Relevance of Nasal Potential Difference in Diagnosis of Cystic Fibrosis Among Children

Arūnas Valiulis[1], Iveta Skurvydienė[2], Valdonė Misevičienė[3], Jūratė Kasnauskienė[4], Laimutė Vaidelienė[3], Algirdas Utkus[4]

[1]Clinic of Children's Diseases, Medical Faculty, Vilnius University, [2]Tauragė District Hospital, [3]Department of Children's Diseases, Medical Academy, Lithuanian University of Health Sciences, [4]Department of Human and Medical Genetics, Medical Faculty, Vilnius University, Lithuania

Key Words: *cystic fibrosis; obstructive lung diseases; diagnosis; basal nasal potential difference; children.*

Summary. *Objective. The aim of this study was to estimate the significance of nasal potential difference (NPD) in the diagnosis of cystic fibrosis (CF) in children with clinical symptoms suggestive of the disease, positive sweat test results, and/or genetically confirmed diagnosis.*

Material and Methods. NPD measurements according to the modifications by Alton were performed in 50 children with clinical CF symptoms supported by positive sweat test results, 50 children with other obstructive lung diseases, and 50 healthy children. A subgroup of 17 children with the diagnosis confirmed by 2 identified mutations in the CF transmembrane regulatory gene was analyzed individually.

Results. The mean NPD value recorded in 50 children with clinical symptoms of CF supported by positive sweat test results and/or genetic analysis was –28.0 mV [SD, 10.2]. The mean NPD value in the subgroup of children with 2 identified mutations in the CF gene (n=17) was more negative than in the subgroup of children with unrecognized mutations (n=33) (–37.1 mV [SD, 7.0] vs. –23.4 mV [SD, 8.3], P<0.001). The mean NPD value in patients with other obstructive lung diseases and healthy children was significantly more positive than in the group of CF children with positive sweat test results and/or identified mutations (–18.1 mV [SD, 3.6] and –15.5 mV [SD, 4.3] vs. –28.0 mV [SD, 10.2], P<0.001). The NPD cut point value for the genetically confirmed diagnosis of CF was –35.0 mV (sensitivity, 93.9%; specificity, 88.2%), while in general, the NPD prognostic value was –24.0 mV (sensitivity, 58.0%; specificity, 98.0%)

Conclusions. The NPD measurement is a valuable tool for the diagnosis of CF in children, but further studies are necessary to establish NPD values related to the CF genotype and to reduce the intrasubject variability of this test.

Introduction

Cystic fibrosis (CF) is characterized by severe chronic obstructive lung disease, chronic fat malabsorption, and malnutrition (1, 2). A sweat chloride concentration above 60 mmol/L and/or the presence of 2 mutations in the cystic fibrosis transmembrane regulatory (*CFTR*) protein-encoding gene is uniformly accepted as the diagnostic criteria for the classical form of the disease (2–4).

Positive sweat test results are documented in approximately 98% of patients with typical clinical features of the disease, but sometimes patients carrying particular genotypes with a combination of 2 CF-related mutations may have a sweat chloride value in the intermediate range (30–60 mmol/L) (5, 6). Contrary to a rather clearly defined range for the sweat chloride concentration, it is very difficult to identify the most common combination of CF-causing mutations as more than 1700 *CFTR* mutations in CF patients have been described (1, 7, 8).

The defective *CFTR* gene frequency for the populations of Caucasian origin is 1 in 50, so more than 640 000 *CFTR* genotypes can be identified (9, 10). The most frequent mutation in the Caucasian population, particularly in North Europe, is F508del (67% in the United Kingdom, 82% in Denmark), and the most frequent genotype is [F508del]+[F508del] (57% in the United Kingdom, 74% in Denmark, and 34% in Lithuania) (10, 11).

Nasal potential difference (NPD) measurement was proposed as an alternative method to diagnose CF. This measurement provides a direct and sensitive evaluation of sodium (Na^+) and chloride (Cl^-) transport in nasal epithelial cells by the assessment of their bioelectric properties caused by *CFTR* mutations (5, 12–15). The NPD values of –30 mV and more negative are accepted as sufficient for the diagnosis of CF (16, 17).

Correspondence to A. Valiulis, Clinic of Children's Diseases, Vilnius City Clinical Hospital, Antakalnio 57, 10207 Vilnius, Lithuania. E-mail: arval@vmul.lt

Although it is recommended to include the NPD measurement in the list of principal CF diagnostic criteria, discussion on the use of NPD for diagnostic purposes, standardization of operating procedures, and establishment of uniform normal NPD values is still relevant and needs further investigations (1, 3, 5, 15).

The aim of this study was to carry out NPD measurements in children with CF and other obstructive lung diseases, and to estimate a diagnostic value of NPD.

Material and Methods

A total of 204 children (115 boys and 89 girls) aged 4 months to 17 years and diagnosed with CF and other chronic obstructive lung diseases (bronchial asthma, recurrent and chronic obstructive bronchitis) from different regions of Lithuania were examined. Inclusion criteria for the CF group in the study were set according to the International Cystic Fibrosis Association Consensus Recommendations: 1) prestudy diagnosis of CF; and 2) two or more clinical features of CF and at least two positive (chloride concentration, >60 mmol/L) or borderline (chloride concentration, 40–60 mmol/L) sweat test results during the last year (5).

The study participants were selected from 204 children who were referred to the tertiary level institution for a consultation with a pediatric pulmonologist to confirm the diagnosis of CF. Of the 204 patients, 50 met the inclusion criteria for the CF group. Other 50 patients who had only one positive sweat test result during the last year and obstructive lung disease (bronchial asthma, recurrent obstructive bronchitis, chronic obstructive bronchitis, and bronchiectases) were included into the diseased control group. The remaining 104 children had negative sweat test results and were excluded from the study.

Fifty healthy volunteers (30 boys and 20 girls) aged 8 to 10 years from Tauragė were randomly selected to participate in the study after their annual check-up; these children enrolled into the healthy control group.

Anthropometric measurements and the sweat test were performed in all the patients of the CF and diseased control groups. All the patients in CF group underwent genetic testing for *CFTR* gene mutations. NPD measurements were carried in all 3 study groups.

Written informed consent to participate in the study was signed by parents of all the children examined. All the procedures listed above were accomplished in accordance with the ethical standards and national legislation requirements.

Weight-for-age and height-for-age percentiles for all children were calculated from the national growth charts (18).

Two consequent sweat tests were performed in all children from the CF group and the diseased control group according to the pilocarpine iontophoresis technique described by Gibson and Cooke, and in some cases, according to the quantitative Wescor Macroduct Sweat Collection System (Wescor, Inc., Logan, Utah, USA; Chemlab Scientific Products, Hornchurch, Essex, UK). Chloride concentration values of more than 60 mmol/L by the Gibson and Cooke method and 80 mmol/L by the Wescor Macroduct method were considered as positive and highly interrelated with the diagnosis of CF. The sweat chloride concentration within the range of 40–60 mmol/L was considered as borderline and less suggestive of CF diagnosis (6).

Blood DNA samples were taken and analyzed for the most common mutations of the *CFTR* gene in North Europe (F508del, R553X, N1303K, I507del, N1282X, G542X, G551D, 1717-(G)A, and 349delTT) (2) in the Center for Medical Genetics of Vilnius University Hospital using an INNO LiPA CF2 assay (19). The entire *CFTR* gene sequencing was performed in one heterozygous child with severe CF, carrying one F508del mutation and one unidentified mutation in the *CFTR* gene.

The baseline NPD measurement was done in all patients: 50 patients from the CF group, 50 patients from the diseased control group (16 patients with bronchial asthma and 34 patients with chronic and recurrent bronchitis), and 50 children from the healthy control group. It was measured according to the recommendations by Alton et al., where it was reported that NPD values below –30 mV (more negative) were suggestive of the diagnosis of CF (20).

The baseline NPD was measured across the floor of the nasal cavity. Ag/AgCl EEG electrodes and a high impedance voltmeter were used to perform the measurements. A reference electrode was placed epicutaneously on the abraded skin of the forearm. A pediatric urethral Foley catheter, filled with an exploring bridge (mixture of ECG cream and Ringer solution, 1:2), was used as a nasal catheter. A registering electrode was placed in the Foley catheter. The exploring bridge was also placed between the reference electrode and abraded skin of the forearm by using a 22-gauge needle. The NPD was measured in each nostril by gradually pushing the Foley catheter along the nasal floor 7–10 cm and then recording a potential difference for 5 seconds until a stable value was achieved. Two measurements from each nostril were made, and the mean of each side was calculated; then, the overall mean potential difference for each subject was determined.

Statistical analysis of the data was performed using software packages SPSS 13.0 (Statistical Package for the Social Sciences 13 for Windows) and

Statistica 6.0. All parametric data were expressed as mean (SD). The Kolmogorov-Smirnov test was used to check the distribution of quantitative data. When the distribution of data was normal, the Student *t* test was used for the comparison of quantitative values of two independent samples. One-way analysis of variance (ANOVA) was used to compare more than 2 independent groups. The Bonferroni post hoc test was used for multiple paired comparisons. Binary logistic regression analysis for the prediction of probability of the event was used.

The Pearson or the Spearmen correlation was used to assess a linear relationship between the variables considering their distribution. In order to assess minimally false negative and minimally false positive results with greatest accuracy, the ROC curve was used.

Differences between the groups were considered significant when $P<0.05$.

Results

Of the 50 patients from the CF group, 45 had elevated sweat chloride levels with a mean value of 91.7 mmol/L (SD, 24.0), and the remaining 5 patients showed borderline sweat test results with a mean value of 56.6 mmol/L (SD, 5.6).

The *CFTR* gene mutations were identified in 25 patients (50.0%). The *CFTR* genotype was confirmed in 17 patients (34.0%): 15 (88.0%) were homozygotes for the F508del mutation ([F508del]+[F508del]) and 2 were compound heterozygotes ([R553X]+[F508del]). Of the 25 patients, 8 were wound to carry only a single *CFTR* gene mutation (F508del). According to the criteria of the Cystic Fibrosis Foundation Consensus for the CF diagnosis, those 8 patients were qualified as having unrecognized *CFTR* genotypes. The entire *CFTR* gene sequencing was performed in all of them. In one case, a large deletion (21 kb) in exons 2 and 3 was detected, and the patient's genotype was confirmed to be [F508del]+[CFTRdele 2,3 (21 kb)].

The mean NPD value in the CF group was –28.0 mV (SD, 10.2). The mean NPD value in the CF subgroup with the confirmed *CFTR* genotypes (n=17) was significantly more negative than in the subgroup of 33 patients with unknown genotypes (–37.1 mV [SD, 7.0] vs. –23.4 mV [SD, 8.]; $P<0.001$). Two subgroups of the CF group were compared regarding height-for-age and weight-for-age percentiles, sweat chloride concentrations, and NPD values. The patients with the confirmed *CFTR* genotypes (subgroup g^+) had significantly lower weight-for-age and height-for-age percentiles, but higher sweat chloride concentrations and more negative NPD values than the patients with the unrecognized *CFTR* genotypes (subgroup g^-) (Table 1).

In the diseased control group, the mean sweat chloride concentration was 44.0 mmol/L (SD, 23.3), and the mean NPD value was –18.1 mV (SD, 3.6). These values significantly differed from those in the CF group (91.7 mmol/L [SD, 24.0] and –28.0 mV [SD, 10.2], respectively; $P<0.001$) (Table 2). In patients with bronchial asthma (n=16) and those with recurrent or chronic obstructive bronchitis (n=34), the mean NPD values were –16.9 mV (SD, 2.4) and –18.8 mV (SD, 4.2), respectively.

Table 1. Comparison of Height-for-Age and Weight-for-Age Percentiles, Sweat Chloride Concentration, and Nasal Potential Difference by Different Cystic Fibrosis (CF) Subgroups

Variable	CF Group (n=50)	CF Subgroup g^+ (n=17)	CF Subgroup g^- (n=33)
Height-for-age percentiles	38.1 (17.1)	23.7 (14.7)	45.5 (13.2)*
Weight-for-age percentiles	34.9 (18.3)	20.5 (13.6)	42.3 (15.9)*
Sweat chloride concentration, mmol/L†	91.7 (24.0)	107.2 (22.7)	83.6 (20.7)*
Nasal potential difference, mV	–28.0 (10.2)	–37.1 (7.0)	–23.4 (8.3)*

Values are mean (standard deviation).
CF subgroup g^+, cystic fibrosis patients with recognized *CFTR* genotypes; CF subgroup g^-, cystic fibrosis patients with unknown *CFTR* genotypes.
*$P<0.001$ as compared with the CF subgroup g^+. †According to the Gibson and Cooke method.

Table 2. Comparison of Height-for-Age and Weight-for-Age Percentiles, Sweat Chloride Concentration, and Nasal Potential Difference by Different Groups

Variable	Cystic Fibrosis Group (n=50)	Diseased Control Group (n=50)	Healthy Control Group (n=50)
Height-for-age percentiles	38.1 (17.1)	48.6 (14.2)*	51.0 (17.0)*†
Weight-for-age percentiles	34.9 (18.3)	41.7 (14.2)*	53.1 (14.9)*†
Sweat chloride concentration, mmol/L‡	91.7 (24.0)	44.0 (23.3)*	ND
Nasal potential difference, mV	–28.0 (10.2)	–18.1 (3.6)*	–15.5 (4.3)*†

Values are mean (standard deviation). ND, not done.
*$P<0.001$ as compared with the cystic fibrosis group. †$P<0.001$ as compared with the diseased control group.
‡According to the Gibson and Cooke method.

The analysis of data between the CF subgroups (subgroup g^+ and subgroup g^-) and the diseased and healthy control groups revealed the mean NPD values to be significantly more negative in both the CF subgroups than the control groups (–37.1 mV [SD, 7.0] and –23.4 mV [SD, 8.3] vs. –18.1 mV [SD, 3.6] and –15.5 mV [SD, 4.3]; $P<0.001$). Moreover, there was a significant difference in the sweat chloride level between the diseased control group and the CF subgroup with the unrecognized phenotype (44.0 mmol/L [SD, 23.3] vs. 83.6 mmol/L [SD, 20.7], $P<0.001$).

Correlation between sweat chloride levels and NPD values in the study groups (CF and diseased control groups) were evaluated separately. A significant negative correlation between these variables was found in the CF group ($r=–0.5$; $P<0.001$; y=–8.132–0.217x), but the analysis of data in the subgroups showed a significant correlation only in the subgroup with the identified genotypes ($r=–0.5$, $P<0.001$) (Fig. 1). The overall correlation between NPD values and sweat chloride levels was also negative and statistically significant ($r=–0.6$, $P<0.001$) (Fig. 2).

The NPD cut point value defined by the ROC curve for the genetically confirmed diagnosis of CF was –35.0 mV (sensitivity, 93.9%; specificity, 88.2%), while in general, the NPD prognostic value for the diagnosis of CF was –24.0 mV (sensitivity, 58.0%; specificity, 98.0) (Figs. 3 and 4). Binary logistic regression analysis showed that patients with a defined NPD value of –35 mV were more likely to be diagnosed with CF than those CF patients with a mean NPD value of –24.0 mV (OR, 69.0; 95% CI, 8.8–540.4; $P=0.008$), and if the diagnosis was confirmed genetically, the likelihood was even greater (OR, 116.3; 95% CI, 14.9–907.2).

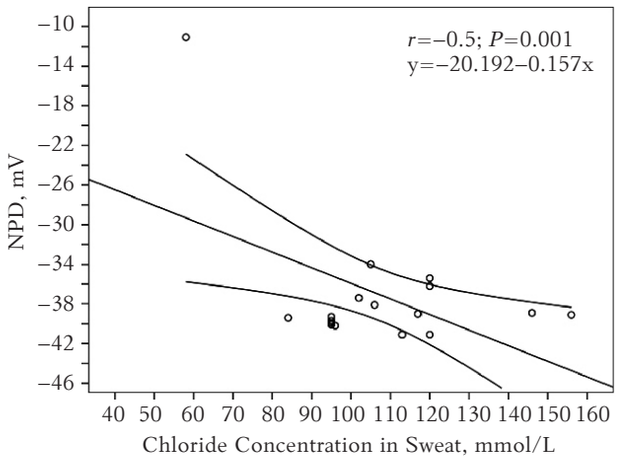

Fig. 1. Correlation between sweat chloride concentrations and nasal potential difference (NPD) values in the genetically confirmed cystic fibrosis subgroup

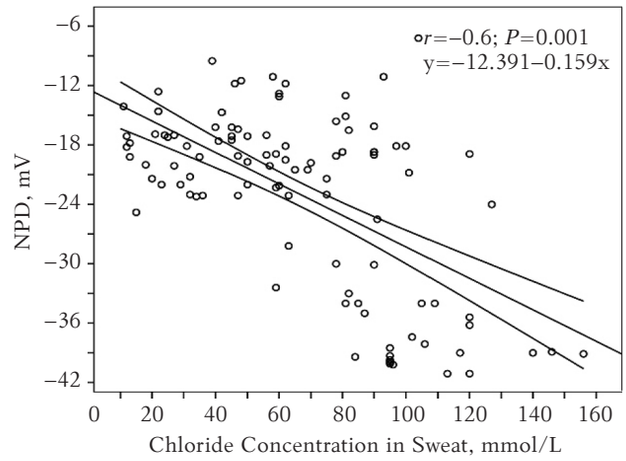

Fig. 2. Correlation between sweat chloride concentrations and nasal potential difference (NPD) values in the general group of patients with cystic fibrosis and diseased controls

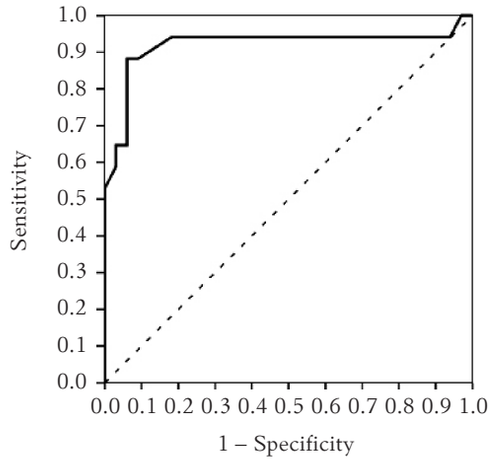

Fig. 3. The ROC curve for a prognostic NPD value in cystic fibrosis patients with identified genotypes (area under the ROC curve, 91.9%)

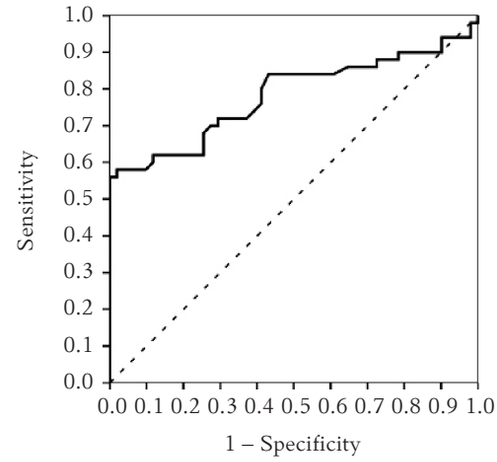

Fig. 4. The ROC curve for a prognostic NPD value in patients with cystic fibrosis (area under the ROC curve, 78.2%)

Discussion

In the majority of cases, there is no difficulty in diagnosing CF. However, there may be a wide variation in signs and symptoms among individuals that encourages the scientific community constantly to improve the diagnostic tests available and develop better methods to establish a final diagnosis (1, 15).

The detection of mutations in the CF gene and the measurement of transepithelial bioelectric properties associated directly with mutations have broadened the spectrum of CF enormously in recent years. Although NPD and intestinal current measurements are being employed extensively, studies on this topic are scarce especially in children (14, 15).

Our data showed that the NPD measurement is a valuable tool for distinguishing children with CF from healthy children and those with other chronic obstructive lung diseases.

A significant correlation between the *CFTR* genotypes causing a severe form of the disease and lower body height-for-age and weight-for-age percentiles as well as higher sweat chloride concentrations and NPD values was found. Most studies employing NDP measurements have also demonstrated that patients with an abnormal NPD tend to develop more severe pulmonary disease and carry CF-causing mutations more frequently (1, 21).

Our data are in line with the results reported by Delmarco et al. (22). Their data showed that more negative baseline NPD values from CF patients (mean NPD –39±8 mV, n=37) correlated with a higher sweat chloride concentration. In non-CF patients, the mean NPD value was –15±4 mV and it significantly differed from that of CF patients (n=24, $P<0.0001$).

Some investigators reported even more negative NPD values as diagnostic for CF patients (14, 15, 23). Knowles et al. (12, 17, 24) demonstrated that the NDP values in healthy individuals were around –20 mV, whereas in patients with CF, the values were more negative, i.e., –50 mV. According to the results by Hoffman et al. (25), the NPD measurement is a valuable procedure to discriminate CF patients from healthy controls and non-CF diseased patients. The cut point for a possible diagnosis of CF should be –40 mV.

Though the mean NPD value in the general CF group was –28.0±10.2 mV and was significantly more negative than in other study groups, it was still lower than now accepted value (–30 mV) as sufficient for the diagnosis of CF (16, 17). The mean NPD value was even less negative in the CF subgroup with the unrecognized genotypes, but according to our results, the difference between this subgroup and control groups as well as other diagnostic parameters was statistically significant and it is obvious evidence for NPD measurements to be a good and suitable test for the diagnosis of CF.

Only in the CF subgroup with the identified genotypes, the mean NPD value was negative enough and suitable for an accurate diagnosis of CF. In agreement with our results, Groman et al. (26) reported that individuals with the CF phenotype in the absence of *CFTR* mutations had NPD values distinct from those for CF patients with 2 *CFTR* mutations.

Recently, it has been reported that patients with mild or atypical forms of CF are found to have intermediate values; therefore, considering sweat chloride concentration values, the attitude toward their diagnostic range is changing especially in cases with milder forms of the disease (15, 22, 27, 28). For example, Pradal et al. (29) hypothesized that the *CFTR* gene mutations causing mild CF (R117H, A455E, 27789+5G>A or 3849+10 kbC>T) could be associated with a delay in the formation of bronchiectases, congenital bilateral absence of the *vas deferens* development before birth and less negative NPD values.

It is evident that consensus agreement on the borderline NPD values in the diagnosis of CF is needed. Considering our results in the CF subgroups, patients with possible CF symptoms and NPD values more negative than –24 mV should be consistently examined and may be treated as having mild CF.

A carrier frequency for the most frequent F508del mutation of the *CFTR* gene in Lithuania (1 in 118) is lower than in other North Europe countries (1 in 38 in Denmark and 1 in 66 in Sweden) (10); therefore, the probability of this mutation is lower in our patients. The support to this suggestion is that the *CFTR* genotype in our study was confirmed only in 17 of the 50 CF patients.

Furthermore, no *CFTR* gene mutations causing a mild form of the disease that might be associated with the development of congenital bilateral absence of the *vas deferens* were found in our population. In the future, we expect to identify new genotypes in our patients, both with classic and atypical CF phenotypes.

Referring to the data available from our study, we suggest that none of atypical CF case should be omitted. In some cases, the CF phenotype is obvious, but sweat test results are uninformative (borderline). The NPD measurement and the entire *CFTR* gene sequencing should be considered in such cases.

On the other hand, despite numerous studies those have sought to assess the role of NPD as a diagnostic tool in CF, the test has neither been standardized nor validated for diagnostic accuracy

(1). In Europe, the application of the NPD method as a diagnostic tool has been implemented by individual CF centers without centralized procedures. We still lack rigorous case-control studies to prove the sensitivity and specificity of NPD for the diagnosis of CF and to define the best cut-off point for differentiating CF patients (various genotypes and phenotypes) from diseased controls and healthy subjects (15).

Conclusions

NPD measurement is a valuable tool in the diagnosis of CF in children, but further studies are necessary to establish NPD values associated with the CF genotype and to reduce intrasubject variability of this test.

Statement of Conflicts of Interest

The authors state no conflict of interest.

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