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Mini-symposium: Biomarkers and Phenotype

Biomarkers in Paediatric Cystic Fibrosis Lung Disease

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EDUCATIONAL AIMS:

After reading this article the reader should be able to:

1. Describe biomarkers of cystic fibrosis transmembrane regulator function.
2. Understand the advantages and disadvantages of available sampling methods in paediatrics.
3. Know the biomarkers (available and in development) of airway inflammation, infection, and exacerbations of paediatric cystic fibrosis lung disease.

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SUMMARY

Biomarkers in cystic fibrosis are used i. for the measurement of cystic fibrosis transmembrane regulator function in order to diagnose cystic fibrosis, and ii. to assess aspects of lung disease severity (e.g. inflammation, infection). Effective biomarkers can aid disease monitoring and contribute to the development of new therapies. The tests of cystic fibrosis transmembrane regulator function each have unique strengths and weaknesses, and biomarkers of inflammation, infection and tissue destruction have the potential to enhance the management of cystic fibrosis through the early detection of disease processes. The development of biomarkers of cystic fibrosis lung disease, in particular airway inflammation and infection, is influenced by the challenges of obtaining relevant samples from infants and children for whom early detection and treatment of disease might have the greatest long term benefits.

INTRODUCTION

An important advance in the field of cystic fibrosis (CF) has been the development of drugs that improve the function of abnormal cystic fibrosis transmembrane regulator (CFTR) proteins. CFTR potentiators and correctors, and other novel drugs in development improve disease pathophysiology at the basic cellular level. As the search for and development of new drugs continues, there is a need for biomarkers of CFTR activity which can accurately quantify CFTR function early in the drug development process, for identifying treatment responders, and for dose selection.

Biomarkers are also needed for the monitoring of lung disease in infants and children. Lung disease starts early in life and progresses rapidly during childhood despite the absence of apparent clinical symptoms [1,2]. Early intervention is the key to improving long term outcomes in CF. Consequently there is a need for accurate, non-invasive biomarkers of early disease processes for disease monitoring, to aid treatment decisions and importantly, as outcome measures in clinical trials.

Biomarkers of CFTR function will be discussed in the first part of this review, and biomarkers of disease outcomes will be discussed in the second part of the review, with particular emphasis on infants and young children.

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BIOMARKERS OF CFTR FUNCTION

Cystic fibrosis is caused by mutations in the gene coding for the CFTR protein, a membrane spanning protein at the apical surface of epithelial cells. CFTR has a number of functions, including the regulation of anion transport. The development of CFTR targeted drugs represents an important milestone in the personalised treatment of patients with CF. A vital component of providing personalised medicine is the identification of biomarkers that can accurately assess the disease extent while measuring the response to treatment [3,4]. Further development of *in-vitro* biomarkers will be essential for the assessment of new and combination drug therapies in CF [5]. One topical example includes biomarkers measured in intestinal organoids, which involves the three dimensional culture and outgrowth of human and mouse intestinal crypt stem cells fashioned into organ-like structures. Forskolin can induce rapid swelling of organoids that is completely dependent on apical CFTR expression [5]. Important functional assays of CFTR include the measures of sweat chloride, nasal potential difference (NPD), and intestinal current (IC). These assays measure both CFTR presence and ion transport activity, each with unique strengths and weaknesses (Table 1).

Sweat chloride: The measurement of sweat chloride is the most commonly used biomarker of CFTR function. Sweat chloride levels in CF are elevated due to a decrease in chloride reabsorption via CFTR in the sweat ducts [6]. Cystic fibrosis sweat chloride levels are commonly used to confirm a diagnosis of CF [7,8]. Sweat chloride accurately discriminates between individuals with and without CF, and reflects, to a limited degree, the intrinsic severity of CF disease [9]. Sweat chloride responds rapidly to treatment with CFTR potentiator ivacaftor [10,11] and corrector lumicaftor [12]. The measurement of sweat chloride is non-invasive, generally easy to perform, not expensive and available in most centres. However, obtaining sufficient amounts of sweat for this test can be challenging in very young children. Reliability data for sweat chloride testing is inconclusive [9], with reports of high between-patient variability in clinical trials [13]. An often cited benefit of sweat chloride measurement is that CFTR function is assessed in an organ not damaged by the disease. However, sweat chloride levels do not appear to respond to the read-through agent, ataluren. The failure of sweat chloride levels to detect the CFTR altering effect of ataluren is thought to be due to an organ specific effect of ataluren, as ataluren use can be detected by another measure of CFTR function, nasal potential difference [14,15].

Nasal potential difference: Nasal potential difference (NPD) involves the *in vivo* measurement of voltage potential in the nose resulting from epithelial ion flux at the mucosal surface. This test provides information on both sodium absorption and chloride secretion, and reflects CFTR function directly in the upper respiratory tract [16–18]. Data on reliability of NPD suggests a large intra-subject variability [9]. In terms of validity, NPD has the ability to discriminate between individuals with and without CF [9]. In clinical trials, NPD has been shown to be responsive to ataluren [14,15], ivacaftor [10], gene therapy [9], but not lumacaftor, a CFTR corrector [12]. There are a limited number of centres with the expertise to perform NPD, and measurements are not possible when the patient has an upper respiratory tract infection, nasal polyps, or sinus issues. In infants and young children, the use of NPD measurements can be limited by the need for cooperation to perform the test.

Intestinal current measurements: A test of CFTR function that can be performed with relative ease in all age groups is intestinal current measurement through rectal biopsy. The evaluation of intestinal current (IC) involves the measure of ion flux in intestinal tissue obtained from biopsy [19–21], usually rectal biopsy. Intestinal current measurements have been shown to correlate with CFTR mutation analysis and sweat chloride levels [20]. These measurements can distinguish between individuals with and without CF, and between mild and severe phenotypes of CF disease [20,22]. The response of IC to effective treatment can be rapid [23]. Rectal tissue will also remain unchanged in the presence of airway infection or advanced airway disease, and tissue can be used *ex vivo* to study response to potential treatments [24]. The expertise to utilise IC is only available in specialised centres but standardised guidelines for its use have recently been published [25].

BIOMARKERS OF DISEASE OUTCOMES

The hallmarks for cystic fibrosis lung disease including neutrophil dominated airway inflammation, respiratory infection, and structural airway disease (small airway obstruction and bronchiectasis) can be present from infancy. Biomarkers that detect early disease processes can potentially guide treatment and

Table 1
Biomarkers of CFTR function

Test	Strengths	Weaknesses
Sweat chloride measurement	• Non-invasive and relatively easy to perform • Widely available • Discriminates between cystic fibrosis and non-cystic fibrosis with high degree of accuracy • Reflects (limited) intrinsic severity of cystic fibrosis disease • Responds rapidly to treatment • Assesses organ not damaged by cystic fibrosis disease	• Cannot detect change in CFTR function if effect is organ specific • High between-patient variability • Inconclusive reliability data
Nasal potential difference	• Reflects CFTR function in the respiratory tract • Accurately discriminated between cystic fibrosis and non-cystic fibrosis	• Large intra-subject variability • Does not detect change in CFTR function for all drugs (lumacaftor) • Not tolerated by all children • Limited use during upper respiratory tract infection or nasal pathology • Requires expertise not widely available
Intestinal current measurements	• Correlates with CFTR mutation analysis • Distinguish between mild and severe cystic fibrosis phenotypes • Can be performed in all age groups • Rectal tissue not affected by airway disease • Can be used to study disease and therapies *ex vivo*	• Undesirable procedure for some patients • Requires expertise not widely available