TECHNIQUES AND PROCEDURES

Nasal Potential Difference Test to Diagnose Cystic Fibrosis

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Cystic fibrosis is usually diagnosed based on suspicion arising from a typical clinical picture and must be confirmed by either a finding of high chloride concentrations in sweat tests on 2 separate days or detection of 2 gene mutations. The nasal potential difference (NPD) test has been proposed to provide evidence of abnormal function of the cystic fibrosis transmembrane conductance regulator (CFTR), a receptor that forms a chloride ion channel. The test is especially useful for patients who have normal chloride concentrations in sweat tests and in whom 2 gene mutations related to cystic fibrosis have not been detected. The NPD test requires 2 electrodes connected to a voltmeter (a Tholy-Medicap® device). One is placed on the nasal mucosa of the inferior turbinate and the other is placed subcutaneously on the forearm. A reading less than –40 mV is considered abnormal, as values under that cut point are never found in healthy individuals. Two abnormal NPD findings on separate days are required for a diagnosis of CFTR dysfunction. False negatives arise when the integrity of the epithelium is altered. After application of amiloride, NPD decreases more markedly in cystic fibrosis patients than in healthy individuals and applying isoproterenol or fenoterol after amiloride provokes no response in patients with the genetic defect that prevents chloride ion channel activation.

Key words: *Cystic fibrosis. Diagnosis. Nasal potential difference.*

Prueba de la diferencia de potencial nasal para el diagnóstico de la fibrosis quística

En la gran mayoría de los pacientes con fibrosis quística (FQ), el diagnóstico se sospecha por unos síntomas clínicos típicos y debe confirmarse mediante la determinación en sudor de una concentración de cloro elevada en 2 días separados o mediante la identificación de 2 mutaciones en un estudio genético. Para evidenciar el anormal comportamiento de la proteína de membrana CFTR (*cystic fibrosis transmembrane conductance regulator*), encargada del transporte de cloro, se ha ideado la prueba de la diferencia de potencial nasal (DPN), especialmente útil en pacientes con concentraciones de cloro normales y en los que no se identifican las 2 mutaciones del gen de la FQ. Para la realización de la DPN se requieren 2 electrodos conectados a un voltímetro (dispositivo de medida Tholy-Medicap®), uno colocado sobre la mucosa nasal del cornete inferior, y otro en el tejido celular subcutáneo del antebrazo. Un valor inferior a –40 mV se considera patológico. Los valores obtenidos en sujetos sanos no sobrepasan nunca este valor. Se precisan 2 determinaciones anormales de DPN registradas en 2 días separados para aceptar la disfunción de la CFTR. Pueden observarse falsos negativos cuando la integridad del epitelio está alterada. En la FQ, tras la aplicación de amilorida la diferencia de potencial se reduce de modo más llamativo que en sanos, y la aplicación de isoproterenol o fenoterol después de amilorida no provoca respuesta debido al defecto genético que impide la activación de los canales de cloro.

Palabras clave: *Fibrosis quística. Diagnóstico. Diferencia de potencial nasal.*

Introduction

Cystic fibrosis, with a prevalence of 1 in 1500 to 2000 infants born in central Europe and in the United States of America, is the most common genetic disease in the white population.[1,2] In Catalonia, Spain, the prevalence is 1 in 5750 live births.[3] Researchers have known since 1985 that the cystic fibrosis gene is located in the long arm of chromosome 7, but it was not until 1986 that a group of Canadian researchers specified the genetic defect.[4] At present, however, we know that no single defect accounts for the disease: gene sequencing has identified over 1000 mutations related to cystic fibrosis symptoms,[4] although the frequency and types of mutations vary by race and ethnicity.

What is generally known is that abnormal function of the cystic fibrosis gene involves the cystic fibrosis transmembrane conductance regulator (CFTR), a receptor that forms a chloride ion channel that can be activated by cyclical adenosine monophosphate (cAMP).

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The membrane of affected ciliated epithelial cells is impermeable to chloride not because chloride ion channels are completely absent, but rather because of a genetic defect in the activation of the cAMP-regulated chloride ion channels. Abnormal regulation means that fewer chloride ions are secreted from the cell and more sodium ions are absorbed. An ionic gradient develops as the concentration outside the cell, in mucous, increases. Alternative chloride channels are regulated by intracellular calcium in both cystic fibrosis patients and healthy individuals, but they can not compensate fully for reduced chloride secretion. Patients have a greater nasal potential difference (NPD) because too much sodium is reabsorbed, with loss of positive valences in the bronchial lumen. The potential difference between nasal cells and the interstitium becomes more negative in patients. CFTR is expressed in the epithelial cells of the lung, pancreas, sweat glands, and vas deferens, where alterations occur that are associated with varying clinical signs, as ion transport does not affect all organs in the same way.

Advantages and Disadvantages of Standard Diagnostic Methods

Until the cystic fibrosis neonatal screening test was introduced, most patients—71% of whom were in the USA—were diagnosed in the first year of life, though in 8% of patients a diagnosis was not established until the age of 10 years. Differential diagnosis when respiratory symptoms are present includes primary immune deficiency, primary ciliary dyskinesia, or Young syndrome.[1,5] Testing for sodium and chloride ion concentrations in sweat after pilocarpine stimulation was introduced by Gibson and Cooke[6-8] in 1959 and has become the standard diagnostic tool, referred to as the "sweat test." We now know, however, that a negative sweat test does not rule out the disease: false positive results are found in 10% of healthy adolescents,[9] and 2% of patients with an "typical" phenotype have normal sweat tests.[2] Proof of a cystic fibrosis gene mutation affecting CFTR in such cases gives a definitive diagnosis, but such testing is time-consuming and expensive. New diagnostic methods that are more sensitive and specific are therefore being investigated to give in vivo evidence of abnormal ion transport due to dysfunction of the CFTR protein in some epithelial cell location of the organism: the NPD test is such a method. As a result, there was consensus in 1998 for a series of changes in the diagnostic criteria for cystic fibrosis.[10] Moreover, the increase in the index of suspicion thanks to the NPD test, along with more and better technical developments in genetic testing, have made it possible to detect a growing number of new and unsuspected mutations in the cystic fibrosis gene that had not previously been noted as characteristic of the disease. This has obliged us to change our ideas about cystic fibrosis and consider that it encompasses a wide clinical spectrum, increasing the number of diagnoses, which involve a growing number of adults. NPD testing was recently introduced in Spain and seems to be increasingly useful for orienting cystic fibrosis diagnosis given its high sensitivity, specificity and prognostic value.[11]

Finally, cystic fibrosis is currently looked for during the neonatal period in many countries, as immunoreactive trypsin testing figures in screening programs. Confirmation comes with the detection of 2 mutations in genetic tests or 2 positive sweat tests. Although most infants are symptom free at the moment of neonatal screening, they will later experience clinical signs of disease.

Measurement of NPD

Clinical Interest

Detection of mutations in the cystic fibrosis gene and the measurement of transepithelial bioelectric properties that arise directly from the mutations has broadened the spectrum of cystic fibrosis enormously in recent years. In 1981 Knowles and colleagues[12] developed the NPD test expressly for diagnosing cystic fibrosis.

The ciliated epithelium of the respiratory tract, including the nasal epithelium, regulates fluids on airway surfaces by way of sodium and chloride ion transport. A nasal transepithelial potential difference is generated and can be measured in vivo in millivolts as a result of differences in ionic concentrations, which will be negative for the submucosa. Basal epithelial cells are isoelectric, as are subcutaneous tissues. Therefore it is possible to take subcutaneous tissue at any part of the body as a reference to establish transepithelial potential difference.

Abnormal ion transport in the respiratory epithelium of patients with cystic fibrosis is associated with NPD values that can be distinguished from those of normal individuals. That is the underlying justification for the diagnostic application of NPD testing. There are 3 traits that distinguish cystic fibrosis:

1. A greater absolute potential difference—the value is more negative—reflecting greater sodium transport across the membrane in comparison to the membrane's relative impermeability to chloride conductance. A NPD value that is evidently high is diagnostic. In healthy individuals values are around –20 mV whereas they are around –50 mV in patients with cystic fibrosis.

2. A major reduction in potential difference can be observed after perfusion of a sodium channel blocker (amiloride), a reflection of accelerated inhibition of sodium transport typical of patients with cystic fibrosis.[13,14] Sodium channel blockers like amiloride prevent selective sodium ion flow in epithelial cells after nasal inhalation. When sodium ions stay on the luminal surface of the ciliated epithelium, the positive charge increases, bringing about a decrease in the NPD, in the form of less negative values.

3. Minimal change or absence of change in NPD in response to perfusion of a chloride-free solution and a β-agonist (isoproterenol or fenoterol) on the nasal surface, reflecting lack of CFTR mediated chloride secretion.[14,15]

Sympathomimetic agents that activate adenyl cyclase and cAMP stimulate chloride channels in healthy individuals but not in cystic fibrosis patients.

The advantages and limitations of the technique are presented in Tables 1 and 2.

Material and Method

Material

Devices to measure NPD: a validated high impedance voltmeter is needed to perform the measurement procedure properly. Various devices have been used. Knowles et al[16] described the first and Hofmann et al,[17] under the direction of Lindemann, later developed one that is simpler to use (Tholy-Medicap®, Ulrichstein, Germany). NPD is measured with an electrode to capture the bioelectric signal from the epithelial surface and another reference electrode is placed intravenously or subcutaneously. Knowles et al[16] demonstrated that the ciliated epithelial cell of the nasal mucosa corresponds qualitatively to that of the bronchial mucosa. Placing the electrode on the ciliated epithelium below the inferior turbinate is easy and just as useful as it would be to place it on the bronchial mucosa. Moreover, it allows changes in NPD to be observed after the administration of local agents.

Needed Material

1. A Tholy-Medicap device for measuring NPD, with a probe to record signals from the exploring electrode and a cable to connect it to the reference electrode (Figure 1). The machine is small and can be set up wherever there is an electrical power source (either 110 or 220 V). A transformer sits between the power outlet and the device.

2. Two electrodes of silver–silver chloride (Figures 2a and 2b):

– Exploring electrode inside the exploring probe.

– Reference electrode (smaller), the tip of which can be connected to an intravenous catheter inserted subcutaneously.

The electrodes are kept moist in plastic tubes filled with a saturated silver chloride solution. After each use the solution is discarded and a new silver chloride solution is provided.

3. Isotonic saline solution (0.9%).

4. Adhesive material to fix the catheter to the skin, cotton, alcohol for disinfection, and syringes.

5. Cold light source.

6. Nasal speculum.

7. Thermal printing paper.

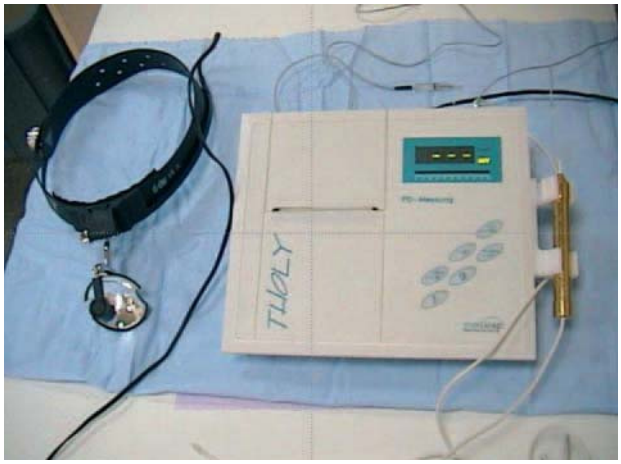


Figure 1. The Tholy-Medicap® device (Ulrichstein, Germany).

Installation: description of the measurement device. The principle the simplified Tholy-Medicap measurement device is based on is the direct connection of an exploring electrode and a fixed reference electrode to a high resistance voltmeter, so that transepithelial potential difference can be easily measured.

An integrated microprocessor and memory chip records the data and there is a built-in thermal printer.

Calibration of the voltmeter. Before a test, check that the electrodes are working and the machine is calibrated. The procedure is as follows: bring the tips of

TABLE 1
Advantages of the Nasal Potential Difference Test

It is an easily reproducible, minimally invasive technique
It provides a direct measurement of the pathophysiological mechanisms of the disease
Technical training is neither long nor costly
Tolerance for the test is excellent

TABLE 2
Limitations of the Nasal Potential Difference Test

The test is unreliable in case of:
Inflammation or nasal infection (sinusitis or acute rhinitis)
Certain treatments, such as aerosol therapy with hypertonic saline, inhaled antibiotics, amiloride and DNAse that tend to normalize the potential difference in cystic fibrosis
Repeated polyp removals, repeated nasal scarring, etc, that can alter or contraindicate exploration
The test can be difficult to carry out in case of:
A large nasal polyp that obstructs visualization
Young age: children younger than 5 years old require slight sedation and a smaller caliber catheter; however, the technique can be performed at any age
Nasal potential difference values correlate with:
The patient's clinical status—respiratory or other exacerbations depending on what system is principally involved (respiratory or digestive)
Genetic mutation

the 2 electrodes together to create a short circuit, such that the voltmeter detects no current. This indicates that the electrodes are functioning properly. If the screen shows that a few millivolts of current are flowing, press the key labeled "0" to balance the electrodes, which should still be in contact with each other. The screen should then show a value of 0 mV, whenever the electrodes are in contact.

Procedure for Measuring NPD

Preparing the apparatus and instruments. To ensure that the electrodes do not dry out, prepare all material just before performing a test. Connect the voltmeter to the power source and the electrodes. If the test can not be carried out immediately, protect the electrodes from drying out by immersing them in the solution provided for that purpose.

Preparing the patient. Disinfect an area on the outside of the patient's upper arm and using aseptic procedures, insert the catheter into adipose tissue, extract the needle from the set, and fasten the inserted line to the skin. To improve contact, inject a small amount of saline solution (2 mL) into the catheter. Then connect the catheter to the reference electrode. That catheter stays in contact with subcutaneous tissue infused with saline solution (because the catheter is filled). The screen should register a current of a few millivolts that will fluctuate when the electrode is moved, possibly alternating between negative and positive values. Should that not be the case, fill the catheter with saline solution again.

Measuring NPD. Precise anatomical placement of the exploring electrode is essential. Bend the tip approximately 20°, depending on the shape of the nose, without touching the ceramic part of the electrode. Aided by the special nasal speculum equipped with a

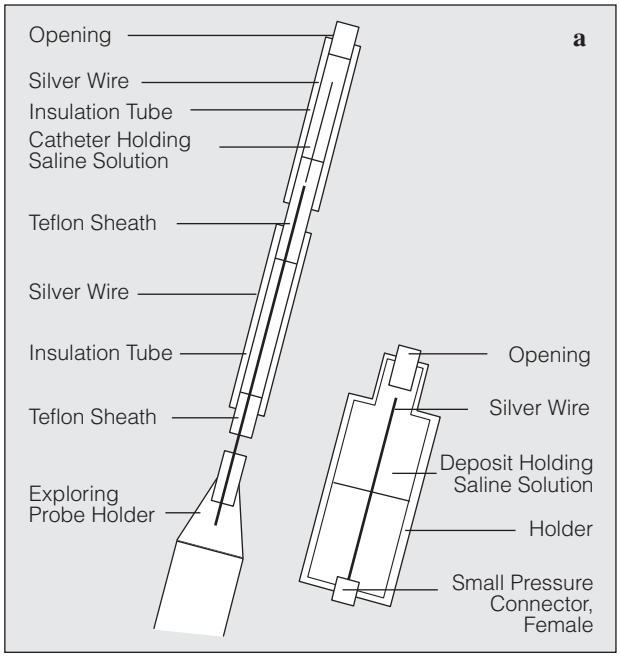
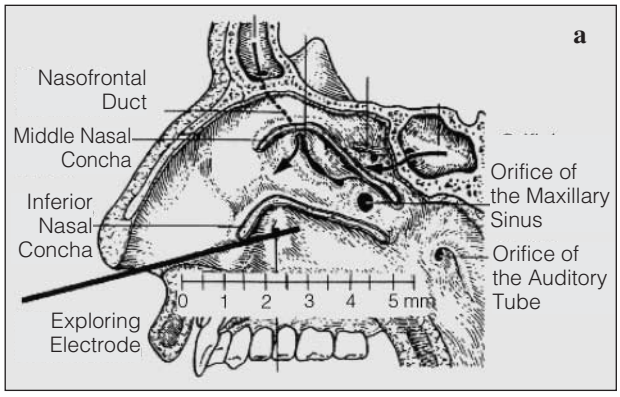


Figure 2. The drawing (a) shows the 2 electrode assemblies: the longer one is the exploring probe that makes contact with the nasal mucosa and the shorter one is inserted subcutaneously or intravenously. In (b), observe how the electrodes are connected to the Tholy measurement device.



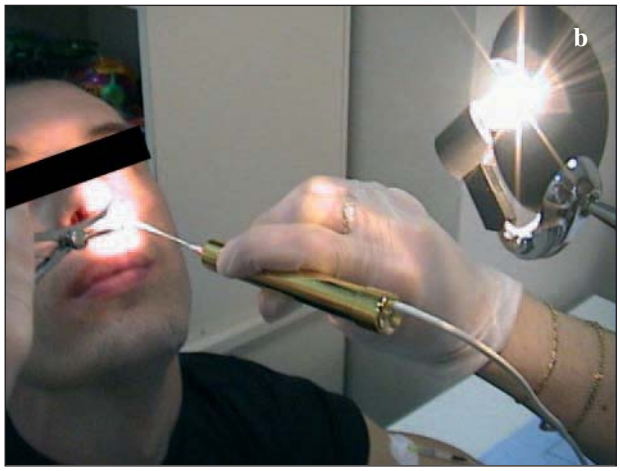


Figure 3. Drawing showing (a) the nasal structure and path of the exploring electrode until it comes in contact with the inferior turbinate (concha), and (b) the position of the patient during the measurement procedure.

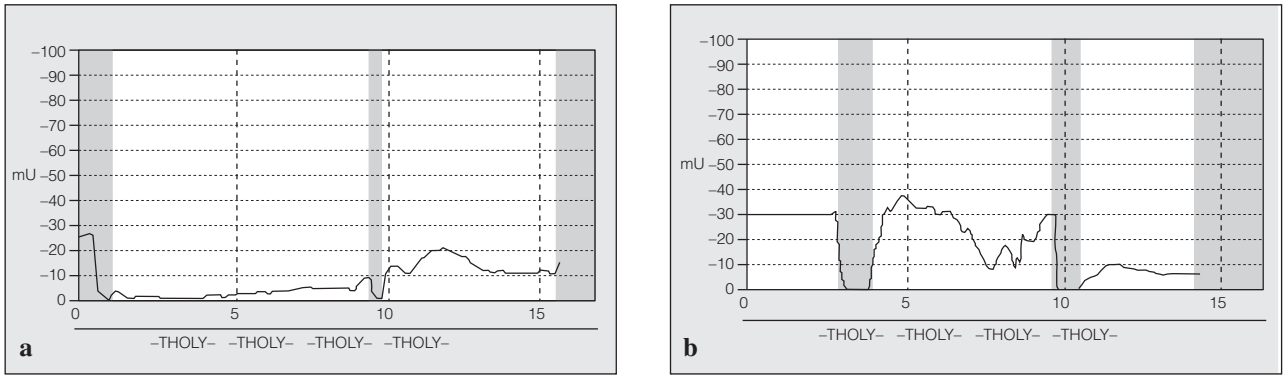


Figure 4. The curve formed by a plot of NPD measurements is as important for supporting a diagnosis of cystic fibrosis as are the potential difference values themselves. The first tracing (a) shows the flat curve of a healthy person. The second (b) shows a curve typical of a patient with cystic fibrosis.

cold light source, examine the interior surface of the patient's nose. The patient should keep still and recline slightly so that the inferior turbinate can be recognized (Figures 3a and 3b). Introduce the exploring electrode approximately 3 to 5 cm into the nose, until the tip can be seen to be under the inferior turbinate. As soon as measurement starts, upon touching the posterior part of the inferior turbinate with the electrode tip, the screen should begin to show values between –5 and –10 mV. If that is not the case, adjust placement of the tip and/or recalibrate the machine. If the machine is properly calibrated, check that the exploring electrode is really in contact with the nasal mucosa. Move the electrode slightly to maintain maximum contact with the mucosa, searching for the point where measured values are greatest and remain stable for at least 2 seconds. When that happens, press the key labeled "End" to obtain a data printout. It is best to have a second person ready to end measurement so the person holding the exploring electrode can keep it in contact with the epithelium. If contact is lost before the "End" key is pressed, the maximum NPD will not be recorded. This procedure should not take longer than 15 to 20 seconds for each nostril. The procedure is repeated in the other nostril with the same electrode. Only the direction of the angle at the tip needs to be changed.

The shape of the curve in the graphic display (Figures 4a and 4b) is as important as the differential value itself. NPD values foreseen are of the order of 0 to –100 mV. If these values are exceeded, the placement of the exploring and reference electrodes should be checked and the procedure repeated.

Interpreting Results

As with any laboratory test for confirming a diagnosis, the NPD procedure should be repeated at least once if it is to be considered diagnostic. Furthermore, any laboratory that plans to establish NPD testing as a diagnostic tool should carry out a sufficient number of studies to provide reference values and guarantee rigor. Between 300 and 400 determinations would be necessary.[18]

Hoffman et al[17] reported on 312 determinations of NPD in cystic fibrosis patients and 269 in healthy controls. The controls had a mean (SD) value of –22.1 (7.3) mV whereas the patients' mean value was –58.8 (13.7) mV. The cut point for a possible diagnosis of cystic fibrosis would be –40 mV. Values higher (less negative) than –30 mV would not support such a diagnosis. Values between –30 and –40 mV would indicate that a test should be repeated at least once, especially if there is infection present.

Values suggesting disease for only one nostril would be insufficient for diagnosis. False negatives can occur in cystic fibrosis patients when the integrity of the epithelium is altered; values in control subjects, on the other hand, are never below –40 mV. When results are inconclusive, it should be possible to distinguish between healthy individuals and those who have cystic fibrosis by applying isoproterenol or fenoterol after amiloride (an agent that blocks sodium flow and the accumulation of positive valence in the lumen). The potential difference then decreases in all persons but more markedly in patients with cystic fibrosis.

Isoproterenol or fenoterol is applied next to induce chloride secretion through cAMP-dependent channels. Patients with cystic fibrosis show no response to fenoterol because the channel can not be activated, or can be only partially activated, because of the genetic defect.[14,15]

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