Journal of Cystic Fibrosis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Human epididymis protein 4 (HE4) plasma concentration inversely correlates with the improvement of cystic fibrosis lung disease in p.Phe508del-CFTR homozygous cases treated with the CFTR modulator *lumacaftor/ivacaftor* combination

Marianna Pócsi[a,b], Zsolt Fejes[a], Zsolt Bene[c], Attila Nagy[d], István Balogh[a,e], Margarida D. Amaral[f], Milan Macek Jr.[g], Béla Nagy Jr.[a,*]

[a] *Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[b] *Laki Kálmán Doctoral School, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[c] *Department of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[d] *Department of Preventive Medicine, Faculty of Public Health, University of Debrecen, Debrecen, Hungary*
[e] *Division of Clinical Genetics, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[f] *BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal*
[g] *Department of Biology and Medical Genetics, 2nd Faculty of Medicine of Charles University and Motol University Hospital, Prague, Czech Republic*

ARTICLE INFO

Article history:
Received 28 November 2022
Revised 30 March 2023
Accepted 6 April 2023
Available online xxx

Keywords:
Biomarker
Cystic fibrosis
HE4
Lumacaftor/ivacaftor
ppFEV1
Sweat chloride

ABSTRACT

Background: We previously documented that elevated HE4 plasma concentration decreased in people with CF (pwCF) bearing the p.Gly551Asp-CFTR variant in response to CFTR modulator (CFTRm) *ivacaftor* (IVA), and this level was inversely correlated with the FEV1% predicted values (ppFEV1). Although the effectiveness of *lumacaftor* (LUM)/IVA in pwCF homozygous for the p.Phe508del-CFTR variant has been evaluated, plasma biomarkers were not used to monitor treatment efficacy thus far.
Methods: Plasma HE4 concentration was examined in 68 pwCF drawn from the PROSPECT study who were homozygous for the p.Phe508del-CFTR variant before treatment and at 1, 3, 6 and 12 months after administration of LUM/IVA therapy. Plasma HE4 was correlated with ppFEV1 using their absolute and delta values. The discriminatory power of delta HE4 was evaluated for the detection of lung function improvements based on ROC-AUC analysis and multiple regression test.
Results: HE4 plasma concentration was significantly reduced below baseline following LUM/IVA administration during the entire study period. The mean change of ppFEV1 was 2.6% (95% CI, 0.6 to 4.5) by 6 months of therapy in this sub-cohort. A significant inverse correlation between delta values of HE4 and ppFEV1 was observed especially in children with CF (r=-0.7053; p<0.0001). Delta HE4 predicted a 2.6% mean change in ppFEV1 (AUC: 0.7898 [95% CI 0.6823–0.8972]; $P < 0.0001$) at a cut-off value of -10.7 pmol/L. Moreover, delta HE4 independently represented the likelihood of being a responder with ≥ 5% delta ppFEV1 at 6 months (OR: 0.89, 95% CI: 0.82–0.95; $P = 0.001$).
Conclusions: Plasma HE4 level negatively correlates with lung function improvement assessed by ppFEV1 in pwCF undergoing LUM/IVA CFTRm treatment.

1. Introduction

Cystic fibrosis (CF) is an autosomal rare monogenic disease caused by pathogenic variants (legacy term mutations) in the *CFTR* gene encoding the CFTR (CF transmembrane conductance regulator) protein. CFTR primarily functions as an anion channel at the apical surface of epithelial cells that transports chloride and bicarbonate and regulates ion and fluid transport in an organ-specific manner [1]. More than 2100 variants have been identified in *CFTR* with the major p.Phe508del (legacy nomenclature F508del) *CFTR* allele accounting for approximately 80% of all CF-causing mutations worldwide [2]. CFTR dysfunction results in a multisystem disease, which is characterized by airway clogging by thick mucus

* Corresponding author.
E-mail address: nagyb80@gmail.com (B. Nagy Jr.).

https://doi.org/10.1016/j.jcf.2023.04.001

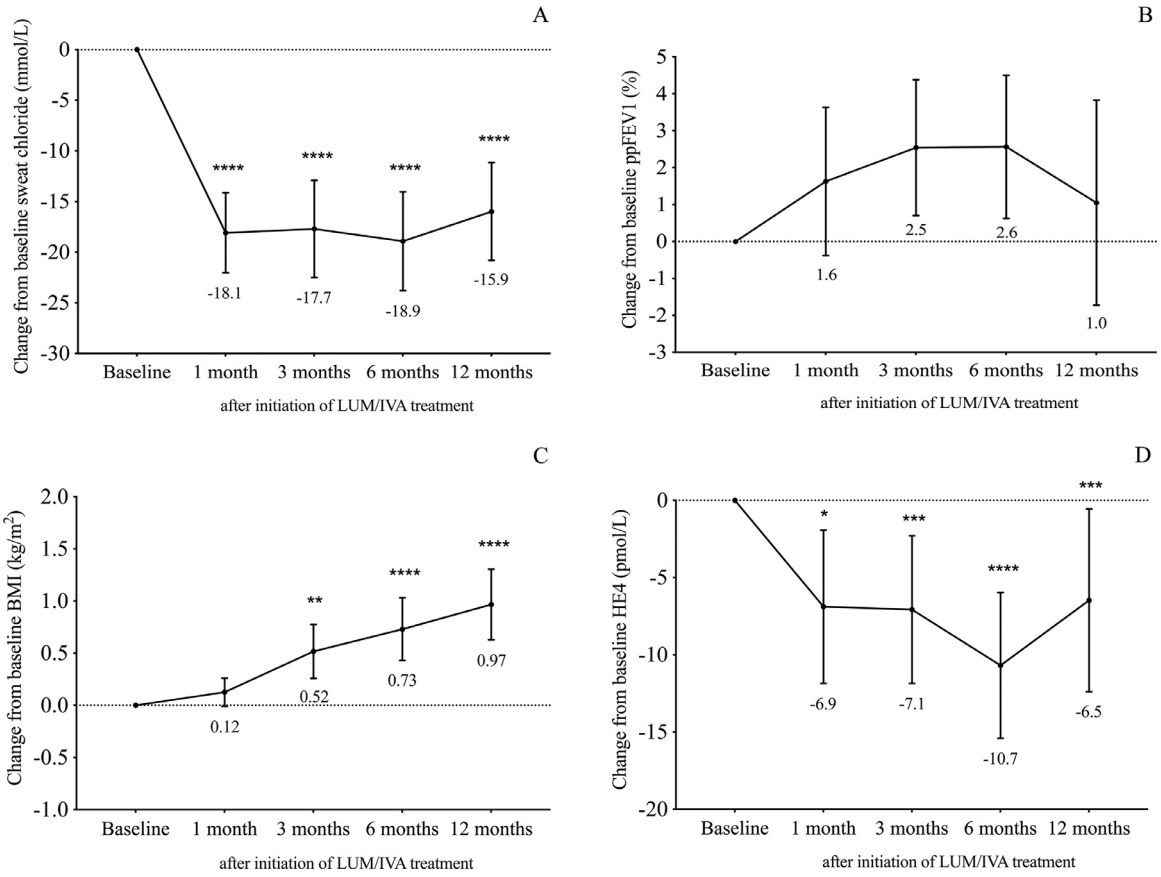

Fig. 1. The mean change (with 95% CI) of sweat chloride concentrations (A), ppFEV1 (B), BMI (C) and plasma HE4 concentrations measured in CF patients ($n = 68$) before LUM/IVA therapy and after 1, 3, 6 and 12 months. Statistical analyses were performed using the Friedman test with Dunn's *post hoc* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ compared to baseline.

secretion associated with progressive sino-bronchial disease, and chronic lung colonization with pathognomonic bacteria, such as *P. aeruginosa* [1].

In the last decade, variant-specific treatments of the basic CFTR defect termed CFTR modulation (henceforward CFTRm), are utilizing small molecules that foster the restoration of CFTR channel function by potentiation and/or correction of its function [3]. The potentiator *ivacaftor* (IVA) rescues the gating function of CFTR at the cell surface [4], and it was approved for CF patients with at least one Class III mutation to improve CFTR-mediated chloride transport by increasing the open probability of apically located channels [5]. The corrector *lumacaftor* (LUM) enhances trafficking of the "mutated" p.Phe508del-CFTR protein to the apical cell surface [6], and is used to treat CF subjects who are homozygous for the p.Phe508del-CFTR mutation [7].

The first large-scale clinical trials revealed that combined treatment with LUM and IVA improves lung function, patients' nutritional status, and thus the quality of their life. This combination also reduces the rate of pulmonary exacerbations in individuals homozygous for the p.Phe508del-CFTR allele [8,9]. The clinical effectiveness and safety of LUM/IVA have been evaluated in subsequent clinical trials [10–13]. Bioactivity of LUM/IVA was established by the substantial decrease of sweat chloride concentrations that were evident already at 1-month post-administration and remained as such until 24 weeks of the trial [10,11,13]. In contrast, non-significant changes [11,13] or only modest improvement in FEV1% predicted values (ppFEV1) [8-10,13] were observed in subjects undergoing LUM/IVA therapy. This discrepancy does not mean that LUM/IVA does not sufficiently improve lung function, but it may be caused by the heterogeneity of these studies which included people with CF (pwCF) with a wide range of lung function values and different study designs [13,14].

Recently, we commenced systematic analysis of human epididymis protein 4 (HE4) plasma concentrations since HE4 appears to be a novel blood-based biomarker in pwCF [15,16]. In addition, we investigated its altered expression in CF in vitro [17]. This protein is encoded by the *WFDC2* (MIM: 617,548) gene [18], and has been utilised in tumor profiling in epithelial ovarian cancers [19] and lung tumours [20]. It is a member of the whey acidic protein (WAP) family that is homologous to other serine proteinase inhibitors, comprising elafin and secretory leukocyte protease inhibitors (SLPIs) [21]. Similarly to these family members, HE4 also displays a variety of functions in the lung. For instance, it acts as an anti-proteinase in the frame of epithelial host defences of the respiratory tract [22]. Before our investigations, a possible association between HE4 and CF was described by immunohistochemistry techniques aimed at the assessment of enhanced *WFDC2* gene expression (hence HE4) in CF lung biopsy samples containing mainly tracheobronchial epithelial cells [23]. We then further demonstrated that elevated HE4 concentrations were positively associated with the degree of pulmonary dysfunction and with the overall CF disease severity in ethnically unrelated pwCF cohorts [15]. On the other hand, plasma HE4 concentrations inversely correlated with lung function improvement in pwCF bearing at least one p.Gly551Asp (G551D) CFTR variant on IVA medication [16]. Furthermore, in response to *in vitro* rescue of CFTR function by LUM/IVA, HE4 expression was lowered in CFBE 41o− cells expressing p.Phe508del-CFTR [17].

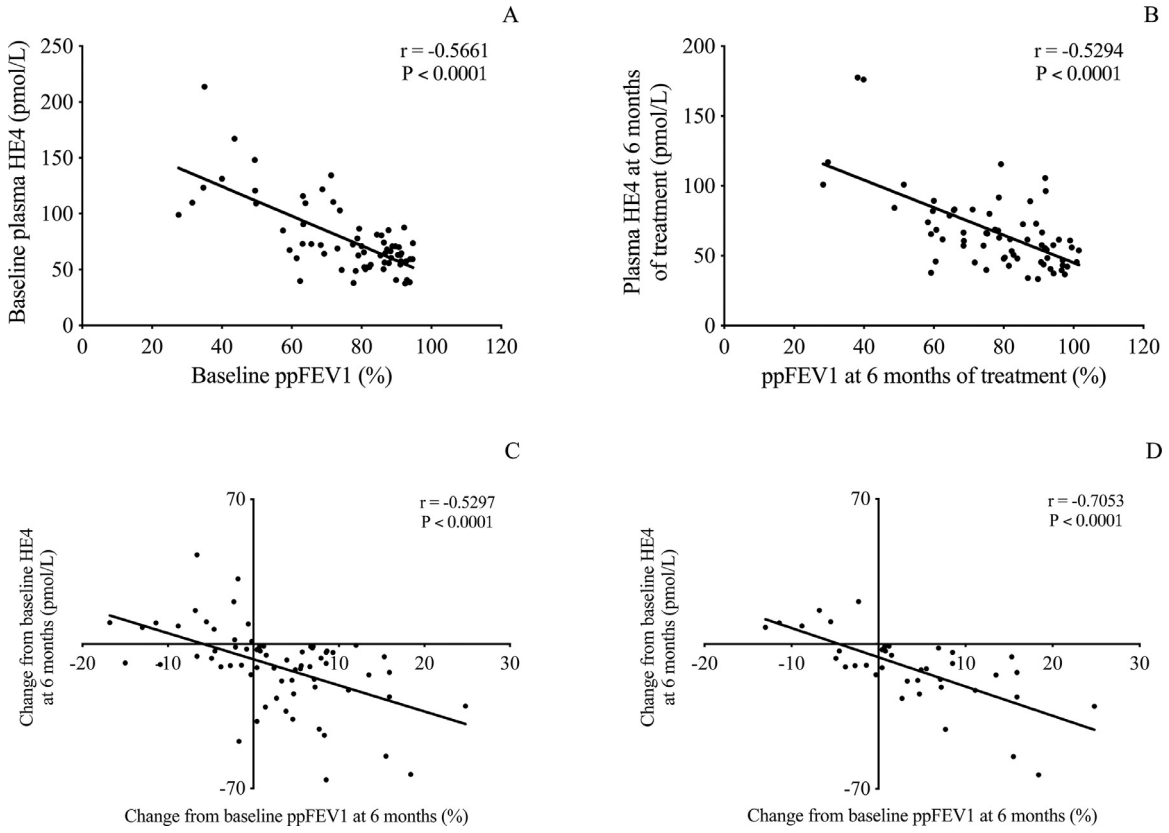

Fig. 2. Analysis of correlation between HE4 concentrations and ppFEV1 values determined at baseline (A) and 6 months after the initiation of LUM/IVA treatment (B). The relationship between the mean change of HE4 and delta ppFEV1 in all study participants (C). We separately analyzed this later association among pwCF under 18 years of age (D). All correlations were studied by the Spearman test.

In this study we evaluated the utility of *in vivo* examination of plasma HE4 to investigate whether this protein may serve as a potential plasma-based biomarker in pwCF undergoing LUM/IVA therapy. For this purpose, a large sub-cohort of pwCF homozygous for the p.Phe508del-CFTR variant, who previously participated in the PROSPECT study [13], was examined for their plasma HE4 concentrations. Hereby, we aimed to confirm our recent findings on the inverse correlation between changes in HE4 concentrations and ppFEV1 values in patients treated with LUM/IVA.

2. Methods

2.1. Cohort of pwCF

Overall, 68 pwCF with two p.Phe508del-CFTR mutations were randomly selected from the pre-existing cohort drawn from the PROSPECT study [13]. These pwCF aged 6 years or older were treated with LUM/IVA (Orkambi®, Vertex Pharmaceuticals, Boston, MA, USA). Inclusion and exclusion criteria were formerly described by the study organizers (data not shown). Five study visits were performed: at baseline (pre-LUM/IVA), and at 1, 3, 6 and 12 months after the initiation of LUM/IVA. K_3-EDTA anticoagulated plasma specimens were requested from the Cystic Fibrosis Foundation Therapeutics (CFFT) Biorepository (Bethesda, MD, USA) to retrospectively measure plasma HE4 concentrations.

2.2. Baseline demographic and clinical parameters of enrolled pwCF

In this cohort, 39 females (57.4%) and 29 males (42.6%) were enrolled, and the median age was 16 years (min, 6 years; max, 29 years). Of 68 pwCF, there were 38 participants under 18 years of age (children and adolescents), while 30 adults were recruited. At baseline, lung function was relatively high, median ppFEV1 = 80.8% (27.6–94.7), and sweat chloride concentrations were over 60 mmol/L in all pwCF, median (min-max), 100.8 mmol/L (64–116). Pre-treatment BMI values (median, 20.1 kg/m^2) were within the range of (min-max), 17.9–21.8 kg/m^2. Finally, *P. aeruginosa* positivity 1 year before and after initiation of LUM/IVA therapy was detected in 32 cases (47%) among these subjects (Suppl. Table 1).

Based on the baseline ppFEV1 categories set by Sagel et al. [13], recruited pwCF were split into three sub-groups: $<$ 50% ppFEV1 (n = 9), 50–89% ppFEV1 (n = 45), and $\geq$ 90% ppFEV1 (n = 14). Furthermore, study participants were sub-grouped as: 1) "responders" if they experienced an increase in ppFEV1 greater than 5% at 6 months (26 of 68 [38.2%]), and 2) "non-responders" if they showed an increase in ppFEV1 of less than 5% (42 of 68 [61.8%]). None of these pwCF had an acute exacerbation of their CF lung disease during the study period.

2.3. Laboratory analysis

Plasma HE4 concentrations were examined in an analyst-blinded manner and were correlated with ppFEV1, sweat chloride concentrations, and BMI. Chemiluminescent microparticle immunoassay (Architect-i1000SR®, Abbott Diagnostics, Wiesbaden, Germany) was used to analyze HE4 plasma concentrations as was formerly done in other CF study cohorts [15–17].

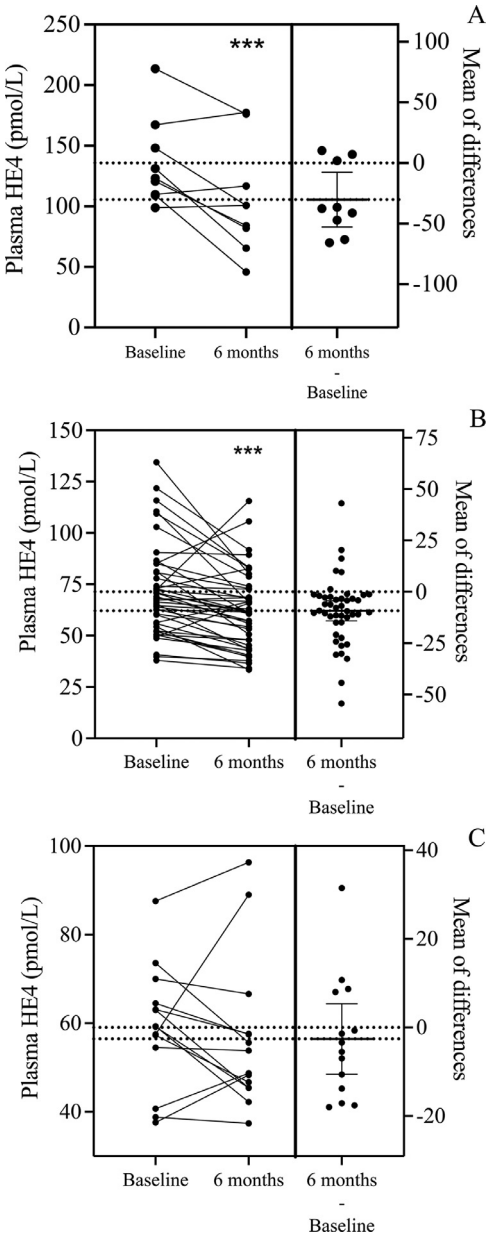

Fig. 3. The mean difference of plasma HE4 concentrations was determined based on the baseline ppFEV1 categories of < 50% (A), 50–89% (B), and ≥ 90% (C), respectively. On the left, baseline values and results at 6 months of LUM/IVA treatment are depicted demonstrating the tendency of HE4 change in each sub-cohort, while the mean difference values with single values (dots) are shown on the right. Values are expressed as mean (95% CI) values. Baseline and 6-month HE4 values were compared by paired *t*-test. $^{***}P < 0.001$ vs baseline.

2.4. Ethics statement

This study was approved by the Regional Ethics Committee of the University of Debrecen (permit number: 4813–2017) following the World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects. The biorepository application was reviewed and accepted by CFFT providing fully anonymized pwCF samples.

2.5. Statistical analyses

Statistical tests are described in the Supplementary materials. Analyses were performed using GraphPad Prism, version 9 (GraphPad Software, La Jolla, CA, USA) and SPSS Statistics software, version 26.0 (IBM Corporation).

3. Results

3.1. Changes in clinical parameters and plasma HE4 in response to LUM/IVA treatment

Sweat chloride decreased by an average of −18.1 mmol/L (95% CI, −22.3 to −15.0) from baseline by 1 month following LUM/IVA administration and such decreased values remained at this level at 6 months (mean value of −18.9 mmol/L; 95% CI, −24.5 to −14.8) compared to pre-treatment data (Fig. 1A). In ppFEV1, a moderate, statistically not significant change was observed from baseline to 1 to 6 months, with a mean change at 1 month of 1.6% (95% CI, −0.4 to 3.6) and at 6 months of 2.6% (95% CI, 0.6 to 4.5) in this sub-cohort (Fig. 1B) in contrast to the results of the entire study population [13]. Nutritional status substantially improved, as indicated by steadily increased BMI values throughout the study with a mean change of 0.73 kg/m^2 (95% CI, 0.42 to 0.98) at 6 months (Fig. 1C).

Plasma HE4 concentrations before the introduction of LUM/IVA therapy were similar (median, IQR, 68.5 (56.6–87.3) pmol/L) in other CF cases before IVA treatment (63.1 [47.9–97.5] pmol/L) in our previous study [16] (Suppl. Fig. 1A). Plasma HE4 levels were reduced at all post-treatment time points showing the largest delta value at 6 months, i.e., −10.7 mmol/L (95% CI, −15.4 to −5.9) vs baseline (Fig. 1D). Interestingly, the mean change of HE4 (−6.5 pmol/L, 95% CI, −12.4 to −0.5) was smaller at 12 months among these pwCF, which was related to less improved lung function (delta ppFEV1: 1.0% (95% CI, −1.7 to 3.8) by the end of study course.

Using Spearman tests, the comparison of change in ppFEV1 at 1 and 6 months against changes in sweat chloride concentration at 1 month ($r = 0.0595$; $P = 0.6403$) and 6 months ($r = 0.0148$, $P = 0.9117$) did not reveal consistent trends (Suppl. Fig. 1B and C). Thus, we sought to investigate the potential of the HE4 plasma-based biomarker to monitor the effect of LUM/IVA CFTRm treatment throughout the study visits.

3.2. HE4 plasma concentration strongly correlates with ppFEV1

Initially, we correlated the HE4 concentrations with the ppFEV1 values determined at baseline and 6 months after the initiation of LUM/IVA therapy (i.e., when the largest improvement in ppFEV1 was detected) to observe whether absolute values of plasma HE4 reflected the lung function status. A significant inverse correlation was detected between pre-treatment values of plasma HE4 and ppFEV$_1$ ($r = −0.5661$; $P < 0.0001$) (Fig. 2A). Likewise, there was a significant negative relationship between plasma HE4 concentrations and ppFEV1 at 6 months of LUM/IVA therapy ($r = −0.5294$; $P < 0.0001$) (Fig. 2B). In parallel, the mean change of HE4 from baseline (i.e., delta HE4 of its plasma level) similarly correlated with delta ppFEV1 ($r = −0.5297$; $P < 0.0001$) in all study participants (Fig. 2C). However, when we separately analyzed this association among pwCF under 18 years of age, a stronger correlation was found in childhood ($r = −0.7053$; $P < 0.0001$) (Fig. 2D).

When the absolute HE4 concentrations measured at baseline and 6 months were evaluated using the baseline ppFEV1 categories of < 50%, 50–89%, and ≥ 90%, respectively, as previously set by Sagel et al. [13], the baseline and 6-month HE4 concentrations were significantly higher in those with < 50% baseline ppFEV1 compared to the other two sub-groups (Suppl. Fig. 2A and B). More importantly, the largest, statistically significant mean difference in HE4 was determined in these (mostly adult) pwCF with severely impaired lung function before treatment (Fig. 3A and B),

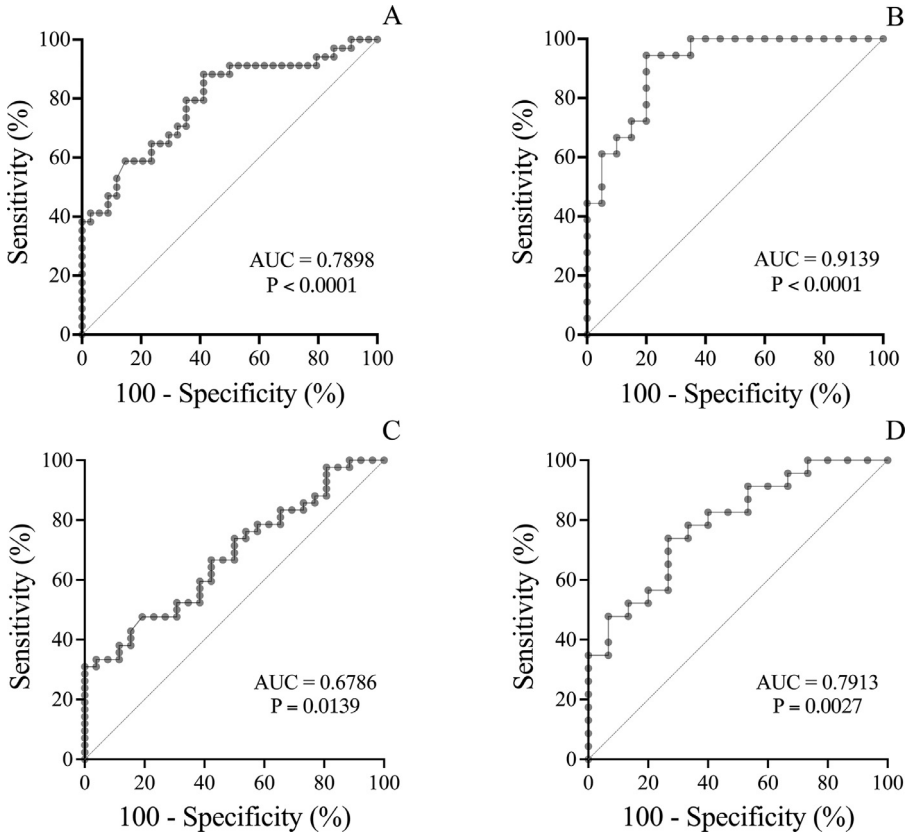

Fig. 4. Determination of the discriminatory power of mean change of HE4 if 2.6% (A, B) or 5% of delta ppFEV1 (C, D) at 6 months of treatment was used as the binary classifier using ROC-AUC curve analyses. Compared to the data of the entire cohort (A), the mean change of HE4 concentrations was more pronounced in children using the same cut-off value (B). When 5% of delta ppFEV1 was the classifier, the AUC value for HE4 moderately indicated corresponding lung function improvement in all pwCF (C) and children with CF (D).

while the lowest, non-significant changes were observed in subjects with baseline ppFEV1 ≥ 90% (Fig. 3C).

3.3. Change in plasma HE4 predicts the improvement of CF lung disease under LUM/IVA therapy

We then investigated whether decreasing HE4 concentrations in response to LUM/IVA predicted the improvement of CF lung function as assessed by delta ppFEV1 values. Thus, we calculated the discriminative power of the mean change of HE4 if 2.6% delta ppFEV1 at 6 months of treatment (i.e., the mean value of this pwCF cohort) was used as the binary classifier. Considerable AUC value of delta HE4 (0.7898 [95% CI 0.6823–0.8972], $P < 0.0001$) was found with its cut-off value of −10.7 pmol/L demonstrating 62% sensitivity and 79% specificity for the entire pwCF population (Fig. 4A). The mean change of HE4 was more powerful in CF children (0.9139 [95% CI 0.8268–1.0000], $P < 0.0001$) and using the same cut-off value sensitivity (80%) and specificity (83%) were much higher (Fig. 4B). This value for HE4, when 5% delta ppFEV1 was the classifier being the expected lung function improvement from a "good responder", was 0.6786 (95% CI 0.5523–0.8048), $P = 0.0139$) in all pwCF using a cut-off value of −11.8 pmol/L with a 73% sensitivity and 74% specificity (Fig. 4C). On the other hand, the AUC value of delta HE4 in children was higher as 0.7913 (95% CI 0.6481–0.9345), $P = 0.0027$), but at the same cut-off value, we achieved 50% sensitivity and 74% specificity (Fig. 4D).

When pwCF were sub-grouped according to their responder status, i.e., with ≥ 5% mean change of ppFEV1 at 6 months of treatment or their non-responder status with lower delta ppFEV1, we found that significantly decreased plasma HE4 concentrations were observed only in case of responders with expected improved lung function by 6 months of LUM/IVA treatment (Fig. 5A). In contrast, sweat chloride concentrations (measured in mM) and BMI values were significantly altered in both sub-cohorts (Fig. 5B and C). These latter values indicate the overall effect of LUM/IVA medication but cannot facilitate the assessment of responsiveness to the combined CFTR modulator treatment.

Finally, multiple logistic regression analysis was used to evaluate the likelihood of being a responder against the following covariates: delta HE4, age, sex, baseline ppFEV1, sweat chloride concentration, BMI, and *P. aeruginosa* positivity at 6 months after initiation of LUM/IVA treatment. Delta HE4 (OR: 0.89, 95%CI: 0.82–0.95; $P = 0.001$) and baseline ppFEV1 (OR: 1.09, 95%CI: 1.02–1.16; $P = 0.005$) were independently associated in responders compared to non-responders (Suppl. Table 2).

4. Discussion

To the best of our knowledge, this is the first study which provides evidence of the correlation of the plasma HE4 levels with the improvement of lung function (i.e., delta ppFEV1) in response to LUM/IVA (Orkambi®) treatment in pwCF. Recently, the beneficial effect of LUM/IVA was largely monitored using ppFEV1, BMI values and sweat chloride in adults [8,11], in children/adolescents [10,24,25] or in both age groups combined [9,12,13]. Importantly, these functional and laboratory parameters have inherent limitations in terms of monitoring CFTRm. For instance, the discriminatory value of ppFEV1 can be limited within the moderate to normal lung function range [26]. In addition, significant variability was observed in ppFEV1 as related to the age and population-specific

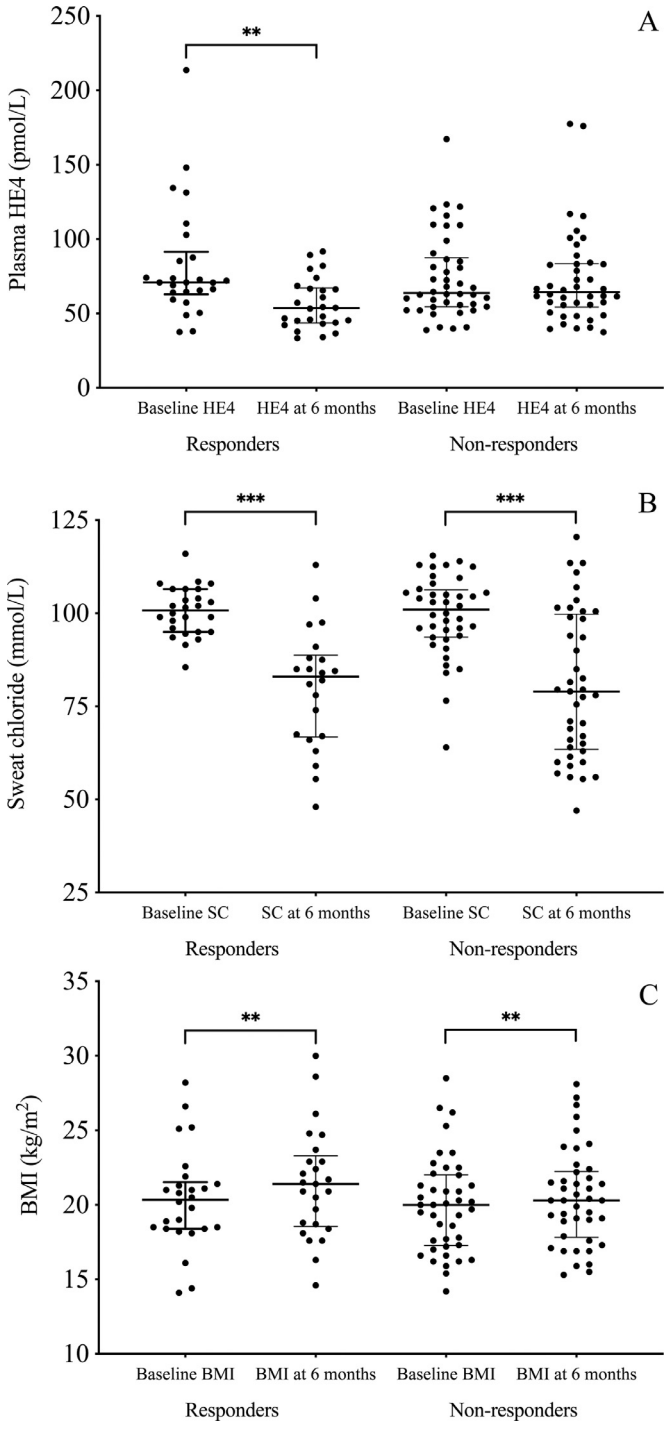

Fig. 5. Analysis of plasma HE4 concentrations (A), sweat chloride concentrations (B), and BMI values (C) when pwCF were sub-grouped as 1) "responders" with ≥ 5% mean change of ppFEV1 at 6 months of treatment, or 2) "non-responders" if they showed an increase in ppFEV1 of less than 5%. In contrast to sweat chloride and BMI, plasma HE4 concentrations altered only in the case of responders. Values are expressed as median (IQR). The comparison of two groups of data was done with Wilcoxon matched pairs signed rank test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs baseline. SC means sweat chloride; BMI means body mass index.

standards, and there can also be practical difficulties in the reliable performance of spirometric examinations, especially in younger CF patients [26]. Sweat chloride concentrations are used as a secondary outcome parameter in clinical studies [27]. However, sweat conductivity measurements still lack standardization [28]. Significant reductions in sweat chloride were reported already by day 15 after LUM/IVA therapy that was sustained up to 24 weeks underscoring the sensitivity of sequential examination of sweat chloride concentrations in the monitoring of treatment efficacy of CFTRm [10,11,24]. On the other hand, the limited correlation between absolute or relative changes in sweat chloride concentrations and ppFEV1 was described despite the clear improvement in lung function at 1–6 months after LUM/IVA therapy [13,29]. Therefore, there remains a clear unmet need to find other routinely available parameters, such as a suitable blood-based biomarkers, which can reliably improve the monitoring of CFTRm in clinical practice [30].

Some biomarkers in peripheral blood have been investigated in this regard, such as C-reactive protein (CRP) in plasma which lowered by 2 months of IVA treatment and correlated with sweat chloride concentrations [31]. Recently, interleukin-18 (IL-18) and tumor necrosis factor-α (TNF-α) concentrations in serum demonstrated a significant gradual decrease by 1–3 months of LUM/IVA medication, while IL-1β concentrations were reduced only after tezacaftor/IVA treatment [32].

In the last several years, our group proved the utility of examining serum HE4 concentrations for the assessment of the severity of CF lung disease in Czech and Hungarian pwCF before the administration of CFTRm [15]. Importantly, a positive correlation of HE4 concentration with the severity of the CF lung disease was independent of patients' age [15]. However, HE4 concentrations could be affected by patient age, smoking and impaired renal function in non-CF populations [33]. Therefore, plasma HE4 levels were efficiently utilized to monitor the alterations of lung function due to the efficacy of IVA in three independent cohorts of pwCF, importantly without impaired renal function [16]. CFTR dysfunction has been reported to contribute to abnormal HE4 expression via the activation of pro-inflammatory NF-κB pathway in CF [17]. The effect of LUM/IVA treatment on HE4 expression has been evaluated in CFBE 41o− cells expressing F508del-CFTR *in vitro* [17], thereby attesting the downregulation of the aforementioned pro-inflammatory pathway. However, *in vivo* plasma HE4 concentrations have not been thoroughly studied in a large CF cohort treated with LUM/IVA thus far.

Here we analyzed well-selected plasma specimens for plasma HE4 measurements in a sub-cohort of pwCF homozygous for the p.Phe508del-CFTR variant, who previously participated in the PROSPECT study [13]. In total, 68 pwCF were enrolled in this study, who demonstrated a modest mean change (2.6%) observed from baseline to 6 months of LUM/IVA therapy in contrast to the results of the whole study population for whom Sagel et al. reported non-significant changes in the mean ppFEV1 (−0.2%) by 6 months [13]. In response to LUM/IVA therapy, HE4 concentrations were significantly lower already at the first follow-up examination and remained that way through the end of respective study periods. The changes of plasma HE4 demonstrated the largest delta value (−10.7 mmol/L) with the lowest absolute HE4 levels (61.5 [47.0–79.7] pmol/L) by 6 months (Suppl. Fig. 1A), i.e., representing a level which approached - but did not reach - the reference interval of HE4 (36.3 [31.1–43.4] pmol/L) previously determined in another study [15]. This observation was corroborated by our recent *in vitro* experiments in CFBE 41o− cells expressing p.Phe508del-CFTR [17], where treatment of these cells with LUM/IVA showed significantly decreased HE4 expression compared to untreated cells, but overall HE4 concentrations did not normalize. All these data underline that HE4 do not reach normal (i.e., "wild-type levels") in pwCF even in the presence of improved CFTR function following administration of CFTRm.

Simultaneously, the mean change of HE4 from the baseline correlated with delta ppFEV1 in all study participants. However, when we separately analyzed this association among CF individuals under 18 years of age, a stronger correlation was found at younger ages. Furthermore, the mean change in plasma HE4 was also evaluated based on the baseline ppFEV1 categories. The largest

mean difference of HE4 was determined in pwCF with severely impaired lung function (< 50%) before CFTRm treatment, while the least prominent changes were observed in subjects with baseline ppFEV1 ≥ 90%. These two observations are not in contradiction due to the following reasoning: a) although there was no difference in baseline ppFEV1 among CF adults and children, a much higher mean delta ppFEV1 was achieved in children (3.7%) versus adults (1.1%) by 6 months, with a higher ratio in responders (Suppl. Table 1). Consequently, due to the strong correlation between plasma HE4 and ppFEV1, the HE4 biomarker showed better discriminatory power in children; b) on the other hand, regardless of age, those study participants who had worse pre-treatment lung function parameters showed a more substantial pulmonary improvement by 6 months of CFTRm therapy leading to higher alterations of HE4 plasma concentration. In the PROSPECT study, stratification by age and baseline lung function reported the same, i.e., that significant improvements in ppFEV1 were only observed in adolescents and young adults at the 3-month post-LUM/IVA administration time point and in those with a baseline ppFEV1 between 50 and 89% at the 3- and 6-month time points [13]. Previously, IVA alone could cause a larger alteration in plasma HE4 (−14.4 pmol/L vs −10.7 pmol/L) in a cohort with CF patients bearing at least one p.Gly551Asp-CFTR mutation in *trans* (being the Class III *CFTR* variant), since the mean delta ppFEV1 was also higher (7.0%) by 6 months of treatment in selected GOAL study participants [16], compared to the PROSPECT study participants (bearing the Class II *CFTR* variant p.Phe508del) with a mean delta ppFEV1 of only 2.6%. Nevertheless, these data are in accordance with our original hypothesis that plasma HE4 level sensitively follow even minor improvements in lung function in pwCF treated with various CFTRm.

The discriminatory "power" of mean change of plasma HE4 was determined by ROC-curve analysis at two different degrees of lung function improvement (i.e., 2.6% and 5% by 6 months of treatment, retrospectively). A considerable AUC value of delta HE4 (0.9139) was found in the entire CF patient population. Furthermore, it was more pronounced in children with a high sensitivity (80%) and specificity (83%). When the 5% of delta ppFEV1 became the classifier, the AUC value was 0.6786 in all pwCF, while again, it was higher in children (0.7913). Recently, a similar AUC value of delta HE4 (0.806) was found especially after 1–2 months of medication with 81% sensitivity and 89% specificity [16]. Responders (≥ 5%) and non-responders (< 5%) were sub-grouped based on mean change of ppFEV1 at 6 months following commencement of treatment. Significantly decreased plasma HE4 concentrations were observed only in the case of responders.

Finally, multiple logistic regression analysis was used to evaluate the likelihood of being a responder against different covariates. Delta HE4 and baseline ppFEV1 were independently associated with responders in terms of change in their ppFEV1 (≥ 5%) compared with non-responders. Similarly, none of the baseline covariates (i.e., age, sex, sweat chloride concentrations, and BMI) was statistically associated with the rate of response to treatment in the original clinical trial [13]. In our previous study, a significant independent relationship was shown between delta ppFEV1 and delta HE4 in pwCF with Class III variants who are treated with IVA [16].

In conclusion, plasma HE4 negatively correlates with lung function improvement in pwCF receiving LUM/IVA therapy. Based on this evidence, we propose that CF lung disease could be monitored in cases treated with LUM/IVA by the examination of both absolute and delta values of plasma HE4, which are inversely correlated with absolute and delta FEV1 values, especially in CF children and in adolescents. Overall, continuous examinations of HE4 plasma concentration at pre-defined time intervals could facilitate monitoring of CFTRm treatment efficacy in routine clinical practice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Marianna Pócsi: Investigation, Methodology, Formal analysis, Data curation, Visualization, Writing – review & editing. **Zsolt Fejes:** Investigation, Data curation. **Zsolt Bene:** Investigation, Project administration. **Attila Nagy:** Formal analysis. **István Balogh:** Validation, Resources. **Margarida D. Amaral:** Supervision, Writing – review & editing. **Milan Macek Jr.:** Supervision, Writing – review & editing. **Béla Nagy Jr.:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Funding acquisition.

Financial disclosure

This study was supported by the Hungarian Research Fund (FK 135327) by the National Research, Development, and Innovation Office to BN, and by the Czech Ministry of Health 00064203/6003 and LM2018132 from the Czech Ministry of Education, Youth and Sports to MM.

Acknowledgments

The authors thank the CF Foundation (Bethesda, MD, USA) to supply the samples for this study. Work in the MDA lab is supported by UIDB/04046/2020 and UIDP/04046/2020 centre grants from FCT Portugal (to BioISI). The authors are grateful to Ms. Abigél Balla MSc student and Erika Szakács Szilágyi technician for their excellent technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.04.001.

References

[1] Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005;352:1992–2001.
[2] ECFS Patient Registry Annual Report, 2020. (https://www.ecfs.eu/projects/ecfs-patient-egistry/annual-reports)
[3] De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. Lancet Respir Med 2016;4:662–74.
[4] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci USA 2009;106:18825–30.
[5] De Boeck K, Munck A, Walker S, Faro A, Hiatt P, Gilmartin G, et al. Efficacy and safety of ivacaftor in patients with cystic fibrosis and a non-G551D gating mutation. J Cyst Fibros 2014;13:674–80.
[6] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci USA 2011;108:18843–8.
[7] Regard L, Martin C, Burnet E, Da Silva J, Burgel PR. CFTR modulators in people with cystic fibrosis: real-world evidence in France. Cells 2022;11(11):1769.
[8] Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E. et al.; VX09-809-102 Study Group. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. Lancet Respir Med 2014;2:527–38.
[9] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, Colombo C, Davies JC, De Boeck K, Flume PA, Konstan MW, McColley SA, McCoy K, McKone EF, Munck A, Ratjen F, Rowe SM, Waltz D, Boyle MPTRAFFIC Study Group; TRANSPORT Study Group. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. N Engl J Med 2015;373(3):220–31.
[10] Milla CE, Ratjen F, Marigowda G, Liu F, Waltz D, Rosenfeld M. VX13-809-011 part B investigator group. Lumacaftor/ivacaftor in patients aged 6-11 years with cystic fibrosis and homozygous for F508del-CFTR. Am J Respir Crit Care Med 2017;195(7):912–20.

[11] Taylor-Cousar JL, Jain M, Barto TL, Haddad T, Atkinson J, Tian S, Tang R, Marigowda G, Waltz D, Pilewski J. VX14-809-106 Investigator Group. Lumacaftor/ivacaftor in patients with cystic fibrosis and advanced lung disease homozygous for F508del-CFTR. J Cyst Fibros 2018;17(2):228–35.
[12] Burgel PR, Munck A, Durieu I, Chiron R, Mely L, Prevotat A, Murris-Espin M, Porzio M, Abely M, Reix P, Marguet C, Macey J, Sermet-Gaudelus I, Corvol H, Bui S, Lemonnier L, Dehillotte C, J Da Silva, Paillasseur JL, D; Hubert. French cystic fibrosis reference network study group. Real-life safety and effectiveness of Lumacaftor-ivacaftor in patients with cystic fibrosis. Am J Respir Crit Care Med 2020;201(2):188–97.
[13] Sagel SD, Khan U, Heltshe SL, Clancy JP, Borowitz D, Gelfond D, Donaldson SH, Moran A, Ratjen F, VanDalfsen JM, Rowe SM. Clinical effectiveness of Lumacaftor/Ivacaftor in patients with cystic fibrosis homozygous for F508del-CFTR. A clinical trial. Ann Am Thorac Soc 2021;18(1):75–83.
[14] Montemayor K, Lechtzin N. The PROSPECT is bright for CFTR modulators. Ann Am Thorac Soc 2021;18(1):32–3.
[15] Nagy B Jr, Nagy B, Fila L, Clarke LA, Gönczy F, Bede O, Nagy D, Újhelyi R, Szabó Á, Anghelyi A, Major M, Bene Z, Fejes Z, Antal-Szalmás P, Bhattoa HP, Balla G, Kappelmayer J, Amaral MD, Macek M Jr, Balogh I. Human epididymis protein 4: a novel serum inflammatory biomarker in cystic fibrosis. Chest 2016;150(3):661–72.
[16] Nagy B Jr, Bene Z, Fejes Z, Heltshe SL, Reid D, Ronan NJ, McCarthy Y, Smith D, Nagy A, Joseloff E, Balla G, Kappelmayer J, Macek M Jr, Bell SC, Plant BJ, Amaral MD, Balogh I. Human epididymis protein 4 (HE4) levels inversely correlate with lung function improvement (delta FEV1) in cystic fibrosis patients receiving ivacaftor treatment. J Cyst Fibros 2019;18(2):271–7.
[17] Bene Z, Fejes Z, Szanto TG, Fenyvesi F, Váradi J, Clarke LA, Panyi G, Macek M Jr, Amaral MD, Balogh I, Nagy B Jr. Enhanced expression of human epididymis protein 4 (HE4) reflecting pro-inflammatory status is regulated by CFTR in cystic fibrosis bronchial epithelial cells. Front Pharmacol 2021;12:592184.
[18] Kirchhoff C, Habben I, Ivell R, Krull N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod 1991;45(2):350–7.
[19] Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, Drescher C, Urban N, Hellström KE. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003;63(13):3695–700.
[20] Nagy B Jr, Bhattoa HP, Steiber Z, Csobán M, Szilasi M, Méhes G, Müller M, Lázár J, Kappelmayer J, Antal-Szalmás P. Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer. Clin Chem Lab Med 2014;52(11):1639–48.
[21] Clauss A, Lilja H, Lundwall A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. Biochem J 2002;368(1):233–42 pt.
[22] Small DM, Doherty DF, Dougan CM, Weldon S, Taggart CC. The role of whey acidic protein four-disulfide-core proteins in respiratory health and disease. Biol Chem 2017;398(4):425–40.
[23] Bingle L, Cross SS, High AS, Wallace WA, Rassl D, Yuan G, Hellstrom I, Campos MA, Bingle CD. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. Respir Res 2006;7:61.
[24] Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, Milla CE, Robinson PD, Waltz D, Davies JC. VX14-809-109 investigator group. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. Lancet Respir Med 2017;5(7):557–67.
[25] Hoppe JE, Chilvers M, Ratjen F, McNamara JJ, Owen CA, Tian S, Zahigian R, Cornell AG, McColley SA. Long-term safety of lumacaftor-ivacaftor in children aged 2-5 years with cystic fibrosis homozygous for the F508del-CFTR mutation: a multicentre, phase 3, open-label, extension study. Lancet Respir Med 2021;9(9):977–88.
[26] Szczesniak R, Heltshe SL, Stanojevic S, Mayer-Hamblett N. Use of FEV1 in cystic fibrosis epidemiologic studies and clinical trials: a statistical perspective for the clinical researcher. J Cyst Fibros 2017;16:318–26.
[27] Accurso FJ, Van Goor F, Zha J, Stone AJ, Dong Q, Ordonez CL, et al. Sweat chloride as a biomarker of CFTR activity: proof of concept and ivacaftor clinical trial data. J Cyst Fibros 2014;13:139–47.
[28] Greaves RF, Jolly L, Massie J, Scott S, Wiley VC, Metz MP, et al. Laboratory performance of sweat conductivity for the screening of cystic fibrosis. Clin Chem Lab Med 2018;28(56):554–9.
[29] Masson A, Schneider-Futschik EK, Baatallah N, Nguyen-Khoa T, Girodon E, Hatton A, Flament T, Le Bourgeois M, Chedevergne F, Bailly C, Kyrilli S, Achimastos D, Hinzpeter A, Edelman A, Sermet-Gaudelus I. Predictive factors for lumacaftor/ivacaftor clinical response. J Cyst Fibros 2019;18(3):368–74.
[30] Bene Z, Fejes Z, Macek M Jr, Amaral MD, Balogh I, Nagy B Jr. Laboratory biomarkers for lung disease severity and progression in cystic fibrosis. Clin Chim Acta 2020;508:277–86.
[31] Guerra L, D'Oria S, Favia M, Castellani S, Santostasi T, Polizzi AM, et al. CFTR-dependent chloride efflux in cystic fibrosis mononuclear cells is increased by ivacaftor therapy. Pediatr. Pulmonol 2017;52:900–8.
[32] Jarosz-Griffiths HH, Scambler T, Wong CH, Lara-Reyna S, Holbrook J, Martinon F, et al. Different CFTR modulator combinations downregulate inflammation differently in cystic fibrosis. Elife 2020;9:e54556 pii.
[33] Lycke M, Ulfenborg B, Malchau Lauesgaard J, Kristjansdottir B, Sundfeldt K. Consideration should be given to smoking, endometriosis, renal function (eGFR) and age when interpreting CA125 and HE4 in ovarian tumor diagnostics. Clin Chem Lab Med 2021;59(12):1954–62.