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Original Article

The predictive potential of the sweat chloride test in cystic fibrosis patients with the *G551D* mutation

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Abstract

Background: Ivacaftor, a cystic fibrosis transmembrane regulator (CFTR) potentiator, decreased sweat chloride concentrations and improved clinical measures in cystic fibrosis (CF) patients with the *G551D* mutation.
Results: Sweat chloride measurements at day 15 had an overall positive predictive value (PPV) of 86.3%, a negative predictive value (NPV) of 65.5%, sensitivity of 73.9%, and specificity of 80.9% for an FEV_1 improvement of ≥5% from baseline at week 16. For ivacaftor patients the median FEV_1 improvement was 16.7%; for placebo patients 0.4%. For patients aged 6–11 years who received ivacaftor and who had a sweat chloride decrease of ≥40 mmol/L from baseline at day 15, a median weight gain of 11.2% at week 16, compared to 6% for those with a smaller decrease was observed.
Conclusions: Changes in sweat chloride concentration at day 15 following treatment with ivacaftor may have sufficient predictive potential to identify individuals that show improvement in pulmonary function and weight gain after 16 weeks of treatment.
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Keywords: PPV; Clinical outcomes; Ivacaftor; FEV_1; Weight

1. Introduction

Cystic fibrosis (CF), an autosomal recessive hereditary disorder, results from reduced chloride transport across the epithelia due to mutations in the gene encoding the CF transmembrane regulator (CFTR) protein, a chloride ion channel [1,2]. The loss of chloride transport results in a range of clinical manifestations, including a high sweat chloride concentration, pancreatic and intestinal disorders, and progressive lung disease, leading to a shortened life expectancy [3]. CF lung disease is characterized by poor mucociliary clearance, resulting in chronic infection and inflammation, airway plugging and bronchiectasis [4,5].

Approximately 5% of the CF population carries a copy of the *G551D CFTR* mutation [6] and most of these patients carry the *F508del CFTR* mutation on the other allele [7,8]. The *G551D CFTR* mutation is a gating defect that causes loss of chloride transport across epithelial membranes by preventing the CFTR channel from opening [7,9–11]. The loss of chloride transport in patients with *G551D* mutations is reflected by a high sweat chloride concentration (mean ~108 mmol/L [12]), a high incidence (>90%) of pancreatic insufficiency [13,14], early clinical manifestations, and a rapid decline in lung function as measured by forced expiratory volume in one second (FEV_1) [13].

CFTR potentiators enhance chloride transport by acting directly on CFTR to increase the gating activity of the channel [8]. In clinical studies in patients carrying the *G551D* mutation [6,15–18] administration of ivacaftor (VX-770, KALYDECO™; Vertex Pharmaceuticals Incorporated, Cambridge, MA, USA) [19], the only currently-approved CFTR potentiator, resulted in a

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substantial mean decrease in sweat chloride concentration. Ivacaftor also improved lung function as measured by FEV_1 and nutritional status as measured by weight gain [6,15–18].

The current post-hoc analysis was designed to determine whether a short-term (15 day) change in sweat chloride concentration could be used to predict a long-term (16 week) improvement in FEV_1 or weight gain.

2. Methods

2.1. Source of data

Data were obtained from two double blind, multicenter studies in CF patients with *G551D* mutations described previously [6,15–18]. Study 1 involved a cohort of 161 CF patients ≥12 years, and study 2 involved 52 patients, of age 6–11 years. Across the two studies, 109 CF patients received ivacaftor 150 mg every 12 h and 104 received placebo.

Sweat chloride concentration was measured throughout each study. This post-hoc analysis focused on the values obtained at day 1 (baseline) and on day 15, the first post-treatment sweat chloride measurement.

FEV_1 and weight were measured throughout each study. This post-hoc analysis focused on the data at screening, day −14, day 1 and at week 16 values. In the study in children 6–11 years of age, data were not collected at day −14.

2.2. Study population

All CF patients included in the current analyses carried the *G551D* mutation. The analysis for FEV_1 compared the two groups that received ivacaftor or placebo. The analysis of weight gain considered age groups: age 6–11 years or age 12 years and older at enrollment.

2.3. Statistical analysis

Baseline values were calculated as the values determined for sweat chloride, FEV_1, and weight, as mean of all available individual measurements before the first administration of ivacaftor or placebo.

The raw change of sweat chloride concentration was determined by calculating: (day 15 measurement − baseline).

The value of FEV_1 and weight percent change at week 16 was obtained as: ((week 16 measurement − baseline) / baseline) · 100.

Patients were excluded from the analysis, if the following were not available: values for the sweat chloride concentration or sweat chloride raw change in concentration at day 15, or values of percent change in FEV_1, or in weight at week 16.

The mean, median, and the standard deviation were calculated for sweat chloride, FEV_1, and weight.

Statistical comparisons between those receiving ivacaftor or placebo were performed using two-sided t-tests with $\alpha = 0.05$.

In analysis 1 an FEV_1 improvement of ≥5% from baseline at week 16 is clinically regarded as being indicative of providing clinical benefit in lung function. In analysis 2, a weight gain of ≥10% from baseline at week 16 is clinically regarded as being indicative of providing clinical benefit in weight gain.

The positive predictive value (PPV) for both an improvement in FEV_1 of ≥5% and a weight gain of ≥10% at week 16 was calculated for the binned intervals (10 mmol/L) of sweat chloride concentration at day 15 (first interval 130–121, last interval 30–21) and the binned intervals (10 mmol/L) of sweat chloride raw change in concentration at day 15 (first interval 0–9, last interval 50–59) with sweat chloride concentration as predictor.

The results of the 'binned interval calculation' were used as a threshold determination for the sweat chloride concentration and for the sweat chloride raw change in concentration as a predictor for the further calculation of the statistics, including PPV, negative predictive value (NPV), sensitivity, and specificity.

Pearson's correlation was calculated for sweat chloride, FEV_1, and weight.

Statistical analyses were performed using Base SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Short-term changes in sweat chloride concentration and long-term improvement in FEV_1

The relationship between raw and raw changes in sweat chloride concentration on day 15 and relative changes in FEV_1 at week 16 after the first dose of ivacaftor or placebo were evaluated. A significant ($p < 0.0001$) decrease in both the mean sweat chloride concentration and the mean raw change from baseline in sweat chloride concentration at day 15 was observed for the full cohort of patients across the two studies (Fig. 1A and B). Significant increases in mean FEV_1 ($p = 0.0006$) and in the mean percent change from baseline ($p < 0.0001$) in FEV_1 were observed at week 16 (Fig. 1C and D).

No statistical correlation was observed between change from baseline in FEV_1 at week 16 and sweat chloride concentration (ivacaftor: correlation: −0.05, $p = 0.63$; placebo: correlation: −0.006, $p = 0.96$; Fig. 2A), or reduction in sweat chloride (ivacaftor: correlation: −0.06, $p = 0.56$; placebo: correlation: −0.04, $p = 0.74$; Fig. 2B) at day 15. However, scatter plots show two distinct groupings of patients.

3.2. Likelihood of a meaningful improvement in FEV_1 at week 16 based on sweat chloride concentration measures at day 15

Since no statistically significant correlation was found between FEV_1 and sweat chloride measures, long-term prediction of an improvement in $FEV_1 \geq 5\%$ from baseline based on short-term change in sweat chloride was investigated using an algorithm (Fig. 3). An improvement of ≥5% in FEV_1 from baseline at week 16 is clinically regarded as being indicative of providing clinical benefit in lung function in CF patients. The binned intervals of 10 mmol/L in sweat chloride concentration and 10 mmol/L raw change in sweat chloride concentration from baseline were used to calculate PPVs for an improvement

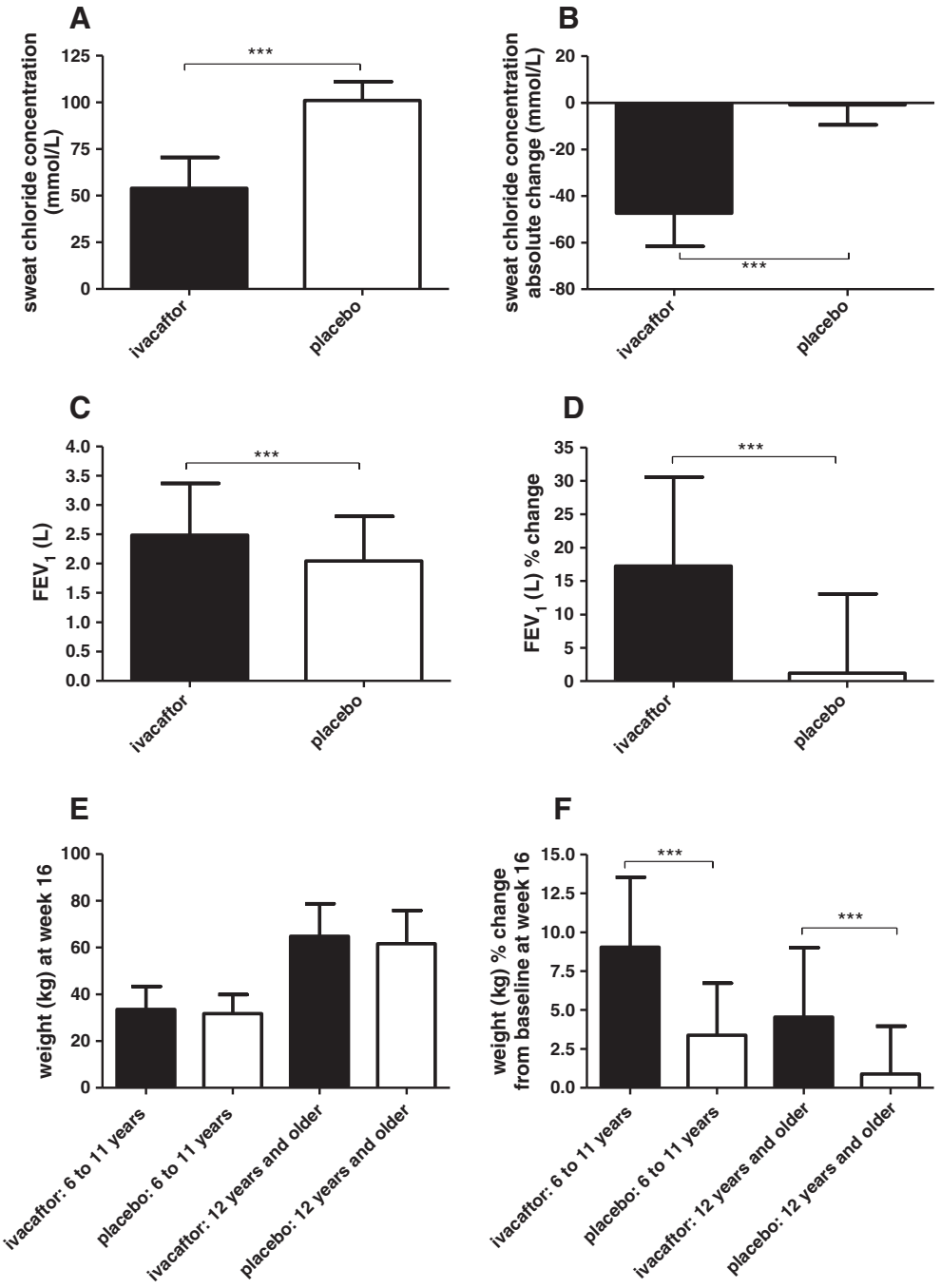

Fig. 1. Values and changes in sweat chloride concentrations (day 15), FEV_1 (week 16), and weight (week 16) for ivacaftor and placebo treated patients. A: mean sweat chloride concentration (mmol/L) at day 15 with a $p < 0.0001$ for ivacaftor treated patients (n = 93; mean at baseline: 101.3 mmol/L) versus placebo treated patients (n = 86; mean at baseline: 102.0 mmol/L). B: mean sweat chloride concentration (mmol/L) raw change from baseline at day 15 with a $p < 0.0001$ for ivacaftor treated patients (n = 93; mean at baseline: 101.3 mmol/L) versus placebo treated patients (n = 86; mean at baseline: 102.0 mmol/L). C: mean FEV_1 (L) level at week 16 with a $p = 0.0006$ for ivacaftor treated patients (n = 93; mean at baseline: 2.1 L) versus placebo treated patients (n = 86; mean at baseline: 2.0 L). D: mean FEV_1 (L) percent change at week 16 with a $p < 0.0001$ for ivacaftor treated patients (n = 93) versus placebo treated patients (n = 86). E: mean weight level (kg) at week 16 for patients who are 6 to 11 years old (n = 41) and patients who are 12 years and older (n = 138), separated into two subgroups. Subgroup 1: patients who are 6 to 11 years old who receive ivacaftor (n = 21; mean at baseline: 30.7 kg) and patients who receive placebo (n = 20; mean at baseline: 30.7 kg) and subgroup 2: patients who are 12 years and older who receive ivacaftor (n = 72; mean at baseline: 62.1 kg) and patients who receive placebo (n = 66; mean at baseline: 61.2 kg). F: mean percent weight gain from baseline of the subgroups of patients with age 6 to 11 years and patients age 12 years and older at week 16 with a $p < 0.0001$ for ivacaftor treated patients versus placebo treated patients who are 6 to 11 years old, and a $p < 0.0001$ for ivacaftor treated patients versus placebo treated patients of age 12 years and older. The I bars show the standard deviation in A–F.

in $FEV_1 \geq 5\%$ at week 16. The threshold for the sweat chloride concentration was 80 mmol/L, and the threshold for the raw change in sweat chloride concentration was 20 mmol/L. These thresholds were used to calculate PPV (86.3%), sensitivity (73.9%), NPV (65.5%), and specificity (80.9%) for an improvement in FEV_1 of $\geq 5\%$ from baseline at week 16

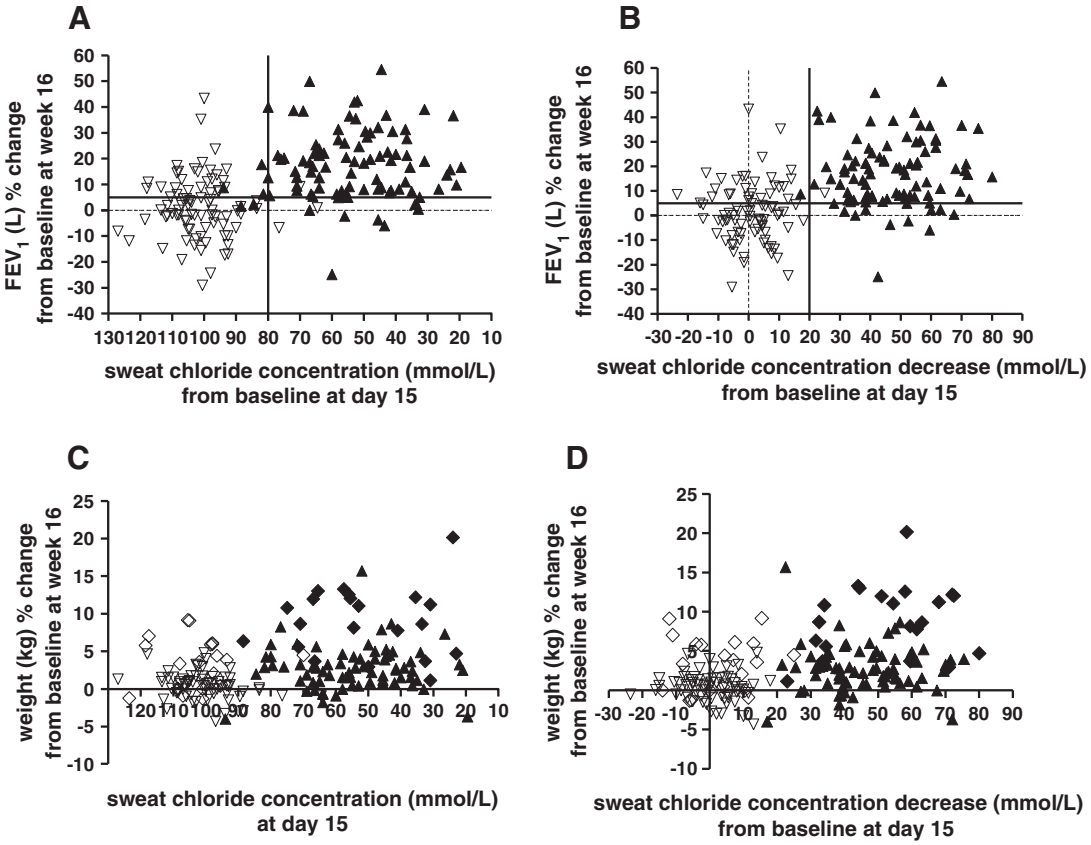

Fig. 2. Relationship between short-term (15-day) change in sweat chloride concentration and long-term (16-week) FEV_1 percent changes and weight percent changes for the individual patients. A shows a scatter plot of the individual patients who received ivacaftor (closed triangle) (n = 93) and who received placebo (open triangle) (n = 86). The x-axis shows the sweat chloride concentration (mmol/L) at day 15 and the y-axis shows the FEV_1 percent change from baseline at week 16. B shows a scatter plot of the individual patients who received ivacaftor (closed triangle) (n = 93) and who received placebo (open triangle) (n = 86). The x-axis shows the absolute change in sweat chloride concentration from baseline at day 15 and the y-axis shows the FEV_1 percent change from baseline at week 16. C: scatter plot of the individual patients who are 6 to 11 years old (n = 41) and who are 12 years and older (n = 138). The patients of age 6 to 11 years and the patients 12 years and older were additionally separated into two subgroups: patients who received ivacaftor and patients who received placebo. The patients who are 6 to 11 years old and received ivacaftor are displayed as closed triangles and those who received placebo are displayed as open triangles. The patients who are 12 years and older and received ivacaftor are displayed as closed diamonds and those who received placebo as open diamonds. The x-axis shows the sweat chloride concentration (mmol/L) at day 15 and the y-axis shows the weight percent change from baseline at week 16. D: scatter plot of the individual patients who are 6 to 11 years old and those who are 12 years and older. The x-axis shows the absolute change in sweat chloride concentration from baseline at day 15 and the y-axis shows the weight percent change from baseline at week 16.

(Table 1). PPV and sensitivity improved for the ivacaftor group, to 88.0% and 98.8%, respectively, when a combination of both thresholds was taken into account in the calculations (Table 1). The PPV was slightly higher for children (6–11 years) (90.9%; 90.5% only ivacaftor) in comparison with adults (≥ 12 years) (84.9%; 87.3% only ivacaftor). The NPV and specificity for the placebo group, calculated separately, were 66.3% and 96.5%, respectively (Table 1). Among those who reached either of the sweat chloride concentration threshold noted above, the median improvement in FEV_1 was 16.7% (n = 95; mean = 16.8%; std = 13.5) versus 0.4% (n = 85; mean = 1.3%; std = 11.9) for those who reached neither threshold.

3.3. Short-term changes in sweat chloride concentration and long-term increase in weight

To investigate the relationship between raw values and raw changes in sweat chloride concentration at day 15 and relative changes in weight at week 16 after the first dose of ivacaftor or placebo, groups were considered by age at initial enrollment: 6–11 years of age or ≥ 12 years. No significant difference was seen between ivacaftor and placebo groups for mean weight at week 16 in either age group (Fig. 1E). However, the mean percent weight gain at week 16 was significantly higher ($p < 0.0001$) among ivacaftor patients compared with placebo patients in both age cohorts (Fig. 1F).

No statistically significant Pearson's correlation was found for weight gain at week 16 and sweat chloride concentration at day 15 in the 6–11 year old patients (ivacaftor: correlation: −0.027, p = 0.91; placebo: correlation: −0.10, p = 0.66) or the group of CF patients ≥ 12 years (ivacaftor: correlation: −0.09, p = 0.45; placebo: correlation: 0.27, p = 0.03). Similarly, no statistically significant Pearson's correlation was found for patients 6–11 years of age for weight gain at 16 weeks and the change from baseline in sweat chloride concentration at day 15 (ivacaftor: correlation: −0.21, p = 0.36; placebo: −0.07, p = 0.76) or for those ≥ 12 years (ivacaftor: correlation: –0.05,

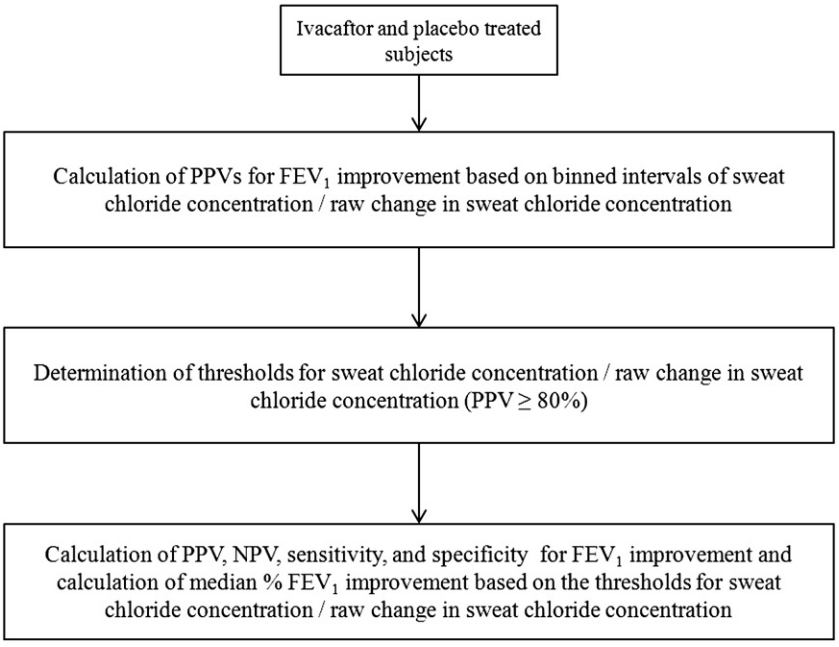

Fig. 3. Algorithm for calculation of prediction of FEV_1 improvement ≥5% from baseline at week 16 based on sweat chloride concentration at day 15 and sweat chloride concentration absolute change from baseline at day 15.

p = 0.70; placebo: correlation: 0.06, p = 0.62). However, scatter plots show two distinct clusters for placebo and ivacaftor groups for both comparisons (Fig. 2C and D).

3.4. Likelihood of a clinically meaningful weight gain at week 16 based on sweat chloride concentration measures at day 15

As no statistically significant correlation between percent weight change from baseline and sweat chloride concentration measures was found, an algorithm (Fig. 4) was used to calculate the likelihood of weight gain at week 16 based on sweat chloride concentration and on raw change in sweat chloride concentration from baseline at day 15. Calculation of PPVs for a weight gain ≥10% from baseline at week 16 was based on the binned intervals of 10 mmol/L sweat chloride concentration or 10 mmol/L raw change from baseline in sweat chloride concentration and was performed separately for the cohorts 6–11 years of age and ≥12 years at enrollment. A threshold of 60 mmol/L in sweat chloride concentration, and a threshold of 40 mmol/L decrease in sweat chloride concentration at day 15 were found for patients 6–11 years. No threshold was found for patients ≥12 years. For patients 6–11 years the median percent weight gain was calculated for those patients who had a sweat chloride decrease of 40 mmol/L or greater and for those patients who had a sweat chloride decrease <40 mmol/L. All patients who had a decrease of ≥40 mmol/L had received ivacaftor. Their median percent weight gain from baseline was 11.2% (3.1 kg) at week 16. The patients who had a decrease

Table 1
Positive and negative predictive value, sensitivity, and specificity for FEV_1 ≥ 5% in 179 patients who carry the *G551D* mutation on at least one allele.

Sweat chloride threshold	True positive (n)	True positive (%)	Positive predictive value	Sensitivity	True negative (n)	True negative (%)	Negative predictive value	Specificity
Pooled: ivacaftor and placebo, 179 patients								
Decrease ≥ 20 mmol/L	82	45.8	88.2	73.9	57	31.8	66.3	83.8
Concentration ≤ 80 mmol/L	80	44.7	87.9	72.1	57	31.8	64.8	83.8
Decrease ≥ 20 mmol/L or concentration ≤ 80 mmol/L	82	45.8	86.3	73.9	55	30.7	65.5	80.9
Ivacaftor, 93 patients								
Decrease ≥ 20 mmol/L	81	87.1	88.0	98.8	0	0.0	0.0	0.0
Concentration ≤ 80 mmol/L	79	85.0	89.8	96.3	2	2.2	40.0	18.2
Decrease ≥ 20 mmol/L or concentration ≤ 80 mmol/L	81	87.1	88.0	98.8	0	0.0	0.0	0.0
Placebo, 86 patients								
Decrease ≥ 20 mmol/L	1	1.2	100.0	3.5	57	66.3	67.1	100.0
Concentration ≤ 80 mmol/L	1	1.2	33.3	3.5	55	64.0	66.3	96.5
Decrease ≥ 20 mmol/L or concentration ≤ 80 mmol/L	1	1.2	33.3	3.5	55	64.0	66.3	96.5

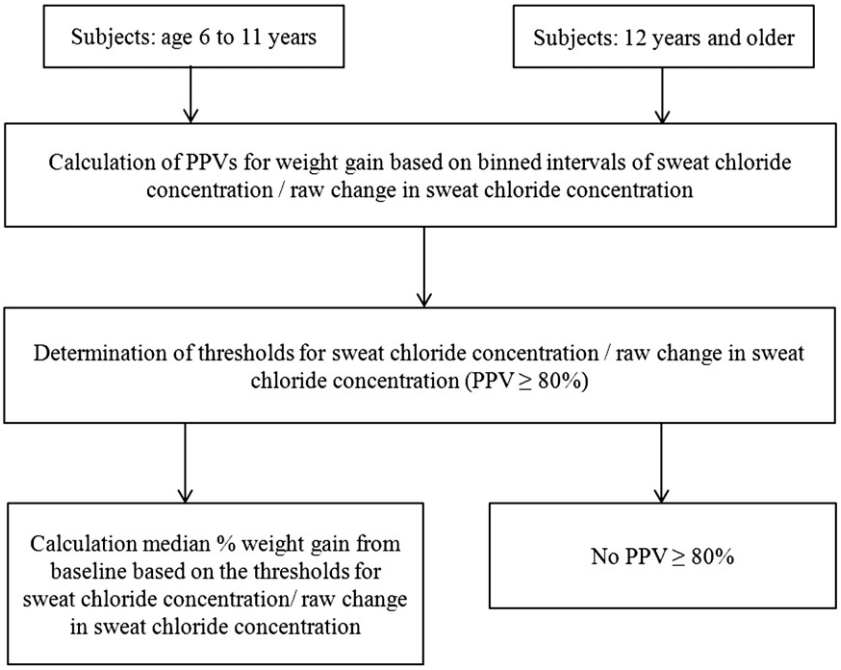

Fig. 4. Algorithm for calculation of prediction of median % weight gain from baseline at week 16. A weight gain of ≥10% from baseline at week 16 and sweat chloride concentration at day 15 and absolute change in concentration from baseline at day 15 were used for the calculation of the positive predicted values (PPVs).

<40 mmol/L were separated into those patients, who had received ivacaftor and those who had received placebo during the course of the original study. The median percent weight gain for the patients of age 6–11 years who received ivacaftor was 6.0% (1.5 kg) and for the patients who received placebo 3.7% (1.2 kg).

Weight gain was also examined for patients using the threshold of a sweat chloride concentration of 60 mmol/L. All patients who had a sweat chloride concentration of ≤60 mmol/L had received ivacaftor. Their median percent weight gain from baseline was 9.8% (2.8 kg). The patients who had a sweat chloride concentration >60 mmol/L at day 15 were separated into two groups, patients who received either ivacaftor or placebo during the course of the original study. The median percent weight gain for the patients of age 6 to 11 years who received ivacaftor was 8.7% (2.0 kg) and for the patients who received placebo 3.7% (1.2 kg).

4. Discussion

Cystic fibrosis is a 'whole body' disease with many clinical manifestations [20]. Markers are derived from several of these disease manifestations to assess and predict the clinical course. Each marker is individually valuable; however, attempts to correlate the results obtained from different markers have been only marginally successful, especially for those with differing physiological bases [21]. In a recent publication [22] Durmowicz et al. claim that there is no correlation for ivacaftor treated patients between decrease in sweat chloride concentration and improvement of FEV_1, as has also been observed by the authors. Dumrowicz et al. conclude that this devaluates sweat chloride measurements as a marker for clinical endpoint determination. This post-hoc analysis found that short-term changes in sweat chloride concentration show a high likelihood for longer-term improvement in pulmonary function and weight gain.

In the post-hoc analysis no formal statistical correlation between sweat chloride concentration measurements and FEV_1 outcomes was observed. The progress of lung disease depends on several factors. The primary factor, airway surface dehydration, coexists with viral and bacterial infections leading to mucus plugging, inflammation, and structural damage in the lung [4,5]. FEV_1 reflects pulmonary function, affected by all adverse factors present. In contrast, defective CFTR function appears to be the only defect in the sweat gland, and no known structural damage to the gland that impedes its function in CF patients.

The malfunction of the pancreas and intestinal systems in patients is directly related to their inability to produce adequate or functional CFTR. The low levels of CFTR function in CF patients with the *G551D* mutation leads to pancreatic insufficiency in the majority of these patients [13]. The intestines of these CF patients are often affected by thick mucus and/or motility problems resulting from inadequate epithelial chloride transport [14,23]. Here again, many different factors influence inability to digest food and absorb nutrients. As with FEV_1, another clinical measure affected by multiple physiological factors, weight gain does not correlate with sweat chloride concentrations.

Although Pearson's correlations were not successful with sweat chloride and these outcomes, it is still possible that sweat chloride could be predictive of clinical improvements following pharmaceutical interventions. Based on the characterization of CF as a 'whole body' disease we hypothesized, that any improvement in the function of CFTR channels that could be measured by a decrease in sweat chloride concentration would also be reflected by a status improvement of different organ defects that are measured by FEV_1 and weight gain.

We analyzed the effect of ivacaftor, a new CFTR potentiator drug for CF patients carrying the *G551D* mutation, because it is the only therapeutic agent that has been shown to have clinically meaningful effects on lung function and weight gain. In clinical studies, ivacaftor treatment decreased sweat chloride concentration, and was associated with improvements in lung function, increased body weight and improvements in other clinical symptoms of CF [6,15–18]. We demonstrated that decreases in sweat chloride concentrations, improvements in FEV_1 and weight gain, are functionally linked during ivacaftor therapy.

Individual patient responses to therapy will differ even within a population sharing the same genotype likely as a result of various factors, such as age, progress of irreversible airway destruction, infection status, and the influence of the genetic or metabolic status. We examined individual responses in scatter plots of individual correlations which showed a distinct separation into two clusters, those who received ivacaftor and those who received placebo. Individuals who received ivacaftor and responded to this treatment by a rapid decrease of sweat chloride concentration within two weeks, had a long-term improvement of their lung status as measured by FEV_1 at 16 weeks.

This separation of clusters allowed the identification of thresholds, of 80 mmol/L sweat chloride concentration or 20 mmol/L sweat chloride concentration decrease from baseline at day 15. Notably, a sweat chloride concentration of 80 mmol/L is approximately 20 mmol/L lower than the mean sweat chloride concentration at baseline in the populations studied. These thresholds could be used for calculating the improvement in FEV_1 to be expected at 16 weeks. The relevance of this prediction is indicated by the high PPV (86.3%) and sensitivity of 73.9%, and specificity of 65.5% and NPV of 80.9% for the total cohort of CF patients studied.

We also assessed whether an improvement in weight could also be predicted for CF patients who received ivacaftor and had a decrease in sweat chloride concentration within 15 days. Sweat chloride had predictive value only in children age 6–11 years at enrollment, who could be assumed to be growing. All children age 6–11 years who received ivacaftor had a significant increase in weight relative to those who received placebo. For children who received ivacaftor, a sweat chloride decrease of ≥ 40 mmol/L at day 15 predicted a much more substantial increase in weight relative to those who had a smaller decrease. Additionally, for the children 6–11 years a threshold of 60 mmol/L of sweat chloride concentration was found. However, there was no substantial difference in weight gain between children who were above or below this threshold and who received ivacaftor.

The present post-hoc analysis demonstrates a predictive value for trends as opposed to the raw value at baseline of a marker representing the status of a disease. The simple fact that a certain short-term decrease of the patient's sweat chloride concentration was achieved at 15 days of CFTR potentiator treatment with ivacaftor, predisposed patients to long-term improvements in pulmonary function as quantified by FEV_1, independent of medical history and health status when treatment was initiated. A similar predictive value was identified for weight gain in children 6–11 years suffering from CF, although no such relationship was apparent in CF patients ≥ 12 years.

These findings are of interest not only as diagnostic tools or predictors of disease progression, but also in drug development. The models we present open the possibility to design response-based clinical studies using sweat chloride concentration as a recruitment biomarker. Relevance to clinical decision making is limited due to the relatively poor negative predictive value. Therefore, while a fall in sweat chloride ≥ 20 mmol/L has good positive predictive value, some ivacaftor responsive patients may not demonstrate this sweat chloride decline. Therefore, sweat chloride cannot be recommended as a tool to identify ivacaftor non-responsive patients.

Role of authors

Verena I. Seliger, Dr. David Rodman, Dr. Fredrick Van Goor and Professor Dr. Peter Mueller were involved with the conception of the post-hoc analysis, data analysis and interpretation of the data. Verena I. Seliger wrote the draft of the manuscript. All authors were involved with the critical revision of the manuscript and approved the final version of the manuscript. Verena I. Seliger would like to thank Professor Dr. Peter Mueller for supervision of the post-hoc analysis.

Conflict of interest statement

All authors, except Associate Professor Dr. A. Schmelz, are employees of Vertex Pharmaceuticals Incorporated and own stock in Vertex Pharmaceuticals Incorporated.

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