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Nasal potential difference in cystic fibrosis patients presenting borderline sweat test

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ABSTRACT: The diagnosis of cystic fibrosis (CF) can be difficult if the sweat test and routine deoxyribonucleic acid (DNA) analysis are inconclusive. Under these circumstances, measurement of nasal potential difference (NPD) was proposed as a complementary diagnostic tool, as demonstrated in subjects bearing the G551S or 3849+10KbC→T mutations. The purpose of the present study was to verify the diagnostic value of this technique in CF patients with a borderline sweat test.

NPD was measured in 18 patients with a borderline sweat test, in whom CF diagnosis was based on the presence of one CF gene mutation in each chromosome (CF borderline). These patients were compared both to non-CF controls and CF patients with an abnormal sweat test (CF controls).

Basal NPD values of CF borderline patients (mean value -39±6 mV, range -29 to -52 mV; n=18) were in the pathological range of CF controls (-39±8 mV, range -28 to -57 mV; n=37), and both were statistically different from values obtained in non-CF controls (-15±4 mV, range -6 to -23 mV; n=24; p<0.0001). Mutation analysis confirmed a high frequency of the 3849+10KbC→T mutation in this group of CF borderline patients (positive in 14 out of 18 subjects), whereas other mutations, such as ΔF508, Q552X, N1303K and R1162X, were also found to be associated with this atypical CF phenotype.

These results confirm the presence of pathological values of basal NPD in CF patients with borderline sweat test, and also extend this finding to subjects bearing genotypes other than the G551S and 3849+10KbC→T mutations. The present findings, therefore, confirm the usefulness of measurement of basal nasal potential difference in all those patients in whom diagnosis of cystic fibrosis can be suspected but the sweat test remains inconclusive.

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Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians [1]. The basic defect of CF leads to altered ion and water transport across respiratory epithelia [2], which ultimately results in chronic lung inflammation and respiratory failure. The impaired ion transport of CF respiratory epithelial function can be studied *in vivo* by measuring the potential difference in nasal mucosa (NPD), a noninvasive technique first proposed by KNOWLES *et al*. [3] and, subsequently, by others [4, 5]. NPD makes it possible to discriminate CF patients from normal subjects, CF obligate heterozygotes, and patients with a variety of respiratory disorders [4], so that the technique has been proposed as a useful diagnostic tool in CF [6].

In clinical practice, measurement of NPD is seldom necessary, since the sweat test provides an accurate diagnosis of CF in most instances. However, patients with symptoms consistent with the disease in whom the sweat test results in normal or borderline values still represent a major clinical problem. Since CF gene mutations on both chromosomes may not be found in all CF patients, with a false-negative rate ranging 10–30% depending on the geographical area, CF disease can sometimes only be suspected from clinical status, pancreatic function, chronic lung disease, *etc*. [7]. In these subjects, the possibility of a simple complementary technique overcoming the limitations of the sweat test and mutation analysis would be extremely useful. Abnormal NPD values have been reported in CF homozygotes carrying the G551S or the 3849+10KbC→T mutations on at least one chromosome and presenting a normal sweat test [8–10]. It must, therefore, be determined whether a normal sweat electrolyte concentration in the presence of abnormal ion transport in the respiratory nasal epithelium of some CF subjects is associated only with the presence of G551S or 3849+ 10KbC→T mutations.

In view of these considerations, we undertook this study to assess whether the simple basal NPD measurement could be helpful in achieving a correct diagnosis in atypical cases of CF. We measured basal NPD in a group of patients with borderline sweat test, who carried a known CF gene mutation on both chromosomes.

Methods

Subjects

Informed consent was obtained from all patients or from their parents, when necessary. The study was approved by the ethics authorities of our institution.

The subjects evaluated in the present study were divided into three groups: Group 1 ("CF controls") comprised 37 patients with typical CF (17 males and 20 females; median age 22 yrs, range 11–38 yrs). Diagnosis was confirmed by at least two abnormal sweat tests, according to GIBSON and COOKE [11], as well as compatible clinical features and genotype, when possible. In these patients, the sweat Cl^- concentration was >80 mmol·L^{-1}.

Group 2 ("CF borderline") comprised 18 patients with CF diagnosed by deoxyribonucleic acid (DNA) analysis (9 males and 9 females; median age 24 yrs, range 9–30 yrs). These patients had clinical symptoms suggesting CF but a borderline sweat test. Patients were enrolled from the out-patient clinic of our centre and were selected following sweat Cl^- values <70 mmol·L^{-1} on at least three occasions, independently of Na^+ concentration. In these patients, pancreatic exocrine function was studied by measuring the stool concentration of chymotrypsin and the level of faecal fats over 72 h, as well as, in most cases, by pancreatic stimulation test with secretin/pancreozymin-cholecystokinin oktapeptide and output determination of enzymes and bicarbonate. Routine clinical evaluation included: 1) lung function tests performed according to standard techniques and procedures and compared to reference values [12]; 2) chest radiograph evaluated by means of the CHRISPIN and NORMAN [13] score; 3) sputum culture for microbiological evaluation; and 4) nutritional assessment according to the Cystic Fibrosis Foundation Guidelines for CF patients; in particular, the weight-to-height ratio (Wt/Ht), representing the actual weight expressed as a percentage of the ideal weight for height, age and gender, was considered to determine the nutritional status of these patients [14].

Group 3 ("non-CF controls") comprised 24 subjects selected from patients admitted to our centre for other well-documented diseases, and normal volunteers (14 males and 10 females; median age 26 yrs, range 12–43 yrs). In these subjects, the sweat test was always within reference values. The presence of the 13 most frequent CF gene mutations detected in our geographical area (see DNA analysis) was excluded in these subjects.

Sweat test

The sweat test was performed according to GIBSON and COOKE [11]. Provided that more than 50 mg of sweat were collected on a 3.2×3.2 cm filter paper, abnormal values were assumed for Cl^- concentrations ≥70 mmol·L^{-1} (Group 1). A borderline or normal sweat test was considered when Cl^- concentrations were <70 mmol·L^{-1} or <40 mmol·L^{-1}, respectively [7, 15–17]. Each test consisted of two reproducible determinations. Patients with normal or borderline results repeated the sweat test at least three times at different periods, and the mean Cl^- concentration was considered.

DNA analysis

The cystic fibrosis transmembrane (conductance) regulator (CFTR) gene mutations ΔF508, R1162X, 2183AA → G, N1303K, 3849+10KbC→T, G542X, 1717-1G→A, R553X, Q552X, G85E, 711+5G→A, 3132delTG, and 2789+5G→A were tested by combined assays, as detailed previously [18]. These mutations are known to account for 85% of the chromosomes of CF patients of our geographical area [18].

NPD measurement

In order to avoid possible faulty NPD recordings, exclusion criteria for measurement of NPD were: the presence of nasal polyps; acute upper airway infection; and previous nasal surgery [3, 19]. Young children were also excluded, as this technique is difficult to perform in that age group. NPD was measured according to ALTON and co-workers [5]. Briefly, an Ag/AgCl exploring electrode (SLE Instruments, South Croydon, Surrey, UK) was positioned into a Foley catheter filled with electrode gel and connected to a high impedance voltmeter (Beckman, Schiller Park, IL, USA). The instrument's calibration provided accuracy at all levels of electrical activity. The second reference Ag/AgCl electrode was positioned on the patient's forearm, after adequate skin abrasion, and then connected to the voltmeter. Calibration was performed previously, considering as acceptable offset values ±5 mV. The Foley catheter was then introduced into one nostril and advanced and slowly withdrawn along the floor of the nose, in order to detect the point of maximum potential difference (PD). The maximum value had to be stable for at least 10 s, and the procedure was repeated at least four times in each nostril.

The values recorded were averaged for each nostril and the mean value between nostrils was calculated. The total duration of the procedure was approximately 15 min. Statistical analysis involved Mann-Whitney test for unpaired data in order to compare NPD values between the three groups. A p-value of less than 0.05 was considered significant.

Results

Individual values of sweat electrolytes, NPD, genotype and main clinical characteristics of patients with CF and borderline sweat test are reported in table 1. All patients showed sweat Cl^- concentrations <70 mmol·L^{-1}. Sweat Na^+ was ≤70 mmol·L^{-1} in all but one of the patients. In this subject (patient no. 7 in table 1) the $[Na^+]/[Cl^-]$ ratio was higher than 1, *i.e.* not typical for CF. Moreover, none of the subjects in the present study showed a sum of sodium plus chloride sweat concentration higher than 140 mmol·L^{-1}, which would be considered typical for CF patients [20, 21].

In the majority of these subjects (14 out of 18), the 3849+10KbC→T mutation, which is known to be associated with pancreatic sufficiency and normal or borderline sweat test [22, 23], was detected on at least one of the two chromosomes. Furthermore, the 3849+10KbC→T mutation CF borderline subgroup showed pancreatic

Table 1. – Clinical features and genotype of 18 cystic fibrosis (CF) patients with borderline sweat chloride

Pts No.	Sex	Age yrs	NPD mV	Sweat weight mg	Sweat Cl^- $mmol \cdot L^{-1}$	Sweat Na^+ $mmol \cdot L^{-1}$	Genotype	Pancreatic function	Wt/Ht %	Sputum culture	FEV_1 % pred	CXR score
1	M	23	-42	212	64	51	ΔF508/Q552X	PI	98	*P. aeruginosa*	98	10
2	M	30	-52	128	44	38	G542X/3849	PS	104	*P. aeruginosa*	52	13
3	M	20	-44	131	67	53	R1162X/R1162X	PI	79	*P. aeruginosa*	30	20
4	M	25	-30	238	53	56	ΔF508/3849	PS	86	*S. aureus*	33	22
5	F	24	-39	54	61	57	ΔF508/ΔF508	PI	89	*B. cepacia*	31	12
6	M	25	-39	333	67	70	ΔF508/N1303K	PI	87	*P. aeruginosa*	39	17
7	F	16	-35	118	60	77	W128X/3849	PS	107	*B. cepacia*	27	25
8	F	17	-36	112	53	58	1717-1G→A/3849	PS	76	*B. cepacia*	54	24
9	M	10	-43	86	47	56	ΔF508/3849	PS	87	*S. aureus*	117	3
10	F	24	-40	103	66	63	ΔF508/3849	PS	89	*P. aeruginosa*	41	20
11	F	28	-35	84	67	66	ΔF508/3849	PS	76	*B. cepacia*	40	16
12	F	26	-29	106	40	54	ΔF508/3849	PS	104	*P. aeruginosa*	57	10
13	M	28	-33	158	48	44	ΔF508/3849	PS	109	*P. aeruginosa*	68	9
14	M	24	-36	115	42	49	ΔF508/3849	PS	105	*P. aeruginosa*	32	18
15	F	9	-42	80	41	43	G542X/3849	PS	94	*P. aeruginosa*	60	13
16	M	27	-47	131	66	65	R1162X/3849	PS	92	*P. aeruginosa*	56	16
17	F	22	-35	191	49	46	ΔF508/3849	PS	84	*P. aeruginosa*	47	13
18	F	22	-38	106	56	52	R1162X/3849	PS	94	*P. aeruginosa*	72	12
Mean		22	-39	138	54	55			92		53	15
SD		6	6	68	10	10			10		24	6

Pts: patients; M: male; F: female; NPD: nasal potential difference; 3849: 3849+10KbC→T; PI: pancreatic insufficiency; PS: pancreatic sufficiency; Wt/Ht: weight as a percentage of ideal weight for height; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *B. cepacia*: *Burkholderia cepacia*; FEV_1: forced expiratory volume in one second; CXR: chest radiography (score computed according to [13]).

sufficiency. Interestingly, other CF borderline subjects were carrying mutations usually associated with pancreatic insufficiency, such as ΔF508, N1303K, Q552X, and R1162X.

All patients had lower airway colonization by pathogens usually detected in CF patients, *i.e. Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia*.

On the whole, this group showed moderate airway obstruction, with a mean forced expiratory volume in one second (FEV_1) of 53±24% predicted, ranging from severe airway impairment (FEV_1 27% pred) to normal values (FEV_1 117% pred). Only two patients out of 18 showed normal lung function. Three patients showed mild airway obstruction, as indicated by FEV_1 ≥60% pred. Seven patients showed moderate airway obstruction, with FEV_1 ranging 40–60% pred. In six patients, FEV_1 was <40% pred, reflecting severe airway impairment. Chest radiographic studies were also characterized by abnormal findings usually observed in CF.

When considering nutritional status as expressed by the Wt/Ht ratio, three patients showed moderate malnutrition (Wt/Ht 75–79%), six patients were underweight (Wt/Ht 85–89%), and in nine patients Wt/Ht was within normal limits (90–110%). Considering all these parameters, the subgroup of CF borderline subjects bearing the 3849+10KbC→T mutation differed consistently from the other subgroup only by the presence of pancreatic sufficiency.

All NPD measurements were well-tolerated and performed with minimal discomfort to the subjects. Reproducibility of results was tested in 35 patients with CF, 16 control subjects and 18 borderline patients of the present study. The mean coefficient of variation (CV) within subjects for each test was 6.4 for CF patients, 9.5 for controls, and 10.5 for the borderline group. When the test was repeated for three times on alternate days, the mean CV was 8.9 and 10.5 for 9 CF patients and 10 control subjects, respectively. Figure 1 shows NPD values obtained in the three groups of subjects. From this figure, it is evident that, despite nonpathological sweat Cl^- concentrations, patients from Group 2 had NPD values typical of CF patients with an abnormal sweat test. In fact, NPD values in non-CF controls ranged -6 to -23 mV (mean value -15±4 mV), and were statistically different from those obtained in CF patients with an abnormal sweat test, ranging -28 to -57 mV (mean value -39±8; $p<0.0001$), as described previously [4, 5]. CF patients with a borderline sweat test had NDP

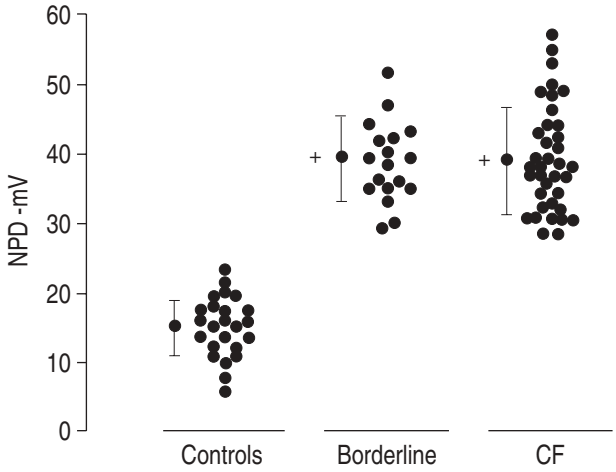

Fig. 1. – Values of nasal potential difference (NPD) obtained in non-CF control subjects (Controls), patients with cystic fibrosis and a borderline sweat test diagnosed by means of deoxyribonucleic acid (DNA) analysis (Borderline), and CF controls with abnormal sweat electrolytes (CF). Mean±SD are represented beside the data for each group. +: $p<0.0001$, with respect to non-CF controls. CF: cystic fibrosis.

values in the pathological range of our study population, *i.e.* more negative than -28 mV (mean value -39± 6 mV, range -29 to -52 mV). Therefore, in these patients, NPD values were statistically different from those obtained in non-CF subjects ($p<0.0001$).

Discussion

The results of this study show that measurement of baseline NPD can detect CF patients bearing different gene mutations, presenting clinical features compatible with CF and a borderline sweat test. We chose to study only patients with a well-defined genotype, with the purpose of verifying NPD measurement in an unequivocal CF condition. In fact, at present, only DNA analysis can be considered as a gold standard to assess the diagnostic value of NPD in doubtful cases of CF. Even if measurement of NPD has been advocated as a complementary diagnostic test in patients with a doubtful diagnosis of CF [6], at present, little experience is available in such cases. For instance, to our knowledge, this is the first report of pathological NPD values in a group of CF patients with a borderline sweat test and carrying CF gene mutations other than 3849+10KbC→T and G551S.

Previous papers have provided evidence that these two CFTR mutations can be associated with a non-pathological sweat test and abnormal NPD values. In 1991, STRONG *et al.* [8] described two sisters carrying the G551S mutation, who showed a normal sweat test, clinical features suggesting mild CF, and abnormal bioelectric properties of the nasal epithelium. HIGHSMITH *et al.* [9] studied 23 patients carrying the 3849+10KbC →T mutation, with clinical features suggesting CF but with normal sweat electrolyte concentrations; in eight patients, NPD was measured and revealed abnormal bioelectric features. Similar findings were reported by STEWART *et al.* [10], who studied two patients with CF with the ΔF508/3849+10KbC→T genotype, in whom NPD values were found to be in the pathological range.

In the present study, 14 out of 18 CF patients carried the 3849+10KbC→T mutation. However 4 of the 18 had different gene mutations. From our results and those of others, we cannot state that the detection of abnormal NPD values is conclusive for a diagnosis of CF in all circumstances, but there is a growing body of evidence to suggest that the CF nasal mucosa is both representative and specific of the basic electrolyte defect expressed on the surface of the CF bronchial epithelium. In this respect, NPD measurement can be considered a useful and attractive diagnostic tool, mainly because of its reliability, simplicity and noninvasive nature, particularly in those cases in which a diagnosis of CF can be suspected in the absence of sweat gland involvement and positivity of CF gene mutations.

In this study, patients with a borderline sweat test were selected, considering a cut-off value for sweat chloride of 70 $mmol \cdot L^{-1}$. In our laboratory, about 900 sweat tests are currently performed every year, with more than 20,000 tests over a 32 year experience, and we consider values >70 $mmol \cdot L^{-1}$ to be consistent with a diagnosis of CF, on the basis of previously reported data [7, 17]. Patients with sweat chloride concentrations below this cut-off level require further consideration, in agreement with the suggestions of HALL *et al.* [15] and KIRK *et al.* [16].

In the present study, we evaluated basal NPD according to the methods and procedure suggested by ALTON and co-workers [5]. This technique is easy to perform, rapidly carried out, and usually well tolerated. With this procedure, it is possible to discriminate clearly between classic CF and non-CF patients, as we observed in this study population. In our experience, NPD values obtained in CF patients and control subjects are, in fact, similar to those of previous studies in which this method was applied [4, 5]. However, it is worth mentioning that it is important that the individuals performing this test should be well-trained, in order to avoid false-positive and -negative results.

Another procedure was proposed to evaluate Cl^- secretion and Na^+ transport when measuring NPD after perfusion with different solutions containing ion channel inhibitors and activators [24, 25]. The method is rather difficult and takes more time to perform; and we use it for research purposes. Moreover, skilled operators with a great deal of experience are necessary to carry out the procedure reliably. It is noteworthy that the procedure makes it possible to evaluate different components of ion transport across the respiratory epithelia, and it must be stressed that, in some circumstances, such as the attempt to normalize ion transport with gene transfer or other therapies, the procedure remains of major importance. However, in the clinical setting, the possibility of obtaining useful diagnostic information with a test which is easier to perform is of significant interest. In this context, it is noteworthy that in previous studies it was found that patients with a normal sweat test and abnormal NPD after superinfusion with various drugs also had abnormal basal or maximal NPD values [8, 9]. The results of these studies, as well as the present findings, suggest that, at least for clinical purposes, measurements of basal NPD provide diagnostic information in patients with suspected CF and a nondiagnostic sweat test.

In conclusion, the results obtained in this study and the subsequent clinical implications can be summarized as follows. Firstly, in the patients with clinical features suggesting cystic fibrosis and with a borderline sweat test, basal values of nasal potential difference were in the pathological range, in agreement with diagnosis confirmed by deoxyribonucleic acid analysis. These patients were characterized by different genotypes. Therefore, it is reasonable to suggest measurement of nasal potential difference in all cases of doubtful diagnosis, particularly when sweat test and deoxyribonucleic acid analysis are not conclusive. Secondly, so far, assay of the basal nasal potential difference appears sufficient to detect cystic fibrosis patients with a borderline sweat test. However, the measurement of nasal potential difference after superinfusion with various drugs remains of major importance in research studies, in particular to assess the effect of gene transfer, but does not appear to be mandatory when used as a complementary diagnostic tool in atypical cases of cystic fibrosis.

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