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ORIGINAL ARTICLE

Infection Dynamics of *Aspergillus fumigatus* in Adults with Cystic Fibrosis (CF)

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Abstract

Objective *Aspergillus fumigatus* (*A. fumigatus*) has emerged as a significant pathogen in patients with cystic fibrosis (CF) and currently is within the top five isolated organisms reported in several international CF patient registries. *A. fumigatus* has been attributed to disease progression, although its role remains controversial. There is a paucity of reports on its infection dynamics, it was the aim of this study to examine time to first laboratory reports of *A. fumigatus* acquisition and to correlate this with patient gender and cystic fibrosis transmembrane conductance regulator (CFTR) mutation type.

Methods One hundred adult (≥ 18 years) CF patients were examined (50 females, 50 males; mean age 24.6 years ± 6.25 (SD), median age 24 years; maximum age 76 years). CFTR mutation groups consisted (i) F508del/F508del homozygous (n = 45), (ii) F508del/other heterozygous (n = 45) and (iii) others (n = 10). CFTR mutation type, patient gender, presence/absence of *A. fumigatus* and time (months) to first isolation of *A. fumigatus* were examined.

Results Microbiological data was examined from 100 patients from birth to present (31/12/2021), equating to 2455 patient years. *A. fumigatus* was isolated from 66/100 (66%) adult CF patients; (i) F508del/F508del homozygous (82%; 37/45), (ii) F508del/other heterozygous (56%; 25/45) and (iii) others (40%; 4/10). Within the F508del/other heterozygous group, 14 mutations were noted on the second allele, with R560T and R117H collectively accounting for 36% of the second mutations. Four unique allele/allele mutations were noted in the Other Mutations category. There was a trend to a higher *A. fumigatus* acquisition in F508del/F508del homozygous patients than with F508del/other patients (p = 0.0529). Of the 66 patients who were positive for *A. fumigatus*, 35(53%) were male and 31(47%) were female. The median and mean time to first isolation of *A. fumigatus* in all *A. fumigatus*-positive

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patients was 119.5 months and 128 months, respectively, shortest time was 12 months, longest time 288 months. There was a statistical significance in time-to-first isolation in relation to CFTR mutation group ($p = 0.0272$), whereby F508del homozygous individuals had their first isolation of *A. fumigatus* at 116.8 ± 7.9 months (mean ± standard error of the mean (SEM)) and F508del heterozygous patients had their first isolate of *A. fumigatus* at 150.4 months ± 13.7 months (mean ± SEM), approximately 2.75 years after their F508del homozygous peers. There was no significant difference ($p = 0.12$) in time to first acquisiton between males and females, whereby males had their first *A. fumigatus* isolate at 118 ± 9.4 months, whereas females had their first *A. fumigatus* isolate at 140 ± 10.8 months. The highest rate of first *A. fumigatus* isolation was from 4 years until 16 years and by the age of 16 years, approximately 85% of *A. fumigatus*-positive patients had recorded their first *A. fumigatus* isolate.

Conclusion To minimise the risk of first acquisition of *A. fumigatus*, it is important that infection prevention educational messaging is delivered in the paediatric clinic, to enhance health literacy around *A. fumigatus* acquisition.

Keywords Allergic bronchopulmonary aspergillosis · ABPA · *Aspergillus fumigatus* · Cystic fibrosis · Fungi · Infection

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease of mainly Caucasian populations of European ancestry, representing the most common life-limiting genetic disease within this population [1]. This disease is exacerbated by a continuous cycle of respiratory inflammation and lung infection, which may become chronic, leading to increasing disease severity, manifesting in increased morbidity and mortality [1]. The production of thick viscous sputum as a result of the physiological problems of transporting chloride ions allows for the entrapment of environmental bacterial and fungal organisms, which may eventually lead to chronic colonisation and infection. Therefore, people with cystic fibrosis are vulnerable to acquiring new environmental organisms in the lower respiratory tract, as well as clinical isolates from other patients with cystic fibrosis, through cross infection events [2].

The ascomycota filamentous fungus, *Aspergillus fumigatus* (*A. fumigatus*), has now emerged as the most commonly isolated filamentous fungus from respiratory specimens from the lower respiratory tract of people with cystic fibrosis (PwCF) [3]. For a seminal review on filamentous fungi in CF, please see Pihet and colleagues [4] and King and colleagues [5]. More recently in 2021, Schwarz and colleagues in this journal published a review which discussed cultural detection methods and susceptibility testing, as well as examining the problem of increasing azole resistance in *A. fumigatus* and epidemiological risk factors for fungal diseases in patients with CF [6].

Many international CF patient registries detail the prevalence of *Aspergillus* in respiratory specimens. The Canadian CF Registry 2020 report quotes *A. fumigatus* as the third most commonly isolated organism cultured from the airways of individuals with CF, after *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with a prevalence of 13%, which has declined year-on-year since 2016 [7]. Additionally, this report shows that *Aspergillus* was most prevalent in the 18–24 years old age group [8]. Likewise, these metrics are mirrored by individuals with CF from Australia in the 2020 Australian CF Registry report [8]. In the UK, *Aspergillus* mirrors a similar prevalence to both Canadian and Australian CF patients, with an overall prevalence of 17.2% in CF adults (≥ 16 years) [9], however peak prevalence is in a younger population of 16–19 years old at 22.8% [9].

Whilst there are numerous reports describing the clinical consequences of AF colonisation and infection, particularly in relation to allergic bronchopulmonary aspergillosis (ABPA) [10], currently, there is a paucity of reports on the infection dynamics of *A. fumigatus* in PwCF, hence it was the aim of this study to (i). examine time-to-first laboratory reports of *A. fumigatus* acquisition, (ii) gender and (iii) cystic fibrosis transmembrane conductance regulator (CFTR) mutation type, in association with initial *A. fumigatus* isolation in a population of adults with cystic fibrosis.

Methods

Patient Demographics and Microbiology Analyses

One hundred adult CF patients (≥ 18 years old) patients who attended the Northern Ireland Adult Cystic Fibrosis Centre, Belfast City Hospital, were included in this retrospective analysis for the first presence of *A. fumigatus* in their respiratory specimen, since birth until the present (31 December 2021). Patients were placed into three groups, according to CFTR mutation type, namely (i) F508del/F508del homozygous, (ii) F508del/other heterozygous and (iii) others. CFTR mutation type, sex, presence/absence of *A. fumigatus* and time (months) to first isolation of *A. fumigatus* was noted.

Statistical Analyses

Descriptive statistics were reported in relation to the patient cohort, in terms of mean, standard deviation, median, upper and lower quartile ranges. The Kolmorogov-Smirnov test was employed to test for normality, as well as one way analysis of variance (ANOVA) and Fisher's Exact test (odds ratio), using GraphPad PRISM version 9.4.1 (San Diego, USA).

Results

Patient Demographics

One hundred adult CF patients (≥ 18 years old) were examined in this study, with a mean age of 24.6 years (standard deviation 6.25 years), median age 24 years and a minimum age of 18 years and a maximum age of 76 years. This patient cohort consisted of 50 females and 50 males. CFTR mutation groups consisted (i) F508del/F508del homozygous (n = 45), (ii) F508del/other heterozygous (n = 45) and (iii) others (n = 10).

Microbiology Analyses

Microbiological data was examined from 100 patients from birth to present (31/12/2021), equating to 2455 patient years. *A. fumigatus* was isolated from 66/100 (66%) adult CF patients; (i) F508del/F508del homozygous (82%; 37/45), (ii) F508del/other heterozygous (56%; 25/45) and (iii) others (40%; 4/10). Within the F508del/other heterozygous group, 14 mutations were noted on the second allele, with R560T and R117H collectively accounting for 36% of the second mutations. Four unique allele/allele mutations were noted in the Other Mutations category. Whilst not statistically significant, there was a trend to a higher *A. fumigatus* acquisition in F508del/F508del homozygous patients than with F508del/other patients (p = 0.0529). Of the 66 patients who were positive for *A. fumigatus*, 35(53%) were male and 31(47%) were female.

The median and mean time to first isolation of *A. fumigatus* in all *A. fumigatus* -positive patients was 119.5 months and 128 months, respectively, shortest time was 12 months, longest time 288 months. There was a statistical significance in time to first isolation in relation to CFTR mutation group (Fig. 1; p = 0.0272), whereby F508del homozygous individuals had their first isolation of *A. fumigatus* at 116.8 ± 7.9 months (mean ± standard error of the mean (SEM)) and F508del heterozygous patients had their first isolate of *A. fumigatus* at 150.4 months ± 13.7 months

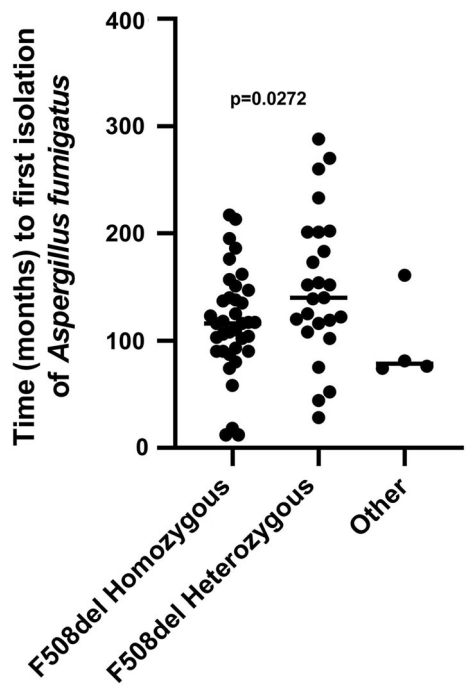

Fig. 1 A comparison of time to first isolation (months) of *Aspergillus fumigatus* from respiratory specimens from patients with cystic fibrosis (n = 66) in relation to CFTR mutation type

(mean ± SEM), approximately 2.75 years after their F508del homozygous peers.

There was no significant difference (p = 0.12) in time to first acquisiton between males and females (Fig. 2), whereby males had their first *A. fumigatus* isolate at 118 ± 9.4 months, whereas females had their first *A. fumigatus* isolate at 140 ± 10.8 months.

Figure 3 shows the cumulative isolation of *A. fumigatus* by patient age and stages of education. The steepest gradient on the curve and hence the highest rate of first *A. fumigatus* isolation was from 4 years until 16 years and by the age of 16 years, approximately 85% of *A. fumigatus* -positive patients had recorded their first *A. fumigatus* isolate.

Discussion

Many international CF registries and reports detail the prevalence of *A. fumigatus* at various patient ages [7–9]. Whilst this information is important to know, particularly from a surveillance and epidemiological perspective, such a description does not offer any value in establishing when the organism was first acquired. The data in this current study is presented in a novel format, namely the age of the patient at first isolation of *A. fumigatus*, thereby offering further insight and value to infection control and prevention consideration, in relation to *A. fumigatus* acquisition.

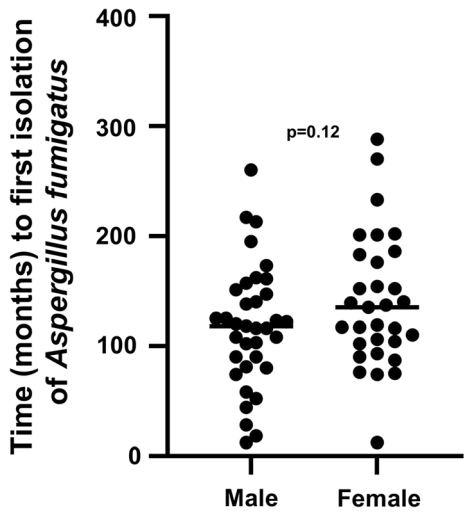

Fig. 2 A comparison of time to first isolation (months) of *Aspergillus fumigatus* from respiratory specimens from patients with cystic fibrosis (n = 66) in relation to gender

Knowing the patient's age at first acquisition is important to educate patients as to when they are most likely to acquire this organism, so that preventative mitigations can be established during this period of heightened risk, in an attempt to help avoid *A. fumigatus* acquisition and the potential for early colonisation to develop into chronic colonisation and infection.

Results from the current study showed that initial acquisition of *A. fumigatus* was not until approximately 10 years of age (119.5 months median time). This time is slightly younger by 2.3 years than previously published in data from two centres in France (Angers and Giens), where the mean age of the patients at date of first isolation from respiratory specimens of *A. fumigatus* was about 12.3 years [10]. As first acquisition of *A. fumigatus* usually succeeds to bacterial infections, it was suggested that bacterial proteases or leukocyte elastase released during the inflammatory response are necessary for adherence of airborne fungal spores to the host epithelium and that receptors on the surface of *A. fumigatus* conidia may facilitate attachment via laminin or fibronectin exposed after epithelial tissue damage [10].

The present study demonstrates a significant difference in time-to-first acquisition of *A. fumigatus* between F508del homozygous patients and patients heterozygous for the F508del mutation. Similar conclusions were reported regarding time-to-first acquisition of the *Ascomycota* fungus, *Geosmithia argillacea*, now called the *Rasamsonia argillacea* species complex [11]. Giraud and colleagues reported that first isolation of *G. argillacea* occurred from F508del-homozygous patients at a mean age of 10 years (range 6–17 years) and from heterozygous patients at a mean age of 36 years (range 23–48 years) [11]. The findings of the study by Giraud and colleagues is similar to our study with *A. fumigatus*, in that first isolation was earlier in F508del homozygous patients that in F508del heterozygous patients, with time-to-first-isolation identical for *Geosmithia argillacea* and *A. fumigatus*, both 10 years of age. These data indicate that there is a not a "one size fits all" with regard to fungal acquistion in patients with CF, rather the results of these two studies adds to the evidence-base that CFTR mutation type can influence the time-to-first acquistion of fungal pathogens.

In conclusion, this study showed that median time to first respiratory culture of *Aspergillus* in CF patients

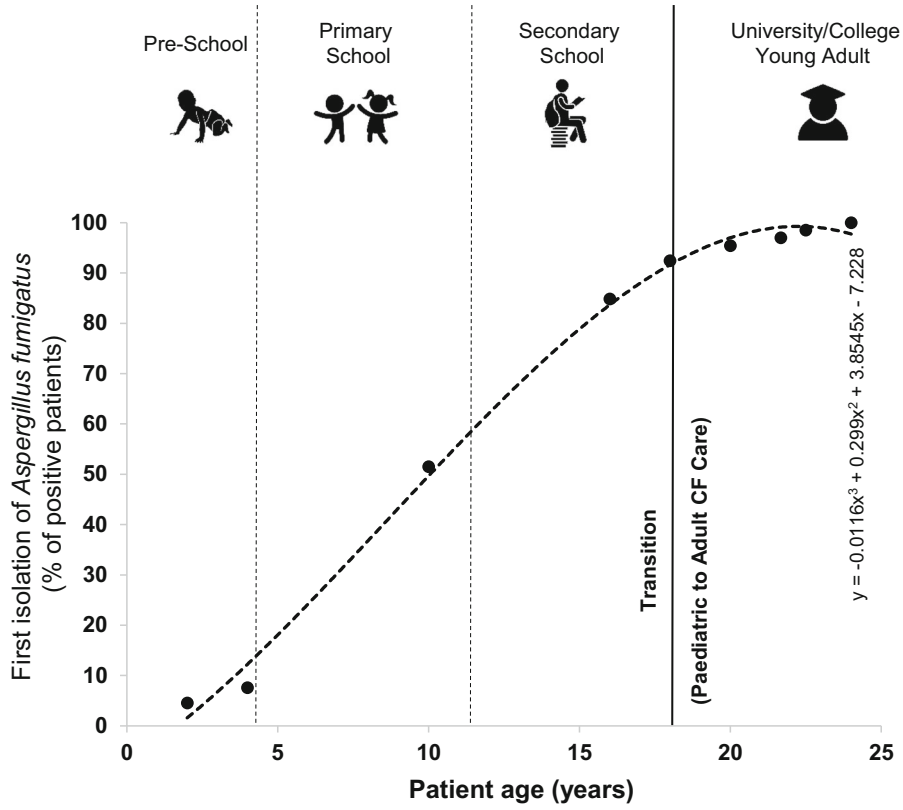

Fig. 3 Cumulative percentage of patients with cystic fibrosis (n = 66) in relation to time to first isolation (months) of *Aspergillus fumigatus* from respiratory specimens

occurs around 10 years of age. To minimise the risk of first acquisition of *A. fumigatus*, it is important that infection prevention educational messaging is delivered in the paediatric clinic, to enhance health literacy around *A. fumigatus* acquisition. Further investigation is now required to determine how previous colonisation/infection with bacterial pathogens may influence fungal colonisation.

Author contributions CRediT authorship contribution statement; BCM: Conceptualization; formal analysis; methodology; roles/writing—original draft; writing—review and editing; JCR: Writing—review and editing; JEM: Conceptualization; formal analysis; methodology; roles/writing—original draft; writing—review and editing.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial, financial or other relationships that could be construed as a potential conflict of interest.

Ethics Statement This study did not have any involvement or interaction with human or animal subjects.

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