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Nasal potential difference: a clinical diagnostic test for cystic fibrosis

E.W.F.W. Alton, D. Currie, R. Logan-Sinclair, J.O. Warner, M.E. Hodson, D.M. Geddes

Nasal potential difference: a clinical diagnostic test for cystic fibrosis. E.W.F.W. Alton, D. Currie, R. Logan-Sinclair, J.O. Warner, M.E. Hodson, D.M. Geddes.
ABSTRACT: Patients with cystic fibrosis (CF) demonstrate a markedly more negative potential difference (PD) across respiratory epithelia than normal or "diseased" controls. A technique is described for the measurement of nasal PD in both children and adults. 145 non-CF subjects showed a mean PD of -19.0 mV (range -2 to -36) in comparison to 60 patients with cystic fibrosis with mean of -46.0 mV (range -32 to -77). Amongst the latter group those with more severe disease had a more negative PD. Measurement of nasal PD is easily learnt and rapidly performed and may provide an additional means of diagnosis for CF.
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The sweat test remains the single most important procedure for the diagnosis of cystic fibrosis (CF) since its introduction in 1959 [1]. In children, repeated studies have confirmed that 98–99% of homozygous cystic fibrosis children have sweat chloride and sodium levels well above 70 and 60 $mmol \cdot l^{-1}$, respectively [2–4]. However, it is accepted that the investigation is fraught with errors and should only be undertaken by specialist units with suitably trained personnel. Sweat electrolyte content is increased in normal adolescents and adults compared with children and there is a considerable overlap with the cystic fibrosis cases. Clearly, in equivocal situations, it would be useful to have a second technique available which could be easily learnt without special training. Such a test should at least match the sensitivity and specificity of the sweat test and be related as closely as possible to our present understanding of the defect in cystic fibrosis.

In 1981, KNOWLES *et al.* [5] described a technique for the measurement of nasal potential difference. They demonstrated that for recordings from the inferior surface of the inferior turbinate no overlap of values existed between a population with CF and any other group studied [6]. We [7] and other workers [8] have been able to reproduce these results to varying degrees, but the technique was not suitable for use as a routine diagnostic test. In particular the degree of co-operation required of the subjects, coupled with the difficulty in learning the procedure, preclude its clinical use.

We have recently described measurements of potential difference from the floor of the nasal cavity as a means of discriminating adult CF patients from normal or "diseased" controls [9]. With further modifications we have developed a technique suitable for use in children of all ages as well as in adults, which is easily and quickly learnt. We report here on the assessment of its diagnostic use in 205 subjects comprising patients with known CF, and "diseased" or normal controls.

Materials and methods

Subjects

Two hundred and five subjects were studied:
1) One hundred and forty five normal or "diseased" controls. Mean age 33.2 yrs (range 4 mths to 73 yrs) with 64 females and 81 males. These included 64 normal volunteers and 41 subjects with persistent daily purulent sputum production including patients with bronchiectasis and Young's syndrome. No patient was studied during an acute exacerbation. The remainder (n=40) included patients with asthma, hypertension, sarcoidosis, diabetes mellitus, coeliac disease, Kartagener's syndrome, hay fever, normal pregnancy and known CF heterozygotes.
2) Sixty patients with an established diagnosis of CF. All fulfilled accepted criteria of an abnormally elevated sweat sodium, as well as characteristic radiological and clinical abnormalities. The mean was 19.0 yrs (range 2–58 yrs), 34 being female. Severity of disease ranged from patients requiring frequent hospital admission to those seen only at regular outpatient follow-up. The mean forced expiratory volume in one second (FEV_1) of the group was 53% and mean forced vital capacity (FVC) 68% of predicted values.

To record intra-subject variability repeated measurements were made in 7 normal volunteers, with a mean of 6 readings in each subject over a period of 7 wks.

nostril and the tip of the catheter passed along the floor of the nose (fig. 1) without direct vision. The catheter was advanced until the maximum PD value had been passed (approximately 4–6 cm inside the nose), and then

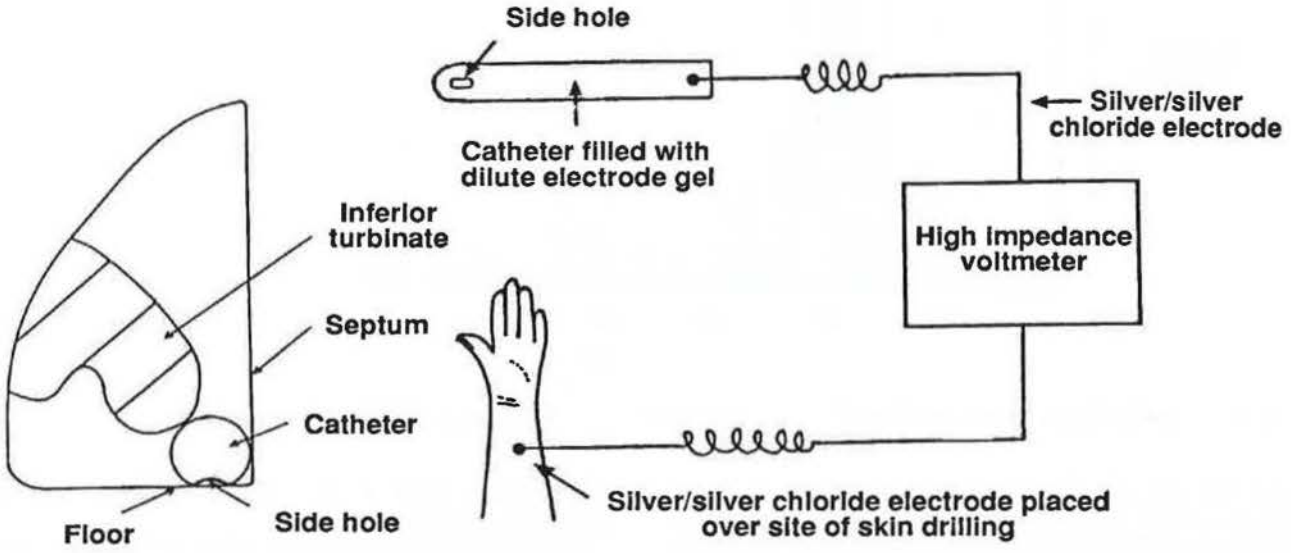

Fig. 1. – Schematic representation of equipment for recording nasal PD and anterior view of right nasal cavity showing site of measurement of PD. PD: potential difference.

Materials

The exploring electrode was a Foley size 8 rubber urinary catheter (Arterial Medical Supplies) filled with diluted electrode gel (electrocardiograph electrode cream/ Ringers saline 1:2 by volume). This contained a silver/ silver chloride electrode (SLE Instruments) with a wire connection to a high impedance voltmeter (World Precision Instruments) (fig. 1). The catheter was used without inflation of the balloon, one hole at the tip being closed with cyanoacrylic glue, and the other used for measurements. To create a suitable site of zero potential for the reference electrode, the epidermal surface of the skin of the forearm was abraded [10] using a hand-held motor unit (SLE Instruments), containing a diamond-tipped dental burr (Hi Di 541, Ash Instruments).

This procedure lasted approximately 5 s and was completely painless. The reference silver/silver chloride electrode was taped over this site and dilute electrode gel injected through the hole in the electrode to allow electrical contact.

Prior to use, the electrode were calibrated by placing the reference electrode in close contact with the exploring electrode in the catheter. Values >±5 mV were judged unacceptable and the electrodes replaced. Suitable adjustments were made to recorded values for this offset. To ensure correct functioning of the equipment prior to insertion into the nose, the potential difference (PD) of the tip of the index finger was noted, acceptable values ranging from -30mV upwards.

A plastic auroscope speculum was inserted into one

slowly withdrawn whilst readings were noted from the voltmeter. The maximum (most negative) stable PD (±1 mV over 10 s) was recorded and readings repeated between 2 and 4 times in each nostril. Each measurement was rounded to the nearest whole mV and a mean taken for each nostril. An overall mean maximum value was then calculated as the average of the two means. The total duration of the procedure was approximately 10 min.

The first 94 patients were studied using our previously described technique with a subcutaneous cannula as a reference electrode [9]. Thereafter all measurements were made using the technique described above. Concurrent measurement of nasal PD in four subjects using the two types of reference showed a mean difference of approximately 1 mV. An overall comparison of recordings using the two types of reference showed: subcutaneous -18.3 mV, SD 4.9, n=74; abraded surface -19.8 mV, SD 6.4, n=71, p=NS).

The Student t-test was used to assess differences between means, the null hypothesis being rejected at $p<0.05$. Goodness-of-fit to a normal distribution was assessed by the Lilliefour test, and correlation calculated using the Pearson product-moment correlation. Significance of the latter was assessed using the Fischer one-tail test.

Results

Values for maximal PD in the control population formed a normal distribution around the mean of -19.0 mV (range -2 to -36) with 95% confidence limits of -7.6

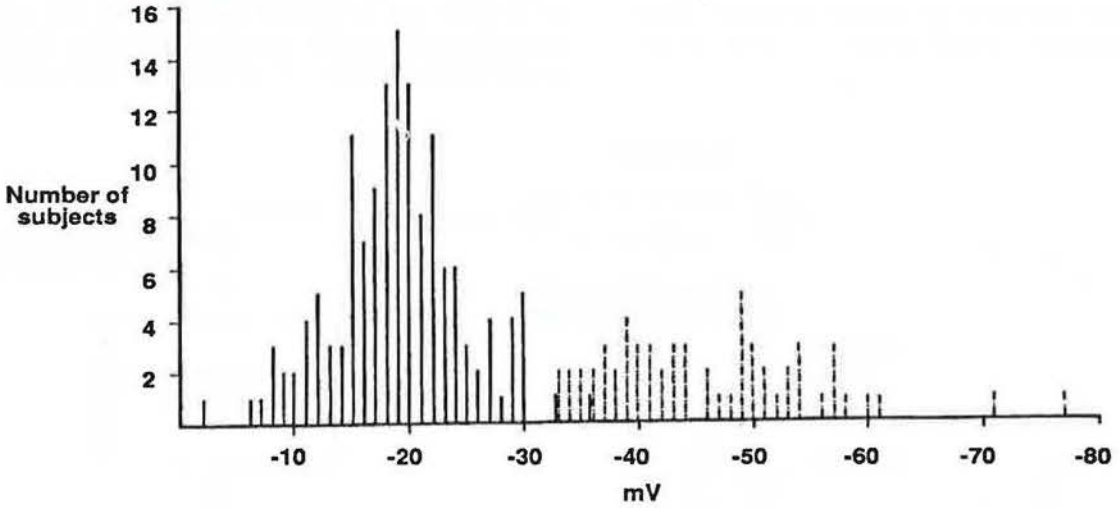

Fig. 2. – Frequency distribution of nasal PD in CF (– · – ·) and controls (——). PD: potential difference; CF: cystic fibrosis.

and -30.4 mV (fig. 2). No significant difference was noted between left or right sides, the mean intra-subject difference between nostrils being 3.6 mV (SD 3.5, range 0–13). Neither the age (r=-0.06) or sex of the subject significantly influenced the measurements, nor was there an effect of concurrent therapy with β_2 agonists, inhaled steroids, ipratropium bromide, amiloride, frusemide or β-blockers.

Within the control population the normal volunteers (n=64) had a mean maximal PD of -19.0 mV (SD 5.1) and range -8 to -30 mV, not different from the value for "diseased" controls (n=81) of -19.0 mV (SD 6.2) and range -2 to -36 mV. There was no overlap in values between the normal volunteers and the CF population. Patients with chronic bronchial sepsis (n=41) had a mean PD of -18.9 mV (SD 7.0) and range -6 to -36 mV with two subjects demonstrating values within the CF range. One of these patients had principally upper lobe disease with sweat sodiums of 89 and 91 on two occasions, but in whom values fell to 64 with fludrocortisone suppression. The other repeatedly grew *Pseudomonas* from sputum culture, and had a sweat sodium of 54. Interestingly, asthmatics (n=10) had a lower mean PD (-16.8 mV, SD 5.2), than either of the above groups, although this did not reach significance (p=0.1).

The mean maximal PD amongst patients with CF formed a normal distribution around a mean of -46.1 mV (range -33 to -77) with 95% confidence limits of -27.7 and -64.5 mV. Again the age or sex of the patient, or drug therapy had no effect on measured values. There was no significant correlation between nasal PD and sweat sodium (r=-0.005, n=33) nor with a past history of meconium ileus. However, disease severity as measured by percentage predicted FEV_1 (r=-0.27, n=53, p<0.05), FVC (r=-0.26, n=53, p<0.05) and weight (r=-0.37, n=41, p<0.01) was inversely correlated with nasal PD.

Intra-subject variability recorded in 7 normal volunteers showed a 2 standard deviation variation of 6.4 mV around a mean of -20.1 mV (42 readings) with a coefficient of variation of 16%.

It was not possible to record values in 7 subjects. Six were children aged 18 mths to 10 yrs in whom either the catheter would not fit into the nose, or it was not possible to maintain stable values because of poor co-operation. With respect to the latter, particular difficulty was noted amongst children around the age of 2 yrs. One adult disliked the sensation of the catheter within the external nares and would not allow a measurement to be made.

Data for 4 patients with CF were excluded from analysis. In 3, readings were made at the time of an acute upper respiratory infection, previously shown to markedly reduce values [11]. The fourth had a history of 5 recent operations for nasal polyps with recurrence at the time of testing. The epithelial lining of polyps *in vivo* is known to produce a lower than normal PD [12], and surgery further reduces these values [8]. All four of these patients had values within the non-CF range. Two were retested after resolution of the acute infection and showed typically raised values (-53 and -66 mV).

Discussion

Since the first elegant recordings of nasal potential *in vivo* in humans by KNOWLES *et al.* [5], two other groups have repeated this work. SAUDER *et al.* [8] were able to reproduce the absolute discrimination of values between patients with CF and any other group using an identical technique to the original authors. However, they concluded that the complexity of the technique and "the need for meticulous attention" probably precludes its use in preference to the sweat test. In our hands [7], using the technique of KNOWLES *et al.* [5], a significant difference was noted between the groups, but there was also a considerable overlap of values. Perhaps more importantly we found the investigation technically difficult, and

uncomfortable even for the most co-operative adult volunteers. We have therefore modified the technique to allow for its possible use in the routine clinical diagnosis of CF.

With the use of a surface electrode the reference area of zero potential can be painlessly created, and the connecting silver/silver chloride wires remain *in situ* despite the movements of children. Potentials are measured along the floor of the nasal cavity by a soft flexible tube with a rounded tip. This causes a tickling sensation but is not in any way uncomfortable. The catheter fits between the midline septum and the medial border of the inferior turbinate, and passes "naturally" along the chosen area of measurement without recourse to direct visual inspection. The need for expertise is markedly reduced by this means, and the technique may be quickly and easily learnt.

In order to validate this technique we have studied 145 normal or "diseased" controls and 60 patients with CF. Amongst the former, disease groups were chosen to include patients with persistent daily purulent sputum production likely to be considered as having CF. Values for both the control and CF populations fell into normal distributions. The sensitivity of measurements in comparison to the sweat test was 100% (allowing for exclusion for acute infections and previous surgery) and specificity 99%. No correlation was noted between PD values and the sex, age or drug therapy of any patient, nor amongst CF patients with sweat sodium performed either concurrently with the nasal PD or at anytime during their lifetime. However, perhaps importantly, objective measures of disease severity namely FEV_1, FVC and body weight were inversely related to PD. This lends support to current thought implicating the abnormal control of chloride [13] and sodium [14] channels, to causation of pulmonary pathology.

It is important to stress the possible problems that may be encountered with this technique. It is well recognized that the epithelial lining of nasal polyps exhibits a much lower PD than normal nasal epithelium both *in vivo* and *in vitro* [12]. Thus, falsely low values may be recorded in these cases. Secondly, we have previously shown that acute upper respiratory tract infections may markedly lower PD [11], and therefore recordings should not be made during such an episode. Finally, because readings are not taken under direct vision, and because of frequent nasal asymmetry, the catheter may measure PD from varying sites in the area bordered by its tip. An asymmetry of up to 13 mV may be recorded between left and right sides, although this value is usually between 0 and 5 mV. In 2 patients this has resulted in one side showing high normal values and one in the CF range. Our experience suggests that in such cases only the highest recorded value should be used. This is also true for patients with CF amongst whom occasionally only "islands" of high voltages may be found, presumably related both to squamous metaplasia from repeated infections and trauma, as well as the presence of polyps.

Recently the CF gene has been identified [15–17] and the predicted sequence for the protein established. Homology with other membrane proteins suggests it may be involved in ion channel regulation, a finding in agreement with documented abnormalities in the control of both sodium and chloride transport [18]. Nasal PD may therefore directly reflect the basic defect in CF. The above technique allows for easy and rapid measurement of nasal PD which may be of value both in the routine diagnosis of CF, and in patients with inconclusive sweat tests or other conflicting data.

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Différence de potentiel nasal: un test clinique pour le diagnostic de la fibrose kystique. E.W.F.W. Alton, D. Currie, R. Logan-Sinclair, J.O. Warner, M.E. Hodson, D.M. Geddes.

RÉSUMÉ: Les patients atteints de fibrose kystique (CF) ont une différence de potentiel (PD) nettement plus négative au travers les épithélia respiratoires que les contrôles normaux ou malades. Nous décrivons une technique de mesure de la PD nasal applicable aux enfants et aux adultes. Chez 145 sujets sans CF, la PD moyenne est de -19.0 mV (extrêmes: -2 à -36), alors que cell de 60 patients atteints de CF est en moyenne de -46.0 mV (extrêmes: -32 à -77). Dans ce dernier groupe, les sujets les plus atteints ont une PD plus négative. La mesure de la PD nasale est aisée à apprendre et rapidement réalisée; elle peut constituer une technique complémentaire pour le diagnostic de la CF.

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