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The contribution of induced sputum sampling to surveillance of lower respiratory tract microbiology in children with Cystic Fibrosis.

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Summary

- Children with Cystic Fibrosis (CF) are thought to be born with normal lungs. Difficulties with secretion clearance and repeated infections cause a gradual decline in lung function. Aggressive management with regular physiotherapy and treatment of infection has improved the prognosis for children with CF.
- Standard of care for children with CF involves regular surveillance of airway microbiology with aggressive treatment of positive airway cultures. The CF Trust guidelines currently recommend that cough swabs are taken routinely at each clinic visit and also during exacerbations. North American guidelines suggest taking a throat swab rather than a cough swab.
- Most children with CF are unable to produce a sputum sample even if they have a productive cough. Cough swabs are used as a surrogate marker for lower airway cultures. Cough swabs at the children's CF centre in Cardiff are routinely taken at 2 monthly intervals.
- Bronchoalveolar lavage (BAL) is the gold standard for identifying lower airway microbiology and is performed in children with CF when there are chronic symptoms unresponsive to empirical antibiotic treatment. BAL cultures taken from multiple sites in the lung are not concordant and the number of lobes sampled sequentially increases microbiological yield.
- Bronchoscopy with BAL is an invasive procedure and usually involves a general anaesthetic.
- There is an emphasis on early aggressive management of lung disease in children with CF. Annual bronchoscopy for children with CF has been raised by the international CF community, as a potentially useful and acceptable approach to airway microbiology surveillance. This is an invasive procedure and usually involves a general anaesthetic.
- Induced sputum is a non-invasive method of obtaining lower airway secretions from children who are not spontaneously productive. Induced sputum has been shown to be more effective than cough swab at identifying CF pathogens.
- There are currently no data comparing microbiological yield from induced sputum and from bronchoalveolar lavage in children with CF. Induced sputum specimens sample the whole lower respiratory tract and may therefore provide a more accurate overview of lower airway microbiology when compared with BAL, which only samples a localised part of the lung.
- Methods for obtaining induced sputum are well described in children as young as 6 months of age.
- The study is designed to assess the benefits of induced sputum as an approach to airway microbiology sampling in CF and will compare 3 modalities of microbiology sampling from the respiratory tract in children who have CF. The three modalities are cough swab, induced sputum and bronchoalveolar lavage (BAL) performed at bronchoscopy.
- Induced sputum is a non-invasive approach to airway sampling and if shown to be effective, may have a contribution both in routine infection surveillance and also in reducing the number of bronchoscopies required in children with CF with acute chest exacerbations.

Safety and tolerability of sputum induction manoeuvre

- Induced sputum is a well established, safe approach to obtaining lower airway samples either for culture or for assessment of cytology and inflammation.
- The evidence that sputum induction is a safe procedure in children comes from 22 papers looking at sputum induction in the context of asthma¹⁻⁹, tuberculosis¹⁰⁻¹³, pneumonia¹⁴⁻¹⁵ and cystic fibrosis¹⁶⁻²². Together these papers report the tolerability of induced sputum in 1834 children. There were no serious side effects in any of the reports but 5% of all patients could not tolerate completion of the procedure.
- Most studies included patients age >6 years who could reliably perform spirometry so that adverse consequences to the sputum induction procedure could be monitored.
- Six reports studied the use of sputum induction in children under the age of 5 years^{11-15,18}. Three of these reports, all published by the same group, concentrated on the use of sputum induction in infants and very young children. These high profile large studies assessed sputum induction in patients with tuberculosis and pneumonia in an HIV prevalent area.^{11-12,14} Together the three studies included a total of 602 patients. The median age in the three studies ranged between 6 and 13 months. Induced sputum was very well tolerated in this age group even in acutely unwell children. Vomiting, wheeze or hypoxia occurred in only 3% of cases. Mild epistaxis was also seen in a number of children.
- The six studies looking at safety and tolerability of induced sputum in children with cystic fibrosis¹⁶⁻²² have generally enrolled older children who can perform reliable spirometry. Together these six studies included a total of 211 patients and identified 16 patients who could not complete the sputum induction protocol because of symptoms (7.5%). In the one study that reported on a wider age range of children (between age 8 months and 8 years), there were less side effects reported in the younger age group who tolerated the procedure well.¹⁸
- No studies have looked specifically at the tolerability and safety of sputum induction in infants and very young children with Cystic fibrosis. However, hypertonic saline as a treatment, rather than as part of a procedure, has been shown to be well tolerated in infants with Cystic Fibrosis.²³⁻²⁶

Bronchoscopy and bronchoalveolar lavage

- A European Respiratory Society (ERS) task force published guidance on Bronchoalveolar lavage (BAL) in children in 2000²⁷. It recommended taking a single BAL from the most affected lobe or if there was diffuse disease, from the right middle lobe.
- A study comparing microbiological yield from BAL collected from the right middle lobe and lingula showed differences, suggesting bacterial distribution is heterogeneous within the lung. An attempt at establishing international consensus in 2007 generated modified guidelines for BAL surveillance of lower airway microbiology in children with Cystic Fibrosis: A three-aliquot BAL should be taken from the right middle lobe and a single-aliquot BAL from the lingula or the most affected lobe²⁸.
- A recent study²⁹ looking at cumulative microbiological yield with increasing BAL sampling showed 6 lobe sampling to be superior to one²⁷ or two²⁸ lobe sampling.

Induced sputum versus bronchoscopy and BAL.

- Bronchoscopy and BAL is the established gold standard method for obtaining lower airway samples.
- The evidence that induced sputum is as good as bronchoscopy and BAL at obtaining lower airways samples comes from 27 papers in adults and 2 papers in children. Adult papers have compared the two approaches in their ability to clarify and monitor diagnostic cytology in sarcoidosis³⁰⁻³⁵, asthma³⁶⁻⁴¹, cystic fibrosis⁴⁰, interstitial lung disease, pulmonary fibrosis and hypersensitivity pneumonitis^{33,42-43}. Microbiological yield using the two approaches has been compared in patients with TB⁴⁴⁻⁴⁹ and HIV⁵⁰⁻⁵³. Two papers have compared induced sputum with bronchoscopy and BAL in children^{20,54}. These papers reported on patients with asthma and cystic fibrosis. Both papers compared inflammatory indices rather than microbiological yield.
- In a small study involving 11 adult patients with Cystic fibrosis, microbiological yield from spontaneous sputum, sputum induction and BAL were compared⁵⁵. Test specific detection rates for the major CF pathogens showed equivalence in sensitivity of isolation between the three approaches, with a non-significant trend to superior sensitivity for sputum induction. There are no equivalent studies in children with CF. Studies are needed to identify whether the relationship between these three approaches holds for young patients with correspondingly milder CF chest disease. The value of sputum induction as a non-invasive surrogate for BAL may be greater in younger children, as spontaneous sputum induction is unusual.

Oropharyngeal cultures or nasal cultures versus bronchoscopy and BAL

- Evidence that oropharyngeal cultures can be used as a surrogate for cultures from lower airways samples in non-expectorating patients with cystic fibrosis comes from 5 reports⁵⁶⁻⁶⁰. Rosenfeld reviewed 3 studies involving 141 children with CF age <5 years. Oropharyngeal cultures carried a sensitivity of 44%, specificity of 95%, PPV 44% and NPV 95%. In this age range, where lung disease is generally mild, and there are few pathogen isolations, this data suggests that a negative oropharyngeal culture can effectively exclude a lower airway infection, whereas a positive oropharyngeal culture does not reliably rule in lower airway infection.
- The PPV and NPV of oropharyngeal cultures in adult patient groups, where there is a high prevalence of airway pathogens, correspondingly change. In this situation, a negative oropharyngeal culture cannot effectively exclude a lower airway infection (NPV is low), but a positive oropharyngeal culture does reliably rule in lower airway infection (PPV is high)⁶¹.

Oropharyngeal cultures or nasal cultures versus induced sputum

- Evidence that induced sputum is more effective at identifying bacterial pathogens in patients with cystic fibrosis when compared to oropharyngeal samples comes from 5 papers^{16-17,22,55,62}. A small study of 10 adults with CF found comparable microbiological yield using spontaneously expectorated sputum, induced sputum and BAL. Two small studies in children found higher yields on induced sputum compared to cough swab^{17,22}. Two larger studies comparing cough swab with induced sputum in patients with CF, enrolled 43 children and 94 children respectively. The two studies identified additional organisms on induced sputum in 30% and 42% of cases respectively^{16,62}.
- The benefits of sputum induction over cough swab have been documented in adults and older children with cystic fibrosis. There are no studies comparing the two approaches early in life. In younger children, CF disease is less severe and fewer children are sputum

producers. The benefits of sputum induction over cough swab in this age group may therefore be yet greater than that seen in older children and adults.

Research hypotheses

Hypothesis 1

Induced sputum in young children with cystic fibrosis age <6 years is safe and well tolerated both in children who are well and in children who are hospitalised with chest exacerbations.

Hypothesis 2

Induced sputum microbiology is as good as microbiology from samples taken directly from the lower airway using bronchoscopy and BAL.

Hypothesis 3

Induced sputum provides a greater yield of CF respiratory pathogens than cough swab or throat swab.

Hypothesis 4

Routine induced sputum sampling taken at annual review, in addition to the normal procedure of two monthly cough swabs, increases the annual yield of respiratory pathogens in children with CF, thereby improving microbiology surveillance in this group of patients, and contributing directly to clinical care.

Hypothesis 5

In young children with mild CF disease who are non-productive even after the induced sputum procedure, a cough swab taken after the induced sputum manoeuvre provides a greater yield of CF respiratory pathogens than a routine cough swab alone.

Hypothesis 6

Culture-independent approaches to microbiological surveillance of the CF lung can identify occult pathogens in non-productive young healthy children with cystic fibrosis who are non-productive, and have a clinical contribution to make in addition to standard microbiological processing.

Hypothesis 7

Nasal carriage of CF pathogens correlates with lower airway microbiology and nasal swabs can be used as a surrogate non-invasive approach to bacterial surveillance

Hypothesis 8

Longitudinal surveillance of patients using culture-independent approaches to microbiological surveillance can identify early life factors associated with the development of healthy or unhealthy microbiomic signatures in later life.

Recruitment of subjects

Children with cystic fibrosis will be recruited in 4 clinical situations

1. Those attending for clinically indicated bronchoscopy
2. Those attending for routine surgery under GA, when surveillance bronchoscopy is standard of care at UHW
3. Those admitted for treatment of a chest exacerbation
4. Those attending for annual review in outpatient clinic

- **Hypothesis 1** will recruit patients from clinical situations 1,2,3,4
- **Hypothesis 2** will recruit from clinical situations 1 and 2
- **Hypothesis 3** will recruit from clinical situations 1,2,3,4
- **Hypothesis 4** will recruit from clinical situation 4
- **Hypothesis 5** will recruit from clinical situation 1,2,3,4
- **Hypothesis 6** will recruit from clinical situations 1,2,3,4
- **Hypothesis 7** will recruit from clinical situations 1,2,3,4

Sampling

Nasal carriage swabs are routine procedure for hospital cross infection purposes and are a non-invasive procedure involving passing a wet swab over the anterior surface of the nostril. This sample will always be taken first.

Taking a throat swab is a routine non-invasive investigation in children and involves placing a swab on the mucosal surface of the pharynx. This sample will always be taken second.

Taking a cough swab involves asking the child to cough while a swab is placed in the oropharynx. This is also a routine, very non-invasive uncomplicated procedure and is unlikely to influence the result of an induced sputum performed afterwards. Induced sputum however is a more involved procedure involving hypertonic saline nebulisation for 15 minutes with physiotherapy in order to mobilise secretions. This is highly likely to influence any cough swab result taken afterwards. The decision not to randomise the order of these two procedures is based on the prior knowledge that induced sputum is highly likely to influence cough swab, whereas cough swab is highly unlikely to influence induced sputum.

Hypothesis 3 and 4 will therefore be tested using comparisons between cough swab taken first followed by an induced sputum afterwards.

Young children often have mild disease and are often non-productive. It is possible therefore that the induced sputum manoeuvre in this age group will sometimes be unsuccessful in producing sputum. However, the induced sputum manoeuvre may have mobilised secretions even if sputum cannot be collected, and this may improve the yield on a cough swab taken afterwards. All induced sputum procedures will therefore be followed by a cough swab. Hypothesis 5 will be tested by comparing yield from the cough swab taken before induced sputum to that taken after.

For the above reasons, it follows that all induced sputum procedures will be preceded and followed by cough swabs.

Hypothesis 1

Induced sputum in young children with cystic fibrosis age <6 years is safe and well tolerated both in children who are well and in children who are hospitalised with chest exacerbations.

Subjects

Children attending for induced sputum in all 4 clinical situations will be included in a safety and tolerability assessment. In total approximately 200 children will be recruited to the study (50 attending for bronchoscopy, 90 attending for annual review and 60 admitted unwell with chest exacerbations – see below for more details)

Methods

- All induced sputum procedures will be carried out by a physiotherapist.
- All children will be monitored during the procedure with oxygen saturations and heart rate, and will be continuously assessed for signs of respiratory distress, degree of cough and wheeze.

- For those children > 7 years old, spirometry will be performed before and after the procedure.
- The physiotherapist will document whether there were mild, moderate, severe or no complications, and subjectively grade whether the procedure was well or poorly tolerated.
- The patient and family will be given a questionnaire relating to tolerability of the induced sputum procedure.

Analysis

- Analysis will be descriptive

Hypothesis 2

Induced sputum microbiology is as good as microbiology from samples taken directly from the lower airway using bronchoscopy and BAL.

Subjects

- Approximately 50 bronchoscopies are performed / year. Approximately 30 procedures are performed to obtain lower airways cultures. In 20 of these there would be the possibility of comparing cough swab, induced sputum and BAL microbiology. The majority of these patients will have cystic fibrosis. We expect to enrol 50 CF patients within a 3 year period.
- Patients of all ages for whom bronchoscopy and BAL are clinically indicated under current standards of care will be approached to take part in the study.
- It is normal practise at UHW for children with CF who are undergoing any routine surgical procedure under GA, to have a bronchoscopy and BAL for microbiological surveillance. These patients will also be approached to take part in the study

Methods

- For patients and their families attending for bronchoscopy, an information sheet outlining the research project will accompany the invitation letter to attend for bronchoscopy.
- For those undergoing routine surgery, there are strong communication links between surgical and CF nurse specialists in preparation for surgery. These patients are identified early and will also be sent an information sheet outlining the research project before the procedure.
- Children are admitted for bronchoscopy and BAL on the afternoon before the procedure. There will be an opportunity to discuss the research on arrival to the ward.
- If happy to participate in the study, consent will be taken.
- A cough swab followed by induced sputum followed by a further cough swab in that order, will be taken on the afternoon before bronchoscopy is performed.
- Taking the samples in this order and across this timeframe will minimise the chance of one procedure affecting the outcome of another. Induced sputum will be performed by a respiratory physiotherapist. Samples will be sent immediately for microbiology processing.
- All children with CF receive a general anaesthetic for bronchoscopy procedure. Bronchoscopy will be performed by a consultant in paediatric respiratory medicine. BAL will be performed from multiple sites in the lung. The first sample will be taken from the site of maximal disease on chest x-ray, the second sample from the contralateral side (either right middle lobe or lingula). In this study, bilateral samples will be taken using two separate bronchoscopes to ensure that cross contamination between sites does not occur and yield from the two sites can be compared. Further samples will be taken from the remaining 4 lobes and combined in a third sample.

Analysis

Conclusions will be drawn on the following

- Relative microbiological yield of induced sputum versus single BAL, bilateral BAL and extensive 6 lobe BAL
- Relative strengths of induced sputum and cough swab in predicting BAL microbiology

Hypothesis 3

Induced sputum provides a greater yield of CF respiratory pathogens than cough swab or throat swab.

Subjects

- All induced sputum procedures will be paired with a preceding throat swab and cough swab. Patients will therefore be recruited for this arm of the study from all 4 clinical situations.

Bronchoscopy and routine surgery patients

- See details above

Outpatients

- Children with CF attend for an extensive annual review in Cardiff where they are seen by the multi-disciplinary team. The opportunity therefore arises for all patients to have an induced sputum sample at annual review in addition to normal microbiology sampling.
- 80 patients attend for annual review each year and we would envisage performing induced sputum on 30 children each year. Induced sputum will be performed by the CF physiotherapist.
- We would aim to obtain induced sputum samples on a minimum of 90 occasions over a three year period.

Inpatients

- Children with CF with chest symptoms refractory to oral antibiotic treatment are admitted to hospital for a two week course of IV antibiotics together with aggressive physiotherapy and dietetic assessment. The opportunity therefore arises for all patients to have an induced sputum sample in addition to normal microbiology sampling when unwell.
- Approximately 30 patients are admitted each year for IV antibiotics. We would envisage performing induced sputum on 20 of these children each year. Induced sputum will be performed by the CF physiotherapist.
- We would aim to obtain induced sputum samples on a minimum of 60 occasions over a three year period.

Methods

Outpatients

- Annual reviews are planned by the paediatric CF nurse specialists in advance. Patients and their families will be sent an information sheet outlining the research project with their invitation for annual review.
- 2 patients are seen for annual review in clinic each week, interspersed with other CF patients attending for routine clinic appointments. We would aim to enrol one of the two patients attending for annual review in the research study. Consent will be taken
- Strict infection control policy exists for the children's CF clinic as transmission of some respiratory organisms can occur between patients with CF. Children are seen in their own clinic room by rotating members of the multidisciplinary team, so that they do not share the same space with other children with CF at any time during their visit to clinic.
- Children enrolled in the study will be seen at the end of the clinic so that the induced sputum manoeuvre does not complicate existing infection control policy.

- Although we aim to enrol one patient per week, capacity in the children's outpatient clinic is sufficient to enrol both children attending for annual review while still observing strict infection control policy.
- The patient and family will be seen by all member of the MDT as normal. At the end of the clinic appointment they will be seen by the physiotherapist for cough swab followed by sputum induction followed by cough swab.

Inpatients

- Children admitted for IV antibiotics routinely receive a cough swab before IV antibiotics are started and are reviewed by the physiotherapist when they arrive.
- Children and parents will be recruited on arrival, given an information leaflet and asked for consent
- We would aim to recruit one patient each week.

Analysis

Conclusions will be drawn on the following

- Relative microbiological yield from induced sputum versus cough swab

Hypothesis 4

Routine induced sputum sampling taken at annual review, in addition to the normal procedure of two monthly cough swabs, increases the *annual* yield of respiratory pathogens in children with CF, thereby improving microbiology surveillance in this group of patients, and contributing directly to clinical care.

Subjects

- Results from cough swabs from patients seen for annual review will be used to test this hypothesis.

Analysis

Conclusions will be drawn on the following

- The added value of an induced sputum at annual review in addition to 2 monthly cough swabs taken over the preceding year, in terms of additional treatments instituted as a consequence

Hypothesis 5

In young children with mild CF disease who are non-productive even after the induced sputum procedure, a cough swab taken after the induced sputum manoeuvre provides a greater yield of CF respiratory pathogens than a routine cough swab alone.

Subjects

- All induced sputum procedures will be combined both with a preceding cough swab and a cough swab afterwards. Patients will be recruited for this arm of the study from all 4 clinical situations.

Analysis

Conclusions will be drawn on the following

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- In those children who do not manage to produce sputum with the induced sputum manoeuvre, microbiology from the cough swab preceding the induced sputum procedure will be compared with the cough swab taken afterwards.

Hypothesis 6

Culture-independent approaches to microbiological surveillance of the CF lung can identify occult pathogens in non-productive young healthy children with cystic fibrosis who are non-productive, and have a clinical contribution to make in addition to standard microbiological processing.

Subjects

- All induced sputum procedures will be combined both with a preceding cough swab and a cough swab afterwards. Patients will be recruited for this arm of the study from all 4 clinical situations.

Analysis

Conclusions will be drawn on the following

- Paired samples will be sent for standard microbiological processing and for culture independent analysis and results compared.
- Conclusions will be drawn as to which sample type (cough swab, induced sputum, BAL) from non-productive young children is most appropriate for culture independent analysis.

Hypothesis 7

Nasal carriage of CF pathogens correlates with lower airway microbiology

Subjects

- All induced sputum procedures and bronchoscopy procedures will be combined with a preceding nasal swab, cough swab and a cough swab afterwards. Patients will be recruited for this arm of the study from all 4 clinical situations.

Analysis

Conclusions will be drawn on the following

- Samples will be sent for standard microbiological processing and for culture independent analysis and results compared.
- Conclusions will be drawn as to which sample type (nasal swab cough swab, induced sputum, BAL) from non-productive young children is most appropriate for culture independent analysis.

Hypothesis 8

Longitudinal surveillance of patients using culture-independent approaches to microbiological surveillance are useful in identifying early life factors associated with the development of healthy or unhealthy microbiomic signatures in later life.

Subjects

- Patients recruited into the CF-SpIT study will be followed longitudinally through childhood in a prospective manner to identify changes in microbiome on sputa and BAL with age and

clinical progression. This will enable the identification of early risk factors in the development of unhealthy microbiomic signatures later in life.

Analysis

Conclusions will be drawn on the following

- Samples from sequential induced sputum and BAL samples will be compared in a longitudinal manner and correlates made with significant clinical outcomes.

Methodology

Protocol for induced sputum sampling¹¹

Induced sputum sampling will be performed by a paediatric physiotherapist

- Induced sputum should be performed after a 2 hour fast.
- Make an initial assessment of the chest. Attach an oxygen saturation monitor and document oxygen saturations, heart rate, respiratory rate. Perform lung function in children older than 7 years old.
- Before the procedure, obtain a cough swab.
- Administer 200mcg salbutamol via metered dose inhaler and spacer to prevent bronchoconstriction.
- Use a jet nebuliser attached to wall oxygen at a flow rate of 5 l/min to deliver 8 ml of 7% sterile hypertonic saline for 15 minutes.
- Make an assessment of the chest every 5 minutes.
- After 15 minutes, apply physiotherapy techniques including chest percussion, vibration, and active cycle breathing.
- Obtain sputum either by expectoration (in children able to cooperate) or by suctioning through the nasopharynx or oropharynx using a sterile, mucus extractor or suction catheter size 6.
- Make a final assessment and document observations, oxygen saturations, and lung function in children over 7 years old.
- Repeat cough swab after procedure even if induced sputum sampling is unsuccessful.

Processing of samples

1) Standard microbiological processing

Cough swabs, induced sputa and BAL samples will all be taken at the University Hospital of Wales (UHW) and processed on site by the Cardiff and Vale microbiology department, who are collaborators in this project (contact: Dr Robin Howe). Protocols for microbiological processing of CF samples are well established in the clinical laboratory, and adhere tightly to the Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis, published by the CF Trust.⁶³ The depth of routine microbiological processing of samples in the clinical laboratory will therefore help answer the hypotheses outlines above, on a clinically pragmatic level as this is the level of processing that would be available in any CF centre. There is interest from microbiology in using these samples to identify optimal processing of samples and this work may involve the microbiology research laboratories.

2) Culture independent microbiology

Airway samples samples will be analysed in a parallel study using culture-independent microbiology. This approach will enable us to retest the current hypothesis using new emerging approaches to microbiology.

Comparisons will be made between the two approaches. The culture independent approach will be performed in collaboration with Professor Eshwar Mahenthalingam, in the Department of Biosciences, University of Cardiff. All samples will remain anonymous to collaborators in Biosciences.

3) Inflammation in the CF lung

Airway samples will be analysed for patterns of inflammation to try and understand the correlation between inflammation and infection in the CF lung. Samples will be processed in the labs of Dr Eamon Mcgreal.

Storage of microbiological samples

- All samples will be supplied anonymously to researchers; only Dr Forton and members of his research group will be able to identify which samples were donated by specific patients. The recipients of the samples will not be supplied with the name or any other identifiable information and will not be able to identify patients from the samples.
- Any residual samples at the end of the study will be stored under a license from the Human Tissue Authority. License no: 12422.
- Samples may be retained at the end of this study for use in future research within the UK and abroad. At this stage we do not know what the research will involve but some of it could include more bacterial genetic research and further research on lung inflammation. The samples will not be sold and will not be used in human genetic research, animal research or the commercial sector.

- **Current Use of samples in this study**

Participation in this study is voluntary and patients are free to withdraw at any time without giving a reason and without medical care or legal rights being affected.

If a patient does withdraw consent, those samples will not be used further in this study and will be destroyed according to locally approved practices. Any samples, or results derived from the samples, that have already been used prior to the withdrawal of consent will continue to be used in this study.

- **Future Use of samples in other related studies**

Consent may be withdrawn at any time for the storage and future use of samples at any point. If a patient does withdraw consent, those samples will not be used in any subsequent studies and will be destroyed according to locally approved practices. Any samples already distributed for use in research prior to the withdrawal of consent will continue to be used in that study and any samples remaining at the end of the study will be destroyed.

Consent

- For all patients less than 18 years of age written parental consent and patient assent will be sought. All patients over 18 years of age written consent will be sought.

Statistical analysis

- Descriptive statistics including range, frequencies, mean, median, standard deviation and/or percents will be used to describe the baseline characteristics of the study population.
- Frequency of positive microbiological yield and more specifically frequency of positive microbiological yield for known CF pathogens will be compared between induced sputum and BAL (standard and extended approach), and between induced sputum and cough swab using the chi squared test.

- For each known CF pathogen, discrepancies in microbiological yield between induced sputum and BAL (standard and extended approach), and between induced sputum and cough swab, will be interrogated using the McNemar test.
- Test specific detection rates for each test (induced sputum, standard or extended BAL, cough swab) will be calculated simply as the number positive by the specified test / number positive by any test.

Power calculation

- **Hypothesis 2**
There is no data comparing BAL to induced sputum in patients with CF except for a small study of 11 adult patients. Such data does exist for TB but is difficult to extrapolate to bacterial isolates in CF. Power calculations for comparison of BAL with induced sputum samples in children with CF can not be calculated in a meaningful way. Data collected will be used to generate power calculations for definitive studies.
- **Hypothesis 3**
There is no available data on induced sputum sampling in children with cystic fibrosis under age 6 years. In a study of 94 children with CF aged >6 years where cough swab was compared with induced sputum, positive cultures were seen in 75% of cases, with discrepant culture results in 27% of patients. More CF pathogens were isolated from induced sputum samples compared with cough swab. In the present study, we aim to recruit 200 paired samples to compare cough swab with induced sputum in children with CF of all ages. Assuming a positive culture result in 75% of cases, and the same discrepancy characteristics between cough swab and induced sputum as described in the study above, this sample size will be capable of detecting a 15% discrepancy in culture results with a power of 80% and probability of type 1 error of 0.05.

Confidentiality and data protection.

- All patient samples will be anonymised by allocation of codes and it is not anticipated that patient identifiable information will be used routinely within the study. All data will nevertheless be handled in line with the NHS Code of Confidentiality. Specifically, all members of the research team will be aware of their responsibilities regarding confidentiality. Patient information will be recorded consistently and accurately. All data will be kept private and stored securely and where that information is used, this will be done with appropriate care.

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