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Original Article

# Improved repeatability of nasal potential difference with a larger surface catheter

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**Abstract**

*Objective:* To increase the power of nasal potential difference (NPD) as a biomarker of CFTR function, improvement of its repeatability is needed. We evaluated the improvement in repeatability resulting from measuring NPD (1) over a larger surface area and (2) at a fixed location.
*Methods:* To assess repeatability, NPD was measured on two occasions with a new method using a larger surface catheter at fixed locations on the nasal floor (LSC-floor$_{5cm}$ and LSC-floor$_{3cm}$) or at the most negative basal potential (LSC-floor$_{max}$); with a sidehole catheter on the nasal floor at 5 cm from the nasal margin (SHC-floor$_{5\ cm}$) or at the most negative potential (SHC-floor$_{max}$); and with an endhole catheter below the inferior surface of the lower turbinate at the most negative potential (EHC-turb$_{max}$).
*Results:* The within-subject standard deviation ($S_w$) for repeated measurements of the total chloride response in the controls was smallest with the LSC-floor at a fixed location (LSC-floor$_{5\ cm}$ 3.1 mV; 95% CI 2.3–4.6 mV) and highest with the SHC-floor (SHC-floor$_{max}$ 14.6 mV; 95% CI 10.9–22.2 mV) or the EHC-turbinate (EHC-turb$_{max}$ 12.5 mV; 95% CI 10.7–23.0 mV) at the most negative basal potential. Measuring with the LSC-floor at the maximal potential increased the $S_w$ (LSC-floor$_{max}$ 8.8 mV, 95% CI 6.0–16.1 mV, p = 0.009 vs LSC-floor$_{5\ cm}$), while measuring with the SHC-floor at a fixed location slightly decreased the $S_w$ (SHC-floor$_{5\ cm}$ 9.8 mV, 95% CI 8.9–20.6 mV, p = 0.06 vs SHC-floor$_{max}$). In patients with cystic fibrosis, the $S_w$ was comparable, between 2.2 mV and 4.3 mV. Sample size calculations for trials using NPD to assess changes in ion transport showed that the number of subjects to be included could be approximately halved measuring with the larger surface catheter at a fixed location vs SHC or EHC at fixed locations.
*Conclusion:* Measuring the NPD at a fixed location and over a larger surface resulted in increased repeatability and thereby also power as a biomarker of CFTR modulation.


*Keywords:* Surrogate endpoint; CFTR function; Biomarker

## 1. Introduction

In patients with cystic fibrosis (CF), defective chloride transport through the cystic fibrosis transmembrane conductance regulator (CFTR) is the initiator of a pathophysiological cascade leading to respiratory disease. The first therapy targeting the basic CF ion transport defect was recently licensed. Ivacaftor improves chloride transport [1] and also improves lung function, increases weight and decreases the exacerbation rate in patients harboring the G551D mutation [2]. Therapies targeting the CFTR defect in patients with other CF mutations are under development. Lumacaftor aims to increase the amount of CFTR protein at the

*Abbreviations:* CF, cystic fibrosis (CF); CFF, the Cystic Fibrosis Foundation; CFF-TDN, Cystic Fibrosis Foundation Therapeutic Development Network; CFTR, cystic fibrosis transmembrane conductance regulator; ECFS, the European Cystic Fibrosis Society; ECFS-CTN, European Cystic Fibrosis Society Clinical Trial Network; NPD, nasal potential difference; SOP, standard operating procedure; Sw, within-subject standard deviation; TCR, total chloride response.

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cell membrane in F508del patients [3], and ataluren promotes the read-through of premature nonsense mutations [4].

These therapies are examples of personalized medicine because they are geared towards patients with a specific mutation class. While phase 3 efficacy trials with CFTR modulators are feasible for frequent *CFTR* mutations, such trials will be more challenging for rare mutations.

Therefore, biomarkers that predict the effects of these new therapies in patients with rare mutations are needed [5]. The nasal potential difference (NPD) directly measures the ion transport in the airways and is consistently abnormal in patients with CF [6]. In early studies, aminoglycosides [7] and ataluren [8] have been shown to improve chloride transport in patients with nonsense mutations. Treatment with ivacaftor partially corrects the chloride and sodium transport defects observed on NPD in G551D patients [1].

However, NPD measurements are highly variable [9–11], leading to decreased power to detect changes with treatment [12]. To improve the repeatability of the measurements, standard operating procedures (SOPs) were devised [13,14], the central reading of tracings during clinical trials was instituted [1], and the processing of the obtained values was optimized [15]. Despite these measures, the NPD measurement variability remains high.

The goal of the present study was to compare a new method of measuring the NPD with previously described measurement methods, with regard to repeatability. Two major changes were introduced in the measurement technique. First, the measurement surface area was increased to average the potentials from a larger area of the epithelium. Second, the repeat measurements were performed at a fixed location instead of at the spot of the most negative basal potential. Our hypothesis was that these technical modifications would increase the repeatability of the NPD measurement and thus the power to detect therapeutic effects.

## 2. Methods

### 2.1. Subjects

Patients with CF were recruited during outpatient clinic visits or during admission. The control subjects were recruited through an advertisement. All of the subjects had to be free of any acute upper airway symptoms for more than two weeks. Smokers were excluded. The ethics committee of the University of Leuven approved the study. Caregivers and/or the participants gave written informed consent. Subjects could contribute to the evaluation of more than one measurement method, but not all methods were evaluated in each subject.

### 2.2. Nasal potential difference

The NPD was measured between an intranasal agar-filled catheter and an agar-filled subcutaneous needle (21G or 23G). Potentials were recorded with calomel electrodes (Calomel Reference Electrode, Radiometer Analytical, Villeurbanne, France) connected through a head stage to a bio-amplifier (ISO-Z Isolated Head-Stage and BMA-200 AC/DC Portable Preamplifier, AD Instruments, New Zealand) and a digital recorder (Powerlab 4/30, AD Instruments, New Zealand), as described in both of the available SOPs [13,14].

The potential was measured sequentially during perfusion at 5 ml/min with Ringer's solution, Ringer's solution with 100 μM amiloride, a zero-chloride solution with 100 μM amiloride and a zero-chloride solution with 100 μM amiloride and 10 μM isoprenalin. Each solution was perfused for 3 min or until a stable potential value was obtained, whichever was longer. Solutions were not warmed, as only a minor effect is expected on the potential measurements [16].

Three main indices were calculated: (1) the total chloride response (TCR) was the sum of the change in the NPD observed after changing from the amiloride in Ringer's solution to the amiloride in zero-chloride solution plus the change after changing to the isoprenalin plus amiloride in zero-chloride solution; (2) the Ringer's PD was the NPD at the end of the perfusion with Ringer's solution; and (3) the delta NPD was the change in the NPD from the end of the perfusion with Ringer's solution to the end of the perfusion with the isoprenalin plus amiloride in zero-chloride solution.

Tracings with both the amiloride response lower than 5 mV and the TCR higher than −5 mV were considered 'flat tracings' and were not used for further analysis.

The following different methods were compared (Table 1).

1. The new 'larger surface catheter' method (LSC-floor$_{5\ cm}$): the NPD was measured with a larger surface agar-coated electrode (agar coating of 2 cm length over 360° with a diameter of 4.5 mm, Fig. 1 and Fig. S1 in the online supplement) secured with the tip 5 cm past the nasal margin.
2. SHC-floor$_{max}$: the NPD was measured along the nasal floor with an 8 Fr single lumen side-hole nasogastric catheter (Fig. 1). The solutions were perfused through polyethylene PE90 tubing ending near the opening of the measuring electrode, attached by a short silicone sheat. The catheter was secured at the site of the most negative basal potential. This measurement method is close but not identical to the ECFS-CTN SOP [13], which advocates the use of a sidehole Marquat catheter while a home-made sidehole catheter was used in the present study.
3. EHC-turb$_{max}$: the NPD was measured at the lower surface of the inferior turbinate with an end-hole 2.5-mm catheter (Marquat Genie Biomedical, Boissy-Saint-Léger, France, Fig. 1). Perfusion of the solutions was achieved through the second lumen of the catheter. The catheter was inserted under visualization with a nasal speculum and a headlamp and was secured when the location with the most negative basal potential was identified. This method is similar to the one used for the ataluren trial [8] and differs from the actual CFF-TDN SOP in two ways: the solutions were not warmed and a Marquat endhole catheter was used instead of a PE90/PE50 home-made catheter.
4. Additional methods were tested to assess the respective contributions of the two modifications — larger surface and fixed location — to the improvement in repeatability:

    ◦ SHC-floor$_{5\ cm}$: the sidehole catheter was tested with the catheter secured at a fixed location on the nasal