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Bronchodilator responsiveness in children with primary ciliary dyskinesia

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Key Words
primary ciliary dyskinesia (PCD), evidence-based medicine and outcomes, pulmonary function testing (PFT)

Take home message
Children with PCD and a positive bronchodilator response are at risk for accelerated lung disease progression and may therefore require additional treatment. This study helps understand the clinical benefit of bronchodilator response testing added to routine spirometry.

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Abstract 239 words
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ABSTRACT

Background

Reversible airways obstruction has been reported to be common in people with primary ciliary dyskinesia (PCD). However, the diagnostic value of bronchodilator (BD) response testing added to routine spirometry is unclear.

Methods

This is a retrospective analysis of pulmonary function test (PFT) results obtained from children with PCD seen as outpatients at the Hospital for Sick Children, Toronto. Spirometry results for every Visit with BD-response testing, the previous (Baseline) and the following (Follow-up) encounters were collected.

Results

A positive BD-response was seen in 86/474 (18.1%) of PFTs from 82 children with PCD. BD-responsiveness was associated with a significant absolute change in percent predicted forced expiratory volume in one second (ppFEV₁) from Baseline to Visit pre-BD ppFEV₁ (-6.5%, SD 10.3, p<0.001), but not from Baseline to Follow-up (0.4%, SD 10.8, p=0.757). Antimicrobial therapy was initiated more commonly following a Visit with a positive BD-response (OR 3.8, 95%CI 2.2 – 6.6) compared to no BD-response. Children with a positive BD-response had a greater annual decline in ppFEV₁ compared to those with no BD-response (-0.9%/year vs -0.5%/year; p<0.001). The annual decline in ppFEV₁ was greater in children with multiple compared to one measured positive BD responses (-1.3/year vs -0.6/year, p<0.001) and in those not treated with antibiotic therapy following a positive BD response compared to those treated with antibiotics (-1.1% vs -0.6%; p<0.001).

Conclusion

A positive BD-response in children with PCD may help identify those at risk for accelerated lung disease progression.
INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare, mainly autosomal recessive inherited multi-organ disease, which is characterized by dysfunctional motile cilia. In the respiratory tract PCD causes impaired muco-ciliary clearance, neonatal respiratory distress, chronic nasal congestion, sinusitis and chronic wet cough [1–3]. Recurrent airway infections and inflammation are common, leading to bronchiectasis and irreversible lung damage [2, 4]. The diagnosis of PCD can be challenging and involves typical clinical features, measurement of nasal nitric oxide, structural and functional analysis of cilia, and genetic testing [5]. PCD has an estimated incidence of 1 per 10,000–20,000 [6–8]. The prevalence of PCD is difficult to determine, largely due to inadequacies of diagnostic methods [9].

Due to paucity of high-quality studies, clinical care practice for people with PCD is mainly adopted from other diseases including cystic fibrosis (CF). Current guidelines for people with PCD recommend at least biannual clinic visits with pulmonary function testing (PFT) to monitor lung disease [5]. In children with PCD, reversible airway obstruction has been reported and inhaled beta2-agonists are frequently prescribed [10, 11]. However, the diagnostic value as well as clinical consequence of a positive BD response in children with PCD remains unclear [12].

The aim of this study was to determine the frequency of reversible airways obstruction in children with PCD, and whether a positive BD response at routine clinical testing helps predict clinical outcomes.

METHODS

Pulmonary function test (PFT) results from children with PCD at the Hospital for Sick Children (SickKids), Toronto, between 2009 and 2021 were analysed. The study was approved by the local Research Ethics Board (REB #1000078911). PCD was diagnosed
according to American Thoracic Society (ATS) guidelines [5]. All PCD patients performing PFTs as part of routine care were eligible for this retrospective single centre study. Participants were identified by searching the SickKids Respiratory Medicine PFT database. Spirometry results (forced expiratory volume in one second, FEV₁, and forced vital capacity, FVC) were calculated using published Global Lung Function Initiative (GLI) equations [13] and expressed as percent predicted (pp). A positive BD response was defined as ≥12% and at least 200 ml change in ppFEV₁ or FVC following inhalation of albuterol using a spacer, as per ATS guidelines [14]. Airway microbiology testing was performed in expectorated sputum or throat swabs as per clinical routine. Demographics and clinical characteristics were abstracted from electronic medical records by chart review. This study consisted of two parts:

For the first part, we collected spirometry results for all encounters with BD response testing (Visit) and the previous (Baseline) as well as the following (Follow up) encounters and created two groups: group 1 had positive BD responsiveness at Visit and group 2 had no BD responsiveness at Visit. We analysed the ppFEV₁ change from Baseline to Visit as well as from Visit to Follow up for each group and compared the results.

Secondly, we compared the annual ppFEV₁ decline of PCD children with at least one positive BD response to those with only negative BD response testing results during the study period.

Statistical analysis was performed using SPSS software V.27.0 (IBM Corporation, NY, USA) and GraphPad Prism V 8.4.3 (GraphPad Software, San Diego, USA). All continuous variables were tested for normal distribution (Shapiro-Wilk test), and data presented as number (percent), mean (standard deviation, SD or 95% confidence interval, CI, as appropriate) or median (interquartile range, IQR). Comparison of categorical variables were calculated using chi square test. Differences between groups were analyzed using two-sample unpaired t-test or Mann-Whitney-U test, where appropriate. Paired t-test was used to compare
related samples. Odds ratio (OR, 95%CI) was calculated to quantify the association between BD responsiveness and initiation of antimicrobial treatment. To calculate annual pre-BD ppFEV₁ decline, linear regression models were used and tested for significance by ANOVA. To test the relationship between the slopes a hypothesis test on the difference between regression coefficients was performed. All models were adjusted for the number of visits with PFTs. A p-value < 0.05 was considered significant.

RESULTS

Ninety-nine children with PCD had performed spirometry testing during the study period. Seventeen children were excluded because they never underwent BD testing. Of the remaining 82 children, a total of 474 PFTs with BD response testing were performed and used for the analysis. Genetic testing was performed in 76 patients; bi-allelic pathogenic variants included DNAH5 (22), DNAH11 (7), CCDC39 (7), LRRC6 (4), CCNO (2), CCDC40 (2), DRC1 (2), SPAG1 (2), DNAAF2/KTU (1), DNAAF3 (1), DNAAF4 (1), DNAH9 (1), DNAI1 (1), DNAI2 (1), HYDIN (1), ZMYND10 (1), DNAAF2/KTU (1), and DNAAF3 (1). For 6 patients testing revealed no pathogenic variants known to cause PCD. Based on the genotype, PCD children were divided into two groups: ‘mild’ (DNAH5, DNAH11, DNAAF3, LRRC6, ZMYND10, DNAH9, DNAI1, DNAAF2/KTU, DNAAF4, and DNAI2) and ‘severe’ (DRC1, HYDIN, CCDC39, CCNO, SPAG1, and CCDC40) [15, 16]. None of the patients included in this study had a physician diagnosis of asthma or allergic rhino-conjunctivitis. Laboratory test results revealed normal blood total IgE levels (median IgE 25.0 kU/L; IQR 15.2-77.0). Clinical and demographic details of the study cohort are listed in Table 1. The median (IQR) number of PFTs with BD response testing per patient was 4.00 (IQR 2.00; 7.25). Airway microbiology culture results at Visit were positive in 305/474 (64.3%); most common were Haemophilus influenzae (36%), Staphylococcus aureus (22%), Streptococcus pneumoniae (12%) and Pseudomonas aeruginosa (9%).
The mean (SD) absolute change in FEV$_1$ from pre- to post-BD testing was 140.1 ml (136.3), mean change in ppFEV$_1$ from pre- to post-BD was 5.0% (5.5) (Figure 1). Fifty (50/82) individuals, at 293/472 (62.1%) encounters, received inhaled corticosteroid (ICS) therapy. The mean change in ppFEV$_1$ from pre- to post-BD was not different whether receiving (n=294/474 encounters) or not receiving ICS (5.0% (SD 4.9) vs 5.1% (SD 6.2), p=0.853). A total of 86/474 (18.1%) tests revealed a positive BD response according to ERS/ATS criteria [14]; when a 12% improvement in ppFEV$_1$ was used as the only criterion, 101/474 (21.3%) tests were positive. Forty-one percent (34/82) of tested patients had at least one recorded positive BD response. BD responsiveness was not associated with sex (p=0.059), ethnicity (p=0.167), airway microbiology culture positivity (p=0.933), or age at diagnosis (p=0.074). BD responsiveness was more common at younger age (Table 2). Data of 428/474 encounters documenting clinical symptoms were available for review. Worsened respiratory symptoms (e.g. cough) were documented for 121 (28.3%) of the visits, and these were not associated with a positive BDR (p=0.471).

BD responsiveness was associated with a significant absolute change in ppFEV$_1$ from Baseline to Visit pre-BD ppFEV$_1$ (-6.5%, SD 10.3, p<0.001), and to Visit post-BD ppFEV$_1$ (6.2, SD 10.7, p<0.001), and was more common in individuals with a drop in ppFEV$_1$ from Baseline to Visit of greater than 10% compared to those with less than 10% change in ppFEV$_1$ (37.6% (32/85) vs 13.8% (52/376); p<0.001). Individuals with no BD responsiveness had no change in ppFEV$_1$ from Baseline to Visit pre-BD ppFEV$_1$ (-0.2, SD 10.2, p=0.724). The ppFEV$_1$ was similar between Baseline and Follow-up regardless of BD responsiveness at Visit (0.4, SD 10.8, p=0.757 vs -0.1%, SD 10.9, p=0.862) (Figure 2). Antimicrobial therapy was initiated more frequently following a Visit with a positive BD response compared to no BD response (OR 3.8, 95% CI 2.2 – 6.6). However, at Follow-up ppFEV$_1$ did not differ whether treated or not treated with antimicrobials for both individuals with (2.6% (SD 11.0)
vs -0.5 (SD 11.0) p=0.486) or without BD response (0.2 (SD 9.5) vs -0.8 (SD 11.4) p=0.442) (Table 2).

Longitudinal analysis of PFT data of all 82 children with PCD during the 12-year observation period revealed an average annual decline in pre-BD ppFEV\textsubscript{1} of -0.6/year (95%CI -0.7, -0.4, p<0.001). Children with one or more positive BD response tests (n=34) had a greater annual decline in pre-BD ppFEV\textsubscript{1} compared to those with no BD response (n=48) (-0.9% (95%CI -1.2, -0.7) vs -0.47% (95%CI -0.3, -0.6); p<0.001). To test for prematurity as a potential risk factor for lung disease progression, preterm birth was added to the regression models as a confounder, and had no effect on the annual PFT decline. The annual decline was more pronounced in children with two or more documented positive BD responses (n=15) compared to one positive BD response (n=19) (-1.3/year (95%CI -1.0, -1.7) vs -0.6/year (95%CI -0.4, -0.7); p<0.001). Further, those not treated with antibiotic therapy following a Visit with a positive BD response had a greater pre-BD ppFEV\textsubscript{1} decline compared to those receiving antibiotic therapy (-1.1% (95%CI -1.6, -0.7) vs -0.6% (95%CI -1.1, -0.2); p<0.001) (Table 3). The annual ppFEV\textsubscript{1} decline did not differ between mild and severe PCD genotypes across children with positive BDR responsiveness (p=0.123)

**DISCUSSION**

In this analysis we found that reversible airways obstruction demonstrated on routine pulmonary function testing was common in a large, well characterized paediatric PCD cohort. BD responsiveness was associated with a temporary decline in (pre-BD) ppFEV\textsubscript{1} compared to previous baseline, and children with positive BD response were more likely prescribed antimicrobial therapies. Longitudinal analysis of PFT data revealed an annual decline in ppFEV\textsubscript{1} of -0.6%/year for the entire cohort but children with reversible airway obstruction on multiple occasions as well as those not receiving antibiotic therapy following a positive BD
test had a significantly greater annual decline in ppFEV₁ (-1.3%/year and -1.1%/year, respectively).

Our observation of a positive BD response in 18% of patients is matching an earlier report by Keenan et al. [11], who found a positive BD response in 17% of 47 children with PCD. In another retrospective study, Levine et al. [10] reported results from BD response testing in 46 children with PCD. Reversible airway obstruction, defined as ≥ 10% decline in ppFEV₁, was present in 26 (56.5%) children. Of note, although the history of recurrent wheezing was present in almost half of these patients, reversible airways obstruction was not associated with markers of asthma such as a positive family history, increased blood eosinophil counts, elevated serum IgE, or atopy. Smaller patient numbers, lower threshold for definition of significance in BD response and a preselection towards children with recurrent wheeze likely contributed to the greater prevalence of BD responsiveness in theirs studies compared to ours.

ICS are commonly prescribed in PCD often without evidence of type 2 airway inflammation [17]; in our cohort at 62.1% of the analysed encounters. While current guidelines recommend ICS therapy in PCD patients with co-existing asthma or wheezing [18, 19], in our cohort no participant had a documented diagnosis of asthma. The rational for prescribing long-term ICS therapy in our cohort remains unclear and was not the focus of this study. It can be speculated that ICS therapy in individual patients was initiated in response to recurrent wheezing episodes following viral respiratory infections.

Our data suggest that BD responsiveness in children with PCD is associated with a temporary decline in lung function that can recover with or without antimicrobial therapy, as ppFEV₁ at next follow-up after 4-5 month was similar to recorded baselines prior to the positive BD response, regardless of antibiotic therapy. It is therefore conceivable, that the observed decline in lung function was not always caused by bacterial infections requiring antibiotic
therapy. Other explanations could be non-infectious exacerbations or viral infections. If the latter, this may help explain the observed association of BD responsiveness with younger age, as viral infections are more common in younger compared to older children [20]. An association of respiratory tract infections with reversible airways obstruction is well documented for adults without underlying chronic respiratory condition [21].

However, the analysis of longitudinal pulmonary function data revealed that a positive BD response not treated with antibiotics was in fact associated with a greater loss in pulmonary function over time. Previous cross-sectional and longitudinal analyses of PFT data in PCD reported a high degree of variation in progression of lung disease. Some authors reported stable lung function once the PCD diagnosis was established and treatments were initiated, whereas others described lung disease progression with increasing age [4, 22–25]. Ellerman and Bisgaard analyzed longitudinally PFT data from 24 PCD patients diagnosed at different ages and concluded that aggressive treatment could prevent further lung damage [26]. Other factors potentially contributing to variability in clinical presentation and lung disease progression include differences in the disease-causing PCD genetics and their consequences for structure or function of the respiratory cilia [16].

In children with PCD and BD responsiveness, further studies are needed to assess the benefit of intensified treatment including chest physical therapy as well as antimicrobial or anti-inflammatory agents. Of note, while the annual decline in ppFEV₁ in those with positive BD response was significantly greater if not treated with antibiotics in our study, there appeared to be no difference in annual PFT decline in children with PCD who were treated with antibiotics comparing those with positive or negative BD response prior to initiation of treatment. We therefore speculated that antibiotic therapy, maybe in combination with optimized airway clearance, does result in measurable long-term benefits not detectable in short-term follow-up.
In a recent analysis of PFT data in a cohort of children with CF, we had shown that a significant BD response in CF pulmonary exacerbation (PEx) was rare, was not related to severity of lung disease or potential recovery of lung function, and did not lead to changes in clinical management [27], demonstrating that routine BD response testing in CF PEx is not clinically meaningful. This was in contrast to our findings in children with PCD where BD responsiveness is common and a relevant correlation with clinical outcomes seems to exist. However, the observation that a positive BD response in PCD was also linked to the magnitude of decline in pre-BD ppFEV₁ from previous baseline may suggest that even though a positive BD response is associated with poorer outcome, a significant drop in ppFEV₁ alone could be indicative of a PCD PEx requiring initiation of therapy.

There are several limitations of this retrospective single center study to be considered. First, standard care of children with PCD does not include routine BD response testing and the rationales for ordering PFTs with BD response testing may vary between physicians and centers. Also, children with more severe pulmonary involvement might be seen more often in clinic and therefore be overrepresented in this study. To overcome this issue, we adjusted the statistical model for the individual number of visits. However, children with PCD in the absence of clinical symptoms not performing PFT might have been excluded from this analysis completely, even though PFTs are usually performed at every visit. Also, patient- or parent-reported symptoms were not included in this analysis, and we cannot comment on the association between respiratory symptoms and PFT findings. Finally, although BD response seemed to be associated with PCD PEx, the mechanisms resulting in reversible airway obstruction remain unclear and contributing factors might have been missed.

In conclusion, our data show that reversible airways obstruction is common in children with PCD and is reflective of a temporary decline in ppFEV₁. BD responsiveness may help
identify children with PCD at risk for lung disease progression. Further studies are needed to assess the benefit of different treatment options.
REFERENCES


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Competing interests

There are no competing interests for any author.
LEGENDS TO THE FIGURES

Figure 1 - The relative change in ppFEV₁ post bronchodilator inhalation presented as frequencies of tests (n=474). The dashed line indicates a 12% change in ppFEV₁.

Figure 2 – Absolute change from Baseline to Visit (pre-BD) and Follow-up in ppFEV₁. Data are representing means and bars 95% confidence intervals. The dashed line indicates baseline.
### Table 1 – Characteristics of study cohort. Data are presented as numbers (percent) or mean (standard deviation)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of included patients</td>
<td>82 (100%)</td>
</tr>
<tr>
<td>Female sex</td>
<td>37 (45.1%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>50 (61%)</td>
</tr>
<tr>
<td>Asian</td>
<td>9 (11.0%)</td>
</tr>
<tr>
<td>Black</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>14 (17.1%)</td>
</tr>
<tr>
<td>Data not available</td>
<td>6 (7.3%)</td>
</tr>
<tr>
<td>Age in years at diagnosis</td>
<td>8.0 (5.2)</td>
</tr>
<tr>
<td>EM defects</td>
<td></td>
</tr>
<tr>
<td>ODA defect</td>
<td>16 (19.5%)</td>
</tr>
<tr>
<td>Combined ODA and IDA defect</td>
<td>21 (25.6%)</td>
</tr>
<tr>
<td>Central pair defect</td>
<td>13 (15.9%)</td>
</tr>
<tr>
<td>No ultrastructural defect</td>
<td>8 (9.8%)</td>
</tr>
<tr>
<td>EM not done or data not available</td>
<td>24 (29.2%)</td>
</tr>
<tr>
<td>Situs inversus</td>
<td>34 (41.5%)</td>
</tr>
<tr>
<td>Pathogenic nNO*</td>
<td>63 (76.8%)</td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
</tr>
<tr>
<td>&lt;32 weeks of gestational age</td>
<td>- (0%)</td>
</tr>
<tr>
<td>32 – 37 weeks of gestational age</td>
<td>23 (28.0%)</td>
</tr>
<tr>
<td>&gt;37 weeks of gestational age</td>
<td>59 (72.0%)</td>
</tr>
</tbody>
</table>

EM = electron microscopy, ODA = outer dynein arm defect, IDA = inner dynein arm defect, nNO = nasal nitric oxide

* Pathogenic nNO was defined as test result being lower than 77 nL/min
Table 2 – Spirometry results of the study cohort

<table>
<thead>
<tr>
<th></th>
<th>Total cohort</th>
<th>positive BDR N=34 (41%)</th>
<th>no BDR N=48 (59%)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of PFTs with BD response testing</td>
<td>474 (100%)</td>
<td>86 (18.1%)</td>
<td>388 (81.9%)</td>
<td>-</td>
</tr>
<tr>
<td>Age (Years) at PFT</td>
<td>12.8 (3.4)</td>
<td>11.8 (3.0)</td>
<td>13.0 (3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline ppFEV₁</td>
<td>76.5 (16.5)</td>
<td>76.8 (16.6)</td>
<td>76.5 (16.5)</td>
<td>0.883</td>
</tr>
<tr>
<td>Visit ppFEV₁, pre BD</td>
<td>75.5 (16.8)</td>
<td>70.2 (15.2)</td>
<td>76.7 (16.8)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Follow up ppFEV₁</td>
<td>76.7 (16.6)</td>
<td>77.2 (15.6)</td>
<td>76.6 (16.8)</td>
<td>0.782</td>
</tr>
<tr>
<td>Delta ppFEV₁ Baseline to Visit, pre BD</td>
<td>-1.0 (10.5)</td>
<td>-6.5 (10.3)</td>
<td>0.2 (10.2)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Delta ppFEV₁ Baseline to Visit, post BD</td>
<td>4.1 (10.8)</td>
<td>6.2 (10.7)</td>
<td>3.6 (10.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delta ppFEV₁ Baseline to Follow up</td>
<td>0.0 (10.9)</td>
<td>0.4 (10.8)</td>
<td>-0.1 (10.9)</td>
<td>0.724</td>
</tr>
<tr>
<td>Months between Baseline and Visit</td>
<td>4.7 (3.6)</td>
<td>4.6 (3.3)</td>
<td>4.8 (3.7)</td>
<td>0.717</td>
</tr>
<tr>
<td>Months between Visit and Follow up</td>
<td>4.9 (4.8)</td>
<td>4.3 (4.0)</td>
<td>5.0 (5.0)</td>
<td>0.232</td>
</tr>
<tr>
<td>Antimicrobial treatment initiated after Visit</td>
<td>114/474 (24.1%)</td>
<td>40/86 (46.5%)</td>
<td>74/388 (19.1%)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

* Comparison between children with positive or no bronchodilator response (BDR). Comparison of categorical variables were calculated using chi square test. All continuous variables were tested for normal distribution (Shapiro-Wilk test), and differences between groups analyzed using two-sample unpaired t-test or Mann-Whitney-U test, as appropriate. Boldface values indicate significant differences.
Table 3 – Annual decline in FEV₁ according to BDR positivity, antimicrobial treatment initiated after visit and number of positive BD.

<table>
<thead>
<tr>
<th>No BD responsiveness</th>
<th>Annual ppFEV₁ decline (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial treatment</td>
<td>-0.5 (-0.3, -0.6)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td>-0.7 (-1.0, -0.3)</td>
<td>p=0.001</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>BD responsiveness</th>
<th>Annual ppFEV₁ decline (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial treatment</td>
<td>-0.9 (-1.2, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td>-1.1 (-1.6, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>One positive BD responsiveness testing</td>
<td>-0.6 (-0.4, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>≥ 2 positive BD responsiveness testing</td>
<td>-1.3 (-1.0, -1.7)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

To calculate annual PFT decline (ppFEV₁, per-BD), linear regression models were used and tested for significance by ANOVA. The models were adjusted for the number of visits per participant. Boldface values indicate significant differences.
Figure 1: Relative % change in FEV₁ from pre to post BD
Bronchodilator responsiveness in children with primary ciliary dyskinesia

Elias Seidl¹, Dvir Gatt¹, Wallace B. Wee¹, David Wilson¹, Felix Ratjen¹,², Hartmut Grasemann¹,³

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ABSTRACT

Background

Reversible airways obstruction has been reported to be common in people with primary ciliary dyskinesia (PCD). However, the diagnostic value of bronchodilator (BD) response testing added to routine spirometry is unclear.

Methods

This is a retrospective analysis of pulmonary function test (PFT) results obtained from children with PCD seen as outpatients at the Hospital for Sick Children, Toronto. Spirometry results for every Visit with BD-response testing, the previous (Baseline) and the following (Follow-up) encounters were collected.

Results

A positive BD-response was seen in 86/474 (18.1%) of PFTs from 82 children with PCD. BD-responsiveness was associated with a significant absolute change in percent predicted forced expiratory volume in one second (ppFEV₁) from Baseline to Visit pre-BD ppFEV₁ (-6.5%, SD 10.3, p<0.001), but not from Baseline to Follow-up (0.4%, SD 10.8, p=0.757). Antimicrobial therapy was initiated more commonly following a Visit with a positive BD-response (OR 3.8, 95%CI 2.2 – 6.6) compared to no BD-response. Children with a positive BD-response had a greater annual decline in ppFEV₁ compared to those with no BD-response (-0.9%/year vs -0.5%/year; p<0.001). The annual decline in ppFEV₁ was greater in children with multiple compared to one measured positive BD responses (-1.3/year vs -0.6/year, p<0.001) and in those not treated with antibiotic therapy following a positive BD response compared to those treated with antibiotics (-1.1% vs -0.6%; p<0.001).

Conclusion

A positive BD-response in children with PCD may help identify those at risk for accelerated lung disease progression.
INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare, mainly autosomal recessive inherited multi-organ disease, which is characterized by dysfunctional motile cilia. In the respiratory tract PCD causes impaired muco-ciliary clearance, neonatal respiratory distress, chronic nasal congestion, sinusitis and chronic wet cough [1–3]. Recurrent airway infections and inflammation are common, leading to bronchiectasis and irreversible lung damage [2, 4]. The diagnosis of PCD can be challenging and involves typical clinical features, measurement of nasal nitric oxide, structural and functional analysis of cilia, and genetic testing [5]. PCD has an estimated incidence of 1 per 10,000–20,000 [6–8]. The prevalence of PCD is difficult to determine, largely due to inadequacies of diagnostic methods [9].

Due to paucity of high-quality studies, clinical care practice for people with PCD is mainly adopted from other diseases including cystic fibrosis (CF). Current guidelines for people with PCD recommend at least biannual clinic visits with pulmonary function testing (PFT) to monitor lung disease [5]. In children with PCD, reversible airway obstruction has been reported and inhaled beta2-agonists are frequently prescribed [10, 11]. However, the diagnostic value as well as clinical consequence of a positive BD response in children with PCD remains unclear [12].

The aim of this study was to determine the frequency of reversible airways obstruction in children with PCD, and whether a positive BD response at routine clinical testing helps predict clinical outcomes.

METHODS

Pulmonary function test (PFT) results from children with PCD at the Hospital for Sick Children (SickKids), Toronto, between 2009 and 2021 were analysed. The study was approved by the local Research Ethics Board (REB #1000078911). PCD was diagnosed
according to American Thoracic Society (ATS) guidelines [5]. All PCD patients performing
PFTs as part of routine care were eligible for this retrospective single centre study.
Participants were identified by searching the SickKids Respiratory Medicine PFT database.
Spirometry results (forced expiratory volume in one second, FEV$_1$, and forced vital capacity,
FVC) were calculated using published Global Lung Function Initiative (GLI) equations [13]
and expressed as percent predicted (pp). A positive BD response was defined as $\geq$12% and at
least 200 ml change in ppFEV$_1$ or FVC following inhalation of albuterol using a spacer, as
per ATS guidelines [14]. Airway microbiology testing was performed in expectorated sputum
or throat swabs as per clinical routine. Demographics and clinical characteristics were
abstracted from electronic medical records by chart review. This study consisted of two parts:
For the first part, we collected spirometry results for all encounters with BD response testing
(Visit) and the previous (Baseline) as well as the following (Follow up) encounters and
created two groups: group 1 had positive BD responsiveness at Visit and group 2 had no BD
responsiveness at Visit. We analysed the ppFEV$_1$ change from Baseline to Visit as well as
from Visit to Follow up for each group and compared the results.
Secondly, we compared the annual ppFEV$_1$ decline of PCD children with at least one positive
BD response to those with only negative BD response testing results during the study period.
Statistical analysis was performed using SPSS software V.27.0 (IBM Corporation, NY, USA)
and GraphPad Prism V 8.4.3 (GraphPad Software, San Diego, USA). All continuous
variables were tested for normal distribution (Shapiro-Wilk test), and data presented as
number (percent), mean (standard deviation, SD or 95% confidence interval, CI, as
appropriate) or median (interquartile range, IQR). Comparison of categorical variables were
calculated using chi square test. Differences between groups were analyzed using two-sample
unpaired t-test or Mann-Whitney-U test, where appropriate. Paired t-test was used to compare
related samples. Odds ratio (OR, 95%CI) was calculated to quantify the association between BD responsiveness and initiation of antimicrobial treatment. To calculate annual pre-BD ppFEV$_1$ decline, linear regression models were used and tested for significance by ANOVA. To test the relationship between the slopes a hypothesis test on the difference between regression coefficients was performed. All models were adjusted for the number of visits with PFTs. A p-value < 0.05 was considered significant.

RESULTS

Ninety-nine children with PCD had performed spirometry testing during the study period. Seventeen children were excluded because they never underwent BD testing. Of the remaining 82 children, a total of 474 PFTs with BD response testing were performed and used for the analysis. Genetic testing was performed in 76 patients; bi-allelic pathogenic variants included DNAH5 (22), DNAH11 (7), CCDC39 (7), LRRC6 (4), CCNO (2), CCDC40 (2), DRC1 (2), SPAG1 (2), DNAAF2/KTU (1), DNAAF3, (1), DNAAF4 (1), DNAH9 (1), DNAI1 (1), DNAI2 (1), HYDIN (1), ZMYND10 (1), DNAAF2/KTU (1), and DNAAF3 (1). For 6 patients testing revealed no pathogenic variants known to cause PCD. Based on the genotype, PCD children were divided into two groups: ‘mild’ (DNAH5, DNAH11, DNAAF3, LRRC6, ZMYND10, DNAH9, DNAI1, DNAAF2/KTU, DNAAF4, and DNAI2) and ‘severe’ (DRC1, HYDIN, CCDC39, CCNO, SPAG1, and CCDC40) [15, 16]. None of the patients included in this study had a physician diagnosis of asthma or allergic rhino-conjunctivitis. Laboratory test results revealed normal blood total IgE levels (median IgE 25.0 kU/L; IQR 15.2-77.0). Clinical and demographic details of the study cohort are listed in Table 1. The median (IQR) number of PFTs with BD response testing per patient was 4.00 (IQR 2.00; 7.25). Airway microbiology culture results at Visit were positive in 305/474 (64.3%); most common were *Haemophilus influenzae* (36%), *Staphylococcus aureus* (22%), *Streptococcus pneumoniae* (12%) and *Pseudomonas aeruginosa* (9%).
The mean (SD) absolute change in FEV₁ from pre- to post-BD testing was 140.1 ml (136.3), mean change in ppFEV₁ from pre- to post-BD was 5.0% (5.5) (Figure 1). Fifty (50/82) individuals, at 293/472 (62.1%) encounters, received inhaled corticosteroid (ICS) therapy. The mean change in ppFEV₁ from pre- to post-BD was not different whether receiving (n=294/474 encounters) or not receiving ICS (5.0% (SD 4.9) vs 5.1% (SD 6.2), p=0.853). A total of 86/474 (18.1%) tests revealed a positive BD response according to ERS/ATS criteria [14]; when a 12% improvement in ppFEV₁ was used as the only criterion, 101/474 (21.3%) tests were positive. Forty-one percent (34/82) of tested patients had at least one recorded positive BD response. BD responsiveness was not associated with sex (p=0.059), ethnicity (p=0.167), airway microbiology culture positivity (p=0.933), or age at diagnosis (p=0.074). BD responsiveness was more common at younger age (Table 2). Data of 428/474 encounters documenting clinical symptoms were available for review. Worsened respiratory symptoms (e.g. cough) were documented for 121 (28.3%) of the visits, and these were not associated with a positive BDR (p=0.471).

BD responsiveness was associated with a significant absolute change in ppFEV₁ from Baseline to Visit pre-BD ppFEV₁ (-6.5%, SD 10.3, p<0.001), and to Visit post-BD ppFEV₁ (6.2, SD 10.7, p<0.001), and was more common in individuals with a drop in ppFEV₁ from Baseline to Visit of greater than 10% compared to those with less than 10% change in ppFEV₁ (37.6% (32/85) vs 13.8% (52/376); p<0.001). Individuals with no BD responsiveness had no change in ppFEV₁ from Baseline to Visit pre-BD ppFEV₁ (-0.2, SD 10.2, p=0.724). The ppFEV₁ was similar between Baseline and Follow-up regardless of BD responsiveness at Visit (0.4, SD 10.8, p=0.757 vs -0.1%, SD 10.9, p=0.862) (Figure 2). Antimicrobial therapy was initiated more frequently following a Visit with a positive BD response compared to no BD response (OR 3.8, 95% CI 2.2 – 6.6). However, at Follow-up ppFEV₁ did not differ whether treated or not treated with antimicrobials for both individuals with (2.6% (SD 11.0)
vs -0.5 (SD 11.0) p=0.486) or without BD response (0.2 (SD 9.5) vs -0.8 (SD 11.4) p=0.442) (Table 2).

Longitudinal analysis of PFT data of all 82 children with PCD during the 12-year observation period revealed an average annual decline in pre-BD ppFEV$_1$ of -0.6/year (95%CI -0.7, -0.4, p<0.001). Children with one or more positive BD response tests (n=34) had a greater annual decline in pre-BD ppFEV$_1$ compared to those with no BD response (n=48) (-0.9% (95%CI -1.2, -0.7) vs -0.47% (95%CI -0.3, -0.6); p<0.001). To test for prematurity as a potential risk factor for lung disease progression, preterm birth was added to the regression models as a confounder, and had no effect on the annual PFT decline. The annual decline was more pronounced in children with two or more documented positive BD responses (n=15) compared to one positive BD response (n=19) (-1.3/year (95%CI -1.0, -1.7) vs -0.6/year (95%CI -0.4, -0.7); p<0.001). Further, those not treated with antibiotic therapy following a Visit with a positive BD response had a greater pre-BD ppFEV$_1$ decline compared to those receiving antibiotic therapy (-1.1% (95%CI -1.6, -0.7) vs -0.6% (95%CI -1.1, -0.2); p<0.001) (Table 3). The annual ppFEV$_1$ decline did not differ between mild and severe PCD genotypes across children with positive BDR responsiveness (p=0.123)

**DISCUSSION**

In this analysis we found that reversible airways obstruction demonstrated on routine pulmonary function testing was common in a large, well characterized paediatric PCD cohort. BD responsiveness was associated with a temporary decline in (pre-BD) ppFEV$_1$ compared to previous baseline, and children with positive BD response were more likely prescribed antimicrobial therapies. Longitudinal analysis of PFT data revealed an annual decline in ppFEV$_1$ of -0.6%/year for the entire cohort but children with reversible airway obstruction on multiple occasions as well as those not receiving antibiotic therapy following a positive BD
test had a significantly greater annual decline in ppFEV₁ (-1.3%/year and -1.1%/year, respectively).

Our observation of a positive BD response in 18% of patients is matching an earlier report by Keenan et al. [11], who found a positive BD response in 17% of 47 children with PCD. In another retrospective study, Levine et al. [10] reported results from BD response testing in 46 children with PCD. Reversible airway obstruction, defined as ≥ 10% decline in ppFEV₁, was present in 26 (56.5%) children. Of note, although the history of recurrent wheezing was present in almost half of these patients, reversible airways obstruction was not associated with markers of asthma such as a positive family history, increased blood eosinophil counts, elevated serum IgE, or atopy. Smaller patient numbers, lower threshold for definition of significance in BD response and a preselection towards children with recurrent wheeze likely contributed to the greater prevalence of BD responsiveness in theirs studies compared to ours.

ICS are commonly prescribed in PCD often without evidence of type 2 airway inflammation [17]; in our cohort at 62.1% of the analysed encounters. While current guidelines recommend ICS therapy in PCD patients with co-existing asthma or wheezing [18, 19], in our cohort no participant had a documented diagnosis of asthma. The rational for prescribing long-term ICS therapy in our cohort remains unclear and was not the focus of this study. It can be speculated that ICS therapy in individual patients was initiated in response to recurrent wheezing episodes following viral respiratory infections.

Our data suggest that BD responsiveness in children with PCD is associated with a temporary decline in lung function that can recover with or without antimicrobial therapy, as ppFEV₁ at next follow-up after 4-5 month was similar to recorded baselines prior to the positive BD response, regardless of antibiotic therapy. It is therefore conceivable, that the observed decline in lung function was not always caused by bacterial infections requiring antibiotic
therapy. Other explanations could be non-infectious exacerbations or viral infections. If the latter, this may help explain the observed association of BD responsiveness with younger age, as viral infections are more common in younger compared to older children [20]. An association of respiratory tract infections with reversible airways obstruction is well documented for adults without underlying chronic respiratory condition [21].

However, the analysis of longitudinal pulmonary function data revealed that a positive BD response not treated with antibiotics was in fact associated with a greater loss in pulmonary function over time. Previous cross-sectional and longitudinal analyses of PFT data in PCD reported a high degree of variation in progression of lung disease. Some authors reported stable lung function once the PCD diagnosis was established and treatments were initiated, whereas others described lung disease progression with increasing age [4, 22–25]. Ellerman and Bisgaard analyzed longitudinally PFT data from 24 PCD patients diagnosed at different ages and concluded that aggressive treatment could prevent further lung damage [26]. Other factors potentially contributing to variability in clinical presentation and lung disease progression include differences in the disease-causing PCD genetics and their consequences for structure or function of the respiratory cilia [16].

In children with PCD and BD responsiveness, further studies are needed to assess the benefit of intensified treatment including chest physical therapy as well as antimicrobial or anti-inflammatory agents. Of note, while the annual decline in ppFEV<sub>1</sub> in those with positive BD response was significantly greater if not treated with antibiotics in our study, there appeared to be no difference in annual PFT decline in children with PCD who were treated with antibiotics comparing those with positive or negative BD response prior to initiation of treatment. We therefore speculated that antibiotic therapy, maybe in combination with optimized airway clearance, does result in measurable long-term benefits not detectable in short-term follow-up.
In a recent analysis of PFT data in a cohort of children with CF, we had shown that a significant BD response in CF pulmonary exacerbation (PEx) was rare, was not related to severity of lung disease or potential recovery of lung function, and did not lead to changes in clinical management [27], demonstrating that routine BD response testing in CF PEx is not clinically meaningful. This was in contrast to our findings in children with PCD where BD responsiveness is common and a relevant correlation with clinical outcomes seems to exist. However, the observation that a positive BD response in PCD was also linked to the magnitude of decline in pre-BD ppFEV\textsubscript{1} from previous baseline may suggest that even though a positive BD response is associated with poorer outcome, a significant drop in ppFEV\textsubscript{1} alone could be indicative of a PCD PEx requiring initiation of therapy.

There are several limitations of this retrospective single center study to be considered. First, standard care of children with PCD does not include routine BD response testing and the rationales for ordering PFTs with BD response testing may vary between physicians and centers. Also, children with more severe pulmonary involvement might be seen more often in clinic and therefore be overrepresented in this study. To overcome this issue, we adjusted the statistical model for the individual number of visits. However, children with PCD in the absence of clinical symptoms not performing PFT might have been excluded from this analysis completely, even though PFTs are usually performed at every visit. Also, patient- or parent-reported symptoms were not included in this analysis, and we cannot comment on the association between respiratory symptoms and PFT findings. Finally, although BD response seemed to be associated with PCD PEx, the mechanisms resulting in reversible airway obstruction remain unclear and contributing factors might have been missed.

In conclusion, our data show that reversible airways obstruction is common in children with PCD and is reflective of a temporary decline in ppFEV\textsubscript{1}. BD responsiveness may help
identify children with PCD at risk for lung disease progression. Further studies are needed to assess the benefit of different treatment options.
REFERENCES


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Competing interests

There are no competing interests for any author.
LEGENDS TO THE FIGURES

Figure 1 - The relative change in ppFEV$_1$ post bronchodilator inhalation presented as frequencies of tests (n=474). The dashed line indicates a 12% change in ppFEV$_1$.

Figure 2 – Absolute change from Baseline to Visit (pre-BD) and Follow-up in ppFEV$_1$. Data are representing means and bars 95% confidence intervals. The dashed line indicates baseline.
Table 1 – Characteristics of study cohort. Data are presented as numbers (percent) or mean (standard deviation).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of included patients</td>
<td>82 (100%)</td>
</tr>
<tr>
<td>Female sex</td>
<td>37 (45.1%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>50 (61%)</td>
</tr>
<tr>
<td>Asian</td>
<td>9 (11.0%)</td>
</tr>
<tr>
<td>Black</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>14 (17.1%)</td>
</tr>
<tr>
<td>Data not available</td>
<td>6 (7.3%)</td>
</tr>
<tr>
<td>Age in years at diagnosis</td>
<td>8.0 (5.2)</td>
</tr>
<tr>
<td>EM defects</td>
<td></td>
</tr>
<tr>
<td>ODA defect</td>
<td>16 (19.5%)</td>
</tr>
<tr>
<td>Combined ODA and IDA defect</td>
<td>21 (25.6%)</td>
</tr>
<tr>
<td>Central pair defect</td>
<td>13 (15.9%)</td>
</tr>
<tr>
<td>No ultrastructural defect</td>
<td>8 (9.8%)</td>
</tr>
<tr>
<td>EM not done or data not available</td>
<td>24 (29.2%)</td>
</tr>
<tr>
<td>Situs inversus</td>
<td>34 (41.5%)</td>
</tr>
<tr>
<td>Pathogenic nNO*</td>
<td>63 (76.8%)</td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
</tr>
<tr>
<td>&lt;32 weeks of gestational age</td>
<td>- (0%)</td>
</tr>
<tr>
<td>32 – 37 weeks of gestational age</td>
<td>23 (28.0%)</td>
</tr>
<tr>
<td>&gt;37 weeks of gestational age</td>
<td>59 (72.0%)</td>
</tr>
</tbody>
</table>

EM = electron microscopy, ODA = outer dynein arm defect, IDA = inner dynein arm defect, nNO = nasal nitric oxide

* Pathogenic nNO was defined as test result being lower than 77 nL/min
Table 2 – Spirometry results of the study cohort

<table>
<thead>
<tr>
<th></th>
<th>Total cohort</th>
<th>positive BDR N=34 (41%)</th>
<th>no BDR N=48 (59%)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of PFTs with BD response testing</td>
<td>474 (100%)</td>
<td>86 (18.1%)</td>
<td>388 (81.9%)</td>
<td>-</td>
</tr>
<tr>
<td>Age (Years) at PFT</td>
<td>12.8 (3.4)</td>
<td>11.8 (3.0)</td>
<td>13.0 (3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline ppFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>76.5 (16.5)</td>
<td>76.8 (16.6)</td>
<td>76.5 (16.5)</td>
<td>0.883</td>
</tr>
<tr>
<td>Visit ppFEV&lt;sub&gt;1&lt;/sub&gt;, pre BD</td>
<td>75.5 (16.8)</td>
<td>70.2 (15.2)</td>
<td>76.7 (16.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Follow up ppFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>76.7 (16.6)</td>
<td>77.2 (15.6)</td>
<td>76.6 (16.8)</td>
<td>0.782</td>
</tr>
<tr>
<td>Delta ppFEV&lt;sub&gt;1&lt;/sub&gt; Baseline to Visit, pre BD</td>
<td>-1.0 (10.5)</td>
<td>-6.5 (10.3)</td>
<td>0.2 (10.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delta ppFEV&lt;sub&gt;1&lt;/sub&gt; Baseline to Visit, post BD</td>
<td>4.1 (10.8)</td>
<td>6.2 (10.7)</td>
<td>3.6 (10.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delta ppFEV&lt;sub&gt;1&lt;/sub&gt; Baseline to Follow up</td>
<td>0.0 (10.9)</td>
<td>0.4 (10.8)</td>
<td>-0.1 (10.9)</td>
<td>0.724</td>
</tr>
<tr>
<td>Months between Baseline and Visit</td>
<td>4.7 (3.6)</td>
<td>4.6 (3.3)</td>
<td>4.8 (3.7)</td>
<td>0.717</td>
</tr>
<tr>
<td>Months between Visit and Follow up</td>
<td>4.9 (4.8)</td>
<td>4.3 (4.0)</td>
<td>5.0 (5.0)</td>
<td>0.232</td>
</tr>
<tr>
<td>Antimicrobial treatment initiated after Visit</td>
<td>114/474 (24.1%)</td>
<td>40/86 (46.5%)</td>
<td>74/388 (19.1%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Comparison between children with positive or no bronchodilator response (BDR). Comparison of categorical variables were calculated using chi square test. All continuous variables were tested for normal distribution (Shapiro-Wilk test), and differences between groups analyzed using two-sample unpaired t-test or Mann-Whitney-U test, as appropriate. Boldface values indicate significant differences.
Table 3 – Annual decline in FEV$_1$ according to BDR positivity, antimicrobial treatment initiated after visit and number of positive BD.

<table>
<thead>
<tr>
<th></th>
<th>Annual ppFEV$_1$ decline (95%CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No BD responsiveness</td>
<td>-0.5 (-0.3, -0.6)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>No antimicrobial treatment</td>
<td>-0.3 (-0.5, -0.1)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td>-0.7 (-1.0, -0.3)</td>
<td>p=0.001</td>
</tr>
<tr>
<td>BD responsiveness</td>
<td>-0.9 (-1.2, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>No antimicrobial treatment</td>
<td>-1.1 (-1.6, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td>-0.6 (-1.1, -0.2)</td>
<td>p=0.009</td>
</tr>
<tr>
<td>One positive BD responsiveness testing</td>
<td>-0.6 (-0.4, -0.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>≥ 2 positive BD responsiveness testing</td>
<td>-1.3 (-1.0, -1.7)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

To calculate annual PFT decline (ppFEV$_1$, per-BD), linear regression models were used and tested for significance by ANOVA. The models were adjusted for the number of visits per participant. Boldface values indicate significant differences.
Figure 1 - The relative change in ppFEV1 post bronchodilator inhalation presented as frequencies of tests (n=474). The dashed line indicates a 12% change in ppFEV1.
Figure 2 – Absolute change from Baseline to Visit (pre-BD) and Follow-up in ppFEV1. Data are representing means and bars 95% confidence intervals. The dashed line indicates baseline.