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# Pseudomonas cepacia colonization in cystic fibrosis : mortality, predictors of poor outcome, and effect on pulmonary function

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PSEUDOMONAS CEPACIA COLONIZATION IN CYSTIC FIBROSIS:  
MORTALITY, PREDICTORS OF POOR OUTCOME,  
AND EFFECT ON PULMONARY FUNCTION

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LINDA ORKIN LEWIN

1987

YALE



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PSEUDOMONAS CEPACIA COLONIZATION IN CYSTIC FIBROSIS:  
MORTALITY, PREDICTORS OF POOR OUTCOME,  
AND EFFECT ON PULMONARY FUNCTION

A Thesis Submitted to the Yale University  
School of Medicine in Partial Fulfillment  
of the Requirements for the Degree of  
Doctor of Medicine

by

Linda Orkin Lewin

1987



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This thesis is dedicated,  
with love and gratitude,  
to Jonathan



## ABSTRACT

Pseudomonas cepacia (PC) is a plant pathogen with multiple antibiotic resistance that has been the cause of nosocomial infections in debilitated patients, and has recently become a significant colonizer of the respiratory tracts of patients with cystic fibrosis (CF). One hundred and nineteen colonized patients with CF and their non-colonized sex and age-matched controls were studied to determine mortality in the first and second years following acquisition of PC, and those colonized patients who died in the first year were compared to those who survived to determine differences in pre-colonization clinical status. The FEV1 and RV/TLC data of 33 matched pairs were studied from 3 years before colonization with PC through 2 years following colonization to determine differences in the decline of pulmonary function. Mortality of colonized patients was higher than that of controls in the first year (chi-square  $<.005$ ), and those who died in the first year had worse pulmonary function one year prior to colonization ( $p=.001$ ). Colonized survivors had a significantly lower FEV1 than their controls over the 5 year period, with no difference in the rate of decline.



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## 1. INTRODUCTION

Cystic Fibrosis is a genetically acquired disease in which progressive pulmonary infection and destruction is a primary clinical manifestation. While Staphylococcus aureus was the most common respiratory pathogen in patients with cystic fibrosis prior to the availability of antibiotics (1,2,59), Pseudomonas aeruginosa is currently the most common organism found in their sputum (56,96). Pseudomonas cepacia is an organism first thought to be a pathogen only in plants, but which has since been described as a nosocomial cause of wound infections, urinary tract infections, bacteremia and pneumonia in humans (2,20,27,30,41,60,69,70,71,78,82,84,86,88,95). It has recently become a more common pathogen in patients with cystic fibrosis, and often is resistant to many antibiotics (3,4,8,19,24,27,31,45,49,62,64,67,81,84,87,93), making it a difficult organism to treat. Its effect on the course of patients with cystic fibrosis is unclear, although some studies suggest that many patients follow a rapidly fatal course after acquisition of the organism



(10,40,91). This study was undertaken to better define the effect of Pseudomonas cepacia has on the course of patients with cystic fibrosis.



## 2. REVIEW OF THE LITERATURE

### PULMONARY BACTERIOLOGY OF CYSTIC FIBROSIS

Cystic Fibrosis of the Pancreas is defined, in the 24th edition of Stedman's Medical Dictionary, as "a congenital metabolic disorder, inherited as an (autosomal) recessive trait, in which secretions of exocrine glands are abnormal; excessively viscid mucus causes obstruction of passageways (including pancreatic and bile ducts, intestines, and bronchi) and the sodium and chloride content of sweat are increased throughout the patient's life; symptoms usually appear in childhood and include meconium ileus, poor growth despite good appetite, foul bulky stools, chronic cough, recurrent pneumonia, emphysema, clubbing of the fingers, and salt depletion in hot weather; the underlying metabolic defect is unknown and survival beyond childhood is uncommon" (85). The pulmonary disease in Cystic Fibrosis (CF) remains the main cause of morbidity and mortality in affected patients (88).

The first pathogen associated with CF lung disease was Staphylococcus aureus (SA), originally described in the first paper written on the subject by



Andersen in 1939 (1). While this association has been repeatedly demonstrated, the reason for the organism's predilection for the lung of patients with CF is not known. It has been proposed that the character of the pulmonary secretions in CF favor the proliferation of this organism, with one study reporting that increased p-hydroxyphenylacetic acid concentration in respiratory secretions promoted the growth of SA (55).

It has also been proposed that Protein A, a constituent of the cell wall of SA, contributes to the organism's prevalence in the lung of patients with CF by binding to the Fc portion of immunoglobulins and inhibiting phagocytosis (34). Protein A has also been found to adsorb to precipitating antibodies to Pseudomonas aeruginosa (PA) in patients with CF, making it a possible mechanism of synergism between the two organisms (32).

The role of SA in the initiation of pulmonary damage in CF patients has also been debated in the literature. Lawson, in 1969, stated that it was the SA itself that damaged the lung, and that invasion by PA could only occur after this damage has been done (50). This hypothesis has since been refuted, most notably by May et al. in 1972, who found PA to be the most common pathogen isolated from the sputum of their 6 to 10 year old patients with CF, with precipitating





antibodies against PA being more common than those against SA in the 0 to 10 year olds, suggesting that SA is not always the initial pathogen and that patients with CF are inherently susceptible to bronchial infection from birth (56).

S. aureus continued to be the primary pathogen in CF until the mid-1960's, although the incidence of PA was increasing (59). Nelson's Textbook of Pediatrics in 1964 stated that PA colonization was becoming more frequent due to the use of antibiotics in treating SA infection (76). This was supported by the finding, in 1961, that 50% of CF patients hospitalized at St. Christopher's Hospital for Children who had received antibiotics had admission sputum or throat cultures positive for PA, while only 13% of those who had not been previously treated with antimicrobial drugs carried the organism (38).

Despite the increased incidence of PA infection, however, it was felt, as stated in a 1967 review article in the New England Journal of Medicine by d'Sant'Agnese et al., that PA was not of major significance in the pathogenesis of pulmonary destruction in CF, an argument supported by the fact that the clinical course and pathologic findings at autopsy had remained essentially constant since the first description of the disease, when SA was essentially the only



pathogen (76).

By 1976, however, PA was clearly the predominant organism infecting the lungs of patients with CF, and was increasingly found to be the initial pathogen (97). This was seen in a 1972 study which also demonstrated a decrease in the isolation of SA in all age groups, including babies who had not received any antibiotics in the past, calling into question the idea that the decreased isolation of SA was caused by increased use of these drugs. They proposed that this change in rates of colonization by PA and SA reflected a change in the flora of the hospital environment, indirectly implicating antibiotics (59). A later study in which the fifty percent of patients who had one or no hospitalizations remained free of PA also implicated the hospital environment in causing acquisition of PA infection (49).

While the serotypes of SA that infect patients with CF are not unique to that disease (89), a large percentage of the PA found in these patients have a characteristic mucoid morphology which is not often found in other patients (77). A study done at the National Institutes of Health in 1975 reported that 60% of PA strains from CF patients were mucoid (98), while a later study done in 1979 found the incidence of mucoid PA to be 78% in patients with CF, as



compared to 2% in non-CF patients infected with PA. The authors did not find the same prevalence of mucoid PA in patients with other chronic pulmonary diseases, and the appearance of these bacteria in the sputum of CF patients often preceded treatment with antibiotics, suggesting that the CF lung itself is more suitable to mucoid PA and that selection of these organisms by antibiotic use did not play a major role (68).

Chronic colonization of patients with CF with mucoid strains of PA was associated with a significantly higher number of anti-PA precipitating antibodies than colonization with non-mucoid strains in a study that postulated that mucoid substance is a virulence factor which inhibits antibody-mediated killing of organisms (89). Another group used the high incidence of infection with mucoid PA at the time of death in their CF patients, and a decreasing prevalence of these organisms in their older patients, to support the idea that mucoid PA is quite virulent, causing patients who harbor it to die at a young age (97).

The predilection of mucoid strains of PA for the lungs of patients with CF seems to be related to the interaction of the mucoid substance and the host's immune system. In one study, the slime from mucoid PA was added in vitro to polymorphonuclear leukocytes along with either E. coli, PA or SA. They found that



phagocytosis of all three organisms was inhibited, with the most inhibition occurring in the phagocytosis of PA (79). Another study showed that a preparation of Pseudomonas capsular polysaccharide increased the virulence of PA in mice, as well as the virulence of SA and E. coli (18).

Other Pseudomonas toxins may also be responsible for damage to pulmonary tissue; Exotoxin A and proteases of PA have been shown to cause exfoliation, disorganization, and inhibition of protein synthesis and ciliary activity in hamster tracheal explants (5). Also, mucoid PA forms microcolonies enveloped in a fibrous matrix of glucuronic and mannuronic acids (48), and can physically clog small airways and impair mucociliary clearance (53).

Interestingly, although E. coli is not a common pathogen in CF, it was found at Boston Children's Hospital in 1981 that 11.8% of the 68 patients with CF that had respiratory tract colonization with that organism harbored mucoid strains, as compared to 0 of 89 patients without CF who had sputum cultures positive for E. coli (52). The mucoid substance was found to be distinct from that of PA, yet its existence suggests that it too imports an advantage to the organisms able to make it.

Hemophilus influenza (HI) has also been reported





to be a common pathogen in CF pulmonary disease, even though its presence may be overlooked due to the rapid growth of other organisms in sputum from patients with CF and its fastidious nutritional requirements in vitro (89). In Denmark in 1976 HI was seen as a predominant colonizer of CF patients, and 38% of the CF population was found to have a single biotype present in only 11% of normal children (35). HI, like PA, is found more commonly in more severely ill patients, but its presence is not chronic like that of PA, due to its sensitivity to antibiotics. For this reason, infection with this organism is intermittent and recurrent (34).

Non-fermentative gram negative rods other than PA were first studied in patients with CF at Yale-New Haven Hospital in 1982. Twenty of 170 CF clinic patients were found to have at least one strain, and 8 had more than one, at least once during the 4 year period of study. The most common was Acinetobacter, appearing in 10 cultures, followed by 7 with P. putida-florescens, 4 with P. maltophilia, 4 with unidentified pseudomonads, 2 with P. cepacia, and one each of Bordetella bronchiseptica, Flavobacterium II-B and Achromobacter xylosoxidans. The authors did not propose a specific clinical role for these species, but did suggest that accurate identification of gram



negative organisms would allow increased recognition of non-PA species, and that this, along with the usual resistance of many of them to common antibiotics, would have to be taken into account in designing therapy for those patients colonized with them (6).

Other bacteria have also been found in the pulmonary secretions of patients with CF, as well as occasional fungi (89). A 1980 paper by Boxerbaum et al. describes the case of a 17 year old girl with a positive PPD and evidence of infection with Mycobacterium chelonae. He also identified 7 other patients with CF, aged 21 to 33 years, with rapidly growing mycobacteria in their sputa (11). Legionella pneumophila antibodies were found in 29.4% of 109 CF patients studied by Katz et al. in 1982, as compared to 11.5% of 26 patients with chronic suppurative bronchitis of childhood and 1.7% of 178 age matched controls without pulmonary disease. The patients with high titers of anti-Legionella antibodies were in worse clinical condition than the others, and the authors concluded that the organism was an opportunistic pathogen in already devastated lung tissue (94). Mention is also made in the literature of CF patients harboring other organisms, often gram negative, but they are also considered to be secondary invaders, and not specific to CF lung disease.



The influence of less commonly encountered non-bacterial pathogens on the course of CF pulmonary disease is also unclear, although it has been postulated that there may be synergism between some of these agents and PA. One study described 116 patients with CF who were followed over 8 months to determine the frequency of exacerbations of pulmonary disease and their cause. Twenty percent were attributed to non-bacterial respiratory tract pathogens; 9% to respiratory syncytial viruses (RSV), 5% to parainfluenza viruses, 3.6% to influenza viruses, 2.4% to adenoviruses, 0.6% to Mycoplasma, and 0.6% to Chlamydia. RSV infections were more common in patients who developed chronic PA infection during the study period, and were frequently associated with a rise in PA antibodies in patients who previously harbored these organisms, suggesting a synergistic pathogenicity between the two (66). It is possible that other examples of synergism between respiratory pathogens occur in the CF lung, helping to explain its continual infection and destruction.

## PSEUDOMONAS CEPACIA

### History

Pseudomonas cepacia (PC) was first described in



1950 as a plant pathogen, causing sour skin in onions (13). It was previously known as P. multivorans or P. kingii, which had previously been called "eugonic oxidizer group number one" (EO-1) (24). Later it was noted to be a human pathogen, associated with urinary tract infections, wound infections, pneumonia, and bacteremia in a variety of patients, and later with CF. Often colonization did not lead to symptomatic disease; when clinical illness did occur, the patients tended to be debilitated or have another cause of impaired host defenses.

In many reported cases the source of the organism was found in the hospital environment, often in contaminated detergents and disinfectants. In 1958, 40 patients who were exposed to needles and catheters stored in cationic surface active detergent which harbored PC developed bacteremia (69). PC also grows well in chlorhexidine and has caused urinary tract infections in patients where this antiseptic was used prior to cystoscopy (60), post-operative wound infections in patients exposed to contaminated 10:1 cetricimide-chlorhexidine solutions (7), and bacteremia when chlorhexidine was used to clean intravenous infusion sites (84). In the latter study, many gram negative organisms were isolated from the infected solution, but only PC was found in cultures from





patients. This was interpreted as evidence for selection of PC in patients receiving antibiotic therapy.

PC has also been shown to grow well in benzylkonium chloride solution, where it has been linked to contamination of intravenous and arterial blood pressure monitoring equipment (95), intravenous infusion lines (20), and skin (41). In 1970 benzylkonium chloride in commercially packaged urinary catheter kits was found to contain PC, and caused bactiuria in 13 patients, two of whom developed symptoms of urinary tract infection (30).

Forty-one patients in one hospital became infected with PC in 1972, 26 with urinary tract infections, one with bacteremia, 12 with respiratory tract infections, and 10 with wound infections, and eventually contaminated benzyl ammonium chloride was identified. All of these patients were debilitated, 69% had been on antibiotics, and 37 of them had prior manipulation of the infected site. The authors concluded that hospitalized persons with serious underlying pathology were essentially the only population at risk for symptomatic PC infection (19).

Topical anesthetics have also been responsible for acquisition of PC by hospital inpatients and outpatients. Twenty-two patients seen in one ENT clinic were found to have respiratory washings positive for



PC in a one year period. The organisms were traced to topical tetracaine and cocaine anesthetics used in this clinic, and ultimately to deionized water used by the pharmacy to make these compounds. No clinical disease was noted despite large inocula (78). Bronchial washing cultures positive for PC have also been linked to viscous lidocaine used during bronchoscopy (71).

In another study, fifty-six patients at a Veterans Administration Hospital were reported to have cultures positive for PC. A very low rate of clinical infection was seen in those whose exposure to the organism was traced to contaminated cocaine used in the ENT clinic or lidocaine used in bronchoscopy. For 26 patients, however, no source of PC was found, and the rate of infection was high, including six cases of bacteremia and two cases of pneumonia. Twenty-three of those 26 patients had been intubated during their hospitalization, suggesting a possible mode of entry for the organism. The authors felt that the serious infections in these patients were related to their overall clinical status, which was worse than that of the others, as well as the increased use of intravascular devices in this group (54).

While the above studies suggest that PC is a relatively benign organism in healthy subjects,



several studies have documented severe disease caused by PC in previously ill patients. In 1975, for example, a twenty-one year old patient with chronic granulomatous disease (CGD) with impaired phagocyte function was reported to have recurrent pneumonitis caused by PC. This patient, while exhibiting a more benign course than the usual person with CGD, does demonstrate that PC pulmonary infection can be recurrent, and suggests that chronic immunocompromise may be responsible (16,17).

The role of PC infection in CGD was also addressed in a report of three patients, one of whom had pleural space cultures positive for PC, another with a lymph node infected with the organism, and a third with abscess cultures positive for PC. The authors felt that the chronic antibiotic therapy to which patients with CGD are exposed played a significant role in allowing this colonization and infection (9).

The clear association of clinically significant PC infection with poor underlying clinical status is emphasized by a 1982 Israeli study which describes non-pathogenic colonization of patients exposed to chlorhexidine that was diluted with contaminated de-ionized water. Only two patients, both of whom were immunocompromised, developed fulminant sepsis; both had growth of PC in urine and blood cultures (82).



In another study, which describes PC bacteremia following open heart surgery due to contaminated pressure transducers, the authors again note that possible differences in host resistance, as determined by absence of the expected leukocytosis in the 24 hours following extracorporeal circulation, determined which of their patients developed clinical infection. While all patients studied had comparable pre-operative WBC's, infected patients were found to have significantly lower mean WBC's in the first day after surgery (95).

Other PC infections reported include macerated keratotic foot lesions in British troops training in swamps where the organism is endemic (88), a lung abscess in a diabetic patient which was linked to contaminated water in an ultrasonic nebulizer (70), bacteremia in 10 patients at the University of Maryland Hospital who were treated with contaminated 25% normal serum albumin (86), and pneumonia in a child following surgical repair of Tetralogy of Fallot (94).

### Antibiotic therapy

The prognostic implications of infection with PC in immunocompromised or debilitated hosts are serious, due to the resistance of this organism to most available antibiotics. In all of the studies sited above,





PC was difficult to treat, almost always exhibiting resistance to ampicillin, tetracycline, streptomycin, colistin, cephalothin, and cefaloridine, and often resisting eradication with gentamicin, tobramycin, carbenicillin, ticarcillin, and other agents used to treat infections with gram negative organisms.

Antibiotic resistance in PC is thought to be caused by the presence of resistance plasmids. These intercellular elements were isolated from 11 of 48 strains of PC in one study, though their function was not studied. The rate of plasmid occurrence may have been higher than this, but the presence of nucleases in the electrophoresis preparation may have broken down these plasmids before they were recovered (58). Antibiotic resistance was again shown to be due, at least in part, to resistance plasmids when they were taken from PC and used to transform E. coli HB101. The resultant transformed E. coli exhibited new ampicillin and tetracycline resistance similar to that of PC (96).

Gentamicin is an example of an antibiotic that was developed for use in serious gram negative bacillary infections which is almost never active against PC. It was tested following its approval for clinical use by the FDA, and while PA was found to be 51% sensitive at a drug concentration of 1 ug/ml, 97%



sensitive at 5 ug/ml, and 100% sensitive at 20 ug/ml, PC was insensitive at each of these concentrations (93). At that time PC was not a commonly found organism, and only two strains were tested, however gentamicin resistance of PC has been reported repeatedly (9,19,67,87), with occasional reports of variable sensitivity (84). Only two investigators have found PC isolates to be sensitive to gentamicin, and in both at least half of the strains tested were resistant (6,62).

The most useful antibiotics in the treatment of PC infection since this organism began appearing in the medical literature have been chloramphenicol and trimethoprim/sufamethoxazole (TMP/SXZ). Throughout the 1970's, in fact, these two compounds were the only consistently effective antibiotics reported. In vitro sensitivity to chloramphenicol has been described repeatedly (8,9,19,24,62,81,84,94), but in vivo effectiveness has been variable, especially in patients with serious underlying illness. For example, a heroin addict with persistent PC tricuspid endocarditis who was treated with high dose chloramphenicol to which his PC was sensitive in vitro, but in whom relapse was not prevented was reported in 1973 (73). In 1977, patients with CF whose PC was reported to be sensitive to chloramphenicol in vitro, showed good



clinical response to treatment with the drug, but the organism was only eradicated in a few cases (49).

Sensitivity of PC to chloramphenicol has been decreasing with time. According to one group, in 1977 only "a few" of the 402 respiratory tract cultures from patients with CF at their institution that grew PC were sensitive to chloramphenicol (49). Half of the eleven gentamicin and tobramycin resistant strains of PC and P. maltophilia isolated from patients with CF at another hospital were resistant to chloramphenicol in 1979 (8), more than half of the 13 strains isolated from patients with CF at another institution between 1/79 and 12/83 were resistant (87), and another group reported that few strains of PC remained sensitive to this drug in 1986 (27).

In many studies TMP/SXZ was the only compound found to be effective in vitro against PC (8,75,84), but more recent reports show a decline in the usefulness of this agent as well (27,87). Resistance to sulfonamide alone is occasionally reported in the literature (84,62), but no reports appeared until the early 1980's in which the combination was found to be ineffective in inhibiting PC in vitro. In fact, the combination of TMP and SXZ has often proved more effective than either of the two drugs alone. In one study, PC was found to be very sensitive to TMP alone,



but the addition of SXZ decreased the occurrence of TMP resistance and potentiated the effects of TMP on organisms that were previously resistant (63). Synergism has also been shown between TMP/SXZ and colistin, an agent which is never effective alone (64).

Interestingly, in 1973 synergism was also described between TMP and polymyxin B, another agent found repeatedly to be ineffective against PC (19,67,94), and this synergism was enhanced by sulfonamide, leading the authors to suggest that combination therapy with these agents may be beneficial (73).

Other antibiotics have sporadically shown limited in vitro activity against PC, but agents such as novobiocin (24), nalidixic acid (19), kanamycin (49,62,84,87), neomycin (9,62), carbenicillin (6,9,67,84,87), cefoperazone (81), tobramycin (6,87) and moxalactam (81,87) have not been consistently active in vitro and have been rarely employed in vivo.

The resistance of PC to penicillins and cephalosporins is related to the presence of an inducible beta-lactamase which hydrolyzes many of these compounds (31). It was hoped, however, that third generation cephalosporins would not be affected by this enzyme. In 1983, a study was done which considered the efficacy of ceftazidime in treating CF patients with PC infection, and although they found





most strains at their hospital to be inhibited by 4 mg/l or less of the drug in vitro, one third of the treatment courses were considered failures in the face of deteriorating clinical status, and only one of 19 patients had significant reduction of colony counts in his/her sputum. These failures were thought to reflect either failure of penetration of the sputum by the drug, failure of delivery of sufficient amounts of drug to severely ectatic lung, overwhelming inocula of PC in bronchial secretions, impaired phagocyte function in the CF lung, antibiotic interference by pus, or some combination of these factors (26).

In 1985 the in vitro activity of cefpiramide, ceftazidime, piperacillin, ticarcillin, and aztreonam, all beta-lactam anti-pseudomonal agents, were compared against PA and PC in children with CF. All were tobramycin and amikacin resistant. They found ceftazidime to be the most effective in vitro agent, inhibiting 90% of the isolates at a concentration of 8 mg/l. Fifty percent were sensitive to 32 mg/l or less of cefpiramide, fifty percent were susceptible to the same concentration of aztreonam, and ticarcillin was the least effective agent tested (4). This apparent activity of ceftazidime against PC, while encouraging, must be viewed in light of the earlier in vivo failure of the drug.



In another recent study, the effects of aztreonam, piperacillin, and ticarcillin, alone and in combination with amikacin, were tested against amikacin resistant PA and PC isolated from the sputum of patients with CF. It was reported that 86.3% of the 22 strains of PC were sensitive to 16 ug or less of piperacillin, and 100% were susceptible to the combination of this drug with amikacin. Twenty-two percent were sensitive to aztreonam alone and 81.8% when amikacin was added, and none of the PC was sensitive to ticarcillin, with or without the second drug. The authors suggested that while in vivo data had not been collected, the in vitro results made combination therapy with these drugs an attractive alternative (3).

Ciprofloxacin, a new oral oxyquinoline derivative with significant anti-pseudomonal activity, has also been studied, along with norfloxacin, mezlocillin, azlocillin, aztreonam, imipinem, ceftazidime, and cefsulodin for in vitro activity against sputum PA and PC isolates from patients with CF. With inocula of one million colony-forming units per ml, ciprofloxacin, norfloxacin, and ceftazidime were inhibitory, while the others were not. At larger inoculum sizes there was decreased inhibitory activity of norfloxacin and ceftazidime, but not of ciprofloxacin. The



authors suggest that oral ciprofloxacin may be a useful agent for treating pseudomonal pulmonary exacerbations, both by PA and PC, in CF patients, its oral form making outpatient therapy possible (4).

#### PSEUDOMONAS CEPACIA IN CYSTIC FIBROSIS

The first report of PC isolation from a patient with CF appeared in the literature in 1972, in a study by Ederer et al. of the incidence of PC infection and colonization of patients at the University of Minnesota Hospital. In their 2 year study, from January, 1968 through December, 1969, PC was isolated 115 times from 41 patients, each of whom had a major debilitating condition, with cancer being the most common diagnosis. Of the 41, one patient suffered from CF, but no details of this individual's course are given, and it is unclear whether or not this patient's colonization was felt to be clinically significant (19).

In 1977 an abstract was published in Pediatric Resident reporting 402 PC isolates out of 6364 respiratory tract cultures obtained between July, 1973 and December, 1976 from CF patients at St. Christopher's Hospital for Children. This report first



raised the question of the significance of that organism specifically in patients with CF (49).

The first case report appeared in 1980, when Rosenstein and Hall described a 17 year old girl who was admitted for the first time to the hospital, whose CF had followed a slowly progressive course until that time. No pseudomonads had been found in her sputum cultures for the four years prior to admission. She had been treated continuously with cloxacillin and tetracycline for the two years prior to admission, and had not been treated with home nebulizers. Her clinical course was rocky, and she required intubation, but her sputum PC eventually cleared after treatment with multiple antibiotics (75). Since that report, PC has become the subject of concern in many CF centers where its prevalence has been increasing.

Many studies have been done which attempt to define the incidence and prevalence of infection with PC in patients with CF. In 1977, 6.3% of sputum cultures from patients with CF at St. Christopher's Hospital in Philadelphia grew PC. Fifty-four patients had positive cultures, their ages ranging from 2.5 to 22 years. Fifty-nine percent of those colonized were female and 41% male; 3.7% had mild underlying lung disease, 27.5% had moderate disease, and in 68.5% advanced pulmonary involvement was noted. Twenty-





eight percent of the colonized females died, as compared to 22.7% of the males. No data was given which compared these numbers to the mortality rate of patients with CF who were not colonized with PC (49).

In contrast, at Yale-New Haven Hospital between 1975 and 1979 only two of 170 patients were found to have PC growth in their sputum. Although the patients carrying PC were not analyzed separately, the authors noted that 75% of the 20 patients found to harbor any species of non-fermentative gram negative rod other than PA were in good to excellent clinical condition when the organisms were first isolated. During their period of study only one patient died; his/her infecting organism was not noted (6).

More recently it was reported that the prevalence of PC colonization among patients with CF at Rainbow Babies' and Children's Hospital in Cleveland (RB&C) had increased from 7 to 15% between 1978 and 1983. The mean age of acquisition was 18.1 plus or minus 4.9 years for males, and 18.8 plus or minus 4.9 years for females. In the first 3 years of this study, 60% of patients who acquired PC were in poor condition at the time of colonization, while in the 5th year only 18% were in poor condition, indicating an increasing trend not only in the prevalence of overall colonization, but in the prevalence among patients in better



clinical condition. Also, there was a trend toward younger ages of colonization; between 7/81 and 6/83 eight patients less than 12 years of age were colonized, while prior to 1981 only 2 in that age group had positive cultures (91).

In a paper by Corey et al., an increasing prevalence of PC colonization among the patients with CF at the Hospital for Sick Children in Toronto was also noted. Between 1970 and 1977, 4-5% of their patients younger than ten years of age, and 12-15% of those older than ten, had positive sputum cultures; in 1981 9% of the younger children and 21% of the older group were colonized. The overall prevalence increased during this time period from 9.6 to 18.1%. In that institution more girls were affected than boys, and the mean age of acquisition was 13.7 plus or minus 7.2 years (14).

A 1985 paper from St. Christopher's showed a different trend at that institution. Between 1/79 and 12/83 the prevalence of PC colonization among patients with CF decreased from 20.4% to 12.2%. The authors suggest that this decreasing trend may be explained by a greater rate of mortality in those patients affected than the rate of colonization among those not affected. In that study the mean age of first isolation of PC was 12.1 years. These studies seem to



define the trend as being toward greater rates of colonization by PC of patients with CF and PC, with an increasing number of younger and less severely debilitated patients becoming affected (87).

Other possible risk factors have also been addressed in the literature, and because most earlier reports of infection with PC were traced to contaminated hospital equipment, investigators have been concerned that hospitalization would be a significant risk factor for PC acquisition in patients with CF. In the 1977 abstract from St. Christopher's cited above, the authors state that PC had been cultured from the sputum of patients with CF who had never been hospitalized and who rarely used aerosol therapy (49). In a later study at that institution, no PC was identified in cultures of water, respiratory therapy equipment, or medicine vials, but despite these negative cultures an association between acquisition of PC and frequent visits to the outpatient clinic of that hospital was noted, raising the question of an obscure nosocomial source (87).

At (RB&C), Thomassen et al. determined that 56% of 85 colonized patients had their first positive culture during or immediately following hospitalization (91). Although multiple cultures were done on hospital equipment, water and other environmental



objects and personnel, the source of the organism was never identified (92). At the Hospital for Sick Children, however, growth of PC from spirometers and other equipment from their pulmonary function laboratory was reported by Isles et al., defining the source of at least some of the PC found in their patients (40).

Another risk factor for PC colonization seems to be the presence in the same home of a sibling with CF who is colonized with PC. In 1985, Tablan et al. found that colonization with PC was significantly more likely in patients who had such a sibling, as well as in those with more severe underlying pulmonary disease and in those who had received aminoglycoside antibiotics in the three months prior to the first positive culture (87). At RB&C, Thomassen et al. followed 16 pairs of siblings with CF in which at least one was colonized with PC, and found that in 6 pairs the other child was also chronically colonized and in 3 pairs the second child was colonized intermittently. This was a marked increase in incidence of PC colonization from about 17% of the overall CF population of that institution to 37.5% in those with affected siblings (91).

The apparently increased incidence of PC colonization among siblings suggests that person-to-person





transmission might occur. In Danish CF clinics in 1982, patients were found to be colonized with predominantly one strain of PC, which could be explained by transmission from patient to patient or by a common source of exposure, perhaps located in the clinic itself (72). It is known that transmission of PA requires prolonged intimate contact, as siblings might have, but only one study addressing that question in PC colonization has been done. In this study, Honicky et al. did not find any increased incidence of PC colonization at the end of one week at a CF camp (37). The short duration of exposure and lack of subsequent follow-up make conclusions difficult to draw.

One study which does support the idea of person-to-person spread of PC is from RB&C, where the patients with CF who are colonized by PC are physically separated from those without the organism in the hospital, and even in separate summer camps. They have found the overall incidence of PC colonization to have decreased with these measures, from 8.2% in 1983 to 1.7% in 1984 (97).

One other risk factor that is suggested in the literature is that of female sex. Boxerbaum et al. propose that post-pubertal females have an increased risk of colonization, in an abstract which reports four such patients with fulminant PC bacteremia (10).



Tablan, however, found no such correlation in an epidemiologic investigation done in 1985, and the matter remains unresolved (87).

While questions of true incidence, risk factors and transmission are all important, the most basic question about infection with PC in patients with CF is what, if any, effect does it have on the clinical course of the patients. Isles et al. describe three different clinical courses that they found in these patients. Those in the first group acquire PC with little change in the progression of their disease, in the second they continue on an already downhill course, and in the third a fulminant downward course developed, leading to sepsis and death (40). The first case report of a CF patient with PC, in fact, was that of a seventeen year old girl who had been in previously good condition who acquired PC and followed this third pattern, developing sepsis with respiratory failure, right ventricular failure, SIADH, hyperglycemia, and pneumothorax (75).

Boxerbaum describes four more cases of female CF patients with mild disease whose becoming colonized with PC was followed by a rapidly fatal course. Three of these patients lived less than three weeks following colonization. Their courses were very different from the more slowly progressive decline associated



with PA infection, and were marked by spiking fevers, leukocytosis, and bacteremia (10).

The trend followed by patients with CF who were colonized with PC at RB&C, reported in 1984, was that of a distinctly faster deterioration in clinical status than non-colonized patients, and the prognosis seemed to be worse for females. Fifty-one percent of females had a decline in status in the year following colonization, regardless of their initial condition, while only 30% of the males followed this trend. Of those patients initially in good clinical condition, 64% of the females had a decline in status concurrent with acquisition of PC, while only 6% of the males did. The proportion of colonized patients following a downhill course after acquiring PC was seen to be increasing; in the first year of the study 9% of all CF deaths were associated with PC while a full 55% of deaths in the fifth year fell into this category. The largest increase was seen in the group of patients that followed a rapid and fulminant course (91).

In contrast, at the Hospital for Sick Children, Isles et al. reported that while the absolute pulmonary function parameters of their CF patients with either PC alone or PC and PA in their sputum were worse than those of patients with PA alone, the slope of decline between the two groups did not



significantly differ. However, among girls with PC, mortality was 50% over one year, while girls with PA had a mortality rate of 6%. Poor prognostic indicators in female patients with PC were the presence of fever and leukocytosis. Sixty-two percent of girls admitted with PC and fever died, and of those who died the mean WBC on admission was 25,500, while it was 14,000 in those who lived. Similar studies were not reported for boys. Among all patients with CF admitted to the hospital with PA, only 18% had fever, while 44% of those with PC were febrile on admission. Also in that paper 7 cases of mild to moderately ill patients with CF who developed fulminant, rapidly progressive pulmonary infection with PC, similar to those reported by Boxerbaum, were described. This study suggests that patients admitted to the hospital with PC pulmonary infection, fever, and leukocytosis are at greater risk of death, and that this risk seems to be increased in females (40).

At St. Christopher's it was found that hospitalized patients who were colonized with PC had an increase in the mean proportion of time that they spent in the hospital for pulmonary symptoms from 9% in the year prior to colonization to 21.3% in the year following colonization. In controls, the proportion during the same time period decreased from 2.8% to





1.9%. For their CF patients, colonization with PC, as well as severity of pulmonary disease and female sex, were found to be independent indicators of poor prognosis (87).

The worsening clinical course reported in these recent studies to be associated with PC colonization and infection is not proven to be caused by this organism. It is also conceivable that deterioration caused by some unrelated factor allowed patients to be more susceptible to PC colonization. The possibility that PC, an organism which is difficult to treat, does cause such devastating deterioration, however, makes further study of its true contribution to the disease process imperative.



### 3. METHODS

Subjects were chosen from the Cystic Fibrosis Database of Rainbow Babies' and Children's Hospital, (RB&C) Cleveland, Ohio, which includes all patients who attend that institution's cystic fibrosis clinic. One hundred and forty-six patients with Pseudomonas cepacia colonization, as determined by three sequential positive sputum cultures, were identified. The date of acquisition was recorded as the date of the first positive culture.

One matched control was chosen from the RB&C database for each colonized subject. The matches were assigned on the basis of sex, age within six months of the subject, and survival at least until the time of the subject's acquisition of PC. Where more than one suitable match existed, an attempt was made to choose that patient who had the most pulmonary function data for the period from three years prior to the date of PC acquisition of his/her match to two years following the acquisition date. Matches were found for one hundred and nineteen of the original subjects;



sixty-three were males and fifty-six were females.

Records of the pulmonary function laboratory were searched for each colonized subject and matched control, and all values of actual forced expiratory volume in one second (FEV1) and residual volume to total lung capacity ratio (RV/TLC) for the three years prior to PC acquisition and two years following acquisition were recorded. In each uncolonized control subject, the date of acquisition of PC the of his/her corresponding colonized subject was used. Each patient's date of birth and height at the time of each test was also recorded.

Fifty-six matched pairs in which one patient had less than three tests during this five year period, and thirty pairs in which one patient's data did not cover at least the time span from one year prior to PC acquisition to one year post acquisition were identified and were excluded from the regression analysis performed below. The remaining 66 patients included twenty male and thirteen female pairs.

From the remaining data each patient's age at the time of each pulmonary function test, and the time between each test and the date of PC acquisition were calculated.

To convert the raw FEV1 values into a standard form, the formulae of Knudson et al. were used to



calculate the predicted FEV1 for each test (46). The actual values were then divided by the predicted values and multiplied by 100 to determine the percent of predicted FEV1 for each test. The formulae employed were as follows:

<u>Sex</u>	<u>Age (yrs)</u>	<u>Expected FEV1</u>
Male	6-11.99	$-2.8142 + 0.0348(\text{ht in cm})$
Male	12-24.99	$-6.1181 + 0.0519(\text{ht}) + 0.0636(\text{age in yrs})$
Male	>25	$-6.5147 + 0.0665(\text{ht}) - 0.0292(\text{age})$
Female	6-10.99	$-2.7578 + 0.0336(\text{ht})$
Female	11-19.99	$-3.7622 + 0.0351(\text{ht}) + 0.0694(\text{age})$
Female	>20	$-1.8210 + 0.0332(\text{ht}) - 0.0190(\text{age})$

To remove some of the variation in each individual's values, the one test with the highest percent of predicted FEV1 was chosen for each one month period within the five years chosen above for each subject. The RV/TLC value for that date was also included. Each patient's highest percent of predicted FEV1 and lowest RV/TLC for each two month period was also identified, to be used in the graphs below.





## MORTALITY

Mortality rates were determined for the colonized subjects and matched controls at one year and two years post-acquisition of PC. The date that each deceased patient was last seen at RB&C was used as an approximate date of death. The mortality rate of the colonized subjects was compared to that of the controls, and the rate of death among the female subjects to that of the males using chi-square analysis.

## RISK FACTORS FOR POOR OUTCOME

The 34 colonized subjects who died within one year of acquisition of PC were identified and the FEV1 and RV/TLC values of the one test date closest to exactly one year prior to the date of PC acquisition was recorded for each. The corresponding data of each of their matched controls was also recorded. Thirteen instances in which either a colonized subject or his/her control did not have any test data between .5 and 1.5 years prior to the date of acquisition were not included. If two test dates were equidistant from the date one year prior to acquisition, that test date which was closer to the actual acquisition date was chosen. The same procedure was followed for the 85



colonized subjects who survived more than one year following acquisition and their controls, and the average FEV1 and RV/TLC values of the two groups were compared using t-test analysis to determine whether statistically significant differences existed between them.

The age of each patient included above when he/she acquired PC were also determined. The average age of acquisition of those patients who survived at least one year after acquisition of PC was compared to that of those who did not survive one year to determine whether a significant difference existed.

#### PULMONARY FUNCTION

To visualize the data for colonized subjects and their controls, a plot was made of the mean percentage of predicted FEV1 of the 33 subjects with sufficient data versus time since PC acquisition, for the five year period defined above. This was plotted on the same axis as the corresponding plot for the control patients. Values included in these plots were each patient's best FEV1 values for each two month interval beginning at 36 months prior to acquisition PC and ending at 24 months after acquisition, as determined above. Similar plots were then made for female and



male patients separately.

A graph of mean RV/TLC versus time since acquisition of PC was also made using all 33 colonized subjects and was plotted on the same axis as the corresponding plot for controls. Values chosen were each patient's lowest RV/TLC reading for each two month period as described above. Separate plots were then made for females and males.

To determine the slopes of the lines generated above, linear regression analysis was done using each patient's best test in each one month period, and the slopes and intercepts of the subjects' lines were compared to those of the controls to determine whether a significant difference existed. These parameters were also studied separately in female and male subjects to determine whether or not gender was related to the pulmonary sequelae of colonization by PC.

To assess whether the slopes of these lines changed following acquisition of PC, regression analysis was employed separately for the portion of each line that fell into the time period from 36 months to 12 months prior to the PC acquisition date and from the acquisition date to two years later. The period of one year just prior to PC acquisition was not included because it was felt that in that time period some of the patients would have already been



colonized, due to the impossibility of determining an exact date of acquisition. These slopes were then compared to determine whether any change had occurred. Comparison was also made between the slopes and intercepts of similar data for male and female patients.

Because the above data included different numbers of tests for each patient, the graphs and regression data generated above were weighted toward those patients with more tests. To eliminate this bias, the slopes and intercepts of each individual's data were determined by linear regression analysis. It was necessary to determine only one slope and intercept for the whole five year period for each patient, as there were not enough individuals with sufficient data to analyze the periods prior to and following acquisition of PC separately. T-tests were then employed to determine whether significant differences exist between the average FEV1 and RV/TLC lines of subjects and controls, and between those of male and female subjects and their respective controls.

Multivariate analysis of variance was then done to confirm the results of the t-tests in determining the statistical effects of gender and colonization by PC on the FEV1 and RV/TLC of subjects and controls.





#### 4. RESULTS

##### MORTALITY

Of the 119 subjects colonized by PC, 28.6% (34/119) died in the first year following its acquisition. None of their 119 matched controls died in the same one year period, a significant difference in mortality (chi-square  $<.005$ ).

In the second year following acquisition of PC, five of the remaining 85 subjects died (5.9%), while five of the 119 controls (4.2%) died in that year. This was not a statistically significant difference in mortality (chi-square  $>.05$ ).

In the first year following the date of acquisition of PC, 25.4% (16/63) of the male subjects and 32.1% (18/56) of the female subjects died, not a statistically significant difference (chi-square  $=.05$ ). There was also no statistically significant difference in mortality of males and females in the second year, with 2.1% (1/47) of the males and 10.5% (4/38) of the



females dying in that period (chi-square  $>.05$ ).

#### RISK FACTORS FOR POOR OUTCOME

The average percent of predicted FEV1 of the 21 colonized patients who died within one year of PC acquisition was 34.0 one year prior to colonization, while that of the colonized patients who survived at least one year was 53.9, ( $p=.001$ ). The mean RV/TLC value of the colonized patients who did not survive one year was 0.53 while that of the survivors was 0.47, ( $p=.002$ ). The mean age of PC acquisition of the survivors was 18.9 years, not significantly different from that of the non-survivors, which was 19.8.

#### PULMONARY FUNCTION

A plot of mean FEV1 (as percent of predicted) vs. time for patients and controls is shown in figure 1. Figures 2 and 3 show the corresponding graphs for boys and girls respectively. Figure 4 shows mean RV/TLC vs. time for all patients and controls, and figures 5 and 6 demonstrate these same curves for boys and girls respectively.



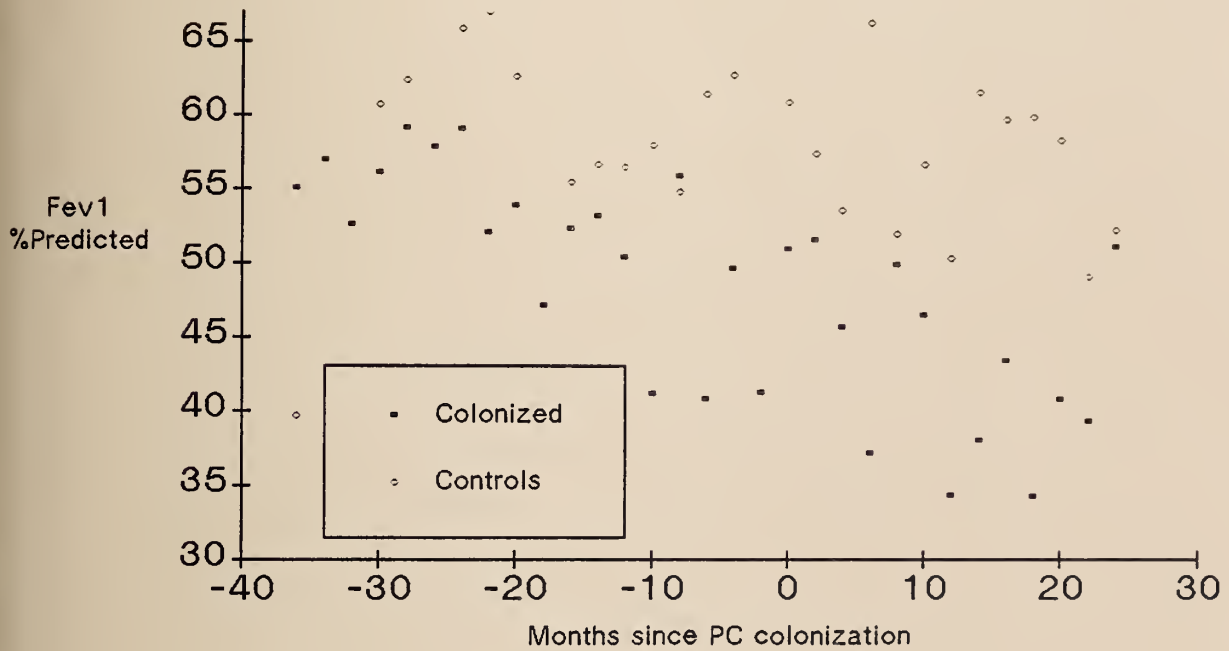


Figure 1. Mean FEV1 as a function of time since colonization with PC in 33 colonized subjects and their controls.



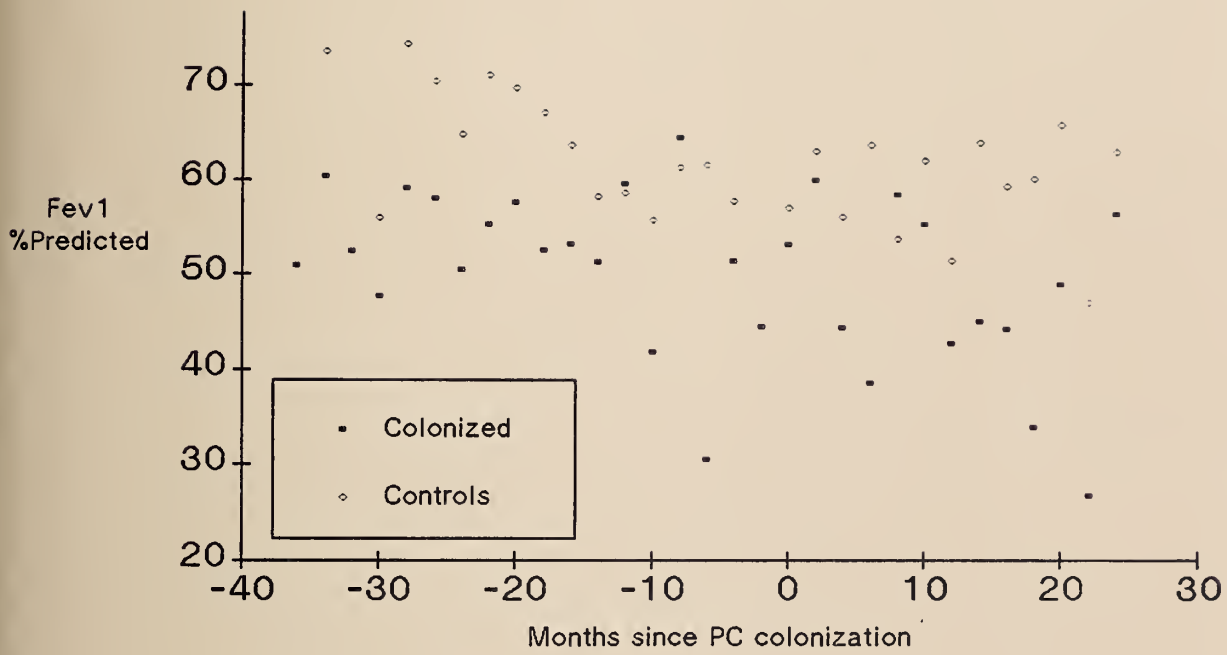


Figure 2. Mean FEV1 as a function of time since colonization with PC in 20 colonized male subjects and their controls.





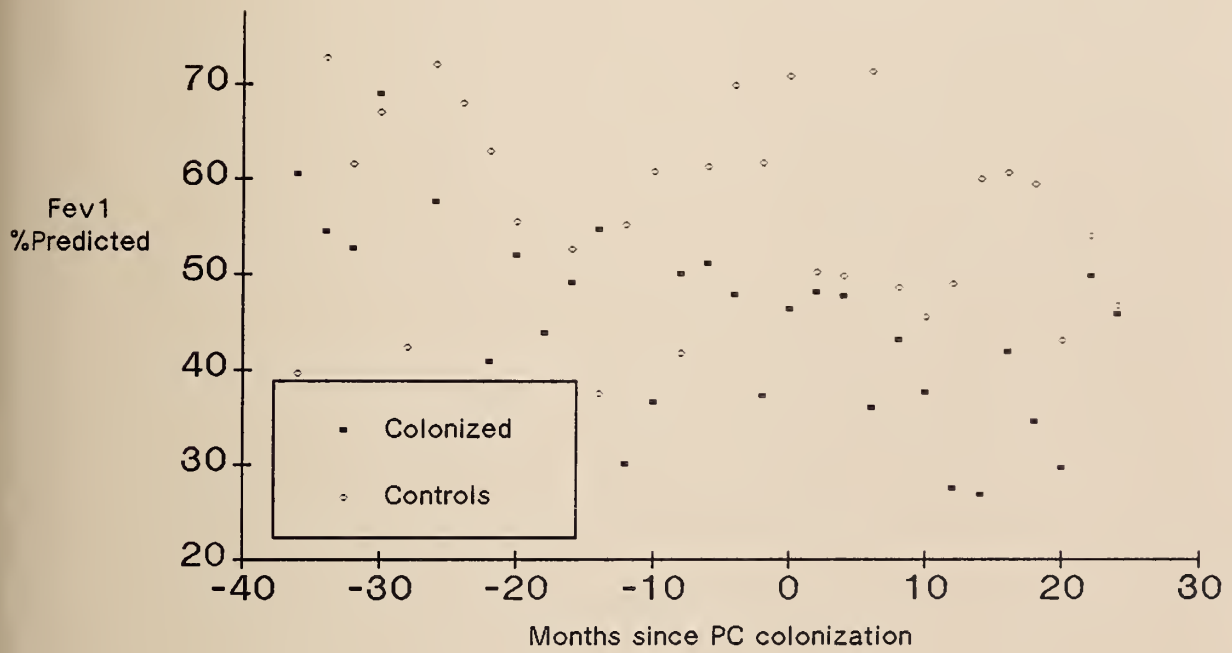


Figure 3. Mean FEV1 as a function of time since colonization with PC in 13 colonized female subjects and their controls.



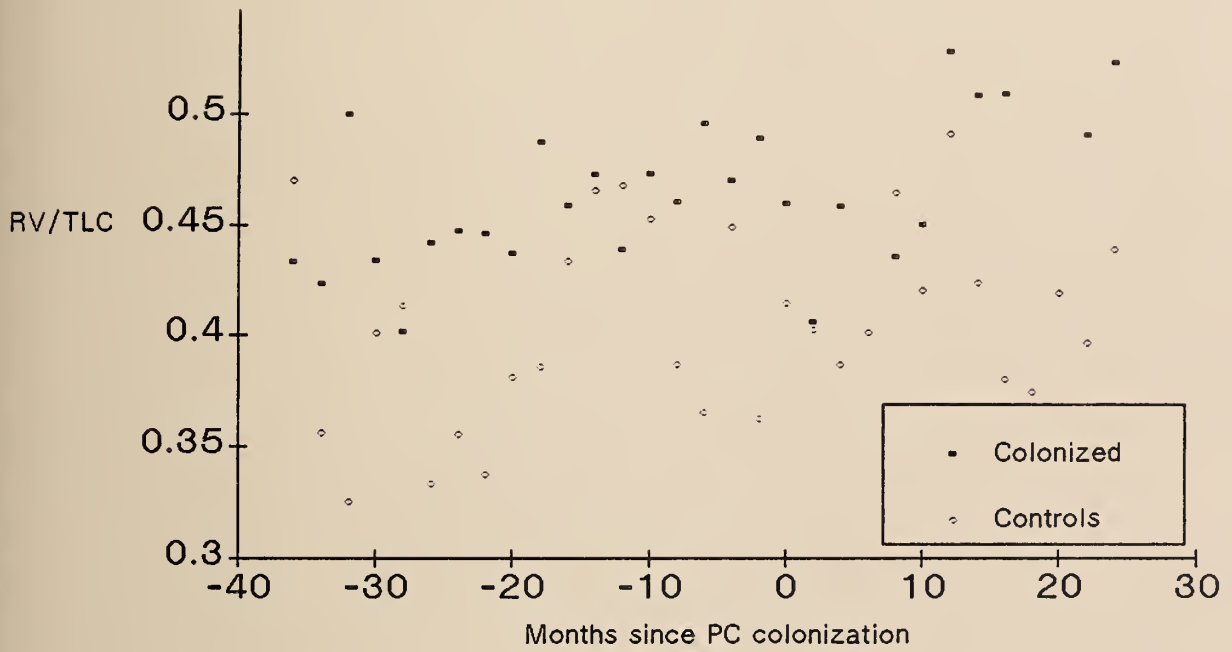


Figure 4. RV/TLC as a function of time since colonization with PC in 33 colonized subjects and their controls.



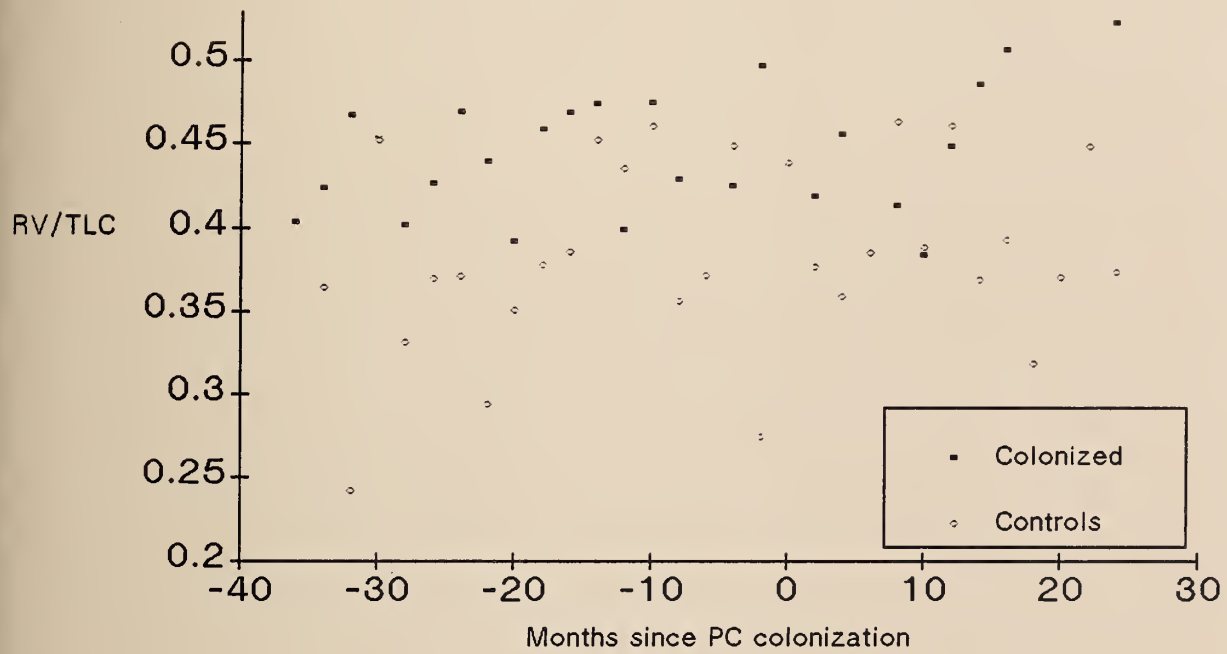


Figure 5. RV/TLC as a function of time since colonization with PC in 20 colonized male subjects and their controls.



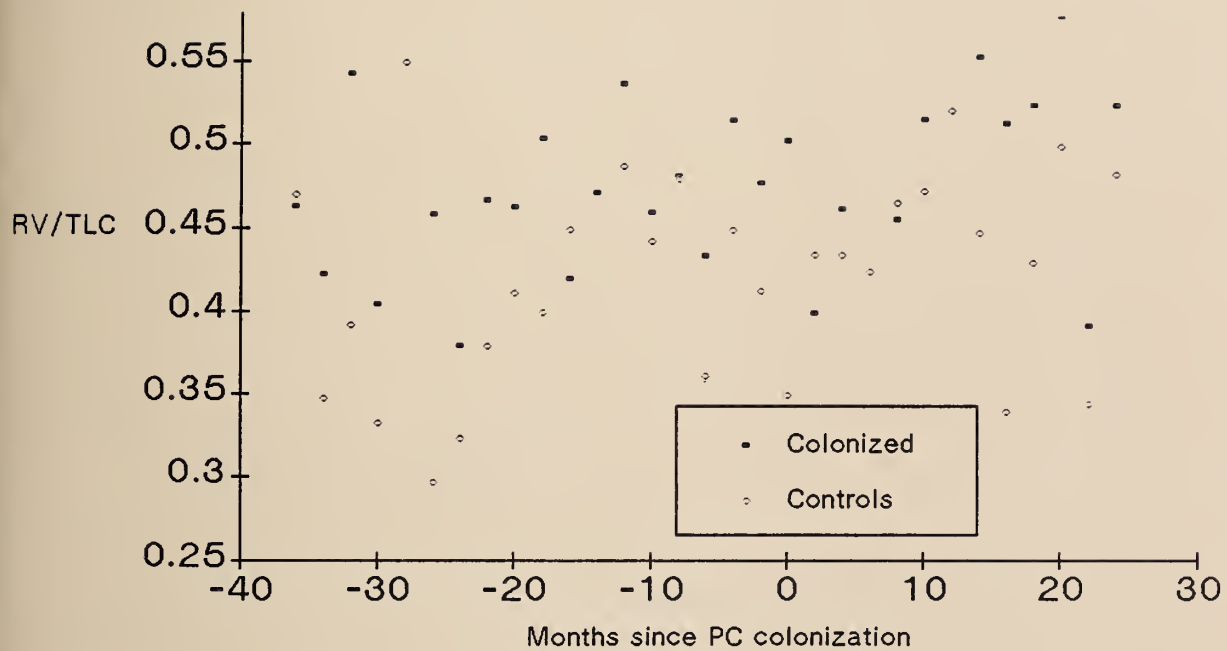


Figure 6. RV/TLC as a function of time since colonization with PC in 13 colonized female subjects and their controls.





The slopes and intercepts of the lines generated by regression analysis of the above graphs are shown in table 1. Significant differences were found between the FEV1 and RV/TLC lines generated for the colonized subjects and controls, with a lower intercept and more negative slope in the subjects' FEV1 data, and a higher intercept and more positive slope in their RV/TLC data over controls. This suggests that the colonized subjects had poorer pulmonary function over those five years than the controls, and that their pulmonary function declined more quickly.

Colonized male subjects, when considered separately, demonstrated a lower FEV1 intercept than their controls, but did not have a significantly different rate of decline, while they had both a higher intercept and faster rate of increase in their RV/TLC than controls. Female subjects were somewhat different in their response, showing both a lower intercept and more negative slope in FEV1 and a higher RV/TLC intercept with no difference in slope from their controls.

When comparing colonized male subjects to their female counterparts, a significantly higher FEV1 intercept and lower RV/TLC intercept were seen in the males, while the slopes of the two parameters did not vary significantly between the two.



TABLE 1

Regression analysis of FEV1 and RV/TLC data of 33 subjects and controls over a five year period

		<u>FEV1</u>	<u>S.E.</u>	<u>RV/TLC</u>	<u>S.E.</u>
<u>COLO</u>	<u>sl</u>	-4.26101	0.76137	0.02100	0.00471
	<u>int</u>	43.66107	1.10397	0.48682	0.00690
<u>CONTROLS</u>	<u>sl</u>	-2.38264	0.85320	0.00868	0.00574
	<u>int</u>	58.16482	1.25967	0.42044	0.00846
<u>M COLO</u>	<u>sl</u>	-3.72658	0.95743	0.02310	0.00612
	<u>int</u>	46.23574	1.41330	0.48181	0.00902
<u>M CONTROLS</u>	<u>sl</u>	-2.74193	1.21641	0.00550	0.00841
	<u>int</u>	59.26513	1.77729	0.40918	0.01222
<u>F COLO</u>	<u>sl</u>	-4.89849	1.21406	0.01835	0.00730
	<u>int</u>	40.55944	1.72097	0.51158	0.01060
<u>F CONTROLS</u>	<u>sl</u>	-2.00608	1.17577	0.01194	0.00768
	<u>int</u>	56.78660	1.75842	0.43346	0.01150

(S.E.=standard error, M=male, F=female, colo=colonized, sl=slope, int=intercept)

The slopes and intercepts of the lines generated by regression analysis of FEV1 and RV/TLC data during the time period from -36 to -12 months and 0 to 24 months are shown in table 2. They show no significant change in either parameter in colonized subjects, and a significantly more negative RV/TLC slope in controls with no difference in FEV1. When considered separately, the males demonstrated a significant decrease in FEV1 slope and increase in RV/TLC slope from the earlier time period to the later, but no change in the



two intercepts. The females showed no change in either parameter over time.

In the earlier time period, neither the FEV1 nor the RV/TLC parameters of the males were significantly different than those of the females, and no significant difference between the genders was seen in the later time period either.

TABLE 2

Regression analysis of FEV1 and RV/TLC data of 33 subjects and controls from 36 to 12 months prior and from 0 months to 24 months following PC acquisition

<u>FEV1</u>		<u>BEFORE</u>	<u>S.E.</u>	<u>AFTER</u>	<u>S.E.</u>
<u>COLO</u>	<u>sl</u>	-3.00273	3.28244	-5.61402	2.55768
	<u>int</u>	46.53508	6.70651	45.41759	2.92495
<u>CONTROLS</u>	<u>sl</u>	-4.95776	3.43811	-0.56106	2.91835
	<u>int</u>	53.33592	6.75844	55.96849	3.40726
<u>M COLO</u>	<u>sl</u>	0.28578	4.05360	-7.94644	3.37762
	<u>int</u>	54.31509	8.30525	51.28824	4.03503
<u>M CONTROLS</u>	<u>sl</u>	-5.77907	5.01846	-0.51430	3.86950
	<u>int</u>	53.86584	9.72063	56.60343	4.44639
<u>F COLO</u>	<u>sl</u>	-6.50609	5.34596	-3.83199	3.84390
	<u>int</u>	38.07909	10.88395	39.84930	4.16326
<u>F CONTROLS</u>	<u>sl</u>	-4.43410	4.56430	-0.42977	4.51520
	<u>int</u>	51.79176	9.13313	54.98022	5.38933
<u>RV/TLC</u>					
<u>COLO</u>	<u>sl</u>	0.01686	0.01758	0.03826	0.01803
	<u>int</u>	0.48773	0.03600	0.47479	0.02054
<u>CONTROLS</u>	<u>sl</u>	0.04535	0.02147	-0.00860	0.02099
	<u>int</u>	0.48993	0.04217	0.43678	0.03678
<u>M COLO</u>	<u>sl</u>	0.01885	0.02406	0.07390	0.02273
	<u>int</u>	0.47935	0.04918	0.42023	0.02714
<u>M CONTROLS</u>	<u>sl</u>	0.04443	0.03133	-0.01867	0.02843
	<u>int</u>	0.48039	0.06057	0.43245	0.03039
<u>F COLO</u>	<u>sl</u>	0.01456	0.02585	-0.00541	0.02848
	<u>int</u>	0.49880	0.05311	0.53664	0.03036
<u>F CONTROLS</u>	<u>sl</u>	0.04878	0.02880	-0.00268	0.03174
	<u>int</u>	0.50628	0.05762	0.44661	0.03751

(Before=36-12 months prior to PC acquisition, After=0-24 months after PC acquisition, S.E.=standard error, Colo=colonized by PC, M=male, F=female, sl=slope, int=intercept)



T-test analysis of the individual regression parameters of each subject and control over the entire 5 year time period revealed a significant difference in the mean FEV1 intercept between subjects and controls ( $p=.020$ ), but no significant difference in the average slope between the two groups. Although the RV/TLC intercept was higher in subjects than controls, the difference was not quite statistically significant ( $p=.051$ ).

Multivariate analysis of variance confirmed that there was no significant association between FEV1 or RV/TLC slope or intercept and sex, and that subjects' FEV1 intercept was significantly lower than that of their controls ( $p=.024$ ). The subjects' RV/TLC intercept was higher than that of the controls, but the difference was not statistically significant ( $p=.053$ ).





## 5. DISCUSSION

Colonization of CF patients with PC has become a common problem in the last decade, yet its implications have not been clearly identified. This study attempts to define the effect of colonization by PC on the mortality and pulmonary function of patients with CF. Colonized patients who survived longer than one year following acquisition of PC were compared to those who died within a year, and differences between these groups prior to colonization which could predict clinical outcome were studied. In addition, differences between the survivors and age and sex matched controls with respect to pulmonary function several years before and after acquisition of PC were sought.

The mortality in the first year of colonization with PC is significantly increased over sex and age-matched controls who were not colonized. No difference in mortality was seen in the second year, suggesting that patients colonized with PC who survive the first year stabilize and do not sustain increased mortality. These findings support, in part, the three



clinical courses of PC colonized CF patients proposed by Isles et al. (40). Their first group, which followed a fulminant and quickly fatal course, corresponds to those patients in this study who died in the first year, while their other two groups, who asymptotically harbored the organism or progressively deteriorated over a protracted period, correspond to the group in this study who survived the first year. No attempt was made in this study to distinguish between these last two groups.

Patients who did not survive at least one year after colonization with PC represent the worrisome clinical problem posed by this organism. In this study, mortality was the only measure of clinical outcome in these patients; they were excluded from regression analysis used to define the rate of decline in pulmonary function of the patients who survived longer because their data spanned such a short period of time that it made that analysis difficult to interpret.

The quickly deteriorating group in this study was found in retrospect to differ from the others even before acquisition of PC. One year prior to colonization the mean percent of predicted FEV1 of those destined to survive more than one year following PC acquisition was significantly higher than that of



the others, and their mean RV/TLC was significantly lower, suggesting that poor pulmonary function prior to PC colonization predisposes to poor outcome. This may also support the hypothesis that PC does not change the downhill course of disease in these patients who were clearly in poor clinical condition prior to acquiring the organism.

The average age that PC was acquired did not differ between those patients who survived one year and those who did not. In this study, however, the acquisition dates ranged from 1978 to 1984, and ages of PC acquisition have been changing with time, (14,87,91,92), possibly confounding these findings. It would be best to study the effect of age of acquisition of PC on clinical course in patients who became colonized by the organism in a short time span, a design that was not possible with the data collected here.

In the literature, other predictors of poor outcome have been noted. Isles et al. found that the presence of fever and leukocytosis in female CF patients at the time of hospitalization for a pulmonary exacerbation due to PC correlated well with poor outcome, but did not identify characteristics present prior to colonization which predicted poor outcome



(40). Some studies have addressed the question of increased risk of colonization (6,10,14,37,72,87, 91,92) but not increased risk of poor outcome once a patient is colonized; others compare the clinical courses of colonized patients to those who are not colonized (10,87,91), rather than those of colonized patients who follow different courses.

It would be useful to study other clinical characteristics of CF patients with PC in the patients who survived a year and those who did not. Differences in overall clinical status, immune status, previous therapeutic regimens, and nutritional status are some of the many parameters not addressed by this study which may be useful in predicting clinical outcome.

The hypothesis that females colonized with PC have a poorer prognosis than males was tested and rejected. Thomassen et al. proposed that females had a more rapid decline in clinical status in the year following PC acquisition than did males, but did not address their relative mortality (91). While the present study did not examine the rate of decline of pulmonary function over the first year after colonization in all men compared to all women, it would be expected that if a more rapid decline occurred in females, increased mortality would accompany it. No





such difference was seen in the first or second years following colonization, suggesting that no difference in prognosis exists, or that small sample size made determination of such a difference impossible.

The second group of subjects, those who survived more than one year, were studied to determine whether their pulmonary function differed markedly from controls who were not colonized with PC, despite their comparable mortality. Before regression analysis was done, figures 1 through 6 were plotted to get a general idea of the differences in data between subjects and controls. The highest FEV1 and lowest RV/TLC for each two month period was chosen to try to delete some of the variation within each patient's data due to poor effort or other confounding influences. A great deal of variation remains, as evidenced by these graphs. Ideally, each point on these plots should represent the mean FEV1 or RV/TLC of the same number of patients, but in this study not all patients had tests at the same intervals, so the number of patients represented by each point is variable.

The regression analysis of these graphs showed poorer FEV1 and RV/TLC and more marked decline in pulmonary function in colonized subjects than controls, and significant differences in FEV1 and RV/TLC intercepts but not slopes between males and females.



The data used included the best FEV1 and RV/TLC values for each one month period, so as to include more points than the every two month values plotted in the graphs. The slopes and intercepts generated, however, included different numbers of data points for each patient, depending on the number of tests he/she had in the five years under study.

In order to overcome this bias, regression analysis was performed on the data of each individual patient, and the slopes and intercepts generated were then analyzed to identify differences between colonized subjects and controls, and differences between males and females. The results of this analysis were somewhat different, showing no difference in slope for either the FEV1 or RV/TLC data between subjects and controls, and a statistically significant difference only in the intercept of the FEV1 data, not in that of the RV/TLC, although it was very close to significant. No difference between males and females were noted either.

These findings support those of Corey et al., who found significantly lower absolute values of several pulmonary function parameters but comparable rates of change in pulmonary function in patients with PC as compared to those without the organism (14).

While the above data gives an indication of the



clinical courses of colonized subjects and their controls over five years, it does not distinguish between their courses before and after acquisition of PC. In this study, there were not enough patients with sufficient data to do separate linear regression before and after PC acquisition on each individual and then compare them as above. Therefore, linear regression was done on data from all patients before and after colonization and is biased toward those with more tests.

Overall there was no significant change in pulmonary function in patients following colonization with PC. A change was seen, however, in the rates of change of FEV1 and RV/TLC of male patients when considered separately. This suggests that male survivors may actually have a worse course when colonized by PC than females, although the slopes were not significantly different from those of the females in either time period. As can be seen in table 2, however, with the small number of data points there is a large standard error, which may account for the lack of statistical significance of several seemingly large differences.



## 6. CONCLUSIONS

Patients with cystic fibrosis who became colonized with Pseudomonas cepacia were more likely to die in the year following acquisition of the organism than were age and sex-matched controls without PC. Those who did not die in the first year were not more likely than controls to die in the second year following colonization. No difference in mortality between males and females was observed.

Those patients colonized with PC who died in the first year had lower mean FEV1 and higher mean RV/TLC values one year prior to acquisition of the organism than colonized patients who survived at least one year. Mean age of acquisition of PC was not useful in predicting whether death would ensue in the first year.

Those patients who survived at least one year following PC colonization had significantly lower FEV1 values than age and sex-matched controls without PC over the five year period from three years prior to colonization to two years post-colonization, but had





a comparable rate of decline in FEV1. Their RV/TLC values and rates of increase were not significantly different from those of controls.

There were no significant differences in either the absolute values of FEV1 and RV/TLC or in the rates of change of these parameters following acquisition of PC in patients who survived at least one year. When considered separately, males demonstrated a significant change for the worse in the slopes of their FEV1 and RV/TLC data, a finding that was not observed in females.



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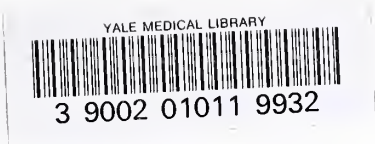












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