Scientific Collaboration: scientific research of pneumocystosis, an important opportunistic infectious disease.

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Pneumocystis jirovecii, formerly known as *Pneumocystis carinii* f. sp. *hominis*, is an ubiquitous distribution opportunistic pathogen which often causes severe interstitial pneumonia known as pneumocystosis or *P. jirovecii* pneumonia (PCP). Within the study of infectious diseases, PCP stands out as an important opportunistic infection and is a significant factor of morbidity and mortality, especially in patients infected with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) [1-3].

With the AIDS pandemic, PCP has become one of the key concerns in the monitoring of HIV positive patients. Before effective prophylaxis was introduced, 80% of HIV infected patients developed at least one episode of PCP in the course of infection, which in 60% of cases represented AIDS, leading to a death rate of between 20-25 per cent of patients. In recent years, there has been significant progress in the prevention, treatment and diagnosis of PCP, leading to an increase in the average life expectancy

of patients with this pathology. In industrialized countries, the widespread use of chemoprophylaxis established in the early 1990s, and the introduction of combination antiretroviral therapy in the middle of that decade, led to the decrease in the incidence of PCP. Still, PCP remains a significant clinical problem, and one of the main opportunistic diseases affecting HIV-positive patients. On the other hand, presently a few cases of PCP denote inequalities in access to medical care of some populations and social groups in various regions of the globe. This means that the timely and effective diagnosis of HIV infection is complicated, and only carried out when patients already present symptoms [1,3].

The laboratory diagnosis of PCP is based on cytochemical staining methods, indirect immunofluorescence using monoclonal antibodies (Fig. 1), and polymerase chain reaction (PCR). The biological samples collected by invasive means are not easy to obtain or repeat in patients with PCP, particularly in patients suffering from respiratory failure or already with AIDS. The rapid and effective detection of the agent in question resorting to inexpensive methods and to samples obtained noninvasively, such as blood, is a pressing need. Several serological markers have been studied and their importance discussed in terms of their application in the diagnosis of infection. The ß-glucan (BG), which is a major component of the cyst wall of *P. jirovecii*, appears to be the most promising candidate. Several studies have shown good correlation between the levels of BG in serum and the severity of the disease, also showing a potential to differentiate between infection by *P. jirovecii* and other fungal infections typical in immunocompromised patients. [4].

Moreover, due to the lack of a P *jirovecii* cultivation sustained process, molecular typing methods have been widely used to characterize this etiological agent. To date, attempts to associate *P. jirovecii* specific genotypes with clinical information have been inconclusive. However, progress in the study of the genetic diversity of *P. jirovecii* has led to important issues, like some organisms may be more pathogenic than others, and more virulent or resistant to drugs genotypes can be transmitted between hosts [2,5].

Given this state of the art, and taking as a starting point the common interest of researchers and health professionals from two important centennial institutions, there has been a long-standing important and ongoing collaboration between the Institute of Hygiene and Tropical Medicine of the New University of Lisbon (IHMT/UNL) and the Faculty of Medicine of the University of (FM/UL). This interdisciplinary scientific collaboration has led to the preparation and conduction of several studies and publications in the area of opportunistic infections, particularly PCP. As a result of a common strategy, the research project entitled “Importance of ß-glucan in *Pneumocystis jirovecii* pneumonia (PCP): a new diagnosis tool” was initiated in 2010,

financed by FCT (Ref. PTDC/SAL-MII/104231/2008). The aim of this study is to demonstrate that the detection of BG in blood and pulmonary samples of *P. jirovecii* infected patients presenting distinct clinical characteristics allows defining the BC levels that are characteristics of PCP, thus facilitating the diagnosis of this infection. As a result of this fruitful cooperation and common effort, a new research project was started in the beginning of the present year, entitled "Clinical relevance of multiple genetic markers in *Pneumocystis jirovecii* pneumonia (PCP): new high-throughput methodologies for application to molecular epidemiology and diagnosis", equally funded by FCT (Ref. PTDC/SAU-MIC/116716/2010). This new study intends to identify the multiple polymorphic sequences of *P. jirovecii* that are potentially associated with clinical parameters of HIV/ *P. jirovecii* co-infection and the development of high-throughput genotyping methodologies, such as multiplex PCR (mPCR) with single-base extension (SBE), associated with DNA pooling technology (mPCR/SBE-DNA pooling). This technology will be applied in diagnosis, prognosis and in the molecular epidemiology of PCP in different geographic regions.

These scientific research projects in the area of Biomedical Sciences/Infectious Diseases are based on a tripartite approach, and stem from the close collaboration between the Opportunistic Protozoa Group, Medical Parasitology Unit, Centre for Malaria and Tropical Diseases – Associate Laboratory (CMDT-LA), IHMT/UNL (the proposing institution) and the University Clinic of Infectious Diseases, FM/UL and the Department of Genetics, Faculty of Medical Sciences, UNL (participating institutions). The common goal is to bridge the gap in knowledge in the area of PCP, and allow the interconnection and the sharing of knowledge between applied research and clinic. In addition to researchers from these three institutions, the internationalization of the research teams must be stressed, as they include scientists from several parts of the world (Spain, Chile, USA) with recognized experience in epidemiology, diagnosis and *P. jirovecii* immunology.

All these new integrated and interdisciplinary approaches may lead to the development of new methodologies in diagnosing *P. jirovecii* infection, using biological samples obtained with less invasive techniques, such as blood, and to the identification of PCP cases with more pathogenic/virulent/resistant *P. jirovecii* genotypes. The ultimate goal of this approach is to help choose the most effective treatment for each case and thus improve the control of PCP globally.

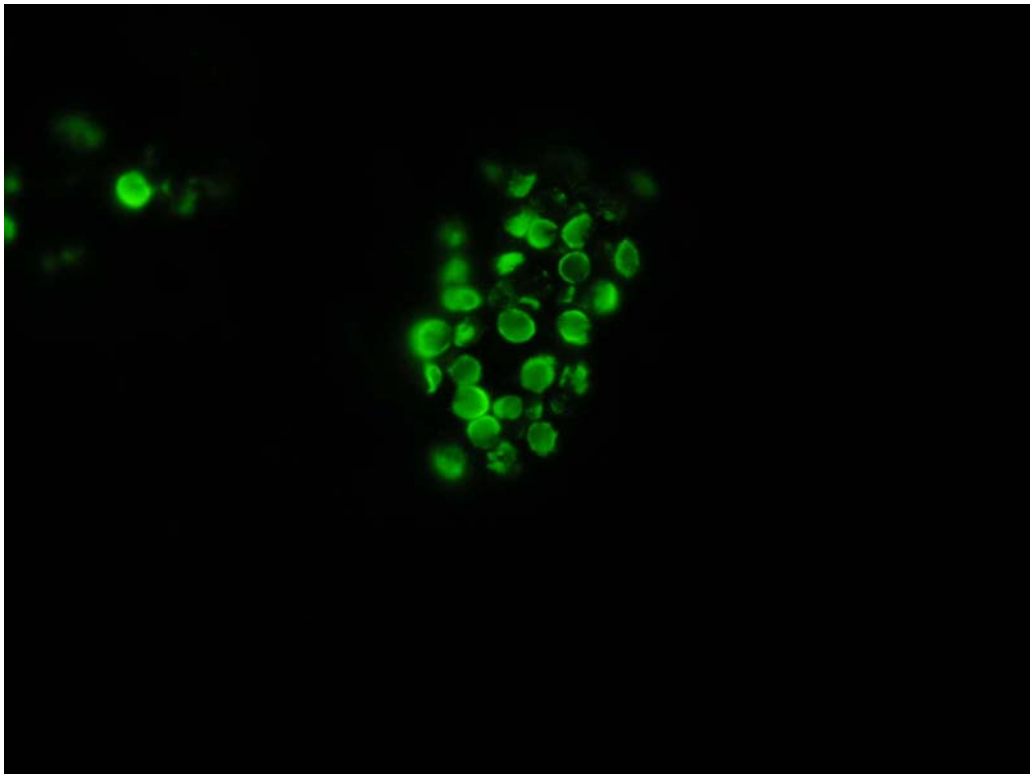

Fig. 1. *P. jirovecii* cysts stained by indirect immunofluorescence technique with monoclonal antibodies (IFI/AcM) (x1000), in a sample of lung secretions (authors' original).

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