ELSEVIER
Journal of Cystic Fibrosis xx (2017) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Short Communication

Ratiometric sweat secretion optical test in cystic fibrosis, carriers and healthy subjects

Gabriella Bergamini [a], Gloria Tridello [b], Elisa Calcaterra [a], Stefano Ceri [c], Marco Tagliasacchi [c], Federico Bianchi [c], Federico Monti [c], Andrea Masciadri [c], Eugenia Laudanna [b], Denise Peserico [b], Elena Sorio [a], Valeria Esposito [a], Teresinha Leal [d], Baroukh Maurice Assael [b,e], Claudio Sorio [a], Paola Melotti [b,*]

[a] Department of Medicine, Section of General Pathology, University of Verona, Verona, Italy
[b] Cystic Fibrosis Centre, Azienda Ospedaliera Universitaria Integrata Verona, Verona, Italy
[c] Department of Electronics, Computer Science and Engineering and Bioengineering, Politecnico di Milano, Milano, Italy
[d] Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium
[e] Department of Pathophysiology and Transplantation, University of Milan, Internal Medicine Department, Respiratory Unit and Cystic Fibrosis Adult Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Received 14 September 2017; revised 16 November 2017; accepted 12 December 2017
Available online xxxx

Abstract

We have simplified the published procedure (5) for measuring sweat rates in individual human sweat glands.

Sweat secretion rates were obtained from sweat drops secreted on the forearm by multiple individual glands. We computed a ratio between CFTR-dependent (by intradermal microinjection of a β adrenergic cocktail) and CFTR-independent (by methacoline as cholinergic stimulus) sweat secretion rates.

We obtained a reproducible, approximately linear readout of CFTR function with measurements performed by two different independent teams. We considered three groups (CF subjects, CF carriers and non-CF controls, n = 22 in each group); their mean ratios was respectively 0.000, 0.104 and 0.205 The average ratio of CF subjects was consistent with diagnosis in 3 additional cases clinically resembling CF. All groups were clearly discriminated, with sensibility and specificity ranging from 82% to 100%. A software was developed for detecting sweat droplets.

This bioassay is suitabile for multicentre studies focusing on CFTR targeted therapies, controversial diagnosis and functional relevance of rare CFTR mutations.

Keywords: Cystic fibrosis; Sweat rate; Image analysis; Cystic fibrosis transmembrane conductance regulator; Sweat glands

1. Introduction

Sweat chloride levels, the gold standard parameter for diagnosis of cystic fibrosis (CF) [1,2,3], have shown to be robust, even not very sensitive, outcomes for molecules targeting the CF Transmembrane Conductance Regulator (CFTR) anion channel [4]. A bioassay for in vivo CFTR function describing a ratio calculated between CFTR-dependent sweating, evoked by a β-adrenergic cocktail (C-sweat), and CFTR-independent sweating, induced by cholinergic methacholine (M-sweat), was reported in small cohorts of subjects [5,6]. Secretion rates by multiple individual glands were quantified by changes of volume of sweat droplets formed under a thin oil layer covering the skin. Specificity of C-sweat induction was confirmed by full inhibition of sweat droplet formation under long-term therapy with β–blocker eye drops [7].

* Corresponding author.
E-mail address: paola.melotti@aovr.veneto.it (P. Melotti).

https://doi.org/10.1016/j.jcf.2017.12.003

Please cite this article as: Bergamini G, et al, Ratiometric sweat secretion optical test in cystic fibrosis, carriers and healthy subjects, J Cyst Fibros (2017), https://doi.org/10.1016/j.jcf.2017.12.003

Multicenter validation of in vivo CFTR assays supporting controversial diagnosis and detection of CFTR function improvement during CFTR targeted therapy require a reproducible procedure as well as reliable readouts. Personalized therapies in CF require standardized outcomes for CFTR function in vivo [8]. Two different in vivo CFTR function assays based on the ratiometric evaluation of β adrenergic- versus cholinergic-induced sweating have been developed [5,9]. The imaging version, but not the evaporimetry version, was found able to detect improvement of CFTR function by ivacaftor [6,10,11]. Data analysis of volume of droplets as initially reported [5] is labor-intensive and operator-dependent. This work was designed to assess the capability of discriminating CF patients, non-CF subjects and healthy carriers, to update the image recording setup and to automatically map single sweat droplets on the skin, for data analysis.

2. Methods

Image-based ratiometric measurements of β-adrenergic/cholinergic sweating in human sweat glands were performed following the procedure described by Wine et al. [5] with modified equipment and staining of sweat droplets during both phases of the test. The local Ethics Committee approved the study (project 304CESC) and each subject signed informed consent.

2.1. Experimental setup

In order to simplify, standardize and improve reproducibility of the recording procedure, the original equipment was replaced by purchasable items. We used a flash ring (Electronic Flash Macro EM-140 DG E0-ETTLII SIGMA, Japan) and a Kaiser RS2 (Germany) stand for image capture instead of the LED ring and camera holder developed at Stanford University [5]. The digital camera was replaced by a Canon EOS 550D Reflex fitted with a macro lens (SP AF90mmF/2.8 Di Macro 1:1, Tamron, Japan) (Fig. 1).

2.2. Staining sweat droplets with blue dye

In order to improve detection of sweat droplets, we used the blue dye (erioglaucine disodium crystals, CAS No. 3844-45-9 also known as Brilliant Blue FCF, FD&C Blue No.1, Acid Blue 9, E133) [6] for both the M and the C phases. Besides

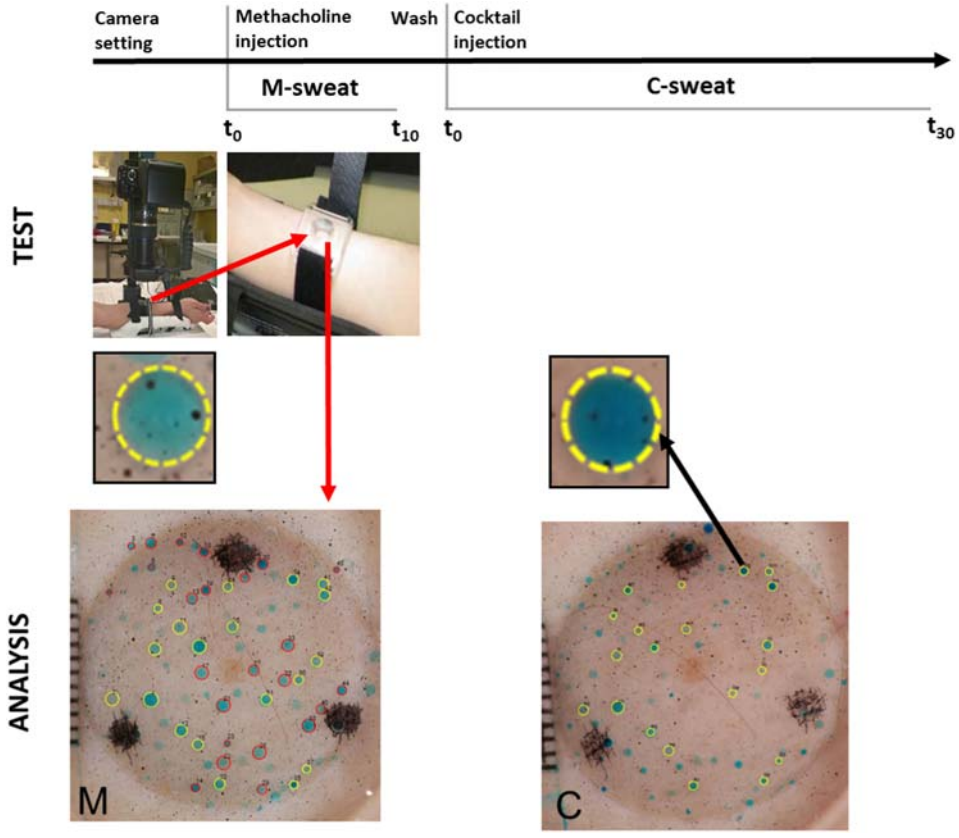

Fig. 1. Test procedure. Phases of the test: 10 min for cholinergic phase (M-sweat) and 30 min for β adrenergic induction (C-sweat) (top); equipment (middle); image analysis (bottom). Automated droplets mapping using a software (pictures on the bottom) with an enlarged sweat droplet (black arrow) from an individual sweat gland. The oil reservoir on the forearm consists of Sylgard in a hard plastic shell; it was prepared according to the method followed in Stanford (available upon request). Analysis by the software requires prior mapping of the tested site on the forearm with three black dots, usually at equal distances of a central freckle, if present. This procedure allows repeating the test on the same glands. The software gets as an input the images of the M and the C phases and performs the following tasks: 1) identify the three black markers defining the field of the test in each image; 2) find the geometrical affine transform that allows correlating the two images; 3) detect the droplets in both images; 4) compute their pairings. Software development is still work in progress: no results of automated analysis are available yet to support its performance.

Please cite this article as: Bergamini G, et al, Ratiometric sweat secretion optical test in cystic fibrosis, carriers and healthy subjects, J Cyst Fibros (2017), https://doi.org/10.1016/j.jcf.2017.12.003