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Original Article

Pulmonary endpoints in clinical trials for children with cystic fibrosis under two years of age

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Abbreviations: ACT, airway clearance techniques; ALARA, as low as reasonably achievable; BA, bronchus-artery; BAL bronchoalveolar lavage' BILD, Basel Bern Infant Lung Development Cohort; BO, bronchiolitis obliterans; BPD, bronchopulmonary dysplasia; CEV, cumulative expired volume; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CFTRm, cystic fibrosis transmembrane conductance regulator modulator(s); CKD, chronic kidney disease; CLD, chronic lung disease; COPD, chronic obstructive pulmonary disease; CO_2, carbon dioxide; CPAM, congenital pulmonary airway malformation; CT, computed tomography; ECFS-CTN, European CF Society Clinical Trials Network; EMA, European Medicines Agency; Enpr-EMA, European network for paediatric research of the European Medicines Agency; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; GI, gastrointestinal; He, helium; HLH, hemophagocytic lymphohistiocytosis; hsCRP, high sensitivity C-reactive protein; HTS, hypertonic saline; IEI, inborn errors of immunity; ILD, interstitial lung disease; IRT, immunoreactive trypsin; IS, isotonic saline; LAR, low attenuation regions; LCI, lung clearance index; MACE, Mother and Child in the Environment; MBW, multiple breath washout; MRI, magnetic resonance imaging; N2, nitrogen; O_2, oxygen; PCV, pressure controlled volume; PD, pharmacodynamic; PD-w MRI, Proton Density weighted magnetic resonance imaging; pwCF, people with cystic fibrosis; PMA, postmenstrual age; RDS, respiratory distress syndrome; rhDNase, recombinant human deoxyribonuclease; SEPAGES, Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effet sur la Santé; SF6, sulphur hexafluoride; SNR, signal to noise ratio; TLC, total lung capacity; VLBW, very low birth weight.

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ABSTRACT

Cystic fibrosis is a lifelong progressive disease in which lung disease is the main prognostic factor, where starting early treatment is crucial for improving long-term outcomes. Therefore, new treatment should be available as early as possible. However, choosing appropriate and feasible clinical trial endpoints in children under 2 years of age presents significant challenges. Most studies in this age group have extrapolated pulmonary efficacy from older age groups, focusing on safety, pharmacokinetics, and biomarker response. As lung health is near normal in infants, demonstrating absence of pulmonary decline requires large sample sizes and extended study duration, which may not be feasible for standard regulatory trials. To address this gap, the European Cystic Fibrosis Society Clinical Trials Network developed a consensus document evaluating direct pulmonary endpoints for therapeutic pulmonary studies in this young age group. The pulmonary endpoints evaluated include multiple-breath washout (MBW); chest computed tomography (CT); chest magnetic resonance imaging (MRI); airway infection and inflammation. Relevant literature, pitfalls, practice guidelines, and recommendations are presented. None of the pulmonary endpoints evaluated are currently suitable to serve as a primary efficacy endpoint in children below 2 years of age, as this will require large numbers and long follow-up. For clinical trials in infants with CF, pharmacokinetics, pharmacodynamics, safety and tolerability should remain the primary endpoints, with pulmonary endpoints as secondary or exploratory outcomes. Post authorization studies are essential to evaluate long-term pulmonary benefits, including MBW, structural lung assessment (e.g. CT and MRI), and markers of pulmonary inflammation to fully understand the impact of early therapy initiation in this young population.

1. Introduction

Cystic Fibrosis (CF) is a severe life-shortening disease characterised by recurrent and chronic chest infections associated with progressive lung damage ultimately leading to respiratory failure, which is the most common cause of death [1]. Several studies suggest that even asymptomatic young children with CF, identified through newborn screening, exhibit detectable changes in lung health through various assessments [2]. This emphasises the importance of clinical trials in very young children with CF to demonstrate the safety and efficacy of new treatments, enabling early intervention to maximise life expectancy and long-term health outcomes. Evidence from CF patient registries supports this approach, suggesting that the earlier CF therapies are started, the greater the benefit in terms of lung function preservation [3] and projected life expectancy [4]. Recent therapeutic developments include the so-called CFTR modulators (CFTRm), which improve the function of the defective CF transmembrane conductance regulator (CFTR) protein. For systemic CFTRm, such as Ivacaftor, Lumacaftor-Ivacaftor, and Elexacaftor-Tezacaftor-Ivacaftor, studies in young children <2 years have primarily focussed on safety assessment and pharmacokinetics, with efficacy assessments limited to biomarker (sweat chloride), and pancreatic measures (faecal elastase and immune reactive trypsin) [5]. Pulmonary efficacy in this age group is usually extrapolated from studies performed in older children and adults.

From 1997 onwards global regulators have issued clear legislation to sponsors encouraging clinical trials in young children to directly evaluate the risk/benefit balance of new drugs to support authorisation for this subpopulation [6]. Often the submission and commitment to a paediatric investigation plan is a requisite for approval of a drug in adults (unless there are clear reasons for a waiver), giving a clear commercial incentive for appropriate studies to occur in children, previously a less revenue-generating priority for pharma.

Conducting clinical trials in children under 2 years with CF presents unique challenges, as common pulmonary endpoints used in older children and adults are not applicable. Techniques requiring cooperation, such as multiple breath washout (MBW), computed tomography (CT) and magnetic resonance imaging (MRI) are difficult due to the need for prolonged immobilization, often necessitating sedation to improve assessment quality. Infant forced expiratory volume techniques have limitations and have been largely abandoned by most paediatric research centres [7]. Additionally, many children in this age group have minimal pulmonary symptoms, complicating the detection of therapeutic changes.

To address these challenges, trials in this age group would benefit from robust validated pulmonary outcome measures that can demonstrate clinically meaningful changes in response to new CF therapies. These measures should be tolerated by young children and feasible for sponsors to implement without requiring prohibitively large studies. Guidance on the development of Paediatric Investigation Plans produced by the European Medicines Agency (EMA) explores this balance of gaining age-group specific data, against feasibility, and details the procedure for extrapolation of data from older age groups [8].

2. Rationale

The European Cystic Fibrosis Society Clinical Trials Network (ECFS-CTN) was approached by the European Network for Paediatric Research of the European Medicines Agency (Enpr-EMA) to provide guidance on pulmonary endpoints in clinical trials involving children under two years of age. EMA specifically sought advice on the feasibility of performing MBW in these trials, as well as exploring other potential assessments as pulmonary efficacy endpoints.

In response, authors from ECFS-CTN with relevant expertise were selected based on both ECFS-CTN certification for MBW, involvement in ECFS-CTN development of standard operating procedures of imaging and induced sputum and/or for publication record. Robust search strategies for each endpoint were conducted. The consensus document outlines potential pulmonary endpoints for clinical trials in children with cystic fibrosis under two years of age. The manuscript contains a summary of literature, feasibility, pitfalls/limitations, practice guidelines and recommendations for each putative endpoint.

3. Methodology

Experts included experienced paediatric pulmonologists, clinical and laboratory researchers with expertise in the specific endpoints, as well as respiratory physiology, physiotherapy, and radiology representation. A comprehensive search strategy was undertaken for each endpoint (see supplemental information). The searches yielded 833 references, of which 662 were discarded as not relevant or overlapping. The remaining 171 references were reviewed by the core group and independently assessed for quality using OCEBM Levels of Evidence [9] (See Appendix 1).

The comprehensive approach addresses the unique challenges of assessing treatment outcomes in this young patient population. The document was produced through regular web conferences and group work on a shared document, with TL, KH and HMJ producing the final draft. There were no voting rounds. All authors have approved the final version.

4. Multiple breath washout

4.1. What is MBW, what does it measure?

Over the last 15–20 years, MBW has developed as a clinical and research tool to detect and monitor early CF lung disease. MBW is performed either having a subject breathe in a trace gas mixture until it is evenly distributed in the lungs, then switching to breathing room air while a device measures the washout tracer gas over several breaths or measuring the washout of resident nitrogen (N_2) whilst the subject breathes 100 % oxygen (O_2). This method assesses the efficiency of ventilation distribution, which is heavily influenced by peripheral airway function, the primary site of gas mixing. The most commonly used outcome parameter in MBW is the lung clearance index (LCI, also termed $LCI_{2.5}$). LCI is calculated as the volume (cumulative expired volume, CEV) needed to decrease the concentration of an inert gas from the start of the washout until the end-tidal concentration reaches 1/40th of the initial concentration, divided by the functional residual capacity (FRC), CEV/FRC. A higher LCI indicates a less efficient gas mixing (or ventilation distribution) within the lung [10]. MBW is more sensitive method than spirometry to detect early CF lung disease in children, and LCI correlates with structural changes observed in both CT and MRI [10–12]. In pre-school children, LCI is predictive of later lung function at school age [13,14], correlates with inflammatory parameters in bronchoalveolar lavage (BAL) [15] and increases over time, reflecting CF disease progression [16,17]. LCI has also shown utility in detecting treatment responses to therapies, such as recombinant human DNase, hypertonic saline, antibiotics, and CFTRm, in childhood [10,18–22].

4.2. Supporting evidence, validation and summary of literature

Several studies have been performed in infants aged zero to two years using MBW as an outcome measure, including healthy children, children with CF, pre-term infants, and infants with other lung diseases (see Suppl Table 1). Overall, these studies demonstrate a moderate to good feasibility of MBW, mainly influenced by the setting in which the test is performed. While MBW is feasible during natural sleep with newborns, term infants during the first months of life and pre-terms up to a postmenstrual age (PMA) of about 50 weeks, the feasibility in older infants and toddlers is improved by light sedation. To date, most studies have been cross-sectional, but those performed with repeated measurements or in a true longitudinal fashion were mostly done in sedated infants to allow for comparable conditions for later MBW tests (See Suppl table 1). A small number of studies demonstrated that repeatability and reproducibility can be good using standardized protocols. Although most studies using LCI in infants and preschool children with CF demonstrated an abnormal LCI compared to healthy controls on a group level, the proportion of infants and preschool children with CF and an abnormal LCI varies between studies. The longitudinal follow-up within the London Cystic Fibrosis Collaboration study revealed that a majority of those infants with an abnormal LCI at 3 months remained abnormal at 12 months [23]. However, the same group found that LCI does not track well across infancy [24], and indeed LCI at 2 years may predict pulmonary function at pre-school age better than LCI at earlier infant timepoints [25,26]. MBW with LCI as an endpoint has been used in a limited number of interventional studies in infants with CF and has been sensitive in detecting treatment responses to inhalation with hypertonic saline (HTS) compared to isotonic saline (IS) [17]. However, the minimal meaningful clinical difference has not yet been established for infants.

4.3. Feasibility of MBW in children under two years of age

MBW testing in infants has generally been limited to the research setting and is performed in only a limited number of specialist centres across Europe. Even fewer perform infant MBW as part of routine care [27], but centres with experience in the technique with trained personnel have demonstrated good feasibility in this age group [28]. There is some evidence to support the feasibility of infant LCI as a primary outcome measure for efficacy in multicentre clinical trials [20], but studies across multiple sites remain challenging. The regulatory focus on safety rather than efficacy endpoints in infant CF clinical trials has been associated with less prominence of infant LCI in trial protocols, limiting knowledge and experience of the technique in this group. Measurement of infant LCI is more resource intense than measurement in older age groups, with two operators trained in infant MBW required for each test. Success rates for achieving LCI test results are influenced by testing protocol (natural sleep vs. sedation) and technical factors relating to test acceptability. Testing in natural sleep is less successful beyond the first 1–2 years of life, and re-testing may cause additional burden for families [29]. Conversely, use of sedation introduces associated risks and extends the duration of study visits (reflecting recovery in addition to testing time), as well as requiring appropriate clinical supervision. Intercurrent respiratory tract infection may influence LCI, as well as contributing to the risks of sedation, hence the importance of confirming clinical stability prior to testing. Quality control parameters for test acceptability are based on recommendations for pre-school tests in the absence of specific recommendations for infants [30]. Additionally, professional accreditation for infant MBW testing is lacking, for example in the UK this is only possible for testing at pre-school age and above. The potential opportunities for using infant LCI as an endpoint in clinical trials should be considered alongside limitations.

4.4. Pitfalls/Limitations

As MBW does not need the child to perform forced breathing manoeuvres, but is performed in tidal breathing, the method per se is suitable for infants and toddlers. There are questions around which inert gas should be used, mainly focussing on N_2 and sulphur hexafluoride (SF_6), the results of which are not interchangeable, and neither are LCI data from the different pieces of commercial equipment [30]. Therefore, normative data derived with various tracer gases also differ, and so far, there are no universal standard values. Techniques have evolved over the years, making even transition between different software versions from the same manufacturer challenging. Other possible problems specific for the age group focussed upon in this recommendation are i) the demonstrated decrease of LCI and wider variation in *healthy children* over the first 2 years of life and ii) the effect of 100 % O_2 during $MBWN_2$ on tidal breathing in infants, possibly affecting the outcome parameters. However, over recent years, correction for sensor errors in a commercially available MBW device suggests that both variability and difference between inert gases has been reduced. Two recent studies though show a closer agreement between LCI performed with SF_6 and N_2 MBW in infants with the corrected measurement of N_2 and with seemingly negligible effect on LCI of decreasing tidal volumes during 100 % O_2 in infants with CF while the decreasing LCI over the first 1–2 years remains [31]. Also, one study shows that end-expiratory lung volumes during breathing 100 % O_2 are unchanged in infants in spontaneous sleep despite lower tidal volumes [32].

The possibility of performing the test during natural sleep decreases with age and is more difficult after 12 months, leading to a discussion on when to do the test in sedation and which sedative to use. There have been two consensus statements published for older children and preschoolers describing the technical aspects that need to be fulfilled by equipment measuring MBW, but there is no recent guideline on performance of MBW in infants under 2 years of age [30,33,34]. All the above challenges need to be considered when using LCI as an endpoint in clinical studies in infants below 2 years of age.

4.5. Practice guidelines for MBW in children under two years of age

An open-circuit device, designed specifically for infants, measuring

respiratory flow and gas concentrations, is the preferred equipment to perform the MBW measurement. The equipment is more complex than for older people and the specifics of both the integrated and individual parts must be considered[35]. It is challenging, yet feasible, to achieve the desired equipment dead space volume to <2 ml/kg [20]. Monitoring of heart rate and oxygen saturation of the infant is necessary to ensure a stable tidal breathing pattern is achieved and that no side effects of sedation are present. Commercially available apparatus utilizes different methods in assessing ventilation distribution; a) a wash-in/wash-out of an inert tracer gas such as SF_6 or b) a wash-in of 100 % O_2 to measure expired resident N_2. Calibration procedures, applying good lab practice, are performed prior to testing with environmental conditions recorded. The infant is then positioned supine with the head in midline, either under sedation or in natural sleep, depending on protocol. Appropriate personnel trained in the test procedure and airway management must be present throughout to assess suitability for sedation and to monitor oxygen saturation and heart rate. Resuscitation equipment must be present. A facemask chosen according to the size of the face to allow for a maximal external dead space of 2 ml/kg body weight is positioned over the infant's nose and mouth, ensuring a leak-free seal, and is connected to the device via a filter and flowmeter [20]. A constant bias flow is set to exceed the maximum inspiratory flow so to prevent rebreathing. In the SF_6 method, the infant inspires a gas mix of (usually) 4 % tracer gas with 21 % O_2. Time is allowed for the normalization of breathing pattern and a minimal difference between end-inspiration and end-expiration of tracer gas across a minimum of five stable tidal breaths signifies adequate wash-in of tracer gas concentration within the lungs. The tracer gas is subsequently 'washed' out until the inert gas concentration falls below 2.5 % of the starting end-tidal concentration for around five tidal breaths. In the N_2 washout method, the infant breathes medical air until a tidal breathing pattern is established for a minimum of five breaths before switching to inspiring 100 % O_2 to washout the N_2. For both methods, washout continues until the gas concentration falls below 2.5 % of the starting concentration for around five tidal breaths. Dedicated analysis software displays the infant's respiratory flow rate, volume, and gas concentrations, providing data acquisition of several parameters of ventilation distribution. Minimal disturbance of the infant is essential to achieve satisfactory results. Post-testing quality control is then performed to confirm acceptability of leak-free quiet tidal breathing in at least two trials. Fig. 1

4.6. Recommendations and future directions

A coordinated research effort has advanced MBW in preschool children with CF substantially over the last couple of decades, and to a lesser extent in those aged 0–2 years. However, in parallel with the growing knowledge and skills base there have been significant improvements in diagnosis and care leading to better clinical outcomes: centre-based care provision via skilled multidisciplinary teams, emphasis on optimised infant nutrition and early interventions to maintain pulmonary health and the widespread implementation of newborn screening programmes in many regions. Infants have been largely ineligible for CFTRm (except for ivacaftor for a small proportion), so these improvements have occurred on a backdrop of conventional standards of care.

What this means is that past outcome measures which have shown clinical and/or research utility, may pose increasing future challenges, when an anticipated majority of children in this age group will receive CFTRm. A greater proportion will have normal values at baseline, meaning that group improvements from an intervention may be diluted. Some trials in older children have required an abnormal LCI at baseline (pre-treatment) providing a greater chance of detecting an efficacy signal. However, such an inclusion criterion limits the pool of available participants and adds to the burden of the trial protocol, particularly if sedation is involved. An alternative to seeking improvements would be to focus on slowing or prevention in LCI worsening longitudinally; this likely requires an extended period of observation during which the operational requirements of MBW may change, for example requirement for sedation and optimal interface as the child ages. Furthermore, interpreting a signal over time would require a well-powered control group.

There are study designs in which this may be possible, such as multicentre, high number interventional or observational studies conducted over a period of year(s). In this context, well-standardised MBW could provide a primary outcome. The PRESIS study indeed achieved significance in change in LCI with a sample size of 21 children less than 4 months of age per arm [36]. What seems less likely is that LCI would be utilised as a primary outcome in this age group within a commercially led drug study. Firstly, to date, there is increasing weight given to safety data and extrapolation of efficacy from older subjects (often with supportive pharmacokinetics / pharmacodynamics), meaning a placebo-control group can be avoided. Whilst this may be understandable for very small populations (e.g. those possessing rare *CFTR* variants), it is, in our view, disappointing that the opportunity for rigorous efficacy data is not taken. In some of these trials, MBW is an optional exploratory endpoint; this means the group size will likely be small and in the absence of a control arm, at risk of being more challenging to interpret.

The natural history of CF is changing and may do so more dramatically once very young children are treated with CFTRm. Taking opportunities, both in clinical and research settings to obtain high quality outcome measure data will underpin not only an enhanced understanding of disease course, but also the powering and design of future interventional studies.

'Real-world' data complements that arising from interventional

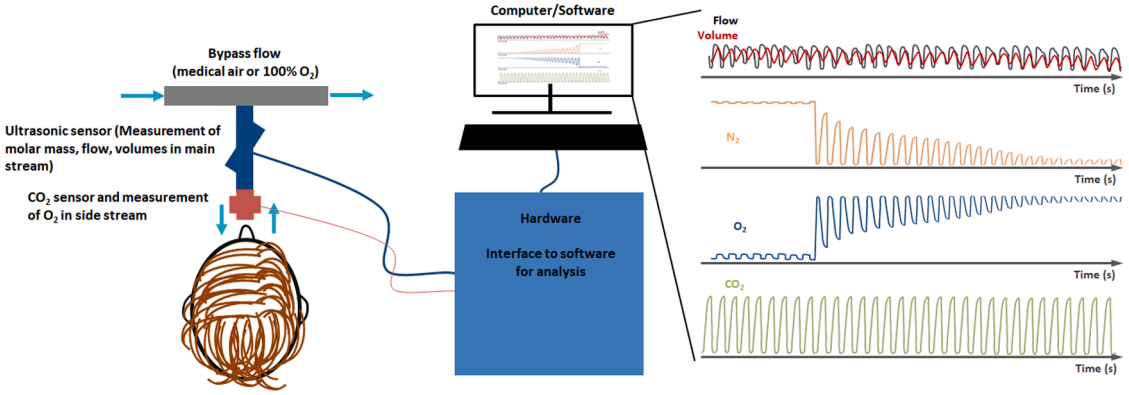

Fig. 1. Schematic structure of a setup for a MBW measurement (left) and enlarged example of a tracing (right) (Adapted from Fuchs, 2017; reprinted with permission Monatsschr Kinderheikd):[36]
Abbreviations: O_2 oxygen; CO_2 carbon dioxide; N_2 nitrogen.

studies, whilst being relevant for a broader proportion of the population, standardisation of techniques, as has been achieved in older children and adults through the global Core Facilities [37], would allow pooling of datasets, enhancing its utility.

Ensuring that the burdens associated with MBW testing are worthwhile for children with CF under 2 years of age and their parents places an imperative on being able to interpret the data; studies including this outcome measure should have a minimum (sub-)group size and an adequate control group.

5. Chest computed tomography

5.1. Chest-CT: what do you measure?

CF lung disease typically leads to structural abnormalities within the first years of life and chest Computed Tomography (CT) is considered the most effective radiology technique for assessing these changes [38,39]. Over 70 % of the European CF centres use chest CT for monitoring CF lung disease [40].

In young children with CF, chest CT is the most sensitive and feasible modality for diagnosing and monitoring structural lung abnormalities [38]. The most significant structural changes detected on chest CT include diffuse bronchus wall thickening, bronchus widening (both key features of bronchiectasis), atelectasis and low attenuation regions (LAR) [41–43]. LAR is a combination of mosaic perfusion and air trapping, reflecting small airways disease. Bronchus wall thickening and LAR often appear as early as three months of age in children with CF [42,43]. In cooperative children bronchial widening (or bronchiectasis) and bronchial wall thickening are detected on inspiratory CT by comparing the outer diameter of the bronchus and its thickness to the diameter of the adjacent pulmonary artery. A broncho-arterial (BA) ratio exceeding 1.1 is considered widened [44,45]. In non-cooperative young children, chest CTs are mostly performed while the child is free breathing. On these CT scans at FRC level, widened airways and/or airway wall thickening can be detected, but the sensitivity is less compared to CT taken at total lung capacity (TLC). Expiratory scans obtained at FRC or below are important to detect and quantify LAR [46–48].

To use chest CT as endpoint, structural abnormalities need to be quantified. Different visual scoring systems, such as the Brody II [49], Bhalla [50] and PRAGMA-CF [51,52] scores have been used for chest CTs in children from the age of 3 months and older with CF. The scoring systems commonly express the presence and severity of abnormalities, such as bronchiectasis, bronchial wall thickening, mucus plugging, atelectasis and LAR as sum of subscores or a percentage of total lung volume. The PRAGMA-CF is the only scoring system used to date as a primary outcome in randomized controlled trials in children below the age of 5 years. All these scores are visual scoring systems, and therefore labour-intensive. Scorers need to be trained, and intra- and inter observer variability needs to be tested.

Several fully automated image analysis programs are available. YACTA is a fully automated lobe segmentation algorithm used to score abnormalities in various lung diseases, such as chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD). Only one study in CF has used YACTA, and this was in children aged 8–14 years of age [53]. More recently, a fully automated, artificial intelligence (AI)-based image analysis system (LungQ™, Thirona, Nijmegen) was developed and validated to measure BA dimensions and ratios of a large number of BA pairs on a chest-CT [54]. LungQ™ BA-outcomes correlate strongly with similar PRAGMA-CF outcomes and have shown higher sensitivity in assessing bronchial widening and wall thickening [55]. Another automatic AI-based scoring system recently tested in a multicentre study involving school-age children and young adult CF patients treated with ETI is the NOVAA-CT. This scoring system provides a normalized volume of airway abnormalities showing good reliability and reproducibility in tracking effect to ETI treatment [56,57]. One key advantage of automated systems is the elimination of observer bias, enabling precise monitoring of treatment effects.

5.2. Supporting evidence, validation and summary of literature

The PRAGMA-CF score is a well validated, sensitive, and quantitative image analysis scoring system used to quantify bronchiectasis, bronchial wall thickening, mucus plugs, and LAR [46,48,52,58–64]. The PRAGMA-CF %Disease metric, which reflects the percentage of lung volume occupied by abnormal airways, has proven sensitive in tracking disease progression in pre-school and older children. PRAGMA-CF is relatively insensitive to variations in CT scanning techniques and protocols. A standardized training program is available for PRAGMA-CF scoring. Furthermore, PRAGMA-CF %Disease in pre-school children with CF has been shown to predict bronchiectasis and lung function outcomes in school-aged children with CF [65]. PRAGMA-CF score has demonstrated greater sensitivity for milder disease in young children compared to the Brody CF-CT score [49,52] and better correlated with airway inflammation.

Two randomized controlled trials (SHIP-CT and COMBAT) have used chest CT outcomes as primary endpoints in pre-school children with CF [61,64]. In the SHIP-CT trial, significant treatment effects were detected using both PRAGMA-CF and BA-analysis, highlighting the sensitivity and validity of chest CT in monitoring lung disease progression in children with CF aged 3–6 years [54]. Specifically, PRAGMA-CF %Disease and BA-analysis demonstrated the ability to track structural lung changes, promoting their use in clinical trials. Conversely, the COMBAT-CF trial, which assessed azithromycin in children aged 3–36 months, found no positive effect on PRAGMA-CF outcomes by the age of 3 years [64]. However, BA-analysis detected a positive effect on airway wall thickness, indicating that this method is more sensitive to early structural changes [66].

In the SHIP-CT study, a treatment effect in PRAGMA-CF %Disease of 0.67 was observed after 48 weeks between the two treatment groups [61]. Previous cohort studies have shown that annual progression in % Disease among CF patients ranges from 0.14 to 1.23 % [63,67,68]. This suggests that the treatment effect observed in SHIP-CT corresponds to an equivalent of 0.5 to 4 years of disease progression, which is considered a meaningful minimal clinically important difference (MCID). Similarly, BA outcomes can be used to assess the treatment effect of an intervention, providing another metric to quantify the annual progression of BA outcomes.

The Brody II scoring system and its upgraded CF-CT version is comprised of 5 subscores for the presence and severity of CF lung abnormalities per lung lobe, namely: bronchiectasis, mucus plugging, peribronchial thickening, opacity/ground glass/cysts/bullae, and air trapping. The sum of the subscores of these components gives a total score, with higher scores indicating more severe disease. This manual scoring system was developed to provide a reproducible method for evaluating the lobar location, severity, and extent of chest CT abnormalities of CF lung disease, especially in children [49]. However, structural changes detected on chest CT in infants aged 1 year were generally very mild and inter- and intra-observer agreements on CT scores were poor using the Brody-II scoring system, except for air trapping [69]. Subsequently, it was shown that Brody-II score for air trapping was only weakly associated with inflammation, assessed with neutrophil elastase in bronchoalveolar lavage in one year old infants [70].

Other scores such as the Castile, Helbich, Santamaria and Bhalla scores have been used in school aged children with CF, but not in infants [50,71,72]. The modified Bhalla score has been shown to have a positive correlation with pseudomonas colonization in a study for one year old children with CF [73].

Despite these encouraging results, it remains unclear whether chest-CT outcomes will be sensitive enough to detect the effects of clinical interventions in infants aged 0–2 years, given the challenges related to limited cooperation, smaller airway size and the lower prevalence of

structural abnormalities in this age group. Structural lung disease in CF before age 2 as assessed by volume-controlled CT appears to be very mild as reported by the London Cystic Fibrosis Collaboration Cohort (LCFC) [70] and other authors [37]. Some studies using free breathing chest-CT's have reported abnormal lung imaging in most infants, however, the severity score remained low [50]. Furthermore, chest CT performed two years apart in children between 1 and 3 years, remained stable under current standard of care, as shown by PRAGMA-CF %Disease [51].

5.3. Feasibility of chest CT in children under two years of age

Performing chest CT is feasible in all ECFS-CTN CF centres equipped with a CT scanner. Over the last decade, significant efforts have been made to educate CF-centres on standardized chest CT acquisition protocols and scoring methods, particularly for use in clinical studies. Almost all 57 ECFS-CTN centers (to 2024) have been trained, characterized, and certified by the Erasmus Medical Centre Lung Analysis CT core laboratory to conduct standardized chest CT protocols following up on a previous standardization initiative [74].

Cooperative children aged 5 years and older can be trained to perform voluntary breath hold manoeuvres after a deep inspiration at TLC and after a deep expiration between functional residual capacity (FRC) and residual volume (RV) [61,75]. Unfortunately, in pre-school children, including infants, breath hold manoeuvres are usually not feasible. In this age group, chest CT can be performed during free breathing at FRC using high speed CT techniques (i.e. high pitch imaging), vacuum mattresses and without anaesthesia. While FRC scans have been shown to be less sensitive than TLC scans in detecting bronchiectasis [42], their specificity remains high, making them an adequate option for assessing structural lung changes.

Another technique to acquire a CT scan in non-cooperative preschool children with CF is the Pressure Controlled Volume (PCV) technique, which is performed under general anaesthesia or sedation [42,64]. This technique is not routinely used in CF centers, due to its invasive nature, as it requires anaesthesia. While PCV-based CT offers high image quality, the additional risks and procedural complexity limit its widespread application in clinical studies for young children with CF.

5.4. Pitfalls/Limitations

While chest CT is well-established for use in children with CF of 3 years and older, its use in younger children remains debated due to several limitations. Firstly, chest CT exposes children to ionising radiation, which is associated with a small increased life-long risk of cancer [76]. This risk is particularly concerning in very young paediatric patients, as they have a higher number of actively dividing cells compared to adults. Although, the risks posed by state-of-the-art low-dose chest CT protocols are uncertain, they are generally considered to be small [77]. Recent advances in CT technology, such as the introduction of Photon-Counting-Detector (PCD) CT scanners, offer significant reduction in radiation dose, while increasing the spatial resolution [78]. Nonetheless, the radiation dose for each CT scan must be justified based on clinical and/or scientific grounds and minimized according to the 'as low as reasonably achievable' (ALARA) principle. Consequently, this radiation exposure restricts the number of CTs that can be justified for a subject over the course of a clinical study. Importantly, the CT protocol for a clinical study should be tailored to the size and weight of the patient and radiation dose must be reduced to a level that still allows for diagnostic image quality and sensitive image analysis.

Secondly, structural lung changes in infants are typically minimal and CT images tend to remain stable in the first two years of life [79,80]. This limits the utility of CT in detecting early disease progression in this age group, but with a longer follow-up chest CT outcomes have proven to be sensitive enough to pick up differences. Thirdly, a limitation specific to infants and young children under 3 years is the detection of fewer BA-pairs than in older children due to smaller airway size and early stage of structural lung changes. Possibly, the advent of PCD-CT technology may significantly increase spatial resolution, improving the visibility of smaller BA-pairs, making those techniques more feasible for younger patients. These factors highlight the need for cautious consideration when using chest CT in infants, balancing the benefits of early disease detection against the limitations of radiation exposure and image sensitivity. Strengths and limitations for chest CT as a pulmonary endpoint are depicted in Table 1.

5.5. Practice guidelines for making a chest-CT in children under two years of age

CF lung disease is heterogeneously distributed throughout the lungs, making a spiral or volumetric CT protocol mandatory to scan the entire lung [38]. Modern CT scanners are fast enough to scan the full lung in less than a second, minimizing or eliminating motion artifacts even in non-cooperative infants and young children. High resolution is crucial for sensitive analysis of small airway dimensions, and when reconstructing images, an isotropic slice thickness of 1 mm or less must be selected. Resolution is also influenced by reconstruction kernel, a reconstruction parameter that influences the resolution and noise of the image. Smooth kernels are usually preferred for automatic imaging analysis. For the detection of LAR in expiratory or free breathing scans, a lower level of resolution is generally sufficient, using thicker slices (>1.5 mm). The CT reconstruction protocol should define reconstruction planes (axial, coronal, sagittal), slice thickness, windowing, and kernel. At least one series of thin-slice images ($\leq$ 1 mm) in the axial plane should be stored using a predefined reconstruction kernel to evaluate the airways with maximum sensitivity.

Most modern scanners can acquire motion-free chest CT images in non-cooperative free breathing young children without the need for sedation or general anaesthesia [75]. Ideally, these scans should be acquired during natural sleep, using method such as the "swaddle and cuddle" technique [81] to reduce movement artifacts. For children below the age of 3 years, a vacuum mattress may be helpful to limit movements during the scan, but care should be taken not to deform the thorax. Scanning begins at the lung apices moving caudally towards the diaphragm. For young children, scanning can be reversed, starting at the diaphragm moving towards the apices to reduce movement artefacts.

The PCV technique under general anaesthesia starts by hyperventilating the child with a series of augmented breaths using high positive pressure delivered via a facemask, laryngeal mask, or endotracheal tube. This recruits all lung areas and allow for a respiratory pause. For inspiratory images, the lung is inflated to a positive transpulmonary pressure of 25 cm H_2O to image the lung at TLC, while maintaining pressure. For expiratory images, no pressure is applied, allowing the lung to deflate to a volume level near FRC. The PCV technique produces highly reproducible lung volumes, but its primary disadvantage is the requirement of general anaesthesia, which may not be considered acceptable particularly for research purposes. Furthermore, anaesthesia can induce atelectasis in the lower lung lobes, which can complicate the interpretation of CT images, as these regions cannot be evaluated for bronchiectasis or other structural abnormalities during the scan.

5.6. Recommendations and future directions

Chest CT has been shown to be a valuable primary endpoint for clinical trials aimed at evaluating interventions designed to prevent or mitigate the progression of structural lung disease in children with CF from the age of 3 years and older. CT outcomes at this age have demonstrated reliability in both clinical and research setting for the majority of children. Realistic sample size, such as 60 patients per arm used in the SHIP-CT study, can provide sufficient statistical power to detect significant effect on primary outcomes, like %Disease or

Table 1
Strengths and limitations of endpoints.

Strengths	Limitations
MBW	
• LCI is more sensitive than other lung function outcomes (e.g. forced expiratory volumes) in detecting functional deficits. • Testing involves tidal breathing with no requirement for forced respiratory manoeuvres. • Potential opportunity for longitudinal measurement. • No radiation burden, as with some forms of imaging. • LCI has been used as a primary and secondary outcome in clinical trials (limited experience to date).	• Weaker association between LCI and structural outcomes than observed in older children; transient increases may be seen e.g. with airway infection. • Most infants with CF have a LCI within the normal range for their age. • LCI may be impacted by test set-up and equipment used (including gas). • Available in highly specialised centres only. • Unknown minimal clinically important difference for LCI. • Test burden and risks of sedation (if used).
CHEST CT	
• CT available and routinely used in most CF centers. • Detect lung damages prior lung function alteration. • Validated scoring system. • Automatic artificial intelligence (AI) tools for quantification of CF features. • Able to detect changes after therapeutic intervention. • Can be performed in a free breathing infant. • No need of anesthesia in infants.	• Radiation exposure. • Some relevant CT outcomes (i.e. air trapping) require pressure-controlled technique under general anesthesia. • Less bronchial/artery pairs visualized in infants. • Inability to assess pulmonary perfusion without the use of contrast. • Few abnormalities in infants in first years of life, this requires long follow-up time
MRI	
• MRI does not involve ionizing radiation, safer than CT for repeated use, especially in children and young patients. • MRI provides excellent soft tissue contrast, allowing for detailed visualization of lung structures and CF-related changes. • Unlike CT, MRI can evaluate lung perfusion without the need for contrast agents. • MRI offers the ability to perform dynamic imaging to assess lung function, including ventilation and perfusion, in real time. • MRI can detect early inflammatory changes and mucus plugging, even before significant structural damage occurs. • MRI allows the use of non-invasive contrast options, such as hyperpolarized gases to assess lung ventilation and gas exchange.	• MRI is not as widely available or routinely used in CF centers as CT, and access to specialized lung MRI techniques may be restricted. • MRI typically requires longer acquisition times compared to CT, which can be challenging for some patients, especially young children. • MRI generally has lower spatial resolution compared to CT, especially Photon Counting CT, that can limit the visualization of small airway structures. • MRI is more sensitive to patient movement, which can affect image quality, particularly in young children who may have difficulty staying still during the procedure. • MRI in infants may require sedation to ensure they remain still, unlike free-breathing CT scans. • MRI requires specialized protocols and experienced radiologists for optimal use in CF imaging, limiting its widespread adoption. • MRI is typically more expensive than CT, which may limit its use in some healthcare settings. • Few abnormalities in infants in first years of life, this requires long follow-up time.
INFECTION & INFLAMMATION BIOMARKERS	
• Infection and inflammation are key pathological pathways in CF. Direct sampling and measurement are critical to understanding treatment impacts • Induced sputum is a non-invasive, repeatable measure	• Lack of standardised procedures for collection, processing and analysis • Limited Information on normative values and natural variability in infants • Intercurrent viral and bacterial infection can adversely impact on measured outcomes • BAL is invasive and not easily repeated

bronchial-arterial dimensions and ratios on chest CT. However, use of CT as a primary outcome in children younger than 3 years remains challenging due to several limitations, including reduced sensitivity to detect bronchial wall thickening and restrictions in relation to radiation exposure.

To address these challenges, future advancements in imaging technology, such as improved spatial resolution with novel PCD-CT scanners, promise to enhance the ability to detect early structural changes in the smaller airways of non-cooperative infants and to further reduce radiation dose. Furthermore, follow up beyond the age of 3 years, may increase the differences in chest-CT scores. These advancements could potentially make chest CT a more viable option for younger children in clinical trials.

In conclusion, while chest CT remains a key tool for assessing lung disease in children above 3 years of age, ongoing advancements in imaging technology and methods will be crucial in overcoming current limitations, particularly for younger patients. Future research should focus on optimizing imaging protocols and minimizing radiation dose to enhance the utility of chest CT in all age groups with CF.

6. Chest-Magnetic resonance imaging (MRI)

6.1. Chest MRI: what is it, what do you measure?

MRI has the ability to provide both structural and functional information about CF lung disease, without the radiation exposure associated with chest CT scans. It is a particularly promising endpoint for clinical trials in young pre-school children with CF as it requires limited patient cooperation. Recent advances in MRI technology and sufficient clinical data support its consideration as an endpoint for clinical trials in children under two years of age. However, challenges remain due to variable image quality between MRI brands, limited commercially available tools for automatic quantification of structural CF lung disease and license-limited software for assessment of functional CF lung disease on MRI.

6.2. Typical findings in chest MRI in CF

6.2.1. Structural changes

Characteristic airway changes in CF are bronchial wall thickening and bronchiectasis, which increase significantly in the course of the disease and can also take on cystic forms. Similarly to CT, on MRI, bronchiectasis is defined as an irreversible enlargement of the bronchus diameter beyond that of the accompanying artery [82,83]. These changes can be observed as some of the first structural abnormalities in CF and are often irreversible. They can be detected in 30 % of 3-month--old and 60 % of 3-year-old CF patients via CT and up to 100 % in infants and preschool children by MRI [41,84–86]. "Mucus plugging" is the second most common morphological finding on lung imaging in about 47.9 to 71.4 % of cases in clinically stable children (≤4 years) [84]. It often occurs together with bronchiectasis, a finding called bronchocele [82,87]. In MRI, "mucus plugging" is easily recognizable due to the high T2-weighted (T2-w) signal in all age groups. A special form of "mucus plugging" caused by Aspergillus superinfection presents an inverse signal behaviour on T2-w imaging due to mineral deposition (corresponding to the high-attenuation mucus sign on CT) [88,89]. Atelectasis and true consolidations, visible during pulmonary exacerbations, are often reversible with therapy and are easy to recognize due to the high

T2-w signal. In the context of pulmonary exacerbations, consolidations were found by MRI in 50 % of preschool children and in 80 % of adolescents, significantly more frequently than in clinically stable lung disease (13 % and 21 %, respectively) [10,85]. Chronic consolidations can affect an entire lobe and are usually associated with a reduction in volume and inlaid bronchiectasis. In such cases, they are usually irreversible and are often referred to as "destroyed lobe".

6.2.2. Functional changes

Due to the obstruction of the peripheral airways and the resulting lower oxygen content beyond the obstacle, there is a physiological constriction of the pulmonary arterial and sometimes pulmonary venous vessels. This reflex, known as Euler-Liljestrand or hypoxic pulmonary vasoconstriction reflex, normally serves to redistribute blood flow to better-ventilated areas, optimizing global oxygen supply. In CF, diffuse "mucus plugging" or consolidations cause perfusion failures, which can be detected with MRI through contrast enhanced or non-contrast enhanced imaging [90,91]. These hypoperfused areas indirectly reflect regional ventilation disturbances that MRI can primarily detect. In contrast, inflammatory infiltrates can also be found with local hyperperfusion. Perfusion changes represent diagnostically independent findings and can occur in areas of the lung that initially appear structurally normal. They are likely the result of obstructions in the peripheral airways that have not yet led to detectable structural changes [85]. LAR, a combination of mosaic perfusion and air trapping, seen on CT as circumscribed reduced lung parenchymal density [82,87], can be detected on Proton Density weighted (PD-w) MR images as a mosaic pattern with normal and reduced lung low signal intensity regions (LIR). These LIRs can be understood as a surrogate for lung perfusion, without the need for contrast medium administration [92].

6.3. Supporting evidence, validation and summary of literature

6.3.1. Structural chest MRI outcomes

To date, structural abnormalities in CF have been predominantly assessed using semi-quantitative scoring systems, such as the Eichinger scoring system [93] or the CF-MRI scoring system [91]. These scoring systems categorize each abnormality based on volume distribution: either more or less than 50 % in the Eichinger score, or 33 %, 33–66 %, and more than 66 % in the CF-MRI score [91]. These scored abnormalities include bronchiectasis/bronchial wall thickening, mucus plugging, atelectasis/consolidation, hypoperfusion, and air trapping/emphysema. The total score is the sum of the individual scores for each category. Both scoring systems have been utilized in numerous CF studies and validated against lung function tests [92,93]. Currently, there is no fully quantitative structural scoring system in place. Dournes et al.'s group has developed in-house software for the automatic quantification of T2-w signal abnormalities on MRI as a measure of inflammation/infection in patients during pulmonary exacerbations [94].

6.3.2. Functional chest MRI outcomes

Functional outcomes involve assessing perfusion using both contrast and non-contrast-enhanced techniques, as well as ventilation-related techniques [95]. Contrast-enhanced techniques are more invasive requiring the positioning of an intravenous cannula and the administration of gadolinium [96]. Routine use of gadolinium is usually avoided in paediatric imaging due to concerns about potential tissue deposition [95–97]. Non-contrast-enhanced techniques for assessing pulmonary perfusion include Fourier Decomposition, which enables the evaluation of changes in signal intensity within the lung parenchyma over time, allowing differentiation between signal changes resulting from perfusion and ventilation. Various algorithms have been proposed [98–100]. These techniques offer the advantage of being performed without the need for patient cooperation [101]. A fully quantitative analysis pipeline for these software applications is available, although it is still primarily in a research setting. So far there is little published on Xenon-MRI in infants with CF but this may be a functional measure of interest in the future.

6.3.3. Evidence from studies on disease progression

In the respective age group, specific alterations of pulmonary structure and perfusion are observed, reflective of early disease manifestations [85,92]. MRI is associated with other parameters of pulmonary disease involvement, such as MBW in young children with CF [10,102]. MRI reflects disease progression in children with CF followed up after newborn screening, where it is associated with known determinants of pulmonary disease severity [28,84]. Longitudinal MRI assessments can identify responses to antibiotic therapy [10,85]. Functional MRI techniques can be performed in very young children [10,90,103,104] and identify disease-specific differences compared to healthy controls [90].

6.3.4. Evidence from interventional trials

MRI can be sufficiently standardized across multiple centers [105,106], also in the age group in question [99]. However, published data on the use of MRI as an endpoint in clinical trials for this age group are scarce, mostly due to difficulties in harmonizing image quality between different MRI brands. Nonetheless, MRI was feasible in infants diagnosed clinically or by newborn screening in the first four months of life in a clinical trial to assess response to inhalation of hypertonic saline (HTS), although no improvements were noted in HTS vs. isotonic saline (IS) [36]. Furthermore, in children aged 2–5 years, MRI detected improvements in structural lung involvement compared to baseline after 48 weeks of dual CFTRm therapy [107,108].

6.3.5. Evidence from lung diseases other than CF

MRI has been successfully employed to identify changes associated with bronchopulmonary dysplasia in young infants [109,110] providing further evidence for feasibility in the age stratum concerned.

6.4. Feasibility of chest MRI in children under two years of age

Chest MRI techniques vary according to the age of the infant [111,112]. In general, in infants up to 6 months of age, chest MRI can be conducted without the need for anaesthesia, utilizing the "swaddle and cuddle" technique, depending on local choice and feasibility [111]. This technique involves fasting the neonate before the examination and, just prior to the MRI scan, providing breastfeeding to induce natural somnolence, which minimizes motion artifacts during the examination. To further minimize movement, the infant is securely wrapped, often using vacuum mattresses. However, this technique requires dedicated nursing personnel, time, and space for breastfeeding and setting the child to sleep, only available in tertiary care paediatric hospitals. For older children, aged 6 months to 5 years, MRI can be performed with either moderate sedation or full anaesthesia. Moderate sedation is achieved through the nasal or rectal administration of sedatives, such as benzodiazepines or dexmedetomidine, with the latter being a newer agent with both sedative and analgesic effects and a short duration of action (approximately 2 h) [111]. MRI in preschool-aged children is non-interactive, meaning it does not require the patient's cooperation [111,112]. Images are acquired using either prospective or retrospective triggering. Prospective triggering involves initiating image acquisition at the end of expiration, employing either a pneumobelt or a navigated echo technique. More recent sequences also allow for retrospective triggering, where the patient can breathe normally, and subsequently, inspiratory, and expiratory phases are reconstructed separately [113].

6.5. Pitfalls/Limitations

MRI availability and protocol heterogeneity across different sites makes multi-centric implementation in clinical trials challenging [105]. Besides availability, costs per imaging session, including scanning time

and also personnel requirements are further challenges to be considered for implementation of MRI in observational or interventional trials.

One of the main clinical limitations of MRI in comparison to CT is the duration of the scan. An average standard MRI protocol typically lasts between 15 and 30 min. This longer duration is more time-consuming and restricts the number of examinations that can be conducted compared to CT. Prolonged scan times, particularly when coupled with anaesthesia, can heighten the risk of atelectasis development, primarily in the lower lung lobes. If anaesthesia is administered, patients often require additional observation time for recovery after the MRI scan, subsequently escalating overall examination costs. In order to reduce anesthesia time, such standardization should prioritize standardized protocols with the shortest sequences, which still provide sufficient resolution in infants.

Presently, the spatial resolution of MRI is lower than what can be achieved with CT especially with the latest photon counting CT scanners, resulting in reduced sensitivity to detect peripheral airways disease, particularly in this age group with very mild disease. Furthermore, there are currently no dedicated automated MRI scoring systems available to quantify structural abnormalities in pwCF. However, recent advancements in assessing bronchopulmonary dysplasia in infants show promise, indicating the potential development of similar software for CF lung disease in the future [114]. Strengths and limitations for chest MRI as pulmonary endpoints are depicted in Table 1.

6.6. Practice guidelines for conducting chest-MRI in children under two years of age

6.6.1. Standard MRI protocols

Standard MRI protocols for chest MRI encompass both T2-w and T1/PD-w sequences [112,113,115]. T2-w sequences are employed to visualize mucus plugging, bronchial wall thickening, and consolidation, while T1/PD-w sequences are typically used to assess bronchiectasis and LIRs, which represent either air trapping or hypoperfusion [112]. Currently, the most used techniques are the PROPELLER/-BLADE/MultiVane sequence (Brand names for GE Healthcare, SIEMENS Healthineers, and Philips, respectively) [95], which features helicoidal K-space reconstruction to reduce sensitivity to motion artifacts. The second most frequently used technique involves radial or spiral K-space reconstruction coupled with ultra or zero echo-time (UTE/ZTE) pulse sequences, enabling optimal signal-to-noise ratio (SNR) and resolution almost comparable to CT imaging [95,116,117]. Currently, UTE/ZTE sequences provide the highest image quality in terms of spatial resolution and SNR, which can be further optimized by the use of deep learning filters [118]. ZTE/UTE sequences could be optimized and standardized for imaging in infants, being available for all major MRI brands. Standardization across multiple centers, which has been feasible in older patient populations, is a prerequisite to improve comparability and thus power in clinical trials [119].

Use of sedation or anaesthesia according to local guidelines and preferences from the age of 6 months to 5 years is recommended for most settings.

6.7. Recommendations/Future directions

The full integration of chest MRI for infants in CF centres necessitates the development and adoption of high-speed techniques that can potentially obviate the need for anaesthesia. A promising recent innovation is the introduction of a real-time technique with exceptional temporal resolution, rendering anaesthesia unnecessary [120]. Furthermore, enhancing the consistency and standardization of chest MRI protocols across different CF centers will significantly improve image quality and the applicability of this technique in infants.

In addition, substantial efforts should be directed towards the creation of comprehensive automatic quantitative scoring systems that can accurately quantify imaging findings in both structural and functional chest MRI. This undertaking will be crucial for advancing the clinical utility of this imaging modality in the evaluation of infants with CF. The use of chest MRI in clinical trials involving children with CF under the age of 3 is feasible, despite acknowledging its greater complexity and lower sensitivity when compared to chest CT imaging.

7. Airway infection and inflammation

7.1. Airway infection and inflammation: what do you measure?

A key mechanism contributing to progressive respiratory failure in CF is the abnormal cycle of infection and inflammation, leading to irreversible airway wall damage [121–123]. This starts early in life and is progressive. Several markers of inflammation in bronchoalveolar lavage (BAL) have either predictive value or an association with structural lung damage [41,79]. These markers reflect the presence of neutrophilic inflammation and oxidative stress. Inflammatory markers in serum have also been studied in CF, but markers have yet to be validated for use in clinical care [124]. The relationship between infection and inflammation in CF is complex, and the impact of each, in isolation and in concert, on outcomes in CF can be hard to disentangle [125]. One of the key challenges in using data in relation to airway infection or inflammation as outcome measures for clinical trials in children with CF (particularly in younger children with mild disease) is the lack of a clear understanding of the natural short-term variability in both, and the likely considerable impact that transient viral or bacterial airway infections have on these outcomes.

7.1.1. Collection of specimens

Considerable evidence exists around the utility of sputum (induced or expectorated) and BAL to measure infection and inflammation in the CF airway [126]. Induced sputum and BAL are considerably more representative and useful than oropharyngeal swab sampling [5,127]. Sputum induction can be performed in young children [128,129]. Unlike for the analysis of blood biomarkers in humans, in which the process of collection, and the concentration and constituents of the biological material are reasonably consistent, collection of airway specimens poses two challenges. Firstly, the concentration of the substance can vary (sputum diluted with saliva, airway lining fluid diluted with saline (BAL) and oropharyngeal and nasal secretions contaminating the sputum). Secondly, the mode of collection can be operator dependent (procedure and duration of sputum induction, installation volume and location of BAL and percentage return). Progress has been made in the standardisation of approaches for sputum induction [128–130] at the ECFS CTN, for infants, young children and adolescents/adults.

7.1.2. Measurement of outcome: infection

Measurement of infection in people with CF can be understood in terms of 1) determining presence or absence of an organism, 2) measuring the extent of the infection [127] and 3) assessing microbiome in upper or lower airways. In general, in research studies, presence/absence is utilised more commonly than quantification. The organism most studied in CF is *P. aeruginosa*, given its link to adverse outcomes, although the prevalence of this is declining, particularly in children [131]. Presence of *A. fumigatus* in BAL has also been shown to be associated with progression of structural lung disease in children aged 1–5 years [132]. Still prevalence of both *P. aeruginosa* and *A. fumigatus* is low in this age group, limiting their utility as pulmonary endpoints in clinical trials, which would require large numbers of subjects. The most frequent airway pathogen in infants with CF is *S. aureus* which has been demonstrated to impact the evolution of lung disease [133]. Microbiome assessment in children under 2 years may also be a way to study the relation of microorganisms and early lung disease development. Studies suggested that shifts in airway microbial composition are associated with inflammation, structural lung damage and antibiotic use [134–138]. However, the current evidence is limited by

methodological variability and challenges in sampling the lower airways in this age group, which hamper the reliability and clinical utility of microbiome profiling as a standalone pulmonary endpoint in young children.

7.1.3. Measurement of outcome: inflammation

The inflammatory environment in the CF airway is complex. Multiple measures of inflammation have been studied in young children with CF, but most revolve around neutrophil recruitment and activation. Some of the key measures that are most used and widely accepted include inflammatory cell counts, Interleukin-8 (IL-8), neutrophil elastase (NE), tumour necrosis factor-alpha (TNF-α), IL-1β and calprotectin [130]. The ECFS-CTN has developed guidance for sputum inflammatory markers and is currently developing guidance for blood biomarkers [130]. Some serum markers have shown potential to be used as a biomarker for lung inflammation in patients with CF, namely IL-6, IL-1β, TNF-α, high sensitivity C-reactive protein (hsCRP), soluble CD14 (sCD14), calprotectin, high mobility group box 1 protein (HGMB-1) and amyloid A [139–141]. However, none of these markers have been studied in children below 2 years of age.

7.2. Supporting evidence, validation and summary of literature

Given the degree of short-term variability in both infection and inflammation in the airways of children with CF, the link between infection and inflammation and outcomes in CF likely to be best judged in terms of the impact of repeated or persistent abnormalities on outcomes. Rosenow et al. specifically examined this in preschool children with CF, concluding that, although there is a temporal association between abnormal airway infection, inflammation and imaging in the short term, repeated episodes of infection are not correlated with abnormal outcomes, whereas repeated episodes of airway inflammation are [58]. This is confirmed in several other studies using BAL from infants with CF showing that increased inflammatory markers such as bioactive lipids, IL-8, myeloperoxidase (MPO), neutrophil percentage and NE are predictors of structural lung damage a few years later [41,60,79,86]. The use of a multifaceted tool (including infection and inflammation) to predict bronchiectasis in children with CF has utility when examined cross sectionally, but not at the patient level, underlining the limitations of measures of airway disease at a single point in time [65]. The COMBAT study sought to determine whether the use of azithromycin over three years would lead to a reduction in the extent of structural lung disease at age 3 in children with CF [64]. As part of this study, the investigators used BAL inflammatory markers (IL-8 and NE) as secondary endpoints. While no effect of azithromycin was seen on structural lung disease, there was an effect on IL-8 and NE at 36 months, but not at 12 months, suggesting that markers of airway inflammation might be better suited to studies with longer follow up.

As for use of BAL endpoint trials in children, Fayon and colleagues conducted an extensive review on behalf of the ECFS-CTN Standardisation Committee on the clinimetric properties of BAL inflammatory markers, advocating for their use in selected centres in early phase trials and in preschool children with CF [142]. Individual markers of infection or inflammation (in BAL or induced sputum) have not been validated specifically for use as outcome measures in young children with CF, and no relevant MCID has been determined for these outcomes, suggesting that the use of these may be better suited to secondary or exploratory endpoints in younger children. Although a small number of specialist centres internationally regularly perform BAL in infants, unfortunately, limited consensus is established in relation to the specifics of the procedure itself (lobes sampled, instillation volume, processing), and this remains unstandardised. Considerable regional variability is seen in pulmonary inflammation in lungs of preschool children with CF [143], supporting the concept of pooling of BAL, an approach that has been shown not to interfere with the ability to successfully detect infection and inflammation in children with CF [143]. The CF-SPIT trial demonstrated the superior sensitivity of six-lobe over two-lobe BAL in terms of detection of infection and inflammation in children with CF (aged down to 4 years) and showed that induced sputum is a good surrogate for BAL in terms of inflammatory endpoints [127].

7.3. Feasibility infection and inflammation markers in children under two years of age

Both induced sputum and BAL are feasible in infants in most centres [128,129,144], however the relatively time-consuming nature of sputum induction means that it is not performed routinely in many centres. BAL, while feasible in many centres is invasive as it requires general anaesthesia and is usually not performed on a longitudinal basis but mostly performed sparingly in most CF centres dictated by clinical need. Several centres have employed annual surveillance BAL, although this is performed less frequently than in the past. A programme of targeted BAL surveillance looking for airway infection does not contribute to the outcome of children in terms of lung function and structural lung disease on chest-CT [145]. Still, in consideration of adding BAL as an endpoint measure, it is good to know that repeated exposure to anaesthesia during infancy does not lead to long term adverse outcomes for cognitive development but may be poorly tolerated and needs day-case hospital attendance [146]. Although BAL is usually feasible, processing, storage and analysis of inflammatory specimens is not a widespread skill, is not standardised and is limited to specific centres [147]. Blood samples can be easily taken; however, the choice of inflammatory outcomes and laboratory measurements of these are not yet standardized and there is no clear validation of the most sensitive biomarker.

7.4. Pitfalls/limitations

The use of induced sputum has been standardised across ECFS-CTN centres [128,129] and can be performed by trained and motivated specialist physiotherapists. However, even when conducted by trained personnel, the procedure is not always well tolerated by young children, particularly the suctioning of sputum from the throat, which can be distressing. Bronchoscopy in infants is a considerably invasive procedure requiring general anaesthesia and day-case hospital attendance, thereby limiting its applicability. Subjecting young children to bronchoscopy solely for research purposes warrants careful ethical consideration. The use of such invasive procedures must be thoroughly justified or limited to situations where there is a clear clinical indication. Importantly, explicit and well-informed parental consent outlining risks and benefits along with approval by institutional review boards is mandatory. However, as clinical outcomes in children with CF continue to improve, the clinical indications for BAL in infants have become rare.

Despite existence of the standardised, agreed protocol of the ECFS-CTN for conducting BAL, including collection processing and analysis of samples [144], variability in both sample acquisition and interpretation remains a significant limitation. Additionally, regional heterogeneity in infection and inflammation in the airways, along with short-term fluctuations in airway infections, both viral and bacterial, can independently impact measured outcomes (see also Table 1).

7.5. Practice guidelines

7.5.1. Induced sputum

Obtaining induced sputum in infants involves several steps to ensure safety and effectiveness. The procedure starts with pre-medication using a bronchodilator to prevent bronchospasm. Next the infant inhales a hypertonic saline solution (preferably 7 %, but in case of known intolerance 3 %) via a nebulizer, which helps to liquefy and mobilize airway secretions. This inhalation typically lasts for 10 min. Following nebulization, airway clearance techniques (ACT) like chest compressions should be applied to further aid sputum mobilisation. This is recommended to be conducted by an experienced physiotherapist. Finally, the

sputum is collected using gentle suctioning from the oropharynx. It is important to minimize contamination by not suctioning while inserting or withdrawing the suctioning tube from the pharynx. Detailed SOPs are available on induced sputum in young children from ECFS-CTN, including instruction videos [128,129].

7.5.2. Bronchoalveolar lavage

BAL fluid is obtained by flexible bronchoscopy and should be performed under general anaesthesia. The bronchoscope can be inserted via an endotracheal tube but more usually a laryngeal mask airway. Typically, 1–3 lavages of 1 mL/kg (max 20 ml) of body weight of 0.9 % saline are instilled per lobe and then aspirated with a maximum of three aliquots. The most sampled segment is the right middle lobe, but it may be decided to include other lobes on the left side, or the most affected lobes, as determined by imaging. The collected BAL fluid (BALF) should be promptly processed to avoid degradation of markers. Immediate cooling on ice, rapid transport to the laboratory and quick transfer to storage at −80 °C are recommended to preserve sample integrity. A detailed SOP on inflammatory mediators in CF bronchoalveolar lavage through flexible bronchoscopy has been developed by the ECFS-CTN [138].

7.6. Recommendations and future directions

Infection may be a useful endpoint when the long-term use of antibiotics is studied [64]. Inflammatory markers in induced sputum [133] and BALF may be used as secondary endpoints in trials, preferably in placebo-controlled trials where comparator groups are available, and with longer intervals between measures to smoothen out temporal variations [79].

What is the number needed for a trial?

There are currently insufficient data on markers of airway infection and inflammation on which to reliably base numbers for trials. Initial studies such as the COMBAT CF study successfully demonstrated differences in inflammatory markers with $n = 50$ (treatment group) vs $n = 44$ (placebo group) [64].

Can the endpoint be used in clinical practice?

Measurement of infection is routinely used in clinical practice, however, given the issues with lack of normative values, standardisation of measurements and lack of availability at many centres sputum inflammation requires further research. Serum inflammation investigation deserves future research, as the clinically relevant threshold is unknown for the biomarkers considered.

Future directions:

Consideration must be given to the invasiveness, burden, representability and transient alterations of infection and inflammation, however, sputum induction and BAL in young children is feasible and standardised [128,129,144]. Less invasive approaches that can be repeated frequently, such as the analysis of markers in exhaled breath, may hold promise [148,149].

8. Conclusions

Conducting interventional studies for children with CF under 2 years of age is challenging, due to the limited availability of clinically meaningful endpoints suitable for assessing efficacy. Pulmonary endpoints traditionally used in older children and adults are not applicable in this age group. Furthermore, with increasingly better care, children often have a ‘near normal’ baseline, making it difficult to demonstrate a clinically meaningful difference.

Candidate endpoints

Potential endpoints for young children 0–2 years of age include MBW, chest-CT, chest MRI, and markers of inflammation and infection.

Multiple breath washout

MBW may serve as a primary pulmonary outcome measure in interventional studies, but light sedation will be necessary in this age group. In an era of CFTRm, studies will likely require higher subject numbers than current approaches used in this age group, as well as an adequate control group. MBW would be valuable in post-marketing observational studies, as differences in MBW between treated and untreated groups may become more apparent over time.

Imaging endpoints

Although there is robust evidence for the feasibility and sensitivity of chest CT as a primary endpoint for children of 3 to 6 years of age, its sensitivity as an outcome measure for children younger than three years is less clear. Currently, for the use of chest CT-related outcomes, a randomized controlled trial could best start after diagnosis and be evaluated with a CT at the age of three years. The introduction of automated image analysis systems and the use of PCD-CT is likely to lower this age threshold.

Chest MRI can evaluate lung ventilation in detail but limited access to MRI facilities in most centers and the need for sedation for 6 months to 2-years limits the feasibility of this endpoint for clinical trial use at present.

Infection and inflammatory endpoints

Currently, none of the infection and inflammatory endpoints have enough evidence to serve as a primary endpoint for children below the age of 2 years. However, infection and inflammatory markers in induced sputum or bronchoalveolar lavage fluid may be useful secondary endpoints, particularly if compared to untreated controls.

Minimal Meaningful Clinical Difference

For all discussed endpoints, the minimal meaningful clinical difference has not yet been established in the 0–2-year age group.

Recommendations for trial design

Children under two years of age represent a particularly vulnerable population for clinical trials. Study designs must aim to minimize burden on participants by carefully selecting the least invasive procedures that still provide the most informative and clinically relevant data.

Current trial designs for new drugs for children with CF under 2 years of age should continue to focus on pharmacokinetics and where appropriate, pharmacodynamic markers such as sweat chloride in response to modulators, with safety and tolerability as primary endpoints. This approach facilitates the timely introduction of new therapies in this vulnerable age group.

Pulmonary endpoints should be included as secondary or exploratory outcomes. Long-term post authorization studies exploring pulmonary outcome measures such as MBW, measures of structural lung disease such as CT and MRI, and measures of pulmonary infection and inflammation will be vital to understanding the pulmonary benefits of commencing therapies in this young cohort.

Credit author statement

Lee et al. Pulmonary endpoints in clinical trials for children with cystic fibrosis under two years of age.

Hettie Janssens and Tim Lee: Conceptualization; Project administration; Methodology, Visualization; Roles/Writing - original draft; and Writing - review & editing. Kate Hill: Project administration; Methodology, Visualization; Roles/Writing - original draft; and Writing - review & editing. All authors: Visualization; Roles/Writing - original draft; and Writing - review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

TL has received consulting fees & payment or honoraria for lectures, presentations from Vertex Pharmaceuticals.

KH, DC, CS & AL have no conflicts of interest to declare.

PC has received research grants from General Electrics 2022 & payment or honoraria for lectures, presentations from Siemens Healthineers and vertex Pharmaceuticals

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Supplementary materials

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