Co-colonisation with Aspergillus Fumigatus and Pseudomonas Aeruginosa Is Associated With Poorer Health In Cystic Fibrosis Patients: an Irish registry analysis

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Co-colonisation with Aspergillus fumigatus and Pseudomonas aeruginosa is associated with poorer health in cystic fibrosis patients: an Irish registry analysis

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Abstract

Background: Pulmonary infection is the main cause of death in cystic fibrosis (CF). Aspergillus fumigatus (AF) and Pseudomonas aeruginosa (PA) are the most prevalent fungal and bacterial pathogens isolated from the CF airway, respectively. Our aim was to determine the effect of different colonisation profiles of AF and PA on the clinical status of patients with CF.

Methods: A retrospective analysis of data from the Cystic Fibrosis Registry of Ireland from 2013 was performed to determine the effect of intermittent and persistent colonisation with AF or PA or co-colonisation with both microorganisms on clinical outcome measures in patients with CF. Key outcomes measured included forced expiratory volume in one second (FEV₁), number of hospitalisations, respiratory exacerbations and antimicrobials prescribed, and complications of CF, including CF related diabetes (CFRD) and allergic bronchopulmonary aspergillosis (ABPA).

Results: The prevalence of AF and PA colonisation were 11% (5% persistent, 6% intermittent) and 31% (19% persistent, 12% intermittent) in the Irish CF population, respectively. Co-colonisation with both pathogens was associated with a 13.8% reduction in FEV₁ (p = 0.016), higher levels of exacerbations (p = 0.042), hospitalisations (p = 0.023) and antimicrobial usage (p = 0.014) compared to non-colonised patients and these clinical outcomes were comparable to those persistently colonised with PA. Intermittent and persistent AF colonisation were not associated with poorer clinical outcomes or ABPA. Patients with persistent PA had a higher prevalence of CFRD diagnosis (p = 0.012).

Conclusions: CF patients co-colonised with AF and PA had poor clinical outcomes comparable to patients persistently colonised with PA, emphasising the clinical significance of co-colonisation with these microorganisms.

Keywords: Co-colonisation, Cystic Fibrosis, Aspergillus fumigatus, Pseudomonas aeruginosa, Pulmonary function
are colonised with a community of microorganisms from an early age [2–4]. Microorganisms previously not considered to be significant in CF microbiology are emerging as having roles in disease progression [4, 5]. The discovery of complex and diverse microbial communities in the airways is forcing a paradigm shift in the treatment of airway infections. Considering this, research into co-infections and polymicrobial infections is imminent. Here we aimed to determine the clinical implications of co-infection with the most common fungal and bacterial pathogen isolated from the CF airways.

*Aspergillus fumigatus* (AF) is the most common fungal pathogen isolated from the CF airways with a reported prevalence between 16 and 58% [6–10]. AF rarely causes invasive infections in CF however it can cause allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity reaction to AF with detrimental consequences on airway inflammation. Generally intermittent or persistent AF colonisation is not treated unless ABPA is confirmed. Criteria for diagnosis of ABPA are based on the CF Foundation consensus [10]. At present there is no consensus for the diagnosis or treatment of asymptomatic AF colonisation in CF. Recent studies have utilized a combination of AF qPCR, galactomannan, AF specific IgE and IgG to decipher the complexity of AF colonisation status in CF patients. Classifying patients into proposed subgroups and delineating those with ABPA from those who are AF sensitised, AF non-sensitised and those with aspergillus bronchitis [11]. Some studies have provided valuable insights into how AF may influence the progression of CF lung disease [9, 12, 13] however the true extent of the impact on disease progression is unknown.

*Pseudomonas aeruginosa* (PA) is the most common bacterial pathogen in CF [14] and in 2013, the Cystic Fibrosis Foundation in the US reported 48.7% of patients were colonised with PA [15]. Early PA colonisation of the CF airway is frequently intermittent and eradication is possible using inhaled antibiotics. However re-colonisation can occur and in some cases despite intense efforts to re-eradicate the organism, the colonisation becomes persistent and a decline in lung function ensues [16, 17]. The transition from intermittent to chronic or persistent PA colonisation is a seminal clinical event in a CF patient’s life and persistent PA colonisation is associated with increased mortality [18, 19].

Despite substantial improvement in median survival, CF remains a life-shortening disease with the mean age of death in Ireland being only 22.8 years [20] and pulmonary insufficiency is the main cause of death. There is a paucity of published data on the prevalence of persistent AF colonisation or AF and PA co-colonisation in CF patients. We aimed to determine the prevalence of persistent and intermittent colonisation with PA or AF and co-colonisation with both pathogens in the Irish CF population. Furthermore we aimed to determine the association of these different colonisation patterns with the clinical status of patients with CF.

**Methods**

**Study population**

Data from patients with CF registered in the Cystic Fibrosis Registry of Ireland (CFRI) database in 2013 was used in this study. The number of live patients registered with the CFRI was 1158 on the last day of 2013 (92.5% of the Irish CF population) and data from 749 patients was included in this study (409 patients with incomplete data sets were excluded). A retrospective cohort study was carried out to establish whether colonisation with AF and/or PA impacts on patient health. The primary clinical outcome measure was forced expiratory volume in one second percent predicted (FEV\(_1\)% and secondary clinical outcome measures were the number of hospitalisations, respiratory exacerbations (treated with intravenous antibiotics), prescribed antimicrobials and complications of CF, including pancreatic insufficiency resulting in CFRD and ABPA. CFRD diagnosis was defined as being treated with insulin for CF-related diabetes, ABPA diagnosis was reported by the physician.

Sputum (\(n = 1859\)), throat swabs (\(n = 31\)), cough swabs (\(n = 52\)), nasal swab (\(n = 1\)) and bronchoalveolar lavage (\(n = 10\)) samples were recorded as positive for PA and/or AF by the individual hospitals. We employed stringent criteria to classify colonisation status: greater than 2 but less than 4 respiratory samples positive within the year was considered intermittent colonisation and greater than 4 samples positive within the year was considered persistent colonisation, in line with the Leeds criteria [21]. Patients were separated into 6 cohorts depending on their colonisation status: 1) intermittent PA colonisation (PA), 2) persistent PA colonisation (PAP), 3) intermittent AF colonisation (AF), 4) persistent AF colonisation (APF), 5) colonised with both pathogens (AF + PA) and 6) negative for both pathogens (clear). Clinical outcome measures were compared between our six cohorts.

**Statistical analysis**

D’Agostino and Pearson omnibus normality test was performed on FEV\(_1\)% predicted data. FEV\(_1\) data was not normally distributed and outcomes comparisons between groups were made using the Kruskal-Wallis test with Dunn’s multiple comparisons. In order to adjust for the potential confounders: age, gender and CFTR mutation, linear regression was used for FEV\(_1\)% predicted and logistic regression models were used for steroid use, ABPA and CFRD positivity yielding Odds Ratios (ORs) for the “poorer” outcomes.

The number of hospitalisations, respiratory exacerbations and the number of antimicrobials were modelled with a Poisson or Negative Binomial regression. The degree of
Results and Discussion

Demographics of the patient population

The male cohort made up the bigger subset of the CF population at 58.6%, reflecting higher mortality rates in females in adolescence and early adulthood [22]. The age distribution in our dataset was 4–69 years and the median age was 18.1 years. The demographic and clinical data of the cohorts are depicted in Table 1. As expected the FEV₁ percent predicted was lower in older patients reflecting a gradual decline with age (Fig. 1).

Prevalence of AF and PA colonisation in the Irish CF population

The prevalence of AF and PA co-colonisation was 3.1% in the Irish CF population. The AF + PA group included AFp + PA (n = 4), AF + PA (n = 5), AF + PAp (n = 7) and AFp + PAp (n = 8) subgroups. No significant differences in FEV₁, number of hospitalisations, number of respiratory exacerbations and antimicrobial prescribing were noted between these subgroups (Additional file 2: Table S1). AF colonisation was most prevalent in pre-adolescents and adolescents (Fig. 1), which contradicts current opinion that AF is more commonly an organism encountered in adulthood. To our knowledge this has not been previously reported. The prevalence of AF colonisation in the Irish CF population was 11%, of which 5% had persistent and 6% intermittent colonisation (Table 1). Previously AF prevalence rates in CF have been reported to be up to 58% using sputum culture [8]. PA becomes more prevalent with age and FEV₁ decline, corroborating previous findings (Fig. 1) [23]. The prevalence of PA in the Irish CF population was 31%, of which 19% had persistent and 12% had intermittent colonisation (Table 1). The prevalence of PA colonisation in Ireland is lower than the 48.7% prevalence reported in the US [17]. Employing similar criteria for the classification of persistent and intermittent PA colonisation as our study, Lee et al., (2003) [21] determined the prevalence of persistent PA to be 18% consistent with our data and intermittent colonisation to be 34%, which is higher than our findings. Proesmans et al., (2006) [24] determined the prevalence of persistent and intermittent PA colonisation to be 31 and 18% respectively which are higher than our prevalence rates. Many of these studies differ in the criteria employed for categorising colonisation status making comparisons between them difficult. We employed stringent criteria to classify colonisation status in line with the Leeds criteria [21] as outlined in our methods. The Leeds criteria have been independently evaluated [24] and are the classification criteria recommended in the European CF Society patient registry guidelines [25]. We did not consider positivity of AF and PA in one sputum sample in a year as intermittent, as other studies have done [6–8, 26], to rule out inclusion of chance events, false positives or sputum contamination. For these reasons our prevalence for AF and PA colonisation may be lower than reported elsewhere [6–9, 15, 21, 24, 26]. Consensus criteria for the definition of intermittent and persistent AF colonisation, comparable to the Leeds criteria for PA, need to be formulated. We acknowledge that a limitation of this study is its cross-sectional design and multi-year analysis of registry data would provide further detail on transition from intermittent to persistent colonisation status and the impact on disease outcome measures.

Persistent colonisation with PA or co-colonisation with AF was associated with reduced lung function

Patients co-colonised with both AF and PA had an FEV₁ 13.8% lower than non-colonised patients (p = 0.016)

Table 1 Demographic and clinical data of the study cohorts

|                | PA          | PAp         | AF          | AFp         | AF + PA     | Clear       |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Number (%)     | 82 (10.9%)  | 130 (17.4%) | 36 (4.8%)   | 26 (3.5%)   | 23 (3.1%)   | 452 (60.3%) |
| Median Age (range) | 26 (7–69)  | 25 (6–48)  | 15 (5–36)  | 15 (5–29)  | 20 (9–45)  | 14 (4–56)  |
| CFTR Genotype  |             |             |             |             |             |             |
| phe508del/phe508del | 6.8%       | 10.3%      | 2.8%       | 2.9%       | 1.7%       | 30.8%       |
| phe508del/other | 3.8%       | 6%         | 1.6%       | 0.4%       | 1.1%       | 24.3%       |
| other          | 0.5%       | 0.8%       | 0.27%      | 0.13%      | 0.27%      | 4%          |
| unknown        | 0.13%      | 0.27%      | 0.13%      | 0%         | 0%         | 1.2%        |
| Median BMI (range) | 21.2± (13.1–34.9) | 20 (9–30.2) | 19 (14.3–26.8) | 18.7 (14.3–26.1) | 19 (12.3–25.7) | 18.9 (10.4–33.7) |

*indicates missing data
(Fig. 2) and co-colonised patients had comparable reductions in lung function to patients persistently colonised with PA. Consistent with our data, Amin et al. (2010) [9] reported that persistent infection with both AF and PA was associated with lower lung function. The transition of PA colonisation from intermittent to persistent is a disease milestone that cannot be reversed and is associated with worse prognosis [18, 27]. We have shown that patients co-colonised with PA and AF had similar reductions in lung function as patients with persistent PA. This is the first time this has been reported. In a recent longitudinal study the sputum microbiology and clinical outcomes of 770 adolescents with CF were recorded and AF was the only species that was associated with an increased risk for infection with PA [28]. This highlights the importance of monitoring the co-colonisation status of CF patients. Perhaps AF colonisation establishes a milieu that enables PA to become persistent and attention to AF during this phase may prevent progression to persistent PA. Further data is required to explore this hypothesis.

Patients with persistent PA colonisation had an FEV$_1$ 11.9% lower than patients who were intermittently colonised with PA (unadjusted $p = 0.0074$), which remained significant following adjustment for confounding factors ($p < 0.001$) (Fig. 2). Previously intermittent PA colonisation has been associated with lower FEV$_1$ [21] (when adjusted for age and gender only). Following data adjustment for confounding factors (age, gender and CFTR mutation) the FEV$_1$ of patients intermittently colonised with PA were not significantly different from the FEV$_1$ of non-colonised patients. Persistent PA colonisation negatively impacted on lung function in agreement with others [21, 23]. Early onset PA colonisation can be eradicated in over 90% of colonised patients by antibiotic therapy [29], therefore efforts should be targeted towards early detection of PA colonisation and preventing establishment of persistent colonisation. Interestingly 2 studies have now shown that polymerase chain reaction methods can detect PA 4.5 months [30] to 8 months [31] in advance of current culture-based techniques used commonly in diagnostic

![Fig. 1](image1.png)

**Fig. 1** Average FEV$_1$ % predicted and prevalence of PA and AF colonisation in patients stratified by age. Error bars represent standard error of the mean.

![Fig. 2](image2.png)

**Fig. 2** Average FEV$_1$ percent predicted in the six colonisation cohorts. Average FEV$_1$/% predicted for patients with no PA or AF colonisation (Clear), intermittent AF colonisation (AF), intermittent PA colonisation (PA), persistent AF colonisation (AFp), intermittent PA colonisation (PA), persistent PA colonisation (PAp) and both AF and PA co-colonisation (AF + PA). Error bars represent min and max values. Asterisks refer to comparison with Clear, unless over a horizontal bar, ** $p \leq 0.01$, *** $p \leq 0.001$ (adjusted data). ∞ indicates significance only prior to data adjustment.
laboratories. This provides evidence to support the use of molecular-based methods for superior detection of PA.

Patients with intermittent and persistent AF colonisation had 4 and 11% lower FEV\(_1\), respectively, than non-colonised patients however these were not statistically significant. A previous study showed patients with AF sensitisation or persistent carriage had 16.5% lower FEV\(_1\) than the control group [32]. Amin et al., (2010) [9] showed that patients persistently colonised with AF had a 3.6% decrease in FEV\(_1\) compared to patients who were clear of AF; however when the data was adjusted for baseline pulmonary function, this decrease was not significant. In agreement with our data, other studies suggest no correlation between AF colonisation and a decline in pulmonary function [33, 34].

**Persistent colonisation with PA or co-colonisation with both PA and AF was associated with more frequent hospitalisations, more respiratory exacerbations and higher use of antimicrobials**

For the first time we have shown that being culture positive for both AF and PA is associated with similar levels of hospital admissions, exacerbations and antimicrobial prescribing as being persistently colonised with PA (Table 2). Patients co-colonised with both AF and PA had a 165% increase in hospital admissions per person (\(p = 0.023\)), an 112% increase in the number of respiratory exacerbations per person (\(p = 0.042\)) and 48% increase in antimicrobial prescribing (\(p = 0.009\)) when compared to patients who were clear of both pathogens (Table 2). Co-colonised patients had a 64% increase in respiratory exacerbations compared to patients intermittently colonised with PA (\(p = 0.015\)) (Table 2).

Patients persistently colonised with PA had an 89% increase in the number of hospital admissions per person within the year (\(p = 0.004\)), a 91% increase in respiratory exacerbations (\(p < 0.001\)) and a 55% increase in antimicrobial prescribing (\(p < 0.001\)) compared to non-colonised patients (Table 2). In comparison to intermittently colonised patients, those persistently colonised with PA had a 60% increase in the number of exacerbations (\(p = 0.001\)) and 23% increase in antibiotic prescribing (\(p = 0.014\)) (Table 2). There was no increase in the number of hospitalisations, respiratory exacerbations or antimicrobial prescribing in patients who were intermittently colonised with PA compared to non-colonised patients.

Patients intermittently or persistently colonised with AF showed no increase in the number of hospitalisations or respiratory exacerbations per person compared to non-colonised patients. However intermittently and persistently colonised patients had a 63% and 90% increase in antimicrobial prescribing compared to non-colonised patients (\(p = <0.001\) and \(p = 0.001\) respectively) (Table 2). The prevalence of *Aspergillus* sp was previously shown to be higher in CF patients receiving prophylactic antibiotic therapy [33] and we also observed an association between AF colonisation and antimicrobial prescribing. We speculate that this finding may reflect antimicrobial suppression of bacteria facilitating fungal growth. It is also possible that AF colonisation establishes a milieu suitable for bacterial colonisation, which would impact on antimicrobial prescribing. No differences were observed between the cohorts regarding inhaled steroid use, steroids taken daily and steroids taken alternative days (Additional file 3: Figure S1).

**Patients colonised with PA had a higher prevalence of CFRD**

A total of 14.7% of patients with CF had diabetes requiring insulin in 2013. Patients persistently colonised with PA had a higher prevalence of CFRD compared to those intermittently colonised or non-colonised (\(p = 0.001\)). Amin et al., (2010) [9] showed that patients persistently colonised with PA had a 3.6% decrease in FEV\(_1\) compared to patients who were clear of PA; however when the data was adjusted for baseline pulmonary function, this decrease was not significant. In agreement with our data, other studies suggest no correlation between PA colonisation and a decline in pulmonary function [33, 34].

### Table 2: Incident Rate Ratio (IRR) of the incidence of hospitalisations, respiratory exacerbations and prescribed antimicrobials between cohorts

| Comparison          | Hospitalisations (IRR 95% CI) | p-value | Respiratory Exacerbations (IRR 95% CI) | p-value | Antimicrobials (IRR 95% CI) | p-value |
|---------------------|-------------------------------|---------|----------------------------------------|---------|-----------------------------|---------|
| PA vs Clear         | 1.00 (0.44, 2.30)             | 1       | 0.76 (0.37, 1.55)                      | 1       | 1.19 (0.93, 1.51)           | 0.259   |
| PAp vs Clear        | 1.89 (1.11, 3.22)             | **0.004** | 1.91 (1.19, 3.05)                     | <0.001  | 1.55 (1.29, 1.86)           | <0.001  |
| AF vs Clear         | 1.16 (0.54, 2.48)             | 1       | 0.95 (0.45, 1.98)                      | 1       | 1.63 (1.19, 2.21)           | <0.001  |
| Afp vs Clear        | 1.97 (0.75, 5.20)             | 0.271   | 1.59 (0.74, 3.44)                      | 0.494   | 1.9 (1.38, 2.62)            | <0.001  |
| AF + PA vs Clear    | 2.65 (1.01, 6.97)             | **0.023** | 2.12 (0.95, 4.72)                     | **0.042** | 1.48 (1.04, 2.11)           | **0.009** |
| PA vs PAp           | 0.53 (0.23, 1.23)             | 0.189   | 0.4 (0.19, 0.81)                       | **0.001** | 0.77 (0.60, 0.98)           | **0.014** |
| AF vs Afp           | 0.59 (0.21, 1.67)             | 0.872   | 0.59 (0.24, 1.47)                      | 0.775   | 0.86 (0.56, 1.30)           | 1       |
| AF + PA vs PA       | 0.38 (0.12, 1.21)             | 0.099   | 0.36 (0.13, 0.95)                      | **0.015** | 0.8 (0.54, 1.19)            | 0.668   |
| AF + PA vs PAp      | 1.40 (0.53, 3.73)             | 1       | 1.11 (0.51, 2.43)                      | 1       | 0.96 (0.66, 1.38)           | 1       |
| AF + PA vs AF       | 0.45 (0.15, 1.33)             | 0.209   | 0.47 (0.17, 1.31)                      | 0.205   | 1.09 (0.69, 1.74)           | 1       |
| AF + PA vs Afp      | 1.30 (0.38, 4.44)             | 1       | 0.79 (0.28, 2.21)                      | 1       | 0.78 (0.49, 1.25)           | 0.8     |

Italicized bold text highlights significance of <0.05.
PA had a higher prevalence of CFRD diagnosis than non-colonised patients \( (p = 0.012) \) (Additional file 4: Table S2). A direct link between insulin deficiency and clinical decline has been shown and patients with CFRD were more likely to be persistently colonised with *Pseudomonas sp.* [35].

**AF colonisation was not associated with prevalence of ABPA**

The overall prevalence of ABPA was 5.9%. Patients co-colonised with AF and PA had the highest prevalence of ABPA (17.4%) but this was not significantly higher than the other groups (Additional file 4: Table S2). Interestingly, patients intermittently or persistently colonised with AF showed no increased prevalence of ABPA. It is not currently understood why some AF colonised patients are vulnerable to developing ABPA and others are not. A number of single nucleotide polymorphisms (SNPs) have been linked to susceptibility to ABPA. SNPs in the IL-4 receptor alpha chain (IL-4R\(\alpha\)) gene [36], the mannose-binding lectin (MBL) 2 gene [37, 38], the toll-like receptor 9 (TLR9) gene [39] and the pulmonary surfactant protein (SPA-2) gene [40] have been identified as genetic risk factors for the development of ABPA. In particular, SNPs in the promoter region of the IL-10 gene were found to be associated with *Aspergillus* colonisation and ABPA in patients with CF. Further studies in the CF setting could yield interesting insights into the susceptibility of CF patients to *Aspergillus* colonisation and development of allergic disease. Culture of AF from respiratory secretions of CF is only a secondary criterion for the diagnosis of ABPA, due to the large number of asymptotically AF colonised CF patients. A retrospective study on 236 patients with CF found that 25% of patients had positive AF culture results but of these only 15 patients (6.5% of the total population) were ABPA positive [41]. In parallel with our findings, positive culture results for *Aspergillus* did not seem to represent a specific risk factor for ABPA [40, 42].

The CF airways are colonised with a diverse community of microorganisms from an early age [2–4]. Interactions between the varieties of dominant and low abundance microorganisms in these communities are likely to impact patient health. The most commonly isolated bacteria and fungi from CF samples are PA and AF, respectively and we have shown that co-colonisation with these two microorganisms is associated with poor clinical outcomes. Previous studies have shown that PA and AF are capable of interacting and competing, which may have implications on CF airway disease. Pyocyanin and 1-hydroxy-phenazine produced by PA both have anti-AF activity [43] and PA has been shown to inhibit AF biofilm formation by direct cell contact and by excreted molecules [44, 45]. More recently the Pf4 bacteriophage produced by PA was shown to reduce both biofilm formation and pre-formed biofilms of AF and this effect was linked to inhibition of AF metabolism via Pf4 iron sequestration [46]. AF has been shown to enhance the production of PA elastase when in co-culture and these co-culture supernatants were more damaging to lung epithelial cell lines than PA supernatants alone [47]. We hypothesize that these PA-AF interactions may play a role in the damaging pathology associated with CF airway disease and may explain why patients who are co-colonised with PA and AF have a poorer prognosis than those who are clear of both pathogens. Further studies on the interaction of these two microorganisms are warranted to expand our understanding of how co-colonisation may impact the CF airways.

**Conclusions**

Persistent PA colonisation was associated with more respiratory exacerbations, more hospitalisations and lower lung function than non-colonised patients. For the first time we have shown that co-colonisation with AF and PA resulted in comparable levels of hospitalisations, respiratory exacerbations and lung function reduction as persistent PA colonisation. Patients with persistent PA had a higher prevalence of CFRD diagnosis. Intermittent and persistent AF colonisation was not associated with poorer clinical outcomes or ABPA. This is a cross-sectional registry analysis therefore a prospective longitudinal study is warranted to confirm these findings.

**Additional files**

| Additional file 1: Supplementary Methods. Further details on the statistics performed on this data (DOCX 140 kb) |
| Additional file 2: Table S1. Comparison of disease outcome measures between the co-colonised sub-groups (PNG 74 kb) |
| Additional file 3: Figure S1. Percentage of patients prescribed steroids in each cohort. Percentage of patients prescribed inhaled steroids; oral steroids every day and oral steroids every alternative day for patients within the 6 cohorts (PNG 109 kb) |
| Additional file 4: Table S2. Odds ratios are presented to determine the odds of a particular colonisation status being linked to development of CFRD and/or ABPA. (PNG 75 kb) |

**Abbreviations**

ABPA: Allergic Bronchopulmonary Aspergillosis; AF: Aspergillus fumigatus; AF + PA: AF and PA co-colonised; Afp: Persistent AF; CF: Cystic Fibrosis; CFRD: CF related diabetes; CFRI: CF registry of Ireland; CFTR: CF transmembrane conductance regulator; FEV1: Forced Expiratory Volume in 1 second; IRR: Incident Rate Ratios; OR: Odds Ratio; PA: Pseudomonas aeruginosa; PAp: Persistent PA; qPCR: quantitive Polymerase Chain Reaction; SNP: Single nucleotide polymorphism; ZINB: Zero-inflated Negative Binomial

**Acknowledgements**

We would like to thank the Cystic Fibrosis Registry of Ireland for providing access to patient data, the individual CF centres for allowing the use of data, the parents/guardians of children, and adults with CF participating in the registry. We thank the National Children’s Hospital for funding this research (http://thenationalchildrenshospital.ie/).
Funding
This work was funded by the National Children’s Hospital, Tallaght hospital, Dublin 24, Ireland (http://thenationalchildrenshospital.ie/). The funding body had no role in designing the study or in collecting, analysing, and interpreting the data or in writing the manuscript.

Availability of data and materials
All the relevant data is presented within the main manuscript and supplementary files. The CFRI dataset is not a publicly available dataset. Requests for anonymous data/information from the CFRI are considered, and are subjected to a data application process. Data/information requests are considered by CFRI’s Research Committee whose decision whether to provide data or information is final.

Authors’ contributions
ER was involved in study design, data analysis, data interpretation and writing and editing the manuscript. RS performed the data analysis and edited the manuscript. AI extracted data from the CFRI, helped with editing the manuscript and performed sub-analysis in response to reviewer’s comments. SMCC was involved in data interpretation and manuscript editing. JR was involved in study design, data analysis, data interpretation and writing and editing the manuscript. PG was involved in study design, data interpretation and manuscript editing. All authors read and approved the final manuscript.

Competing interests
PG has served on advisory boards and received honoraria from Vertex, Novartis, Pharmaxis and PTC pharmaceuticals in the past however none of these organisations have contributed financially or in any other way to this work. For all other co-authors there are no conflicts of interest to report.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Cystic fibrosis registry of Ireland (CFRI) data were used in this study, and the registry was given ethical approval by each Irish cystic fibrosis centre and clinic to collect data on cystic fibrosis patients. Informed consent is provided by registry participants and/or their parent/guardian at registration. The individual ethics committees are as follows: University hospital Galway, University hospital Limerick, Cork University hospital, Beaumont hospital, St Vincents hospital, Tallaght hospital, Our Lady’s Children’s Hospital, Crumlin, Temple Street hospital, Our Lady of Lourdes hospital, Drogheda, Cavan & Monaghan hospital, Sligo University hospital, Mayo General hospital and Waterford Regional hospital.

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Received: 21 December 2016 Accepted: 18 April 2017
Published online: 21 April 2017

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