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Short Communication

Nasal potential difference outcomes support diagnostic decisions in cystic fibrosis

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Abstract

Background: When cystic fibrosis (CF) is suspected Nasal Potential Difference (NPD) measurements are proposed to support controversial diagnosis: we investigated appropriate outcomes at the CF Centre of Verona.
Subjects/methods: NPD were measured in 196 subjects: 50 non-CF, 65 classical CF (the reference group) and 81 with uncertain CF (case group). Discriminating power was determined by comparison between several outcomes from the CF reference group versus non-CF: basal, amiloride, 0Cl, isoproterenol, ATP, Delta-amiloride, Delta-0Cl, Delta-isoproterenol, Delta-ATP, Delta-isoproterenol + Delta-0Cl, Wilschanski Index (WI) and Sermet score (SS). The most appropriate cut-off values for variables with the best discriminating power were then applied to the case group. Descriptive statistics, logistic regression models and ROC curve analysis were applied.
Results: WI and SS were the most powerful in discriminating CF from non-CF subjects. In the reference group sensitivity of the 0.82 WI cut-off was 98%, specificity 96%; both sensitivity and specificity of the −0.44 SS cut-off value were 100%. For the case group, WI and SS were, respectively, consistent with CF diagnosis in 94% and 92% of the cases.
Conclusions: Formulae have the highest discriminating power and can support the diagnosis in uncertain cases; they should be utilized for standardized interpretation of NPD for diagnosis and possibly for clinical research.

Keywords: Nasal potential difference measurements; Cystic fibrosis transmembrane conductance regulator; Cystic fibrosis; Diagnosis; CFTR related disorders; Sweat test; CFTR genetic test

Cystic fibrosis (CF) is the most severe, autosomal recessive disease in the Caucasian population, affecting approximately 1 among 2500–3600 individuals with frequency of healthy carriers approximately 1 among 25–30 individuals.

"Classical" forms of CF cause respiratory and digestive symptoms, with progressive lung damage often leading to respiratory failure. In the "non-classical" forms, respiratory symptoms are slight, if present at all, and their progression is slow. Pancreatic exocrine function is often sufficient and sometimes only a single organ is affected, as in cystic fibrosis transmembrane conductance regulator (CFTR)-related disorders [1]. In these 'non-classical' forms sweat test and genotype are often inconclusive.

Since NPD [2] and intestinal current measurements (ICM) [3] in rectal biopsies are used to evaluate CFTR function, these tools are valuable when sweat-testing and genotyping results are inconclusive. Following the latest diagnostic Standard

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Table 1
Main characteristics of subjects.

		Healthy/carriers	CF	Uncertain diagnosis
		N = 50	N = 65	N = 81
Gender	M	12 (24%)	28 (43%)	39 (48%)
	F	38 (76%)	37 (57%)	42 (52%)
Age at NPD (years)		41.4 (23.9–54.0)	28.4 (8.2–52.3)	30.0 (6.1–62.8)
Age at diagnosis (years)			0.4 (0–40.3)	26.2 (0.1–59.4)
Chloride		–	102 (31–144)	47.5 (9–132)
Pancreatic status	PI	0 (0%)	51 (78%)	5 (7%)
	PS	50 (100%)	14 (22%)	68 (93%)
FEV1%			43 (13–104)	93 (22–131)

Operating Procedures (SOPs) for both ICM and NPD, available from the European Cystic Fibrosis Society, is essential. Moreover it will be important to utilize common outcomes for these tests.

This study was designed to test the capability of NPD outcomes to distinguish CF patients from non-CF patients in a cohort of subjects with uncertain diagnosis.

1. Population

A total of 65 patients with classical CF (28 males and 37 females), 34 non-CF subjects (9 males and 25 females) and 16 healthy carriers (3 males and 13 females) were considered as reference group, with a median age at the time of NPD assessment of 34.7 years (12.2 to 56.0). 81 subjects with uncertain diagnosis were included in the case group, 39 males and 42 females, with median age at NPD assessment of 34.2 years, range 8.0–66.0 (Table 1). Non–CF subjects were healthy volunteers without CFTR genetic test available. All groups had more females than males according to volunteer's availability with non-homogeneous distribution. Difference in main characteristics according to gender was tested, non differences were detected.

Written informed consent to participate in the study was obtained in accordance with the rules of the local Ethics Committee (prot. 1606).

2. NPD measurements

We measured NPD in accordance with accepted standards [4,5]. The potential difference was measured between an agar-filled exploration catheter on the nasal mucosa and a subcutaneous reference agar-bridge (21-gauge needle) in the forearm. Both nostrils were examined with an otoscope; the inferior turbinate was explored for measuring at the site of the most negative voltage using an endhole catheter (Marquat, France) connected to a calomel electrode [6].

We acquired characteristic electro-physiological tracings for classical CF and healthy controls. Responses to the various membrane ion-exchange modulators were analysed to identify which index was most discriminating for diagnosis of CF and to calculate diagnostic thresholds.

We considered values in basal conditions and after perfusions with amiloride, low Cl^- solution (0Cl), isoproterenol (means of a 10 s period when the signal was stable for 30 s for each perfusion) and ATP (peak value within 1 min after starting the ATP phase). We calculated the responses to each perfusion expressed as the difference (delta) between the voltage value obtained at the end and at the beginning of each phase (Table 2). We calculated means of both nostrils; when $\Delta 0Cl^- + \Delta Iso$ differed more than 3 mV the nostril with highest value was considered. Wilschanski Index (WI) and Sermet Score (SS) were calculated as previously described: $WI = \exp.[(\Delta Iso + \Delta 0Cl\text{-})/\Delta Ami]$; $SS = -0.11 * (\Delta 0Cl^- + \Delta Iso) - 0.05 * \Delta Ami$ [7,8].

3. Statistical analysis

Various indices correlated with the NPD parameters were analysed (with the statistical software SAS, version 9.2) to identify the variable that best discriminated between CF and non-CF subjects. For each parameter a logistic regression model

Table 2
Descriptive statistics of the NPD variables.

	Healthy/carriers N = 49 Median (range)/(Mean (SD))	CF N = 64 Median (range)/(Mean (SD))	Uncertain diagnosis N = 81 Median (range)/(Mean (SD))
BASAL	−19.7 (−62.3 to −6.2)/−23.1 (12.6)	−40.4 (−70.6 to −12.6)/−39.3 (14.4)	−24.2 (−64.6 to −8.5)/−25.5 (11.0)
AMI	−11.6 (−48.7–3.0)/−13.3 (9.2)	−18.6 (−55.3–2.0)/−19.6 (9.9)	−11.6 (−51.7 to −3.5)/−14.7 (10.0)
$0Cl^-$	−18.1 (−50.9 to −2.0)/−20.4 (12.1)	−16.7 (−56.9 to −2.6)/−18.2 (11.0)	−13.3 (−64.6 to −1.5)/−16.2 (11.7)
ISO	−28.6 (−63.0 to −3.3)/−29.2 (14.7)	−15.0 (−56.9 to −2.5)/−17.8 (10.4)	−18.9 (−65.9 to −1.9)/−23.1 (15.4)
ATP	−29.9 (−72.5 to −3.6)/−32.0 (14.9)	−17.2 (−62.0 to −2.6)/−20.10 (11.4)	−18.0 (−68.0 to −1.2)/−23.3 (15.5)
DELTA AMI	9.0 (2.2–24.5)/9.12 (4.7)	18.3 (3.7–42.6)/18.7 (9.4)	10.4 (−0.6–29.1)/10.9 (5.3)
DELTA Cl^-	−5.5 (−29.0–7.2)/−7.0 (8.3)	3.8 (−6.5–11.5)/3.4 (3.6)	0.1 (−24.0–10.4)/−1.6 (6.5)
DELTA ISO	−8.7 (−24.8–9.8)/−8.4 (6.7)	0.5 (−4.3–7.6)/0.4 (2.7)	−4.5 (−34.1–8.6)/−6.8 (7.6)
Delta ISO + Delta 0 Cl^-	−14.3 (−45.5–1.2)/−15.6 (11.1)	3.6 (−8.1–19.1)/3.8 (5.2)	−5.7 (−47.4–14.5)/−8.4 (12.8)
Delta ATP	−1.2 (−32.9–8.0)/−1.9 (5.9)	−1.7 (−11.8–4.1)/−2.6 (2.9)	−0.3 (−7.6–16.1)/−0.3 (2.6)
WI	0.2 (0.0–1.2)/0.3 (0.3)	1.2 (0.8–6.9)/1.6 (1.1)	0.5 (0.0–3.4)/0.8 (0.7)
SS	−115.9 (−450.8–43.0)/−127.0 (112.3)	127.8 (43.9–378.9)/134.5 (61.5)	0.2 (−2.3–4.7)/0.38 (1.44)