A step forward for an intermediate cystic fibrosis population

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Shareable abstract (@ERSpublications)
Thao Nguyen-Khoa and colleagues investigated the benefit of using beta-adrenergic (β-adr) sweat secretion (SST) measured by evaporimetry to determine the CFTR functional status, an important report that presents a useful novel method. https://bit.ly/3MEsHhc

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Cystic fibrosis (CF) is a life-long and life-shortening disease, best treated with early interventions to prevent complications across multiple organs and to extend the lifespan of this population. In the USA and Europe, the diagnosis of CF occurs primarily through newborn screening [1]. Newborns with two defective copies of the cystic fibrosis transmembrane conductance regulator (*Cftr*) gene have elevated levels of immuno-reactive trypsinogen (IRT) in their serum. A high IRT result at birth triggers a confirmatory test for diagnosis that involves a functional assay, such as sweat chloride concentration measured by evaporimetry. Normal sweat chloride concentration by this method falls in a range of 29 $mmol \cdot L^{-1}$ or lower while a CF diagnosis is made when sweat chloride reaches values of 60 $mmol \cdot L^{-1}$ or higher [2]. However, some patients fall into an intermediate range with sweat chloride results ranging between 30 and 59 $mmol \cdot L^{-1}$. For these patients, it is not known if they are carriers of a mutation to the gene or if they have two mutated copies of the *Cftr* [3]. Because the rare mutations can be difficult to identify [4], this distinction is important.

In addition to the two options presented above, some patients may fall into a category known as CFTR-related metabolic syndrome (CRMS). Detection of these individuals has risen as newborn screening has expanded [5]. CRMS is defined as a sweat chloride concentration in the intermediate range on at least two occasions and fewer than two identified CF-causing mutations; or a sweat chloride concentration in the normal range and two CFTR mutations, which may or may not have known clinical significance. The European designation for this group is CF screen positive, inconclusive diagnosis (CFSPID) [6]. Individuals who fall into these two categories may be either carriers of the disease who may never develop symptoms, or they may develop progressive symptoms as they age. Those patients who may develop symptoms are at risk for the same life-shortening disease complications as patients with a clear CF diagnosis, and therefore would benefit from early identification and initiation of therapies [7]. Therefore, a reliable method of performing CFTR functional analysis is important to establish.

The commonly recommended CFTR functional analyses include nasal potential difference (NPD) or rectal intestinal current measurements (ICM) [8, 9]. These assays are difficult to perform and interpret and can be quite invasive or intolerable for the patients. As reported in this issue of the *European Respiratory Journal*, Nguyen-Khoa *et al.* [10] investigated the benefit of using beta-adrenergic (β-adr) sweat secretion (SST) measured by evaporimetry to determine the CFTR functional status in lieu of the difficult alternatives. In a cohort of individuals with a query diagnosis of CF (Q), SST was compared to NPD results and combined with genotyping to determine the utility in identifying patients at risk of illness. The authors here sought to determine if the response to beta-adrenergic stimulation could assess CFTR function and differentiate between carriers and those with CF or CF-Related disorders. Results were compared to NPD, ICM and extensive CFTR gene sequencing. Overall, SST showed improved discriminatory ability compared to NPD and ICM. In this study, the authors focused on patients with CF-like symptoms, but no clear diagnosis of CF. The Q subjects underwent β-adr SST, NPD and extensive analysis of the CFTR gene by next generation sequencing. The authors found that β-adr SST was very consistent with CFTR

genotypes and is therefore a useful method of discriminating between carriers and those with CFTR dysfunction, either causing CF or CRMS.

Evaporimetry offers a more accessible method of evaluation. This is a non-invasive test that can be conducted in a short period of time and relies on local administration of secretion stimulatory agents [11], reducing the risk of complications possible in NPD or ICM tests [12, 13]. Furthermore, evaporimetry is routinely conducted in clinical trials for assessment of new CFTR-targeting therapeutics [14, 15], although these outcomes focus on the traditional chloride concentration measurement. In 1984, SATO and SATO [16] reported that human sweat glands of people with CF failed to respond to adrenergic agonists, such as isoproterenol, but had normal responses to cholinergic stimulation. Therefore, applying an adrenergic cocktail, including atropine to block the cholinergic response, reveals a CFTR-dependent mechanism of secretion [17]. Used in the NPD protocol to determine CFTR-dependent potential difference [18], isoproterenol was applied to evaporimetry [11] and found to be highly reliable and accurate at determining CF-disease status, compared to traditional chloride concentrations. This method has since been applied to detecting CFTR functional defects in non-CF smokers [19]. Therefore, the current application to use this method to identify patients in the intermediate or undetermined range for diagnostic purposes is a supported step forward that is likely to be reliable and easy to implement worldwide [20].

There are some limitations to the study presented here. The sample size of the study is small, although the previous reports indicate that the data remains reliable. Additionally, 14% of the study population was excluded due to subject refusal; it is not clear if the widespread community will be similarly averse to participating, although this test remains easier than the alternatives. Additionally, all the subjects included in the study were symptomatic, while many of the patients in the community who might be in need of this level of discriminatory diagnostic method may be asymptomatic. And lastly, it is clear from the manuscript that next generation sequencing approaches may lead to the same result, or may continue to be required, especially as mutations become critical to prescription with novel therapeutics [21]. However, despite these limitations, β-adr SST remains a critical step forward to confirm or rule out a CF diagnosis in a borderline population.

Overall, this is an important report that presents a useful method to help determine patients at risk of developing CF or a CF-related disease. As the authors presented, SST showed better discriminatory ability toward diagnosis compared to NPD and ICM.

Conflict of interest: S.E. Birket has nothing to disclose.

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