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Original Article

Is infection with hypermutable *Pseudomonas aeruginosa* clinically significant?☆

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Abstract

Background: Hypermutable *Pseudomonas aeruginosa* (HPA) with high mutation rate due to defects in the DNA mismatch repair genes are frequently isolated in the sputum of cystic fibrosis (CF) patients. These isolates tend to be multidrug resistant and may be better adapted to the CF lung environment. However, the clinical significance of this infection has not been determined.
Methods: This prospective study enrolled patients with PA infection attending CF clinics in Jerusalem between 2010 and 2011. Mutation frequency of pseudomonas isolates was determined by quantification of colonies resistant to rifampicin.
Results: Of the 73 patients enrolled, 22 (30%) were infected with HPA. Average mutation frequency was 2.95×10^{-4} in HPA and 1×10^{-7} in non-HPA.

Pulmonary function tests, number of pulmonary exacerbations and the response to antibiotic therapy were similar between patients infected with HPA and non-HPA isolates. The only predictors for infection with HPA were resistance to multiple antimicrobial categories (OR = 4.8, 95% CI: 1.8–12.4) and previous use of inhaled colistin (OR = 8.1, 95% CI: 2–30). Resistant mutant subpopulation analysis was a poor screening test for identifying HPA isolates.
Conclusions: Infection with hypermutable strains represents the marked ability of PA to adapt to the lung environment, but was not associated with worse clinical outcome.

Keywords: Cystic fibrosis; Hypermutable; *Pseudomonas aeruginosa*

1. Background

Progressive pulmonary disease is the cause of death for over 90% of cystic fibrosis (CF) patients. The observed decline in pulmonary function with increasing age is associated with *Pseudomonas aeruginosa* (PA) infection of the airways [1–3]. Although PA infection is associated with increased morbidity and mortality, variable disease severity and progression have been observed. Differences in PA virulence can account for some of this variability, such as infection with mucoid PA which is associated with poorer clinical outcome [4,5]. Other bacterial factors that affect the clinical outcome are currently unknown.

Recently, the presence of hypermutable PA (HPA) has been reported in patients with CF [6]. These strains are characterized by increased (up to 1000-fold) spontaneous mutation rates due to defects in genes involved in DNA repair or error avoidance systems [7]. Various mutations in the PA mismatch repair system

Abbreviations: CF, cystic fibrosis; PA, *Pseudomonas aeruginosa*; HPA, hypermutable *Pseudomonas aeruginosa*; MDR, multi-drug resistant; FEV1%, forced expiratory volume in 1 s; MEF 25–75%, maximal mid-expiratory flow.

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in the mutator genes *mutS*, *mutL* and *uvrD* have been identified in these mutator strains [8]. Hogardt et al. have found a significant down-regulation of virulence associated traits in mutator strains isolated from CF patients with end stage lung disease. These mutator isolates lost their ability to live freely (e.g. in tap water) and were less cytotoxic for bronchial epithelial cells, thus were optimally adapted for long term survival in the deteriorating CF lung [9].

The clinical significance of HPA in patients with CF is not well studied.

Hypermutable strains were isolated in 36 to 54% of sputum samples from CF patients [5,9]. These patients were older and had a significantly longer duration of chronic lung infection compared with patients without HPA. A longitudinal analysis of sputum samples found that HPA prevalence increases significantly with time from onset of infection. In the first five years after infection none of the isolates was hypermutable compared with 32% and 65% of isolates 20 and 25 years after the first PA isolation, respectively [9].

The vast majority of patients receive multiple courses of antimicrobial agents. PA strains resistant to multiple antimicrobial drugs almost invariably evolve in the CF lung. Several studies have shown that hypermutable strains are associated with significantly increased resistance to antimicrobial agents [5,10].

Intuitively, increased resistance will lead to treatment failure and worse outcome. Nevertheless, it was shown that in many cases there was no association between the PA susceptibility and clinical response [11]. The extent to which mutator strains lead to treatment failures in CF patients was not systematically studied.

The aim of this study was to examine the clinical significance of HPA infection in CF patients. We hypothesized that this infection will be associated with worse lung function, more frequent pulmonary exacerbations and failure of antibiotic therapy.

2. Materials and methods

2.1. Study population

This is an observational study examining patients with a documented diagnosis of CF and pulmonary PA infection. The study population consisted of patients attending the CF clinics at Hadassah Hebrew University (HHUMC) and Shaare Zedek Medical (SZMC) Centers between 2010 and 2011. These two centers cover all CF patients in the Jerusalem area.

Inclusion criteria were PA infection and patient/guardian informed consent. Patients co-infected with *Burkholderia cepacia* or after lung transplantation were excluded.

Study protocol was approved by the Ethics Committees of SZMC and HHUMC (Approvals #08/11, 0414-09-HMO).

2.2. Clinical data

Demographic and clinical parameters were retrieved from patients' medical charts. Lung function tests recorded for each patient included forced expiratory volume in 1 s (FEV1) and maximal mid-expiratory flow (MEF 25–75%) presented as a percentage of predicted value. Lung function tests 3 months prior to strain isolation, at the time of the culture and 3 months afterwards were recorded. Average values were used for each patient.

PA infection was classified as chronic or intermittent based on criteria proposed by Lee at. al (Leeds criteria) [12], and adopted by the EuroCareCF working group [13]. According to these criteria, chronic infection was defined when more than 50% of sputum samples were positive for PA in the preceding 12 months and intermittent when ≤50% of sputum cultures were positive.

Pulmonary exacerbations requiring parenteral antibiotics in the year prior to strain isolation were recorded. Treatment failure was defined as either a failure to improve FEV1% (FEV1% post-therapy/FEV1% pre-therapy ≤ 1), a decision to change intravenous (IV) antibiotic therapy by the treating physician due to lack of clinical response, or the need for an additional IV course of antibiotics within two weeks.

Treatment with oral or inhaled antibiotics and infection with *Haemophilus influenzae* (HI) or *Staphylococcus aureus* (SA) in the year prior to strain isolation were recorded as well.

2.3. Culture collection and PA isolation

Sputum specimens from each patient were collected during routine clinic visits and coded. A matching code was assigned to each sample during collection, and was thereafter used to refer to that sample, in order to both protect patient confidentiality and perform a blinded study. Clinicians caring for CF patients did not have access to information regarding the hypermutable strains in patients' sputum, nor did the laboratory personnel have access to any of the patients' clinical information. Collected sputum was routinely processed by the clinical microbiology laboratory, using standard protocols to speciate bacterial isolates [14] and perform susceptibility testing [15]. If colonies of different morphologies were present in the sputum culture, three representative colonies from each morphotype were tested. The first sputum culture obtained from each patient during the study period was tested. Subsequent cultures were tested only if the antibiotic susceptibility profile of the new PA strain was different compared with the previous isolate.

2.3.1. Mutation frequency measurement

Bacterial cells incubated overnight at 37 °C were collected and re-suspended. Aliquots from serial dilutions were plated onto LB agar plates, with and without rifampicin (300 μg/ml). In all cases, strains were originally susceptible to such concentrations of rifampicin. Colony counting was performed in plates containing <1000 colonies after 48 h of culture. All experiments were performed in triplicate, and the mean value was recorded. As previously defined by Oliver and colleagues [6], strains were considered hypermutable when the mutation frequency was at least 20-fold higher than that obtained for the control strain PAO1.

Three replicates of PAO1 were used as negative controls.

For comparison, we have used the disk diffusion method to identify resistant mutant subpopulations (RMSs). Inhibition zone