Evaluation of the risk of fungal colonisation/infection in patients with cystic fibrosis: an international prospective study comparing the performances of mycological culture media - MycoFong International Project (mfip)

Acronym : « mfip »

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SYNOPSIS

<table>
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<tr>
<th>ORGANISATION RESPONSIBLE FOR THE RESEARCH</th>
<th>CHRU Lille</th>
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<tr>
<td>TITLE</td>
<td>“Evaluation of the risk of fungal infection in patients with cystic fibrosis: an international prospective study comparing the performances of mycological culture media, the MucoFong International Project”</td>
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<td>CO-ORDINATOR/INVESTIGATOR</td>
<td>Dr Laurence Delhaes</td>
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| NUMBER OF CENTRES | - 10 French centres  
- 11 centres in other European countries, and Australia |
| TYPE OF STUDY | Current care with secondary use of biological samples |
| OBJECTIVES | To evaluate the performance of various culture media for mycological investigation of sputum samples from patients with cystic fibrosis, in order to standardising this analysis.  
This project and its objective are based on an international study designed in the frame of the ECMM/ISHAM Working Group on filamentous fungi and chronic respiratory infections in cystic fibrosis” (convenor: Prof. Jean-Philippe Bouchara, CHU Angers) |
| JUDGEMENT CRITERIA | - Sensitivity and predictive value of the culture media  
- Estimates of the prevalence of each fungal species  
- Identification and implementation of the optimal combination of these media by the CHAID method |
| INCLUSION CRITERIA | - Patient with a diagnosis of cystic fibrosis validated by the criteria currently in use  
- Patient routinely followed up at one of the centres participating in the study  
- Patient able to expectorate  
- Patient for whom a mycological examination is carried out as part of routine microbiological monitoring (good clinical practice) |
- Patient with cystic fibrosis for whom a mycological examination is carried out due to clinical aggravation requiring biological exploration
- Signature of an informed consent form at the time of inclusion.
- Patient covered by the social security system

**EXCLUSION CRITERIA**

- Lung transplant recipients
- Patients or their parents (if the child is a minor) refusing to participate in the study
- Incapable adult subject, with a guardian or a ward of court
- Refusal or inability to give informed consent

**NUMBER OF PARTICIPANTS**

*Between 500 and 700 (25 to 30 per centre)*

**EVIDENCE OF CURRENT PRACTICE FOR THE ACTIONS CARRIED OUT AND/OR THE PRODUCTS USED**

Patients with cystic fibrosis undergo regular mycological examinations of their sputum samples, either as part of their routine annual follow-up or during disease exacerbation. Our study aims to make use of these sputum samples to carry out the examination requested (on a standard medium, such as Sabouraud medium), and also, if the volume is sufficient, including semi-selective media specific to mycology shown to be of value in the national PHRC 1902 “MucoFong” or in published studies.

This project will therefore require the use only of practices and products that are already routinely used.

**PARTICULAR MODES OF SURVEILLANCE**

The media will be inoculated and incubated and then examined twice weekly. For each medium:
- The day on which a positive result is first obtained will be noted
- The fungal species present will be identified
- Each species will be quantified (scored from 0 to +++) on days 8 and 15 of culture.

Each centre will collate its data in an Excel spreadsheet, which will be sent monthly to Dr Delhaes, for the regrouping, checking and analysis of data.

**EVALUATION OF THE BENEFITS, RISKS AND CONSTRAINTS ASSOCIATED WITH THE RESEARCH**

In the short term, the principal benefits will be improvements in fungal detection and identification, leading to improvements in therapeutic management.

In the long term, the benefits will include a better knowledge of the fungi colonising the respiratory tracts of patients and the definition of an international consensus protocol for the mycological examination of these samples.

These benefits are associated with no particular risk, because no additional samples will be required over and above those taken for routine follow-up.

**EXCLUSION PERIOD**

*None*

**STATISTICAL ANALYSIS**

The following statistical analyses will be carried out:
- Data description and checking
- Determination of the sensitivity and negative predictive value (NPV) of each medium
- The CHAID method

**STUDY DURATION**

*1 YEAR* with:
- 3 to 6 months of biological analysis
- 6 months for analysis of the results and the constitution of a fungal strain collection
MFIP ABSTRACT

Introduction: Prognostic of Cystic fibrosis (CF) depends essentially on the lung impairments. While considerable attention has therefore been paid over recent decades to prevent and treat bacterial respiratory infections, we observed emergence of fungi colonization in CF respiratory tract. Among filamentous fungi, Aspergillus fumigatus is the most common, but other species are reported increasingly, such as Scedosporium apiospermum, Aspergillus terreus, Exophiala dermatitidis and Scedosporium prolificans. However, recent publication (Borman et al. 2010) has reported widely variations among the range of fungal pathogens isolated and the species prevalence, as well as a lack of standardization in the method used to isolate fungal pathogens. One of the major aims of the ECMM/ISHAM Working Group on "Filamentous fungi and chronic respiratory infections in cystic fibrosis" coordinated by Pr Jean-Philippe Bouchara is to clarify the prevalence of filamentous fungi in the context of CF airways colonization.

Aims: In this context, our international and multidisciplinary network will take advantage of working together to improve our knowledge on the epidemiology, the physiopathology and clinical relevance of the colonization of the airways by filamentous fungi in patients with CF. Here, we propose to organize an international study with the purpose of comparing performances of different media used for fungal culture, and developing a standardized approach for the mycological examination of respiratory samples from CF patients (MFIP for "MucoFong International Project").

Methods: In order to define the best set of media (including semi-selective media specific for detecting fungi / moulds) able to isolate 99.99% of the fungal potentially pathogens in CF population, we will organized a large international study using the same protocol with the same mycological media to isolate fungi according to the questionnaire we made and analyzed (Appendix 1).

All the media will be prepared in Angers and Lille and send to the different laboratories involved in mfip study. Each mycological laboratory will use the same protocol to plate CF sputum (Appendix 2), with respect of the patient anonymous (center codification plus patient codification (X-00Y)). As the samples analysed in the framework of this study will be obtained from those that patient supply during routine check-up visits (secondary use of sample), this protocol is considered as “current care with secondary use of biological samples”. According to the French Bioethic rules, only the unless patient’s object during the information notice for patient participating to mfip-study is required (Appendixes 3 and 4).

Each center will have the same period (3 months) to include 25-30 sputa from CF patients, and will use a prepared Excel table to summarize results (Appendix 2). Dr Laurence Delhaes will synthesize the whole data for statistically analyzing the results in collaboration with Lille Statistics Department (Pr A Duhamel).

Fungal isolates will be collected in each center (using 1.5 ml of sterile water plus small section of colony growth in agar) and send to Pr Jean-Philippe Bouchara for mass spectrometry analysis (Appendix 2).

Expected results: About 500 to 700 sputa might be included and analyzed during a short period of 3 months with the objective to get an exhaustive analysis of fungal prevalence in sputa from CF patients. The major data of the international survey will be analyzed, presented, and discussed during our next working meeting group with the purpose of developing a standardized approach to the culture of respiratory samples from CF patients.

In conclusion, this concerted effort towards defining the optimal method for the mycological analysis of the fungal component of CF microbiology is becoming a major requirement, not only to analyze the role of some “rarer” filamentous species in CF exacerbation or the existence of geographic variations in the fungal species that colonize CF patients, but also to be able to study the complexity of CF lung microbial ecology as well as its dynamics.

Keywords: Cystic fibrosis, filamentous fungi, mold, yeast, respiratory samples, sputum, culture methods