Universidade Federal do Rio Grande do Sul
Faculdade de Medicina
Programa de Pós-Graduação em Ciências Pneumológicas

Tese de Doutorado

# Associação de pneumocistose e criptococose em pacientes imunocomprometidos: Estudo em serviço especializado

Guilherme Watte

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Tese apresentada ao Programa de Pós-Graduação em Ciências Pneumológicas da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de doutor.

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*À Sicléria.*

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**Lista de abreviaturas e símbolos**

AIDS - *Acquired immunodeficiency syndrome*

BCVA - *Best corrected visual acuity*

BAL - *Bronchoalveolar lavage*

CGB - *Canavanine–glycine–bromothymol*

CNS - *Central nervous system*

CSF - *Cerebrospinal fluid*

CT - *Computed tomography*

CI - *Confidence interval*

CrAg - *Cryptococcal antigen*

IOP - *Intraocular pressure*

IQR - *Interquartile range*

HAART *Terapia antirretroviral de alta potência*

HRCT - *High-resolution computed tomography*

OR - *Odds ratio*

PAD - *Peripheral artery disease*

SDA - *Sabouraud's dextrose agar*

PcP - *Pneumocystis pneumonia*

TARV - *Terapia antirretroviral*

TMP-SMZ - *Trimethoprim-sulfamethoxazole*

UNAIDS - *Joint United Nations Programme on HIV/AIDS*

VDRL - *Venereal disease research laboratory*

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**Resumo**

O objetivo deste trabalho foi o de avaliar a associação da pneumocistose e criptococose a relacionando a características clínicas e epidemiológicas em pacientes infectados pelo HIV. Encontramos dez pacientes com casos confirmados de infecção por *P. jirocecii*, dentre eles, sete com infecção simultânea de *C. neoformans* e três com infecção por *C. gattii* (todos os pacientes infectados com o HIV e com valores de CD4+ inferiores a 200 cel/µL). Estes casos correspondem a um terço das coinfecções da casuística de pneumocistose e 1% da casuística de criptococose da nossa instituição. Esta população teve o diagnóstico simultâneo das doenças durante a internação (desconhecia o diagnóstico de HIV previamente). Dois pacientes com meningite por *C. neoformans* não apresentavam sintomatologia prévia ou durante a internação. A mortalidade alcançou a 30% dos casos, sendo todos os pacientes diagnosticados com infecção por *C. gattii*. Dessa forma, recomendamos que aqueles pacientes com diagnóstico de HIV na internação hospitalar (prévia ou diagnosticada recentemente) com valores de CD4+ inferiores a 200 cel/µL achados de imagéticos com infiltrados pulmonares difusos, independentemente de sua apresentação clínica (mesmo que assintomáticos) prossigam a investigação para infecções fúngicas por pesquisa de fungos pela prata, cultivo, pesquisa de testes de aglutinação em latex ou imunocromatográfico (padronizado), microscopia e imunodifusão.

**Abstract**

The aim of this paper is to identify the double infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans* / *Cryptococcus gattii* in a human immunodeficiency virus (HIV) disease-endemic region. We included data from all patients diagnosed with *P. jirovecii* and Cryptococcus infection from January 1985 to December 2014 by patient medical records. We found 10 patients of double infection by *P. jirovecii* and *C. neoformans* / *C. gattii* in a hospital in a HIV disease-endemic region. Some cases of our sample had asymptomatic infections and all of *C. gattii*-infected patients died during the hospitalization. This paper calls the attention to the correct and rapid diagnoses in suspected PcP with HIV, CD4 levels less than 200 cel/μL and bilateral fibronodular infiltrates independent of clinical presentation. In this way, it is recommended to perform extensive investigation for fungal infections by rapid methenamine silver stain, culture, immunochromatography or latex agglutination tests, microscopy and immunodiffusion in order to provide an efficient clinical benefit.

**Resumo para leigos**

Coorte prospectiva com objetivo de avaliar a associação de pneumocistose e criptococose, relacionando-as a características clínicas e epidemiológicas em pacientes imunocomprometidos. Tese vinculada à Irmandade da Santa Casa de Misericórdia de Porto Alegre [CEP-ISCMPA: 1282/06; 1460/06; 00002509], Pró-Reitoria de Pesquisa, [PROPESQ/UFRGS: 11294] e aos Diretórios dos Grupos de Pesquisa no Brasil Lattes, Grupo de pesquisa: Micologia Clinica – UFRGS, link:

<*dgp.cnpq.br/dgp/espelholinha/00009824176602856839*5>

A coleta dos dados foi realizada entre toda a população diagnosticada de janeiro de 1984 a julho de 2014, ISCMPA, RS-Brasil.

Este projeto contou com apoio nacional e internacional para organização, tabulação e redação de artigos realizado no *Liverpool Heart and Chest Hospital*, LHCH, Liverpool, Grã-Bretanha, sob orientação do Prof. Dr. Klaus Irion (Anexo 1 e 2), contando com apoio do Programa de Pós-Graduação em Ciências Pneumológicas da Universidade Federal do Rio Grande do Sul (Anexo 3 a 6), sendo custeado pelo Governo Federal do Brasil através do Programa de Doutorado Sanduíche no Exterior, Fundação CAPES, Ministério da Educação do Brasil, Processo: BEX 3494/14–4.

Durante o período de junho 2013 até maio de 2017, o aluno bolsista desenvolveu três documentos relacionados à Tese descritos ao longo deste volume.

## 1 Introdução

A pneumonia causada por *Pneumocystis jirovecii* (PcP) (*P. carinii*) tem sido já há algum tempo reconhecida em pacientes com imunidade comprometida. Inicialmente descrita na literatura como epidemia de pneumonia intersticial causada em prematuros e desnutridos, nos anos 80 a PcP era incomum, ocorrendo em pacientes imuno comprometidos, na presença de doenças malignas, de terapia imunossupressora ou em imunodeficiências congênitas. Atualmente, no entanto, a taxa de infecção por *P. jirovecii* vem aumentando em decorrência da infecção pelo vírus da imunodeficiência humana (HIV) (1, 2).

Embora se observe queda na incidência de PcP pelo surgimento da terapia antirretroviral de alta potência (HAART), ela segue como uma doença grave e oportunista em indivíduos infectados pelo HIV. Nos países desenvolvidos, a epidemiologia e a apresentação clínica da PcP foram amplamente definidas e documentadas, contrastando com o número limitado de estudos epidemiológicos acerca da doença nos países em desenvolvimento (1, 3).

A incidência de pneumonia por *Pneumocystis jirovecii* tem sofrido um declínio progressivo e significativo após a disponibilidade dessa terapia mais moderna, mas a mortalidade tem se mantido praticamente inalterada. Entretanto, apesar da eficácia da quimiprofilaxia primária, a PcP continua sendo a infecção oportunista grave mais comum em pacientes com HIV. Muitos desses casos têm ocorrido em indivíduos que desconhecem a própria infecção pelo vírus HIV (muitas vezes recebendo os dois diagnósticos simultaneamente, descobrindo serem HIV+ pela presença da PcP) ou em pacientes com pobre aderência ao esquema HAART. Outro dado importante é que, a mortalidade intra-hospitalar desses pacientes tem permanecido estável (em torno de 10%), mesmo considerando todos os avanços dos cuidados intensivos.

Em países de baixa e média renda, como os da América Latina, a situação da PcP pode ser vista de modo diferente em relação aos países desenvolvidos, devido à falta de meios de diagnóstico e/ou acesso limitado a eles e aos cuidados de saúde e de tratamentos (1, 4).

Vendo sob a percepção da América Latina, observa-se uma grande heterogeneidade regional na prevalência de HIV/Aids (5), citando-se, como exemplo, Panamá e Brasil como tendo os maiores índices de incidência, enquanto que os menores

são vistos na Bolívia e Nicarágua. Salienta-se a complexidade de se estimarem as medidas devido a complexidades regionais, incluindo vários grupos populacionais com comportamentos de risco diversos, bem como, grandes diferenças geográficas (6), como por exemplo, no Brasil.

Este quadro também pode ser exemplificado pela inconsistência da cobertura de terapia antirretroviral (TARV): estima-se que próximo de um terço das pessoas elegíveis para o tratamento não o estava recebendo em 2011, e o número de países latino-americanos que alcançaram 80% de cobertura foi de apenas três no ano de 2010 (6).

Estimou-se que antes da introdução da profilaxia sistematizada anti-*Pneumocystis*, o número de pacientes infectados pelo HIV que desenvolveram PcP era de 20%, dos quais 25% morreram (7). Em países desenvolvidos, onde pacientes com HIV tinham acesso à TARV, as incidências do PcP e a de Aids relacionada diminuindo consideravelmente na Europa (3, 8). Em paralelo, todavia, como conseqüência de terapias imunossupressoras utilizadas por diferentes circunstâncias clínicas, a incidência da PcP tem mostrado tendência de aumento entre os doentes soronegativos (9-11).

Outra infecção oportunística de elevada importância em pacientes com HIV é a criptococose, causada por duas espécies de *Cryptococcus* (*neoformans* e *gattii*), que vem sendo encontrada com maior frequência em pacientes com PcP (12).

A literatura existente em relação à epidemiologia da incidência da PcP em países latino-americanos é escassa, limitada, e de aspecto metodológico distinto (13-19), bem como os trabalhos que relacionam as coinfecções entre PcP e outros microrganismos (12). A inconsistência da forma metodológica com que esses trabalhos foram realizados abre a possibilidade de questionamento dos resultados encontrados, e quanto à direção e à magnitude das tendências do PcP entre pacientes infectados pelo HIV na América Latina (1).

Em virtude destes fatos apresentados, muitos países latino-americanos apresentam alto risco de desenvolver PcP ao contrair Aids, principalmente nos países em que a prevalência é elevada e baixa a cobertura pela TARV.

O presente estudo, efetuado em um serviço especializado em doenças respiratórias do sul do Brasil, teve como objetivo avaliar a associação de pneumocistose e criptococose, relacionando a características clínicas e epidemiológicas observadas em pacientes imunocomprometidos.

## 2 Referencial Teórico

Tabela 1 expõe relação publicações atrelada a *Pneumocystis* no último século.

***Tabela 1**. Apresentação da relação de publicações por autor, ano e importância histórica do entendimento do Pneumocystis.*

| ***Autor, Ano*** | ***País*** | ***Ref*** | ***Registro*** |
|---|---|---|---|
| *Chagas C, 1909* | *Brasil* | (20-22) | *Primeira identificada (na época) como nova forma de vida tripanossomal no pulmão de cobaias por meio de experimento infectadas com Trypanosoma cruzi.* |
| *Delanoe P, 1912* | *França* | (23) | *Por observaram por meio dos registros do Prof. A. Carini cistos no pulmão de ratos de esgoto, concluindo que estes eram um organismo único e uma espécie separada de Trypanosoma, e chamou-o Pneumocystis (tropismo pulmonar e patogênese do organismo) carinii (homenagem a A. Carini). Descrição feita a partir desta data no ICZN como protozoário.* |
| *Ammich O, 1938* | *Alemanha* | (24) | *Antes do inicio da Segunda Guerra Mundial foi descrita forma epidêmica de pneumonia intersticial de células plasmáticas de etiologia desconhecida em crianças desnutridas* |
| *Van der Meer G, 1942* | *Alemanha* | (25) | *Primeira vez identificada infecção por Pneumocystis em seções pulmonares em crianças com pneumonia (descoberta ignorada).* |
| *Vanek J, 1952* | *República Tcheca* | (26) | *Associação do Pneumocystis no pulmão entre crianças prematuras e desnutridas com pneumonia intersticial de células plasmáticas.* |
| *Weller RW, 1955* | *Alemanha* | (27) | *Modelos experimentais utilizando corticosteroides em altas doses resultaram em pneumocistose.* |
| *Junger PW, 1959* | *Canadá* | (28) | *Associação do Pneumocystis como infecção oportunista em crianças imunossuprimidas.* |
| *Post Cornelius, 1964* | *Iran* | (29) | *Associação do Pneumocystis como infecção oportunista em crianças com doenças congênitas institucionalizadas.* |
| *Le Clair RA, 1969* | *Estados Unidos* | (30) | *Pneumocystis começou a ser reconhecido como patógeno oportunista em crianças imunossuprimidas com leucemia aguda.* |
| *Hendley JO, 1971* | *Estados Unidos* | (31) | *Experimentos em ratos utilizando tratamento prolongado com corticosteroides com dexametasona em altas doses resultou em pneumocistose.* |

*ICZN, International Code of Zoological Nomenclature.*

***Continuação da Tabela 1.*** *Apresentação da relação de publicações por autor, ano e importância histórica do entendimento do Pneumocystis.*

| ***Autor, Ano*** | ***País*** | ***Ref*** | ***Registro*** |
|---|---|---|---|
| *Walzer PD, 1976* | *Estados Unidos* | (32) | *Pneumocystis começou a ser reconhecido como patógeno oportunista em pacientes com deficiência imunológica deficiente em função de linfócitos T.* |
| *Frenkel JK, 1976* | *Estados Unidos* | (33) | *Primeiro a sugerir a mudança do nome de P. Carinii para P. jiroveci (não aceita na época) e modificação da classificação para a denominação da doença para fungo.* |
| *Gottlieb MS, 1981 Masur H, 1981* | *Estados Unidos* | (34, 35) | *PcP foi a primeira infecção oportunista relatada em homens homossexuais nos Estados Unidos, denominada como "Síndrome de Gay", posteriormente conhecida como síndrome da imunodeficiência adquirida (Aids).* |
| *Edman JC, 1988* | *Estados Unidos* | (36) | *Sequência de RNA ribossômico mostrou que P. carinii com um membro dos fungos ascomicetos.* |
| *Frenkel JK, 1999* | *Estados Unidos* | (37) | *Primeiro a sugerir a mudança do nome de P. Carinii para P. jiroveci (não aceita na época) e modificação da classificação para a denominação da doença para fungo.* |
| *Stringer JR, 2002* | *Estados Unidos* | (38) | *Mudança da nomenclatura P. Carinii para P. jiroveci.* |
| *San-Andres FJ, 2003* | *Espanha* | (39) | *Profilaxia de pneumocistose em 1989 e a terapia antiretroviral de combinação poderosa em 1996 levaram a declínios substanciais na incidência de PcP nas pessoas infectadas pelo HIV.* |
| *Bonnet F, 2005* | *França* | (40) | *Causa importante de morbidade e mortalidade em pessoas infectadas pelo HIV que não estão recebendo ou não respondendo a HAART e entre aqueles que desconhecem seu estado HIV.* |
| *Calderón EJ, 2007* | *Espanha* | (41) | *Pneumocystis pode desempenhar um papel na progressão da doença pulmonar obstrutiva crônica.* |
| *Stringer JR, 2009* | *Estados Unidos* | (42) | *Correção da nomenclatura P. jiroveci para P. jirovecii.* |
| *Buchacz K, 2016* | *Estados Unidos* | (43) | *Manutenção do declínio da PcP devido a HAART.* |
| *Eddens T, 2016* | *Estados Unidos* | (44) | *Pneumocystis é considerado um alergênico de via aérea capaz de induzir uma patologia pulmonar semelhante a asma.* |

De 1909 até 2016 muito já se evoluiu no entendimento do *Pneumocystis* (Tabela 1). Hoje é sabido que este microorganismo pertence à classe dos fungos (33, 36-38), e que há diferenciação dele entre as espécies (26). Emergiu ligado ao surgimento da deficiência imunológica e de linfócitos T em crianças (28), e doença associada definidora da Aids (34, 35). Com advento da HAART e a profilaxia da PcP, a doença vem apresentando declínio ao longo de décadas em locais onde existe gerenciamento adequado de saúde (39, 40, 43).

Em relação ao cenário do HIV/AIDS na América Latina, segundo dados da *Joint United Nations Programme on HIV/AIDS* (UNAIDS) (45), o Brasil lidera os índices de prevalência, morte e novos casos de no continente (Figuras 1-3). Neste aspecto, caminha-se para o quadro já conhecido de morbidade e mortalidade em pessoas infectadas pelo HIV, que não estão recebendo ou não respondendo a HAART, e entre aqueles que desconhecem seu estado HIV (40).

Em 2013, Calderon EJ e colaboradores (46), publicaram um elegante artigo fazendo o seguinte questionamento: "*Pneumocystis jirovecii pneumonia in Latin America. A public health problem?*". Na Tabela 2, apresentam a proporção de PcP em diferentes países da América Latina. Relatam da grande disparidade metodológica que limita o entendimento sobre a doença nesses países.

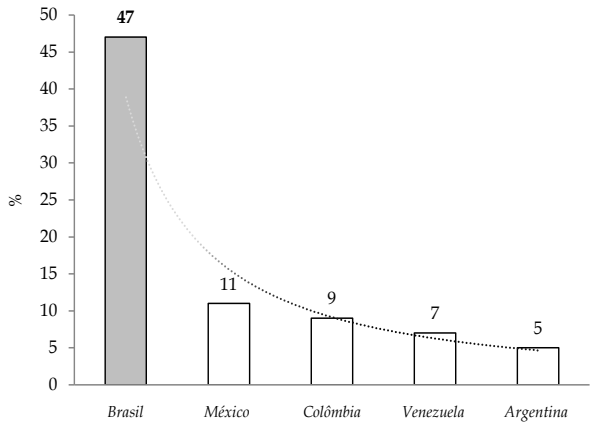


***Figura 1.*** *Pessoas vivendo com HIV na América Latina, 2013. Adaptado de* UNAIDS, 2014 (45).

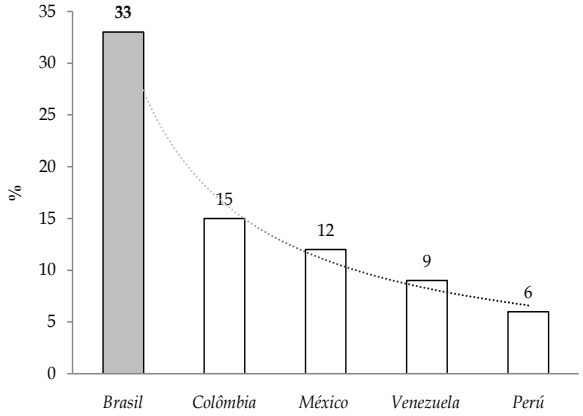


***Figura 2.*** *Mortes relacionadas à Aids na América Latina. Adaptado de* UNAIDS, 2014 (45).

Em uma de suas conclusões relataram que a América Latina pode, num futuro próximo, apresentar diferentes cenários, ou seja, alguns países atingirão um padrão de acesso universal ao TARV, e terão ainda que lidar com o surgimento do PcP entre indivíduos não imunossuprimidos com HIV.

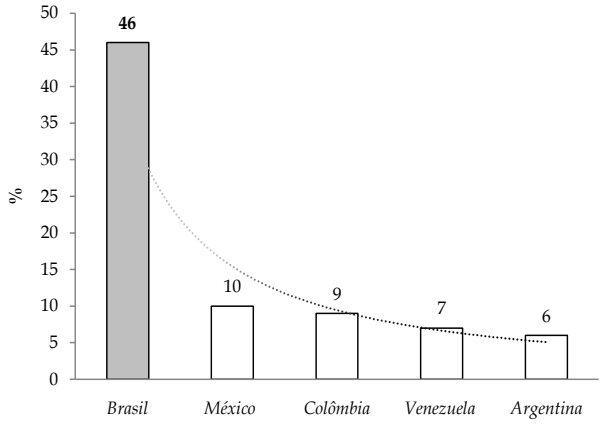


***Figura 3.*** *Novos casos de HIV na América Latina, 2013. Adaptado de* UNAIDS, 2014 (45).

Por outro lado, outros países ainda enfrentarão ambiente de diagnóstico tardio e a falta de acesso sustentado ao TARV resultando em alta incidência de Aids associadas a infecções oportunistas como PcP.

***Tabela 2. Proporção de pneumonia por Pneumocystis em pacientes com Aids em países Latino Americanos.***

| *País* | *Ref.* | *Autor, ano* | *População* | *N* | *PcP-%* | *Período* |
|---|---|---|---|---|---|---|
| *Argentina* | | | | | | |
| | (13) | *Perez E, 2005* | *Adultos internados* | *289* | *5,9%* | *1995-96* |
| | (13) | *Perez E, 2005* | *Adultos internados* | *233* | *9,4%* | *2001-02* |
| *Brasil* | | | | | | |
| | (14) | *Neres AN, 2009* | *Adultos internados* | *266* | *26,3%* | *2000-02* |
| | (19) | *Soeiro AM, 2008* | *Crian.+ adul. Internados* | *828* | *22,2%* | *2001-04* |
| | (17) | *Soares VY, 2008* | *Autópsia* | *250* | *27,0%* | *1990-00* |
| | (47) | *Cury PM, 2003* | *Autópsia* | *92* | *17,3%* | *1993-00* |
| | (15) | *Pereira SA, 2002* | *Autópsia* | *40* | *19,1%* | *1990-00* |
| | (16) | *Santos B, 1994* | *Adultos internados* | *224* | *16,5%* | *1986-91* |
| | (18) | *Weinberg A, 1993* | *Adultos + sintomas resp.* | *35* | *55,0%* | *1988-89* |
| *Chile* | | | | | | |
| | (48) | *Perez C, 2011* | *Adultos + pneumonia* | *57* | *52,6%* | *1999-02* |
| | (49) | *Chernilo S, 2005* | *Adultos + sintomas resp.* | *171* | *37,7%* | *1999-03* |
| *Colômbia* | | | | | | |
| | (50) | *Rodiño J, 2011* | *Adultos + sintomas resp.* | *131* | *17,5%* | *2007-09* |
| *Cuba* | | | | | | |
| | (51) | *Hernandez EA, 1998* | *Autópsia* | *211* | *32,0%* | *1986-95* |
| *Guatemala* | | | | | | |
| | (52) | *Estrada Y, 1992* | *Coorte* | *92* | *15,2%* | *1991-92* |
| *México* | | | | | | |
| | (53) | *Villasis-Keever A, 2001* | *Adultos internados* | *909* | *24,8%* | *1984-88* |
| | (53) | *Villasis-Keever A, 2001* | *Adultos internados* | *909* | *17,0%* | *1989-92* |
| | (53) | *Villasis-Keever A, 2001* | *Adultos internados* | *909* | *14,0%* | *1993-95* |
| | (54) | *Mohar A, 1992* | *Autópsia* | *177* | *24,0%* | *1984-89* |
| *Panamá* | | | | | | |
| | (55) | *Rodriguez AF, 1996* | *Adultos + sintomas resp.* | *55* | *45,4%* | *1995* |
| *Perú* | | | | | | |
| | (56) | *Eza D, 2006* | *Autópsia* | *16* | *12,5%* | *1999-04* |
| *Porto Rico* | | | | | | |
| | (57) | *Jesus-Berrios Y, 2005* | *Adultos + sintomas resp.* | *32* | *15,6%* | *1998-00* |
| | (58) | *Gomez MA, 2000* | *Coorte* | *1520* | *28,1%* | *1992-96* |
| | (59) | *Climent C, 1994* | *Autópsia* | *100* | *49,0%* | *1982-91* |
| *Venezuela* | | | | | | |
| | (60) | *Panizo MA, 2008* | *Adultos + sintomas resp.* | *41* | *36.6%* | *2001-06* |

*Adaptado de Calderon EJ et al. Pneumocystis jirovecii pneumonia in Latin America. A public health problem? Expert Rev Anti Infect Ther. 2013;11(6):565-70.*

Lowe DM e colaboradores em 2013 (12) avaliaram em uma metanalise em estudos de países tropicais de baixa ou média renda a relação da PcP e outros microorganismos. Encontraram um valor expressivo de coinfecções nos estudos avaliados (444 episódios/1425 casos de PcP). A  infecção fúngica mais prevalente foi a criptococose. Entretanto, defechos de sobrevida não foram avaliados neste artigo.

## 3 Justificativa

Indiscutivelmente, o Brasil é um dos países expoentes na América Latina na assistência de pacientes com o vírus HIV. Entretanto, isso não se reflete na contenção do avanço alarmante da incidência de novos casos e das mortes pela doença no continente.

Cerca de 2% dos casos de HIV diagnosticados no mundo são de pacientes que vivem no Brasil, bem como 47% dos novos casos de HIV na América Latina. Em relação às mortes por HIV, o Brasil ocupa a primeira posição no continente (33% em relação aos países da região).

Estes fatos nos remetem ao cenário existente antes da profilaxia com TARV/casos de HIV, quando elevou-se de forma exponencial o número de casos de infecção por PcP no mundo.

Hoje, muitos dos indivíduos por não fazerem exames de rotina, e assim, desconhecendo o seu estado de saúde, acabam tardiamente diagnosticados com HIV e, simultaneamente, com outras infecções como *P. jirovecii* e *Criptococcus*, em uma condição de saúde muito delicada, necessitando de assistência de alta complexidade para a manutenção da vida. Entender o envolvimento deste cenário, no que diz respeito ao diagnóstico, tratamento e desfecho, se faz necessário para tomada de medidas em saúde pública.

# 4 Objetivos

## 4.1 Objetivo geral

Avaliar a associação da pneumocistose e criptococose, relacionando a características clínicas e epidemiológicas em pacientes imunocomprometidos por Aids.

## 4.2 Objetivos específicos

- Verificar a mortalidade.
- Caracterizar padrões de imagem das infecções fúngicas.
- Relatar caso raro.

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## 6 Artigos científicos

*Segue a lista dos trabalhos desenvolvidos neste doutorado por título e periódico onde foi submetido até o presente momento:*

1. **Título:** Double Infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans/Cryptococcus gattii* in HIV patients
   **Periódico:** Mycopathologia (Springer)

2. **Título:** *Cryptococcus gattii* infection: clinical and imaging features and mortality risk factors in a cohort in Southern Brazil
   **Periódico:** Mycoses **(**John Wiley & Sons**)**

3. **Título:** *Pneumocystis* choroiditis: The First Case in Southern America
   **Periódico:** Eye **(**Nature**)**

## 6.1 Artigo 1

# Double Infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans/Cryptococcus gattii* in HIV patients

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**Abstract**

The aim of this paper is to identify the double infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans* / *Cryptococcus gattii* in a human immunodeficiency virus (HIV) disease-endemic region. We included data from all patients diagnosed with *P. jirovecii* and Cryptococcus infection from January 1985 to December 2014 by patient medical records. We found 10 patients of double infection by *P. jirovecii* and *C. neoformans* / *C. gattii* in a hospital in a HIV disease-endemic region. Some cases of our sample had asymptomatic infections and all of *C. gattii*-infected patients died during the hospitalization. This paper calls the attention to the correct and rapid diagnoses in suspected PcP with HIV, CD4 levels less than 200 cel/μL and bilateral fibronodular infiltrates independent of clinical presentation. In this way, it is recommended to perform extensive investigation for fungal infections by rapid methenamine silver stain, culture, immunochromatography or latex agglutination tests, microscopy and immunodiffusion in order to provide an efficient clinical benefit.

**Keywords:** HIV; *Pneumocystis jirovecii; Cryptococcus neoformans; Cryptococcus gattii.*

## Introduction

The pneumonia caused by *Pneumocystis jirovecii* infection (PcP) has been recognized in patients with compromised immunity (1-3). This was initially described in the literature as an epidemic of interstitial pneumonia caused in premature and malnourished patients (3). However, the rate of *P. jirovecii* infection has continued to increase in countries with uncontrolled human immunodeficiency virus (HIV) disease and limited access to highly active antiretroviral therapy (HAART) and/or health care (2).

Currently, in poor and developing countries such as African nations and Brazil in Latin America, the incidence and mortality of HIV infection are still extremely high (4, 5) and concomitantly the outbreaks of opportunistic fungal infections such as *P. jirovecii* and *Cryptococcus* (6, 7). The literature on the epidemiology of PcP incidence in Latin American countries is limited and has different methodological aspects (8-13), as well as studies that relate the co-infections between PcP and other microorganisms (14). The aim of this paper is to identify the double infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans* / *Cryptococcus gattii* in a HIV disease-endemic region.

## Patients and Methods

We included data from all patients diagnosed with *P. jirovecii* and *Cryptococcus* infection reported to the Department of Mycology of Irmandade da Santa Casa de Misericordia de Porto Alegre (ISCMPA), southern Brazil, from January 1985 to December 2014.

The diagnosis of *P. jirovecii* infection was performed by bronchial brushings and wash/lavage specimens were examined using rapid methenamine silver stain (15) (Grocott) and Papanicolaou stains, the microscopy analysis showed the typical alveolar casts, with a foamy appearance, highly suggestive of *P. jirovecii* pneumonia.

The *Cryptococcus* infection was identified microscopically by demonstration of encapsulated budding yeasts; growth on Sabouraud's dextrose agar (SDA) at 30ºC; urease production on Christensen's urea medium; and sub-cultures on Staib's *Guizotia abyssinica* seed agar showing characteristic brown pigment formation within 5 days. All tests were performed using cerebrospinal fluid (CSF), blood, urine, bronchoalveolar lavage (BAL) or lung biopsy. When a culture was positive, the canavanine–glycine–bromothymol blue agar was used to differentiate *C. neoformans* of *C. gattii* (16). The fungal blood cultures (Isolator) grew *C. gattii* colonies. Leishman's staining method was suitable for staining the smears of the double infection by *P. jirovecii* and *Cryptococcus*.

From patient medical records, the following data were selected for analysis: sex, age, skin color, clinical findings, X-ray films, HIV, CD4+, infected sites/organs, the species identified in the microscopy and culture and antifungal treatments and outcome. The clinical outcome was based on the report of hospital discharge or death during hospitalization.

This study was approved by our institutional review board, ISCMPA Committee, numbers 1282/06, 1460/06 and 00002509.

## Results

The presenting symptoms and physical characteristics of the patients are summarized in Table 1. We found ten HIV patients with *P. jirovecii* confirmed double infection by Cryptococcus, seven of which had a simultaneous infection of *C. neoformans* and three with *C. gattii* infection. The diagnosis was simultaneous of the diseases during hospitalization and all of them presented CD4 levels less than 200 cel/µL. These number of infected patients corresponds to one third of the co-infections of the *pneumocystosis* data and 1% of the *cryptococcosis* data in our centre (ISCMPA). Two of three patients with *C. neoformans* meningitis referred no symptoms during the anamnesis during hospitalization. The mortality occurred in 30% of the cases and all of them with *C. gattii* infection.

The Figure 1 (A) using methenamine silver staining in the Papanicolaou or M-G-G stained material showed the typical alveolar casts, with a foamy appearance, highly suggestive of *P. jirovecii* pneumonia. Figure 1 (B and C), the Papanicolaou or M-G-G stained material showed the typical alveolar casts, with a foamy appearance, highly suggestive of *P. jirovecii* pneumonia. For *Cryptococcus* in M-G-G stained smears the capsule may stain in pink color, due to its high sugar content, and the fungi are more easily seen, even if in low numbers.

The study imaging showed in all X-rays the same finding of bilateral fibronodular infiltrates. The computed tomography (CT) demonstrated diffuse areas of ground glass opacity in both lungs (Figure 2).

## Discussion

To our knowledge, this study presented the largest sample of double infection by *P. jirovecii* and *C. neoformans*/*C. gattii* in HIV patients, all of these diagnoses were performed in the same time during the hospitalization. Moreover, two thirds patients with confirmed brain *C. neoformans* diagnoses had asymptomatic neurological infection and all patients with double infection by *P. jirovecii* and *C. gattii* died.

Asymptomatic neurological infection is a reality in cryptococcal meningitis (17-19) and this finding is commonly associated with poor prognosis, because the patients who have this condition often have less CD4 levels and viral load increased (17). Moreover, the delay to start the correct treatment could affect the patient prognosis.

The *C. gattii* infection is more pathogenic than *C. neoformans* infection (20); the treatment usually is longer and it is a difficult approach to solve this condition. Also, for asymptomatic infections or mild-to-moderate pulmonary infections, fluconazole is the first option of treatment (21). For severe lungs or central nervous system infections, is recommended to treat the patient with a combination of amphotericin B with flucytosine and subsequent fluconazole for an extended time to resolve the infection. In addition, patients that developed cryptococcomas may need to complement the treatment with surgery (20).

In our sample, all of the *C. gattii*-infected patients with *P. jirovecii* and HIV died in the hospitalization. A possible explanation for this outcome is the short time to treat these patients, since the success of the antifungal therapy depends of the cryptococcomas solution (20, 21). Unfortunately, patients of the present study didn't have clinical conditions to perform surgical treatment and showed poorly response of

antifungal therapy, as seen in the literature (18, 19, 22). All of them died in intensive care unit due to sepsis.

In summary, we identified 10 cases of double infection by *P. jirovecii* and *C. neoformans* / *C. gattii* in a hospital in a HIV disease-endemic region. Some cases of our sample had asymptomatic infections and all of *C. gattii*-infected patients died during the hospitalization. This paper calls the attention to the correct and rapid diagnoses in suspected PcP with HIV, CD4 levels less than 200 cel/μL and bilateral fibronodular infiltrates independent of clinical presentation. In this way, it is recommended to perform extensive investigation for fungal infections by rapid methenamine silver stain, culture, immunochromatography or latex agglutination tests, microscopy and immunodiffusion in order to provide an efficient clinical benefit.

## Conflict of interest

No authors have any conflicts of interest.

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**Figure Legends**

**Figure 1.** Case 6; Broncho-alveolar lavage, 34-year old man with HIV. (A) Methenamine silver staining, this is the typical appearance of *P. jirovecii* cysts, with silver staining, with characteristic morphology: spherical or cup shaped cysts with a central thickening of its wall. Some of the cysts may have the appearance of smashed ping pong balls. The Papanicolaou or M-G-G stained material showed the typical alveolar casts, with a foamy appearance, highly suggestive of *P. jirovecii* pneumonia. (B and C) *Pneumocystis*: The Papanicolaou or M-G-G stained material showed the typical alveolar casts, with a foamy appearance, highly suggestive of *P. jirovecii* pneumonia. *Cryptococcus*. The fungus is spherical, and produces a single or rarely double teardrop shaped bud. The thick mucoid capsule produces an unstained halo around the organism, in Papanicolaou stained smears. In M-G-G stained smears the capsule may stain in pink color, due to its high sugar content, and the fungi are more easily seen, even if in low numbers.

**Figure 2.** Case 7; Images from a 59-year-old male, double infection by *Pneumocystis jirovecii* and *Cryptococcus neoformans* in HIV patient with productive cough and dyspnoea. CT scan axial (A) and coronal (B and C) demonstrated diffuse areas of ground glass opacity in both lungs.

Table 1. Summary of clinical, radiological and laboratory findings of ten cases with double infection by *P. jirovecii* and *C. neoformans*/*C. gattii.*

| Case | Sex–Age | White | Clinical findings | X-ray film | HIV | CD4+ T, cel/µL | *P. jirovecii* | | *Cryptococcus* | | | Treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | MD | Localization | MD | Type | Localization | | |
| 1 | F–44 | Yes | Weight loss, fever, productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 153 | Microscopy | Lung | M+C | *Cn* | Lung | TMP/SMX + Fluconazole | Recovery |
| 2 | F–27 | Yes | Productive cough, dyspnoea, headache | Bilateral fibronodular infiltrates | Yes | NP | Microscopy | Lung | M+L | *Cn* | Brain | TMP/SMX + Fluconazole | Recovery |
| 3 | F–41 | No | Weight loss, fever, productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 17 | Microscopy | Lung | M+L | *Cn* | Brain | TMP/SMX + Fluconazole | Recovery |
| 4 | M–62 | Yes | Weight loss, fever, productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 51 | Microscopy | Lung | M+C | *Cn* | Lung | TMP/SMX + Fluconazole | Recovery |
| 5 | F–30 | Yes | Weight loss, fever, productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 97 | Microscopy | Lung | M+L | *Cn* | Brain | TMP/SMX + Fluconazole | Recovery |
| 6 | M–34 | Yes | Weight loss, fever, productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 45 | Microscopy | Lung | M+C | *Cn* | Lung | TMP/SMX + Fluconazole | Recovery |
| 7 | M–59 | Yes | Productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 44 | Microscopy | Lung | M+L | *Cn* | Lung | TMP/SMX + Fluconazole | Recovery |
| 8 | F–29 | Yes | Productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | NP | Microscopy | Lung | M+C | *Cg* | Lung | TMP/SMX + Fluconazole | Died |
| 9 | M–58 | Yes | Productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 169 | Microscopy | Lung | M+L | *Cg* | Lung | TMP/SMX + Fluconazole | Died |
| 10 | F–48 | Yes | Productive cough, dyspnoea | Bilateral fibronodular infiltrates | Yes | 93 | Microscopy | Lung | M+L | *Cg* | Lung | TMP/SMX + Fluconazole | Died |

Note: F, female; M, Male; HIV, human immunodeficiency virus; NP, not performed; MD, mycology diagnosis; M, microscopy; M+C, microscopy and culture; M+L, microscopy and latex; *Cn*, *C. neoformans*; *Cg*, *C. gattii.*

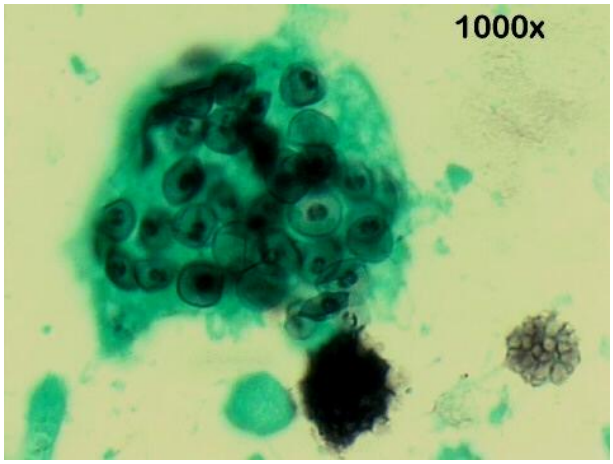


Figure 1A

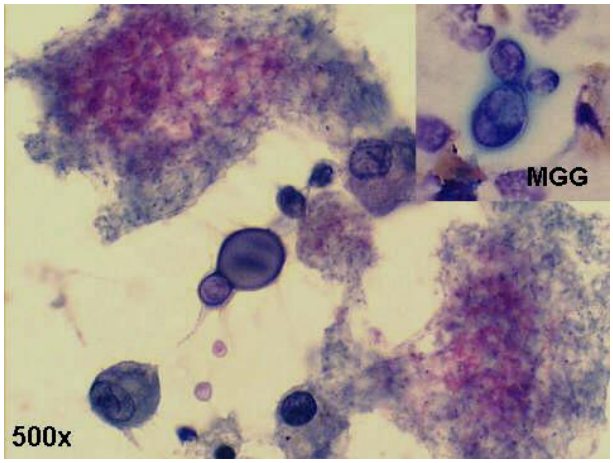


Figure 1B

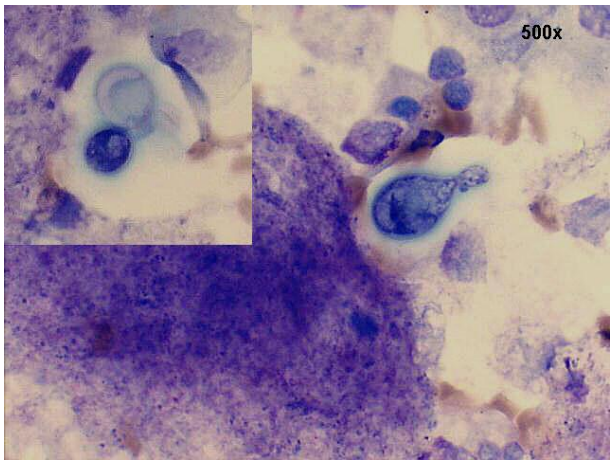


Figure 1C

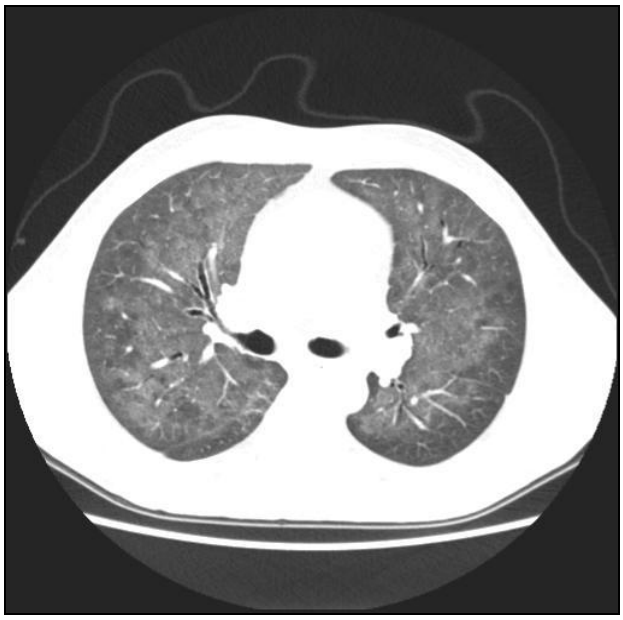

Figure 2A

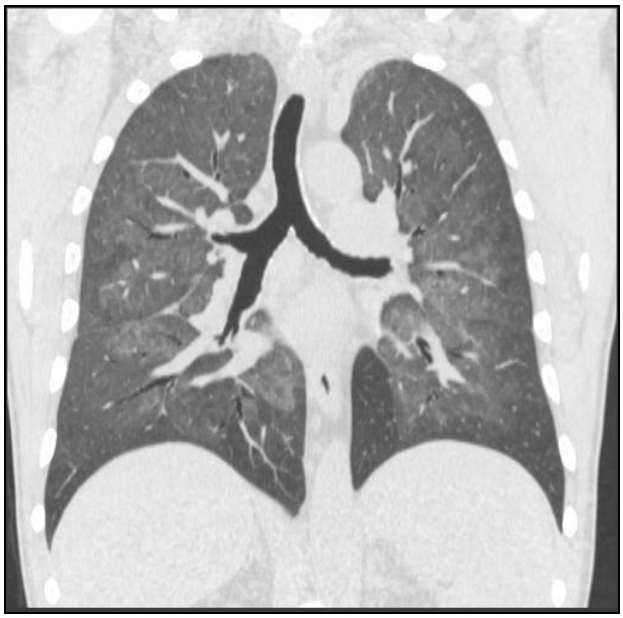

Figure 2B

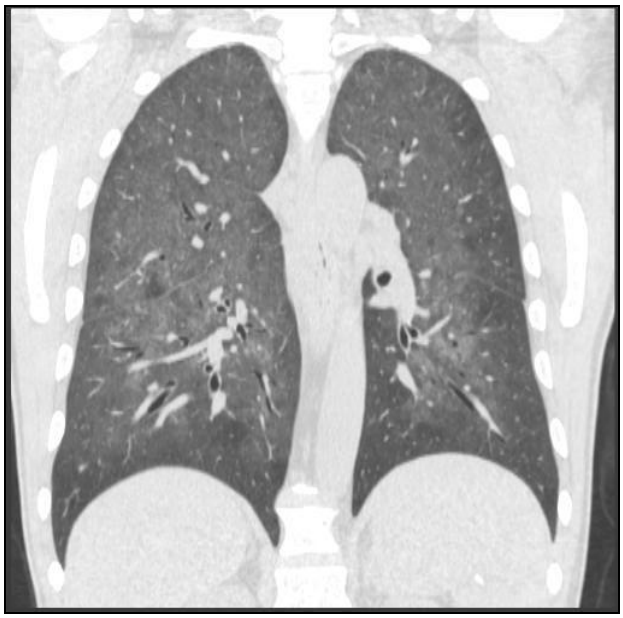

Figure 2C

## 6.2 Artigo 2

**Title page**

# *Cryptococcus gattii* infection: clinical and imaging features and mortality risk factors in a cohort in Southern Brazil

**Short title:**

***Cryptococcus gattii* infection in Brazil**


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**Key words:** Cryptococcosis; epidemiology; *Cryptococcus gattii*.

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**Summary**

**Background:** Data about *Cryptococcus gattii* infection remain limited in South America, including long-term outcomes and predictors of morbidity or mortality. **Objectives:** The aim of this study was to investigate clinical and imaging features, and mortality risk factors for patients infected with *C. gattii* in Brazil. Methods: We retrospectively analyzed data from all patients diagnosed with *C. gattii* infection referred to our hospital from 1985 to 2016. Clinical and imaging findings associated with mortality were analyzed. **Results:** There were 63 cases identified, all were urease positive and showed melanin production on Staib niger seed agar and resistance to canavanine on canavanine–glycine–bromothymol agar. Cryptococcal antigen titers were elevated in 94.44% of cases it was performed (n=36). Chest and brain CT scans were abnormal in 39 and 16 cases, respectively. Masses were the most common finding on both chest (n=18) and brain (n=10) studies. Ten patients did not comply with treatment, and 30 patients died. Higher mortality risks were identified for age ≥50 years (odds ratio [OR], 3.77; 95% confidence interval [CI], 1.02–13.8) and immunocompromised patients (OR, 4.67; 95%CI, 1.06–20.4). **Conclusions:** Key clinical practice points highlighted by this study were the major data basis of *C. gattii* patients in Southern America, characterizing clinical and radiological findings in this population.

## 1. Introduction

*Cryptococcus gattii* has traditionally been considered a pathogen of tropical and subtropical regions (1, 2). Usually, *C. gattii* infection occurs in individuals with normal immune systems. Nevertheless recent researches from British Columbia, Canada, and the US Pacific Northwest have appointed new risk factors for infection including: human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS), cancer, smoking, and oral corticosteroid treatment within the previous year (3-6).

The Southern Brazil is an endemic region of *C. gattii* (7) and it has caused recent outbreaks as other climatic zones in Canada and in the USA (1). There are several longitudinal studies about demographics, clinical presentation, predictors of mortality and comorbidities associated with *C. gattii* infection (3,4,8,9). However, data about long-term outcomes and predictors of morbidity or mortality in Southern America remain limited. The aim of this study was to investigate clinical and imaging features, and mortality risk factors for patients infected with *C. gattii* in Southern Brazil.

## 2. Materials and Methods

### 2.1 Patients

With the approval of our institutional review board (Irmandade da Santa Casa de Misericordia de Porto Alegre Committee, IRB00002509), we included data from all patients diagnosed with *C. gattii* infection referred to the Department of Mycology of

our institution, based in the city of Porto Alegre, Southern Brazil, from January 1985 to December 2016.

*C. gattii* infection was identified by: (a) demonstration of encapsulated budding yeasts microscopically; (b) growth on Sabouraud's dextrose agar (SDA) at 30ºC; (c) urease production on Christensen's urea medium; and (d) sub-cultures on Staib's *Guizotia abyssinica* seed agar showing characteristic brown pigment formation within 5 days. All tests were performed using cerebrospinal fluid (CSF), blood, urine, bronchoalveolar lavage (BAL) or lung biopsy. When a culture was positive, the canavanine–glycine–bromothymol (CGB) blue agar was used to differentiate *C. neoformans* of *C. gattii* (10). Serum and CSF specimens were submitted to clinical laboratory for the detection of cryptococcal antigen (CrAg) (IMMY, Crypto-Latex Antigen Detection System, Immunomycologics Inc., OH, USA). The fungal blood cultures (Isolator) grew *C. gattii* colonies.

From patient medical records, the following data were selected for analysis: gender, age, infected sites/organs, underlying diseases, HIV/AIDS, titers of CrAg, high-resolution computed tomography (HRCT) findings, antifungal treatments, and species identified in culture. Mortality rates were calculated based on medical records. In cases which patient outcome could not be retrieved from these records, phone contact was made.

## 2.2 Imaging protocols

The HRCT examinations were performed using a 64-multidetector scanner (LightSpeed VCT; GE Healthcare, Waukesha, WI, USA) with the following

parameters: 120 kVp; 250 mA; time, 0.8 s; and pitch, 1.375. The technical parameters included inspiratory volumetric acquisition with 1 mm collimation at 1 mm increments using a high-spatial-frequency reconstruction algorithm. Lung images were obtained with mediastinal (width, 350-450 HU; level, 20-40 HU) and parenchymal (width, 1200-1600 HU; level, −500 to −700 HU) window settings, and reconstructions were performed in the axial and coronal planes. The axial 5 mm slices were reviewed using a standard window setting for brain imaging (width, 80 HU; level, 40 HU).

**2.3 Imaging analysis**

Two thoracic radiologists with more than 10 years of experience who were blinded to the patients' clinical information, except for the fungal infection, independently assessed the CT images. After the two radiologists had conducted independent analyses, they reviewed the images together with a third senior thoracic radiologist with more than 20 years of experience to reach final consensus. Images were assessed and patients were divided in 2 groups: a) Normal chest and brain CT images; b) Brain and chest CT images compatible with cryptococcosis (11, 12).

**2.4 Statistical analysis**

Data are expressed as frequency and percentage for categorical variables and as median and interquartile range [IQR] for continuous variables. Shapiro-Wilk test was used to assess normality of the data distribution. We performed multivariate analyses

using logistic regression to investigate whether clinical and imaging findings were associated with mortality. Variables ($p$ less than 0.2) in univariate analysis were included in a multivariate model. Statistical significance was accepted at $p \leq 0.05$. All results were analyzed using commercial software (SPSS ver. 22, SPSS Inc., Chicago, IL, USA; Excel 2010, Microsoft Corporation, Redmond, WA, USA).

## 3. Results

All 63 cases isolated were identified as *Cryptococcus* by API ID 32. All were urease positive and showed melanin production on Staib niger seed agar and resistance to canavanine with glycine utilization on CGB agar. The demographic characteristics and clinical manifestations are summarized on Table 1.

Cryptococcal antigen (CrAg) titers were performed in 36 patients (57.14%) and they were elevated in 34 cases (94.44%). Serum titers were ≤ 1:100 for 3 patients; 1:100 - 1:999 in 6 cases; 1:1000 - 1:9999 in 20 subjects; and ≥ 10000 in 5 cases. *Cryptococcus* antigen was found in the CSF in 40 cases. Titers were ≤ 1:100 for 8 patients; 1:100 - 1:999 in 7 cases; 1:1000 - 1:9999 in 13 subjects; and ≥ 10000 in 5 patients.

The study imaging findings, treatment and outcomes were summarized in Table 2 and 3. Chest and brain CT scans were abnormal in 39 and 16 cases, respectively. Masses were the most common finding on both chest (n=18) (Fig. 1) and brain (n=10) studies. Ten (15.87%) patients did not comply with antifungal treatment, and 30 patients (47.60%) died.

Adjusted multivariate data analysis confirmed results of the univariate analysis (Table 4). There was an approximate four-fold increase in the mortality risk in patients

with *C. gattii* infection who were ≥50 years (odds ratio [OR], 3.77; 95% confidence interval [CI], 1.02–13.8; $p$=0.046). In addition, there was an approximate five-fold increase in the mortality risk for immunocompromised patients (OR, 4.67; 95%CI, 1.06–20.4; $p$=0.046).

## 4. Discussion

This was the largest sample of culture-confirmed infection due to *C. gattii* in the Southern Brazil to our knowledge. Currently, few studies in South America approached the relationship between clinical characteristics and outcomes in *C. gattii*-infected patients, most of them focusing on molecular characterization (13, 14) and on geographic distribution identification (15, 16). However, understanding clinical and imaging features for the assessment of possible mortality risks factors are crucial for optimal patient management. In our study, we identified age ≥50 years and immunocompromised status as mortality risk factors for *C. gattii*-infected patients. These findings are in accordance with a Canadian study that found age ≥50 years (hazard ratio [HR], 15.6; 95% confidence interval [CI], 1.9–130.5) and immunocompromised (HR, 5.8; CI, 1.5–21.6) as risk factors as well (4).

Although most South American studies about *C. gattii* infection reported few AIDS cases in their samples (7, 15, 16), these patients represented 28.60% of our sample. This number could be related to a high incidence of HIV infection in Southern Brazil (17). Another important point is that almost half of patients died in our study. The overall mortality for culture-confirmed cases in Canada was 27.2% (4), and 33% in

the USA (8). A possible reason is the 15.87% noncompliance with treatment in our sample.

In our study, the antifungal treatment was performed in 84% of patients, similarly to the Canadian group (18) that treated 85% with amphotericin-B, 74% in combination with 5-flucytosine, and 15% received fluconazole monotherapy. In a Colombian study, amphotericin-B was the most common drug, used in 96.7% patients (16).

Mass and nodes were most frequent imaging features. Prevalence of chest and brain CT scan findings was similar to previous studies that reported pulmonary nodules in 75% cases and neurological involvement in less than 10% to up to 37% cases (2-4,8,19).

In our study, limitations were the possibility for unrecognized biases due to retrospective study and incomplete data collection including measurement of the time relapsed between the culture and outcomes, impairing multivariate analysis.

In summary, the key clinical practice points highlighted by this study were the major data basis of *C. gattii* patients in Southern America, characterizing clinical and radiological findings in this population. The *C. gatti*-infected patients in Southern Brazil with age ≥50 years and immunocompromised disease had an increased risk of death. Results similar were found previously in other studies. Therefore, these risks factors are very important to improve the health care management in *C. gattii*-infected patients.

**Conflict of interest**

No authors have any conflicts of interest.

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Table 1

Table 1. Demographic, clinical characteristics and underlying conditions of *C. gattii*-infected patients at diagnosis in Southern Brazil. (N=63)

| Variable | Results |
|---|---|
| Age in years, median [IQR] | 47 [37–59] |
| Age ≥50 yrs | 28 (44.4) |
| Sex | |
| Male | 47 (74.60) |
| Female | 16 (25.40) |
| Smoking status | |
| Past or current smoker | 25 (39.70) |
| Underlying conditions | |
| Immunocompromised | 20 (32.79) |
| AIDS | 18 (28.60) |
| Solid organ transplant | 4 (6.30) |
| Diabetes mellitus | 5 (7.90) |
| Co-infections | |
| *Tubeculosis* | 7 (11.13) |
| *Candida albicans* | 6 (9.54) |
| *Pneumocystis jirovecii* | 3 (4.77) |
| *Aspergillus flavus* | 1 (1.59) |
| *Aspergillus fumigates* | 1 (1.59) |
| *Staphylococcus aureus* | 1 (1.59) |
| *Streptococcus viridians* | 1 (1.59) |
| Clinical findings | |
| Neurological | 38 (60.32) |
| Pulmonary | 33 (52.38) |
| Systemic | 55 (87.30) |
| Dermatological | 12 (19.05) |
| Other | 17 (26.98) |
| Asymptomatic | 1 (1.59) |
| Sites of infection | |
| Lung | 45 (71.40) |
| CNS | 23 (36.50) |
| Skin | 6 (9.50) |
| Kidney | 3 (4.80) |
| Lymph node | 2 (3.20) |

Note. Data are presented as No. (%) or median [IQR]. AIDS, acquired immunodeficiency syndrome; CNS, central nervous system.

Table 2

Table 2. Imaging findings of *C. gattii*-infected patients at diagnosis in Southern Brazil. (N=59)

| Variables | Results |
|---|---|
| Chest CT scan | n=44 (74%) |
| Normal | 5 |
| Pulmonary infiltrates | 4 |
| Consolidations | 12 |
| Mass / Nodules | 18 |
| Cavitation | 3 |
| Pleural effusion | 4 |
| Brain CT scan | n=25 (42%) |
| Normal | 9 |
| Mass lesions | 10 |
| Cerebral edema | 3 |
| Hydrocephalus | 2 |

Note Data are presented as No. CT, computed tomography.

Table 3

Table 3. Treatment and outcome among *C. gattii*-infected patients in Southern Brazil. (N=63)

| Variable | Results |
|---|---|
| Treatment | |
| Amphotericin-B and 5-fluorocytosine | 12 (19.00) |
| Amphotericin-B | 30 (47.60) |
| Fluconazole | 30 (47.60) |
| Itraconazole | 6 (9.50) |
| Surgery | 4 (6.3) |
| Non-compliance | 10 (15.87) |
| Outcome | |
| Cure | 29 (46.00) |
| Death | 30 (47.60) |
| NA | 4 (6.30) |

Note. Data are presented as No. (%). NA, not available.

Table 4

Table 4. Logistic regression analysis of clinical manifestations and radiological findings that were associated with risk of death among *C. gattii*-infected patients in Southern Brazil.

| Variable | Univariate Model | *P* | Multivariate Model[a-b] | *P* |
|---|---|---|---|---|
| | Crude OR (95% CI) | | Adjusted OR (95% CI) | |
| Age ≥50 yrs | 2.85 (0.98–8.21) | .052 | 3.77 (1.02–13.8) | .046 |
| CNS disease | 0.59 (0.19–1.84) | .370 | . | |
| Lung imaging findings | 2.33 (0.53–10.2) | .261 | 2.85 (0.51–15.8) | .231 |
| Past or current smoker | 0.45 (0.15–1.33) | .153 | 0.75 (0.21–2.63) | .655 |
| Immunocompromised | 3.06 (0.96–9.73) | .058 | 4.67 (1.06–20.4) | .040 |
| Non-compliance | 0.96 (0.21–4.26) | .959 | . | |

Note. OR, odds ratio; CI, confidence interval; CNS, central nervous system.
[a] Variables (*p* less than 0.2) in univariate analysis were included in a multivariate model.
[b] Adjusted for all variables.

## Figure Legends

**Figure 1.** Images from a 45-year-old male, immunocompetent and smoker, with dyspnea and dry cough. Axial computed tomography images on mediastinal (a) and lung (b) windows and coronal reconstruction (c) demonstrate a 5.7 X 3.2 cm mass in left upper lobe, not enhancing by contrast media. Centrilobular emphysema and bronchial wall thickening is also noted.

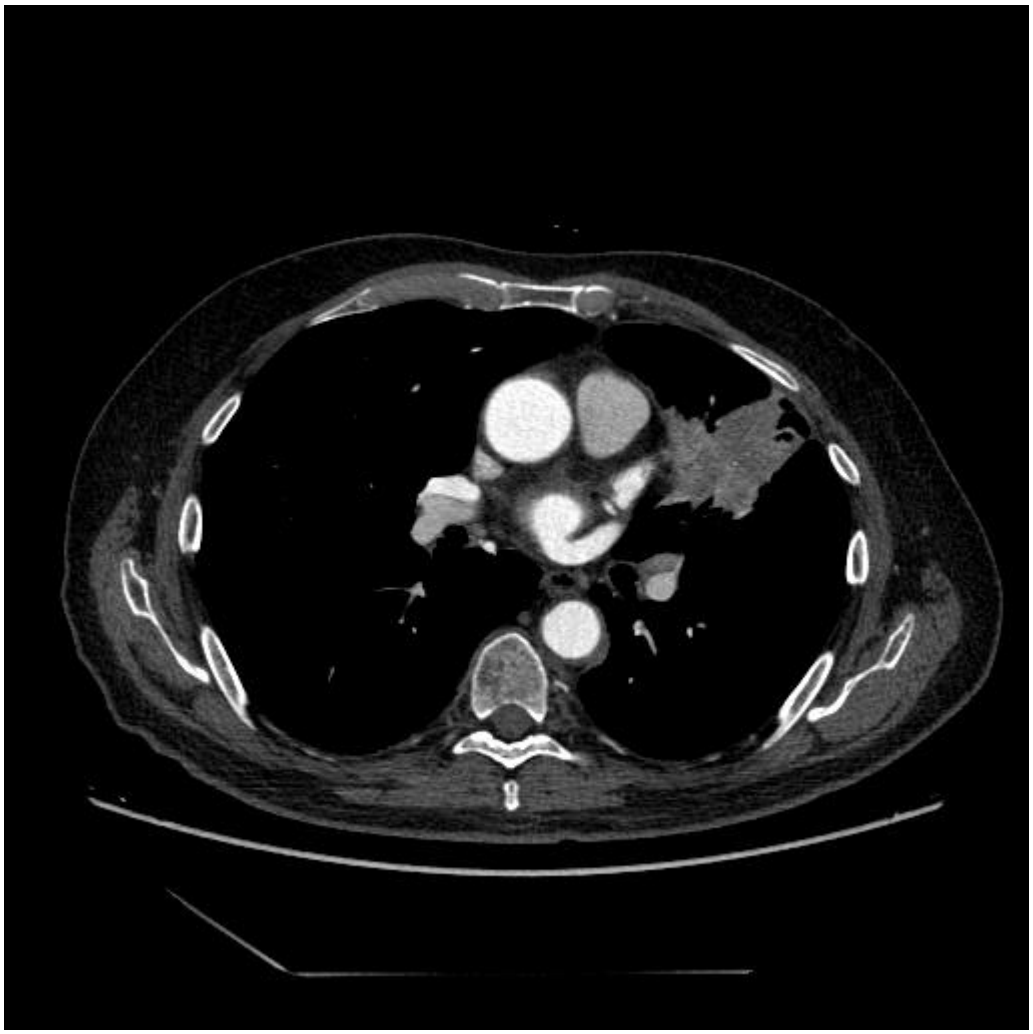

Figure 1A

130x86mm (300 x 300 DPI)

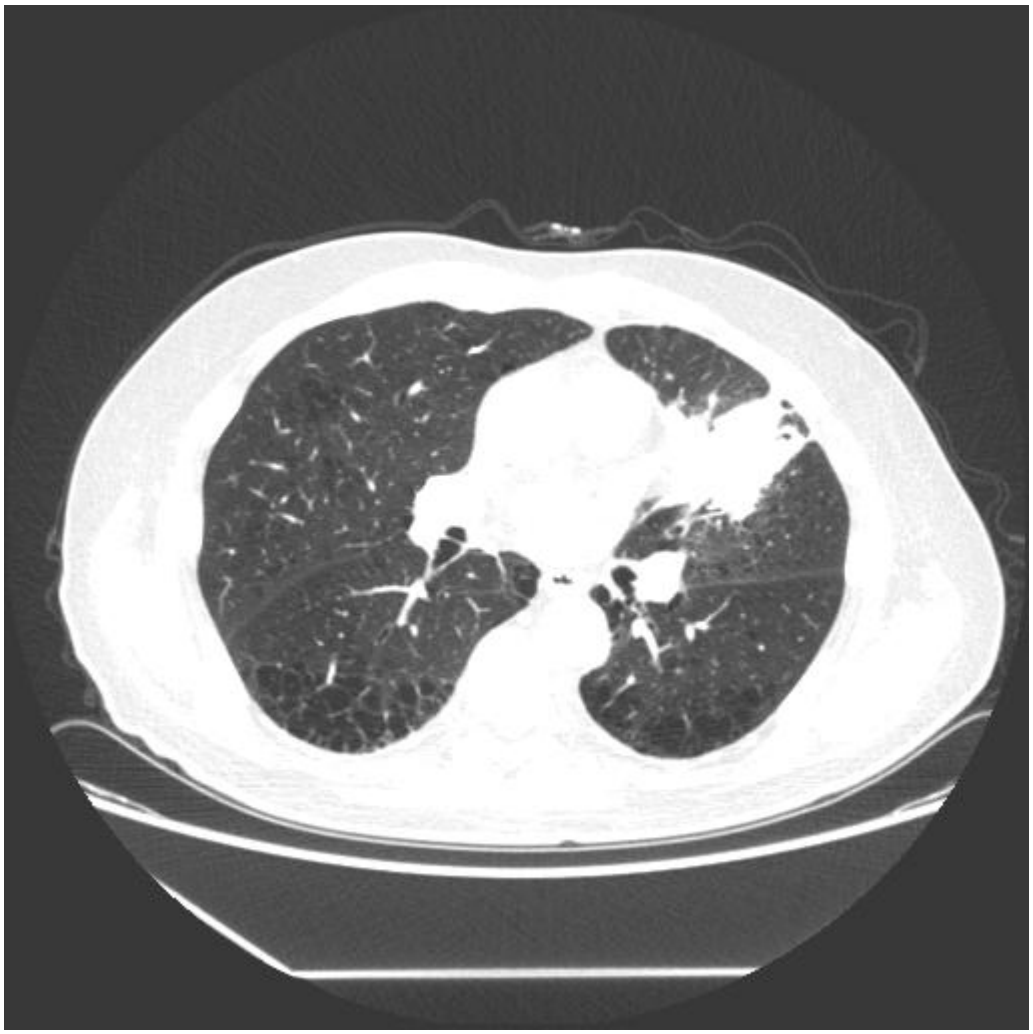

Figure 1B

130x86mm (300 x 300 DPI)

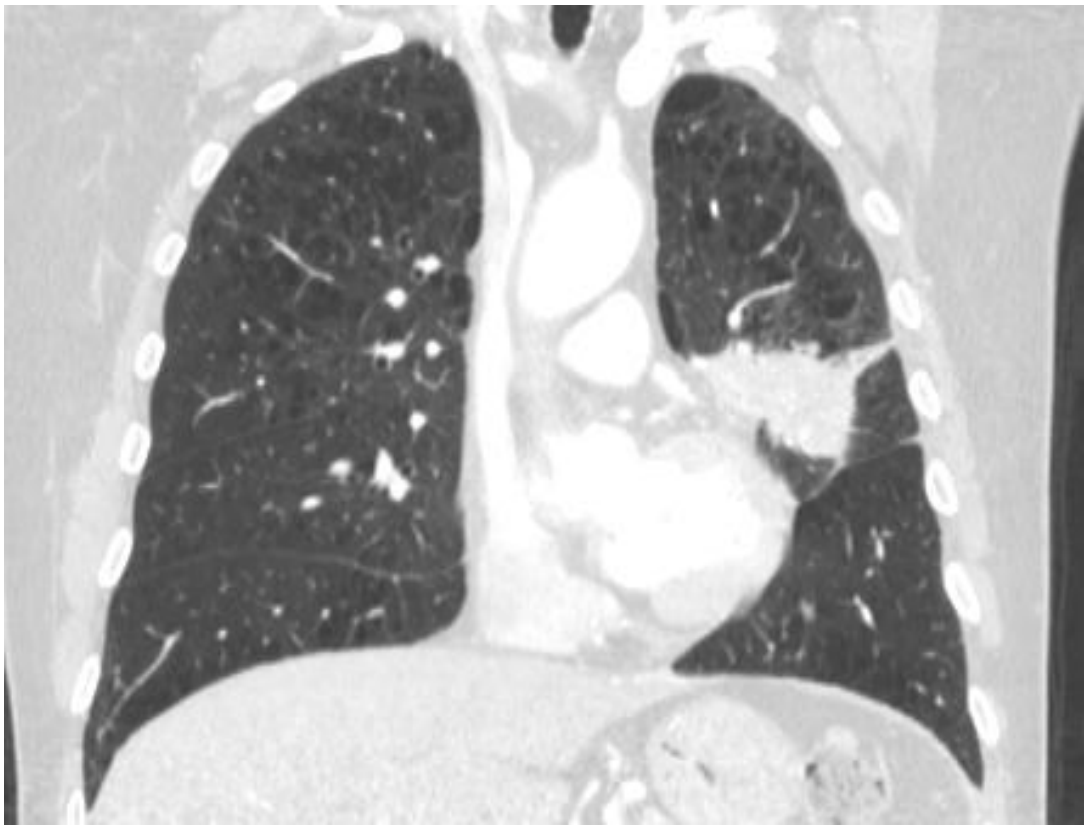

Figure 1C

119x79mm (300 x 300 DPI)

## 6.3 Artigo 3

# *Pneumocystis* choroiditis: The First Case in Southern America

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## *Pneumocystis* choroiditis: The First Case in Southern America

Dear Editor,

We introduce a case of a 38-year-old Brazilian man, diagnosed with human immunodeficiency virus (HIV), presenting blurred vision and increase of conjunctival mass of the left eye. He was ineffective with antiretroviral therapy and had many comorbidities. Viral load was noted to be 83.714 copies/mL and lymphocytes CD4 T cell count was 3 cel/µL. In ophthalmic consultation he was presented cloud vision and conjuctival mass in the left eye that was growing, uncorrected visual acuity (UCVA): 20/40 (-1), best corrected visual acuity (BCVA): 20/40 (+2), presence of peripheral artery disease (PAD), intraocular pressure (IOP): 8mmhg and in anterior chamber mass of 5mm x 2mm.

It was made an incisional biopsy; the diagnosis was made by microscopy for *Pneumocystis jirovecii* and for *Histoplasma capsulatum* by microscopy and culture. Moreover, in order to identify cystic *P. jirovecii* forms and, an immunohistochemical assay was performed (Figure 1) and it confirmed the diagnosis of cystic *Pneumocystis* choroidopathy form yeast-like organisms typical of *H. capsulatum*. Chest radiography and Mantoux test were reported to be normal and venereal disease research laboratory (VDRL) was reagent. The patient was treated with trimethoprim-sulfamethoxazole (TMP-SMZ) for 21 days and intracameral amphotericin B with a total cumulative dose of 500mg. After 4 months, the patient has no iris lesion or other abnormalities.

*P. jirovecii* is considered, up to now, a non-culturable microorganism. *Pneumocystis* pneumonia (PcP) is common in immunodepressed patients and it is

caused by yeast-like fungus called *P. jirovecii* (1). The *P. jirovecii* infection primarily affects the lungs, but extrapulmonary dissemination to other organs, including eyes can occur. To our knowledge, this is the first atypical case of *P. jirovecii* choroiditis in anterior chamber lesion in Southern America.

It is estimated that in the pre highly active antiretroviral therapy (HAART) era, 1% of acquired immunodeficiency syndrome (AIDS) patients with a lymphocytes CD4 T cells below 200 cells/L had *Pneumocystis* choroiditis (2). Patients infected by the HIV frequently have ocular problems.

The incidence of *Pneumocystis* choroidopathy cases was higher in past, because the use of aerosolized pentamidine as prophylaxis for *P. jirovecii* pneumonia (3, 4). Currently there is a different scenario with the TMP-SMZ therapy for the PcP and also responds to treat for *Pneumocystis* choroiditis (2) and could explain the decline of the disease nowadays.

Furthermore, in opportunistic infections an early ophthalmic examination must be performed (5, 6) to access appropriate systemic therapy before widely disseminated infection results in bad outcome.

**Conflict of interest**

The authors declare no conflict of interest.

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**Figure 1**

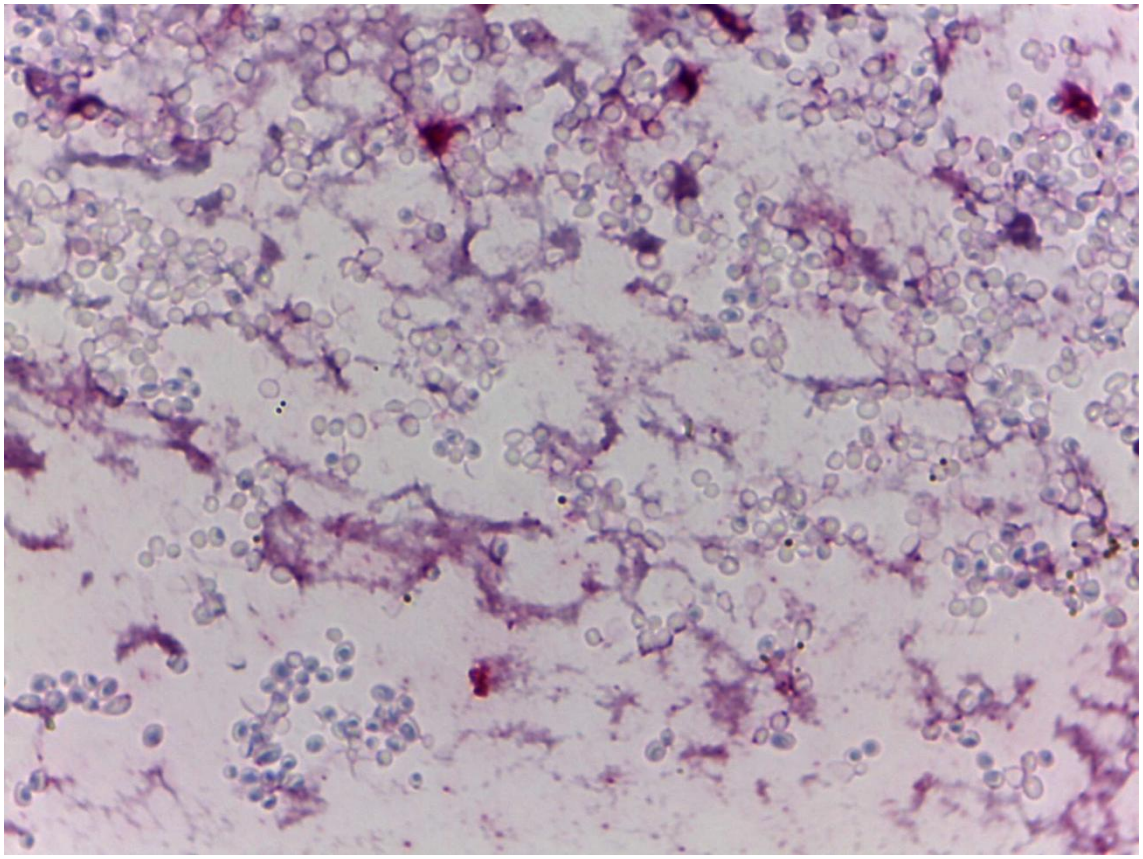

**Figure 1.** Imaging of cystic *P. jirovecii* form and yeast-like organisms typical of *H. capsulatum* by immunohistochemical assay using an anti-*Pneumocystis* mouse monoclonal antibody IgM (*Pneumocystis carinii* (3F6): sc-57980 SANTA CRUZ BIOTECHNOLOGY, INC.) directed against a carbohydrate epitope expressed in *P. carinii* for detection of a 82 kDa glycoproteins. Formalin fixed, paraffin embedded sections of the tissue were incubated overnight in a 1:100 dilution of the antibody preparation, and then stained following a streptavidin-biotin immunoperoxidaseprotocol using Nova Red (Vector Labs, Burlingame, CA) as chromogen.

## 7 Conclusão

No presente estudo foram encontrados dez pacientes com infecção confirmada por *P. jirovecii*, dentre os quais, sete com infecção simultânea de *C. neoformans* e três com infecção por *C. gattii* (todos os pacientes infectados com o HIV e com valores de CD4+ inferiores a 200 cel/µL). Este número corresponde a 10% das coinfecções da casuística de pneumocistose e 1% da casuística de criptococose da ISCMPA. O diagnóstico das doenças foi simultâneo durante a internação. Dois pacientes com meningite por *C. neoformans* não se apresentavam sintomáticos durante a internação. A mortalidade ocorreu em 30% dos casos, sendo todos os pacientes diagnosticados com infecção por *C. gattii*.

Em relação à avaliação de padrões de imagem, foi realizado um estudo sobre a casuística de pacientes confirmados com infecção por *C. gattii a*ssociando achados clínicos e de imagem por tomografia computadorizada e fatores de risco relacionados ao óbito.

Os fatores que aumentam o risco de mortalidade nesta população foram identificados para idade ≥ 50 anos e pacientes imunocomprometidos.

Relata-se neste trabalho o primeiro caso de Coroidite por *Pneumocystis* na América do Sul: após quatro meses de tratamento com TMP-SMZ e anfotericina B o paciente apresentou regressão da lesão de íris e não teve outras anormalidades oculares.

## 8 Considerações finais

*Recomendamos para pacientes com diagnóstico de HIV na internação hospitalar com apresentação de valores de CD4+ inferiores a 200 cel/µL e achados imagéticos com infiltrados pulmonares difusos, independentemente de sua apresentação clínica (inclusive se assintomáticos), a investigação para infecções fúngicas por pesquisa de fungos pela prata, cultivo, pesquisa de testes de aglutinação em latex ou imunocromatográfico (padronizado), microscopia e imunodifusão.*

## Anexo 1 – Carta convite do orientador no exterior

Liverpool Heart and Chest Hospital NHS
NHS Foundation Trust

Klaus Irion, FRCR, PhD
Department of Radiology Lead
Consultant Thoracic Radiologist
Liverpool Heart and Chest Hospital
Thomas Drive, L14 3PE, Liverpool
- Phone +44 151 6001720

Liverpool 22 February 2014

Mr. Guilherme Watte, MsC
Post-Graduation Program in Respiratory Sciences
The Federal University of Rio Grande do Sul
Pulmonary Hypertension Research Centre

Dear Mr Watte

Thank you for your letter describing yours professional and research activities.

We are pleased to know that you have a strong background on public health studies, as we have many research opportunities in this field, particularly on lung cancer and COPD. We are also pleased to learn that you have a special interest on infectious diseases. We have good links with the Department of Tropical Medicine from Liverpool University, which will allow expanding our researches in the field.

I have a strong admiration for your mentors Prof. Luiz Carlos Severo, Prof. Jose da Silva Moreira and Prof. Bruno Hochhegger and it will be a great pleasure to participate as co-supervisor of your PhD thesis.

Your Research plans have been approved, and we would be pleased for you to start your activities in September 2014. We expect that your project should be completed by August 2015. The chronogram of activities you submitted is appropriate and I have approved the length of your project and the number of periods you are planning to return to Liverpool for completion of your studies.

I note that you have proficiency in English Language, which is more than satisfactory for your activities at our department.

It will be a great pleasure and honour having you under my co-supervision and as Research Fellow at our institution.

Best regards

Dr. Klaus Irion, MD, PhD, FRCR, FBIR

*(Electronically signed PDF document)*

## Anexo 2 – Parecer final do orientador no exterior do período no exterior

Liverpool Heart and Chest Hospital NHS
NHS Foundation Trust

Klaus Irion, FRCR, PhD
Consultant Chest Radiologist
Liverpool Heart and Chest Hospital
Thomas Drive, L14 3PE, Liverpool
Klaus.irion@lhch.nhs.uk - Phone +44 151 6001720

Liverpool, 28 September 2015

To the CAPES Foundation, Ministry of Education of Brazil

This letter is to confirm that Mr. Guilherme Watte, M.Sc., from the Post-Graduation Program in Respiratory Sciences of the Federal University of Rio Grande do Sul, has performed all activities scheduled in its work plan under my supervision at Liverpool Heart and Chest Hospital, as part of his PhD thesis under supervision by Professor Luiz Carlos Severo and myself.

It has been a great pleasure to supervise this very intelligent and dedicated Ph.D. Student. Mr. Watte demonstrated an excellent commitment to research and education during this year of his tenure under my supervision. He excelled the expectations, by extending also his activities to other projects and by publishing several papers during the last 12 months. I am impressed with his courtesy and professionalism to all those who requested his support in epidemiology and biostatistics. Mr. Watte has also demonstrated to be very approachable and respectful to colleagues and other staff from all levels.

I am very confident that Mr. Watte will have a brilliant career as a researcher and as an educator, and I am grateful and proud for the opportunity of being his co-supervisor.

If you need and further information, please do not hesitate contacting me on my mobile +4407901545651 or at my hospital extension +01516001720 (LHCH) or +4401517065754 (RLBUTH).

Dr. Klaus Irion, M.D., Ph.D., F.R.C.R., F.B.I.R.

## Anexo 3 – Carta de apresentação

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

CARTA DE APRESENTAÇÃO

Tenho o prazer de apresentar a candidatura do aluno de doutorado Guilherme Watte, regularmente matriculado no Programa de Pós-Graduação em Ciências Pneumológicas da Universidade Federal do Rio Grande do Sul – UFRGS, para realizar parte de seus estudos através do Programa de Doutorado Sanduíche no Exterior – CAPES a convite do Professor Klaus Loureiro Irion do Liverpool Heart and Chest Hospital, NHS Foundation Trust, LHCH, Grã-Bretanha, no período de setembro de 2014 a agosto de 2015, como parte dos estudos que desenvolve no Brasil com a tese intitulada *"Associação da Pneumocistose e Criptococose em Pacientes Imunocomprometidos. Estudo em Serviço Especializado"*.

A participação nesta pesquisa terá impacto direto no projeto do doutorado realizado na UFRGS, mediante ao contexto do tema estudado apresentar aspecto metodológico limitado, distinto e de letalidade na população acometida, podendo traduzir em novas propostas de tratamento para nosso país.

Assumo o compromisso de manter a orientação e o acompanhamento do estudante, durante o período de realização do estágio no exterior, em conjunto com o coorientador da instituição estrangeira, na condução das atividades propostas no plano e cronograma ora aprovados, envidando esforços para que o estudante apresente o empenho desejado, visando tornar proveitosas as atividades desenvolvidas no exterior, que serão avaliadas por meio de relatórios periódicos.

O aluno já possui o número de créditos suficientes para a conclusão do curso em tempo hábil e assumo também a responsabilidade de realçar a relevância de atendimento ao prazo regulamentar de defesa da tese.

Porto Alegre, 27 de fevereiro de 2014.

Prof. Luiz Carlos Severo

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS PNEUMOLÓGICAS
UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
Rua Ramiro Barcelos 2400, 2º andar – CEP: 90035-003 – Porto Alegre – RS – BR
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## Anexo 4 – Termo de seleção de candidatura

**PARA USO DA COORDENAÇÃO DO PROGRAMA**

CAPES

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES
SBN, Quadra 02, Lote 06, Bloco L
70.040-020 Brasília – DF

**TERMO DE SELEÇÃO DE CANDIDATURA AO PDSE**

INSTITUIÇÃO: Universidade Federal do Rio Grande do Sul

PROGRAMA: Programa de Pós-Graduação em Ciências Pneumológicas

DATA: 14/03/2014 LOCAL: Sala de Reuniões – FAMED – UFRGS

**COMISSÃO (Indicar todos os membros da Comissão, inclusive o participante externo*)**

| NOME | INSTITUIÇÃO | CARGO/FUNÇÃO | ASSINATURA |
|---|---|---|---|
| 1. Prof. Sydney Hartz Alves | UFSM | Professor | |
| 2. Prof. José da Silva Moreira | UFRGS | Professor | |
| 3. Prof. João Carlos Prolla | UFRGS | Professor | |
| 4. Elenara da Fonseca Andrade Procianoy | UFRGS | Discente | |

**PARECER FINAL JUSTIFICANDO A ESCOLHA DO CANDIDATO SELECIONADO**

NOME DO(S) CANDIDATO(S): **Guilherme Watte**

PARECER: O Aluno de doutorado acima apresenta os requisitos e a documentação necessária para a candidatura ao PDSE. Assim, confirmamos sua plena qualificação, mediante a comprovação prévia do seu desempenho acadêmico e do seu excepcional potencial científico para o desenvolvimento dos estudos propostos no exterior. Há compatibilidade do plano de suas atividades no exterior com o projeto de tese e sua exequibilidade dentro do cronograma previsto. Verificamos que existe adequação da Instituição de destino e da capacidade técnica científica do co-orientador estrangeiro.

A parceria com o Liverpool Heart and Chest Hospital, Grã-Bretanha contribuirá para reforçar linhas de pesquisa já existentes no Programa de Ciências Pneumológicas da UFRGS e produzirá conhecimento científico relevante nas áreas de Métodos Diagnósticos em Pneumologia: Imagética, Microbiologia, Anatomia Patológica, Recursos e Computação Gráfica e de Doenças Infecciosas e Parasitárias do Pulmão, Vias Aéreas e Pleura.

**Atenção:** para mudar de campo pressione a tecla TAB ou clique com o mouse no campo desejado. **Favor não utilizar a tecla ENTER.**
*** Participante Externo deve ser Doutor e não possuir qualquer vínculo com a Instituição de Ensino Superior da seleção.**

## Anexo 5 – Parecer final do orientador no Brasil do período no exterior

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PARECER ORIENTADOR BRASILEIRO

Tenho o prazer de informar a CAPES que o aluno de doutorado Guilherme Watte, regularmente matriculado no Programa de Pós-Graduação em Ciências Pneumológicas da Universidade Federal do Rio Grande do Sul – UFRGS realizou esplendidamente o período sanduíche de seus estudos realizados através do Programa de Doutorado Sanduíche no Exterior a convite do Professor Klaus Loureiro Irion no Liverpool Heart and Chest Hospital, NHS Foundation Trust, LHCH, Grã-Bretanha, no período de setembro de 2014 a agosto de 2015.

Foi contemplada plenamente a condução das atividades previstas no cronograma de atividades, bem como, manutenção da orientação e o acompanhamento do estudante, durante o período de realização do estágio no exterior, em conjunto com o coorientador da instituição estrangeira.

O estudante apresentou como já se era esperado empenho elevado durante todo período de estágio, sendo de fato um momento muito proveitoso de compartilhamento de saberes entre os centros.

Porto Alegre, 30 de setembro de 2015.

Prof. Luiz Carlos Severo

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## Anexo 6 – Parecer final PPGCP/UFGRS do período no exterior

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Parecer do Coordenador

Venho, através deste documento, comunicar que o aluno de Pós-Graduação do Programa em Ciências Pneumológicas da Universidade Federal do Rio Grande do Sul, Guilherme Watte, realizou seu período de Programa de Doutorado Sanduíche no Exterior no Liverpool Heart and Chest Hospital, NHS Foundation Trust, LHCH, Grã-Bretanha, no período de setembro de 2014 a agosto de 2015.

Durante esse período, o aluno desenvolveu as atividades de pesquisa propostas com o professor Klaus Loureiro Irion. O aluno cumpriu o cronograma de atividades previsto e teve empenho elevado durante o período do estágio.

Esse estágio vincula nosso programa a um dos maiores centros de pesquisa europeu e permitirá o desenvolvimento de novos projetos conjuntos.

Porto Alegre, 12 de Novembro de 2015

Prof. Paulo de Tarso Roth Dalcin
Coord. do PPG - Ciências Pneumológicas
UFRGS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS PNEUMOLÓGICAS
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