in Brewer jars at 37°C., in 95 per cent nitrogen and 5 per cent carbon dioxide.

**Mycoplasma Isolation Techniques**

One-half ml. portions of the exudate and of the ground epithelial currettings were inoculated each into 5 ml. of Difco PPLO broth (pH 7.8), enriched with 10 per cent horse serum and 1 per cent yeast autolysate (Basamin-Busch Powder, Anheuser-Busch, Inc., St. Louis, Mo.) and containing thallium acetate (1:2000) and penicillin (3000 units/ml.). The broth was incubated aerobically in screw-cap tubes, with the caps tightened, for 48 hours at 37°C., 0.2 ml. were then transferred onto PPLO agar plates containing the same enrichment as the broth plus thallium acetate, but lacking penicillin. The agar plates were incubated anaerobically at 37°C. under 95 per cent nitrogen and 5 per cent carbon dioxide. Presence or absence of mycoplasma was recorded after four and 14 days of.
anaerobic incubation. No attempt was made to differentiate mycoplasma from the L-forms of bacteria. Absence of penicillin in the plates, however, would tend to favor reversion of the L-forms back to bacteria in 14 days, had they been present.

**Virological Isolation Techniques**

Specimens were thawed, and 0.2 ml. portions inoculated in duplicate into H. Ep-2 and primary monkey kidney tissue cultures. Tubes were observed daily for a period of 15 days for cytopathic effect (CPE) indicative of presence of virus. A second blind passage was made routinely. On the fifth and fifteenth days of the second passage, all monkey kidney tubes were tested by a hemadsorption technique. This technique consisted of discarding the old maintenance tissue culture medium, and adding 0.2 ml. of a 0.4 per cent suspension of guinea pig erythrocytes. After incubation in a refrigerator at 4°C. for 20 minutes, the tubes were observed for adsorption of the erythrocytes onto the monkey kidney cells. Identification of positive cultures was made by the hemadsorption-inhibition technique using type-specific animal antisera.

**Serological Techniques**

All sera were tested for antibody in a two-fold dilution series using the micro-complement fixation test. The complement fixation tests were performed on serum heated at 56°C. for 30 minutes using veronal buffered saline (pH 7.4), four units of antigen, two exact units of guinea pig complement, and over-night incubation at 6°C. A reading of 4+ was considered as the endpoint. Since the first serologic specimen was collected at the time of bronchoscopy (which was approximately six weeks following the acute respiratory infection), it was considered to be a "convalescent" specimen. It is not surprising, therefore, that differences in titers between this and the two-week "follow-up" specimen were slight and rises and fall of titers could not be used as criteria for a specific infection. Consequently, the data were analyzed in terms of geometric mean antibody titers (−log2) and of the incidence of high titers in the different illness categories.

**Results**

Only two viral isolates have been obtained. Both were influenza B, and both were found in epithelial curretings from two patients with bronchiectasis (Table 1). The highest adenovirus-antibody geometric mean titer was observed in bronchiectasis cases; it was 5.2. Patients with bronchopneumonia, on the other hand, had a titer of 4.3; those with chronic bronchitis 3.5; patients with other pulmonary conditions

<table>
<thead>
<tr>
<th>TABLE 1—ISOLATION OF INFECTIOUS AGENTS FROM BRONCHIAL EXUDATES AND CURRETTINGS, OBTAINED BY BRONCHOSCOPY FROM PATIENTS WITH VARIOUS PULMONARY LESIONS</th>
</tr>
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<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Streptococcus</td>
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<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>No. of Cases</td>
</tr>
<tr>
<td>Bronchiectasis</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
</tr>
<tr>
<td>Bronchitis (Chronic)</td>
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<tr>
<td>Other pulmonary conditions††</td>
</tr>
<tr>
<td>Non-pulmonary conditions††</td>
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<tr>
<td>Totals</td>
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*Final discharge diagnosis; **Isolation limited to bronchial exudates; †Influenza B obtained from bronchial curretings; ††Statistically significant difference; P=<0.03; ‡Histoplasma, bronchogenic carcinoma, coin lesions, etc. (non-recruit Navy personnel or dependents); †††Diagnostic work-up, esophageal carcinoma, vocal cord paresis, etc.
(coin lesions, histoplasmosis) 2.6, and finally those with non-pulmonary conditions, 2.0 (Table 2). The incidence of adenovirus-antibody titers of 1:64 and greater was 46.1 per cent in bronchiectasis; 27 per cent in bronchopneumonia; and 16.7 per cent in chronic bronchitis; while in the other conditions, there were 200 titers of this magnitude. This difference in incidence of high titers between bronchiectasis and bronchopneumonia patients is statistically significant (P value less than 0.03). Antibody titers against other viral agents studied showed no such distribution (Table 2).

Since the majority of patients in this study had been on antibiotics for variable periods of time prior to bronchoscopy, the significance of the bacteriologic results is limited. However, it is of interest that Hemophilus influenzae, group K Streptococcus, and Pseudomonas aeruginosa, were isolated with greater frequency in bronchiectasis than in the non-bronchiectatic conditions (Table 1). Streptococcus salivarius and mitis were isolated in practically all the cases. The results of anaerobic bacterial cultures (when obtained), did not differ qualitatively from aerobic cultures. In some instances, however, a more luxuriant growth of organisms was noted. No fusiform or spirochetal organisms were found.

Anaerobic mycoplasma were isolated in 32.5 per cent (35 of 108) of cases of bronchiectasis, and only in 14.3 per cent (6 of 42) of cases of bronchopneumonia (Table 1). This difference is statistically significant (P value less than 0.03). There were no isolations of the Eaton agent (Mycoplasma pneumoniae). This was also corroborated by serological studies which showed almost identical geometric mean antibody titers against the Eaton agent in all the conditions studied (Table 2).

**Comments**

The dearth of viral isolations, in both the bronchial exudates and superficial mucosal curettings, was disappointing. This may have been due to the toxicity of the exudates for viruses, or to the time interval between bronchoscopy and acute infection. However, the high adenovirus-antibody geometric mean titer (5.2), as well as the high incidence of titers of 1:64 and above (46.1 per cent) found in bronchiectasis, suggests that adenovirus infections may, directly or indirectly, damage bronchial epithelium and thus initiate a series of events leading to the development of bronchiectasis.

The relative lack of the common virulent bacterial isolates should not be considered surprising in view of the antibiotic therapy prior to bronchoscopy, in most patients in this study. The 5.5 per cent isolation incidence of Hemophilus influenzae in bronchiectasis cases is much lower than that reported elsewhere in studies of chronic bronchiectasis, where rates as high as 63 per cent have been described. The high incidence of Streptococcus salivarius and mitis is consistent with previous studies, which have found an almost universal colonization of tracheobronchial tissue with

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>Adenovirus</th>
<th>Influenza B(1760)</th>
<th>C</th>
<th>Parainfluenza 3</th>
<th>Mycoplasma Pneumoniae (Eaton agent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiectasis</td>
<td>5.2</td>
<td>4.0</td>
<td>3.9</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
<td>4.3</td>
<td>4.0</td>
<td>4.4</td>
<td>2.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Bronchitis (chronic)</td>
<td>3.5</td>
<td>4.2</td>
<td>3.6</td>
<td>3.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Other pulmonary conditions**</td>
<td>2.0</td>
<td>3.0</td>
<td>3.3</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>Non-pulmonary conditions†</td>
<td>2.0</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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*Final discharge diagnosis; **Histoplasmosis, bronchogenic carcinoma, coin lesions, etc. (non-recruit Navy personnel or dependents); †Diagnostic work-up, esophageal carcinoma, vocal cord paresis, etc.
"oral commensals" in the presence of bronchial pathology. This is presumably due to damage of the bronchial clearing mechanism. The extensive use of antibiotics in these cases may have also favored growth of resistant organisms. Whether such colonizations with "oral commensals" are entirely innocuous, or whether by perpetuating the inflammatory reaction they contribute to further bronchial damage, has not been elucidated.

The implication of the Eaton agent in the etiology of the non-bacterial bronchopneumonias and its identification as a member of the genus Mycoplasma (or "Pleuropneumonia-like Organisms," "PPLO"), have stimulated interest in the possible etiological importance of mycoplasma in other infectious diseases, especially those of the respiratory tract. Mycoplasma have been shown to be associated with respiratory infections in animals. For example, the prototype PPLO—Mycoplasma mycoides causes pleuropneumonia in cattle (hence the derivation of "PPLO"). Chronic respiratory disease in fowl and a form of bronchiectasis in rats are likewise associated with mycoplasma.

There were no isolations of the Eaton agent in any of the cases reported here. The isolation technique itself, involving incubation under anaerobic conditions, may have contributed to this. Work reported subsequent to this study revealed the necessity of using aerobic conditions, as well as a more enriched medium (20 per cent horse serum, 10 per cent yeast extract), for Eaton agent isolation. The fact that no serologic evidence for the presence of Eaton agent infections was detected in any of the illness categories, however, further indicated a limited etiologic role of this agent in the cases studied.

The higher isolation incidence of anaerobic mycoplasma found in cases of bronchiectasis is of interest. Since only specific antibody rises to these organisms could be taken as unequivocal evidence of infection (which determination has not been carried out in this study for technical reasons), it is impossible to ascribe a primary pathogenetic role to these organisms with certainty. As with oral bacterial commensals, however, they may have contributed indirectly to the pathogenesis of bronchiectasis.

**Summary**

In view of a relatively high incidence of cylindrical bronchiectasis as a complication of bronchopneumonia in Naval recruits, a study was undertaken on the infectious agents associated with this form of bronchiectasis. Isolation data from specimens obtained in 173 cases by bronchoscopy (including 108 cases of cylindrical bronchiectasis), were presented. Only two viral isolations, both of influenza B, were made in two cases of bronchiectasis. Adenovirus-antibody geometric mean titers were highest in cases of bronchiectasis (5.2), and lowest in non-pulmonary conditions (2.0). The incidence of patients with antibody titers against adenovirus of 1:64 and greater was 46.1 per cent in bronchiectasis, 27.0 per cent in bronchopneumonia (P<0.03), 16.7 per cent in chronic bronchitis, and none in the other conditions. Hemophilus influenzae was isolated only in bronchiectasis (5.5 per cent), whereas, oral commensals were present almost universally. Statistically significant (P<0.03) difference in isolation incidence of anaerobic mycoplasma was found between patients with bronchiectasis (32.5 per cent), and those with bronchopneumonia (14.3 per cent). There was no evidence of Eaton agent infection in the population studied.

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**Resumen**

En vista de la relativamente elevada frecuencia de la bronquectasia cilíndrica como complicación de la bronquieumonia en los reclutas navales se emprendió un estudio sobre los microorganismos infecciosos que se encontraron en esta forma de bronquieumonia.
Se presentan los datos aislados de muestras obtenidas en 173 casos por broncoscopia (incluyendo 108 casos de bronquiectasia cilíndrica). Se obtuvieron sólo dos de virus, ambos de influenza B en dos casos de bronquiectasia. Las medidas de geometrías de anticuerpos de adenovirus fueron más elevadas en casos de bronquiectasia (5.2) y más bajas en afecciones no pulmonares (2.0). La frecuencia de enfermos con titulación de anticuerpos contra adenovirus de 1:64, la menor y de 46.1 la mayor en bronquiectasia; 27.0 por ciento en bronconeumonía (P =< 0.03), 16.7 por ciento en bronquitis crónica y ninguna en otras afecciones.

El Hemophilus influenzae se aisló sólo en bronquiectasia (5.5 por ciento) en tanto que se encontraron comensales orales casi en todos. Se encontró una significación estadística en la diferencia en la frecuencia de aislamiento de micoplasma anaerobio entre enfermos con bronquiectasia (32.5 por ciento) y aquellos con bronconeumonía (14.3 por ciento).

No hubo evidencia de infección por el virus Eaton en la población estudiada.

Resumé

Pour expliquer la fréquence relativement éllevée de la bronchiectasie cylindrique comme complication de broncho-pneumonies chez les recrues de la Marine, une étude fut entreprise sur les agents infectieux que l'on rencontre dans cette forme de bronchiectasie. L'auteur présente des données isolées à partir d'échantillons obtenus dans 173 cas par bronchosocie (comprenant 108 cas de bronchiectasie cylindrique). Il n'a pu isoler que deux virus, tous deux de l'influenza B, dans deux cas de bronchiectasie. La moyenne géométrique des titres adénovirus-anticorps fut plus élevée dans les cas de bronchiectasie (5,2) et plus faible dans les atteintes non-pulmonaires (2,0). La fréquence des malades portant des titres d'anticorps contre l'adénovirus au taux de 1/64 et au-delà fut de 46,1% pour la bronchiectasie, 27% dans la bronchoconeumonie (P =< 0.03) 16,7% dans la bronchite chronique, et nulle dans d'autres atteintes.

L'hémophilus influenzae ne fut isolé que dans la bronchiectasie (5,5%) tandis que d'autres germes buccaux associés étaient présents presque partout. On trouva une différence statistiquement marquée (P =< 0.03) dans la fréquence d'isolement de mycoplasma anaérobio entre les malades atteints de bronchiectasie (32,5%) et ceux atteints de bronchoconeumonie (14,3%). Il n'y eut aucune preuve de la présence du virus de Eaton dans le groupe étudié.

References


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Primary Staphylococcal Pericarditis

A case of staphylococcal pericarditis complicating mitral annuloplasty in a 28-year-old woman has been described. This complication, though a rarity, does occur and therefore should be seriously considered in postcardiomyotomy patients with high fever, unremitting symptoms of pain, dyspnea and increasing leukocytosis. Parenterally and intrapericardially administered methicillin and closed surgical drainage effected a bacteriologic cure. Final clinical cure was achieved by partial pericardietomy. Parenterally administered methicillin was found to diffuse into the pericardial sac. Intrapericardial administration of methicillin was not attended by untoward effects. Abu-Nasser, H. J., Yow, E. M., Alexander, J. K. and Lewis, J. M.: "Primary Staphylococcal Pericarditis Complicating Cardiomyotomy," Ann. Int. Med., 60:153, 1964.