ELSEVIER
Journal of Cystic Fibrosis xx (2018) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

One time quantitative PCR detection of *Pseudomonas aeruginosa* to discriminate intermittent from chronic infection in cystic fibrosis

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Received 20 November 2017; revised 18 December 2017; accepted 20 December 2017
Available online xxxx

Abstract

Background: Chronic airway infection with *Pseudomonas aeruginosa* is a major risk factor of progression of lung disease in patients with cystic fibrosis (CF). Chronic *P. aeruginosa* infection evolves from intermittent infection that is amenable to antibiotic eradication, whereas chronically adapted *P. aeruginosa* becomes resistant to antibiotic therapy. Discrimination of intermittent versus chronic infection is therefore of high therapeutic relevance, yet the available diagnostic methods are only partly satisfactory. The aim of the present study was, therefore, to evaluate the usage of quantitative PCR (qPCR) to measure pathogen abundance and to discriminate between intermittent and chronic *Pseudomonas* infection in patients with CF.
Method: Using an established qPCR protocol, we analyzed the abundance of *P. aeruginosa* in 141 throats swabs and 238 sputa from CF patients with intermittent or chronic infection with *P. aeruginosa*, as determined by standard culture based diagnostics.
Results: We observed a large increase of abundance of *P. aeruginosa* in throat swabs and sputum samples from patients with chronic compared to intermittent infections with *P. aeruginosa*. The data show that abundance of *P. aeruginosa* as measured by qPCR is a valuable tool to discriminate intermittent from chronic infection. Of note, *P. aeruginosa* burden seems more sensitive than mucoidity phenotype to discriminate chronic from intermittent strains. Furthermore we observed that molecular detection in throat swabs was linked to a viable culture in the sputum when sputum was available. This result is of special interest in young patients with cystic fibrosis that often cannot expectorate sputum. We also observed that qPCR in comparison to culture detected the infection earlier.
Conclusion: The results suggest that qPCR detection and quantification of *P. aeruginosa* is a precious tool to be added to the diagnostic toolbox in cystic fibrosis.

Keywords: *Pseudomonas aeruginosa*; Quantitative PCR; Diagnostic; Chronic infection

1. Introduction

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene lead to alterations of the airway environment and increase vulnerability to respiratory bacterial infections in cystic fibrosis (CF) [1,2]. One of the major opportunistic pathogens triggering airway inflammation in CF is *Pseudomonas aeruginosa* (Psa) with a prevalence ranging from 50 to 80% among patients with CF in Europe [3,4]. Chronic infection with Psa is considered a major risk factor of severity and progression of CF lung disease [4–6] resulting in decline of lung function, increasing frequency of hospitalization and decreased survival [5,7].

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https://doi.org/10.1016/j.jcf.2017.12.007
1569-1993

Typically, chronic Psa infection evolves mainly in adolescence and develops from a period of intermittent infection with a non adapted strain. Those strains typically show susceptibility to common antibiotics, thus offering a window of opportunity for eradication treatment [4,8–11]. It has been demonstrated that despite the possibility of spontaneous clearance and the risk of resistance acquisition, antibiotic therapy is superior over no treatment at the stage of intermittent infection with Psa [11]. However, once the infection becomes chronic, Psa adapts to the CF airways [12] via a hypermutator phenotype favoring establishment of infection and conferring resistance to antibiotic treatment appears [10]. Then, Psa infection cannot be eradicated anymore and patient care switches to a suppressive therapy for years to reduce the bacterial load triggering inflammation and lung damage [13]. The acquisition of chronic infection is one of the end-point measurements to evaluate severity of the disease [4].

In consequence, it is important to detect the narrow window of opportunity for eradication and to monitor closely the success of antibiotic therapy [14]. The definition of Psa chronic infection in CF evolved to include antibody responses to discriminate intermittent and chronic stages [4,15,16]. However, the classification of chronicity depends on several cultures over time and, in many definitions, on a certain number of sputum samples making this classification unsuitable for young patients which do not produce sputum or in situations when the Psa strain has lost culturability [4,17]. Furthermore, measuring the bacterial burden via conventional microbiology involving colony counting was only included in procedures for broncho-alveolar lavage (BAL).

Therefore, several studies developed molecular quantification via quantitative PCR (qPCR) that can be applied to throat swabs and sputum samples to detect Psa in a quantitative manner [18–20]. The qPCR protocol developed by Héry-Arnaud et al. was applied on a follow up study of 96 patients over 3 years in patients classified as “free” of infection or “never” infected by Psa. This study showed that qPCR can detect earlier first infection or re-infection compared to culture. However, as the cohort was limited to patients without chronic infection, the application of this quantitative measure to discriminate between patients with chronic and intermittent infection remained unsolved.

The hypothesis of an increase of Psa load with development of chronic infection is based on a previous study in which we demonstrated that Psa chronic infection was associated with the dominance of Psa in the lungs while intermittently infected patients only showed low abundance of the pathogen and harbor various microbial ecotypes [21]. Therefore, we hypothesized that quantification of the pathogen via qPCR might be a good diagnostic tool to discriminate between intermittent and chronic infection in order to support therapeutic decisions making. We also compared the molecular detection of Psa by qPCR in the throat with culture-dependent diagnostics in the sputum to evaluate its potential for improved detection of infection in patients not expectorating sputum.

2. Materials & methods

2.1. Subjects

This prospective observational study was approved by the Ethics Committee of the University of Heidelberg (study number S-370/2011) and informed written consent was obtained from the patients, parents or legal guardians of all subjects. Airway samples from CF patients were obtained during routine visits at the CF Center at the University Hospital Heidelberg as previously described [21,22]. The diagnosis of CF was based on established diagnostic criteria [23].

Infection status with Psa was clinically classified according to the following definition: Psa intermittent infection was defined as positive microbial culture of Psa in at least one and <50% of the samples in the last twelve months and no detection of anti-Pseudomonas antibodies (against alkaline protease, elastase and exotoxin A). Psa chronic infection was defined as persistent culture presence of Psa for at least 6 months, or less when combined with a positive finding (titer > 1250) of two or more Psa antibodies [15,24]. Patients were seen for routine visits every 3–4 months plus extra visits during exacerbation or upon specific clinical needs. Patient characteristics are summarized in Table 1.

In order to evaluate the association between mucoidity and chronicity, as it was previously shown [25], we used the culture positive samples from the cohort included in the study for qPCR evaluation (25 throat swabs and 111 sputa) as well as the complete report available from the clinic at the time of the study (847 sputum samples and 147 throat swabs with Psa culture). Only strains with a clear and unique phenotype were included, visits with more than two different phenotypical strains (mucoid and non-mucoid) were discarded. We calculated the percentage of mucoidity per class by the ratio of the number of samples with mucoid or non-mucoid strains/the total number of samples.

2.2. Microbiology

All samples (ESwabs or expectorated sputa) were streaked (10 μL) and cultured for 24 h and 48 h at 36 °C (7% CO_2)

Table 1
Demographics of CF patient cohort.

Infection status for *P. aeruginosa*	Intermittent	Chronic
Number of patients	30	34
Number of samples (throat swab/sputum)	78/88	63/150
Number of matched samples	29	32
Number of patients with longitudinal screening	23	24
Period of observation [days], mean (range)	266.1 (32–914)	328.8 (9–979)
Age [years], mean (range)	17.2 (2–70)	21.2 (3–58)
Male/female	16/14	16/18
F508del/F508del %	46.7	52.9
Pancreatic insufficiency %	90	88.2
BMI [kg/m^2], mean (range)	17.2 (12.5–25.9)	19.1 (12.5–26.1)
FEV1 [%pred], mean (range)	63.0 (20.5–113.8)	51.8 (13.1–114.8)

Please cite this article as: Boutin S, et al, One time quantitative PCR detection of *Pseudomonas aeruginosa* to discriminate intermittent from chronic infection in cystic fibros..., J Cyst Fibros (2018), https://doi.org/10.1016/j.jcf.2017.12.007