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# *In vivo* CFTR function by imaged ratiometric measurement of beta adrenergic/cholinergic sweat rate in human sweat glands

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Sweat secretion rates were given by changes of volume of sweat drops secreted on the forearm in an oil layer, including the presence of a water-soluble blue dye (erioglaucine disodium crystals). We computed a ratio between CFTR-dependent, evoked by intradermal microinjection of a β-adrenergic cocktail (C-sweat), and CFTR-independent, induced by methacoline as cholinergic stimulus (M-sweat), sweat secretion rates by multiple individual glands. The analysis was performed in 22 CF patients, 22 non-CF subjects (CTR), 22 healthy carriers (HTZ) and 3 clinical cases. Sweat secretion rates were given by changes of volume of sweat drops secreted on the forearm in an oil layer, including the presence of a water-soluble blue dye (erioglaucine disodium crystals). We computed a ratio between CFTR-dependent, evoked by intradermal microinjection of a β-adrenergic cocktail (C-sweat), and CFTR-independent, induced by methacoline as cholinergic stimulus (M-sweat), sweat secretion rates by multiple individual glands. The analysis was performed in 22 CF patients, 22 non-CF subjects (CTR), 22 healthy carriers (HTZ) and 3 clinical cases. We obtained an approximately linear readout of CFTR function: the carriers mean ratio was 0.51 the value of non-CF controls while the average ratio of CF subjects was around zero. In a patient affected by CFTR related disorder we found a value in between CF and HTZ mean values. All groups were clearly discriminated with extremely significant differences of C-sweat/M-sweat ratios ($p<0.0001$ for three groups comparison). This method discriminates between CF and non-CF patients (non-CF controls and heterozygotes), providing sensibility and specificity of 100%. It discriminates between heterozygotes and non-CF controls, providing sensibility 82% of specificity of 86%. We obtained reproducible discrimination when different operators performed the test. A software was developed for detecting sweat bubbles, paving the way for automatically mapping and measuring sweat bubbles as required for automated image analysis. This bioassay is capable to clearly discriminate among non CF, healthy carriers and CF individuals at variance with Gibson and Cooke gold standard sweat chloride assay, is minimally invasive and thanks to its exquisite sensitivity and specificity appears suitabile for multicentre studies focusing on CFTR targeted therapies and to assist in the diagnosis of controversial cases. This approach can simplify the analysis and thus promote a better understanding of the functional relevance of rare CFTR mutations.

## Biography

Paola Melotti is currently working Cystic Fibrosis Center of Verona, Italy. Paola Melotti has done progressive work on "*In vivo* CFTR function by imaged ratiometric measurement of beta adrenergic/cholinergic sweat rate in human sweat glands" at University of Verona,Department of Pathology and Diagnostics.

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